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1 **Comparison of cell wall chemical evolution during the**
2 **development of fruits of two contrasting quality from two**
3 **members of the Rosaceae family: apple and sweet cherry**

4
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18

19 **Abstract**

20

21 Cell wall composition was studied during the development of apple cultivars (14-161/182
22 days after full bloom, DAA) maintaining firm fruit (Ariane) or evolving to mealy texture
23 (Rome Beauty) when ripe and in sweet cherry cultivars (21/26-70/75 DAA) to assess their
24 skin-cracking susceptibility (tolerant Regina and susceptible Garnet).

25 Pectin sugar composition and hemicellulose fine structure assessed by enzymatic degradation
26 coupled to MALDI-TOF MS analysis were shown to vary markedly between apples and
27 cherries during fruit development. Apple showed decreasing rhamnogalacturonan I (RGI) and
28 increasing homogalacturonan (HG) pectic domain proportions from young to mature fruit.
29 Hemicellulose-cellulose (HC) sugars peaked at the beginning of fruit expansion
30 corresponding to the maximum cell wall content of glucose and mannose. In contrast, HG
31 peaked very early in the cell wall of young developing cherries and remained constant until
32 ripening whereas RGI content continuously increased. HC content decreased very early and

33 remained low in cell walls. Only the low content of mannose and to a lesser extent fucose
34 increased and then slowly decreased from the beginning of the fruit expansion phase.

35 Hemicellulose structural profiling showed strong varietal differences between cherry
36 cultivars. Both apples and cherries demonstrated a peak of glucomannan oligomers produced
37 by β -glucanase hydrolysis of the cell wall at the onset of cell expansion. The different
38 glucomannan contents and related oligomers released from cell walls are discussed with
39 regard to the contribution of glucomannan to cell wall mechanical properties. These
40 hemicellulose features may prove to be early markers of apple mealiness and cherry skin-
41 cracking susceptibility.

42

43 Keywords: *Malus domestica*, *Prunus avium*, pectin, hemicellulose, fruit-cracking, fruit
44 texture

45 **1 Introduction**

46

47 The Rosaceae family encompasses many economically important fruits, such as apple, cherry,
48 peach, and strawberry, which need to meet several qualitative criteria for consumers and
49 processors. Among them mechanical properties are particularly important with regard to
50 texture and fruit resistance to external stresses. During fruit development, various biotic and
51 abiotic stresses can alter the setup of tissues with consequences for their quality once ripe.
52 The role of these stresses in terms of the variability of quality in cultivars between harvest
53 years is an important question considering climate fluctuations. This variability makes the
54 identification of stable genetic markers useful in breeding programs to improve quality and,
55 notably, texture particularly difficult [1]. Full understanding and control of these variations
56 remain a challenge to date. One issue is understanding the cell wall construction and
57 evolution during fruit development, as these are central in determining tissue mechanical
58 properties involved in fruit quality. Cell walls, composed of an assembly of pectin,
59 hemicellulose and cellulose polysaccharides, provide cell-cell adhesion and mechanical
60 resistance to withstand cell turgor pressure [2]. During apple fruit development from young to
61 mature fruit, firmness declines [3] with the remodeling of cell wall polysaccharides, ending
62 with their disassembly by complex enzyme consortia and nonenzymatic mechanisms [4, 5].
63 Pectin is a major determinant of fruit mechanical properties [6, 7]. As an example, infusion of
64 various specific enzymes targeted toward cell wall polysaccharides degradation showed that
65 cellulose and hemicellulose were involved in the viscoelastic mechanical properties of apple
66 flesh but to a lesser extent than pectin [8]. Moreover, several fruit enzymes degrading pectin
67 are directly related to fruit softening [6, 9-12]. The contribution of pectin structural domains
68 bearing galactan and arabinan side chains (rhamnogalacturonan I, RGI) to fruit texture is also
69 well documented [6]. In fact, homogalacturonan (HG) and side chains of RGI pectin
70 structural domains interact with cellulose and are thought to contribute to cell wall
71 mechanical properties [13]. RGI side chains appear to be beneficial to the firmness of turgid
72 apple fruit but not for plasmolyzed tissue [14]. These side chains were proposed to make
73 analogous contributions to the cell wall mechanical properties, as the minor fraction of
74 xyloglucan hemicellulose (XyG) which interacts with cellulose and forms biomechanical
75 hotspots [15]. The side chains would control the sliding of cellulose microfibrils in the cell
76 wall under the tension stress of turgid cells. HG hydration and cellulose organization are
77 related to cell wall poroviscoelastic mechanical properties [14], for which the water flux
78 controlled by pectin in the porous cell wall contributes to the wall mechanical properties [7].

79 In apple, more pectin is hydrated, more cellulose microfibrils are dispersed, and the storage
80 modulus is higher. In light of these data, the role of bulk hemicellulose in cell wall
81 mechanical properties remains unclear. It is expected that hemicellulose will compete with
82 pectin [16] and interact with other cell wall polymers depending on their fine structure, such
83 as acetyl esterification, which is known to affect hydrogen binding of glucuronoxylan (GuX)
84 or galactoglucomannan (GgM) hemicelluloses to cellulose [17]. The molecular weight of
85 XyG has also been shown to affect cellulose binding and swelling of XyG-cellulose model
86 assembly [18]. As hemicellulose is under continuous remodeling during fruit development
87 [19, 20], it is thought that these genetically and environmentally controlled changes are
88 required during the setup and reorganization of cellulose and pectin in cell wall of the
89 developing fruit. In the present study, the kinetics of cell wall composition and hemicellulose
90 structural changes from young to ripe apple (*Malus domestica*) and sweet cherry (*Prunus*
91 *avium*), two members of Rosaceae, were followed. Hemicellulose structural profiling was
92 realized by coupling the degradation of cell wall polysaccharides by an endo- β -D-glucanase
93 with MALDI-TOF MS analysis of the hydrolysate, an approach that has been previously
94 shown to be sensitive to reveal plant cell wall polysaccharide structural variations according
95 to organ development and plant genetics [19, 21, 22]. Cultivars of apple developing
96 contrasting firmness when ripe were chosen, namely, Ariane (firm) and Rome Beauty (soft to
97 mealy when overripe) [23]. Sweet cherry cultivars differing in their susceptibility to skin
98 cracking were selected, namely, Regina (tolerant) and Garnet (susceptible) [24]. Apple and
99 cherries were also selected for their remarkably different brittle versus soft and melting
100 textures, which are usually related to differential cell wall swelling and pectin metabolism
101 [25]. This analysis allowed testing to determine whether the cell wall setup during early fruit
102 development differed in the texture and skin-cracking of ripe fruits.

103

104

105 **2 Materials and methods**

106

107 *2.1 Fruit*

108 *2.1.1 Apple*

109 Apple fruits were harvested in 2014 from six-year-old apple trees of Ariane and Rome Beauty
110 cultivars trained under normal production conditions in an experimental orchard at the
111 INRAE experimental unit in Beaucouzé, France (47°28'N, 0°33'W). For both cultivars, fruits
112 were harvested at 14, 28, 42, 56, 84, and 98 days after blooming and at commercial harvest

113 (161 and 182 days after blooming for Ariane and Rome Beauty, respectively). At harvest,
114 mature fruit were also kept at 2 °C for 2 months.

115 For each date, fruits were separated into 6 or 12 batches composed of 2 to 5 fruits collected
116 from different trees. For the first harvest date and due to the small size of the fruits, only one
117 batch composed of 9 fruits was formed. For each batch, fruits were cut up to form one sample
118 composed of fruit pieces, immediately frozen in liquid nitrogen and stored at -80 °C before
119 use.

120 Fruit harvest dates were expressed as cumulative growing degree-days (DD) from the date of
121 full bloom to consider the differences between both cultivars in terms of dates of anthesis. For
122 each day, DD was calculated as the difference between the daily mean temperature and a base
123 temperature of 7 °C, as proposed previously [26]. The cumulative DD obtained for a harvest
124 date was then calculated as the sum of DD from the day of full bloom to this harvest day
125 using daily air temperatures (°C) obtained from a local weather station. For cold stored fruits,
126 DD was calculated by adding 120 units (i.e., 60 days at 2 °C) to the DD calculated at
127 commercial harvest.

128

129 2.1.2 Cherries

130 Garnet and Regina cherry cultivars were harvested in 2015 at eight different dates
131 corresponding to 26, 32, 40, 49, 56, 62, 69 and 75 days after blooming for Garnet and 21, 27,
132 35, 44, 51, 57, 64 and 70 days after blooming for Regina. Trees were cultivated at the Tree
133 Experimental Unit (UEA) of the INRAE-Bordeaux research center in Toulence, 50 kms
134 southeast from Bordeaux, France (44°57' N, 0°28' W). The plot where trees were studied was
135 highly homogeneous in terms of soil composition and horticultural practices. For each
136 cultivar, four trees were sampled, two of which were planted together in two different plots.
137 Trees from Regina were 3 and 8 years old and from Garnet were 3 and 9 years old. For each
138 date, fruits from each cultivar and plot were pooled. At harvest, fruits were placed in
139 cryogenic tubes of 40 mL, immediately frozen in liquid nitrogen, and then stored at -80 °C.
140 Pits were removed using mortar and pestle. After pit removal, each sample was ground using
141 an automatic mortar grinder with a high thermic inertia. Ground material was aliquoted with 5
142 g for each sample used for sample analyses. Overall, three technical replicates were made for
143 each biological replicate (or each considered plot). For each cultivar and sample date,
144 between 10 and 20 fruits were individually weighed.

145 For apple, harvest dates were expressed as DD from the date of full bloom. The same base
146 temperature of 7 °C was considered.

147

148 2.2 Cell wall preparation

149 Apple outer cortex and cherry exocarp pieces (free of pit and stone) were freeze-dried and
150 dried at 40 °C for 2 h under vacuum over P₂O₅ before weighing and powdering (FastPrep24,
151 MP Biomedicals; 6.5 m.s⁻¹ for 60 s). Cell walls were prepared as alcohol insoluble material
152 (AIM) from powder using an Automatic Solvent Extractor (ASE® 350, Thermo Scientific) as
153 reported previously [23]. AIMs were dried at 40 °C overnight under vacuum over P₂O₅ before
154 weighing in order to determine the extraction yield. AIMs were ground before chemical
155 analyses.

