Defence gene expression and phloem quality contribute to mesophyll and phloem resistance to aphids in wild barley

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Title:

Defence gene expression and phloem quality contribute to mesophyll and phloem resistance against aphids in wild barley

Highlight:

Wild barley partial-resistance against aphids resides in the mesophyll and phloem tissue, and is likely associated with higher basal defence and phytohormone gene expression and altered phloem amino acid composition.

Abstract:

Aphids, including the bird cherry-oat aphid (*Rhopalosiphum padi*), are significant agricultural pests. The wild relative of barley, *Hordeum spontaneum* 5 (Hsp5) has been described to be partially-resistant against *R. padi,* with this resistance proposed to involve higher thionin and lipoxygenase gene expression. However, the specificity of this resistance against aphids and its underlying mechanistic processes are unknown. In this study, we assessed specificity of Hsp5 resistance against aphids and analysed differences in aphid probing and feeding behaviour on Hsp5 and a susceptible barley cultivar (Concerto). We found that partialresistance in Hsp5 against *R. padi* extends to two other aphid pests of grasses. Using the electrical penetration graph technique we show that partial-resistance is mediated by phloem- and mesophyll-based resistance factors that limit aphid phloem ingestion. To gain insight into plant traits responsible for partial-resistance, we compared non-glandular trichome density, defence gene expression, and phloem composition of Hsp5 with the susceptible barley cultivar Concerto. We show that Hsp5 partial-resistance involves elevated basal expression of thionin and phytohormone signalling genes, and a reduction in phloem quality. This study highlights plant traits that may contribute to broad-spectrum partial-resistance against aphids in barley.

Keywords

Amino Acid Composition, Defence gene expression, Electrical Penetration Graph (EPG), *Hordeum spontaneum*, Partial-resistance, *Rhopalosiphum padi*, *Sitobion avenae*, *Utamphorophora humboldti*

Abbreviations

ABA: Abscisic Acid

Ala: Alanine

Arg: Arginine

Asn: Asparagine

Asp: Aspartic Acid

Concerto: *Hordeum vulgare* cv. Concerto

EPG: Electrical penetration graph

ET: Ethylene

FTIR: Fourier Transform Infrared

Gln: Glutamine

Glu: Glutamic Acid

Gly: Glycine

His: Histidine

Hsp5: *Hordeum spontaneum* 5

Ile: Isoleucine

JA: Jasmonic Acid

Leu: Leucine Lys:

Lysine:

Met: Methionine Phe: Phenylalanine r_m:

the intrinsic rate of population increase

SA: Salicylic Acid

Ser: Serine

Thr: Threonine

Trp: Tryptophan

Tyr: Tyrosine

Val: Valine

Introduction

Barley, *Hordeum vulgare,* is the fourth most agriculturally important cereal by production quantity (Newton et al., 2011). Aphids are major pests of cereals and feed directly from plant phloem using specialised mouthparts known as stylets (Auclair, 1963; Guerrieri and Digilio, 2008). Aphid feeding causes direct plant damage by ingesting phloem sap and indirect damage by transmission of economically important plant viruses (Guerrieri and Digilio, 2008), including *Barley Yellow Dwarf Virus* (*BYDV*). Virus infection can cause yield losses of up to 30% in barley and 80% in wheat (Smith and Sward, 1982; Perry et al., 2000; Murray and Brennan, 2010).

Aphids are primarily controlled by insecticides and as a consequence insecticide use is increasing (Jess et al., 2018), leading to the emergence of insecticide-resistant aphid populations (Chen et al., 2007; Foster et al., 2014), negative impacts on non-target organism (Unal and Jepson, 1991; James et al., 2016), and a potential increase in *BYDV* prevalence (Dewar and Foster, 2017). This has resulted in legislative restrictions on insecticide use and promotion of integrated pest management (Directive, 2009/128/EC). Consequently, there is a growing requirement for more sustainable pest management solutions, and improving crop resistance against aphids is one avenue which could be explored to achieve this.

Plant resistance traits against insect pests can be allocated to one of three categories: 1) chemical deterrence of insect settling and feeding; 2) physical barriers to insect feeding; and 3) reduction in palatability (Mitchell et al., 2016). Partial-resistance against aphids has been associated with each of these categories (respectively, Gibson and Pickett (1983), Tsumuki et al. (1989) and Greenslade et al. (2016)). Aphid resistance in cereals, and many other plant species, is partial in that aphid colonisation is reduced but not completely abolished. Full and partial-resistance can be mediated by Resistance genes (R), which function in a gene-for-gene manner through recognition of specific aphid biotypes within a species. For example, resistance in tomato against the potato aphid, *Macrosiphum euphorbiae,* has been linked to the R gene *Mi-1* (Kaloshian et al., 1995; Cooper et al., 2004) and *RAP1* in *Medicago truncatula* confers resistance against the pea aphid, *Acyrthosiphon pisum* (Kanvil et al., 2015). In cereals, *Dn* and *RA* R genes confer effective resistance against the Russian wheat aphid, *Diuraphis noxia,* and *S. avenae,* respectively (Liu et al., 2001; Liu et al., 2012; Li et al., 2018).

Inherent molecular defences also play a key role in conferring resistance against aphids (Smith and Boyko, 2007; Delp et al., 2009; Guan et al., 2015; Escudero-Martinez et al., 2017).

Defences involved in aphid resistance in *Hordeum* spp. include increased expression of thionin (antimicrobial peptides) genes (Delp et al., 2009; Mehrabi et al., 2014; Escudero-Martinez et al., 2017), increased chitinase and β-1,3-glucanase activity (Forslund et al., 2000), and the presence of plant secondary metabolites (Gianoli and Niemeyer, 1998). Plant phythormone signalling pathways, including Abscisic Acid (ABA), Salicylic Acid (SA), Jasmonic Acid (JA) and Ethylene (ET) signalling, mediate coordinated molecular responses to herbivory via the regulation of defence signalling genes and the biosynthesis of defensive allelochemicals (Smith and Boyko, 2007; Bari and Jones, 2009; Morkunas et al., 2011; Foyer et al., 2016); higher constitutive expression of phytohormone signalling genes can lead to improved resistance against aphids in cereals (Losvik et al., 2017). With a lack of full resistance, especially in cereal crops, the use of partial-resistances to provide crop protection is a powerful approach (Broekgaarden et al., 2011; Dempewolf et al., 2014).

Wild relatives of modern crops feature many resistance traits against herbivorous pests that have been lost during domestication (Moreira et al., 2018). Screening crop wild relatives for (partial-)resistance against aphids provides an opportunity to identify potentially beneficial traits (Zhang et al., 2017) for introduction into agricultural cultivars (Xu et al., 2015; Li et al., 2018; Arora et al., 2019). Screening of wild relatives in wheat (Xu et al., 2015; Aradottir et al., 2017) and maize (Maag et al., 2015) has identified partial-resistance traits associated with reduced palatability and elevated levels of secondary metabolites (Barria et al., 1992; Ahmad et al., 2011; Greenslade et al., 2016; Chandrasekhar et al., 2018; Li et al., 2018). In crops such as wheat, maize, potato, and tomato, partial-resistance has been associated with both singular and interacting epidermal- mesophyll- and phloem-based resistance factors (Alvarez et al., 2006; Greenslade et al., 2016; Machado-Assefh and Alvarez, 2018). The underlying mechanisms of these resistances can involve factors based at the leaf epidermis such as leaf trichomes (Glas et al., 2012) and waxes (Tsumuki et al., 1989; Agrawal et al., 2009), factors residing in the leaf tissue such as allelochemicals (Ahmad et al., 2011; Betsiashvili et al., 2015), elevated phytohomone signalling (Louis et al., 2015), and elevated defence gene expression (Zhai et al., 2017), as well as phloem-based factors:, including reduced phloem quality (Greenslade et al., 2016). Despite this recent progress, our understanding of partial-resistance against aphids in many key crops, including barley, is limited and requires further exploration.

Partial-resistance against *R. padi* was identified in the barley wild relative *H. spontaneum* 5 (Hsp5) (Åhman et al., 2000; Delp et al., 2009). Although Hsp5 features higher tissue concentrations of indole alkaloid gramine, a plant defensive compound, this did not correlate with increased partial-resistance (Åhman et al., 2000). Instead, partial-resistance in Hsp5 is thought to involve increased expression of plant defence genes (Delp et al., 2009; Mehrabi et al., 2014), including higher constitutive expression of thionin genes (Delp et al., 2009) and higher expression of a proteinase inhibitor (Mehrabi et al., 2014). Initial attempts have been made to characterise the molecular processes contributing to partial-resistance in Hsp5 (Delp et al., 2009), with the underlying mechanism(s) of resistance and the tissue location of these resistance factors remaining to be elucidated.

The primary aim of this study was to characterise the plant traits and mechanisms contributing to partial-resistance in Hsp5. To date, Hsp5 partial resistance was reported to be effective against one aphid species, *R. padi*. Here, we show that Hsp5 resistance is broad and effective against two additional aphid species, *S. avenae* (a cereal pest) and *Utamphorophora humboldti* (an invasive species). To investigate where in the plant tissue partial-resistance factors may reside we monitored *R. padi* probing and feeding behaviour on Hsp5 and compared this with aphids feeding on susceptible barley cultivar Concerto using the electrical penetration graph (EPG) technique. This revealed that resistance factors predominantly reside in the mesophyll layers and the phloem. Characterisation of leaf surface architecture, defence and phytohormone signalling, and amino acid composition linked Hsp5 resistance with higher basal gene expression of JA, ABA, and ET signalling markers and defensive thionin genes as well as to reduced availability of essential amino acids in the phloem. Our work highlights the involvement of complex defence mechanisms against aphid pests in wild barley that involves multiple components in different plant tissues and identifies several traits which could be exploited to improve barley resistance against aphids.

Materials and methods

Plant growth and insect rearing conditions

Hordeum vulgare Linnaeus cv. Concerto (Concerto) and *H. spontaneum* 5 Linnaeus (Hsp5) seeds were surface-sterilised by washing in 2% (v/v) hypochlorite and rinsing with d.H_2O. Seeds were kept moist in the dark: Hsp5 seeds were incubated at 4^{o}C for 14 days and Concerto

seeds were kept at room temperature for 48h. Germinated seedlings were planted into a bulrush compost mix (Bulrush, Northern Ireland) and grown under glasshouse conditions with a 16:8h

214 (L:D), light intensity (Photosynthetically Active Radiation: PAR) was a minimum of 150 215 µmol/m^{-2} s^{-1} (facilitated using supplementary lighting when required) and a maximum 400 216 µmol/m^{-2} s^{-1} (with screens employed when light intensity exceeded this), and a 20:15°C 217 day:night temperature. Plants were grown until the first true leaf stage (1.1 – 1.2 on the Zadoks 218 et al. (1974) growth staging key).

219 Asexual laboratory cultures of the bird cherry-oat aphid, *Rhopalosiphum padi* (Linnaeus)
220 (genotype B; Leybourne et al. (2018)), the English grain aphid, *Sitobion avenae* Fabricius and
221 the American grass leaf aphid, *Utamphorophora humboldti* Essig were established from 222 individual apterous adults collected from Dundee, UK. Molecular barcoding of the cytochrome 223 oxidase subunit I gene (Folmer et al., 1994) was used to confirm identity of aphid species.
224 Aphid cultures were reared in controlled growth rooms on one week old barley seedlings (cv.
225 Optic) contained in ventilated cups at 20°C, 16:8h (L:D) with PAR 150 µmol/m^{-2} s^{-1}.

