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Effects of phenolic compounds on walnut bacterial blight in the green husk of Hungarian bred cultivars

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Abstract: The Persian walnut (*Juglans regia* L.) is the most grown fruit species among the nut tree crops in Central Europe. In the frame of this study the full Hungarian walnut assortment having distinct early spring phenology was examined to detect the difference in phenolic profile in their green husks. Furthermore, we investigated the relationship between the presence and concentration of phenolic compounds and tolerance / resistance of the observed cultivars to walnut bacterial blight. Examining the samples, significant differences were found between the concentrations of the different groups of phenolic compounds. Walnut blight immunity tests were also performed to clarify the role of phenolic compounds in the nut derived from non-irrigated orchard. The Hungarian bred local cultivars contained phenolic compounds in higher concentration than the domesticated ones. There was a significant correlation between the budburst, as well as pistillate flowers receptivity and the concentration of juglone. Cultivars with a low concentration of phenolic compounds were the most susceptible to walnut bacterial blight, except 'Bonifác'.

Keywords: phenolic compounds, Hungary, nut development, state approved cultivars, walnut blight immunity test, walnut phenology

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1. Introduction

The Persian walnut (*Juglans regia* L.) is the most grown nut tree crop in Central Europe, its planting area and harvested yield are increasing year on year in this region. All parts of the walnut such as leaves, green husk (mesocarp), pellicle, septum and kernel are rich in phythochemicals [1-5]. The nut is surrounded by the green husk, which contains a lot of bioactive compounds, such as like polyphenols including flavonoids, [6-7], also known as phenolics [8-10], furthermore pectin, glucans [5] and chlorophyll [11], which have antimicrobial, antifungal and antioxidant activities [8, 12-19]. The phenolic compounds play an important role in the plant defence mechanisms against stress caused by many environmental factors and various pathogens [20]. More than 50 compounds, mainly flavonoids and hydroxycinnamic acids, were identified until now, playing a role in this process.

The phenolic content was varied in all parts of walnut [21-22]. Quantity of the compounds depends on the soil and climate conditions [5, 23], the cultivar [8, 10, 24-27], the period inside the growing season [28], and the extracting solvent [29-32].

Unfortunately, the green husk is mostly an agricultural waste, but this valuable organ has some uses. Traditional alcoholic drinks [33-35], bottled fruits, jams [36, 37], cold-pressed oil [38-40] and some cosmetics [41, 42] can be made from it.

In the green husk of walnut from a chemical point of view, one of the most important bioactive compounds is phenolics. The most important groups of phenolics were hydroxybenzoic acids, hydroxycinnamic acids, flavonoids and naphtoquinones which were detected in the green husk samples. Dihydroxybenzoic acid, gallic acid and syringic acid together with their derivatives were hydroxybenzoic acids, while coumaric acid, chlorogenic acid, caffeic acid, ferulic acid and its derivatives or esters are classified in the group of hydroxycinnamic acids. The group of flavonoids includes myricetin, quercetin and naringenin and /or their derivatives, while naphtoquinones includes juglone with its different derivatives, which is a characteristic compound of unripe husks of the nuts [27, 28, 42] .

The phenolic compounds also play an important role in the defence against walnut blight caused by *Xantomonas arboricola* pv. *juglandis*. The gallic acid and the juglone dominated in the nuts compared to the other phenolics [43]. The juglone concentration is usually higher in the green husk and nuts than in the leaves [44]. Matias et al. [45, 46] found a negative correlation between juglone concentration and the susceptibility against walnut blight in the nuts. Juglone can be a discriminating biomarker for Xaj resistance [47, 48], because the juglone concentration is smaller in the healthy areas, than in the infected surfaces [49]. The resistance / tolerance of walnut cultivars against walnut bacterial blight doesn't depend on the density and breadth of stomas, the wax content of the leaves, leaf thickness, thickness of the abaxial epidermis nor stratum corneum [50].

2. Results

In the green husk of walnuts one of the most important bioactive compounds is the phenolics. The studied cultivars were examined to detect the difference in concentration of total phenolics and the various compounds. The total phenolic content of the green husk in the samples ranged between 4420-5740 mg GAE /100 g d.m. A series of studies have looked at the total phenolic content of the green husk, usually examining the samples in the context of antioxidant capacity. Present results are in line with data published by Soto-Madrid and co-workers [7] where the range of the total phenolic compounds was between 3117-10601 mg GAE/100 g d.m. The research group of Soto-Maldonado [42] obtained the total polyphenol content as 1862.9 ± 72.4 mg GAE/100 g d.m. measured in the tested sample, while Oliveira and co-workers [8] determined the total phenolic content of the green husk of different cultivars and found, that their concentration ranged from 32.61 mg/g of GAE (cv.' Mellanaise)' to 74.08 mg/g of GAE (cv. 'Franquette'). Despite the many applications, the Folin-Ciocalteu method [51] is only suitable for a rough estimate because, as Scalbert and co. [52] described, the molar absorbance or otherwise molar absorptivity per reactive group obtained for different

compounds varies, in other words the color generated by the different phenolic compounds depends on the molecular structure.

There is limited information in the scientific literature about detailed analysis of walnut green husk, however it can be important, when we take into account the time of the different developmental stages of the examined cultivars.

When examining the cultivars the difference in concentration of the various compounds was also determined. Figures 2 and 3 represents the chromatograms of the 'Bonifác' sample measured on 280 nm wavelength for the main phenolic components and on 420 nm for juglone determination.

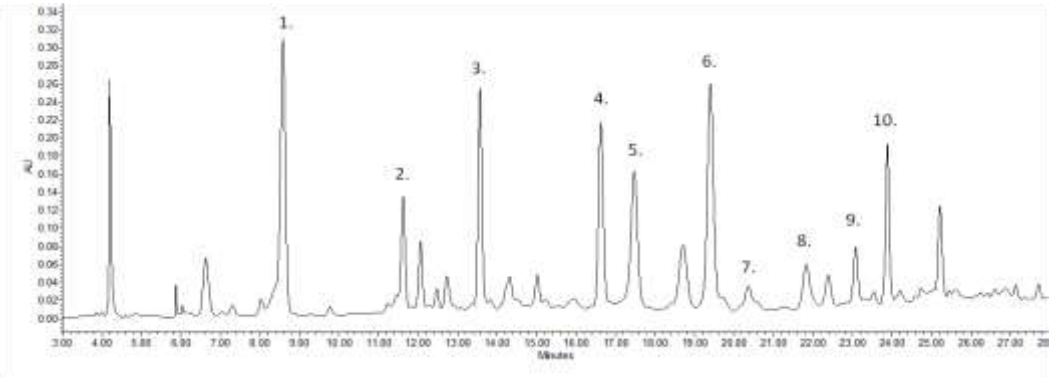


Figure 2. Chromatogram of 'Bonifác' sample for the determination of the main identified phenolic components (λ= 280 nm; 1. gallic acid, 2. neochlorogenic acid, 3. p-coumaric acid, 4. syringic acid, 5. methylmirecitin derivative 1, 6. trihydroxyisoflavanone derivative, 7. quercetin-hexoside; 8. methylmirecitin derivative 2, 9. methoxynaringenin derivative, 10. quercetin-pentoside)

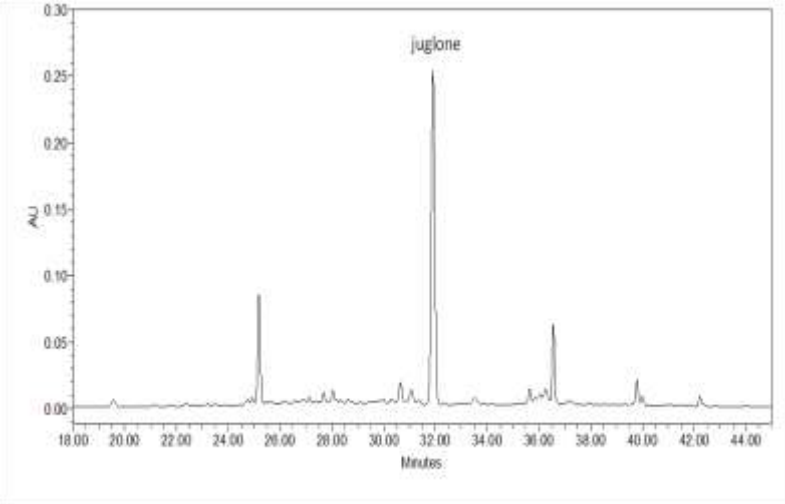


Figure 3 Chromatogram of 'Bonifác' sample for the determination of juglone (λ= 420 nm)