156

157 2.3 Polysaccharide composition

158 Sugar composition in AIM was assessed after two steps of hydrolysis in sulfuric acid (72% at
159 RT for 30 min followed by 2 M at 100 °C for 120 min) [23]. Neutral sugars were analyzed by
160 GC using a Trace GOLD TG-225 GC Column (30 x 0.32 mm ID) on a TRACE™ Ultra Gas
161 Chromatograph (Thermo Scientific; temperature 205 °C, carrier gas H₂) after conversion to
162 alditol acetates, as reported previously [23]. Sugar standard solution and inositol were used
163 for calibration and as internal standards, respectively. Uronic acids in acid hydrolysates were
164 analyzed by colorimetry with meta-hydroxydiphenyl, as reported previously [23].

165 Starch measurement in AIM was performed after amylolysis and HPAEC analyses as
166 described in [27].

167

168 2.4 Hemicellulose enzymatic profiling

169 Cell wall material was degraded by commercial endo-1,4-β-glucanase from *Trichoderma*
170 *longibrachiatum* (Megazyme, Bray, Ireland; 20 U) overnight at 40 °C under agitation, as
171 reported previously [23]. Oligosaccharides in the hydrolysates were analyzed by MALDI-
172 TOF MS in positive mode using an Autoflex III MALDI-TOF/TOF spectrometer (Bruker
173 Daltonics, Bremen, Germany), as reported previously [23]. One or three replicates per
174 hydrolysis were mixed with the ionic liquid matrix DMA/DHB and deposited on MTP 384
175 polished steel. The instrument was externally calibrated using galactomannan oligomers (DP
176 3–9) of known mass. Spectra were recorded in the mass range m/z 500–2000. Spectra were
177 exported to Flex Analysis 3.4 software (Bruker) and preprocessed. Mass lists reporting m/z
178 (monoisotopic masses, after deisotoping with the SNAP algorithm, Bruker) and intensities of
179 detected ions were then exported to R software [28] for statistical analysis and graphical
180 representation. Ion masses and intensities of the spectra of glucanase hydrolysates were

181 normalized to that of the XXXG ion at m/z 1085. Identification of ions was performed by
182 comparison with the m/z list of theoretical masses of the sodium adduct of different
183 oligosaccharides. Ion attribution to xyloglucan structures was performed on the basis of
184 combinations of hexose, methyl-pentose, pentose, hexuronic acid and acetyl ester
185 substituents. The nomenclature of oligomers of xyloglucan released by glucanase followed
186 that of [29] and is summarized in **Fig S1**. Briefly, bare (1,4)-linked β -D- glucose residues
187 were noted as G. Extension of glucose by (1,6)-linked α -D-xylose was noted as X. Further
188 extensions on the xylose residues on O-2 by β -D-galactose or α -D-fucosyl-(1->2)- β -D-
189 galactose were noted as L and F, respectively. When a hexuronic acid residue is present, it is
190 indicated by the letter Y, while the presence of two contiguous xylosyl groups on the glucose
191 backbone is noted as U. For other oligomers, Hex and Pen refer to hexose and pentose,
192 respectively, followed by the number of these residues in the oligomer (see **Table S1**).
193 Acetyl-esterification of structures was noted as “a” while the presence of hexuronic acid and a
194 methyl ether was noted by “u” and “m” respectively. The number following the code
195 corresponds to the number of sugar or substituent groups. For example, Pen2u1m1a1
196 corresponds to a dimer of pentose with one hexuronic acid, one methyl ether and one acetyl
197 group.

198

199 *2.5. Data analysis*

200 All statistical analyses were performed using R [28]. These included standard error of the
201 mean and principal component analysis (PCA) calculation of data replicates. The number of
202 replicates is indicated in figure and table legends. Due to the different degree-days of the
203 samples, significant differences in data between varieties were based on the comparison of the
204 95% confidence interval estimated from the standard error of the mean \times 1.96. Ellipses on
205 individual PCA plots corresponded to the 95% confidence location of the data barycenter
206 according to [30].

207 MS data were analyzed by an in-house script in R used previously [19, 20, 22, 31, 32]. This
208 script tabulated m/z values and peak intensity from spectra exported as text files from the
209 mass spectrometer. The same script associated the m/z value with a known or calculated mass
210 values for oligosaccharides (**Table S1**) prior to statistical analyses.

211 All MS spectra were normalized to the intensity of the XyG ion XXXG (1085 m/z) to correct
212 for differences in intensities between spectra. PCA of MS data from glucanase hydrolysate
213 were also scaled to zero mean and unit variance.

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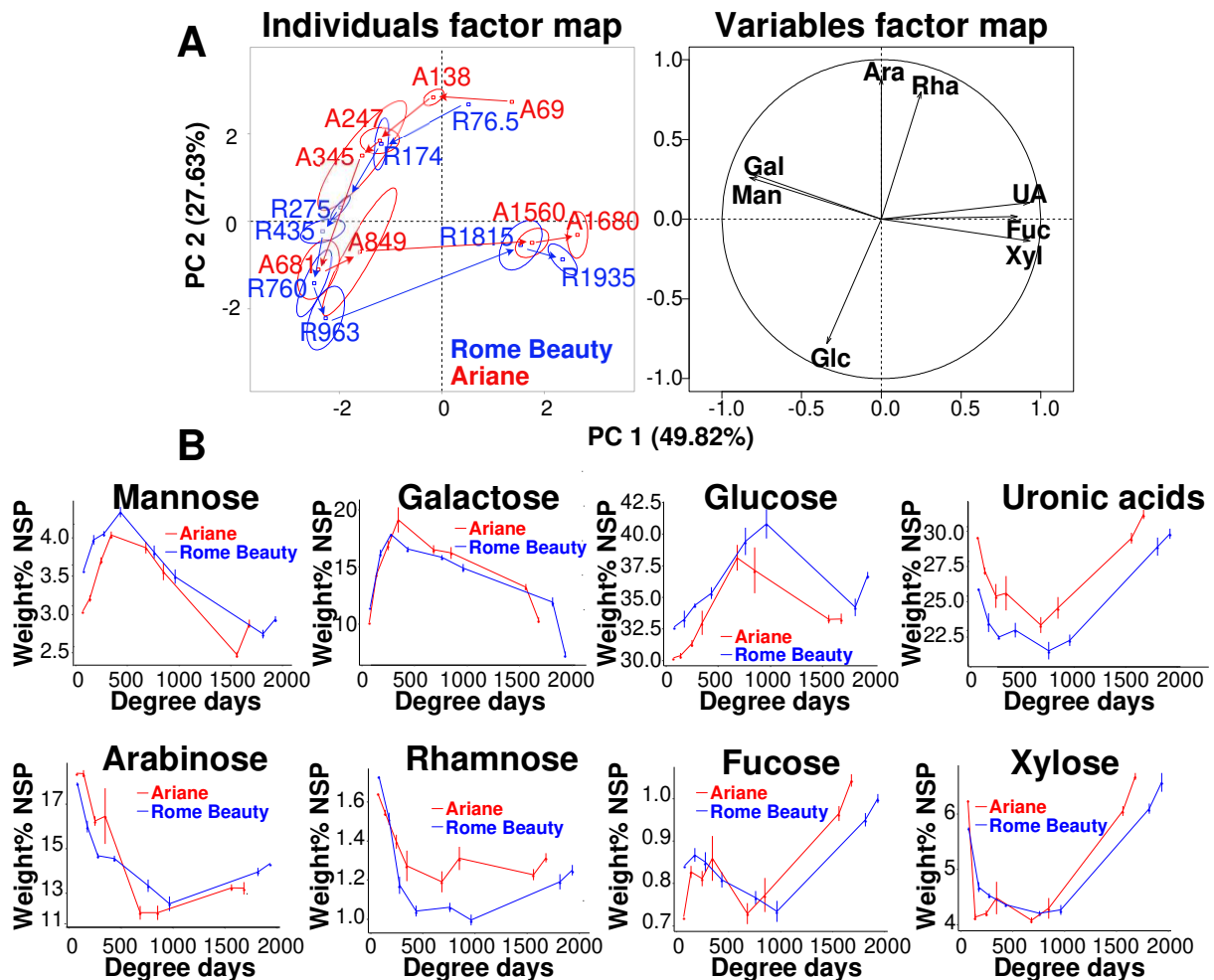
3 Results

3.1 Cell wall compositions of firm and soft apple cultivars differ and are markedly affected by development

Rome Beauty fruits developed into larger fruits than Ariane fruits (**Fig S2A**). The fruit dry weight content first decreased in young fruits of both cultivars until approximately 250 degree-days (DD) and then increased steeply up to approximately 750 DD to increase more slowly later on (**Fig S2B**). After approximately 500 DD, by comparing estimated confidence intervals from the standard error of the means, Ariane was significantly richer in dry matter than Rome Beauty, likely due in part to the lower amount of water compared to the large Rome Beauty fruits. Ariane fruits were also enriched in dry matter during postharvest storage. The alcohol insoluble material representing polymeric material decreased in young fruits along with dry material before a rebound at approximately 250 DD to reach a maximum at approximately 750-1000 DD before a marked decrease during fruit expansion (**Fig S2C**). Nonpolysaccharide polymeric material (most likely mainly protein; AIM subtracted from nonstarch polysaccharides and starch; **Fig S2D**) was at its highest level in the very young fruits (approximately 60% AIM dw) and decreased to approximately 10% after approximately 700 DD. Starch started to accumulate from approximately 250 DD to a maximum at approximately 750-1000 DD before markedly decreasing thereafter and notably during storage (**Fig S2E**). Nonstarch polysaccharides corresponding to cell wall material followed the opposite behavior with an enrichment during storage. Between 500 and 1000 DD starch and cell wall contents in Ariane were significantly higher and lower than Rome Beauty, respectively (**Fig S2F**).

