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The biosynthetic origin of E-resveratrol accumulation in grape canes during post-harvest storage

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1 **The biosynthetic origin of *E*-resveratrol accumulation in grape canes during post-harvest**
2 **storage**

3

4 Resveratrol biosynthesis in pruned vine-wood.

5

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23 **Abstract (100-150 words)**

24 Grape canes are vineyard waste products containing valuable phytochemicals of medicine
25 and agriculture interest. Grape canes storage is critical for the accumulation of these
26 bioactive compounds. In the present study, we investigated the changes in stilbenoid
27 phytochemical composition during grape cane storage and the influence of temperature on
28 final concentrations. A strong increase in the concentration of the monomer *E*-resveratrol
29 (approx. 57-fold) was observed during the first six weeks of storage at 20°C in eight different
30 grape varieties without any change in oligomer concentrations. The *E*-resveratrol
31 accumulation was temperature dependent with an optimal range at 15-20°C. A 2h heat-
32 shock treatment aiming at protein denaturation inhibited *E*-resveratrol accumulation. The
33 constitutive expression of key-genes involved in the stilbene precursor biosynthesis along
34 with an induction of STS (stilbene synthase) expression during the first weeks of storage
35 contribute to a *de novo* biosynthesis of *E*-resveratrol in pruned wood grapes.

36

37 **Keywords:** stilbenoids, grape cane, postharvest storage, stilbene synthase

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42 **INTRODUCTION**

43 Stilbenoids are non-flavonoid polyphenols derived from the phenylpropanoid pathway. From
44 phenylalanine, the first three steps are successively catalyzed by the phenylalanine ammonia
45 lyase (PAL), cinnamate 4-hydroxylase (C4H) and 4-coumarate CoA ligase (4CL) yielding *p*-
46 coumaroyl-CoA. This product is an important metabolic intermediate acting as a substrate
47 for the generation of several phenylpropanoid subclasses such as ubiquitous lignin or
48 flavonoids but also stilbenoids which are present in a few species including grapevine. In
49 these plants, *p*-coumaroyl-CoA is condensed by stilbene synthase (STS) to form *E*-resveratrol
50 the first stilbenoid product.¹ In grapevine, STS is induced under both biotic and abiotic
51 conditions² in accordance with stilbenoid functions in plant resistance.³⁻⁵ For instance,
52 antimicrobial activities of *E*-resveratrol and its dimer *E*- ϵ -viniferin have been reported for
53 downy mildew⁶, powdery mildew⁷ and gray mold.⁸ *E*-Piceatannol, a resveratrol analog, is
54 induced by fungal elicitors⁹ and under UV irradiations.¹⁰ Additionally, resveratrol oligomers
55 display interesting activities against several grape fungal diseases.¹¹⁻¹²

56 A growing interest in stilbenoids arises due to their potential impact on human health.
57 Several reviews have indicated that *E*-resveratrol possesses a number of important
58 pharmacological activities and is known to provide a level of protection against both
59 metabolic and cardiac disorders together with anti-aging, anti-carcinogenic and anti-
60 inflammatory properties.¹³⁻¹⁵ Furthermore, the less studied *E*-piceatannol also exhibits a
61 wide range of biological activities including suppression of tumor proliferation and
62 chemopreventive activities.¹⁶ Recently, bioassays testing oligomers and analogs of *E*-
63 resveratrol highlighted their broad pharmacological potential.¹⁷⁻²²

64 Given the pharmacological importance of stilbenoids, a growing demand is expected for
65 diverse applications in health, nutraceutical, food and cosmetic sectors. Currently, *E*-
66 resveratrol is primarily extracted from field-grown Japanese knotweed (*Fallopia japonica*)
67 and several alternative production strategies have been proposed including
68 biotechnological²³ and synthetic chemistry strategies.²⁴ Whereas the synthesis of *E*-
69 resveratrol and its derivatives remained challenging,²⁵ the bioproduction by yeast, bacterial
70 and plant cell cultures requires scalable methods.^{23,26,27} Additionally, by-products of the wine
71 industry have been recently proposed as a valuable source of stilbenoids.²⁸

72 Grape canes represent a large source of waste derived from the viticulture industry with an
73 estimated volume of 1-5 tons/hectare/year depending on plantation density, climate and
74 vigor of the grape variety. Currently, no or limited valorization of grape canes exists since
75 they are usually burnt in the field or composted.²⁹ Recently, pruned wood from vineyard has
76 been shown to accumulate a broad spectrum of stilbenoids ranging from *E*-resveratrol to
77 complex stilbene oligomers.^{11,28,30} Therefore, grape canes represent a promising bio-
78 resource containing valuable bioactive compounds with potential applications in medicine
79 due to multiple pharmacological properties of stilbenoids³¹ and in agriculture as alternative
80 source of anti-phytopathogenic substances.¹²

81 Despite recent advances in the evaluation of stilbenoid composition of grape canes,³¹⁻³³
82 varying results have been reported. Several factors can explain this variability. First, the
83 variety and the provenance of the grape can greatly influence stilbenoid composition.^{11,30,34}
84 To note, *E*-resveratrol ranged from 0.022 mg kg⁻¹ DW in Turkish cultivars³⁵ to 6533 mg kg⁻¹
85 DW in canes from Chile.³⁰ Secondly, the extraction method is critical to ensure a good
86 recovery of stilbenoids^{32,36} and the range of extraction methods tends to bias the results.

87 Furthermore, an accurate quantification of stilbenoids relies on calibration curves with pure
88 stilbenoid standards since the quantification of stilbenoids as resveratrol equivalent leads to
89 an underestimation of the concentrations of resveratrol oligomers.¹¹ Additionally, a disparity
90 in stilbenoid composition of “Pinot Noir” grape canes has been observed during storage i.e.
91 the time elapsed between vine pruning and the phytochemical analyses.³⁷ An increase in
92 stilbenoid concentration (up to fivefold) after six months at 20°C was observed. Canes were
93 enriched in *E*-resveratrol whereas the level of *E*- ϵ -viniferin did not vary. Although an increase
94 in stilbenoid concentrations has been found in “Pinot Noir” canes in response to storage, the
95 determining factors controlling this accumulation remain unknown and the effect of storage
96 on other grape varieties has not been determined systematically.