226 ***Insect fitness measurements***

227 The performance of all aphid species was assessed in glasshouse conditions (described above) 228 on Hsp5 and Concerto (n = 12) in a randomised block design (each block contained one
229 replicate of each treatment combination). Plants were infested with a single apterous aphid
230 which was allowed to reproduce overnight, then a total of three nymphs were retained on each 231 plant. Nymph mass and survival was recorded at 72h and 168h, aphids were manipulated using
232 a fine horsehair brush and a microbalance (Mettler Toledo MX5, Mettler Toledo, UK) was
233 used to measure aphid mass. After the 168h measurement a random single nymph was returned 234 to the plant; for this nymph, data were collected on the length of the pre-reproductive period 235 (d) and the intrinsic rate of population increase (r_m). Aphids were caged onto the first fully 236 expanded true leaf using Perspex clip-cages of 25mm internal diameter (MacGillivray and 237 Anderson, 1957). Nymph mass gain was calculated as the change in mass between 168 and
238 72h and aphid r_m was calculated using the equation of Wyatt and White (1977): $rm = \frac{\ln(Fd)}{d}$
239 0.74 , where d is the time period between birth and production of first progeny, and Fd
240 is the total progeny produced over a time period equal to d.

241 ***Electrical penetration graph (EPG) monitoring of aphid feeding***

242 The DC-EPG technique (Tjallingii, 1978; Tjallingii, 1988; Tjallingii, 1991; Tjallingii, 2001)

was employed to monitor the probing and feeding behaviour of adult apterous *R. padi* (approximately 7-10 days old) over a 6h period using a Giga-4 DC-EPG device (EPG Systems,

The Netherlands) on plants at the true-leaf stage. A plant probe (copper rod approximately 50 mm long x 5 mm diameter), was soldered to electrical wire extending from the plant voltage output of the Giga-4 device and inserted into the plant soil. An aphid probe was made by soldering a piece of copper wire (30 mm long x 2 mm diameter) to a brass pin (tip diameter 2 mm). Approximately 30 mm of gold wire (20 µm diameter; EPG Systems, The Netherlands) was adhered to the copper end of the aphid probe using water-based silver glue (EPG Systems, The Netherlands) and aphids were connected by adhering the free end of the gold wire onto the aphid dorsum using the same water-based adhesive. Wired aphids were connected to the Giga-4 device by placing the end of the brass pin into the EPG probes with a 1 GΩ input resistance and a 50x gain (Tjallingii, 1988). The order in which *R. padi* – plant combinations were tested and allocated to an EPG probe was randomised. Data were acquired using Stylet+D software (EPG Systems, The Netherlands). A total of 18 and 16 successful recordings (recordings where aphids were probing into plant tissue for at least 80% of the total recording time) were made for aphids feeding on Concerto and Hsp5, respectively. All EPG recordings were obtained within a grounded Faraday cage.

EPG waveforms were annotated using Stylet+A software (EPG Systems, The Netherlands). Waveforms were annotated by assigning waveforms to np (non-probing), C (stylet penetration/pathway), pd (intercellular punctures), E1 (saliva secretion into phloem), E2 (saliva secretion and passive phloem ingestion), F (penetration difficulty) or G (xylem ingestion) phases (Tjallingii, 1988; Alvarez et al., 2006). No E1e (extracellular saliva secretion) phases were detected. Annotated waveforms were converted into time-series data using the excel macro developed by Dr Schliephake (Julius Kühn-Institut, Germany).

Determination of non-glandular trichome density

Non-glandular trichome densities of Hsp5 and Concerto (n = 9) were determined using polarised light microscopy following a procedure adapted from Pomeranz et al. (2013). Briefly, the first true leaf was excised from plant stems and treated to two incubation steps to clear the leaf epidermis. Firstly, leaf area was measured and leaves were soaked in 96% Ethanol (SigmaAldrich, UK) for 48h before being treated with 1.25 M NaOH:EtOh (1:1, v:v) at 70ºC for 2h. Leaves were stored in 50% glycerol prior to trichome visualisation; if NaOH:EtOH treatment did not clear the leaf epidermis leaves were further treated with 80% lactic acid for 6h. To analyse trichome density samples were placed adaxial side up on microscope slides (75 x 25 mm; Corning, UK). A polarising light microscope was created using two 50 mm^2

polarising filters (Sigma-Aldrich, UK), one placed below the stage of a stereo-microscope but above the light source and the second attached below the objective lens. Non-glandular trichomes appeared illuminated under the polarised microscope setup and the number of trichomes per unit area were counted manually. To allow correlation of trichome density with aphid performance, an additional *R. padi* performance experiment (as described above) was carried out on these plants prior to trichome density analysis.

Epicuticular wax analysis

A Fourier Transform Infrared (FTIR) spectrometer (Bruker Vertex 70 FTIR spectrometer, Bruker, Ettlingen, Germany), incorporating a Diamond Attenuated Total Reflection (DATR) sampling accessory, was used to identify the functional groups present in chemical extracts from the surfaces of Hsp5 and Concerto leaves. The waxes from upper and lower leaf surfaces of the first true leaf (n = 4) were extracted with dichloromethane (DCM) by running approximately 1-2 ml of DCM along the leaf surface directly onto the diamond window of the ATR sampling accessory; after evaporation of the DCM, FTIR spectra were recorded of the films deposited on the DATR. Signal-to-noise ratio was enhanced by taking 200 scans for each sample and averaging to obtain a single spectrum. The spectral range scanned was 4000 cm^{-1} to 400 cm^{-1} and a background reading was taken before each sample was analysed.

Analysis of phloem amino acid composition

Hsp5 and Concerto plants were grown under glasshouse conditions (as described above) in a temporally-split randomised block design: three temporal blocks, one initial temporal block comprising two replicate sub-blocks, and two further temporal blocks each comprising four replicate sub-blocks. Each sub-block contained a single replicate of every treatment combination. Plants at the first true-leaf stage were infested with either ten seven day old apterous *R. padi* adults caged onto the plants with microperforated bags (Polybags, UK) or aphid-free bagged controls. Samples were collected at 0 and 24h post-aphid infestation. Phloem sap was collected using the method of King and Zeevaart (1974); leaves (*n = 10*) were excised from the stem by cutting at the base of the leaf blade at the petiole, placed in a dish containing 1 mM EDTA (Sigma-Aldrich, UK) solution and immediately re-cut. The cut surface of the leaf was placed into 200µl of filter-sterilised 1mM EDTA solution (pH 7.5 with NaOH) in a 2 mL Eppendorf™ microcentrifuge tube and incubated for 1.5h at room temperature in a darkened exudation chamber (a polystyrene box containing a dish of 6 M K_2HPO_4 (Sigma-Aldrich, UK),

and moistened paper towels to maximise humidity and decrease leaf transpiration). After incubation, leaves were removed and the samples of EDTA solution containing exuded phloem sap were stored at -80^{o}C until analysis.

Reverse-phase HPLC was used to separate phloem amino acids Asp, Glu, Asn, His, Ser, Gln, Arg, Gly, Thr, Tyr, Ala, Trp, Met, Val, Phe, Ile, Leu and Lys using an Agilent HP1100 series auto-sampling LC system equipped with a ZORBAX™ Eclipse AAA column and fluorescence detector. Amino acids were derivatised using *o*-phthaldialdehyde (Sigma-Aldrich, UK) following the method developed by Jones et al. (1981); all protein amino acids except proline and cysteine could be detected by this method, with a detection limit of approximately 0.5 pmol. Samples were processed in their experimental blocks along with blanks (1 mM EDTA solution that was not exposed to an excised leaf) as controls. Amino acids were identified and quantified by comparison with known concentrations of amino acid standards (AA-S-18 (Sigma, UK), supplemented with Asp, Glu and Trp).

RT-qPCR analysis of plant defence genes

Five sub-blocks over the three temporal blocks (one from the first block and two from each subsequent temporal block) were chosen at random from the phloem exudation experiment described above and leaf tissue was sampled for gene expression analysis. Approximately 25mm length of leaf tissue was cut from the apex of the true leaf and flash-frozen in liquid nitrogen immediately prior to the excision of the leaf for phloem exudation. Plant material was stored at -80^{o}C until RNA extraction. RT-qPCR experiment design followed the MIQE guidelines (Bustin et al., 2009).

Leaf samples were ground to a fine powder under liquid nitrogen using a mortar and pestle and total RNA was extracted with the Norgen Plant/Fungi RNA Extraction Kit (Sigma-Aldrich, UK) following the manufacturer's protocol with additional DNAse I treatment (Qiagen, UK); RNA quality and quantity was assessed with a NanoDrop® ND-1000 Spectrophotometer (ThermoFischer, UK). cDNA synthesis was carried out with approximately 1000 ng RNA using the SuperScript® III cDNA synthesis kit (Sigma-Aldrich, UK), following the manufacturer's protocol. RT-qPCR primers were designed using the Roche Universal Probe Library Assay Design Centre. Reference gene primers were described by Hua et al. (2015). All primer sequences are shown in Supplementary Table 1 and were supplied by Sigma-Aldrich,

UK. Primers were validated for PCR efficiency prior to use, and reference gene primers were tested for stability across all treatments following the geNorm procedure (Vandesompele et al., 2002). Gene expression analysis used SYBR® Green chemistry with GoTaq® qPCR Master Mix (Promega, UK) on a StepOne™ Real-Time PCR Machine (Applied Biosystems, UK). Reactions were carried out in 12.5 µl reactions with a final concentration of 1x GoTaq® qPCR Master Mix, 1µM of each primer, 1.4 mM $MgCl_2$, 2.4 µM CXR reference dye and a cDNA quantity of approx. 12.5 ng (assuming 1:1 RNA:cDNA conversion). The qPCR conditions were as follows: 95ºC for 15 mins followed by 40 cycles of denaturing for 15s at 95ºC, annealing for 30s at 60ºC, and 30s at 72ºC for DNA extension. Fluorescence was recorded at the end of each annealing cycle and a melting curve was incorporated into the end of the qPCR programme. Data were normalised to the geometric mean expression of two reference genes, *HvCYP* (AK253120.1) and *HvUBC* (AK248472.1), and $2^{-\Delta\Delta Ct}$ methodology (Livak and Schmittgen, 2001) was used to determine differential expression, with Concerto at 0h aphid infestation used as the experimental control treatment. Expression levels at 24h were further normalised to uninfested control plants. Samples were processed in experimental blocks with three technical replicates at the PCR level and five biological replicates per treatment.

Statistical analysis

Statistical analyses were carried out using R Studio v.1.0.143 running R v.3.4.3 (R Core Team, 2014) using packages: car v.2.1-4 (Fox and Weisberg, 2011), coxme v.2.2-7 (Therneau, 2018), dunn.test v.1.3.5 (Dinno, 2017)**,** ggplot2 v.2.2.1 (Wickham, 2009), ggpubr v. 0.1.2 (Kassambara, 2017), ggfortify v.0.4.5 (Tang et al., 2016), lawstat v.3.2 (Hui et al., 2008), lme4 v.1.1-13 (Bates et al., 2015), lmerTest v.2.0-33 (Kuznetsova et al., 2017), lsmeans v.2.2762 (Lenth, 2016), survival v.2.41-3 (Therneau and Grambsch, 2000)**,** survminer v.0.4.2 (Kassambara and Kosinski, 2017), vegan v.2.5-3 (Oksanen et al., 2013).

Nymph mass gain and adult r_m, were modelled using linear mixed effects models with experimental block incorporated as a random factor. ANOVA with type III Satterthwaite approximation for degrees of freedom was used to analyse the final models with calculation of Least Squares Means used for *post-hoc* testing. A Cox proportional hazards regression model was used for nymph survival analysis (Therneau and Grambsch, 2000; Therneau, 2018), incorporating experimental block as a random factor; a χ^2 test was used on the final model. A log-rank test with Benjamini-Hochberg correction was used to carry out pairwise comparisons between aphid-plant combinations. Aphid feeding behaviour was assessed globally by fitting a

permutated MANOVA to the dataset. Response variables with normal data distribution were analysed by either ANOVA or general linear models, while non-normally distributed data were analysed with Kruskal-Wallis tests.