Examining the samples, significant differences were found between the concentrations of the different groups of phenolic compounds. In our measurements all components were

calculated in rutin equivalent, except for the naphtoquinones calculated in juglone equivalent. Figure 4 depicts the phenolic compound composition of the samples. The green husk of the 'Alsószentiváni' type samples contained the highest concentration of the components in question, especially 'Bonifác', in which the concentration of hydroxybenzoic acids, hydroxycinnamic acids, flavonoids and naphtoquinones were 3176 mg/100 g d.m., 1947 mg/100 g d.m., 2203 mg/100 g d.m., 1275 mg/100 g d.m., respectively. Comparing these results to the control 'Chandler', the total content of phenolics in the green husk was about 3.7 times higher in 'Bonifac'. The total phenolics varied in the samples between 2329-8601 mg/100 g d.m.

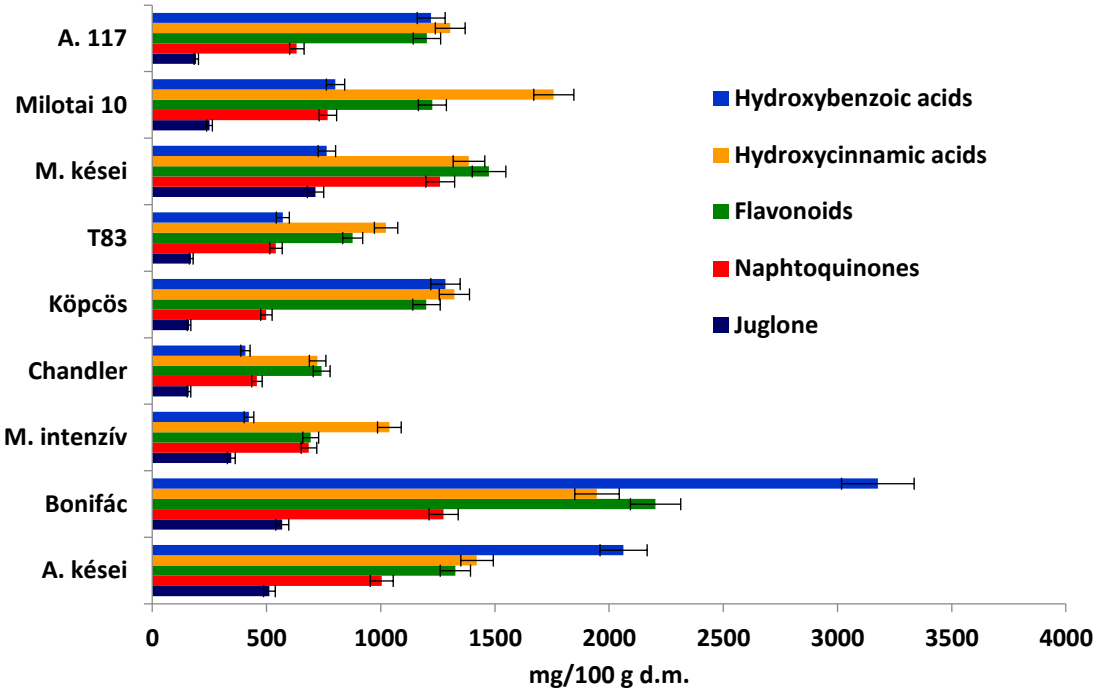
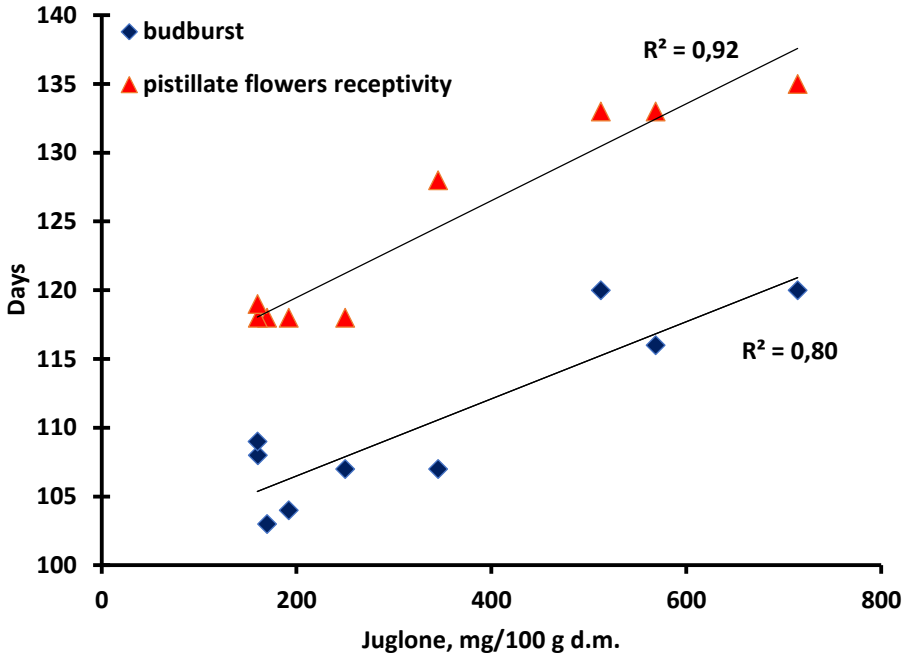


Fig 4. Composition of phenolic compounds measured in the green husk of walnut samples (A.: Alsószentiváni, M.: Milotai)

The concentration of the **hydroxybenzoic acids** varied between 407 – and 3176 mg/100 g d.m., with the samples of higher value being 'Alsószentiváni 117', 'Alsószentiváni kései', 'Bonifác' and 'Köpcös', 1220 mg/100 g d.m., 2063 mg/100 g d.m., 3176 mg/100 g d.m. and 1283 mg/100 g d.m., respectively. 'Milotai 10' and 'Milotai kései' contained 802 mg/100 g d.m. and 764 mg/100 g d.m., while 'Milotai intenzív' and 'Chandler' had the lowest value, 423 mg/100 g d.m. and 407 mg/100 g d.m., respectively. The content of **hydroxycinnamic acids** did not show such great differences, the concentration varied between 723 – 1947 mg/100 g d.m. In terms of **flavonoids** a similar trend is seen, the concentration varied between 741 mg/100 g d.m. and 2203 mg/100 g d.m. The content of **naphtoquinones** was the highest in 'Bonifác' (1275 mg/100 g d.m.) and 'Milotai kései' (1260 mg/100 g d.m.).

131 The lowest concentration of this compound group was detected in 'Köpcös' (499 mg/100
132 g d.m.) and 'Chandler' (458 mg/100 g d.m.) (Fig. 4).
133 The 'Alsószentiváni 117', 'Milotai 10', 'Tiszacsécsi 83', and 'Köpcös' started their
134 budburst and pistillate flowers receptivity almost at the same time as the control cultivar
135 'Chandler'. Budburst of 'Milotai intenzív' was similar to control, but it needed the
136 longest period among the observed cultivars for the pistillate flowers to appear, 7 to 12
137 days later after budburst. Budburst and pistillate flowers receptivity of 'Bonifác',
138 'Milotai kései', and 'Alsószentiváni kései' were 3 to 12 days and 7 to 17 days later than
139 the control.