97 The aim of this study was to better characterize stilbenoid accumulation in pruned grape
98 canes and particularly to i) determine the optimal storage period for 8 grape varieties
99 representative of the Loire valley, ii) evaluate the influence of temperature storage on
100 stilbenoid accumulation and iii) assess the potential biosynthetic activity in pruned grape
101 wood.

102 MATERIALS AND METHODS

103 **Plant material.** Grape canes of *Vitis vinifera* varieties, “Cabernet Franc”, “Chardonnay”,
104 “Chenin”, “Malbec” (Côt), “Gamay”, “Grolleau”, “Pinot Noir” and “Sauvignon Blanc” were
105 collected in vineyards at the wine school of Amboise (France) in December 2012. The canes
106 were cut into 10 cm long sections and stored in a controlled chamber at 20°C for 10 weeks in
107 the dark. In the same way, grape canes of *Vitis vinifera* var. “Malbec” were stored in
108 additional controlled chambers at -20°C, 5°C, 15°C, 20°C and 28°C for 10 weeks in dark. Heat
109 shock treatment with intent to denature enzymes consisted of a treatment at 65°C for 2

110 hours immediately after cane harvest and followed by storage at 20°C. After storage,
111 samples were ground for 2 min with a cooled analytical grinder (Ika-Werke, Staufen,
112 Germany), frozen in liquid nitrogen and stored at -80°C for stilbenoid and gene expression
113 analysis. For time point zero, canes were cut in the vineyards, transported to the laboratory
114 (1 hour) and immediately grinded as described above.

115 **Chemicals.** *E*-Resveratrol, *E*-piceatannol, catechin and epicatechin were purchased from
116 Sigma-Aldrich (St Louis, MI, USA). *E*- ϵ -Viniferin, *Z/E*-vitisin B, ampelopsin A and hopeaphenol
117 were extracted from grape canes.¹⁹ Acetonitrile and ethanol were purchased from Thermo
118 Fisher Scientific (Courtaboeuf, France). Ultrapure water was obtained from a Millipore
119 Milli-Q water purification system.

120 **Stilbenoid analysis.** A second grinding step was performed to obtain a powder with an
121 average particle size of 1 mm using a cutting mill (Polymix PX-MFC 90 D, Kinematica AG,
122 Switzerland). Stilbenoid extraction was adapted from Karacabey and Mazza.³³ Fifty mg of
123 lyophilized powder were extracted in 1 mL ethanol/water solution (60:40; v/v) and shaken
124 for 30 min at 1400 rpm at 83°C and centrifuged at 18 000 $\times g$ for 5 min. HPLC apparatus
125 consisted in a Waters system equipped with a gradient pump (Waters 600 controller,
126 Milford, MA), a cooled autosampler (Waters 717 plus) and a UV-visible photodiode-array
127 detector (Waters 996) set to acquire data from 200 to 400 nm. Empower 2 software from
128 Waters was used for instrument control, data acquisition and data processing. Analyses
129 were performed on a column packed with 3 μm particles (250 \times 4 mm, Multospher 120
130 RP18HP; CS-Service, Langerwehe, Germany) at 24°C. The mobile phase consisted in aqueous
131 phosphoric acid (0.1% w/v; eluent A) and acetonitrile (eluent B) pumped at 0.5 mL min⁻¹
132 into the HPLC system. The gradient started at 5% B and increased linearly to 72.5% in 60

133 min, followed by washing and reconditioning the column. Compounds in extracts were
134 identified according to their UV spectra and retention time by comparison with external
135 standards. Quantification was performed using pure reference standards of *E*-resveratrol, *E*-
136 piceatannol, *E*- ϵ -viniferin, *Z/E*-vitisin B, ampelopsin A and hopeaphenol and 5-point
137 calibration curves (0-100 ppm) using the Maxplot detection mode.

138 **Gene expression analysis.** Total RNA extractions were performed on ground grape canes
139 (approximately 100 mg) using the improved RNA extraction protocol for woody plants,³⁸
140 with RNeasy Plant Mini Kit (Qiagen, UK). RNA quality and concentration were assessed both
141 spectrophotometrically and on agarose gel. Reverse transcription of RNA was carried out
142 using ThermoScript RT (Invitrogen) according to manufacturer's instructions. First-strand
143 cDNA was synthesized using total RNA (200ng) and oligo dT₂₀ primer (2.5 μ M) in presence of
144 RNaseOUT (Invitrogen). Gene expression was analysed by quantitative real-time PCR on
145 retro-transcribed RNA using the following primer pairs: 5'-GAAACCCATCAATTCCGAACC-3'
146 (forward) and 5'-GCACTGAGACAATCCAGAAGAG-3' (reverse) for phenylalanine ammonia
147 lyase (PAL), 5'-AAAGCTCTGTTAGCGGTGTTCTG-3' (forward) and 5'-
148 TCAGGTGGTTCAAGTCATCCC-3' (reverse) for cinnamate 4-hydroxylase (C4H), 5'-
149 CCTGAAATCCCATCTCCAACC-3' (forward) and 5'-GACTTTGCGAGAAATGAGGTG-3' (reverse)
150 for 4-coumaric acid:CoA ligase (4CL), 5'-CAGCAGCCCAAACATTTATTCC-3' (forward) and 5'-
151 GTTCCAATCGCTAATACCAAGTG-3' (reverse) for stilbene synthase (STS) and 5'-
152 CTTCTCTGTATGGGAGCTGCG-3' (forward) and 5'-GCCATAACAACCTGGTACAATCGAC-3'
153 (reverse) for VATP16 reference gene chosen according to Gamm et al.³⁹ Real-time PCR was
154 run on a CFX96 Touch Real- Time PCR System (Bio-Rad) using the SYBR Green I technology.
155 Each PCR mixture of 15 μ L total reaction volume contained equal amount of cDNAs, 0.2 μ M

156 forward and reverse primers and 1x SsoAdvanced SYBR Green Supermix (Bio-Rad). The
157 reaction was initiated by a denaturation step (95°C, 7 min), followed by 40 cycles at 95°C for
158 10 s and 60°C for 30s. Melt curve analysis was performed on the end products of PCR, to
159 determine the specificity of reactions. Absolute quantification of transcript copy number was
160 performed using calibration curves (plasmid dilution series from 10⁸ to 10⁴ copies). The
161 results are expressed as copy number of cDNA normalized using VATP16 reference gene.