Levene's test (Hui et al., 2008) was used to analyse differences in trichome density; a Kendall's rank correlation tau was then used to test for correlations between trichome density and *R. padi* performance. Differences in leaf surface chemistry were analysed using a Welch two sample *t*-test by comparing the total number of identified functional groups in the two plant species.

Phloem amino acid concentrations (pmol/µl) were expressed as relative amino acid composition by converting into mole % of total amino acid content and subjected to principal component analysis with a correlation matrix. Three principal components explained 69% of observed variation and were analysed by fitting a linear mixed effects model incorporating aphid treatment, plant, time-point, plant x time-point interaction, and leaf subsampling for RNA (to test effect on amino acid composition) as explanatory variables with temporal block, randomised block and HPLC batch as random factors. Final models were analysed with a χ^2 test. Differential gene expression ($2^{-\Delta\Delta Ct}$) data were analysed using Kruskall-Wallis rank-sum tests, with subsequent Dunn's test *post-hoc* analysis of the plant x infestation interaction.

Results

A wild relative of barley exhibits partial-resistance against multiple aphid species

Aphid performance experiments were undertaken to assess whether partial-resistance in Hsp5 against *R. padi* (Delp et al., 2009; Leybourne et al., 2018) extends to other aphid species. We assessed the fitness of *R. padi, S. avenae,* and *U. humboldti* on Hsp5 in comparison with a commercial barley cultivar (Concerto) and found evidence for partial-resistance against all three aphid species. Specifically, nymph survival was significantly lower on Hsp5 ($\chi^2_1 = 10.65$; p = 0.001; Fig. 1A; Table 1); pairwise comparison showed that nymph survival was reduced on Hsp5 for *U. humboldti* (p = <0.001), but not for *R. padi* (p = 0.057) or *S. avenae* (p = 0.973). Furthermore, nymph mass gain ($F_{56,1} = 9.14$, p = 0.003; Fig. 1B; Table 1) and the rate of population increase (r_m) ($F_{54,1} = 27.43$, p = <0.001; Fig. 1C; Table 1) were reduced for all aphid species when feeding from Hsp5. Differences were also detected between aphid species, with *R. padi* exhibiting the highest r_m ($F_{54,2} = 99.10$, p = <0.001; Fig. 1B; Table 1) and *S. avenae* the largest mass gain ($F_{56,2} = 49.82$, p = <0.001; Fig. 1C; Table 1). On average *U. humboldti* was the least fit species (Fig. 1A-C).

Partial-resistance against R. padi involves mesophyll and phloem resistance factors

To elucidate the potential underlying partial-resistance mechanism(s) against aphids in Hsp5 *R. padi* feeding behaviour was monitored using the EPG technique. Overall, the feeding behaviour of *R. padi* differed significantly when feeding on Hsp5 compared with Concerto ($F_{1,33}$ = 2.61; p = 0.022; Fig. 2; Table 2). Aphids showed significant differences in feeding behaviour at the leaf epidermis and within mesophyll tissue (Fig. 3; Table 2). We observed an approximate three-fold decrease in the time to the first epidermal probe when feeding on Hsp5 ($F_{1,33}$ = 7.99; p = 0.008), alongside a shorter duration of the first probe ($F_{1,33}$ = 6.94; p = 0.013) and an increased time to the first sieve element puncture ($F_{1,33}$= 6.33; p = 0.017). Aphids feeding on Hsp5 also showed a two-fold delay in initiating passive phloem ingestion ($\chi^2_{1,33}$ = 4.29; p = 0.038) and a decrease in the number of intracellular punctures observed during the first aphid probe of plant tissue (10 on Hsp5 vs. 24 on Concerto; $F_{1,33}$ = 3.95; p = 0.047; Table 2).

Feeding patterns within the vascular tissue also differed (Fig. 4; Table 2). Aphids feeding on Hsp5 showed delayed initiation of sustained phloem ingestion (ingestion for >10 mins) after the first sieve element puncture ($\chi^2_{1,33}$ = 4.29; p = 0.038). The total length of time aphids spent in the sustained feeding phase was also 2.5x shorter ($\chi^2_{1,33}$ = 7.49; p = 0.006), and the ratio of time aphids spent ingesting phloem relative to the length of time probing plant tissue (ingestion:pathway ratio) was four-fold lower ($\chi^2_{1,33}$ = 7.43; p = 0.006). Furthermore, aphids feeding on Hsp5 spent three times longer ingesting xylem ($\chi^2_{1,33}$ = 5.28; p = 0.022). Table 2 reports additional feeding parameters which highlight further mesophyll and vascular-mediated resistance against *R. padi* in Hsp5. The results for the non-significant parameters are reported in Supplementary Table 2 and the full statistical results for the significant parameters are displayed in Supplementary Table 3.

Leaf surface architecture differs between susceptible and partially-resistant plants.

The leaf surface represents a key interface between plants and insects. Many surface factors, including leaf trichomes and epicuticular waxes, can modulate plant-insect interactions, (Agrawal et al., 2009; Glas et al., 2012; Karley et al., 2016). Non-glandular leaf trichome counts revealed Hsp5 had a significantly higher abundance than Concerto ($F_{1,18}$ = 6.24; p = 0.022; Fig. 5). Trichome abundance showed a negative correlation with *R. padi* fecundity (Z = -3.60; p = <0.001; T = -0.56; Adj-R^2 = 0.68; Supplementary Fig. 1A) but no relationship with nymph

mass gain (Z = -0.29; p = 0.770; T = -0.04; Adj-R^2 = -0.05; Supplementary Fig. 1B). In addition, we found differences in the epicuticular wax composition between the two plants. The complexity of the epicuticular wax differed in that aliphatic hydrocarbons were detected in surface extracts of Hsp5 leaves, two Hsp5 replicates also contained weak ester bands (Supplementary Table 4). In Concerto the chemical mixture contained a more consistent mixture of aliphatic hydrocarbons and more pronounced ester bands, alongside a more complex, but variable, mixture of chemical groups, including carboxylates and amides (t = 4.89; df = 6; p = 0.002). Overall, the results were consistent within Hsp5 but more variable in Concerto (Supplementary Table 4). However, the detection of some Nitrogen-containing functional groups in Concerto leaf extracts could be a result of extracting some components within the upper tissue layers, this potentially highlights differences in the thickness of the wax between Hsp5 and Concerto.

Basal expression levels of thionins and multiple phytohormone signalling genes are elevated in Hsp5

To investigate the level of defence gene expression in Concerto versus Hsp5 we selected marker genes for defensive thionins (*HvTHIO1, HvTHIO2, HvβTHIO*) alongside JA (HvLOXA, *HvLOX2* and *HvJAZ*), SA (*HvNPR1*), ET (*HvERF*), and ABA (*HvA1*) signalling pathways. We assessed expression of marker genes in both plant types constitutively and in response to 24h of aphid infestation. We observed 10-(*HvTHIO1)*, 13-(*HvTHIO)* and 7-fold (*HvβTHIO)* higher expression of the three thionin genes in Hsp5 (Fig 6; Table 3). Thionin gene expression levels remained higher in Hsp5 24h after aphid infestation but were not differentially regulated in either plant in response to aphid infestation (Fig 6; Table 3). In addition, *HvLOXA*, *HvLOX2*, *HvA1*, and *HvERF1*, were more highly expressed in Hsp5, with 3.5-, 6-, 10- and 14-fold higher expression levels, respectively (Fig 6; Table 3).

No genes were differentially expressed in response to aphid infestation. However, several of the phytohormone signalling genes were differentially expressed in response to the plant x aphid infestation interaction; namely *HvLOXA*, *HvLOX2*, *HvNPR1*, and *HvERF1* (Table 3). *HvLOXA* was significantly down-regulated in Hsp5 after 24h aphid infestation to levels similar to those observed in Concerto (Fig. 6; Table 4). For *HvLOX2*, *HvNPR1*, and *HvERF*, expression levels were higher in Hsp5 after 24h of aphid infestation compared with levels in Concerto (Fig. 6; Table 4), and *HvNPR1* levels were significantly lower in Concerto 24 h after aphid

infestation compared with constitutive levels (Fig. 6; Table 4). *HvJAZ* was not differentially regulated in response to any treatment factor.

Phloem amino acid composition in Hsp5 is characterised by a reduction in the proportion of essential amino acids and an increased abundance of asparagine

To investigate phloem factors contributing to partial-resistance against aphids (Fig. 4; Table 2) we analysed the amino acid composition and the proportion of essential and non-essential amino acids in phloem exudates (Fig. 7). Principal component analysis of amino acid composition (mol% of Asp, Glu, Asn, His, Ser, Gln, Arg, Gly, Thr, Tyr, Ala, Trp, Met, Val, Phe, Ile, Leu, and Lys) of phloem exudates revealed that three principal components explained 69% of the observed variation in overall amino acid composition, with all three principle components showing a degree of separation between the two plant types (Table 5). Furthermore, >50% of the variation was explained by the first two principal components (Fig. 7C; Table 5) with separation of Hsp5 from Concerto largely occurring along PC1 due to differences in the relative amounts of Asn and His vs. most other essential amino acids (Fig. 7C). The total percentage of essential amino acids (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val; described in Morris (1991)) was higher in Concerto compared with Hsp5 (18.97% vs 11.45%; $\chi^2_1 = 25.43$; p = <0.001; Fig. 7D; Table 6).

The difference in composition of non-essential amino acids was most pronounced between the two plant types, with a higher proportion of Asn and lower proportions of Glu and Gly in Hsp5 (42.75%, 7.34% and 12.69%, respectively) compared with Concerto (7.55%, 15.18% and 22.43%, respectively) (Fig 7A-B). Amino acid composition and percentage of essential amino acids changed over the 24h of the experiment, but not in response to aphid treatment (Table 5; Table 6). Harvesting of leaf material for RT-qPCR analysis had no effect on the phloem amino acid composition (Table 5; Table 6).

Discussion

Resistance factors contributing to partial-resistance in Hsp5 are based primarily in the mesophyll and phloem

Our observation that *R. padi* feeding on Hsp5 spends more time in the pathway phase (probing into plant tissue) and takes longer to reach the phloem is representative of mesophyll-based resistance (Alvarez et al., 2006). In addition, *R. padi* shows a reduction in salivation into and

ingestion of the phloem, in line with resistance factors residing in the phloem (Alvarez et al., 2006).

Partial-resistance against aphids in other plant-aphid systems have similarly pointed to the importance of mesophyll and phloem-based resistance factors (Montllor and Tjallingii, 1989; Caillaud et al., 1995; Pegadaraju et al., 2007; Guo et al., 2012; Greenslade et al., 2016; Simon et al., 2017). Partial resistance in *Triticum monococcum* lines (a wild relative of wheat) against *S. avenae* and *R. padi* involved resistance at the phloem level, as determined by EPG (Caillaud et al., 1995; Greenslade et al., 2016; Simon et al., 2017). Metabolic profiling of resistant and susceptible lines by Greenslade et al. (2016) highlighted contrasting metabolite profiles, including lower levels of primary metabolites and elevated Asn in the partially-resistant lines (Greenslade et al., 2016), in line with our observations in Hsp5. Resistance against *M. persicae* and *Ma. euphorbiae,* in *Solanum stoloniferum* was associated with the up-regulation of a suite of defence genes (Alvarez et al., 2013). For *M. persicae* this resistance was associated with an increased difficulty in accessing and ingesting the phloem sap compared with aphids feeding on susceptible *S. tuberosum* (Alvarez et al., 2013; Machado-Assefh and Alvarez, 2018). In contrast, *Ma. euphorbiae* feeding on *S. stoloniferum* showed an increased period of salivation into the phloem (Alvarez et al., 2013).