140

141 Fig 5. Correlation between the budburst and pistillate flowers receptivity and the
142 concentration of juglone

143

144 Discriminant analysis was performed in order to find out if the different cultivars can
145 be distinguished according the grouping variables, as budburst and pistillate flowers
146 receptivity based on the given analytical tests. We used the sum of the four data groups
147 and juglone concentration as independents (Fig 6 and 7). Based on the results we can
148 summarize, that the date of the budburst is less indicative, than the pistillate flowers
149 receptivity. When analysing the results, the discriminant analysis could not distinguish
150 between 'Milotai 10', 'Milotai intenzív', 'Köpcös' and 'Chandler', as they were very
151 similar in the time of budburst (107-109 calendar days), but differed in analytical values.
152 When using all five data series for discriminant analysis, 89% of the original grouped
153 cases were correctly classified. In contrast, when the data of pistillate flowers receptivity
154 was used for the discriminant analysis, it was found that the amount of flavonoids,
155 naphthoquinones, and juglone as variables already ensures the correct classification of
156 100% of the original grouped cases.

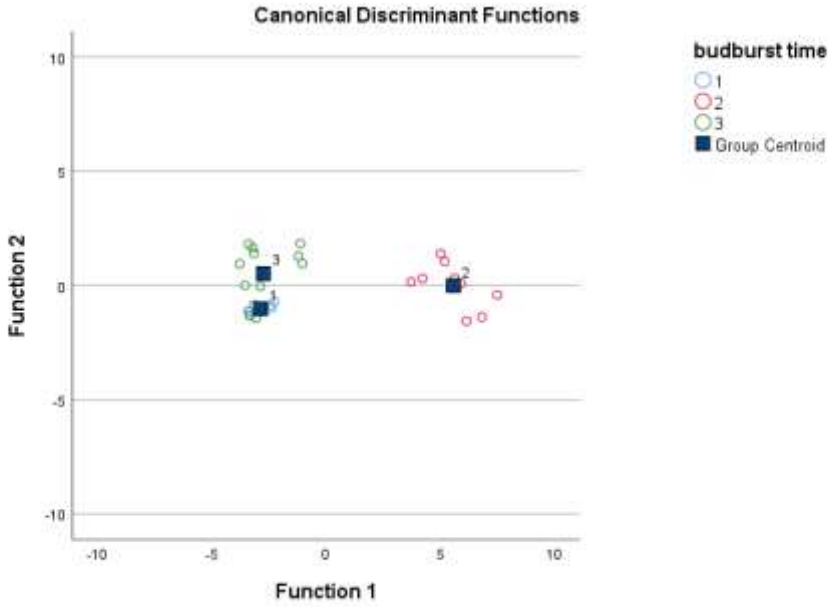


Fig 6. Discriminant analysis by budburst time as a grouping variable; (independents: sum of hydroxybenzoic acids, hydroxycinnamic acids, flavonoids and naphtoquinones, as well as juglone concentration)

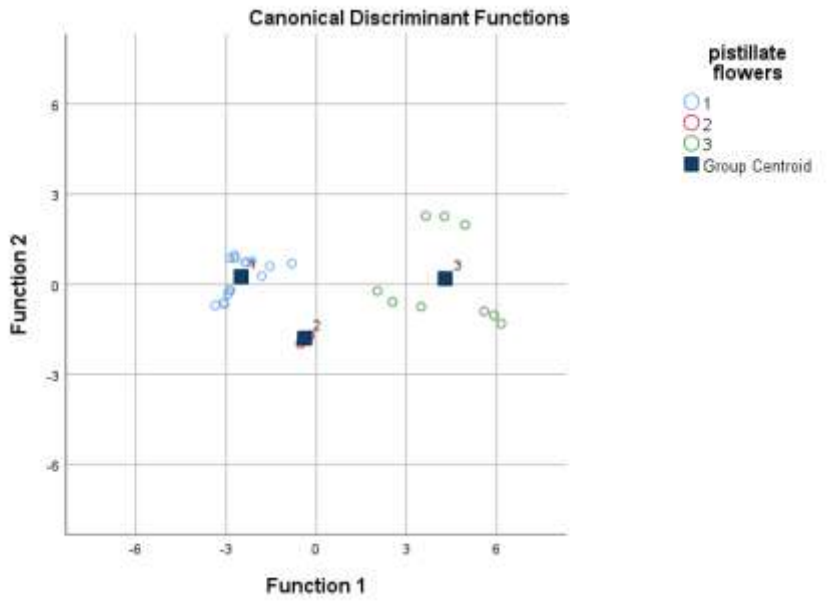


Fig 7. Discriminant analysis by pistillate flowers receptivity time as a grouping variable (independents: sum of flavonoids and naphtoquinones, as well as juglone concentration)

In Table 3 all correlation coefficients of all measured compounds are indicated. All compounds correlated well to the total phenolic acids; the correlation between hydroxybenzoic acid and total phenolic acid was 0.90, between hydroxycinnamic and

171 total phenolic acids 0.79, flavonoids and total phenolic acids 0.95, and naphtoquinones
172 and total phenolic acids 0.63. Content of flavonoids correlated well to content of
173 hydroxybenzoic acids (0.79). Also content of flavonoids showed a strong correlation with
174 hydroxycinnamic (0.76). The naphtoquinones correlated well to the budburst (0.70) and
175 the blossom time (0.78). It was interesting to see that there is a strong correlation between
176 the budburst and the blossom of the pistillate flowers (0.79), because without having a
177 shoot it is not possible for the female flower to develop.
178
179 Table 3. Correlation matrix of measured parameters and observed characteristics*

	hydroxy-cinnamic aids	flavonoids	naphto-quinones	total phenolic acids	bud-burst	blossom time
hydroxy-benzoic acids	0.57	**0.79**	0.41	**0.90**	0.27	0.27
hydroxycinnamic acids		**0.76**	0.47	**0.79**	0.15	0.14
flavonoids			**0.64**	**0.95**	0.37	0.31
naphtoquinones				**0.63**	**0.70**	**0.78**
total phenolic acids					0.40	0.35
budburst						**0.79**

180 *: Samples were collected during lignification of the nuts
181
182 Furthermore, there were strong significant correlations between the budburst, as well as
183 pistillate flowers receptivity and the concentration of **juglone**, where R^2 was 0.80 and 0.92,
184 respectively. The lowest values are measured in samples, which were the earliest in
185 pistillate flowers receptivity, namely 'Alsószentiváni 117' (192 mg/100 g d.m.,), 'Milotai
186 10' (250 mg/100 g d.m), 'Tiszacsécsi 83' (170 mg/100 g d.m.), 'Köpcös' (160 mg/100 g d.m.)
187 and 'Chandler' (160 mg/100 g d.m). (Fig. 4.) The 'Alsószentiváni kései', 'Bonifác', 'Milotai
188 intenzív' and 'Milotai kései' reached a similar state of development about 10-15 days later,
189 and the juglone content was measured much higher (512 mg/100 g d.m., 568 mg/100 g
190 d.m., 345 mg/100 g d.m., and 714 mg/100 g d.m.) compared to those varieties, which had
191 earlier budburst and blossom (Fig. 3.).

192 **3. Discussion**

193 A higher concetration of phenolic compounds were detected in our trial compared to the
194 data found in the literature [9, 10, 53], as the mentioned authors collected their samples
195 before ripening, our samples were collected during the lignification period in late June,
196 when these components' amount reach their peak [51, 53]. The juglone concentration
197 measured in this trial was similar to the results of Solar et al. [54]. This literature source
198 also confirms that the local bred cultivars can produce a higher concentration of
199 compounds compared to the domesticated ones.
200 For juglone, several authors reported data that are consistent with our results.
201 Soto-Maldonado and co. [42] reported the amount of juglone was 169.1 mg/100 g d.m.

and Stampar [53] published values between 218 and 1404 mg/100 g d.m. depending on sampling time, while Cosmolescu and co. [10] measured between 20.56 and 42.78 mg/100 g from different varieties of mature walnut green husk. These results are in the same order of magnitude to ours, but at the same time they indicate the great variation due to the sampling time.