162 **Statistical analysis.** Data were analysed with Statistica, version 6.0 (StatSoft Inc., Tulsa, USA).
163 Statistical significance from different treatments was revealed after one-way analysis of
164 variance (ANOVA) followed by Tukey's test.

165

166 **RESULTS AND DISCUSSION**

167 Ten compounds were identified in grape cane extracts from the varieties, “Cabernet Franc”,
168 “Chardonnay”, “Chenin”, “Malbec” (Côt), “Gamay”, “Grolleau”, “Pinot Noir” and “Sauvignon
169 blanc” corresponding to the flavonoids; catechin and epicatechin, and the stilbenoids;
170 ampelopsin A, *E*-piceatannol, *E*-resveratrol, hopeaphenol, isohopeaphenol, *E*- ϵ -viniferin, *E*-
171 miyabenol C and *Z/E*-vitisin B (Figure 1). HPLC chromatograms of “Sauvignon blanc” grape
172 cane extracts before and after 6-week storage period show striking changes in stilbenoid
173 composition, particularly for *E*-resveratrol and *E*-piceatannol (Figure 2). To confirm those
174 observations, a survey of stilbenoid accumulation in pruned wood was expanded to eight
175 grape varieties stored for 8 weeks at 20°C (Figure 3). The initial concentration of *E*-
176 resveratrol contained in branches before any treatment was determined prior storage. For
177 this, the grape canes were ground and frozen at -80°C within 1 hour (maximum) after
178 pruning in the vineyards. The *E*-resveratrol contents measured at t_0 in all varieties were

179 shown to be very low ($101 \pm 77 \text{ mg kg}^{-1} \text{ DW}$) and corresponded to the values previously
180 reported for unstored grape canes.³⁷ By contrast, an accumulation of *E*-resveratrol was
181 observed in the eight tested grape varieties during storage reaching a peak after six weeks
182 (Figure 3A). On average a 57-fold increase in *E*-resveratrol was observed with a range from
183 19-fold for “Pinot Noir” to 102-fold for “Chenin” (Table 1). At the same time, *E*-piceatannol
184 increased in a similar fashion but on a smaller scale (Figure 3B). The accumulation of *E*-
185 resveratrol during storage in these eight varieties confirms previous results obtained for
186 “Pinot Noir”³⁷ with an additional increase in *E*-piceatannol (3-hydroxyresveratrol). Such an
187 induction of monomeric stilbenoids was also observed in other postharvest plant
188 materials.⁴⁰ Indeed, *E*-resveratrol and *E*-piceatannol were shown to accumulate in sugarcane
189 billets within seven days after harvest. Furthermore, it has been shown that red wines and
190 table grapes can be enriched in stilbenoids by postharvest UV-C irradiation of grapes.⁴¹⁻⁴³ As
191 for other stilbenoid-rich products, the present results highlight the importance of the
192 postharvest process to obtain grape canes with enriched contents in *E*-resveratrol and *E*-
193 piceatannol.

194 Nevertheless, the origin of the accumulated stilbenoids was not elucidated. They might be
195 formed from the degradation of oligomeric stilbenoids or by a biosynthetic activity. For this
196 reason, the concentrations of resveratrol oligomers (ampelopsin A, hopeaphenol,
197 isohopeaphenol, *E*- ϵ -viniferin, *E*-miyabenol C and *Z/E*-vitisin B) were individually quantified
198 during the storage period but no significant changes were observed (data not shown). In
199 summary, Figure 3C presents the total concentration of resveratrol oligomers which do not
200 significantly vary during storage in any of the varieties tested. Therefore, the possibility that
201 *E*-resveratrol and *E*-piceatannol originated from the degradation of resveratrol oligomers
202 was ruled out. To test the hypothesis that the accumulation of *E*-resveratrol originated from

203 a *de novo* biosynthesis, the influence of temperature storage on *E*-resveratrol accumulation
204 was tested on the “Cabernet franc” variety over 10 weeks (Figure 4). Firstly, when the canes
205 were stored at either 5, 15 or 20°C, *E*-resveratrol concentration increased to approximately
206 $6384 \pm 456 \text{ mg kg}^{-1} \text{ DW}$, however the accumulation rate of *E*-resveratrol was shown to be
207 delayed at lower temperatures. Secondly, at 28°C *E*-resveratrol accumulated rapidly during
208 the first two weeks up to a peak of $3923 \pm 584 \text{ mg kg}^{-1} \text{ DW}$, however no further
209 accumulation was observed in the weeks after. Finally when a short heat shock treatment (2
210 hours at 65°C) was applied to grape canes, no *E*-resveratrol accumulation was observed.
211 Such a treatment with intent to denature proteins inhibits the accumulation of *E*-resveratrol
212 during the storage period and suggests the involvement of active enzymes in this
213 phenomenon. Similarly, when canes were stored at -20°C, *E*-resveratrol was not induced.
214 The temperature during the storage process of grape canes is therefore a determining factor
215 for optimal *E*-resveratrol accumulation (observed at 20°C). Moreover, whereas excessive
216 temperature (up to 28°C) rapidly inhibits *E*-resveratrol accumulation, a decrease in the
217 storage temperature only slows down the induction rate and the optimal *E*-resveratrol
218 content is still achieved, albeit at a later time period (Figure 4). These data prompt us to
219 assume that the biosynthetic enzyme activities, and particularly those involved in the
220 stilbene pathway, persist during storage and that the optimal period of storage is directly
221 related to the storage temperature.

222 Given these data, we investigated by real-time PCR the transcriptional activity of key-genes
223 involved in the stilbene biosynthesis during the six weeks of *E*-resveratrol accumulation in
224 pruned grape wood of the “Cabernet franc” variety stored at 20°C (Figure 5). The transcript
225 abundance of the structural genes PAL, 4CL, C4H and STS were assayed by quantitative RT-
226 PCR (Figure 5). PAL, 4CL and C4H the three genes of the general phenylpropanoid pathway

227 were constitutively expressed over the six weeks of storage and in addition STS (forming *E*-
228 resveratrol) was induced during the first four weeks of the storage. These results suggest
229 that grape pruned wood is still transcriptionally active during the storage period.