The mean sugar composition of nonstarch polysaccharides (NSP) considering all the developmental stages and both cultivars was glucose (**Table S2**; 33.9% of AIM dry weight), uronic acid (25.3%), arabinose (14.6%), galactose (14.0%), xylose (5.0%), mannose (3.4%), rhamnose (1.3%) and fucose (0.8%). This composition changed during fruit development, as particularly shown by principal component analysis capturing 77.5% of the variance in the first two components (PCA; **Fig 1A**). Young developing fruit were characterized by an increase in galactose and mannose content up to approximately 500 DD and a concomitant decrease in arabinose, rhamnose, uronic acid and xylose contents (**Fig. 1B**). The glucose content increased up to 850 DD for Ariane and 960 DD for Rome Beauty before decreasing along with mannose and galactose contents to benefit an increase in uronic acids, xylose and

249 fucose. The proportion of the three latter sugars distinguished the late development phases
 250 (Fig 1, Table S2). Of interest are the negative correlations between the glucose content and
 251 those of rhamnose and arabinose, the absence of a relation between galactose and mannose
 252 with rhamnose and glucose (orthogonal positions) and the collinearity of mannose and
 253 galactose contents (Fig 1A). There appears to be antagonism between pectic RGI structural
 254 domains rich in rhamnose and either RGI arabinose side chains and/or arabinogalactan
 255 proteins with cellulose, xyloglucan (XyG) and/or galactoglucomannan (GgM) contents. These
 256 results also indicated a disjunction of galactose metabolism distinguishing RGI galactan side
 257 chains from RGI arabinan side chains. An antagonism between the metabolism of GgM and
 258 XyG hemicelluloses, taking fucose and xylose as markers of XyG appeared with
 259 development. Rome Beauty tended to be richer in glucose and mannose and poorer in uronic
 260 acids, rhamnose, fucose and xylose.



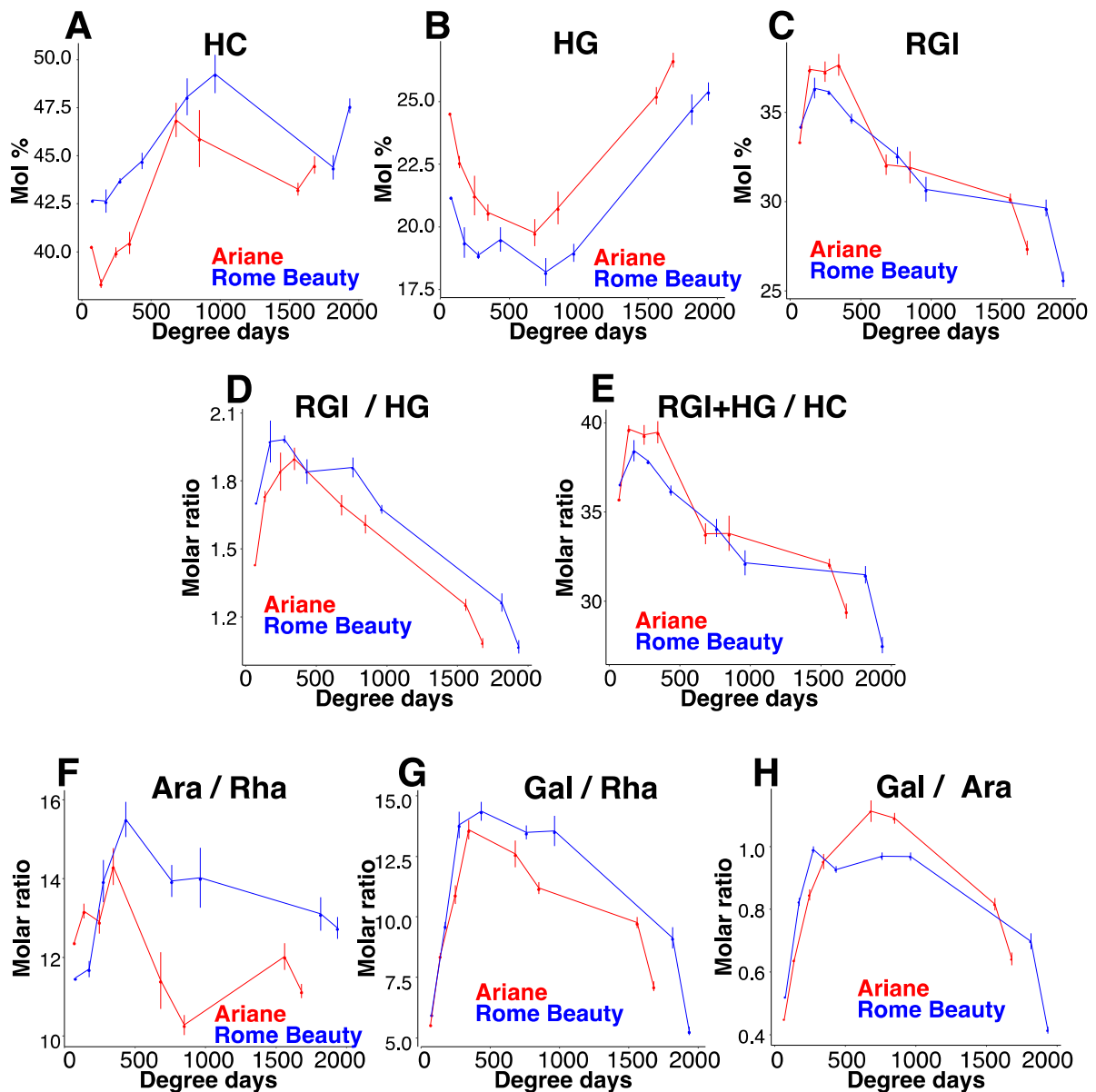
261
 262 **Figure 1** Principal component analysis (A) and variations in sugars content in nonstarch
 263 polysaccharides (B) according to Ariane and Rome Beauty apple development. Ellipses in (A)
 264 are 95% confidence ellipses for the barycenter of individuals; arrows in (A) and lines in (B)

265 are aids for the eyes to follow changes with fruit development; bars: standard error; n = 1
266 (pool of 9 fruits), 6, 6, 6, 6, 6, 12, 12 fruits for both Ariane and Rome Beauty.

267

268 Considering molar proportions of cell wall sugars, the major turning points noticed on the
269 PCA map of individuals at approximately 850 and 960 DD for Ariane and Rome Beauty,
270 respectively, corresponded to the maximum molar proportion of cellulose and hemicellulose
271 sugars (HC) and to the lowest proportion of HG pectic domains (**Fig 2A, 2B**). In agreement
272 with the sugar composition of the NSP, Rome Beauty was richer in HC and poorer in pectic
273 HG domains than to Ariane. The molar proportion of the RGI pectic domain was close in the
274 two cultivars and decreased after a maximum at approximately 250-500 DD (**Fig 2C**).
275 However, considering the HG and RGI pectic domains together, the Rome Beauty cell wall
276 pectin was richer in RGI, and Ariane showed a higher proportion of HG domains (**Fig 2D**).
277 Nevertheless, the ratio of pectin to hemicellulose and cellulose was close for the two
278 cultivars: the richness in HG pectic domains in Ariane compensated for its sparseness in HC
279 (**Fig 2E**). Assuming that arabinose and galactose were all part of pectic RGI neutral side
280 chains linked to rhamnose, the richness in rhamnose for Ariane (**Fig 1B**) led to shorter side
281 chains according to their molar ratio (**Fig 2F and G**). However, considering only these side
282 chains, their ratio indicated that Ariane was richer in galactan side chains from approximately
283 500 DD until harvest (**Fig. 2H**).

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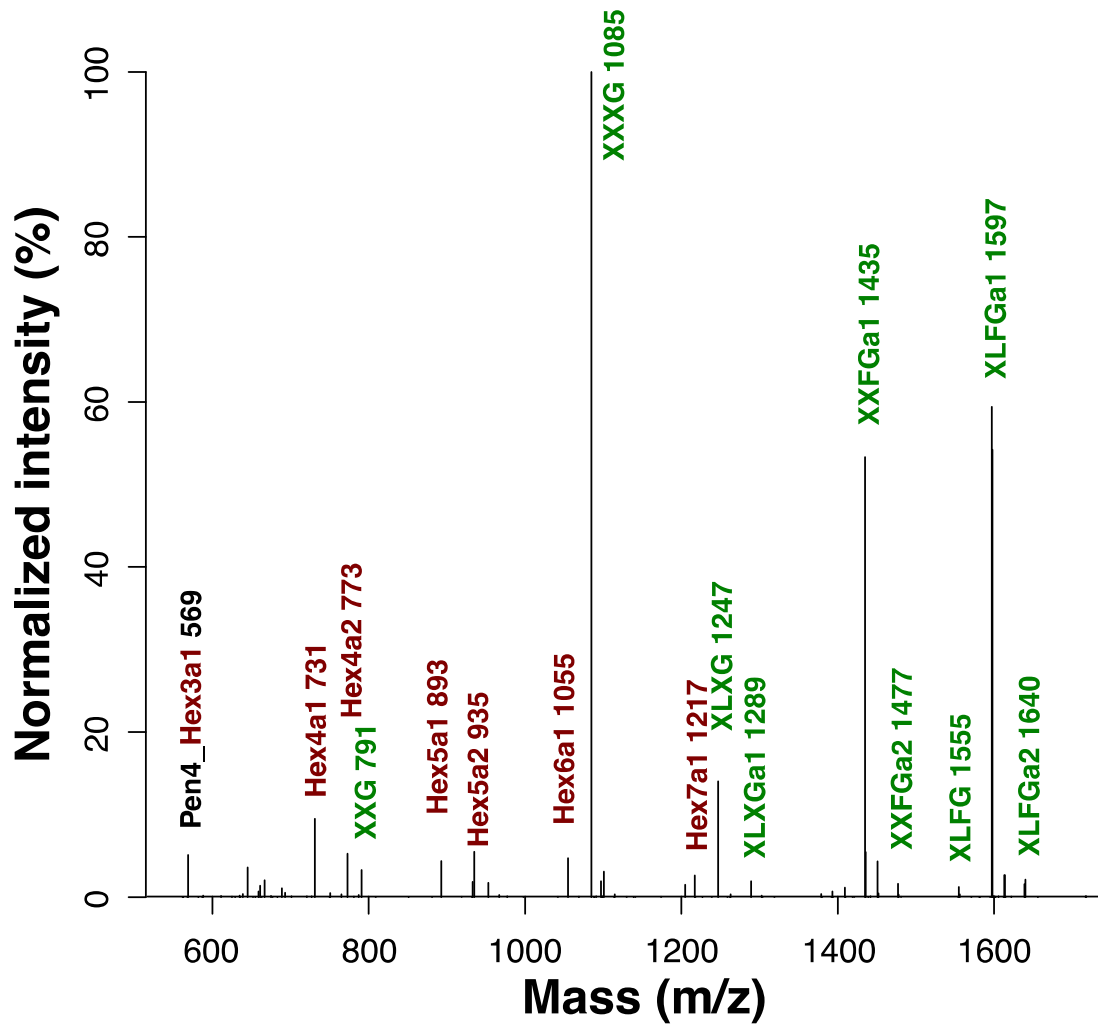


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 286 **Figure 2** Cell wall sugar molar percentage ratio: (A) hemicellulose and cellulose (HC;
 287 Glc+Xyl+Man+Fuc mol%), (B) homogalacturonan (HG; uronic acids – Rha mol%), (C)
 288 rhamnogalacturonan I (RGI; Ara+Gal+Rha mol%), (D) ratio of RGI/HG pectic domains, (E)
 289 ratio of pectin to cellulose and hemicellulose, (F) arabinose to rhamnose mol%, (G) galactose
 290 to rhamnose mol%, and (H) ratio of RGI side chains in Ariane and Rome Beauty according to
 291 development. Lines are aids for the eyes bars: standard errors; n = 1 (pool of 9 fruits), 6, 6, 6,
 292 6, 6, 12, 12 fruits for both Ariane and Rome Beauty.