The dendogram, containing the diameter of the necrotic spots and the disease rate values, differentiated the cultivars belonging to certain susceptibility groups. In 2020 susceptibility/resistance of cultivars showed significant differences. Based on the statistical evaluation of data, 'Milotai intenzív' proved to have a high susceptibility (hS), while susceptibility (S) was detected for 'Bonifác', 'Tiszacsécsi 83', 'Alsószentiváni kései', 'Milotai 10' and 'Chandler'. Moderately susceptible cultivars were 'Milotai kései' and 'Alsószentiváni 117'. The origin of the Hungarian walnut cultivars didn't relate with their susceptibility to walnut blight (Fig. 8.).

In previous research a linkage between the polyphenols content and resistance to walnut bacterial blight was determined [28]. In our case a strong correlation between the high polyphenols content and resistance to walnut blight was observed. Current results confirmed this statement, except in the case of cultivar 'Bonifac'. This cultivar contained the highest concetration of phenolic compounds, but its nuts were susceptible to walnut blight (Fig.8.) as described in cultivars' description [55]. Beside the phenolic compounds there are more factors e.g. protein profile of the green husk [56] to determine the susceptibility of a genotype against walnut blight. There are just some research about susceptivity of 'Alsószentivani 117' and its hybrids to walnut blight, but further research needs to investigate this. Based on the artificial infections the varieties with late budburst had usually better resistance to walnut blight due to a higher amount of phenolic compounds (Fig 3., Fig 4., Fig. 8.). However, high concentration of phenolic compounds doesn't mean only link the resistance. Some cultivars having an early budburst can also have a good resistance such as 'BD6' [57]. This conclusion confirms results of Matias et al. [45, 46] as well as that of Solar et al. [54], that there is a negative correlation between juglone concentration and susceptibility of the walnut genotypes, varieties against blight. The concentration and composition of phenolic compounds in the green husk of walnuts change significantly during crop development, thus a detailed kinetic study would be very important.

Evaluating the results, it can be concluded that the concentration of phenolic compounds of the most susceptible cultivars, as 'Milotai intenzív' 'Tiszacsécsi 83', and 'Chandler'. was the lowest during the measurements.

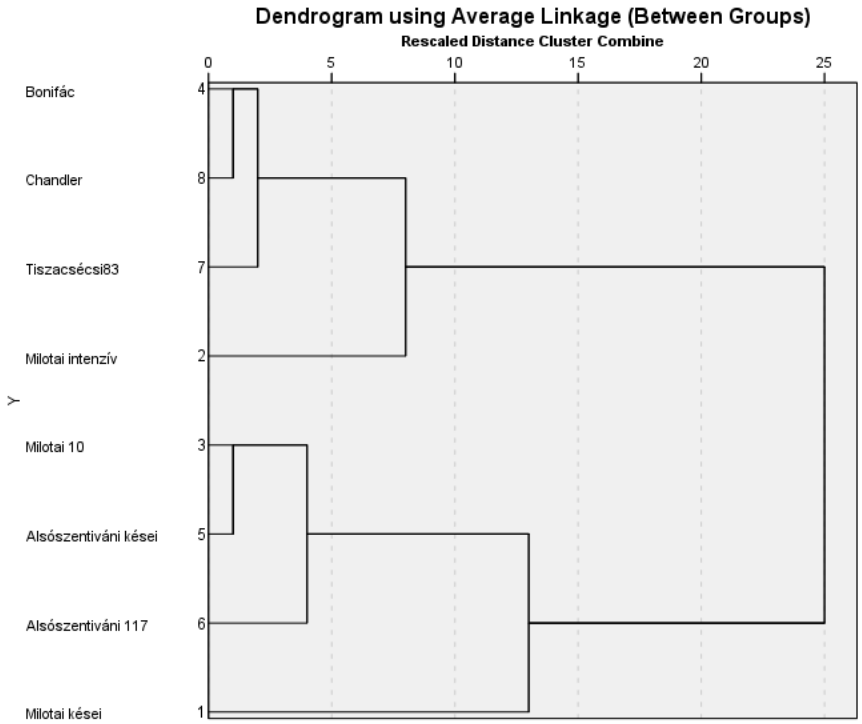


Fig. 8. Dendrogram of Hungarian bred walnut cultivars in the point of view of their suseptibility to walnut blight

4. Materials and Methods

4.1. Plant material

The trial is at the Experimental Fields of the Hungarian University of Agriculture and Life Sciences Research Centre for Fruit Growing (GPS coordinates: N 47°20′11,44″ E 18°51′53,42″). The trial was planted in spring 1990 on chernozem soil with high lime (pH=8, total lime content in the top 60 cm layer 5%) and humus content (2.3-2.5%). Considering the the Arany-type cohesion index [58] the K_A=40 refers to medium compactness. Meteorological conditions of the site are presented in Table 1.

The entire Hungarian walnut assortment (‘Alsószentiváni 117’, ‘Milotai 10’, ‘Tiszacsécsi 83’ selected from the local population; ‘Milotai intenzív’, ‘Milotai kései®’(kései refers to its late leafing time) crossbred between ‘Milotai 10’ and ‘Pedro’; ‘Alsószentiváni kései®’, ‘Bonifác®’ crossbred between ‘Alsószentiváni 117’ and ‘Pedro’) and a genotype for the rootstock ‘Köpcös’ were established in the trial. The control of our trial was the US-bred ‘Chandler’. The trees were grafted on a selected *Juglans regia* seedling, and planted out 10 x 10 m in the row and between the rows. The grafted trees were trained as a central leader canopy, replicated 5 trees of each, and the trial was not irrigated.

Table 1. Meteorological data during the data collection 2020

parameters	value
average yearly temperature	11.4 °C
average yearly temperature during the growing season	16.1 °C

(March - September)	
average yearly luminous flux	1015 l/m²/day
average yearly precipitation	434.1 mm
annual average of sunshine hours	2065

259

260 **4.2. Phenological stages**

261 All the phenological stages were recorded as indicated in the Ctifl scheme [59]. The
262 corresponding stages, when the data were collected, were the budburst stage of "Cf"; the
263 start of pistillate flowers receptivity, the stage of "Ff2". Thus the start of pistillate
264 receptivity period was considered when the stigmas in the earliest pistillate flowers
265 became receptive up. The data were recorded in two to three days intervals, usually in
266 the morning.

267

268 **4.3. Sample collection and preparation**

269 The samples were collected late June (on 30th June), on the 181st day of 2020, during
270 lignification of the nut shell. The time of budburst and pistillate flowers receptivity in
271 days of each samples is summarized in Table 2. At that time of the sample collection, the
272 following periods took from the pistillate flowers receptivity until the sample collection:
273 for 'Alsószentiváni 117', 'Milotai 10', 'Tiszacsécsi 83', 'Köpcös' 63 days, for 'Chandler' 62
274 days, for Milotai intenzív' 53 days, for 'Alsószentiváni kései' and 'Bonifác' 48 days, as
275 well as for 'Milotai kései' 45 days. The collected nuts were delivered to the lab
276 immediately. In the laboratory ten nuts of every type samples were peeled with a 2 mm
277 width blade 125 kitchen peeler and the green husk was lyophilized. The dried samples
278 were grinded to 126 fine powder and stored hermetically sealed at 4 °C until analysis.