230 *In planta*, *E*-resveratrol is a well-known phytoalexin i.e. an anti-microbial substance that
231 rapidly accumulate at the pathogen infection site. *E*-Resveratrol is induced in response to
232 biotic and abiotic stresses such as elicitors⁴⁴ UV-C light,⁴⁵ ozone,⁴⁶ anoxia⁴⁷ and wounding.⁴⁸
233 Following pathogenic infection, the STS transcripts are induced within 16-48 hours^{3,39} and
234 the peak value for *E*-resveratrol accumulation occurs around 24-72 hours.⁶ By contrast, the
235 induction of stilbene metabolism in postharvest material is poorly documented and available
236 data are restricted to grape berries. Pioneering work showing the induction of stilbene
237 metabolism during drying process of postharvest grape berries was reported by Versari et
238 al.⁴⁹ Recently, complementary studies showed that both STS transcripts and *E*-resveratrol
239 biosynthesis are induced in the epicarp of postharvest grape berries and the induction level
240 depends on the dehydration rate.⁵⁰ Our study highlights a similar induction of stilbene
241 biosynthesis in postharvest grape canes where the drying process during storage might be
242 perceived as a stress signal by living tissues. Stilbene metabolism is thus induced and *E*-
243 resveratrol and *E*-piceatannol accumulate until the tissues are dry. Further investigations will
244 help to evaluate the influence of the hygrometric conditions in relation to temperature on
245 stilbenoid accumulation during grape canes storage.

246 In conclusion, at the pruning stage, grape canes contain high levels of oligomeric stilbenoids
247 previously accumulated during the growing season but almost no monomeric stilbenoids.
248 During the first six weeks of storage, *E*-resveratrol and *E*-piceatannol accumulate at various
249 rates depending on storage temperature. This *E*-resveratrol accumulation resulted from a *de*

250 *novo* biosynthesis with induction of STS expression during storage. Grape canes appear to be
251 an original plant by product containing valuable stilbenoids. Because this pruned wood is still
252 metabolically active during the drying process, the storage conditions have to be controlled
253 to obtain stilbene-enriched material for further medicinal or agronomical applications. To
254 further characterize the variability of stilbenoids in grape canes, the impact of grape diseases
255 during the growing season will be required.

256

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260 **Notes**

261 The authors declare no competing financial interest.

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266

267 **REFERENCES**

- 268 (1) Riviere, C.; Pawlus, A. D.; Merillon, J.-M. Natural stilbenoids: Distribution in the plant
269 kingdom and chemotaxonomic interest in *Vitaceae*. *Nat. Prod. Rep.* **2012**, *29*, 1317-1333.
- 270 (2) Vannozzi, A.; Dry, I. B.; Fasoli, M.; Zenoni, S.; Lucchin, M. Genome-wide analysis of
271 the grapevine stilbene synthase multigenic family: Genomic organization and expression
272 profiles upon biotic and abiotic stresses. *BMC Plant Biol.* **2012**, *12*.
- 273 (3) Chong, J.; Poutaraud, A.; Huguency, P. Metabolism and roles of stilbenes in plants.
274 *Plant Sci.* **2009**, *177*, 143-155.
- 275 (4) Jeandet, P.; Clement, C.; Courot, E.; Cordelier, S. Modulation of Phytoalexin
276 Biosynthesis in Engineered Plants for Disease Resistance. *International Journal of Molecular*
277 *Sciences* **2013**, *14*, 14136-14170.
- 278 (5) Jeandet, P.; Hebrard, C.; Deville, M.-A.; Cordelier, S.; Dorey, S.; Aziz, A.; Crouzet, J.
279 Deciphering the Role of Phytoalexins in Plant-Microorganism Interactions and Human
280 Health. *Molecules* **2014**, *19*, 18033-18056.
- 281 (6) Pezet, R.; Gindro, K.; Viret, O.; Richter, H. Effects of resveratrol, viniferins and
282 pterostilbene on *Plasmopara viticola* zoospore mobility and disease development. *Vitis*
283 **2004**, *43*, 145-148.
- 284 (7) Schnee, S.; Viret, O.; Gindro, K. Role of stilbenes in the resistance of grapevine to
285 powdery mildew. *Physiol. Mol. Plant P.* **2008**, *72*, 128-133.
- 286 (8) Adrian, M.; Jeandet, P. Effects of resveratrol on the ultrastructure of *Botrytis cinerea*
287 conidia and biological significance in plant/pathogen interactions. *Fitoterapia* **2012**, *83*,
288 1345-1350.

