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Probing behavior of *Aphis fabae* and *Myzus persicae* on three species of grapevines with analysis of grapevine leaf anatomy and allelochemicals

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Abstract
The peach-potato aphid *Myzus persicae* (Sulzer) and the black bean aphid *Aphis fabae* Scopoli are polyphagous and cosmopolitan hemipterans, therefore they can infest grapevines in all areas of cultivation. Electrical Penetration Graph (EPG) technique was applied to monitor the probing behavior of *A. fabae* and *M. persicae* on *Vitis amurensis* Rupr., *Vitis riparia* Michaux, and *Vitis vinifera* L. The content of major flavonoids and stilbenoids in grapevine leaves and epidermal thickness, distance between abaxial leaf surface and phloem, and the simulated shortest pathway from epidermis to phloem that might have affected aphid probing were also analyzed. Aphid probing was limited mainly to non-vascular tissues on the three studied grapevine species. Phloem phase occurred in 32%, 14%, and 6% of *A. fabae* and in 76%, 39%, and 74% of *M. persicae* on *V. amurensis, V. riparia* and *V. vinifera*, respectively. Phloem phase consisted of only salivation into sieve elements and lasted less than 2.5 minutes on average in all aphids. The time to reach the first phloem phase on grapevines was 5.0 hours in *A. fabae* and 2.6–3.6 hours in *M. persicae*. Of the analyzed flavonoids, catechin, epicatechin, and quercetin occurred in all grapevine species, while rutin – in *V. amurensis* and *V. riparia* and isorhamnetin only in *V. amurensis*. Of the analyzed stilbenoids, piceid occurred in all grapevines, resveratrol in *V. amurensis* and *V. vinifera*, and ε-viniferin only in *V. vinifera*. Aphid behavior demonstrated that *V. amurensis, V. riparia* and *V. vinifera* are not attractive host plants to *A. fabae* and *M. persicae*. It is likely that the content of flavonoids and stilbenoids contributes to the limited susceptibility of the three grapevine species to *A. fabae* and *M. persicae*, while the observed slight differences in the anatomical structure of the leaves seem not significant in this context.

Keywords: *Plant resistance*, *antixenosis*, *flavonoids*, *stilbenoids*, *peach-potato aphid*, *black bean aphid*

Introduction

Grapevines (Vitaceae Juss). include about 950 species, usually shrubs or lianas, assigned to 16 genera, most of which occur in tropical or subtropical zones (Wen et al. 2018). On the other hand, species of the genus *Vitis* occur mainly in the temperate climate zones of the northern hemisphere – in Europe and Asia (Eurasian species, e.g. *Vitis amurensis* Rupr.) and North and Central America (American species, e.g. *Vitis riparia* Michaux). The genus *Vitis* comprises about 60 species. Among them, one of the most important, especially in economic terms, is *Vitis vinifera* L., which gave rise to the vast majority of grapevines cultivated today. Grapevine species differ in terms of morphology and preferred habitat, however, they can easily interbreed, creating interspecies hybrids (Keller 2015a). The term "hybrid" is

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used for grapevines that originate from the crossing of *Vitis* species (e.g. *V. vinifera* and *V. riparia*), and the hybrid grape-based wine is called a "hybrid wine" (Burns et al. 2002). Many attempts of cross-species breeding have been made to combine the positive qualities of some while eliminating the negative of the other. Hybrid varieties were produced to control diseases (downy mildew, powdery mildew caused by *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni and *Erysiphe necator* Schwein. In addition, some species are tolerant to environmental factors, such as *V. amurensis*, which is highly frost resistant (native to cold areas in Northeast China and Russian Siberia) (Kedrina-Okutan et al. 2019). Despite attempts to obtain varieties resistant to biotic and abiotic factors at the same time without losing the quality of grapes, grapevines are still a food source for many insect species including Coleoptera, Diptera (Sekrecka et al. 2015), Hemiptera (Omer et al. 1999; Ocete et al. 2008; Myśliwiec 2009; Baronio et al. 2014), Thysanoptera (Ocete et al. 2008), Lepidoptera (Ocete et al. 2008; Vogelweith et al. 2014), as well as Arachnidae (James & Whitney 1993). Among the grapevine-related herbivores, the occurrence of various species of Aphididae has been observed on various grapevine species, especially *V. vinifera*, e.g., the peach-potato aphid *Myzus persicae* (Sulzer) and the black bean aphid *Aphis fabae* Scopoli (Blackman & Eastop 2006). The presence of *M. persicae* and *A. fabae* on grapevines, including *V. vinifera*, in various areas of Europe was also indicated by Holman (2009), who referred to research by many authors. The occurrence of *M. persicae* on vines was reported in Germany, while *A. fabae* - in Spain, Portugal, France, Serbia, and also outside Europe, in Turkey and Tajikistan (Holman 2009). However, considering the polyphagy and the worldwide distribution of *A. fabae* and *M. persicae*, grapevines can be infested by these aphid species in all areas of cultivation (CABI 2022a, 2022b).

Herbivores with sucking-piercing mouthparts, e.g., Coccoidea, Aphididae, Phylloxeridae, due to the way of feeding, can be vectors of various plant viruses, also transmitted to various species of the genus *Vitis* sp. In the scientific literature, a lot of attention has been paid to the interactions in the grapevine-*Phylloxera* system, also in terms of defense against viruses (Keller 2015b). At the same time, studies on the interactions between grapevines and aphids are scarce. Aphids transmit *Alfalfa Mosaic Virus* (AMV), *Cucumber Mosaic Virus* (CMV), *Bean Common Mosaic Virus* (BCMV), *Broad Bean Wilt Virus* (BBWV), *Grapevine Vein Clearing Virus* (GVCV) or *Grapevine Enamovirus 1* (GEV1) (Basso et al. 2016; Fuchs 2020). The *Sowbane Mosaic Virus* (SoMV) on *Vitis* sp. is transmitted only by *M. persicae* (Chan et al. 1991). Virus transmission is related to the behavior of aphids during the selection of host plants (transmission of non-persistent and semi-persistent viruses during the test punctures of plant tissues) and to the specificity of aphid feeding (aphids uptake the phloem sap directly from the sieve tubes, which enables the transmission of persistent viruses) (Martin et al. 1997). It is therefore important to obtain cultivars resistant to the feeding of the aphids, as they are therefore less likely to be infected by viruses transmitted by these insects. *Vitis amurensis, V. riparia* and *V. vinifera* are important basic species in breeding hybrid grapevine varieties (De la Fuente Lloreda 2018; Teissedre 2018). The probing behavior of aphids on grapevines has not been studied in detail, hitherto.

The resistance of given species or cultivar to the feeding of herbivores may be based on the morphological and anatomical structure of the plant and the presence of allelochemicals (Harborne 1997; Powell et al. 2006; Stout 2013). Secondary metabolites in grapevines are mainly phenolic compounds – flavonoids, phenolic acids, and stilbenoids, as well as phenol derivatives such as tannins (De Rosso et al. 2014; Kedrina-Okutan et al. 2019). The content of phenolic compounds varies depending on the species and cultivar and is related to the variation in the climatic conditions, weather variability, irrigation, soil or environmental stresses (Petrussa et al. 2013). Flavonoids determine the color of plants, the taste of fruits, and are natural repellents for herbivores (Jasiński et al. 2009; Majewska & Czeczot 2009; Goławska et al. 2010, 2014; Golonko et al. 2015). Stilbenoids are derivatives of stilbene (Makowska-Wąs & Janeczko 2008; Jeszka et al. 2010). Depending on the species or cultivar, stilbenoids occur in the entire plant or its individual organs: in fruits, leaves, stems, and roots (Pawlus et al. 2012). Stilbenoids are involved in allelopathic effects and in the plant's response to oxidative stress generated by UV radiation, they can also inhibit the growth of bacteria and fungi (disease-resistant species produce stilbenoids in higher concentrations) and show herbivore repellent properties (Chong et al. 2009; Pawlus et al. 2012; Nopo-Olazabal et al. 2014; Kozłowska & Czekała 2017). The choice of a plant as a food source may depend also on physical barriers. In the case of aphids, the thickness of the sclerenchyma layer surrounding the vascular bundles, which can constitute a barrier during

penetration, may be important: the thicker the sclerenchyma layer, the more difficult it is for the aphids to reach the vascular bundle, especially the phloem from which the aphids take up the sap (Kidd 1976; Boczek 1988; Malinowski 2008; Ammar et al. 2014).