279

280 Table 2. The time of budburst and pistillate flowers receptivity in calendar days starting
281 from 1st January

	budburst (days)	pistillate flowers receptivity (days)	Days after receptivity before sampling
Alsószentiváni 117	104	118	63
Milotai 10	107	118	63
Tiszacsécsi 83	103	118	63
Köpcös	108	118	63
Chandler	109	119	62
Milotai intenzív	107	128	53
Bonifác	116	133	48
Milotai kései	120	135	45
Alószentiváni kései	120	133	48

282

283 **4.4. Chemicals**

The following standards were used for identification and quantification of phenolic compounds: gallic acid, syringic acid, catechin, chlorogenic acid, caffeic acid, p-coumaric acid, quercetin, rutin (quercetin-3-O-rutinoside), hyperoside (quercetin-3-O-galactoside), kaempherol, juglone (Merck, Darmstadt, Germany) ferulic acid and ellagic acid (Fluka-Honeywell, Charlotte, USA). HPLC/MS grade acetonitrile and formic acid, analytical grade methanol and acetic acid were purchased from Avantor (Radnor, USA). The bidistilled water was filtered through a 0.45 μm nylon membrane.

4.5. Total phenolic content

Total phenolic content was determined by the Folin-Ciocalteu's photometric method [53]. Fifty mg of green husk was extracted in 10 ml of 80% methanol, left for 24 h. After 30 min shaking at 25°C the mixture was filtered. One hundred μl of the filtrate was mixed with 3.7 ml distilled water, 0.5 ml Folin-Ciocalteu reagent and 2 ml 20% Na2CO3 solution and filled up to 10 ml with distilled water. Samples were left in a dark place for 30 min in room temperature; the absorbance was measured by spectrophotometer (UY-160 A, Shimadzu, Japan) at 750 nm against blank sample (4.75 ml distilled water and 0.25 ml Folin-Ciocalteu reagent). Total phenolic content was calculated according to a calibration curve prepared with gallic acid in 0-0.5 mg/ml concentration range (Sigma-Aldrich).

4.6. Phenolic compounds determination by HPLC-ESI-DAD

100 mg (milligrams) of dried green husks were weighted in a test tube and 10 ml of the extraction solvent (bidistilled water / 2% acetic acid in methanol, 30/70) added. Mixtures were sonicated (AU 65, ArgoLab, Carpi, Italy) for 20 minutes at room temperature. After centrifugation (5000 rpm, 20 min; Digicen 21, Orto Alresa, Madrid, Spain) the supernatant was filtered through a 0.45 μm PVDF syringe filter before HPLC injection and analysed on the same day.

A Waters Alliance system (Waters, Milford, USA) consisting of a Model e2695 separation module with Model 2998 photodiode array (PDA) detector operated by Empower software (Waters, Milford, USA) was used for the determination of the phenolic compounds. The separation was carried out using a Sphinx 5 μm 250x4.6 mm column (Macherey-Nagel, Düren, Germany) by gradient elution. The mobile phase was (A) 0.1% formic acid in bidistilled water and (B) 0.1% formic acid in acetonitrile. The gradient elution program: 0-9 min, 5-20% B; 9-16 min, 20-22% B; 16-25 min, 22-50% B; 25-28 min 50% B; 28-40 min, 50-100% B; 40-43 min, 100% B; 43-45 min, 100-5% B; 45-50 min, 5% B. The injected volume was 20 μl and the flow rate was 0.7 ml/min. Data acquisition of PDA proceeded the range of 200-600 nm and the detection wavelengths were at 280, 320, 355 and 420 nm.

The HPLC system was coupled to a Model Acquity Mass (QDa) detector (Waters, Milford, USA). The mass spectrometry conditions were set as both negative and positive modes: electrospray ionization (ESI) was used as a source, mass spectra in the m/z range 100 to 1000 were obtained and probe temperature was adjusted to 600°C

(default). In positive the ion mode cone voltage was set to 15 V and capillary voltage was 1.5 kV. In negative ion mode those were 50 V and 0.8 kV respectively.

Identification of phenolic compounds was achieved by comparing their spectral characteristic, retention times, measured mass (m/z) and fragmentation pattern. In addition, previously published literature [26, 60-62] and internet databases [63-65] were applied. The quantification of the identified phenolic compounds was based on calibration curve of rutin at 280, 320 and 355 nm as rutin gives well measurable signal at these wavelengths, and juglone at 420 nm.

4.7. Walnut blight immunity test

The susceptibility test was carried out based on the methods [66-67] using 10 immature green nuts from every individual, collected in 2020. For the artificial infection a mixture of 3 Xaj strains was used, isolated from naturally infected walnut nuts from four different locations in Hungary (Vecsés, Karád, Balatonbolgár and Zánka). Before infection of immature nuts isolates were either inoculated into the intercellular tissue of tobacco leaf ('White Burley') or were inoculated in immature nuts for confirmation capability of them to induce hypersensitive tissue necrosis and also aggressiveness to produce disease symptom. The mixture of the three strains was used for the infection after the virulence test. Before the infection the fruit surface was disinfected with alcohol. Two inoculations of 20µl bacterial suspension were performed in exocarpium for each nut, thus the 10 nuts per cultivar with 2 inoculations each were tested. Sterile distilled water (SDW) was injected into the 10 immature nuts for every cultivar as control negative treatment. After the infection, the nuts were incubated in transparent plastic boxes for 7 days at a temperature of 26-28 °C, with over 90-95% relative humidity. Temperature and RH% were monitored by a micro-sensor placed into one of the plastic boxes. The disease severity was recorded on a scale from 0 to 4 (Fig. 1), from the least to the most, based on the diameter and depth of necrosis reached on the 7th day: 0 - no symptoms; 1 - less than 2.0 mm, superficial and small spots on the inoculation point; 2 - blackening on the inoculation point of nut by 2.1 mm to 3 mm; 3 - blackening on the inoculation point of nut by 3.1 mm to 4 mm; 4 - blackening on the inoculation point of nut more than 4.1 mm; see legend of Fig1

Four groups were formed: Moderately Resistant (MR) ≤2mm; Moderately Susceptible (MS) 2.1-3 mm; Susceptible (S) 3.1-4 mm; High Susceptible (HS) 4.1 mm≤.

The test was performed using the same cultivars as for chemical analysis except for 'Köpcös'.

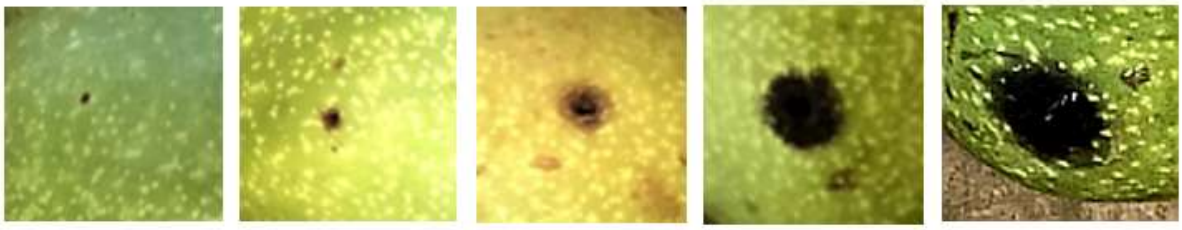


Fig. 1. Evaluation of susceptibility, scale (0-4)

0 - no symptoms; 1 - less than 2.0 mm, superficial and small spots on the inoculation point; 2 - blackening on the inoculation point of nut by 2.1 mm to 2.6 mm; 3 - blackening on the inoculation point of nut by 2.7 mm to 3.1 mm; 4 - blackening on the inoculation point of nut more than 4.1 mm;

4.8. Statistical analysis

To separate the significance factors analysis of variance of IBM SPSS 28 software package (normality analysis (Kolmogorov-Smirnov and Shapiro-Wilk test) and discriminant analysis) was used at P value of 0.05. Variance of data evaluated by one-way analysis of variance (ANOVA) and for testing of significance between means we applied Duncan's homogeneity test. Values represent the mean and standard deviation of three replicates from each sample. Relationships between the observed traits in the correlation matrix were calculated by Pearson correlation coefficient. The same letters indicate values in the same homogeneous subsets without significant differences.

The data derived from the immunity test were evaluated using the SPSS software (IBM SPSS 27.0, Chicago, IL). The statistical analyses were determined based on the sample size, distribution analysis (Kolmogorov Smirnov test) and t-test. It was accepted the p≤95% confidence level. A hierarchical cluster analysis based on one-year data regarding the diameter and disease rate was conducted, in order to classify the cultivars into susceptibility groups. The results were represented in a dendogram (Fig. 8).