- 289 (9) Yang, M.-H.; Kuo, C.-H.; Hsieh, W.-C.; Ku, K.-L. Investigation of microbial elicitation of
290 trans-resveratrol and trans-piceatannol in peanut callus led to the application of chitin as a
291 potential elicitor. *J. Agric. Food Chem.* **2010**, *58*, 9537-9541.
- 292 (10) Ku, K. L.; Chang, P. S.; Cheng, Y. C.; Lien, C. Y. Production of stilbenoids from the callus
293 of *Arachis hypogaea*: a novel source of the anticancer compound piceatannol. *J. Agric. Food*
294 *Chem.* **2005**, *53*, 3877-3881.
- 295 (11) Lambert, C.; Richard, T.; Renouf, E.; Bisson, J.; Waffo-Teguo, P.; Bordenave, L.; Ollat,
296 N.; Merillon, J.-M.; Cluzet, S. Comparative analyses of stilbenoids in canes of major *Vitis*
297 *vinifera* l. cultivars. *J. Agric. Food Chem.* **2013**, *61*, 11392-11399.
- 298 (12) Schnee, S.; Queiroz, E. F.; Voinesco, F.; Marcourt, L.; Dubuis, P.-H.; Wolfender, J.-L.;
299 Gindro, K. *Vitis vinifera* Canes, a New Source of Antifungal Compounds against *Plasmopara*
300 *viticola*, *Erysiphe necator*, and *Botrytis cinerea*. *J. Agric. Food Chem.* **2013**, *61*, 5459-5467.
- 301 (13) Cottart, C.-H.; Nivet-Antoine, V.; Beaudeau, J.-L. Review of recent data on the
302 metabolism, biological effects, and toxicity of resveratrol in humans. *Mol. Nutr. Food Res.*
303 **2014**, *58*, 7-21.
- 304 (14) Smoliga, J. M.; Baur, J. A.; Hausenblas, H. A. Resveratrol and health - A
305 comprehensive review of human clinical trials. *Mol. Nutr. Food Res.* **2011**, *55*, 1129-1141.
- 306 (15) Vang, O.; Ahmad, N.; Baile, C. A.; Baur, J. A.; Brown, K.; Csiszar, A.; Das, D. K.; Delmas,
307 D.; Gottfried, C.; Lin, H.-Y.; Ma, Q.-Y.; Mukhopadhyay, P.; Nalini, N.; Pezzuto, J. M.; Richard,
308 T.; Shukla, Y.; Surh, Y.-J.; Szekeres, T.; Szkudelski, T.; Walle, T.; Wu, J. M. What is new for an
309 old molecule? Systematic review and recommendations on the use of resveratrol. *PLOS ONE*
310 **2011**, *6*, e19881.
- 311 (16) Piotrowska, H.; Kucinska, M.; Murias, M. Biological activity of piceatannol: Leaving
312 the shadow of resveratrol. *Mutat. Res-Rev. Mutat.* **2012**, *750*, 60-82.

- 313 (17) Fu, J.; Jin, J.; Cichewicz, R. H.; Hageman, S. A.; Ellis, T. K.; Xiang, L.; Peng, Q.; Jiang, M.;
314 Arbez, N.; Hotaling, K.; Ross, C. A.; Duan, W. trans(-)- ϵ -Viniferin increases mitochondrial
315 sirtuin 3 (SIRT3), activates AMP-activated protein kinase (AMPK), and protects cells in
316 models of Huntington Disease. *J. Biol. Chem.* **2012**, *287*, 24460-24472.
- 317 (18) Gonzalez-Sarrias, A.; Gromek, S.; Niesen, D.; Seeram, N. P.; Henry, G. E. Resveratrol
318 oligomers isolated from *carex* species inhibit growth of human colon tumorigenic cells
319 mediated by cell cycle arrest. *J. Agric. Food Chem.* **2011**, *59*, 8632-8638.
- 320 (19) Houille, B.; Papon, N.; Boudesocque, L.; Bourdeaud, E.; Besseau, S.; Courdavault, V.;
321 Enguehard-Gueiffier, C.; Delanoue, G.; Guerin, L.; Bouchara, J.-P.; Clastre, M.; Giglioli-
322 Guivarc'h, N.; Guillard, J.; Lanoue, A. Antifungal activity of resveratrol derivatives against
323 *candida* species. *J. Nat. Prod.* **2014**, *77*, 1658-1662.
- 324 (20) Richard, T.; Poupard, P.; Nassra, M.; Papastamoulis, Y.; Iglesias, M.-L.; Krisa, S.;
325 Waffo-Teguo, P.; Merillon, J.-M.; Monti, J.-P. Protective effect of ϵ -viniferin on β -amyloid
326 peptide aggregation investigated by electrospray ionization mass spectrometry. *Bioorg.*
327 *Med. Chem.* **2011**, *19*, 3152-3155.
- 328 (21) Zghonda, N.; Yoshida, S.; Ezaki, S.; Otake, Y.; Murakami, C.; Mliki, A.; Ghorbel, A.;
329 Miyazaki, H. ϵ -Viniferin is more effective than its monomer resveratrol in improving the
330 functions of vascular endothelial cells and the heart. *Biosci. Biotechnol. Biochem.* **2012**, *76*,
331 954-960.
- 332 (22) Zhang, Y.; Yu, B.; Sui, Y.; Gao, X.; Yang, H.; Ma, T. Identification of resveratrol
333 oligomers as inhibitors of cystic fibrosis transmembrane conductance regulator by high-
334 throughput screening of natural products from chinese medicinal plants. *PLOS ONE* **2014**, *9*.
- 335 (23) Donnez, D.; Jeandet, P.; Clement, C.; Courot, E. Bioproduction of resveratrol and
336 stilbene derivatives by plant cells and microorganisms. *Trends Biotechnol.* **2009**, *27*, 706-13.

- 337 (24) Snyder, S. A.; Gollner, A.; Chiriac, M. I. Regioselective reactions for programmable
338 resveratrol oligomer synthesis. *Nature* **2011**, *474*, 461-466.
- 339 (25) Shen, T.; Wang, X.-N.; Lou, H.-X. Natural stilbenes: An overview. *Nat. Prod. Rep.* **2009**,
340 *26*, 916-935.
- 341 (26) Kang, S.-Y.; Lee, J. K.; Choi, O.; Kim, C. Y.; Jang, J.-H.; Hwang, B. Y.; Hong, Y.-S.
342 Biosynthesis of methylated resveratrol analogs through the construction of an artificial
343 biosynthetic pathway in *E. coli*. *BMC Biotechnol.* **2014**, *14*, 67.
- 344 (27) Jeandet, P.; Delaunois, B.; Aziz, A.; Donnez, D.; Vasserot, Y.; Cordelier, S.; Courot, E.
345 Metabolic engineering of yeast and plants for the production of the biologically active
346 hydroxystilbene, resveratrol. *J Biomed. Biotechnol.* **2012**, 579089.
- 347 (28) Rayne, S.; Karacabey, E.; Mazza, G. Grape cane waste as a source of trans-resveratrol
348 and trans-viniferin: High-value phytochemicals with medicinal and anti-phytopathogenic
349 applications. *Ind. Crops Prod.* **2008**, *27*, 335-340.
- 350 (29) Devesa-Rey, R.; Vecino, X.; Varela-Alende, J. L.; Barral, M. T.; Cruz, J. M.; Moldes, A. B.
351 Valorization of winery waste vs. the costs of not recycling. *Waste Manage.* **2011**, *31*, 2327-
352 2335.
- 353 (30) Vergara, C.; von Baer, D.; Mardones, C.; Wilkens, A.; Wernekinck, K.; Damm, A.;
354 Macke, S.; Gorena, T.; Winterhalter, P. Stilbene levels in grape cane of different cultivars in
355 southern Chile: Determination by HPLC-DAD-MS/MS method. *J. Agric. Food Chem.* **2012**, *60*,
356 929-933.
- 357 (31) Pawlus, A. D.; Waffo-Teguo, P.; Saver, J.; Merillon, J.-M. Stilbenoid chemistry from
358 wine and the genus *Vitis*, a review. *J. Int. Sci. Vigne Vin* **2012**, *46*, 57-111.