293

294 The hemicellulose structural profile resulting from glucanase degradation coupled to MALDI-
 295 TOF mass spectrometry consisted of XyG oligomers and acetylated hexose oligomers
 296 attributed in part to GgM and glucan (**Fig 3**). The variation in their relative proportion

297 confirmed their structural evolution with apple development. GgM/glucan oligosaccharides
298 contributed more than XyG to distinguish profiles by PCA in the early developmental stages
299 (**Fig 4**). The PCA of the normalized ion intensity of oligosaccharides relative to that of the ion
300 of the XXXG structure released by glucanase accounted for 61.2% of the variance in the first
301 two components. It clearly showed a turning point at approximately 680 DD for Ariane and
302 between 435-760 DD for Rome Beauty on the individual map and was mainly attributable to
303 the variation in the contribution of GgM/glucan oligomers to the MS profiles. Changes were
304 also noticed at the same developmental stage for XyG structures, such as for XLFGa1, which
305 reached its maximum contribution, while XLFG reached its minimal contribution and
306 plateaued at low relative proportions thereafter (**Fig 4A, 4B; Table S3**). The two cultivars
307 differed: GgM/glucan oligomers contributed more to the Rome Beauty glucanase
308 oligosaccharide profile than Ariane oligomers, while XLXGa1 and XLFGa1 structures
309 distinguished the Ariane and Rome Beauty profiles, respectively (**Fig 4B; Table S3**). The
310 third and fourth components accounting for 14.3% of the variance did not distinguish
311 varieties or development (sup Fig).
312



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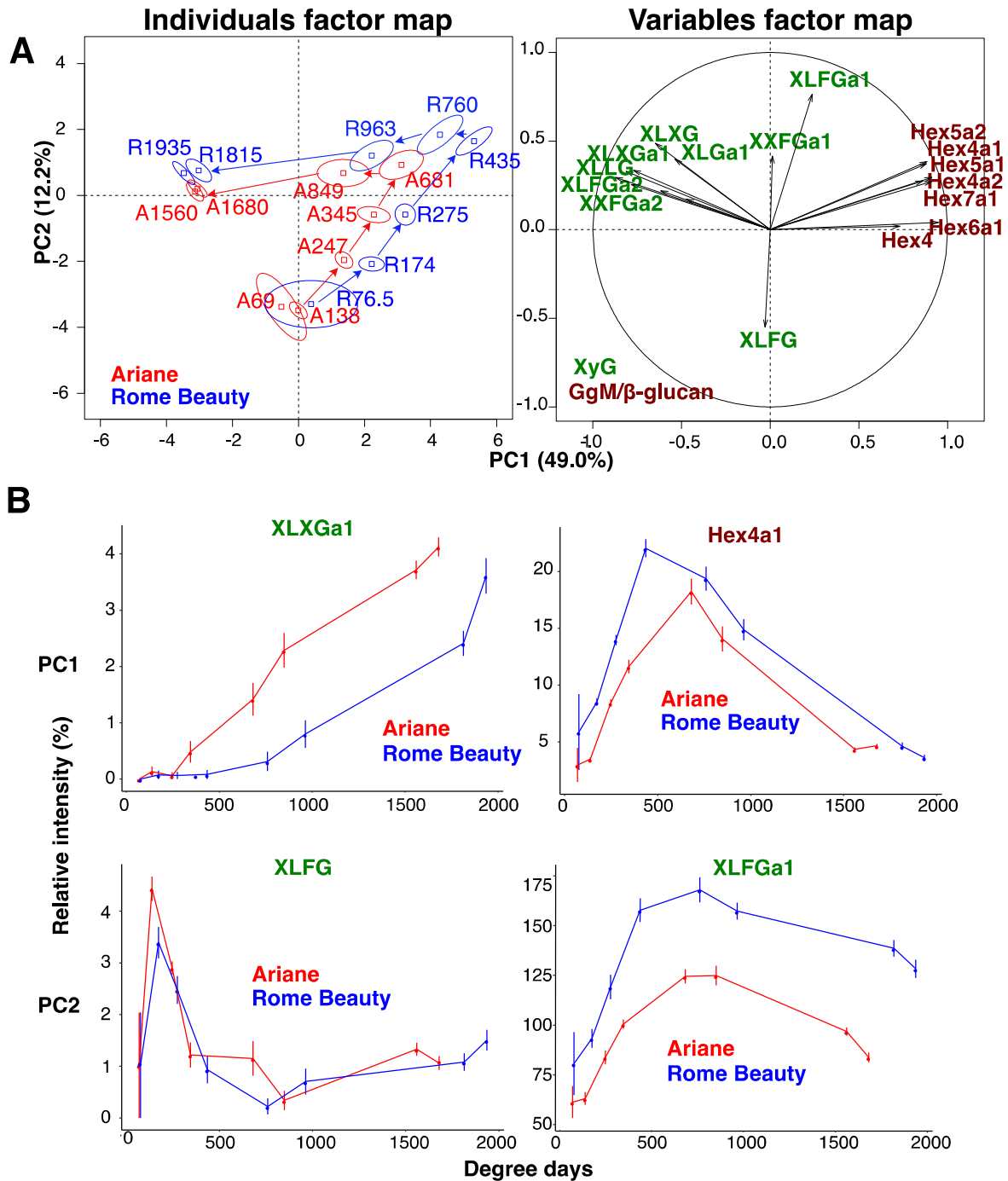
315 **Figure 3** Average MALDI-TOF MS spectrum of apple glucanase hydrolysate from the entire
316 fruit collection (n = 330). The nomenclature is as described in the text, in Table S1 and Figure
317 S1 followed by the m/z value; in brown: hexose-based oligomers attributed to GgM/ β -glucan,
318 in green: XyG oligomer; black: pentose-based oligomers attributed to
319 glucuronoarabinoxylan/glucuronoxylan (GAX/GuX). The intensity of ions was normalized to
320 that at m/z 1085 attributed to the XyG structure XXXG.

321

322

323 Thus, the cell wall composition and the hemicellulose structure differed between the soft and
324 firm cultivars. Rome Beauty had higher proportions of cellulose/hemicellulose, whereas
325 Ariane was richer in HG pectic domains. Further differences were noted in the pectic RGI
326 domains with longer side chains for Rome Beauty but more frequent galactan side chains for
327 Ariane. Aside from these varietal differences, the kinetics of cell wall construction and
328 modification showed differences between the two cultivars. They both showed “turning

329 points” with regard to compositional changes in hemicellulose, cellulose and pectin and
330 hemicellulose structure with fruit development. Up to approximately 500 DD, RGI pectic
331 domains were the highest, while HG domains reached a minimum that remained until 1000
332 DD, at which point they increased to reach their maximum at harvest/postharvest for both
333 cultivars. The main difference with regard to sugars attributed to pectin concerned the molar
334 ratio of arabinose to rhamnose, which increased for the two cultivars up to 400-500 DD but
335 then markedly decreased for Ariane to reach its lowest value at approximately 1000 DD to
336 increase to a second maximum at approximately 1500 DD, while for Rome Beauty, the
337 decrease was less steep and more continuous. Kinetics of hemicellulose and cellulose changes
338 in proportion also distinguished Ariane and Rome Beauty. These polysaccharides increased
339 from approximately 250 DD to reach a maximum at 600 DD for Ariane and 1000 DD for
340 Rome Beauty to decrease thereafter until harvest before another enrichment during storage.
341 With regard to hemicellulose fragments following glucanase hydrolysis of cell walls, no
342 marked difference was observed in the kinetics of changes between Ariane and Rome Beauty.
343 The main “turning point” after approximately 345-850 DD corresponded to the maximum of
344 hemicellulose/cellulose.
345



346

347 **Figure 4** Principal component analysis of MALDI-TOF MS ions attributed to hemicellulose
 348 oligomers in the glucanase hydrolysate of AIM from Rome Beauty (R) and Ariane (A)
 349 according to development (the number following the letter corresponds to the degree-days).
 350 A) Principal components 1 and 2 (PC1, PC2), maps of individuals and variables; B) Variation
 351 in relative intensity of characteristic oligosaccharides contributing to PC1 and PC2.
 352 Nomenclature is as described in the text, Table S1 and Fig S1; ellipses in (A) are 95%
 353 confidence ellipses for the barycenter of individuals ; arrows in (A) and lines in (B) are

354 indicated to guide the eyes to follow changes with fruit development; bars: standard errors; n
355 = 1, 6, 6, 6, 6, 6, 12, 12 fruits x instrumental triplicates for a total of 3, 18 or 36 repetitions for
356 both Ariane and Rome Beauty.