The anatomy of the vascular bundle can also affect aphid feeding and influence plant resistance/susceptibility to aphids. Simon et al. (2017) showed that the vascular bundle of the susceptible *T. monococcum* line MDR037 was wider than the resistant line MDR045. Infection with mycorrhizal fungi increased the width of the vascular bundle in both plants, amplified susceptibility to aphids, and promoted phloem ingestion (Simon et al., 2017). The inorganic chemistry of leaf tissue can also play a role in plant defence (Lane, 2002; Boyd, 2012), with oxalates highlighted as important contributors towards plant defence against pathogens in cereals (Lane, 2002) and against leaf-chewing insects in *Medicago truncatula* (Korth et al., 2006; Park et al., 2009). The inorganic chemistry of Hsp5 and Concerto leaves was not assessed in this study, however comparing leaf chemistry could highlight further chemical groups which could be involved in mediating plant-aphid interactions.

Leaf surface characteristics contribute little to partial-resistance against aphids in Hsp5

Leaf surface architectural differences were detected between Concerto and Hsp5 and included a higher abundance of non-glandular leaf trichomes and a less complex epicuticular chemistry

in Hsp5. Non-glandular trichomes have been reported to decrease *R. padi* fitness in wheat (Roberts and Foster, 1983), but have contrasting effects on herbivore fitness in other plants (Karley et al., 2016).

Although we detected a positive correlation between trichome abundance and *R. padi* r_m, there was no correlation between trichome abundance and juvenile *R. padi* mass gain, indicating that either non-glandular trichomes have differential effects on aphids at different life-stages or that the higher abundance of non-glandular trichomes are not a primary cause of partialresistance against aphids in Hsp5. Indeed, EPG analysis indicated that leaf surface traits contributed little to partial-resistance in Hsp5. Aphids feeding on Hsp5 did not show increased time spent in the non-probing phase (a key indicator of epidermal-mediated resistance: Alvarez et al. (2006)). Moreover, the time taken for aphids to penetrate the leaf surface was shorter on Hsp5, indicating aphids experienced fewer barriers to probing the leaf epidermis on Hsp5. Less complex epicuticular chemistry in Hsp5 may promote aphid probing of plant tissue and previous studies have shown that epicuticular chemical compounds can either promote or deter aphid probing of plant tissue (Powell et al., 1999), however the influence of specific chemical functional groups on aphid behaviour is not well characterised.

Contribution of defensive thionins and multiple phytohormone signalling genes to partialresistance in Hsp5

In line with Delp et al. (2009) and Mehrabi et al. (2014), basal expression levels of thionin genes, which contribute to barley defences against aphids (Escudero-Martinez et al., 2017), and *LOX2*, a JA-signalling marker, were elevated in Hsp5. The feeding parameters exhibited by *R. padi* while feeding on Hsp5 indicated that mesophyll-based mechanisms conferred shortterm (6h) partial-resistance against aphids. Thionins are located in plant cell walls and within the intracellular space (Reimann-Philipp et al., 1989; Stec, 2006). It is therefore likely that aphids are exposed to thionins when probing mesophyll tissue, contributing to the reduced aphid performance on Hsp5.

In addition, several phytohormone signalling genes, *LOX* (JA), *ERF1* (ET) and *A1* (ABA) showed higher basal expression in Hsp5, indicating that Hsp5 can respond more efficiently to biotic stimuli. In line with our data, *HvLOX2* genes and other components of the JA (*HvAOS*), ET (*HvACCO*), and Auxin (*HvTDS*) signalling pathways showed higher basal expression in the barley cultivar Stoneham, which is partially-resistant against *D. noxia,* relative to susceptible

cultivar Otis (Marimuthu and Smith, 2012). Furthermore, overexpression of *HvLOX2* in barley resulted in decreased *R. padi* fecundity (Losvik et al., 2017), pointing to a functional role of this gene and JA signalling in short-term barley defence against aphids. Expression levels of *CmERF1* in early responses (6h) to aphid infestation were around ten-fold higher in a resistant variety of melon compared with a susceptible variety (Anstead et al., 2010), and higher ET levels have been reported to contribute to *R. maidis* resistance in maize (Louis et al., 2015). ABA mediated-processes have been implicated in increasing plant resistance (Zhu-Salzman et al., 2004; Park et al., 2006) and susceptibility (Kerchev et al., 2013; Hillwig et al., 2016) to aphids. Therefore the consequence of elevated *HvA1* in the context of partial aphid resistance is not clear. Guo et al. (2016) reported that elevated ABA levels in response to drought conditions can lead to increased aphid xylem ingestion. This finding suggests that elevated expression of components of the ABA signalling pathway could indirectly contribute to partial-resistance by encouraging xylem ingestion, thereby reducing phloem ingestion. Indeed, longer periods of xylem ingestion were detected from *R. padi* feeding on Hsp5, indicating that there may be potential fitness consequences for aphids as a result of higher basal expression levels.

In contrast, basal expression levels of *HvNPR1*, a SA-signalling marker, were not significantly different between Hsp5 and Concerto. However, levels in Concerto were down-regulated 24h after aphid infestation to levels that were significantly lower than *HvNPR1* levels in Hsp5 after 24h of aphid infestation. Therefore, SA-mediated defences in Concerto, but not Hsp5, may be repressed upon aphid infestation leading to increased susceptibility of this cultivar. Indeed, NPR1 is required for initiation of SA-mediated defence against aphids in *A. thaliana* (Moran and Thompson, 2001; Wu et al., 2012).

Reduced nutritional quality of Hsp5 phloem sap likely contributes to partial-resistance

The lower relative concentration of essential amino acids in the phloem of Hsp5 compared with Concerto likely contributes to the phloem-based resistance factors in Hsp5. Alterations to amino acid composition can reduce plant palatability and nutritional quality and contribute to increased resistance against aphids (Sandström and Pettersson, 1994; Ponder et al., 2000; Karley et al., 2002). Decreased survival and fecundity of *M. persicae* and *Ma. euphorbiae* feeding from older "tuber-filling" potato plants compared with younger "pre-tuber-filling" plants was linked to changes in amino acid composition (Karley et al., 2002). When presented with chemically-defined diets representative of the phloem sap composition of young and mature potato plants, *M. persicae* and *Ma. euphorbiae* showed decreased feeding rate on the

"tuber-filling" diets (Karley et al., 2002). In addition, *R. padi* feeding from barley plants grown under nitrogen-limited conditions exhibited reduced r_m compared with *R. padi* feeding on barley grown under nitrogen-rich conditions (Ponder et al., 2000). Interestingly, plants under nitrogen-limited conditions contained a higher percentage of Asn, a lower percentage of Gly and a small reduction in the concentration of essential amino acids compared with nitrogenfertilised plants (Ponder et al., 2000), similar to the phloem amino acid composition of Hsp5.

Changes in the abundance of specific amino acids cannot be readily associated with increased insect resistance/susceptibility. It is most likely that any observed effect on aphid fitness is due primarily to the ratio of essential:non-essential amino acids as a result of compositional changes. Indeed, essential amino acids are generally elevated in susceptible plants compared with varieties showing increased resistance against aphids (Auclair, 1976). Vogel and Moran (2011) found that the mass of multiple *A. pisum* biotypes was reduced when essential amino acids were removed from aphid artificial diets; this was observed even though aphids have the capacity to synthesise essential amino acids via their essential endosymbiont, *Buchnera aphidicola* (Douglas and Prosser, 1992). Some aphid species actively remobilise plant nutrients to increase the abundance of essential amino acids (Telang et al., 1999; Sandström et al., 2000). However, Sandström et al. (2000) showed that *R. padi* does not remobilise amino acids in the phloem of its host, similar to observations made in our experimental system.

Conclusion

Our work shows that resistance against aphids in the wild barley Hsp5 is not only effective against *R. padi,* as previously shown (Delp et al., 2009), but also against other aphid pests of cereals. By characterising aphid probing and feeding patterns using EPG we were able to show that Hsp5 resistance factors reside predominately within the mesophyll cell layers and the phloem. Subsequent morphological, biochemical, and molecular assessment of the differences between Hsp5 and a susceptible modern barley cultivar, Concerto, indicated that this resistance likely involves higher basal expression levels, and in some cases higher induced expression, of multiple phytohormone signalling genes as well as a reduced nutritional quality of the phloem sap. Thereby this study provides new insight into the determinants and complexity of partialresistance in a wild progenitor of barley and highlights resistance mechanisms of agricultural importance.

Supplementary Data

Supplementary Table 1: primers used in RT-qPCR analysis.

Supplementary Table 2: Additional EPG parameters.

Supplementary Table 3: Full statistical results of significant EPG parameters.

Supplementary Table 4: FTIR results for leaf surface extracts.

Supplementary Fig. 1: Correlation of insect fitness with non-glandular trichome density.

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Author Contributions

JIBB, AJK, and DJL conceived and designed the experiments. DJL, EP-F, and AMM performed the experiments. JIBB, AJK, DJL, and JAHR analysed the data. DJL wrote the manuscript with input from JIBB, AJK, TAV, and JAHR. All authors read and approved the final manuscript.

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Tables

Table 1: Statistical results of aphid performance experiments

Response Variable	Explanatory Variable	Model Basis	Error Distribution or Statistical Method	Statistical Test	Test Statistic	P value

Nymph Survival	Plant	Cox Proportional Hazards Regression	N/A	Type II Wald χ^2 Analysis of Deviance	$X^2_1 = 10.65$	**0.001***
	Aphid				$X^2_2 = 13.94$	**<0.001***
	Plant x Aphid				$X^2_2 = 5.85$	0.054
Nymph mass gain (mg)	Plant	Linear Mixed Effects Model	Restricted Maximum Likelihood	Type III Analysis of Variance with Satterthwaite approximation for degrees of freedom	$F_{1,56} = 9.14$	**0.004***
	Aphid				$F_{2,56} = 49.83$	**<0.001***
	Plant x Aphid				$F_{2,46} = 3.12$	0.054
Rate of population increase (r_m)	Plant				$F_{1,54} = 27.43$	**<0.001***
	Aphid				$F_{2,54} = 99.10$	**<0.001***
	Plant x Aphid				$F_{2,52} = 1.49$	0.234

*** Indicates which variables are significantly different** Table 2: Statistical results of the significant EPG parameters. The mean value for each plant host and the standard error of the mean are also displayed; full statistical results are displayed in Supplementary Table 3

EPG Parameter	**Hypothesised location of resistance factor**	**Mean Value ± Standard Error of the Mean produced waveform**		**Number of individuals which**		**P Value**
		Concerto	**HsP5**	**Concerto**	**Hsp5**	
All parameters (Global analysis)	All tissue	-	-	-	-	**0.022**
Number of potential drops (intracellular punctures) in first probe	Mesophyll	24 ± 5.36	10 ± 2.82	**18/18**	**16/16**	**0.047**
Time to the first phloem phase containing E1 and/or E2	Mesophyll	3761 s ± 675 s	9864 s ± 1884 s	**18/18**	**16/16**	**0.019**
Time to the first phloem ingestion (E2) phase	Mesophyll	4395 s ± 846 s	9946 s ± 1880 s	**18/18**	**16/16**	**0.028**
Number of probes before first E1	Mesophyll	0.66 ± 0.34	2.38 ± 0.76	**18/18**	**16/16**	**0.002**
Number of probes before first E2	Mesophyll	1 ± 0.38	2.5 ± 0.79	**18/18**	**15/16**	**0.042**
Total number of xylem phases	Xylem	0.72 ± 0.13	1.56 ± 0.30	**12/18**	**13/16**	**0.029**