- 359 (32) Karacabey, E.; Bayindirli, L.; Artik, N.; Mazza, G. Modeling solid-liquid extraction
360 kinetics of trans-resveratrol and trans- ϵ -viniferin from grape cane. *J. Food Process Eng.* **2013**,
361 *36*, 103-112.
- 362 (33) Karacabey, E.; Mazza, G. Optimisation of antioxidant activity of grape cane extracts
363 using response surface methodology. *Food Chem.* **2010**, *119*, 343-348.
- 364 (34) Zhang, A.; Fang, Y.; Li, X.; Meng, J.; Wang, H.; Li, H.; Zhang, Z.; Guo, Z. Occurrence and
365 estimation of trans-resveratrol in one-year-old canes from seven major Chinese grape
366 producing regions. *Molecules* **2011**, *16*, 2846-2861.
- 367 (35) Cetin, E. S.; Altinoz, D.; Tarcan, E.; Baydar, N. G. Chemical composition of grape canes.
368 *Ind. Crops Prod.* **2011**, *34*, 994-998.
- 369 (36) Karacabey, E.; Mazza, G. Optimization of solid-liquid extraction of resveratrol and
370 other phenolic compounds from milled grape canes (*Vitis vinifera*). *J. Agric. Food Chem.*
371 **2008**, *56*, 6318-6325.
- 372 (37) Gorena, T.; Saez, V.; Mardones, C.; Vergara, C.; Winterhalter, P.; von Baer, D.
373 Influence of post-pruning storage on stilbenoid levels in *Vitis vinifera* L. canes. *Food Chem.*
374 **2014**, *155*, 256-263.
- 375 (38) MacKenzie, D. J.; McLean, M. A.; Mukerji, S.; Green, M. Improved RNA extraction
376 from woody plants for the detection of viral pathogens by reverse transcription-polymerase
377 chain reaction. *Plant Dis.* **1997**, *81*, 222-226.
- 378 (39) Gamm, M.; Heloir, M.-C.; Kelloniemi, J.; Poinssot, B.; Wendehenne, D.; Adrian, M.
379 Identification of reference genes suitable for qRT-PCR in grapevine and application for the
380 study of the expression of genes involved in pterostilbene synthesis. *Mol. Genet. Genomics*
381 **2011**, *285*, 273-285.

- 382 (40) Boue, S. M.; Shih, B. Y.; Burow, M. E.; Eggleston, G.; Lingle, S.; Pan, Y.-B.; Daigle, K.;
383 Bhatnagar, D. Postharvest accumulation of resveratrol and piceatannol in sugarcane with
384 enhanced antioxidant activity. *J. Agric. Food Chem.* **2013**, *61*, 8412-8419.
- 385 (41) Cantos, E.; Espin, J. C.; Fernandez, M. J.; Oliva, J.; Tomas-Barberan, F. A. Postharvest
386 UV-C-irradiated grapes as a potential source for producing stilbene-enriched red wines. *J.*
387 *Agric. Food Chem.* **2003**, *51*, 1208-1214.
- 388 (42) Crupi, P.; Pichierri, A.; Basile, T.; Antonacci, D. Postharvest stilbenes and flavonoids
389 enrichment of table grape cv Redglobe (*Vitis vinifera* L.) as affected by interactive UV-C
390 exposure and storage conditions. *Food Chem.* **2013**, *141*, 802-808.
- 391 (43) Gonzalez-Barrio, R.; Beltran, D.; Cantos, E.; Gil, M. I.; Espin, J. C.; Tomas-Barberan, F.
392 A. Comparison of ozone and UV-C treatments on the postharvest stilbenoid monomer,
393 dimer, and trimer induction in var. 'Superior' white table grapes. *J. Agric. Food Chem.* **2006**,
394 *54*, 4222-4228.
- 395 (44) Chang, X.; Heene, E.; Qiao, F.; Nick, P. The phytoalexin resveratrol regulates the
396 initiation of hypersensitive cell death in *Vitis* cell. *PLOS ONE* **2011**, *6*, e26405.
- 397 (45) Adrian, M.; Jeandet, P.; Douillet-Breuil, A. C.; Tesson, L.; Bessis, R. Stilbene content of
398 mature *Vitis vinifera* berries in response to UV-C elicitation. *J. Agric. Food Chem.* **2000**, *48*,
399 6103-6105.
- 400 (46) Grimmig, B.; Gonzalez-Perez, M. N.; Welzl, G.; Penuelas, J.; Schubert, R.; Hain, R.;
401 Heidenreich, B.; Betz, C.; Langebartels, C.; Ernst, D.; Sandermann, H. Ethylene- and ozone-
402 induced regulation of a grapevine resveratrol synthase gene: different responsive promoter
403 regions. *Plant Physiol. Biochem.* **2002**, *40*, 865-870.

404 (47) Jimenez, J. B.; Orea, J. M.; Gonzalez Urena, A.; Escribano, P.; Lopez de la Osa, P.;
405 Guadarrama, A. Short anoxic treatments to enhance trans-resveratrol content in grapes and
406 wine. *Eur. Food Res. Technol.* **2007**, *224*, 373-378.

407 (48) Chung, I. M.; Park, M. R.; Chun, J. C.; Yun, S. J. Resveratrol accumulation and
408 resveratrol synthase gene expression in response to abiotic stresses and hormones in peanut
409 plants. *Plant Sci.* **2003**, *164*, 103-109.

410 (49) Versari, A.; Parpinello, G. P.; Tornielli, G. B.; Ferrarini, R.; Giulivo, C. Stilbene
411 compounds and stilbene synthase expression during ripening, wilting, and UV treatment in
412 grape cv. Corvina. *J. Agric. Food Chem.* **2001**, *49*, 5531-5536.