The aim of the present study was to assess the susceptibility of selected grapevine species *V. amurensis*, *V. riparia* and *V. vinifera* to *M. persicae* and *A. fabae*. We monitored aphid stylet penetration activities in plant tissues with the use of the Electrical Penetration Graph (EPG, known also as electropenetrography) technique, which is crucial in determining the impact of antixenosis factors on individual phases of aphid probing in peripheral as well as in vascular plant tissues. In addition, we analyzed the quantitative and qualitative variation in the content of selected major flavonoids and stilbenoids in the leaves of the grapevine species and we measured leaf anatomical parameters that might have affected aphid probing.

Material and methods

Cultures of plants and aphids

Grapevines. The grapevines used in the experiments were derived from Instituto Nacional de Investigacion y Technologia Agraria y Alimentaria (INIA), Madrid, Spain (*Vitis vinifera* L.) and Global Flowers Ltd, Rotherham, UK, through Sklep Ogrodniczy Plumeria (*V. amurensis* Rupr. and *V. riparia* Michx.). Previous studies showed that some natural populations of *V. vinifera* ssp. *sylvestris* in the Iberian Peninsula showed small infestations caused by aphids. Therefore, we have chosen the genotype with assumed tolerance to aphid infestation from Spain. All plants were grown in commercial soil in plastic pots (vol. 4 dm^3), in the laboratory at 20°C, 65% r.h., and L16:8D photoperiod. The plants were watered regularly and no fertilizers were applied. For all experiments, 1-2-years old plants were used.

Aphids. Laboratory cultures of *Aphis fabae* and *Myzus persicae* were maintained as multiclonal colonies on *Vicia faba* L. cv. White Windsor and *Brassica pekinensis* (Lour.) Rupr. cv. Hilton, respectively, in the laboratory at 20°C, 65% r.h., and L16:8D photoperiod in a growing chamber Sanyo MLR-351 H (Sanyo Electronics Co. Ltd.).

Electronic monitoring of aphid probing behavior

Probing behavior of *Aphis fabae* and *Myzus persicae* on *Vitis amurensis*, *V. riparia* and *V. vinifera* was monitored using the technique of electronic registration of aphid behavior known as Electrical Penetration Graph (EPG) technique or electropenetrograhy that is frequently employed in insect–plant relationship studies (Will et al. 2007; Philippi et al. 2015). In the experimental set-up, aphid and plant are made parts of an electric circuit, which is completed when the aphid inserts its stylets into the plant. Weak voltage is supplied in the circuit, and all changing electric properties are recorded as EPG waveforms that can be correlated with aphid activities and stylet position in plant tissues (Tjallingii 1994). The parameters describing aphid behavior during probing and feeding, such as total time of probing, proportion of phloem patterns E1 and E2, number of probes, etc., are good indicators of plant suitability or interference of probing by chemical or physical factors in plant tissues (Mayoral et al. 1996). Based on previous research on aphid behavior during probing on susceptible and resistant plant species and cultivars (Gabryś & Pawluk 1999; Kordan et al. 2018, 2019, 2021; Stec et al. 2021), we established the following levels of grapevine susceptibility: (I) susceptible (phloem phase present and contains phloem sap ingestion); (II) relatively susceptible (over 90% of aphids reach the phloem phase but the phloem phase represented only by the E1 waveform related to the secretion of watery saliva); (III) moderately susceptible (40–75% of aphids reach the phloem phase, the phloem phase represented only by the E1 waveform related to the secretion of watery saliva); (IV) relatively resistant (5–35% of aphids reach the phloem phase, the phloem phase represented only by the E1 waveform related to the secretion of watery saliva); (V) resistant (no phloem phase).

In the present study, aphids for EPG experiments were 2–3 days old (2–3 days after the final molt) viviparous apterous *A. fabae* and *M. persicae*, which were attached to a golden wire electrode with silver paint and starved for 1 h prior to the experiments. The experiments were repeated 24 times for each species of grapevine and each replicate consisted of a freshly prepared aphid and a plant. Incomplete recordings (i.e., recordings that terminated due to aphid falling from the plant or where EPG signal was unclear) were rejected from analysis, so the final number of replications used for analyses was: *A. fabae*/*V. amurensis*, n = 19; *A. fabae*/*V. riparia*, n = 21; *A. fabae*/*V. vinifera*, n = 18 and *M. persicae*/*V. amurensis*, n = 17; *M. persicae*/*V. riparia*, n = 18; *M. persicae*/*V. vinifera*, n = 19. Probing behavior of aphids was monitored for 8 h continuously and all bioassays started at 10:00–11:00 h MEST (Middle European Summer Time). Giga-8 DC EPG system

with a 1 GΩ of input resistance (EPG Systems, Wageningen, The Netherlands) was used to record EPGs. EPGs were recorded and analyzed using Stylet+ Software (EPG Systems, Wageningen, The Netherlands). Signals were saved on the computer and the various behavioral phases were labeled manually using the Stylet+ software.

The following EPG waveform patterns were distinguished: "np" (no penetration, when aphid stylets did not have contact with plant tissues), "ABC" (pathway phase – penetration of parenchyma/mesophyll), "E1e" (salivation into apoplast), "E1" (salivation into sieve elements), "E2" including "E1/E2" transitions (ingestion of phloem sap), "F" (derailed stylet activities representing undetermined difficulties in probing) and "G" (ingestion of xylem sap). The waveform patterns that were not terminated before the end of the experimental period (8 h) were included in the calculations. In sequential parameters, when time to waveforms related to phloem phase (E1 or E2) or xylem phase (G) was calculated, only individuals that showed either of the phases were included in calculations and statistical analysis. In non-sequential parameters, when a given waveform had not been recorded for an individual, the duration of that waveform was given the value of 0.

High-performance liquid chromatography of flavonoids and stilbenoids

The dried grapevine leaves of *Vitis amurensis, V. riparia*, and *V. vinifera* (1.2 g of each) were homogenized in an aqueous ethanol solution (80%) using a Diax 900 homogenizer. The resulting suspension was centrifuged (12,000 rpm, 10 min) and the supernatant solution was collected in a graduated flask and the pellet was reconditioned. This operation was repeated three times, and the obtained extracts were combined. The homogenization procedure in combination with the extraction was carried out in such a way that the final volume of the extract was 100 ml. From the prepared ethanol extracts, 10 ml was taken and evaporated to dryness in a rotary evaporator under reduced pressure at 40°C. The dry extracts were dissolved in 100% methanol to a volume of 1 ml. Resulting methanolic extracts containing flavonoid compounds were analyzed by HPLC–ESI-MS/MS.

The contents of flavonoids apigenin, catechin, epicatechin, hesperetin, isorhamnetin, kaempferol, luteolin, naringin, quercetin, rutin, taxifolin and stilbenoids ampelopsin, piceid, resveratrol, ε-viniferin were determined. The selection of the phenolic compounds for analysis was based on literature data that reported major phenolic constituents of vegetative parts of grapevines (Castillo-Munoz et al. 2010; Pawlus et al. 2012; Portu et al. 2015; Biais et al. 2017; Aliaño-González et al. 2020; Goufo et al. 2020; Baroi et al. 2022).

Individual pure flavonoids and stilbenoids were purchased from Sigma–Aldrich (Poland). Ethanol, HPLC gradient grade methanol and acetonitrile were supplied by Merck (Germany). Formic acid was purchased from Sigma-Aldrich (Poland). Stock standard solutions of individual phenolic compounds (50 mg/l) were prepared by dissolving appropriate amounts of solid reagents in methanol. Mixed working standard solutions of flavonoid compounds at 20, 10, 5, 2.5 and 1 mg/l concentrations were prepared by appropriate dilutions of stock standard solutions.

The chromatographic analysis was carried out with a Shimadzu LC system, comprising a LC20-AD binary pump, a DGU-20A5 degasser, a CTO-20AC column oven and a SIL-20AC autosampler, connected to a 3200 QTRAP hybrid triple quadrupole (Applied Biosystem, MDS SCIEX, USA) with electrospray ionization source (ESI) operated in negative-ion mode. Phenolic compounds were separated on a Phenomenex Luna C-18 column (100 × 2.0 mm × 3.0 μm) with a pre-column, both maintained at 30°C. A 7.4 mmol/l solution of formic acid (pH 2.8, eluent A) and acetonitrile (eluent B) were used. The mobile phase was delivered at 0.2 ml/min in a linear gradient mode as follows: 0–2 min 10% B, 30 min 60% B, 40 min 100% B, 55 min 10% B. Flavonoids and stilbenoids were identified by comparing their retention times and m/z values of precursor and resulting fragmentation product ions in their MS and MS/MS spectra, respectively, to those obtained for respective standard solutions analyzed under the same conditions. The quantification of flavonoids was done using calibration curves obtained in the SRM (single reaction mode) (Biesaga & Pyrzyńska 2013; Sergiel et al. 2014).