Total time ingesting phloem (E2)	Phloem	7965 s ± 1306 s	3299 s ± 1188 s	**18/18**	**15/16**	**0.004**
Average time of phloem ingestion (E2)	Phloem	5317 s ± 1533 s	1717 s ± 702 s	**18/18**	**15/16**	**0.011**
Total time of phloem phases containing E1 and/or E2	Phloem	8824 s ± 1348 s	3620 s ± 1241 s	**18/18**	**15/16**	**0.002**
Maximum time of a single phloem phase with E1 and/or E2	Phloem	7913 s ± 1442 s	3225 s ± 1172 s	**18/18**	**15/16**	**0.002**
Maximum length of a single E2 period	Phloem	7215 s ± 1371 s	2884 s ± 1134 s	**18/18**	**15/16**	**0.004**
Average time of phloem phase containing E1 and/or E2	Phloem	5911 s ± 1653 s	1913 s ± 722 s	**18/18**	**15/16**	**0.008**
Total number of sustained E2 phases	Phloem	1.39 ± 0.16	0.75 ± 0.17	**17/18**	**10/16**	**0.014**
Mean duration of sustained E2	Phloem	6500 s ± 1424 s	2779 s ± 1141 s	**17/18**	**10/16**	**0.008**
Median duration of sustained E2	Phloem	6425 s ± 1440 s	2779 s ± 1141 s	**17/18**	**10/16**	**0.011**

Table 3: Statistical results of plant defence gene expression assessed by RT-qPCR showing χ^2 and p values

Gene	**Explanatory variable**							
	Plant		**Aphid infestation**		**Plant x infestation**		**Experimental Block**	
HvTHIO1	X^2 = 11.06	p = **<0.001***	X^2 = 0.57	p = 0.450	X^2 = 11.67	p = **0.009***	X^2 = 1.10	p = 0.894
HvTHIO2	X^2 = 13.62	p = **<0.001***	X^2 = 0.57	p = 0.449	X^2 = 14.31	p = **0.002***	X^2 = 0.47	p = 0.976
HvβTHIO	X^2 = 14.29	p = **<0.001***	X^2 = 1.12	p = 0.290	X^2 = 16.10	p = **0.001***	X^2 = 0.15	p = 0.997
HvLOXA	X^2 = 4.81	p = **0.028***	X^2 = 4.48	p = **0.034***	X^2 = 8.05	p = **0.045***	X^2 = 3.11	p = 0.539
HvLOX2	X^2 = 5.49	p = **0.019***	X^2 = 2.76	p = 0.096	X^2 = 8.46	p = **0.037***	X^2 = 3.97	p = 0.410
HvJAZ	X^2 = 2.85	p = 0.131	X^2 = 0.57	p = 0.450	X^2 = 3.55	p = 0.315	X^2 = 9.42	p = 0.055
HvNPR1	X^2 = 1.12	p = 0.290	X^2 = 1.85	p = 0.174	X^2 = 8.46	p = **0.037***	X^2 = 3.64	p = 0.457
HvA1	X^2 = 5.77	p = **0.016***	X^2 = 1.46	p = 0.226	X^2 = 4.57	p = 0.210	X^2 = 4.58	p = 0.206
HvERF1	X^2 = 7.00	p = **0.008***	X^2 = 4.81	p = **0.028***	X^2 = 12.26	p = **0.006***	X^2 = 2.24	p = 0.691

***** Indicates which variables are significantly different

Table 4: Dunn's test *post-hoc* analysis of RT-qPCR results significant for plant x time-point interaction, showing *t* and p values for each pairwise comparison; C = Concerto, H = Hsp5, 0 = 0 h infestation, 24 = 24 h aphid infestation

	Pairwise comparison											
Gene	**C0:C24**		**C0:H0**		**C0:H24**		**C24:H0**		**C24:H24**		**H0:H24**	
HvTHIO1	*t* = 0.69	P = 0.244	*t* = -2.19	P = **0.014***	*t* = -1.82	P = **0.034***	*t* = -2.89	P = **0.001***	*t* = -2.51	P = **0.006***	*t* = 0.37	P = 0.354
HvTHIO2	*t* = 1.28	P = 0.099	*t* = -1.76	P = **0.038***	*t* = -1.97	P = **0.024***	*t* = -3.05	P = **0.001***	*t* = -*3.26*	P = <**0.001***	*t* = -0.21	P = 0.415
HvβTHIO	*t* = 1.33	P = 0.090	*t* = -2.08	P = **0.018***	*t* = -1.92	P = **0.022***	*t* = -3.42	P = <**0.001***	*t* =-3.26	P = <**0.001***	*t* = 0.16	P = 0.436
HvLOXA	*t* = 1.12	P = 0.132	*t* = -1.66	P = **0.048***	*t* = 0.21	P = 0.415	*t* = -2.77	P = **0.002***	*t* = -0.91	P = 0.182	*t* = 1.87	P = **0.030***
HvLOX2	*t* = 1.49	P = 0.067	*t* = -1.33	P = 0.091	*t* = -0.48	P = 0.315	*t* = -2.83	P = **0.002***	*t* = -1.97	P = **0.024***	*t* = 0.85	P = 0.196
HvNPR1	*t* = 2.62	P = **0.004***	*t* = 0.91	P = 0.182	*t* = 0.21	P = 0.415	*t* = -1.71	P = **0.043***	*t* = -2.41	P = **0.008***	*t* = -0.69	P = 0.244
HvERF1	*t* = 2.03	P = **0.021***	*t* = -1.38	P = 0.082	*t* = -0.32	P = 0.374	*t* = -3.42	P = <**0.001***	*t* = -2.35	P = <**0.001***	*t* = 1.06	P = 0.143

*Indicates which variables are significantly different

Table 5: Analysis of deviance table for the scores on each principal component (% variation explained is indicated in brackets) derived from Principal Component Analysis of phloem amino acid composition

	PC1 (34.38%)		PC2 (17.80%)		PC3 (10.89%)	
Explanatory Variable	X_2 (df)	p-value	X_2 (df)	p-value	X^2 (df)	p-value
Plant	40.11 (1)	**<0.001***	5.40 (1)	**0.020***	11.98 (1)	**<0.001***
Aphid infestation	0.29 (1)	0.585	0.24 (1)	0.626	2.26 (1)	0.131
Time-point	5.42 (1)	**0.019***	3.05 (1)	0.080	0.08 (1)	0.765
Plant x time-point	0.05 (1)	0.816	2.17 (1)	0.140	1.97 (1)	0.159
RNA Harvest ǂ	0.17 (1)	0.671	1.92 (1)	0.166	0.67 (1)	0.413

*Indicates which variables are significantly different ǂ RNA harvest is a binary variable introduced by the sub-sampling of 50% of the plant material for RT-qPCR analysis.

Table 6: Analysis of deviance table for essential amino acids as a percentage of total amino acids in phloem sap of Hsp5 and Concerto leaves

	Essential Amino acid (%)	
Explanatory Variable	X_2 (df)	p-value
Plant	23.35 (1)	**<0.001***
Aphid infestation	0.13 (1)	0.716
Time-point	8.82 (1)	**0.002***
Plant x time-point	0.39 (1)	0.530
RNA Harvest ǂ	2.01 (1)	0.155

*Indicates which variables are significantly different ǂ RNA harvest is a binary variable introduced by the sub-sampling of 50% of the plant material for RT-qPCR analysis.

Figure Legends

Fig 1. Aphid performance on a susceptible barley cultivar (Concerto) and partially-resistant wild relative (HsP5). A) Survival of aphid nymphs over seven days. Number of model

observations = 216. B) Nymph mass gain and C) intrinsic rate of population increase (rm) of the three aphid species while feeding from the two plant types. Values represent means ± SE. Number of model observations = 72.. *N = 36* for each aphid-plant combination for survival analysis (A), for nymph mass gain (B) *n* = 12 for the *R. padi* – Concerto, *n = 11* for the *S. avenae* – Concerto, *S. avenae* – Hsp5 and *U. humboldti* – Concerto combinations, *n = 10* for the *R. padi* – Hsp5 combination, and *n = 5* for the *U. humboldti* – Hsp5 combination. For r_m (C) *n = 12* for the *R. padi* – Concerto and *R. padi* – Hsp5 combinations, *n = 10* for the *S. avenae* – Concerto, *S. avenae* – Hsp5 and *U. humboldti* – Concerto combinations, and *n = 4* for the *U. humboldti* – Hsp5 combination. Letters indicate which groups are similar to each other based on log-rank testing (A) and least squares means analysis with Tukey correction (B, C). This figure is available in colour at JXB online.

Fig. 2: Graphical representative feeding patterns of *R. padi* feeding on the susceptible (Concerto) and partially-resistant (Hsp5) plant types. A and B are graphical models showing an overview of aphid feeding behaviour on Concerto (A) and Hsp5 (B). Models show the frequency and length of each feeding parameter over a 6h (21600s) period split into the five main waveforms observed: non-probing of plant tissue (np), probing into epidermal and mesophyll tissue (the pathway phase (C)), saliva secretion into phloem (E1), phloem ingestion (E2), and xylem ingestion (G). C and D display representative waveforms collected from aphids feeding on Concerto (C) and Hsp5 (D) and are annotated with the corresponding waveforms: np, C, pd (cellular punctures during C phase), E1 and E2. Waveform G is not displayed in panels C and D.

Fig 3: EPG parameters indicative of epidermal, mesophyll and mesophyll/phloem resistance factors. A) Time (s) from start of EPG recording to first stylet puncture of leaf tissue, *n* = 18 and 16 for aphids feeding on Concerto and Hsp5, respectively. B) Duration (s) of first stylet probe into plant tissue, *n* = 18 and 16 for aphids feeding on Concerto and Hsp5, respectively. C) Time (s) since start of EPG recording until stylet puncture of a sieve tube element, *n* = 18 and 16 for aphids feeding on Concerto and Hsp5, respectively. D) Time (s) from start of EPG recording until sustained phloem ingestion *n* = 17 and 10 for aphids feeding on Concerto and Hsp5, respectively. Panels A, B and C display means ± SE, panel D displays the median and confidence intervals. Number of model observations = 34. This figure is available in colour at JXB online.

Fig 4: EPG parameters indicative of vascular (phloem and xylem) resistance factors. A) Time

(s) from first stylet penetration of a sieve tube element to first sustained phloem ingestion, *n* = 17 and 10 for aphids feeding on Concerto and Hsp5, respectively. B) Total time (s) of sustained phloem feeding, *n* = 17 and 10 for aphids feeding on Concerto and Hsp5, respectively. C) Ratio of time spent in phloem phase relative to the pathway phase, *n* = 18 and 15 for aphids feeding on Concerto and Hsp5, respectively. D) Total time (s) ingesting xylem, *n* = 12 and 13 for aphids feeding on Concerto and Hsp5, respectively. Panels A, B, and D display the median value and confidence intervals, panel C displays the mean ±SE. Number of model observations = 34. This figure is available in colour at JXB online.

Fig. 5: A) Median non-glandular trichome densities (No. cm^{-2}) and confidence intervals for Concerto and Hsp5. B) Polarised light micrograph showing the contrasting leaf hair densities between HsP5 (top) and Concerto (bottom). Number of model observations = 30. This figure is available in colour at JXB online.

Fig 6. Expression patterns of thionin and phytohormone signalling genes 0 h and 24 h after aphid infestation. All gene expression values are relative to the mean expression of two reference genes, *HvUBC* and *HvCYP*, and normalised to uninfested control plants. Foldchange, $2^{-\Delta\Delta Ct}$, is relative to the expression of Concerto at 0 h. Boxplots show median and confidence intervals; total number of model observations for each gene = 20; *n = 5* for each treatment. Letters indicate which groups are significantly different based on Dunn's Test *post-hoc* analysis. This figure is available in colour at JXB online.