413 (50) Bonghi, C.; Rizzini, F. M.; Gambuti, A.; Moio, L.; Chkaiban, L.; Tonutti, P. Phenol
414 compound metabolism and gene expression in the skin of wine grape (*Vitis vinifera* L.)
415 berries subjected to partial postharvest dehydration. *Postharvest Biol. Tech.* **2012**, *67*, 102-
416 109.

417

418 **FIGURE LEGENDS**

419

420 **Figure 1.** Selected structures of typical stilbenoids present in grape canes.

421 **Figure 2.** HPLC chromatograms measured in maxplot detection of hydroalcoholic cane
422 extracts of *Vitis vinifera* "Sauvignon blanc". Grape canes were harvest in the vineyard and
423 stored for six weeks at 20°C (A) or were immediately ground for stilbenoid extraction (B). (1)
424 catechin, (2) epicatechin, (3) ampelopsin A, (4) *E*-piceatannol, (5) *E*-resveratrol, (6)
425 hopeaphenol, (7) isohopeaphenol, (8) *E*- ϵ -viniferin, (9) *E*-miyabenol C, (10) *Z/E*-vitisin B.

426 **Figure 3.** Changes in stilbene concentration during storage of grape canes from eight
427 varieties. The grape varieties are presented by lines with “Cabernet Franc”, “Chardonnay”,
428 “Chenin”, “Malbec”, “Gamay”, “Grolleau”, “Pinot Noir” and “Sauvignon blanc”. The stilbene
429 compounds are presented by column with *E*-resveratrol (A), *E*-piceatannol (B) and the sum
430 of oligomers (C; i. e. addition of concentrations of ampelopsin A, hopeaphenol,
431 isohopeaphenol, *E*- ϵ -viniferin, *E*-miyabenol C and *Z/E*-vitisin B).

432 **Figure 4.** Effect of temperature on *E*-resveratrol accumulation during cane storage of *Vitis*
433 *vinifera* “Cabernet franc”. Grape canes were stored for a period of 10 weeks at -20°C, 5°C,
434 15°C, 20°C and 28°C. Heat shock consisted of a treatment at 65°C for a period of 2 hours
435 immediately after harvest and followed by storage at 20°C.

436 **Figure 5.** Transcript abundance of the stilbene biosynthesis pathway genes PAL, 4CL, C4H
437 and STS measured by quantitative RT-PCR during cane storage of *Vitis vinifera* “Cabernet
438 franc”. Grape canes were collected in the vineyard and directly frozen at -80°C (t_0) or stored
439 at 20°C for 2, 4 and 6 weeks. Transcript abundance of each gene was normalized against
440 VATP16 reference gene. Different letters in the same group of bars indicate significant
441 differences ($P < 0.05$).

442

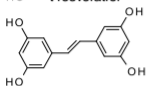
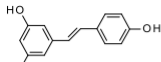
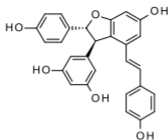
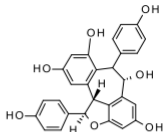
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444 **TABLE**

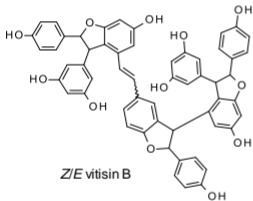
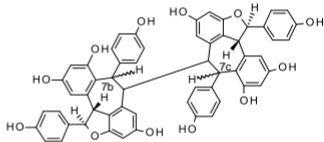
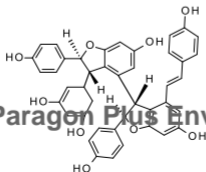
445 **Table 1.** Changes in *E*-resveratrol concentration in grape canes from eight varieties after a 6-
446 week storage period at 20°C.

variety	<i>E</i> -resveratrol concentration (mg kg ⁻¹ DW)		fold induction
	0 weeks	6 weeks	
Sauvignon blanc	60 (31)	2908 (552)	83
Chardonnay	96 (16)	3175 (508)	33
Côt	158 (23)	3316 (762)	102
Grolleau	41 (18)	3913 (704)	21
Chenin	45 (15)	4631 (926)	51
Pinot noir	250 (112)	4725 (897)	95
Cabernet franc	57 (15)	4762 (952)	19
Gamay	100 (33)	5100 (867)	48
Means	101	4066	57

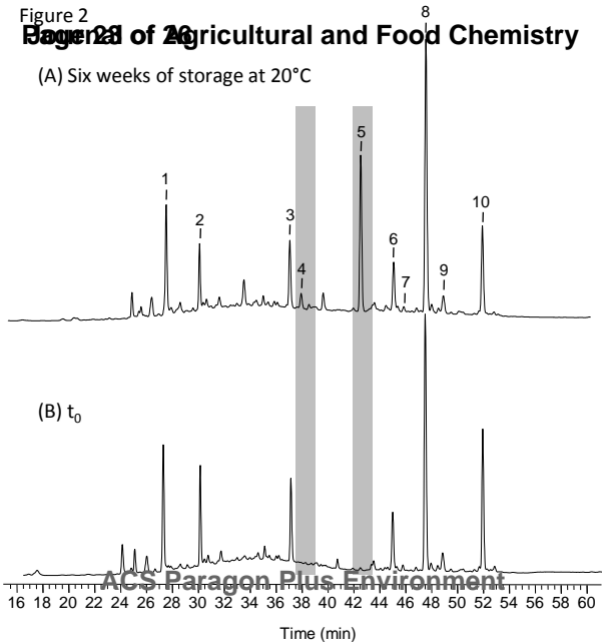
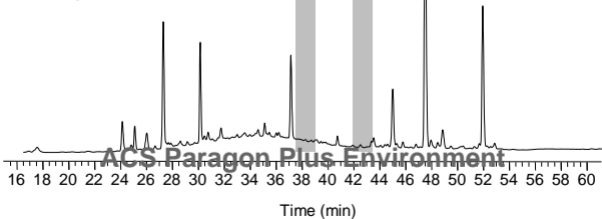
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*E*-piceatannol*E*- ϵ -viniferin