Analysis of leaf anatomy

For anatomical measurements, 0.5 mm thick pieces from distal and proximal parts of the leaves (Fig. S1) were cut and fixed for 24 hours in Karmovsky's fixative containing 10% paraformaldehyde and 25% glutaraldehyde in 0.2 M phosphate buffer (pH 7.2). The fixed material was rinsed three times (15 min. each time) in 0.1 M phosphate buffer (pH 7.4), dehydrated in a graded ethanol series (for 10 min. in 30%, 50%, 70%, and 96% ethanol and 2 × 10 min. in 99.8% ethanol), and placed in 100% polypropylene oxide (2 × 10 min.). The slices were then placed overnight in a 1:1 mixture of epon resin (5.25 ml Poly/Bed 812, 3.25 ml DDSA, 0.75 ml MNA, 0.175 DMP) and

propyl oxide. Next day, the plant samples were embedded in 100% epon resin. Transverse slices (0.5 μm) from distal and proximal sections of the leaves were obtained using a rotary automatic microtome Leica HistoCore Nanocut R. Slices were stained with toluidine blue, mounted on slides and examined with a light microscope Carl Zeiss Axio Imager 2 coupled with Zeiss AxioCam ERc 5 s and ZEN Lite computer programme.

Anatomical evaluation of grapevine leaves included measurements of the abaxial epidermal cell thickness, the distance from the abaxial leaf surface to phloem and the simulation of the shortest aphid stylet penetration pathway from abaxial leaf surface to phloem. The stylet pathway was expressed as intercellular area within epidermis and mesophyll (μm^2). Three measurements at three different sites of the leaf midrib at proximal and distal sections of the leaf were taken (Fig. S2). The average of the three measurements was considered one replication. The number of replications (=analyzed slices) was n = 9.

Statistical analysis

EPG parameters describing aphid probing behavior were calculated manually and individually for every aphid and the mean and standard errors were subsequently calculated using the EPG analysis Excel worksheet created by the authors especially for this study. The data thus obtained were analyzed for the significance of differences among grapevine species using Kruskal–Wallis test and post-hoc multiple comparisons of mean ranks for all groups (Dunn's test). Values of $p < 0.05$ were considered statistically significantly different.

For anatomical measurements, dual comparisons were applied. First, differences between values obtained from distal and proximal parts were evaluated within each grapevine species by Mann-Whitney U-test. Second, differences among grapevine species using values obtained from distal and proximal parts of the leaves were evaluated by Kruskal–Wallis test and post-hoc multiple comparisons of mean ranks for all groups (Dunn's test). Values of $p < 0.05$ were considered statistically significantly different.

All statistical calculations were performed using StatSoft, Inc. (2014) STATISTICA (data analysis software system), version 12.

Results

Aphid probing behavior

The 8-h EPG monitoring of *Aphis fabae* and *Myzus persicae* behavior on grapevines *Vitis amurensis, V. riparia and V. vinifera* revealed activities defined as no-probing and probing. Probing activities embraced pathway phase, xylem phase, and phloem phase. Pathway phase included progressive stylet movements in apoplast with intracellular punctures and watery salivation into apoplast. Phloem phase occurred rarely in both aphid species and in all grapevines studied. When present, the phloem phase consisted only of watery salivation into sieve elements. No phloem sap ingestion was recorded in any aphid on any grapevine species (Tables I and II, Figures 1 and 2).

In *A. fabae*, probing activities predominated on all grapevine species during the entire 8-h monitoring period and occupied from 62.6% of the experimental time in *V. vinifera* to 82.0% in *V. amurensis* (Table I, Figure 2). The average duration of a probe, i.e., the time when aphid stylets were in plant tissues, ranged from 12 minutes on *V. vinifera* to *47* min. in *V. riparia*. In *V. vinifera*, short, less than 2 minutes long, probes were the most common (51% of all probes), while in *V. amurensis* and *V. riparia*, the most common were probes longer than 10 minutes (40% and 38%, respectively). The proportion of probes that included phloem phase ranged from 0.4% of the probes in *V. vinifera* to 1.6% in *V. amurensis*. Probing activity included pathway phase, which occurred in all studied grapevines, and activities in vascular tissues, which occurred with different frequencies, depending on grapevine species. Within non-vascular tissues, the pathway (= the progressive stylet movements within epidermis and mesophyll apoplast with short intracellular punctures) predominated over other probing activities in all grapevine species (from 49.7% in *V. vinifera* to 58.9% in *V. amurensis*). Other activities, i.e., salivation into apoplast of peripheral tissues occupied from 1.4% to 2.3% on V. *vinifera* and *V. amurensis*, respectively, and derailed stylet movements – from 7.2% on *V. vinifera* and *V. amurensis* to 16.9% on *V. riparia*. The vascular tissues were reached within 3–5 hours from the onset of the first probe within the experiment, which was represented by the appearance of xylem phase (3–4 hours) and phloem phase (5 hours). Xylem phase was the most common activity within vascular tissues and it occurred in 76.2%, 36.8%, and 22.2% of aphids on *V. riparia, V. amurensis* and *V. vinifera*, respectively (Figure 3) and occupied from 4.1% to 9.5% of probing time in *V. amurensis* and *V. riparia*, respectively. Phloem phase was rare: it consisted only of watery salivation and occurred in 1 of the 18 studied aphids on *V. vinifera* (6%), 3 of 21 aphids on *V. riparia* (14%) and in 6 of 19 aphids on *V. amurensis* (32%). In *V. amurensis*, where the

Table I. Probing behavior of *Aphis fabae* on *Vitis amurensis*, *Vitis riparia* and *Vitis vinifera*.

EPG variable[1]	*Vitis amurensis*	*Vitis riparia*	*Vitis vinifera*
General aspects	n = 19	n = 21	n = 18
Total duration of no probing (h) [2]	2.7 ± 0.3ab	1.4 ± 0.2b	3.0 ± 0.4a
Total duration of probing in non-phloem tissues C + F + G + E1e (h) [2]	5.8 ± 0.3ab	6.6 ± 0.2b	5.0 ± 0.4a
Total duration of pathway phase C (min) [2]	283.0 ± 15.0	258.0 ± 21.0	238.4 ± 22.2
Total duration of xylem phase G (min) [2]	19.8 ± 8.0ab	45.5 ± 10.1b	20.4 ± 14.7a
Total duration of phase F (min) [2]	36.3 ± 12.4	81.1 ± 24.1	34.7 ± 14.8
Total duration of phase E1e (min) [2]	10.9 ± 5.7	8.8 ± 4.3	6.8 ± 2.3
Total duration of probing in phloem E1+ E2 (min) [2]	0.4 ± 0.2	0.1 ± 0.1	0.0 ± 0.0
Time to the first probe (min) [2]	17.2 ± 9.4	11.6 ± 4.1	5.3 ± 1.7
Number of probes[2]	25.1 ± 3.6ab	18.1 ± 1.9b	28.8 ± 2.8a
Number of probes < 2 min	9.4 ± 2.2b	6.0 ± 1.0b	14.6 ± 1.7a
Number of probes >2 min<10 min	5.6 ± 1.3	5.2 ± 0.8	6.6 ± 0.9
Number of probes > 10 min	10.1 ± 0.8ab	6.8 ± 0.6ac	7.6 ± 0.7a
Number of probes with phloem phase[2]	0.4 ± 0.1	0.1 ± 0.1	0.1 ± 0.01
Mean duration of a probe (min) [2]	22.1 ± 4.9b	47.3 ± 20.1ab	12.1 ± 1.8a
Time from the beginning of experiment to the first xylem phase G (h)[3]	4.0 ± 1.3	3.4 ± 0.5	n.a.
	n = 7	n = 16	n = 4
Probing in non-phloem tissues before first phloem phase	n = 6	n = 3	n = 1
Time to the first probe (min)[4]	3.8 ± 1.5	n.a.	n.a.
Duration of the first probe (min)[4]	0.5 ± 0.1	n.a.	n.a.
Number of probes before the first phloem phase[4]	25.6 ± 7.4	n.a.	n.a.
Time from the first probe to the first phloem phase (h)[4]	5.0 ± 1.1	n.a.	n.a.
Probing in phloem tissues	n = 6	n = 3	n = 1
Duration of the first phloem phase E1 or E1+ E2 (min)[4]	0.6 ± 0.1	n.a.	n.a.
Duration of the first phloem salivation period E1 (min) [4]	0.6 ± 0.1	n.a.	n.a.
Duration of the first phloem sap ingestion period E2 (min) [4]	0.0 ± 0.0	n.a.	n.a.
Number of phloem phases[4]	1.3 ± 0.2	n.a.	n.a.
Mean duration of phloem phase E1+ E2 (min) [4]	0.9 ± 0.3	n.a.	n.a.
Mean duration of phloem salivation phase E1 (min) [4]	0.9 ± 0.3	n.a.	n.a.
Mean duration of phloem sap ingestion phase E2 (min) [4]	0.0 ± 0.0	n.a.	n.a.
Proportion of phloem phase in total probing (E1+ E2)/(C + F + G + E1e+E1+ E2) [4]	0.003 ± 0.002	n.a.	n.a.
Proportion of salivation in phloem phase E1/(E1+ E2) [4]	1.0 ± 0.0	n.a.	n.a.