Fig. 7: Amino acid composition of leaf phloem exudates of Hsp5 and Concerto. A and B) phloem amino acid composition in uninfested leaves of Concerto and Hsp5, respectively, at time zero. C) Biplot of scores on the first two principal components explaining >50% of the detected variation in amino acid composition. D) % proportion of essential and non-essential amino acids in phloem exudates of uninfested Concerto and Hsp5 leaves at time zero. *N = 10* for each treatment.

Figures

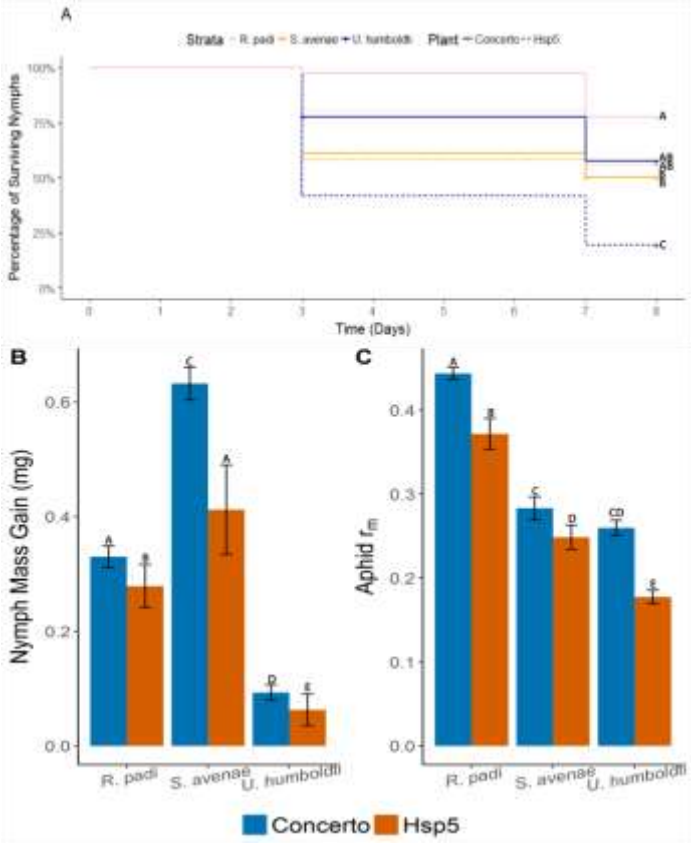


Fig 1. Aphid performance on a susceptible barley cultivar (Concerto) and partially-resistant wild relative (HsP5).

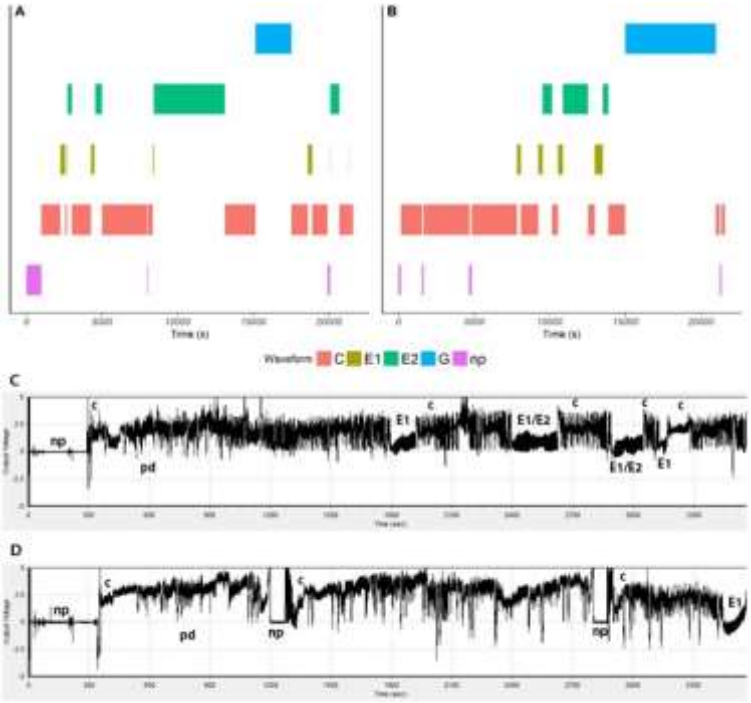


Fig. 2: Graphical representative feeding patterns of *R. padi* feeding on the susceptible (Concerto) and partially-resistant (Hsp5) plant types.

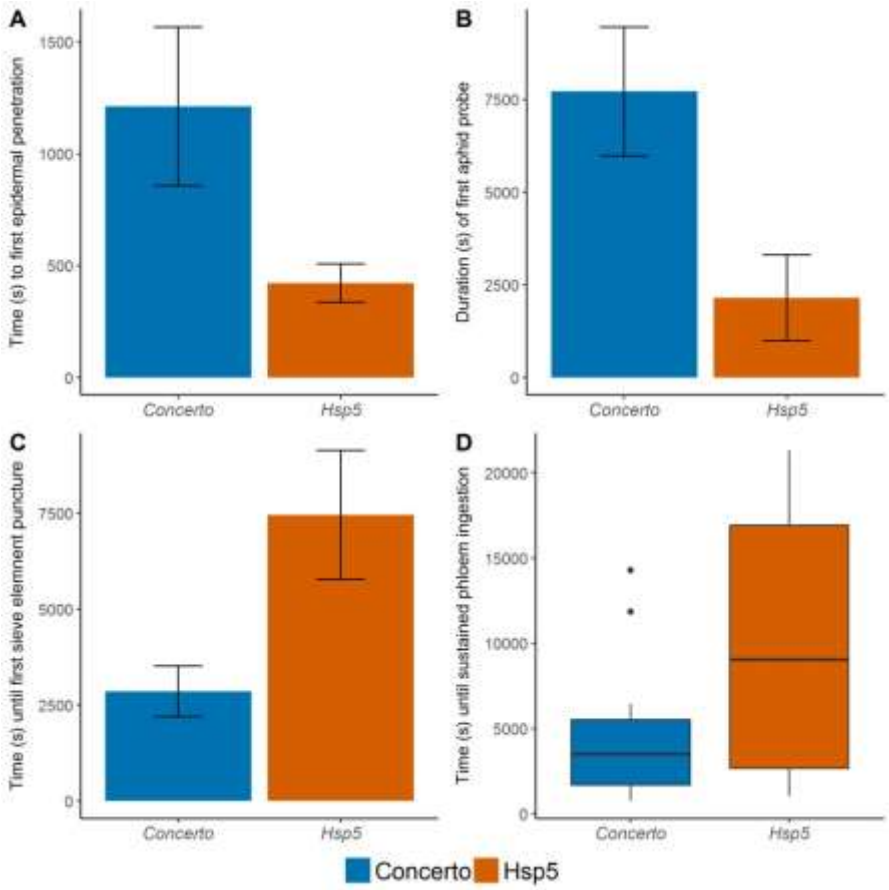


Fig 3: EPG parameters indicative of epidermal, mesophyll and mesophyll/phloem resistance factors.

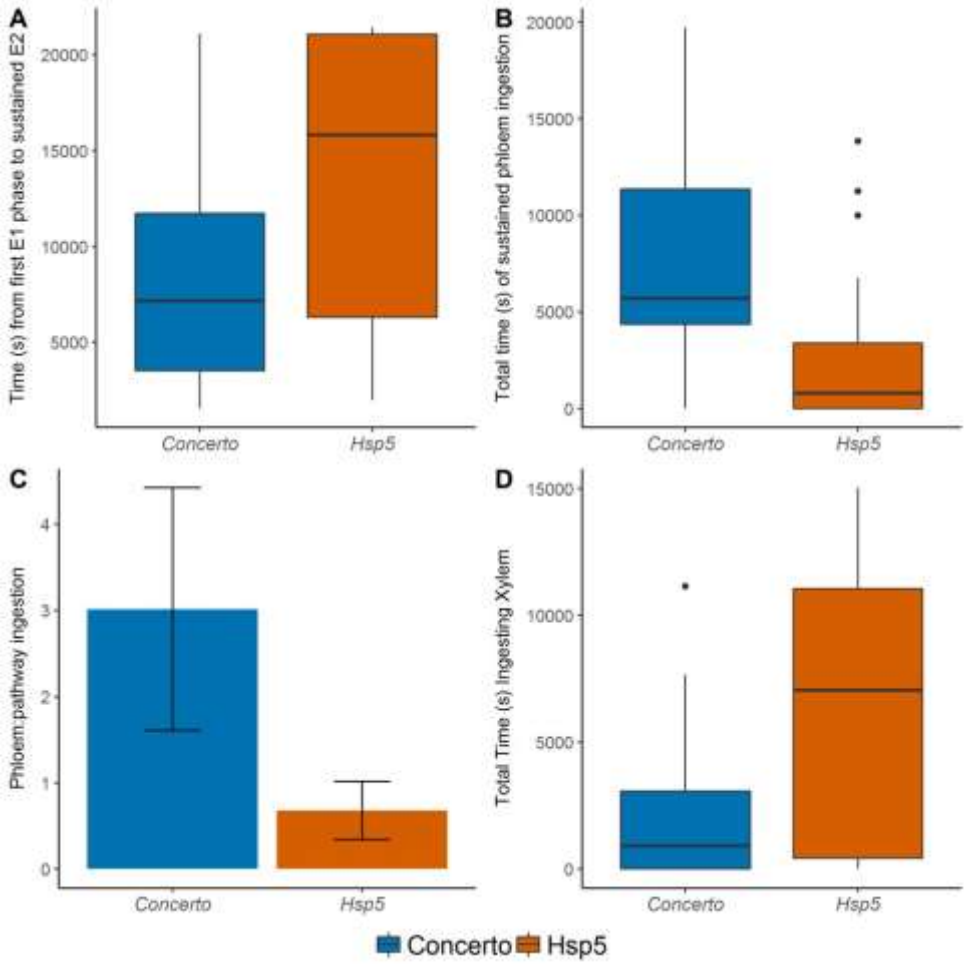


Fig 4: EPG parameters indicative of vascular (phloem and xylem) resistance factors.

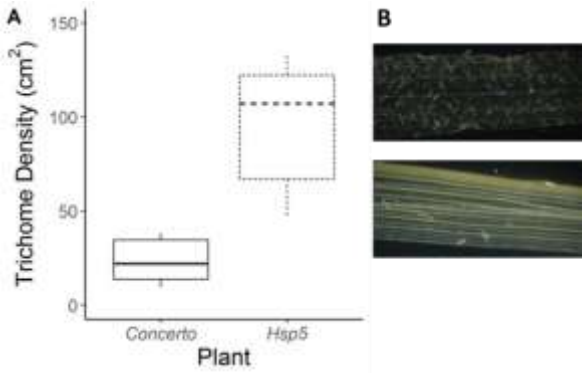


Fig. 5: A) Median non-glandular trichome densities (No. cm^{-2}) and confidence intervals for Concerto and Hsp5.