(+) -Ampelopsin A

*Z/E* vitisin BHopeaphenol: 7b; 7c = α
Isohopeaphenol: 7b; 7c = β *E*-Miyabenol C

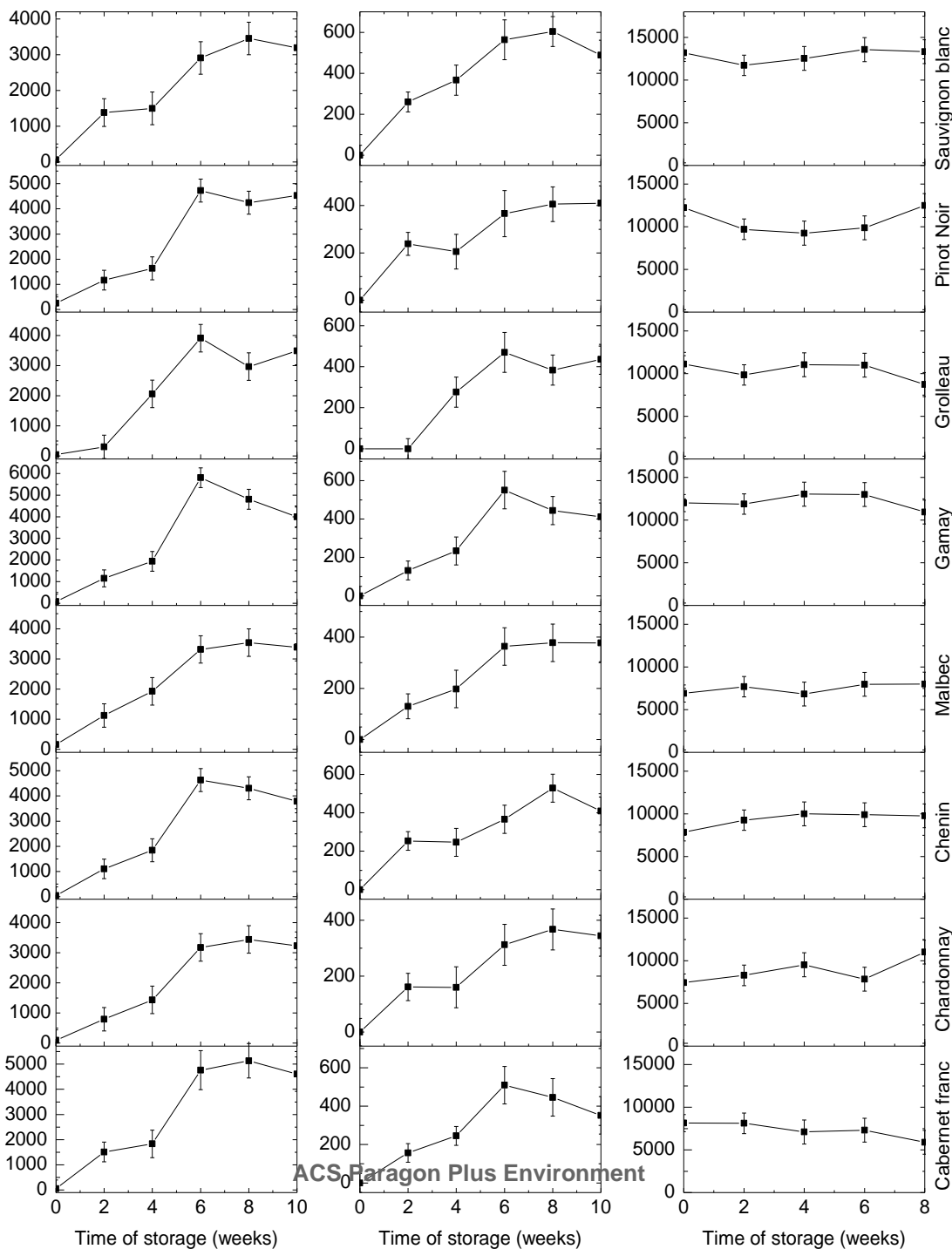
(A) Six weeks of storage at 20°C

(B) t_0 

(A) *E*-resveratrol (mg kg⁻¹ DW)

(B) *E*-piceatannol (mg kg⁻¹ DW)

(C) Sum of oligomers (mg kg⁻¹ DW)



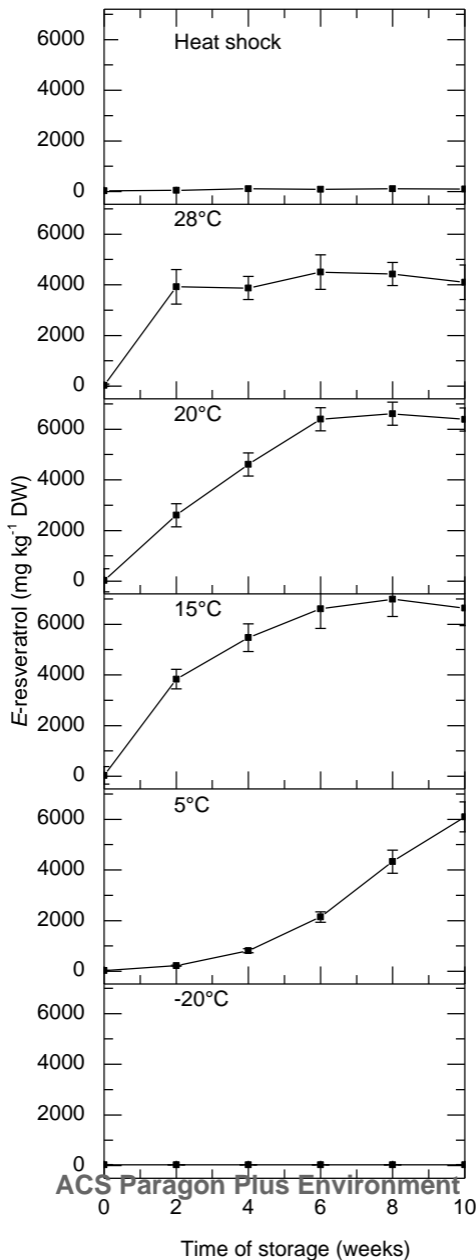


Figure 5