[1]C = pathway, E1e = watery salivation into apoplast, F = unidentified difficulties in penetration, G = xylem sap ingestion, E1 = watery salivation into sieve elements, E2 = phloem sap ingestion, np = no-probing; [2] all replicates (=individual EPG recordings) were included in statistical analysis irrespective of the presence of phloem or xylem phase; [3]only replicates that embraced xylem phase were included in statistical analysis; [4] only replicates that embraced at least phloem phase E1 were included in statistical analysis; n = number of replicates included in statistical analysis; n.a. = not analyzed statistically due to the low number (fewer than 5) of replicates in which the phloem phase occurred. Values represent means ± SE. Different letters in rows denote statistically significant differences among grapevine species (Kruskal–Wallis test, $p < 0.05$).

phloem phase was the most common, individual periods of phloem phase were short, 36 seconds on average and the average proportion of phloem phase in all aphid probing activities was 0.02% (Table I, Figure 2).

In *M. persicae*, probing activities occupied from 74.6% of the 8-h experiment in *V. vinifera* to 77.9% in *V. riparia* (Table II, Figure 2). The average duration of the probe ranged from 17 minutes in *V. amurensis* and *V. vinifera* to 27 min in *V. riparia*. Short, less than 2 minutes long, probes were the most common, from 42% in *V. riparia* to 54% in *V. amurensis*. The proportion of probes that included phloem phase ranged from 3.0% in *V. riparia* to 4.3% in *V. vinifera*. Probing activity included pathway phase, which occurred in all studied grapevines, and activities in vascular tissues, which occurred with different frequencies, depending on grapevine species. Within non-vascular tissues, the pathway activity predominated in all grapevine species and it occupied from 33.9% to 49.5% of all activities in *V. riparia* and *V. amurensis*, respectively. Other activities, i.e., salivation into apoplast of non-vascular tissues, occupied from 7.3% to 14.4% in *V. amurensis* and *V. vinifera*, respectively, and derailed stylet activities – from 7.2% to 20.2% on *V. amurensis* and *V. riparia*, respectively. The vascular tissues were reached within 2.2–3.6 hours from the onset of the first probe within the

Table II. Probing behavior of *Myzus persicae* on *Vitis amurensis*, *Vitis riparia* and *Vitis vinifera*.

EPG variable[1]	*Vitis amurensis*	*Vitis riparia*	*Vitis vinifera*
General aspects	n = 17	n = 18	n = 19
Total duration of no probing (h)[2]	1.9 ± 0.4	1.8 ± 0.4	2.0 ± 0.4
Total duration of probing in non-phloem tissues C + F + G+ E1e (h)[2]	6.0 ± 0.4	6.2 ± 0.4	5.9 ± 0.4
Total duration of pathway phase C (min)[2]	237.4 ± 15.5	162.8 ± 25.4	220.6 ± 18.7
Total duration of xylem phase G (min)[2]	52.4 ± 10.7	52.2 ± 14.0	27.4 ± 5.8
Total duration of phase F (min)[2]	34.7 ± 13.0a	96.9 ± 16.8b	39.3 ± 9.8a
Total duration of phase E1e (min)[2]	34.9 ± 11.6	60.7 ± 17.4	69.0 ± 21.4
Total duration of probing in phloem E1+ E2 (min)[2]	4.8 ± 1.7ab	1.2 ± 0.4ac	2.7 ± 0.6a
Time to the first probe (min)[2]	3.3 ± 1.7	8.7 ± 2.8	4.3 ± 1.4
Number of probes[2]	39.8 ± 5.8ab	22.9 ± 4.6ac	30.2 ± 3.0a
Number of probes < 2 min	21.1 ± 4.6	9.6 ± 2.1	12.2 ± 1.8
Number of probes >2 min<10 min	10.4 ± 2.1	6.3 ± 1.5	8.5 ± 1.4
Number of probes > 10 min	8.3 ± 0.8	7.0 ± 0.9	9.5 ± 0.8
Number of probes with phloem phase[2]	1.5 ± 0.2	0.7 ± 0.3	1.3 ± 0.2
Mean duration of a probe (min)[2]	17.9 ± 4.4	26.8 ± 4.0	17.3 ± 3.9
Time from the beginning of experiment to the first xylem phase G (h)[3]	2.2 ± 0.4	3.1 ± 0.5	3.1 ± 0.4
	n = 16	n = 17	n = 13
Probing in non-phloem tissues before first phloem phase	n = 13	n = 8	n = 14
Time to the first probe (min)[4]	4.3 ± 2.1	5.5 ± 4.0	5.1 ± 1.9
Duration of the first probe (min)[4]	19.3 ± 9.3b	2.4 ± 0.0ab	1.1 ± 0.6a
Number of probes before the first phloem phase[4]	16.7 ± 5.7	18.6 ± 6.9	14.7 ± 0.3
Time from the first probe to the first phloem phase (h)[4]	2.6 ± 0.5	3.6 ± 0.8	3.3 ± 0.4
Probing in phloem tissues	n = 13	n = 8	n = 14
Duration of the first phloem phase E1 or E1+ E2 (min)[4]	2.5 ± 1.0	1.2 ± 0.4	2.0 ± 0.5
Duration of the first phloem salivation period E1 (min)[4]	2.5 ± 1.0	1.2 ± 0.4	2.0 ± 0.5
Duration of the first phloem sap ingestion period E2 (min)[4]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Number of phloem phases[4]	3.2 ± 0.6	2.4 ± 0.5	2.5 ± 0.2
Mean duration of phloem phase E1+ E2 (min)[4]	2.0 ± 0.3	1.3 ± 0.4	1.6 ± 0.3
Mean duration of phloem salivation phase E1 (min)[4]	2.0 ± 0.3	1.3 ± 0.4	1.6 ± 0.3
Mean duration of phloem sap ingestion phase E2 (min)[4]	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Proportion of phloem phase in total probing (E1+ E2)/(C + F + G + E1e+E1+ E2)[4]	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Proportion of salivation in phloem phase E1/(E1+ E2)[4]	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0

[1]C = pathway, E1e = watery salivation into apoplast, F = unidentified difficulties in penetration, G = xylem sap ingestion, E1 = watery salivation into sieve elements, E2 = phloem sap ingestion, np = no-probing; [2] all replicates (=individual EPG recordings) were included in statistical analysis irrespective of the presence of phloem or xylem phase; [3]only replicates that embraced xylem phase were included in statistical analysis; [4] only replicates that embraced at least phloem phase E1 were included in statistical analysis; n = number of replicates included in statistical analysis;. Values represent means ± SE. Different letters in rows denote statistically significant differences among grapevine species (Kruskal–Wallis test, $p < 0.05$).

experiment, which was represented by the appearance of xylem phase (2.2–3.1 hours) and phloem phase (2.6–3.6 hours). Xylem phase was the most common activity within vascular tissues and it occurred in 94.4%, 94.1%, and 68.4% aphids on *V. riparia*, *V. amurensis*, and *V. vinifera*, respectively (Figure 3) and occupied from 5.7% to 10.9% of the activities on *V. vinifera*, and *V. amurensis* and *V. riparia*, respectively. The phloem phase, which consisted of only watery salivation, occurred in 76%, 74%, and 44% aphids on *V. amurensis*, *V. vinifera*, and *V. riparia*, respectively. Individual bouts of the phloem phase were 1–2 minutes long in all grapevine species and the average proportion of phloem phase in all aphid activities ranged from 0.3% in *V. riparia* to 1.0% in *V. amurensis* (Table II, Figure 2).