357

358 *3.2 Sweet cherry cell walls are rich in pectin, and proportions in HG and RGI structural* 359 *domains are markedly affected by fruit development*

360 Cherry fruits, characterized by double sigmoid growth, (**Fig S3A**), yielded alcohol insoluble
361 material (AIM) from 6 to 50% of the initial dry powder (**Fig S3B**). Recovery significantly
362 decreased according to cultivar at the different dates of collection. The increase in the overall
363 fresh weight and the decrease in AIM during fruit growth were similar to those reported [33].
364 From approximately 300 DD to 550 DD, the cultivar Regina yielded significantly more AIM
365 than Garnet ($p < 0.01$). The overall sugar content varied significantly from 56 to 74% of the
366 AIM dry weight according to the collection period but irrespective of the cultivar (**Fig S3C**).
367 The lowest cell wall sugar content was observed in fruit collected at 512 DD for Garnet and at
368 472 DD and 554 DD for Regina.

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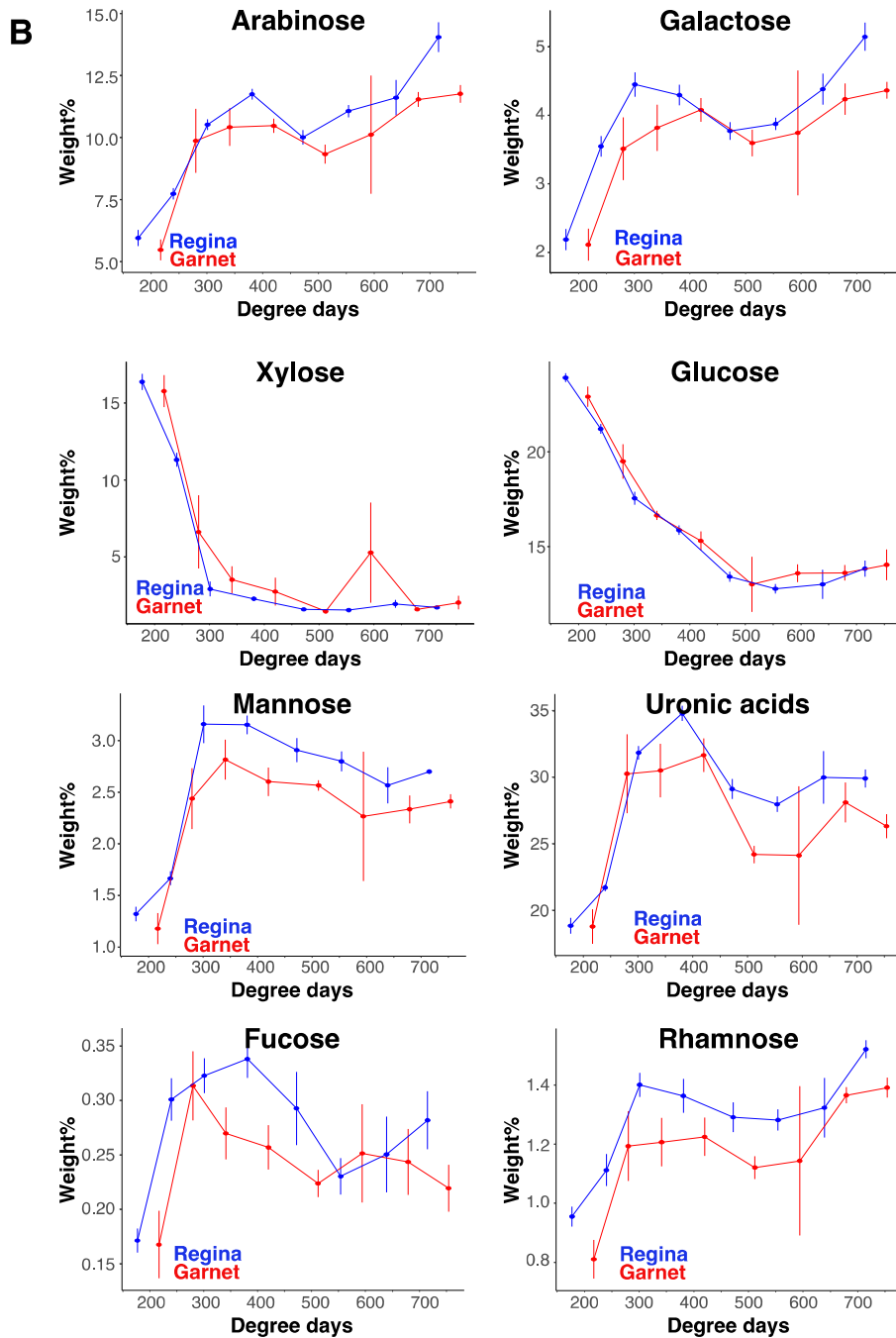
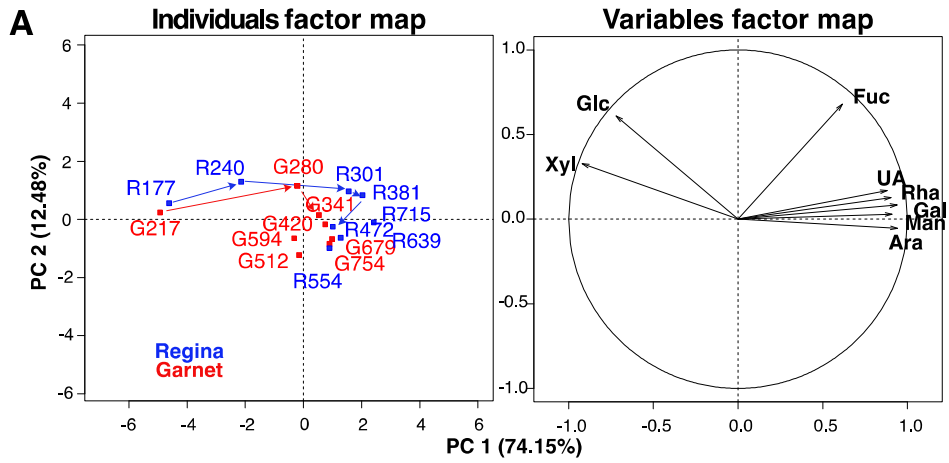
370 The average cherry AIM was composed of uronic acids (27%), glucose (16%), arabinose
371 (10%), xylose (5%), galactose (4%), mannose (2%) rhamnose (1%) and fucose (0.3% on the
372 dry weight basis of AIM; **Table S4**). Principal component analysis of sugar composition
373 captured 86.6% of the variance in the first two components. There were marked changes
374 during fruit development, with a turning point at approximately 250-450 DD (**Fig 5A**). These
375 changes were primarily due to a marked increase in the content of mannose and uronic acids
376 up to date 3 and a marked decrease in glucose and xylose contents up to approximately 300-
377 350 DD (**Fig 5B**). In addition, the contents of arabinose and galactose continuously increased
378 throughout the collection period but showed a transient maximum at 300-350 DD followed by
379 a decrease up to 450-550 DD before increasing until the end of the collection. There was no
380 major difference in sugar content between the two cultivars during development except
381 mannose content, which was higher in Regina compared to Garnet, and, to a lower extent, that
382 of fucose and rhamnose between 300-500 DD.

383 Considering the molar percentage of sugar representative of the
384 hemicellulose/cellulose (HC) and RGI and HG pectic domains, HC was a major contributor to
385 sweet cherry cell walls in young fruit (**Fig 6A**) but rapidly decreased to low values after 300-
386 350 DD to the benefit of the RGI and HG pectic domains (**Fig 6B, 6C**). The HG proportion
387 increased and plateaued after 300-350 DD, while the RGI proportion still progressed until the

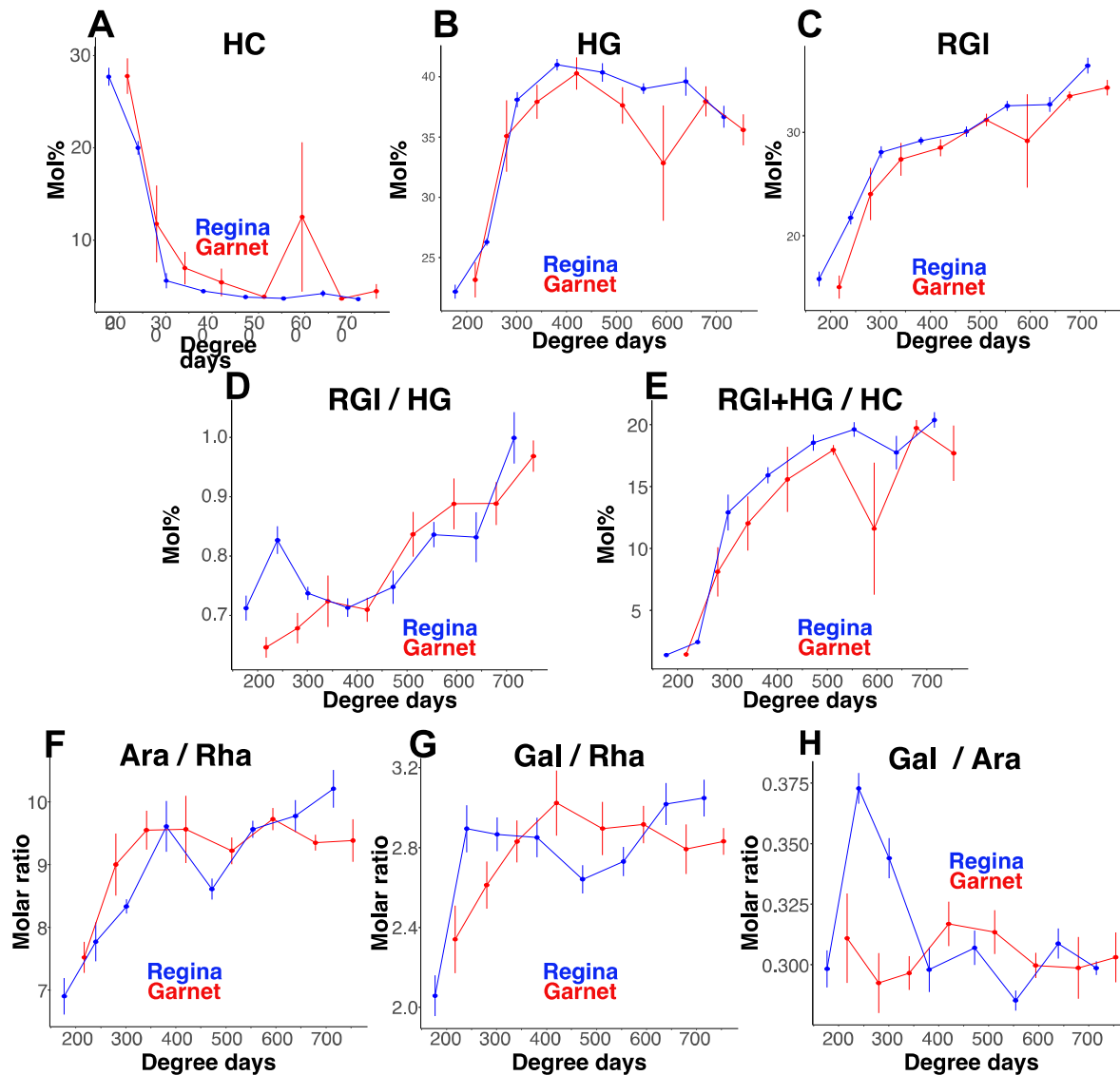
388 end of the collection. There were no major differences in the pectic domains between the two
389 cultivars, and the RGI/HG ratio increased steadily throughout fruit development (**Fig 6D**).
390 Likewise, the molar ratio of sugar attributed to pectin relative to cellulose and hemicellulose
391 steadily increased to level off after approximately 600 DD (**Fig 6E**). With regard to the RGI,
392 assuming that all galactose and arabinose were part of the side chains linked to rhamnose, the
393 proportion of arabinose and galactose to rhamnose progressed until 300-350 DD and then
394 plateaued. There was no major difference in the evolution of these ratios between Regina and
395 Garnet during fruit development (**Fig 6F, 6G**). Considering similar rhamnose contents in the
396 early development of the two cultivars (approximately 300 DD, **Fig 5**), Regina presented
397 longer Gal side chains (**Fig 6H**). Such differences disappeared in later developmental stages.
398 Thus, similar to apple, sweet cherry cell wall composition markedly changed during fruit
399 development. However, unlike apple, the two sweet cherry cultivars did not differ in the
400 kinetics of cell wall composition changes during development. They markedly differed from
401 apple with a much lower contribution of cellulose/hemicellulose to the cell wall
402 (approximately 6 mol% for sweet cherries for approximately 52 mol% in ripe apples),
403 although they shared close global pectin structural domain composition with approximately
404 50 mol% HG for ripe sweet cherries and 48 mol% in ripe apples.