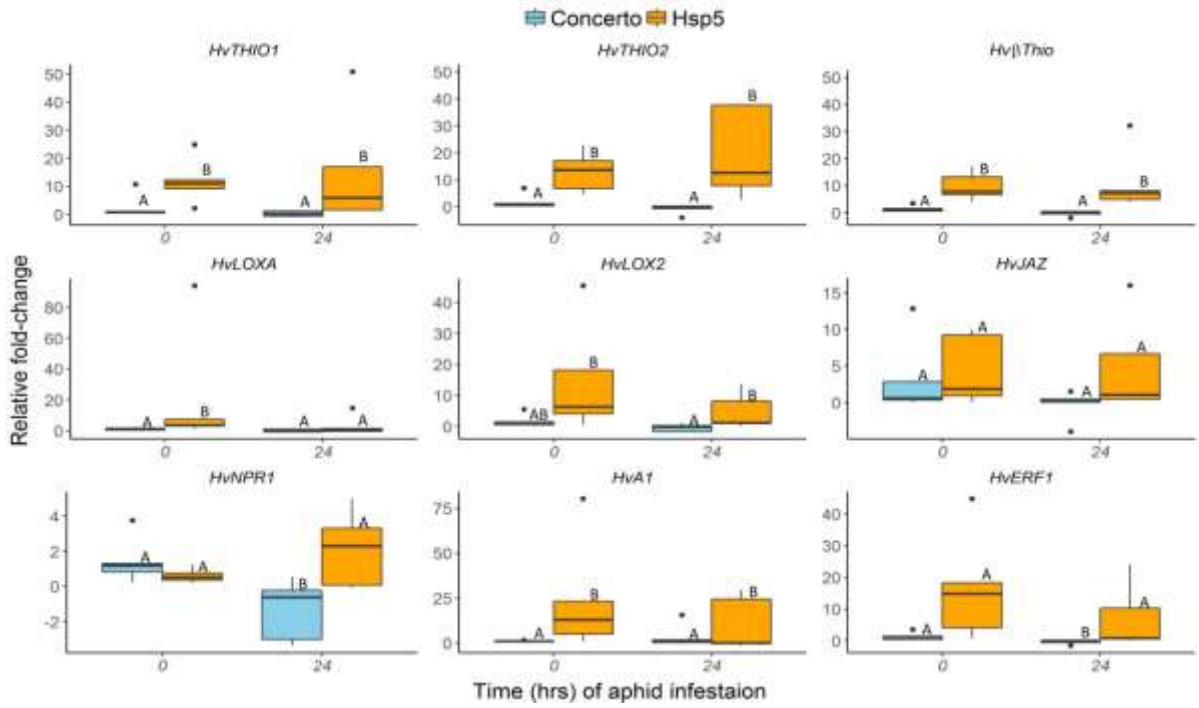


Fig 6. Expression patterns of thionin and phytohormone signalling genes 0 h and 24 h after aphid infestation.

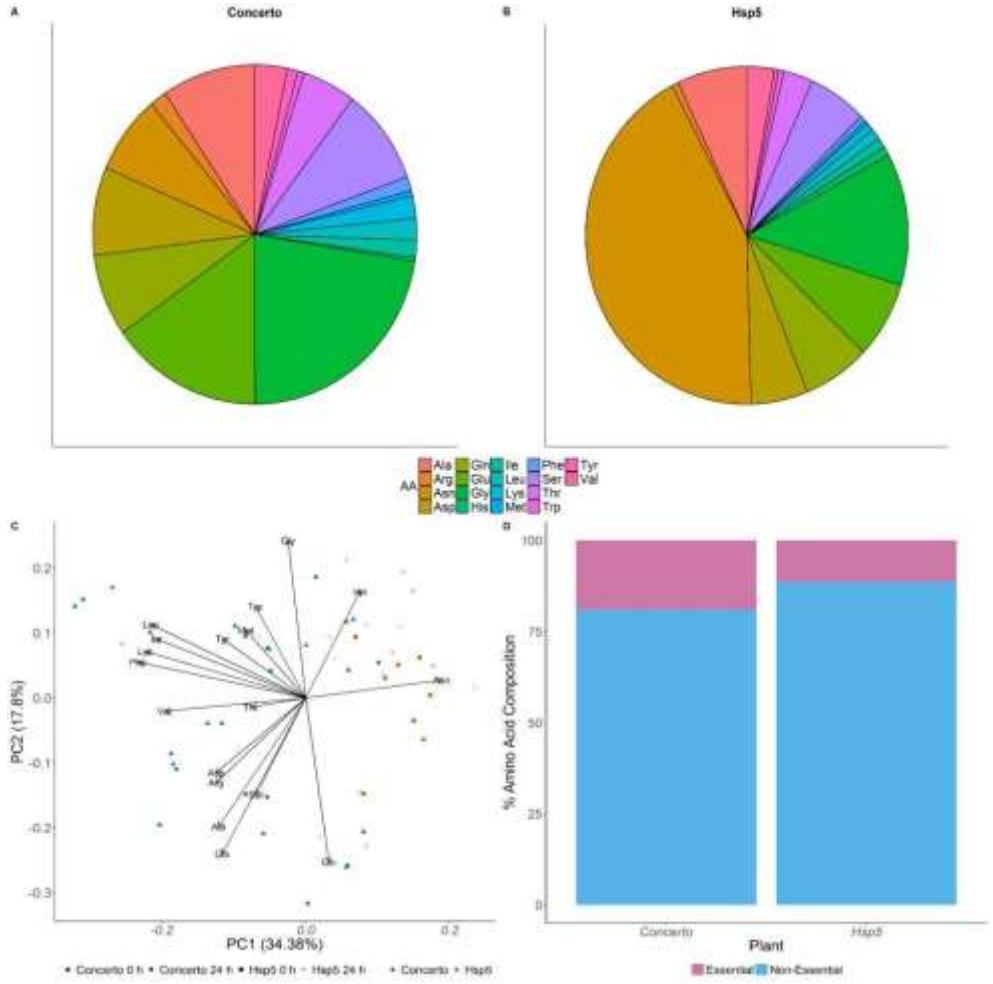


Fig. 7: Amino acid composition of leaf phloem exudates of Hsp5 and Concerto.

Supplementary table 1: primers used in qPCR analysis

Gene	Name	Accession no.	Amplicon Length (bp)	Forward primer	Reverse Primer	Primer Efficiency and R^2 value	Primer Reference
HvUBC	Ubiquitin-conjugating enzyme	AK248472.1	218	tcaattcccgagcagtatcc	agattgcctgagtcgcagtt	87%, 0.98	Hua et al 2014
HvCYP	Cytochrome P450	AK253120.1	237	ctgtcgtgtcgtcggtctaa	tgaaagcgacaaacagatgc	102%, 0.95	Hua et al 2014
HvLOXA	Lipoxygenase 1	AY220737.2	61	gccagatccagaccatcatc	tcggaggagtgcttcgac	108%; 0.94	Designed for this study
HvLOX2	Predicted Lipoxygenase	AK357253.1	60	atgtcctatcccacgacacc	agtgcgtcctcagccagt	103%; 0.91	Escudero-Martinez et al 2017
HvJAZ	Jasmonate ZIM-domain protein 3	MLOC_9995.2	60	atctggagcaatccgttgac	aggaaaagtggtcgtggttg	92%; 0.95	Escudero-Martinez et al 2017
HvA1	ABA-inducible late embryogenesis abundant protein	X13498.1	66	atgggaggggacaacacc	ggaaattaagcgcgaacg	91%; 0.95	Designed for this study
HvNPR1	Non-expresser of pathogenesis-related genes 1-Like	MLOC_64922.1	64	ttgataacatctagaggcaatgct	tgcgtgaaactgttcgagag	84%; 0.93	Designed for this study
HvERF1	Ethylene-response factor 1	HQ328941.1	62	ctatataatgattgggtgcatgtt g	ggcatatgacccaaggtgtt	87%; 0.98	Designed for this study
HvTHIO1	Thionin 1	AK359149	84	tatggccaaggtcgttttgt	cataactaagatgatacatttgct tcg	118%; 0.94	Escudero-Martinez et al 2017
HvTHIO2	Thionin 2	AK357884	88	gcggttcaaaatgtcctagtg	ccaatggtgcagtactgagtg	112%; 0.97	Escudero-Martinez et al 2017
HvβTHIO	β Purothionin	AK252675.1	90	tactgggtttagttctggagcag	acgtgtccttgcagcaactt	115%; 0.95	Escudero-Martinez et al 2017

Supplementary table 2: Additional EPG parameters

EPG Parameter	Hypothesised location of resistance factor	Transformation	Mean Value Concerto	Mean Value Hsp5	Statistical Test	Test Statistic	p value
average non probing (period duration)	Epidermis	-	804.11 s	442.26 s	K.W	0.87	0.352
median non probing (period duration)	Epidermis	-	654.92 s	380.85 s	K.W	0.80	0.352
sum of non probing	Epidermis	sqrt	2552.92 s	2095.83 s	ANOVA	0.51	0.483
number of non probing periods	Epidermis	log[2]	5.22	0.89	GLM	0.40	0.534
number of brief probes < 3 min before 1st E	Epidermis, Mesophyll	-	0.11	0.81	K.W	2.17	0.141
number of probes before 1st pd	Epidermis, Mesophyll	-	0.66	0.87	K.W	1.98	0.159
number of probes before the first G	Epidermis, Mesophyll	-	1.94	1.87	K.W	0.50	0.480
number of probes	Epidermis, Mesophyll	log[2]	5.22	5.62	GLM	0.40	0.534
sum of probing	Epidermis, Mesophyll	-	19057.08 s	19504.17 s	K.W	0.43	0.558
sum of C	Epidermis, Mesophyll	sqrt	7357.38 s	8051.17 s	ANOVA	0.30	0.590
median time to 1st pd in all probes with a pd	Epidermis, Mesophyll	sqrt	112.47 s	118.31 s	ANOVA	0.28	0.605
number of brief probes (probes < 180 s)	Epidermis, Mesophyll	-	0.61	1.06	K.W	0.80	0.949
number of C periods	Epidermis, Mesophyll	-	11.00	11.12	GLM	0.01	0.950
median probe	Epidermis, Mesophyll	-	5691.88 s	3627.73 s	K.W	0.01	0.973
min. time to 1st pd in 1st probe	Epidermis, Mesophyll	-	60.47 s	60.53 s	K.W	0.00	0.973
no. pd per min C , only C_phases with pd	Mesophyll	-	0.54	0.67	GLM	1.85	0.183
sum of pd	Mesophyll	sqrt	353.79	416.04	ANOVA	1.54	0.223
duration of the first pd	Mesophyll	-	4.27 s	5.33 s	K.W	1.46	0.227
no. pd per min C	Mesophyll	-	0.65	0.75	GLM	0.97	0.332
median duration of pd	Mesophyll	-	4.05 s	4.72 s	K.W	0.93	0.334
number of F	Mesophyll	-	0.17	0.06	K.W	0.86	0.354
time to 1st pd (from start of 1st probe)	Mesophyll	sqrt	220.34 s	289.85 s	ANOVA	0.80	0.378
mean duration of the first 5 pd	Mesophyll	-	4.30 s	5.07 s	K.W	0.74	0.388
average F	Mesophyll	-	492.66 s	825.67 s	K.W	0.69	0.405
median F	Mesophyll	-	492.66 s	825.67 s	K.W	0.69	0.405
sum of F	Mesophyll	-	492.66 s	825.67 s	K.W	0.69	0.405
average duration of pd	Mesophyll	-	4.34 s	4.97 s	K.W	0.68	0.408
number of pd	Mesophyll	-	72.61	83.12	K.W	0.58	0.448
median C	Mesophyll	log[2]	502.91 s	459.90 s	ANOVA	0.25	0.618
average probe	Mesophyll	log[2]	6802.31 s	4884.68 s	ANOVA	0.25	0.622
duration of the second pd	Mesophyll	-	4.45 s	5.29 s	K.W	0.22	0.641
average C; with pd without E1e, F and G	Mesophyll	-	742.72 s	763.48 s	ANOVA	0.03	0.866
time to 1st pd in 1st probe with a pd	Mesophyll	sqrt	205.05 s	160.95 s	ANOVA	0.01	0.894
average time to 1st pd in all probes with a pd	Mesophyll	sqrt	153.11 s	131.63 s	ANOVA	0.01	0.924
time to 1st E within the 1st probe with E	Mesophyll, Phloem	log[10]	1018.99 s	1453.15 s	ANOVA	1.35	0.253
number of probes before 1st sE2	Mesophyll, Phloem	-	1.72	1.68	K.W	0.59	0.442
number of probes after 1st sE2	Mesophyll, Phloem	-	2.17	1362.00	K.W	0.59	0.442
time from the 1st E1 to 1st E2	Mesophyll, Phloem	-	1538.73 s	2493.86 s	K.W	0.39	0.535
minimum time to 1st E within probes	Mesophyll, Phloem	-	687.47 s	1043.44 s	K.W	0.17	0.678
average time to 1st E within probes	Mesophyll, Phloem	-	912.91 s	1581.33 s	K.W	0.12	0.729
number of all E1 periods (sgE1 + frE1)	Mesophyll, Phloem	log[2]	5.44	4.56	GLM	0.11	0.749
Total time in E1 before E2	Phloem	-	825.60 s	300.07 s	K.W	3.37	0.066
E2 index: %	Phloem	-	0.50	0.35	K.W	3.11	0.078
Total time in E1	Phloem	log[10]	1029.75 s	459.12 s	ANOVA	3.08	0.089
sum of fractions of E1	Phloem	-	858.83	320.75	K.W	2.86	0.091
number of E12 phloem periods i.e. with both E1 and E2	Phloem	sqrt	2.88	1.93	GLM	3.03	0.091
median E2	Phloem	-	4803.10	1601.25	K.W	2.52	0.113
number of E2 periods	Phloem	sqrt	2.88	2.00	GLM	2.62	0.115
median E12 (with both E1 and/or E2)	Phloem	-	5363.61 s	1751.12 s	K.W	2.30	0.129
number of fractions of E1;: E1followed/preceded by E2	Phloem	sqrt	2.88	2.06	GLM	2.26	0.142
maximum E1 period (either sgE1 or frE1)	Phloem	-	762.95 s	225.69 s	K.W	2.10	0.147
maximum duration of a fraction of E1	Phloem	-	738.92 s	215.04 s	K.W	2.00	0.157
average E1	Phloem	-	334.70 s	87.26 s	K.W	2.00	0.157
median E1 (sgE1 and E1fr)	Phloem	log[10]	290.67 s	69.61 s	ANOVA	1.70	0.202
E1 index:duration E1/ allE as %	Phloem	-	0.16	0.25	K.W	1.46	0.227
average fraction of E1	Phloem	-	593.51	117.22	K.W	0.69	0.408
median fraction of E1	Phloem	-	550.17	95.72	K.W	0.53	0.469
median single E1	Phloem	sqrt	47.17 s	47.32 s	ANOVA	0.44	0.513
number of single E1 (without E2) periods	Phloem	sqrt	2.55	2.50	GLM	0.37	0.545
average single E1	Phloem	sqrt	52.66 s	49.33 s	ANOVA	0.20	0.658
number of E2 before the 1st sE2	Phloem	-	0.72	0.56	K.W	0.16	0.685
sum of E2 before 1st sE2	Phloem	-	123.11 s	127.56 s	K.W	0.09	0.762
maximum duration of a single E1 period	Phloem	sqrt	85.07 s	75.23 s	ANOVA	0.08	0.774
sum of sgE1	Phloem	-	170.92 s	138.37 s	K.W	0.05	0.809