Flavonoids and stilbenoids in grapevine leaves

In grapevine leaves studied, both, flavonoids and stilbenoids, were identified. Of the analyzed flavonoids, the flavan-3-ols catechin and epicatechin and flavonols isorhamnetin, kaempferol, quercetin and rutin, which occurred in different amounts and proportions in individual grapevine species, were detected (Table S1, Figure 4). Catechin, epicatechin, and quercetin occurred in all species, kaempferol and rutin were detected in *V. amurensis* and *V. riparia*, and isorhamnetin occurred only in *V. amurensis*. Quercetin was the most abundant flavonoid in *V. amurensis* and *V. riparia* (52% and 58% of all flavonoids, respectively), while in *V. vinifera* – catechin was the most common (71%) (Figure 4). Of the analyzed stilbenoids, piceid, resveratrol and ε-viniferin were detected. Piceid occurred in all

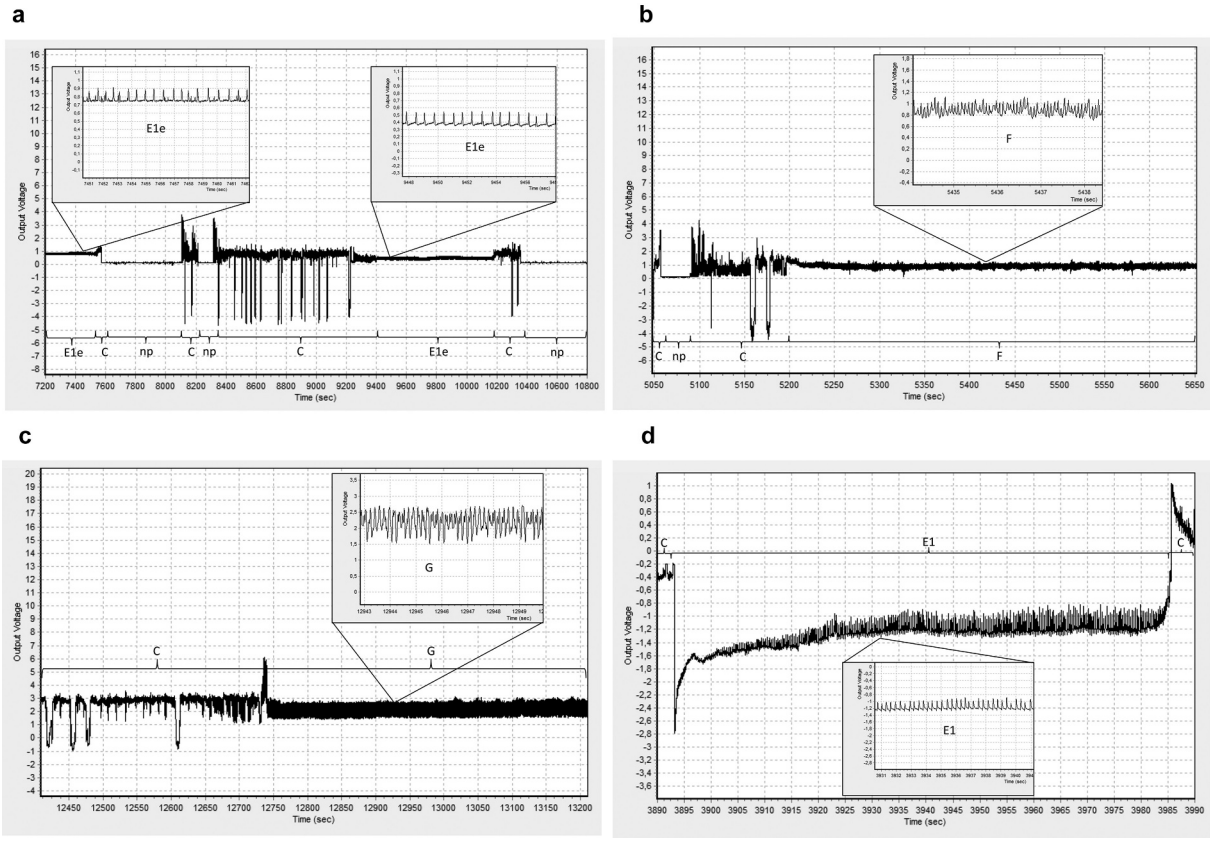

Figure 1. Visualization of characteristic aphid probing activities derived from Electrical Penetration Graphs (EPGs) technique. The illustration is composed of representative samples from EPG recordings in the present study. The images for panels a, c and d were obtained from EPG recordings of *Aphis fabae* on *Vitis amurensis* and the image for panel b was obtained from EPG recording of *Myzus persicae* on *V. amurensis*. "np" – no probing, "C" – pathway activity in apoplast with intracellular punctures, "E1e" – watery salivation into apoplast, "E1" – watery salivation into sieve elements, "F" – derailed stylet activities in apoplast, "G" – sap ingestion from xylem vessels.

grapevine species, while resveratrol in *V. amurensis* and *V. vinifera* and ε-viniferin only in *V. vinifera*. Piceid was the most abundant stilbenoid in all grapevine species (76%, 100%, and 64% of all analyzed stilbenoid compounds in *V. amurensis, V. riparia*, and *V. vinifera*, respectively) (Table S1, Figure 4).

Anatomy of grapevine leaves

The anatomical structure of grapevine leaves was analyzed based on transverse sections of proximal and distal parts of the leaves, at the region of the midrib (Fig. S1). The visual examination revealed adaxial epidermis, collenchyma, parenchyma, and the vascular bundle surrounded by a sheath (Figure 5), which was typical for grapevines (Keller 2015a). No visible differences in the anatomical organization of leaf tissues occurred among the studied grapevine species (Figure 5). The thickness of abaxial epidermis in grapevine leaves ranged from 5.1 μm to 6.1 μm in proximal parts of the leaf and from 5.0 μm to 8.1 μm in distal parts in *V. amurensis* and *V. riparia*, respectively (Table III). The distance from abaxial leaf surface to the closest phloem vessel ranged from 62.3 μm to 86.6 μm in proximal parts in *V. vinifera* and *V. riparia*, respectively, and from 58.1 μm to 109.3 μm in distal parts in *V. vinifera* and *V. amurensis*, respectively. The length of the simulated shortest aphid stylet pathway from abaxial leaf surface to phloem, expressed as intercellular area within epidermis and mesophyll, ranged from 181.6 μm^2 in *V. vinifera* to 279 μm^2 in *V. riparia* in proximal parts of the leaf and from 146.8 μm^2 in *V. vinifera* to 384,7 μm^2 in *V. amurensis* in distal parts (Table III).

Discussion

The analysis of parameters describing the behavior of *A. fabae* and *M. persicae* during stylet penetration