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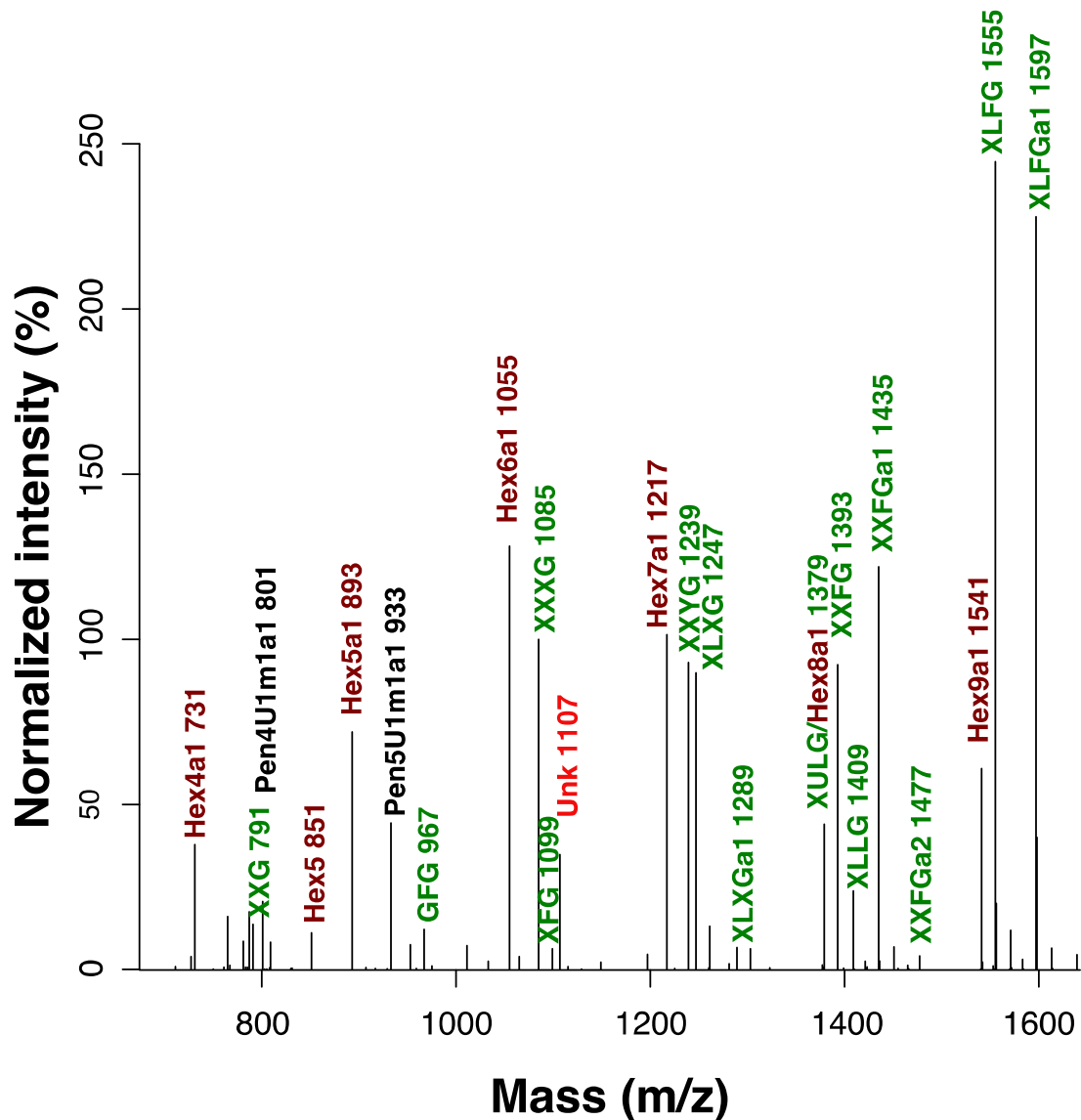


408 **Figure 5** Principal component analysis (A) and variations in sugars content in nonstarch
 409 polysaccharides (B) according to Regina and Garnet sweet cherry development. Arrows in
 410 (A) and lines in (B) are aids for the eyes; bars: standard error; n = 6 except G4 n = 3 and G6 n
 411 = 5.
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 414 **Figure 6** Cell wall sugar molar percentage ratio: (A) hemicellulose and cellulose (HC;
 415 Glc+Xyl+Man+Fuc mol%), (B) homogalacturonan (HG; uronic acids – Rha mol%), (C)
 416 rhamnogalacturonan I (RGI; Ara+Gal+Rha mol%), (D) ratio of RGI/HG pectic domains, (E)
 417 ratio of pectin to cellulose and hemicellulose, (F) arabinose to rhamnose mol%, (G) galactose
 418 to rhamnose mol%, and (H) ratio of RGI side chains in Regina and Garnet according to
 419 development. Lines are aids for the eyes, bars: standard error; n = 6 except G4 n = 3 and G6 n
 420 = 5.
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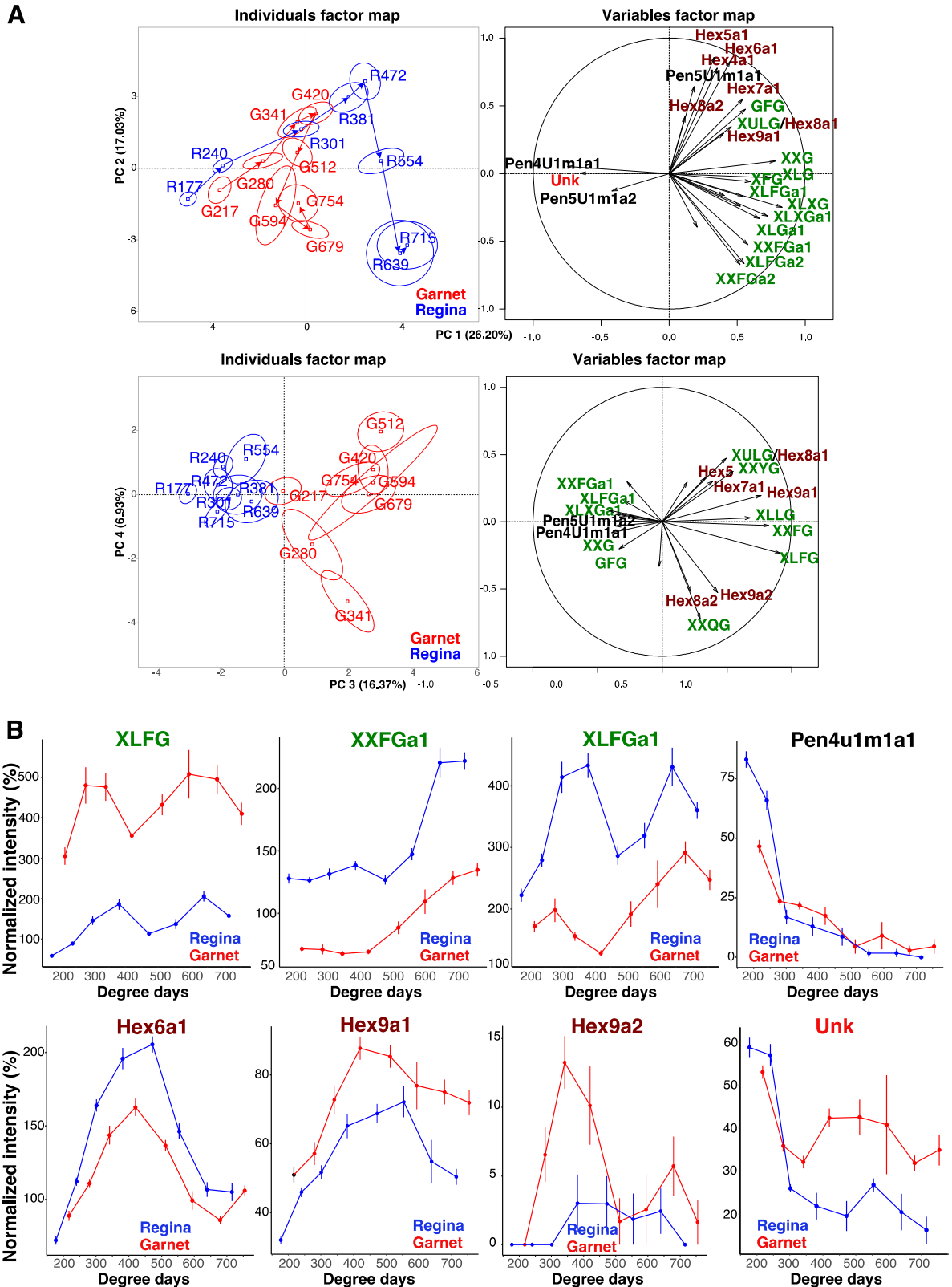
422 Hemicellulose degradation by endoglucanase released oligomers that were attributed by
 423 MALDI-TOF MS on the basis of m/z . A typical MS spectrum is shown in **Fig. 7**. It differed
 424 from apple by the presence of unusual oligomers containing uronic acid (XXYG and
 425 potentially XULG, which is isobar with Hex8a1) and an unknown oligosaccharide at m/z
 426 1107.
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 429 **Figure 7** Average MALDI-TOF MS spectrum of sweet cherry glucanase hydrolysate from the
 430 entire fruit collection ($n=183$). The nomenclature is as described in the text, in Table S1 and
 431 Figure S1 followed by the m/z value; in brown: hexose-based oligomers attributed to GgM/ β -
 432 glucan, in green: XyG oligomer; black: pentose-based oligomers attributed to
 433 glucuronoarabinoxylan/glucuronoxylan (GAX/GuX), in red: unknown oligomer. The
 434 intensity of ions was normalized to that at m/z 1085 attributed to the XyG structure XXXG.