5. Conclusions

Solar and co-workers [54] indicated in their study that the concentration of phenolics in the walnut crop varies with time. It can be assumed, that the concentration and composition of phenolic compounds in the green husk of walnuts change also significantly during crop development, thus a detailed kinetic study would be very important in the future.

Concentration of compounds was higher in the local bred cultivars compared to the foreign bred 'Chandler', grown and collected among Hungarian climatic conditions. The concentration of total phenol acids are correlated well to the concentration of the phenolic compounds, therefore we got strong correlations between total phenolic acids and hydroxybenzoic acid, hydroxycinnamic acids, flavonoids, and naphtoquinones. The flavonoids had a strong correlation with other phenolic compounds, such as hydroxybenzoic acid, hydroxycinnamic acids, and naphtoquinones, playing an

important role in the protection against diseases produced naturally. The flavonoids are one of the initiating materials of the hydroxycinnamic acids [68-69]. Naphtoquinones showed strong correlations between budburst and blossom time, which are related to their yearly fluctuation; the more early the budburst and blossom, the more early their peak concentration can be reached [54]. It is important to mention that after reaching the annual peak quantity of the naphtoquinones their concentration decreases. In the late part of the growing season, the varieties reach their peak polyphenol concentration, delaying the decrease of these compounds. From the point of view of this phenomenon, the cultivars with late budburst and blossom time are the best due to their susceptibility to walnut blight as described in some previous papers [70-72]. Fortunately, there is a strong correlation between the budburst and blossom time (0.79) too as described by breeders [70-72].

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References

References must be numbered in order of appearance in the text (including citations in tables and legends) and listed individually at the end of the manuscript. We recommend preparing the references with a bibliography software package, such as EndNote, ReferenceManager or Zotero to avoid typing mistakes and duplicated references. Include the digital object identifier (DOI) for all references where available.

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References

1. Rahimipanah, M.; Hamedi, M.; Mirzapour, M. Antioxidant activity and phenolic contents of Persian walnut (*Juglans regia* L.) green husk extract. *African Journal of Food Science and Technology* **2010**, *4*, 105-111.

2. Al-Nadaf, A.H. Bastoni, H.M.; Hamdan, D.F. Microwave-assisted efficient extraction of phenolics from *Juglans regia* L.: pellicle; kernel unripe fruits; and leaves in different solvents. *International Journal of Green Pharmacy* **2018**, *3*, 182-190. doi: 10.22377/ijgp.v12i03.1950
3. Marius, R.E.; Gheldiu, A-M., Moldovan A.M.C.; Popa D-S.; Tomuta, I.; Vlase, L. Process Optimization for Improved Phenolic Compounds Recovery from Walnut (*Juglans regia* L.) Septum: Phytochemical Profile and Biological Activities. *Molecules* **2018**. *11*, 2814. doi: 10.3390/molecules23112814.
4. Acquaviva, R., D'Angeli, F.; Malfa, G. A.; Ronsisvalle, S.; Garozzo, A.; Stivala, A., Ragusa, S.; Nicolosi, D.; Salmeri, M., Genovese, C. Antibacterial and anti-biofilm activities of walnut pellicle extract (*Juglans regia* L.) against coagulase-negative staphylococci. *Nat Prod Res* **2021**, *12*, 2076-2081. doi:10.1080/14786419.2019.1650352
5. La Torre, C.; Caputo, P.; Plastina, P.; Cione, E.; Fazio, A. Green Husk of Walnuts (*Juglans regia* L.) from Southern Italy as a Valuable Source for the Recovery of Glucans and Pectins. *Fermentation* **2021**, *7*, 305. doi:10.3390/fermentation7040305
6. Croitoru, A.; Ficai, D.; Craciun, L.; Ficai, A.; Andronescu, E. Evaluation and Exploitation of Bioactive Compounds of Walnut, *Juglans regia*. *Curr Pharm Des* **2019**, *2*, 119-131. doi:10.2174/1381612825666190329150825.
7. Soto-Madrid, D.; Gutiérrez-Cutiño, M.; Pozo-Martínez, J.; Zúñiga-López, M.C.; Olea-Azar, C.; Matiacevich, S. Dependence of the Ripeness Stage on the Antioxidant and Antimicrobial Properties of Walnut (*Juglans regia* L.) Green Husk Extracts from Industrial By-Products. *Molecules* **2021**, *10*, 2878. doi:10.3390/molecules26102878.
8. Oliveira I.; Sousa A., Ferreira I.C.; Bento A.; Estevinho L.; Pereira J.A. Total phenols, antioxidant potential and antimicrobial activity of walnut (*Juglans regia* L.) green husks. *Food Chem Toxicol*. **2008**, *7*, 2326-31. doi:10.1016/j.fct.2008.03.017.
9. Carvalho M.; Ferreira P.J.; Mendes V.S.; Silva R.; Pereira J.A.; Jerónimo C.; Silva B.M. Human cancer cell antiproliferative and antioxidant activities of *Juglans regia* L. *Food Chem Toxico*l. **2010**, *1*, 441-7. doi:10.1016/j.fct.2009.10.043.
10. Cosmulesscu, S.N.; Trandafir, I.; Achim, G.; Botu, M.; Baciu, A.; Gruia, M. Phenolics of Green Husk in Mature Walnut Fruits. *Not Bot Horti Agrobo* **2010**, *1*, 53-56. doi:10.15835/nbha3814624
11. Popovici, C.; Gîtin, L.; Alexe, P. Characterization of walnut (*Juglans regia* L.) green husk extract obtained by supercritical carbon dioxide fluid extraction. *Journal of Food and Packaging Science, Technique and Technologies* **2012**, *1*, 5-9.
12. Ding, C.-B.; Wu, S.-Z.; Li, G.-Q.; Jia, C.-H.; Chang, L.-X. Anti-fungal activity of walnut green husk extracts. *Modern Food Science and Technology* **2013**, *29*, 722-724+755.
13. Zhang, W.; Kaize; H.E.; Qiang, P.U. Antimicrobial and antioxidant activities of extracts from walnut green husks. *Chinese Journal of Applied & Environmental Biology* **2014**, *1*, 87-92, doi:10.3724/SP.J.1145.2014.00087
14. Salejda, A.; Janiewicz, U.; Korzeniowska, M.; Kolniak-Ostek, J.; Grazyna, K. Effect of walnut green husk addition on some quality properties of cooked sausages. *LWT - Food Sci Technol.* **2015**, *61*, 751-757. doi:10.1016/j.lwt.2015.08.069
15. Panth, N.; Paudel, K.R.; Karki, R. Phytochemical profile and biological activity of *Juglans regia*. *J Integr Med*. **2016**, *5*, 359-373. doi:10.1016/S2095-4964(16)60274-1.
16. Wianowska, D.; Garbaczewska, S.; Cieniecka-Roslonkiewicz, A., Dawidowicz, A.L.; Jankowska, A. Comparison of antifungal activity of extracts from different *Juglans regia* cultivars and juglone. *Microb Pathogenesis* **2016**, *100*, 263-267, doi:10.1016/j.micpath.2016.10.009
17. Sadeghi-Kiakhani, M.; Tehrani-Bagha, A.; Gharanjig, K.; Hashemi, E. Use of Pomegranate peels and Walnut Green husks as the green antimicrobial agents to reduce the consumption of inorganic nanoparticles on wool yarns. *J Clean Prod* **2019**, 231. doi:10.1016/j.jclepro.2019.05.283.
18. Wianowska, D.; Garbaczewska, S.; Cieniecka-Roslonkiewicz, A.; Dawidowicz, A.; Typek, R.; Kielczewska, A. Influence of the Extraction Conditions on the Antifungal Properties of Walnut Green Husk Isolates. *Anal Lett* **2020**, *53* 1-12. doi:10.1080/00032719.2020.1725889.
19. Dehghani, S.; Nouri, M.; Baghi, M. The effect of adding walnut green husk extract on antioxidant and antimicrobial properties of ketchup. *Journal of Food and Bioprocess Engineering* **2020**, *2, 93-100.*
20. Medic, A.; Jakopič, J.; Hudina, M.; Solar, A.; Veberic, R. Identification and quantification of major phenolic constituents in *Juglans regia* L. leaves: healthy vs. infected leaves with *Xanthomonas campestris* pv. *juglandis* using HPLC- MS/MS. *Journal of King Saud University – Science* **2022**, 34. doi:101890.10.1016/j.jksus.2022.101890.
21. Colarič M.; Veberic, R.; Solar, A.; Hudina, M.; Stampar, F. Phenolic Acids, Syringaldehyde, and Juglone in Fruits of Different Cultivars of *Juglans regia* L. *J Agric Food Chem* **2005**, *53*, 6390-6396. doi:10.1021/jf050721n.
22. Hama, J.; Anwar, R.; Rashid, R.; Mohammed, N.; Thoss, V. The Diversity of Phenolic Compounds along Defatted Kernel, Green Husk and Leaves of Walnut (*Juglans regia* L.), *Anal. Chem. Lett*., **2016**, *1*, 35-46. doi:10.1080/22297928.2016.1152912
23. Ghasemi, K.; Ghasemi, Y.; Ehtesham N.; Ab, Nabavi, S.M.; & Nabavi, S.F.; Ebrahimzadeh, .; Pourmorad, F. Influence of environmental factors on antioxidant activity, phenol and flavonoids contents of walnut (*Juglans regia* L.) green husks. *J Med Plants Res* **2011**, *5*, 1128-1133.
24. Cosmulescu, S.; Trandafir, I.; Achim, G.; Baciu, A.; Juglone Content in Leaf and Green Husk of Five Walnut (*Juglans regia* L.) Cultivars. *Not Bot Horti Agrobo* **2011**, *39*. 237-240. doi:10.15835/nbha3915728.