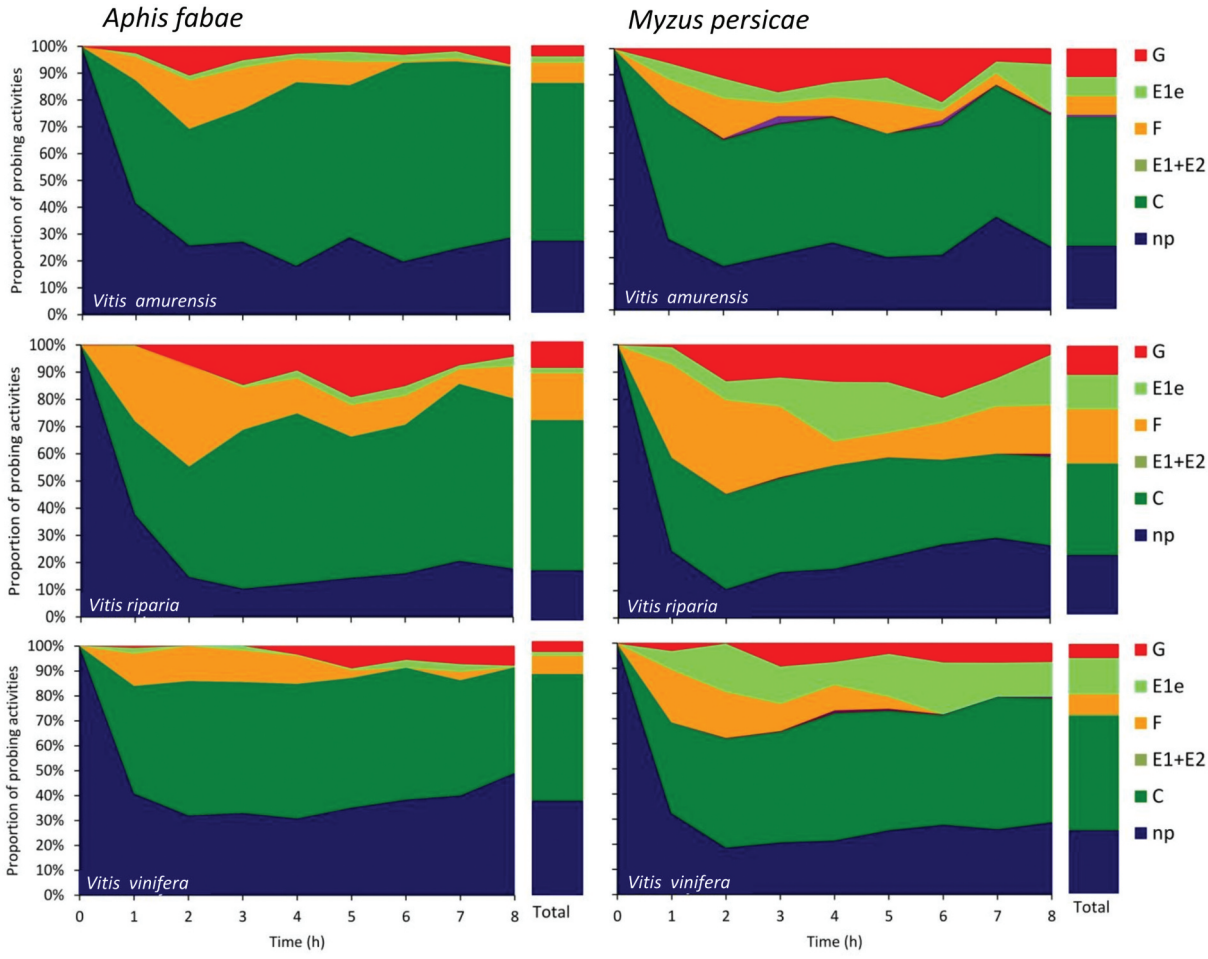

Figure 2. Temporal changes in probing behavior of *Aphis fabae* and *Myzus persicae* on grapevines *Vitis amurensis, Vitis riparia* and *Vitis vinifera*. G = xylem sap ingestion; E1e = watery salivation into apoplast; F = unidentified difficulties in penetration, E1 = watery salivation into sieve elements, E2 = phloem sap ingestion, C = pathway, np = no-probing. "Total" = proportion of all aphid activities during 8-hour EPG monitoring.

in leaf tissues showed that the two aphid species responded to the studied grapevine species differently. The recorded significant differences were related both to general aspects of probing and when probing was analyzed individually in non-vascular and vascular tissues.

The total time of no-probing did not differ in the case of *M. persicae* on each of the examined vines, where it amounted to 2 h in total during the 8-h experiment. In contrast, in *A. fabae*, no-probing was shortest on *V. riparia* (1.4 hr) and twice as long on *V. riparia* and *V. vinifera*. Consequently, the total duration of *A. fabae* probing was longest on *V. riparia* as opposed to the two other grapevine species. The prolonged no-probing times may indicate the presence of factors that discourage the continuation of probing, such as secondary metabolites (Mayoral et al. 1996; Prado & Tjallingii 1997).

The main activity in non-vascular tissues in all aphids on all grapevines was the progressive movement of stylets towards vascular tissues, classified as "C": 50–60% in *A. fabae* and 34–50% in *M. persicae*. This activity is mainly extracellular, with brief, 5–10 sec punctures of cells adjacent to stylet path (Tjallingii 1978). During these punctures, samples of cell contents are ingested for gustatory purposes (Tjallingii & Esch 1993; Mayoral et al. 1996; Pettersson et al. 2007). Aphids use the information gathered during these punctures to continue or terminate the probes (Tjallingii 2001, 2006). In the present study, the probing activity of aphids on grapevines was frequently interrupted: during the 8-h EPG monitoring, there were from 18.2 to 28.8 probes per aphid in *A. fabae* and from 22.9 to 39.8 probes per aphid in *M. persicae*. In *A. fabae*, significantly more probes (1.5 times more) occurred in

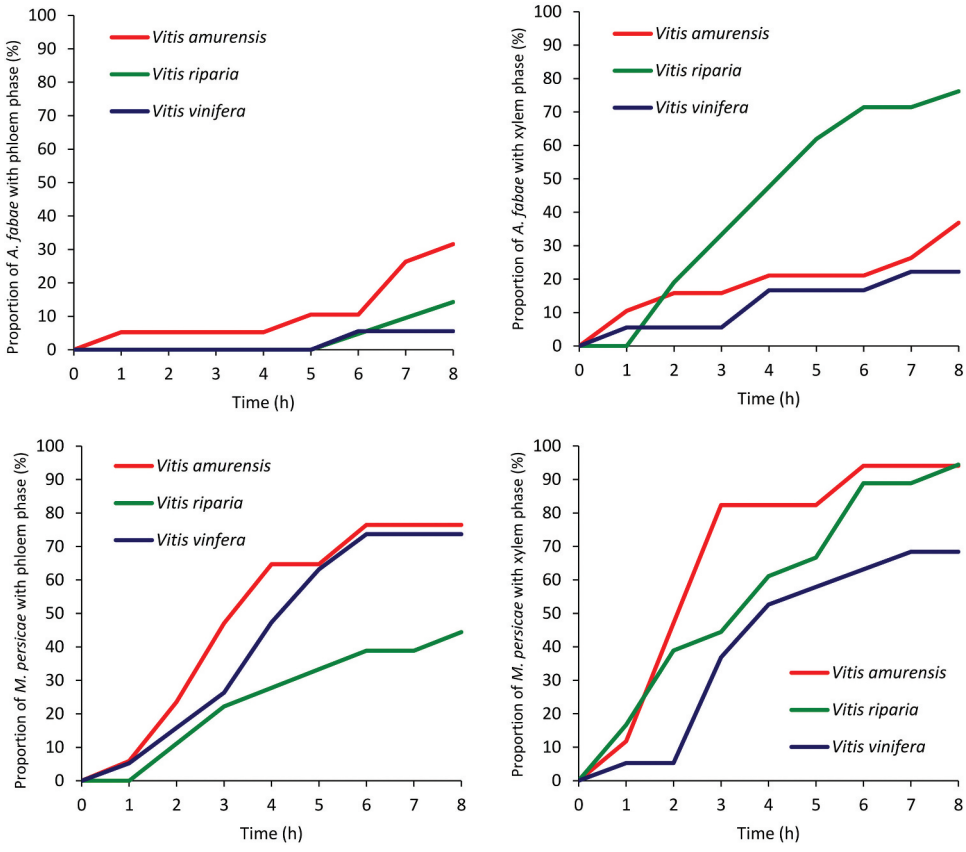

Figure 3. Proportion of *Aphis fabae* and *Myzus persicae* that reached vascular tissues, phloem and xylem, in the leaves of *Vitis amurensis*, *Vitis riparia* and *Vitis vinifera*.

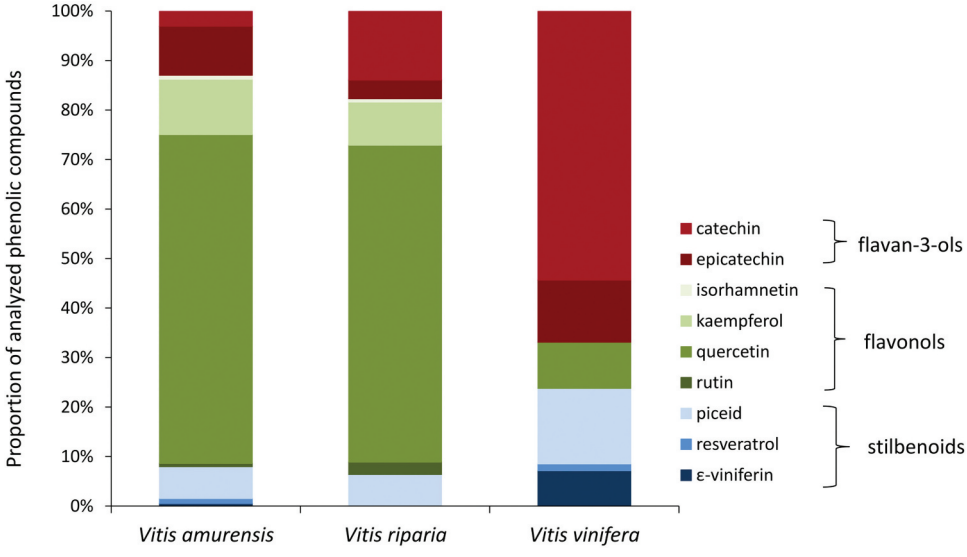

Figure 4. Proportion of phenolic compounds analyzed in grapevines *Vitis amurensis*, *V. riparia* and *V. vinifera*.