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The PCA of the normalized ion intensities of oligosaccharides relative to that of the ion of the XXXG structure released by glucanase accounted for 43.2% of the variance in the first two components and for 23.3% of the variance in the third and fourth components (Fig. 8A). The first two components distinguished among development stages while the third and fourth components differentiated the two varieties. In the first two components, the contribution of acetylated hexose oligomers was proposed to be related to galactoglucomannan structures: Hex4a1 to Hex9a1 increased up to 420 DD for Garnet and approximately 470 DD for Regina and then decreased (**Fig 8B, Table S5**). GuX oligomers (Pen4u1m1a1) were at their highest levels in the early developing fruit and then decreased to reach a plateau after approximately 300 DD (**Fig 8B, Table S5**). The unidentified oligomer (m/z 1107) had a similar contribution to the profile as Pen4u1m1a1 in Regina (**Fig 8B**). Xyloglucan oligomers contributed more to the oligosaccharide profiles in the later days (**Fig 8A, 8B, Table S5**). Interestingly, a bimodal contribution of XLFG and XLFGa1 structures was detected showing a minimum at approximately 400-600 DD, which is opposite to the maximum of the acetylated hexose oligomers attributed to GgM oligosaccharides (**Fig 8B, Table S5**). The third and fourth components distinguished Garnet from Regina with more important contributions of XyG (XLFG, XXFG) and GgM oligomers of high degree of polymerization (Hex8a1, Hex9a1) in Garnet (**Fig 8A, B**). Besides XLFG, the contribution of XLFGa1 and XXFGa1 differed markedly between in the two cherry cultivars, whereas, as regards ions attributed to GgM (i.e., Hex6a1, Hex9a1), differences were higher between 300-500 DD and opposite for low and high degree of polymerization. GuX oligomers (Pen4u1m1a1), the unknown structure (unk m/z 1107) and diacetylated GgM structures (Hex8a2, Hex9a2) distinguished the two cultivars, particularly during early stages of their development.



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Figure 8 Principal component analysis of MALDI-TOF MS ions attributed to hemicellulose oligomers in the glucanase hydrolysate of AIM from Regina (R) and Garnet (A) according to development (the number following the letter corresponds to the degree-days). A) Principal components 1, 2, 3, 4 (PC1, PC2, PC3, PC4), maps of individuals and variables; B) variation

465 in relative intensity of characteristic oligosaccharides contributing to PC axes. Nomenclature
466 is as described in the text, Table S1 and Fig S1; Unk: nonidentified oligomer; ellipses in (A)
467 are 95% confidence ellipses for the barycenter of individuals; arrows in (A) and lines in (B)
468 are indicated to guide the eyes to follow changes with fruit development; bars: standard
469 errors; n = 12 except G4 n = 10, G6 n = 6, R7 n = 11.

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472 **4 Discussion**

473 *4.1 Pectin structural domains markedly differ in the set setup and evolution of apple and* 474 *cherry cell walls during fruit development*

475 The cell wall compositions were within the literature values for apple [19, 22] and cherry
476 [33]. However, they were markedly affected by fruit development: the proportion in the RGI
477 pectin domain increased very early in apple and then decreased to the benefit of the HG
478 domain, while the opposite was observed for cherry after a decrease in
479 cellulose/hemicellulose in very early developmental stages. RGI galactan and arabinan side
480 chains have been related to fruit texture and have long been known to be markedly modified
481 during the late developmental stages (ripening) [6]. Galactan and arabinan are often related to
482 fruit texture (firmness, mealiness) [12, 14, 34], possibly involving both hydrogen binding to
483 cellulose [13] and cell wall water flow since galactan was proposed to limit it [7], while
484 arabinan was found to be more hydrophilic [35]. These side chains are related to apple
485 firmness when the cells are turgid, possibly by limiting slippage of cellulose fibers under
486 tension due to cell turgor pressure, but are detrimental to residual viscoelasticity of the
487 plasmolyzed tissue [14]. Furthermore, the arabinan contribution to apple firmness does not
488 seem to be linked to interactions between linear arabinan and cellulose but more collectively
489 to different types of arabinose linkages in the cell wall [14], including those that may be
490 involved in arabinogalactan proteins reported to participate in fruit softening [36].
491 Considering that cell expansion starts after approximately 240-270 DD (approximately 45
492 DAA) [3] in apple, RGI side chains are first very short in very early developmental stages,
493 such as in tomato [20]. RGI then peaked at the beginning of the cell expansion phase to
494 slowly decrease and more noticeably after approximately 1000 DD (after approximately 100
495 DAA), which is within the apple ripening phase [3]. In contrast, for cherries, after a decrease
496 in hemicellulose/cellulose to a very low proportion (3-5 mol%) after approximately 240-280
497 DD, the pectin HG structure dominated rapidly (35-40 mol%) after approximately 280-300
498 DD, while RGI domains increased steadily with fruit development to reach approximately 35

499 mol% in the ripe fruit. A similar dilution of cellulose content in pectin has already been
500 reported in the cell wall of growing cherry [37]. Fruit softening perception has been related to
501 pectin metabolism/solubilization during ripening [2]. Brittle and soft/melty perception of an
502 apple and a cherry, respectively, can also be related to distinct cell wall organization. In apple,
503 pectin solubilization in ripe fruit is limited and forms a contiguous solid complex remaining
504 when the cellulose-hemicellulose network is removed by enzymatic treatment [38]. Its
505 distribution and hydration within the cellulose-hemicellulose network would control fruit
506 firmness [14]. The richness in HG over RGI domains and a higher ratio of galactose over
507 arabinose as side chains of RGI in the firm Ariane compared to Rome Beauty would agree
508 with a model of cell wall where RGI galactan binding to cellulose contributes to
509 strengthening the cell wall, possibly by limiting cellulose slippage, while hydrated pectin
510 within dispersed cellulose-hemicellulose increases resistance to cell wall compression and by
511 extension, that of the tissue. In contrast, in cherry, the increasing RGI/HG ratio with fruit
512 development with particularly high arabinose content (ratio of Gal/Ara ~ 0.3) would favor of
513 a particularly hydrated cell wall due in part to the presence of hydrophilic arabinan. However,
514 uronic acid richness compared with neutral sugar content in cherry cell walls also appears to
515 be related to firmer texture [39]. Together with a limited cellulose-hemicellulose network, in
516 agreement with [33], the RGI- and arabinan-rich cell wall would be responsible for the soft-
517 to-melting texture of cherry. The increase in pectin rich in RGI domains with arabinan side
518 chains during cherry development would agree with the increase in the ability of the fruit cell
519 wall to take up and retain water (swelling, water holding capacity) during development [37].
520 However, there was no clear difference supporting the skin cracking susceptibility of the two
521 cultivars. This was most likely due to the global scale at which the present study was realized.
522 The cell wall analyzed (AIM) mainly originated from mesocarp tissue and only slightly
523 originated from epicarp tissue, which is the site of skin cracking. Skin cracking results from
524 microcrack propagation at the cuticle level. It involves the epidermal anticlinal cell wall at the
525 middle lamella and cell wall swelling [40]. The cuticle is a thin complex assembly of cutin,
526 wax, phenolic compounds and polysaccharides [41]. Specific variations in the composition
527 and structure of this barrier leading to different cracking susceptibilities represent a future
528 area of research.