25. Akbari V.; Jamei R.; Heidari R.; Esfahlan A.J. Antiradical activity of different parts of Walnut (*Juglans regia* L.) fruit as a function of genotype. *Food Chem*. **2012,** *135*, 2404-2410. doi: 10.1016/j.foodchem.2012.07.030.
26. Rahmani, F.; Dehganiasl, M.; Heidari, R.; Rezaee, R.; Darvishzadeh, R. Genotype impact on antioxidant potential of hull and kernel in Persian walnut (*Juglans regia* L.). *Int. Food Res. J.* **2018**, *25*, 35–42
27. Jahanban-Esfahlan, A.; Ostadrahimi, A.; Tabibiazar, M.; Amarowicz, R. A Comprehensive Review on the Chemical Constituents and Functional Uses of Walnut (*Juglans* spp.) Husk. *Int. J. Mol. Sci.* **2019**, *20*, 3920. doi:10.3390/ijms20163920
28. Jakopic, J.; Solar, A.; Colaric, M.; Hudina, M.; Veberic, R.; Stampar, F. The influence of ethanol concentration on content of total and individual phenolics in walnut alcoholic drink. *Acta Aliment* **2008**, *37*, 233–239. doi:10.1556/AAlim.37.2008.2.9
29. Jakopic J., Veberic R. Extraction of phenolic compounds from green walnut fruits in different solvents. *Acta Agric Slov* **2009**; *93*, 11-15. doi:10.2478/v10014-009-0002-4
30. Fernández-Agulló, A.; Pereira, E.; Freire, M.S.; Valentão, P.; Andrade, P.B.; González-Álvarez, J.; Pereira, J.A. Influence of solvent on the antioxidant and antimicrobial properties of walnut (*Juglans regia* L.) green husk extracts. *Ind. Crops Prod*. **2013**, *42*, 126–132
31. Oluwaseun, R.A.; Nour H.A.; Chinonso I.U.; Extraction of phenolic compounds: A review. *Current Research in Food Science*, **2021**, *4*, 200-214, doi:10.1016/j.crfs.2021.03.011.
32. Jakopic, J.; Colaric, M.; Veberic, R.; Hudina, M.; Solar, A.; Stampar, F. How much do cultivar and preparation time influence on phenolics content in walnut liqueur? *Food Chem.* **2007**, *104*, 100–105. doi:10.1016/j.foodchem.2006.11.008
33. Cosmulescu, S.; Trandafir, I.; Nour, V.; Ionica, M.; Tutulescu, F. Phenolics content, antioxidant activity and color of green walnut extracts for preparing walnut liquor. *Not Bot Horti Agrobo* **2014**, *42*, 551–555. doi: 10.15835/nbha.42.2.9649
34. Uğurlu, S.; Okumuş, E.; Bakkalbaşı, E.; Reduction Of Bitterness In Green Walnuts By Conventional And Ultrasound-Assisted Maceration. *Ultrason Sonochem* **2020**, *66*, 105094. doi:10.1016/j.ultsonch.2020.105094.
35. Binici, H.; Şat, İ.; Aoudeh, E. Nutritional Composition and Health Benefits of Walnut and its Products. Atatürk *Üniversitesi Ziraat Fakültesi Dergisi*. **2021**, 224-230. doi:10.17097/ataunizfd.843028.
36. Cindrić, I.; Zeiner, M.; Hlebec, D.; Mineral Composition of Elements in Walnuts and Walnut Oils. *Int J Env Res Pub He* **2018**, *15*, 2674. doi:10.3390/ijerph15122674.
37. Grosso, A.; Asensio, C.; Nepote, V.; Grosso, N. Antioxidant Activity Displayed by Phenolic Compounds Obtained from Walnut Oil Cake used for Walnut Oil Preservation. J Am Oil Chem Soc **2018**, *95*. doi:10.1002/aocs.12145.
38. Rabrenović, B.B.; Natić, M.; Dabić Zagorac, D.; Meland, M.; Fotirić Akšić, M. Bioactive Phytochemicals from Walnut (*Juglans* spp.) Oil Processing By-Products. *Reference Series in Phytochemistry*, **2021**, 189-197, doi:10.1007/978-3-030-63961-7_25-1.
39. Beiki, T.; Najafpour, G.D.; Hosseini, M. Evaluation of antimicrobial and dyeing properties of walnut (*Juglans regia* L.) green husk extract for cosmetics. *Color. Technol.* **2018**, *134*, 71–81. doi:**10.1111/cote.12322**
40. Tsiantos J., Vagelas I.K., Rumbos C.I., Chatzaki A., Rouskas D. And Gravanis F.T. (2008): Evaluation of resistance of cultivated walnut varieties and selections to *Xanthomonas arboricola* pv.*juglandis* in Greece. COST873 Management Committee Meeting, Athens, Greece. http://www.cost873.ch/uploads/files/GRXajSheetWalnut.pdf (accessed on 26th April 2020).
41. Han, M.M.; Shi, S.Y.; Wang, F.; Pang, M.X.; Qu, C.; Qi, J.H. Extraction and Dyeing Propertie of Juglone from Walnut Green Husk. *IOP Conference Series: Earth and Environmental Science* **2018**, *199, 042008*. doi:10.1088/1755-1315/199/4/042008.
42. Soto-Maldonado, C.; Vergara-Castro, M.; Jara-Quezada, J.; Caballero-Valdés E.; Müller-Pavez, A.; Zúñiga-Hansen, M. E.; Altamirano, C. Polyphenolic extracts of walnut (Juglans regia) green husk containing juglone inhibit the growth of HL-60 cells and induce apoptosis. Electronic *J Biotechnol*, **2019**, *39*, 1–7. doi:10.1016/j.ejbt.2019.02.001
43. Frutos, D.; Ortega, G. Search for *Juglans regia* genotypes resistant/tolerant to *Xanthomonas arboricola* pv. *Juglandis* in the framework of COST Action 873. *J Plant Pathol* **2012**, 94. doi:10.4454/jpp.v94i1sup.007.
44. Pereira, J.A.; Oliveira, I.; Sousa, A.; Valentão, P.; Andrade, P.B., Ferreira, I.C.F.R., Ferreres, F., Bento, A., Seabra, R., Estevinho, L. Walnut (*Juglans regia* L.) leaves: Phenolic com-pounds, antibacterial activity and antioxidant potential of different cultivars. Food Chem Toxicol **2007**, *45*, 2287-2295. doi: 10.1016/j.fct.2007.06.004
45. Matias, J.; Jakopic ,J.; Solar, A.; Rovira, M.; Aletà, N. Walnut varietal patterns of polyphenol contents. Cost 873,WG3/WG4 Joint Meeting, Murcia, Spain. http://www.cost873.ch/_uploads/_files/m_Matias_2007_10.pdf , 2007 (accessed on 10th May 2022)
46. Matias, J.; Aletà, N.; Rovira, C.; Moragrega, C.; Phenoliccomposition in *Juglans regia* commercial cultivars. Relationship with blight susceptibility. Annual Cost 873 Meeting, Cetara, Italy. http://www.cost873.ch/_uploads/_files/JMatias_Walnut-Phenolics_Italy_1.pdf, 2009 (accessed on 10th May 2022)
47. Solar, A.; Jakopic, J.; Veberic, R.; Stampar, F.; Phenolic compounds as a potential marker of walnut resistance against *Xanthomonas arboricola* pv. *juglandis.* Cost 873,WG3/WG4 Joint Meeting, Murcia, Spain. **2007**, http://www.cost873.ch/_up_loads/_files/m_Solar_murcia (accessed on 10th May 2022)
48. Dirlewanger, E. Genetic mapping and MAS of resistancegenes in stone fruit and nut trees at INRA. Cost 873 STF Meeting, Barcelona, Spain. **2010**, http://www.cost873.ch/_uploads/_files/EDirlewanger_GeneticMappingPeach. (accessed on 10th May 2022)
49. Solar, A.; Jakopic, J.; Mikulic-Petkovs˘ek, M.; Veberic ,R.; Dreo, T.; Zadravec, P.; Stampar, F.; Validation of polyphenol contents as biochemical markers for walnut blight (*Xanthomonas arboricola* pv. *juglandis*) resistance. Cost 873, WGand Management