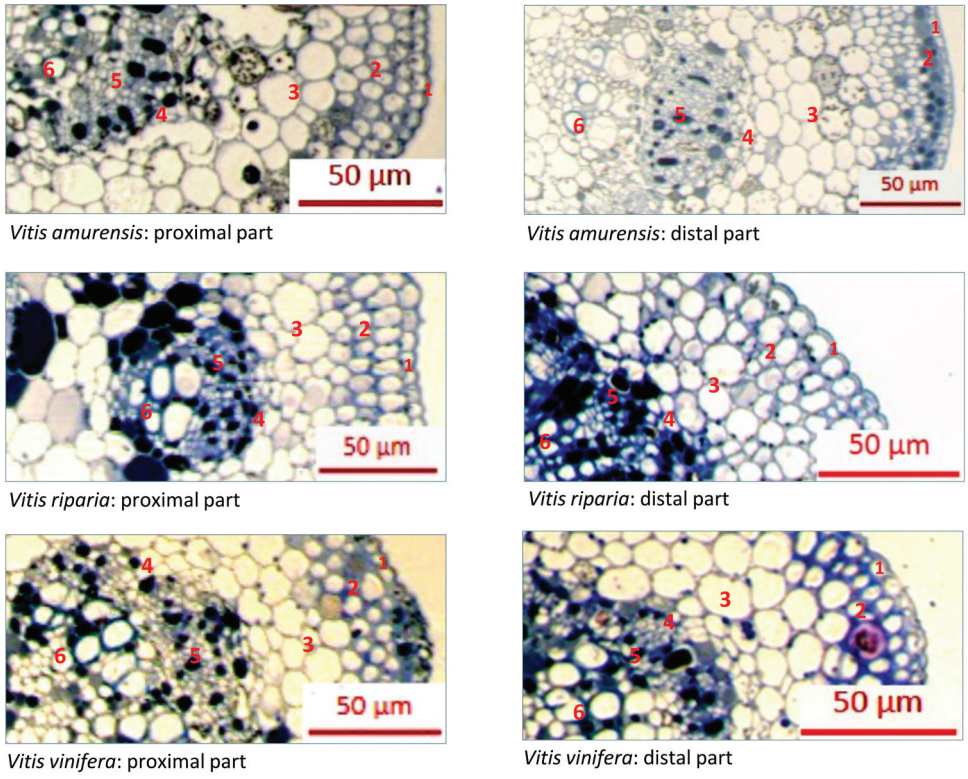

Figure 5. Representative samples of transverse sections of grapevine leaves at main veins of the leaves of *Vitis amurensis, V. riparia* and *V. vinifera* observed under light microscope Carl Zeiss Axio Imager 2 coupled with Zeiss AxioCam ERc 5s and ZEN Lite computer programme (400x magnification). 1 – adaxial epidermis; 2 – collenchyma; 3 – parenchyma; 4 – vascular bundle sheath; 5 – phloem; 6 – xylem.

Table III. Anatomical measurements of leaves of *Vitis amurensis, Vitis riparia* and *Vitis vinifera.*

	Vitis amurensis	*Vitis riparia*	*Vitis vinifera*
Epidermis			
Proximal	5.1 ± 0.4	6.1 ± 0.2 a	5.7 ± 0.3
Distal	5.0 ± 0.2 A	8.1 ± 0.2 b B	5.6 ± 0.3 A
Epidermis-Phloem			
Proximal	80.9 ± 5.0 a B	86.6 ± 6.2 B	62.3 ± 3.4 A
Distal	109.3 ± 9.1 b B	68.4 ± 3.7 A	58.1 ± 2.9 A
Pathway			
Proximal	275.6 ± 40.9	279.0 ± 37.8 a	181.6 ± 17.7
Distal	384.7 ± 54.1 B	156.7 ± 12.0 b A	146.8 ± 15.7 A

"Epidermis" = abaxial epidermal thickness (µm); "Epidermis-phloem" = distance from leaf surface to phloem (= thickness of mesophyll and epidermis, i.e., the distance between abaxial leaf surface and the closest phloem vessel; in µm); "Pathway" = simulation of the shortest aphid stylet penetration pathway from abaxial leaf surface to phloem (μm^2). Values represent means ± SE; n = 9. Different small letters in columns indicate significant differences between proximal and distal parts of the leaf within a grapevine species (Mann-Whitney U-test, $p < 0.05$); different capital letters in rows indicate significant differences among grapevine species (Kruskal–Wallis test, $p < 0.05$)

V. vinifera than in *V. riparia* and in *M. persicae* – 1.5 times more probes occurred in *V. amurensis* than in *V. riparia*. It must be stressed that in both *M. persicae* and *A. fabae*, short penetrations lasting less than 2 minutes prevailed on all plants. The occurrence and frequency of short probes are good indicators of plant suitability. As the time required by aphid stylets to pass one layer of cells is approximately 2–3 minutes (Van Hoof 1958), it means that a large number of short penetrations in relation to the total number of probes may indicate the presence of factors in the epidermis and outer

mesophyll that discouraged the continuation of penetration in the tissues of the studied grapevines. Such relationship is typical of incompatible plant-aphid associations (Tjallingii 2001; Alvarez et al. 2006; Halarewicz & Gabryś 2012; Tetreault et al. 2019; Souza & Davis 2020). The presence of factors limiting probing within mesophyll is also indicated by the time from the beginning of penetration to the first phloem or xylem phase, i.e. the time to reach the vascular bundle in the leaves (Tjallingii 2001; Alvarez et al. 2006). During probing in grapevine leaf tissues, the phloem and xylem phases appeared in EPG recordings 2.6–3.6 and 2.2–3.1 hours, respectively, after the start of the first penetration in *M. persicae*, and after 5 and 3.4–4 hours, respectively, in *A. fabae*. On suitable hosts, aphids reach phloem usually after 2.4 hours of probing in non-vascular tissues (Wróblewska-Kurdyk et al. 2019). Moreover, the prolonged penetration time in the non-phloem compartments in relation to the penetration of phloem tissues may result from negative factors present in the phloem itself (Mayoral et al. 1996). Studies on the susceptibility of the varieties of two lupine species – yellow lupine *Lupinus luteus* L. and narrow-leaf lupine *Lupinus angustifolius* L. to the feeding of the pea aphid *Acyrthosiphon pisum* Harris have shown that the presence of various alkaloids in the tissues of lupines might have caused the extension of the duration of no-probing and an increase in the number of short probes (Kordan et al. 2008; Wróblewska et al. 2012). On the other hand, the reason for the occurrence of a large number of short penetrations may be the specificity of the method used to assess the behavior of aphids during the penetration of plant tissues (EPG technique), in which the aphids attached to an electrode of a certain length cannot leave the plant that is not suitable for them (Tjallingii 1986; Halarewicz & Gabryś 2012). Nevertheless, the EPG technique is indispensable to monitor aphid stylet penetration within plant tissues, as this activity is hidden from the human eye (Dancewicz et al. 2016; Zhang et al. 2017; Stec et al. 2021). This technique allows also a separate analysis of aphid behavior at pre-ingestive (within non-phloem tissues before the first phloem or xylem phase) and ingestive (within the phloem or xylem) phases of probing and localization of natural plant resistance factors (Pettersson et al. 2007). Apart from typical pathway activity "C", aphids on grapevine leaves also showed two other activities in non-vascular tissues which occurred quite commonly: "F" and "E1e". Activity "F" visualizes probably the "derailment" of the stylets in plant tissues (Alvarez et al. 2006; Silva-Sanzana et al. 2020) and its occurrence is usually linked to the aphid or plant physiological state or plant resistance level (Alvarez et al. 2007; Marchetti et al. 2009; Pompon & Pelletier 2012; Machado-Assefh & Alvarez 2018). In the present study, the significant increase in the duration of "F" was noted in *M. persicae* on *V. riparia* in relation to other species (more than 2 times longer duration of "F"). In *A. fabae*, no significant differences in the duration of "F" were observed, but on *V. riparia, A. fabae* also showed a tendency to increase the incidence of "F" in comparison to other grapevine species. The occurrence of "E1e" in EPG recordings is rare, and this activity is not fully understood. Most likely, it is associated with the secretion of watery saliva in the apoplast (Huang et al. 2012; Wu et al. 2013). Watery saliva contains a variety of detoxifying enzymes (Klinger et al. 1998; Miles 1999), hence the presence of this extracellular salivation activity and its increased proportion in aphid probing may indicate the presence of factors in epidermis and mesophyll that do not favor aphid penetration of plant tissues (Huang et al. 2012; Wu et al. 2013). In the present study, "E1e" occurred quite commonly in all aphids on all grapevines.