529

530 *4.2 Apple and cherry share common glucomannan enrichment at the onset of cell expansion*
531 *during early fruit development*

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533 Besides global cell wall composition, there was a focus was made on the evolution of
534 hemicellulose structure. In apple, developmental regulation of hemicellulose synthesis and
535 metabolism, notably galactoglucomannan, was observed in early developmental stages [19].
536 This report confirmed this observation and improved the time-scale resolution, specifying that
537 there was a marked change between approximately 435-760 DD (56-84 DAA) for Rome
538 Beauty and approximately 681 DD (84 DAA) for Ariane, corresponding to the maximum
539 contribution of GgM oligomers to the MALDI-TOF spectra of glucanase hydrolysates. It also
540 corresponded to the maximum mannose and galactose in the cell wall composition. There was
541 a concomitant change in the contribution of the XLFG XyG structure, which peaked very
542 early at approximately 138-174 DD for Ariane and Rome Beauty and then markedly
543 decreased to reach a minimum starting after 345-435 DD for Ariane and Rome Beauty, while
544 its acetylated counterpart, XLFGa1, peaked. It would be of interest to establish whether these
545 nonacetylated and acetylated XyG structures represent markers for dividing versus expanding
546 cells and to identify a role for XyG acetyl esterification in the regulation of apple fruit
547 development, particularly with regard to its interaction with cellulose. Cell expansion is also
548 associated with the XLXGa1 structure, which started increasing in the glucanase hydrolysate
549 during the same period. In fact, this developmental stage appears determinant, as it was close
550 to changes reported in the mechanical properties of apple flesh measured by compression and
551 shear puncture forces [3]. Volz et al. [3] reported a continuous decrease in the compression
552 puncture force in growing Gala apple from young fruits 16 days after full bloom (DAA) to
553 ripe fruit (153 DAA). However, they observed an increase in shear puncture force occurring
554 as two peaks, one after anthesis and a second one at approximately 109-119 DAA depending
555 on the harvest year, followed each time by a decline. If increased cell density may be
556 responsible for the first peak, the authors interpreted the second increase as resulting from a
557 different cell wall setup during this growing phase since there was less wall under the probe
558 as the cell markedly expanded. The peak of GgM oligomers released in the glucanase cell
559 wall hydrolysate and of mannose and galactose in Ariane and Rome Beauty occurring within
560 the same growth period as reported for increased shear puncture force strongly suggest a role
561 of GgM in tissue mechanical properties. Along with this hemicellulose, the peak in the pectin
562 RGI domain and its galactan and arabinan side chains could also contribute to the specific cell
563 wall mechanical properties at the initiation of cell expansion. Of interest is a similar peak in
564 hemicellulose structure attributed to GgM observed in the cell wall glucanase hydrolysate of
565 cherry cell walls at approximately 340-472 DD (40-50 DAA). This period corresponds to
566 stage III in cherry development and is associated with a marked cell expansion [37]. Unlike

567 apple, XLFG and XLFGa1 XyG oligomers did not peak distinctly with the onset of
568 expansion. Instead, their proportion decreased during this period and resumed during later
569 stages to finally decrease in late fruit development. In contrast with apple, for which the
570 XLXGa1 XyG oligomer proportion increased with cell expansion, it was the proportions of
571 XXFGa1 and its nonacetylated derivative that increased with cell expansion. Marked
572 differences were noted between the hemicellulose oligosaccharide profiles of the two cherry
573 cultivars, as already noted for other Rosaceae [31], confirming the genetic control of XyG
574 structure [22].

575 The function of GgM in the plant cell wall is diverse. In tomato fruit, it was proposed to be
576 associated with cell-cell adhesion [42], and in a mutant oversynthesizing cell wall mannose,
577 defects were observed in cell division and expansion with softer fruits and fragile stems [43].
578 GgM interacts with cellulose fibers, which in a model system composed of bacterial cellulose
579 and secondary cell wall GgM led to an increase in the elastic and shear modulus of the
580 composite [17]. This is in line with the reported increase in shear puncture behavior in Gala
581 apple [3] occurring during the same period when the synthesis of GgM was observed to be
582 high [19], and as reported here. GgM helps with cellulose crystallization, aggregation and
583 toughening of bundles, but as observed in composite models, its biomechanical impact also
584 relies on the presence of other cell wall polymers, such as other xylans in the secondary cell
585 wall, and on the fine chemical structure of the hemicelluloses [17]. In the present case, pectin,
586 notably RGI and its side chains together with XyG, which showed remarkable structural
587 changes when GgM oligomers were released most from the cell wall, likely contributed to
588 adapting the cell wall mechanical properties to the cell function. With regard to the
589 contrasting texture in apple, the presence of GgM and its lower metabolism in Rome Beauty,
590 since it contained significantly more mannose and more GgM oligomers were released
591 throughout fruit development compared to Ariane, may lead to cell wall that is resistant to
592 tension and less able to tear apart and to free cellular juice when ripe. Such a situation may
593 contribute to the development of fruit mealiness perception often reported for overripe Rome
594 Beauty. Mealiness involves cell separation instead of cell breakage during flesh destruction
595 [44]. Analogously increased cell wall mechanical resistance may result in the cracking-
596 tolerant cherry cultivar Regina, in which the mesocarp/epicarp cell walls richer in mannose
597 and GgM, than in the susceptible cultivar Garnet. Such mechanical resistance to tension may
598 limit microcrack propagation into macrocracks. Other specific contributions of XyG
599 structures that markedly differed between the two cultivars may also participate in
600 distinguishing the cultivars with regard to their cell wall mechanical properties. Thus, in

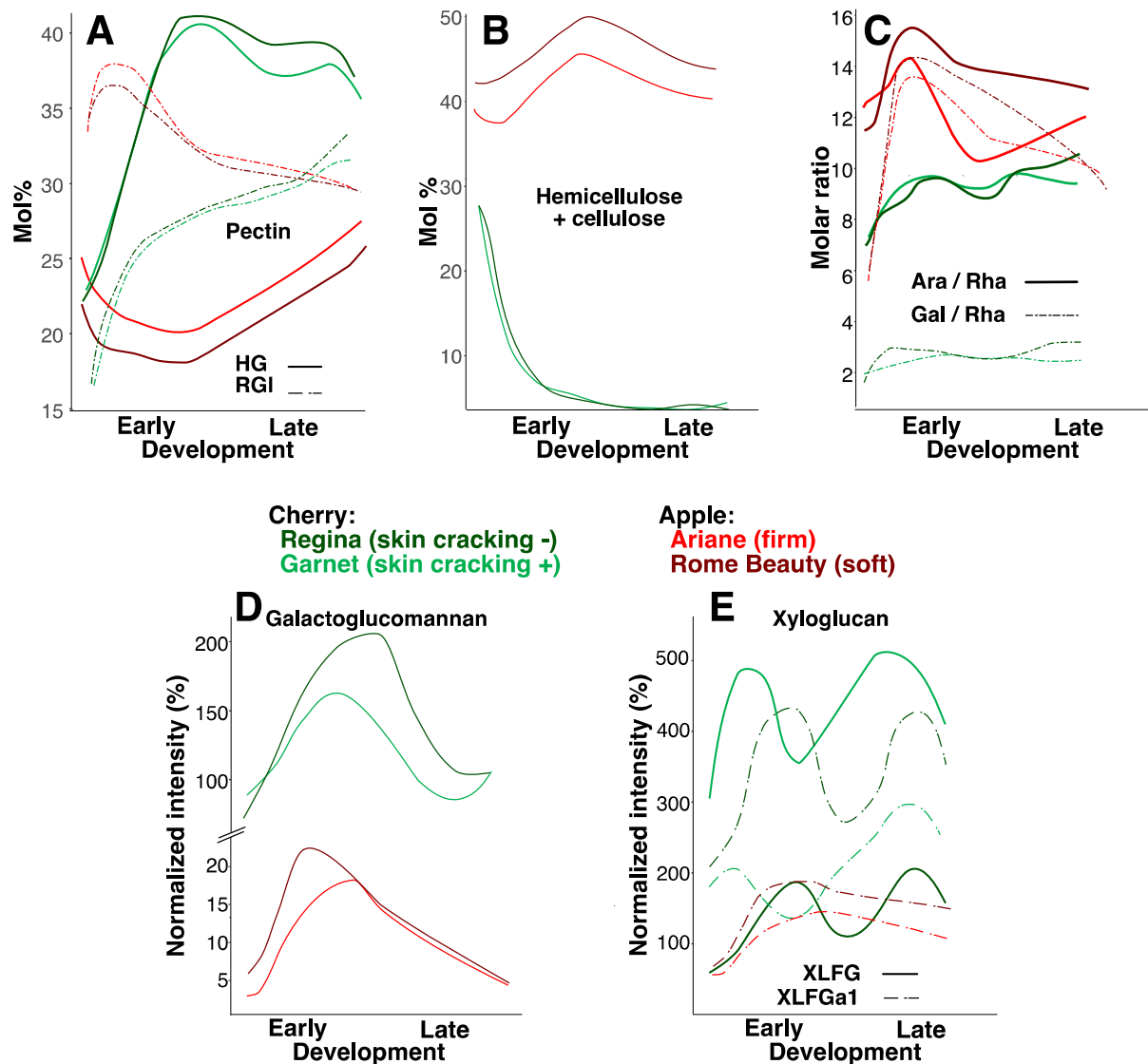
601 addition to other structural differences in the cuticle composition and structure that may be
602 critical for microcrack occurrence and that remain to be established, further work is needed to
603 support the contributions of hemicellulose biosynthesis and metabolism, particularly GgM, to
604 the control of mechanical properties underlying skin cracking in cherry.

605

606 **5 Conclusion**

607 Analysis of cell wall polysaccharides from brittle apple and soft-textured cherry from the
608 young fruit stage to the ripe fruit allowed the characterization of several features summarized
609 in Figure 9. These merit further studies with regard to contrasting flesh textures and their
610 prediction: the content of cellulose/hemicellulose versus pectin, the ratio of HG and RGI
611 pectin structural domains with the nature of the side chains, and particularly, the fine structure
612 profile of the hemicellulose as revealed by the coupling of cell wall glucanase degradation
613 and MALDI-TOF MS analysis of hydrolysates. This is particularly true for XyG and GgM
614 hemicelluloses, which need to be further assessed as possible markers for cell expansion and
615 whose metabolism may distinguish the texture of the ripe fruit or fruit susceptibility to
616 cracking. In this respect, genes coding for mannan synthase (CLSA), GH9 putative glucanase
617 and galactosidase specific for early fruit development [19] may be worth testing as early
618 markers whose expression levels may help predict apple texture when ripe according to
619 cultivar and growth conditions. Similar approaches could be pursued in cherries following the
620 first gene expression report of developing sweet cherry [45] with regard to the involvement of
621 hemicellulose in skin cracking susceptibility. In addition to the fine structure of GgM, XyG,
622 their acetylation remains to be further assessed concerning their function with regard to cell
623 development and their role in cell wall organization and mechanical properties.

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625

626 **Figure 9** Schematic plots of pectin HG and RGI (A), hemicellulose/cellulose (B) mol
 627 proportions, molar ratio of arabinose and galactose to rhamnose representing pectic RGI side
 628 chains (C) and evolution of galactoglucomannan (D) and xyloglucan (E) fine structures with
 629 fruit development of texture-contrasted apple and sweet cherry with different skin-cracking
 630 susceptibilities.

631

632

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642

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