mean duration of E2 periods before the 1st sE2	Phloem	-	59.25 s	65.46 s	K.W	0.02	0.900
time to the first G (after first penetration)	Mesophyll, Xylem	-	10761.03 s	6577.95 s	K.W	1.22	0.270
Average xylem ingestion time	Xylem	-	2117.75 s	4043.78 s	K.W	3.03	0.082
median G	Xylem	-	2117.75 s	3770.59 s	K.W	2.24	0.134

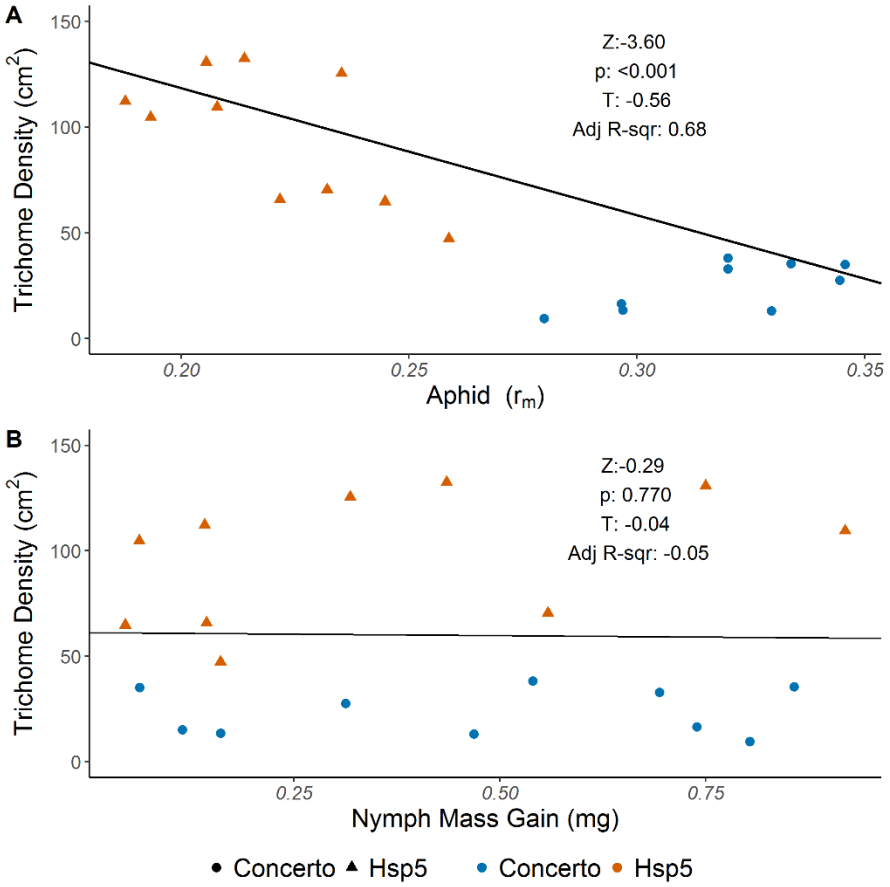
Abbreviations in the table: np - non-probing of plant tissue, C phase – stylet probing into epidermal and mesophyll tissue (the pathway phase), pd – potential drop (stylet puncture into plant cells during C phase), E1 phase - saliva secretion into phloem, E1e – extracellular saliva secretion, E2 phase - phloem ingestion, sE2 phase – sustained E2 (period of ingestion >10 mins), E – any phase involving stylet activities in the phloem, sgE1 – single E1 phase, frE1 – fraction of E1 phase from E phase containing E1 and E2, G phase -xylem ingestion, and F phase – stylet penetration difficulties

Supplementary Table 3: Statistical results of the significant EPG parameters; in the test statistic column χ^2 values are italicised and F values are underlined.

EPG Parameter	Hypothesised location of resistance factor	Transformation	Statistical Test	Test Statistic and Degrees of Freedom (Residuals)	P Value
All parameters (Global analysis)	All tissue	-	Permutated Multiple Analysis of Variance	$\underline{2.61}_{1(33)}$	**0.022**
Number of potential drops (intracellular punctures) in first probe	Mesophyll	Sqrt	ANOVA on glm	$\textit{3.95}_{1(33)}$	**0.047**
Time to the first phloem phase containing E1 and/or E2	Mesophyll	-	Kruskall-Wallis Test (KW)	$\textit{5.50}_{1(33)}$	**0.019**
Time to the first phloem ingestion (E2) phase	Mesophyll	-	ANOVA	$\underline{5.32}_{1(33)}$	**0.028**
Number of probes before first E1	Mesophyll	-	KW	$\textit{9.36}_{1(33)}$	**0.002**
Number of probes before first E2	Mesophyll	-	KW	$\textit{4.14}_{1(33)}$	**0.042**
Total number of xylem phases	Xylem	-	KW	$\textit{4.77}_{1(33)}$	**0.029**
Total time ingesting phloem (E2)	Phloem	-	KW	$\textit{8.00}_{1(33)}$	**0.004**
Average time of phloem ingestion (E2)	Phloem	-	KW	$\textit{6.52}_{1(33)}$	**0.011**
Total time of phloem phases containing E1 and/or E2	Phloem	-	KW	$\textit{9.22}_{1(33)}$	**0.002**
Maximum time of a single phloem phase with E1 and/or E2	Phloem	-	KW	$\textit{9.01}_{1(33)}$	**0.002**
Maximum length of a single E2 period	Phloem	-	KW	$\textit{8.40}_{1(33)}$	**0.004**
Average time of phloem phase containing E1 and/or E2	Phloem	-	KW	$\textit{6.88}_{1(33)}$	**0.008**
Total number of sustained E2 phases	Phloem	-	KW	$\textit{6.06}_{1(33)}$	**0.014**
Mean duration of sustained E2	Phloem	-	KW	$\textit{7.12}_{1(33)}$	**0.008**
Median duration of sustained E2	Phloem	-	KW	$\textit{6.40}_{1(33)}$	**0.011**

Supplementary table 4: Functional groups identified using FTIR spectral analysis on dichloromethane leaf surface extracts for Hsp5 and Concerto, showing presence of band (cm^{-1}) in the spectra and allocated functional group

Plant	Rep	Number of functional groups	Identified Bands (cm^{-1}) and Functional Groups								
HsP5	1	3	2959 CH_3 stretching	2920/2852 CH_2 stretching	1736 C=O stretch for ester group (v. weak)					1473/1462 CH_2 deformation	731/719 CH_2 wag
	2	2	2959 CH_3 stretching	2920/2852 CH_2 stretching						1473/1462 CH_2 deformation	731/719 CH_2 wag
	3	2	2959 CH_3 stretching	2920/2852 CH_2 stretching						1473/1462 CH_2 deformation	731/719 CH_2 wag
	4	3	2959 CH_3 stretching	2920/2852 CH_2 stretching	1736 C=O stretch for ester group (v. weak)					1473/1462 CH_2 deformation	731/719 CH_2 wag
Concerto	1	4	2959 CH_3 stretching	2920/2852 CH_2 stretching	1736 C=O stretch for ester group			1578/1540 $RCOO^-$ carboxylic acid salt		1473/1462 CH_2 deformation	731/719 CH_2 wag
	2	5	2959 CH_3 stretching	2920/2852 CH_2 stretching	1736 C=O stretch for ester group		1605/1515 C=C stretch aromatic compound	1581/1390/ 1263 $RCOO^-$ carboxylate		1473/1462 CH_2 deformation	731/719 CH_2 wag
	3	5	2959 CH_3 stretching	2920/2852 CH_2 stretching	1736 C=O stretch for ester group	1653/1549 amide I and II protein or amide			1354 nitro containing component (possibly NO_3)	1473/1462 CH_2 deformation	731/719 CH_2 wag
	4	3	2959 CH_3 stretching	2920/2852 CH_2 stretching	1736 C=O stretch for ester group					1473/1462 CH_2 deformation	731/719 CH_2 wag



Supplementary Fig. 1: Comparison of insect fitness with plant trichomes. A) Correlation between leaf non-glandular trichome density (cm^2) and *R. padi* r_m. Line represents model correlation coefficient. Number of model observations = 19. B) Correlation between leaf non-glandular trichome density (cm^2) and *R. padi* nymph mass gain. Line represents model correlation coefficient. Number of model observations = 20. Plots also display test statistic (Z value), p value, correlation (T) and adjusted R^2 value.