The success rate in reaching vascular bundles varied and it depended on both the aphid and the grapevine species. The proportion of *A. fabae* reaching xylem was highest on *V. riparia* (76%) and lowest on *V. vinifera* (22%) and phloem – highest on *V. amurensis* (31.6%) and lowest on *V. vinifera* (5.6%). The proportion of *M. persicae* reaching xylem was equally high on *V. amurensis* and *V. riparia* (94%) and lower on *V. vinifera* (68.4%) and phloem – comparable on *V. amurensis and V, vinifera* (76.5% and 73.7%, respectively) and lower on *V. amurensis* (44.4%). The duration of xylem phase "G" varied depending on the species of grapevine. In *A. fabae*, the duration of "G" was significantly longer on *V. riparia* in comparison to *V. amurensis* and *V. vinifera*. In *M. persicae*, the duration of "G" did not differ significantly among grapevine species. The increased proportion of xylem phase occurs in fasted individuals and may be caused by the presence of negative factors in the phloem tissues, which make it impossible to use phloem resources (Spiller et al. 1990; Leszczyński 1996). On the other hand, the increased xylem phase activity may also be caused by the inability to use phloem resources due to the presence of negative factors in the plant at the stage before the aphids reach the phloem (Liang et al. 2012; Souza & Davis 2020). The incidence of phloem phase in the present study was low and no phloem sap ingestion was recorded in any aphid that did reach sieve elements; phloem phase was represented of only watery

salivation "E1". A low percentage of aphids showing the phloem or xylem phase during probing may be due to the presence of negative factors in mesophyll, indicating difficulties in reaching vascular elements (Jiang et al. 2001; Kordan et al. 2019). The reduced uptake of phloem sap or complete absence of sap ingestion is the main feature related to plant resistance to aphid feeding: on susceptible plants, aphids have the ability to quickly initiate phloem sap uptake and achieve the continuous feeding phase (Sauge et al. 1998). As a rule, in susceptible (or host) plants, sap ingestion occurs shortly after the start of the phloem phase, moreover, in susceptible plants, the ingestion of phloem sap may last for many hours continuously (Akbar et al. 2014; Kordan et al. 2019). The presence of only E1 model may indicate the presence of factors in the phloem that negatively affect the behavior and feeding of aphids (Alvarez et al. 2006). These factors may make it difficult or impossible for aphids to take phloem sap. As stated earlier, saliva secreted by aphids may play a role in responses to plant defense mechanisms present in sieve elements, e.g. take part in detoxification of phenolic compounds produced by plants (Klinger et al. 1998; Canassa et al. 2020).

The studied grapevines show the features of resistant plants, described in numerous studies. (Sauge et al. 1998; Kordan et al. 2008, 2021; Philippi et al. 2015; Sun et al. 2018; Stec et al. 2021). Aphids on the resistant plant species or cultivars have difficulties in finding phloem vessels and/or start and continue feeding in a sustained manner. Usually, the observed resistance is linked to the variation in the content of plant-specific allelochemicals (Kordan et al. 2008, 2021; Philippi et al. 2015; Stec et al. 2021). Grapevine resistance to *A. fabae* and *M. persicae* detected in the present study, is most likely related to the presence of the antixenosis mechanism at the level of mesophyll and also phloem tissues: the phloem phase constituted a negligible proportion in the entire probing activity, the ingestion of phloem sap was non-existent, not all aphids reached the phloem elements, and those that reached the phloem phase did that at the late stages of the experiment. A high proportion of pathway and xylem phases during the entire aphid activity was also demonstrated, and a high proportion of G, F, and E1e activities was also observed. Antixenosis-based plant resistance to aphid feeding may result from the presence and concentration of secondary metabolites in plant tissues (Smith & Boyko 2007; Dogimont et al. 2010; Smith & Clement 2012; Smith & Chuang 2014). Among secondary metabolites, phenolic compounds are frequently reported as associated with plant resistance to herbivores (Lattanzio et al. 2000; Simmonds 2003; Buer et al. 2008; Pawlus et al. 2012; Ahmed et al. 2019). The studied grapevines differed in the quantitative and qualitative content of the analyzed group of phenolic compounds, flavonoids and stilbenoids. In all grapevines, flavonoids predominated among the analyzed phenolic compounds: in *V. amurensis* and *V. riparia* flavonoids amounted to 90% of the major phenolic compounds analyzed while in *V. vinifera* – 76%. The flavonoids belonged to two groups: flavan-3-ols and flavonols. Flavan-3-ols - catechin and epicatechin were present in the tissues of all studied grapevines. Of the flavonols analyzed, kaempferol, quercetin and rutin occurred in *V. amurensis* and *V. riparia*, while isorhamnetin occurred only in *V. amurensis*, and in *V. vinifera* only quercetin occurred. The stilbenoids occurred in all grapevines. Of the stilbenoids analyzed, piceid, resveratrol and ε-viniferin were detected in *V. vinifera*, piceid and resveratrol occurred in *V. amurensis*, and only piceid was recorded in *V. riparia*. The relationship between the susceptibility/resistance to aphids and the content of quercetin, kaempferol and isorhamnetin in plants was reported by Lattanzio et al. (2000), Togola et al. (2020), Kozak et al. (2015); Goławska and Łukasik (2009). In addition, the application of flavonoids to artificial diets confirmed the deterrent effect of flavonoids on the probing and feeding of several aphid species (Goławska & Łukasik 2012; Goławska et al. 2014; Stec et al. 2021).

In the anatomical studies of grapevine leaves, the following characters were determined: epidermal thickness, distance between abaxial leaf surface and phloem, and the simulated shortest pathway from epidermis to phloem. The analysis of data showed differences in the anatomical structure of leaves of individual grapevine species and varieties. Measurements were made in two parts of the leaf: the proximal part and the distal part. Significant differences occurred in the distance between epidermis and phloem between distal and proximal part of the leaf in *V. amurensis* and in the epidermal thickness and the simulated pathway in *V. riparia*. The epidermal thickness was highest in the distal part of *V. riparia* and the distance between leaf surface and phloem, and the value of the simulated pathway were highest in the distal parts of *V. amurensis* leaves and lowest in *V. vinifera* distal parts of the leaves. Aphids were able to move across the plant for a distance determined by the length of the electrode attached to the body (about 3 cm); therefore, during the EPG monitoring, they could penetrate tissues in various parts of the leaf (proximal and distal parts).

Considering the results of the EPG monitoring of aphid behavior on grapevines, it can be inferred that the anatomical parameters did not have a significant impact on the time to reach the phloem and xylem elements by *M. persicae* and *A. fabae*. The differences in aphid behavior on grapevines, especially those related to probing in non-phloem tissues did not correspond with the differences in plant anatomy. When analyzing the data in studies to assess the susceptibility of grapevine species to the feeding of *A. fabae* and *M. persicae*, the main EPG variables indicating difficulties in reaching the vascular tissue, both phloem and xylem, were the time from the beginning of the experiment to reaching phloem and/or xylem elements, or the percentage of aphids that reached the vascular elements. In *V. amurensis*, where a large distance between plant surface and phloem was noted and the simulated pathway was the longest, no significant differences in the values of EPG variables were observed. Similarly, no differences were found during the penetration of *V. vinifera* tissues, where the above two parameters had a lower value (the lowest epidermis-phloem distance, the shortest simulated pathway). The findings in the present study are in accordance with the results obtained by other authors who determined that the resistance of the studied plant cultivars did not depend on the anatomical structure of the leaves (Singh et al. 2020)

In conclusion, the present study revealed that all studied species of grapevines, *V. amurensis*, *V. riparia* and *V. vinifera* are relatively resistant to *A. fabae* and *M. persicae* (class "IV"). These grapevines showed features related to the activity of antixenosis factors at the level of mesophyll and phloem tissues, which was manifested in difficulties in tissue penetration by aphids, little success in reaching the phloem phase and its short duration. *Vitis vinifera* was the least susceptible grapevine species to *A. fabae*. No differences in the susceptibility among grapevines occurred against *M. persicae*. However, considering the higher success rate in reaching phloem phase and longer duration of probing, it can be inferred that *M. persicae* poses greater threat to the studied grapevines than *A. fabae*. The longer duration of probing increases the risk of virus transmission. Differences in anatomical structure of the leaves did not relate with aphid probing behavior. However, flavonoids and stilbenoids may have caused the reduction of probing in phloem tissues. The present study revealed that all studied species of grapevines, *V. amurensis*, *V. riparia* and *V. vinifera* showed features related to the activity of antixenosis factors at the level of mesophyll and phloem tissues, which was manifested in difficulties in leaf tissue penetration by aphids, little success in reaching the phloem phase and its short duration. It can be assumed that anatomical structure of the leaves did not affect aphid probing behavior but flavonoids and stilbenoids might have caused the reduction of probing in phloem tissues. However, unambiguous determination of the direct influence of individual flavonoids and stilbenoids and particularly, piceid, on the behavior of *M. persicae* and *A. fabae* requires further, more detailed studies involving the application of artificial diets.

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