Committee Meeting, Athens, Greece. http://www.cost873.ch/ uploads/ files/m Athens Abstracts FinalBook.pdf, **2008**, (accessed on 10th May 2022)

50. Yang, H. Resistance evaluation of walnut (*Juglans* spp.) against *Xanthomonas arboricola* and the correlation between leaf structure and resistance. *Forest Pathol* **2021**, *51*. doi:10.1111/efp.12659.
51. Singleton, V.L. & Rossi, J.A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic*. **, 1999,** 16, 144–158.
52. Scalbert, A.; Monties, B.; Janin, G. :Tannins in Wood: Comparison of Different Estimation Methods. *J Agric Food Chem*, **1989**, *5*, 1324-1329.
53. Stampar, F.; Solar, A.; Hudina, M.; Veberic, R.; Colaric, M. Traditional walnut liqueur—Cocktail of phenolics. *Food Chem.* **2006**, *95*, 627–631. doi:10.1016/j.foodchem.2005.01.035
54. Solar, A.; Colarič, M.; Usenik, V.; Stampar, F. Seasonal variations of selected flavonoids, phenolic acids, and quinones in annual shoots of common walnut (*Juglans regia* L.) *Plant Sci* **2006**, *170*, 453–461. doi:10.1016/j.plantsci.2005.09.012
55. Szentiványi, P.; Kállay T. (eds). Dió [Walnut]. Diófajták leírása. [Description of walnut cultivars]. Mezőgazda Kiadó, Budapest, (Hungary), 2006, pp. 62-65.
56. Sagawa, H. D. C; de Assis, A. B. R; Zaini, P.A; Wilmarth, P.A; Phinney, B.S; Moreira, L.M; Dandekar, A.M. Proteome Analysis of Walnut Bacterial Blight Disease. *Int J Mol Sci*, **2020** ,21, 7453. doi: 10.3390/ijms21207453.
57. Bujdosó, G.; Fodor, A.; Végh, A. BD6 walnut. *Hortscience*, **2020**, *8*, 1393-1394. doi:10.21273/HORTSCI15148-20
58. Dobos, E.; Bialko, T.; Micheli, E.; Kobra, J. Legacy soil data harmonization and database development. In: Boettinger, J.L.; Howell, D.W.; Moore, A.C.; Hartemink, A. E; Kienast-Brown, S. Eds. Digital Soil Mapping. Progress in Soil Science, vol 2. Springer, Dordrecht, Germany, 2010, pp: 215-223.
59. Germain, E.; Prunet, J-P.; Garcin, A. Le Noyer. [The walnut]. Biologie florale [Flower biology]. Centr'Imprim, Issoudun (France), 1999, pp.43-56.
60. Singleton, V.L; Orthofer, R.; Lamuela-Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Method Enzymol* **1999**, *299*, 152-178.
61. Nour, V.; Trandafir, I.; Cosmulescu, S. HPLC Determination of Phenolic Acids, Flavonoids and Juglone in Walnut Leaves. *J Chromatogr Sci* **2012**, *51*, 883–890 doi:10.1093/chromsci/bms180
62. Huo, J-H.; Du, X-W.; Sun, G-D.;, Dong, W-T,;, Wang W-M. Identification and characterization of major constituents in *Juglans mandshurica* using ultra performance liquid chromatography coupled with time-of-flight mass spectrometry (UPLC-ESI-Q-TOF/MS). *Chin J Nat Medicines*, **2018**, *7*, 0525-0545
63. Phenol-Explorer, http://phenol-explorer.eu/ (accessed on 10th May 2022.).
64. MassBank of North America, https://mona.fiehnlab.ucdavis.edu/ (accessed on 10th May 2022.).
65. PubChem, https://pubchem.ncbi.nlm.nih.gov/ (accessed on 10th May 2022.).
66. Ozaktan, H.; Erdal, M.; Akkopru, A.; Aslan, E. Evaluation of susceptibility of some walnut cultivars to *Xanthomonas arboricola* pv.*juglandis* by immature nut test. COST873 Management Committee Meeting, Athens, Greece. **2008**, http://www.cost873.ch/uploads/files/GRXapFactSheetWalnut.pdf (accessed on 26th April 2020).
67. Solar, A.; Jakopič, Je.; Veberic, R.; Stampar, F. Correlations between *Xanthomonas arboricola* pv. *juglandis* severity and endogenous juglone and phenolic acids in walnut. *J Plant Pathol*, **2012**, *94*, doi: 10.4454/jpp.fa.2012.013
68. Pavic, K.; Zorc, B.. Cinnamic acid and cinnamic acid derivatives. *Farmaceutski Glasnik*, **2018**, *74*, 415-428.
69. Soldatov, D.; Esmaeili, M.; Wedde, E.; Mandato, J.; King, C. Photoreactivity and polymorphism of cinnamic acid revisited. *Acta Crystallographica Section A Foundations and Advances*, **2021**, 77. a137-a137. doi: 10.1107/S0108767321098627
70. Botu, M.; Tudor, M.; Papachatzis, A. Evaluation of some walnut cultivars with different bearing habits in the ecological conditions of Oltenia – Romania. *Acta Hortic*. **2010**, *861*, 119-126. doi 10.17660/ActaHortic.2010.861.15
71. Bujdosó, G.; Izsépi, F.; Szügyiné Bartha, K.; Varjas, V.; Szentiványi, P. Persian walnut breeding program at Naric Fruticulture Research Institute in Hungary. *Acta Hortic*. **2020**, *1280*, 89-94. DOI:10.17660/ActaHortic.2020.1280.13
72. Solar, A.; Stampar, F. Evaluation of some perspective walnut genotypes in Slovenia. *Acta Hortic*, **2005**, *705*. 131-136.