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1 Sodium arsenite effect on *Vitis vinifera* L. physiology.

2

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21

22

23 **Abstract**

24

25 Sodium arsenite ( $\text{NaAsO}_2$ ) was especially used as a dormant spray to control grapevine trunk  
26 diseases (GTDs) in European vineyards until 2003 when it was banned. It was an efficient  
27 product but it was banned due to high risk for human health and the environment. Now, as  
28 one of the consequences with climatic changes, GTDs threaten the sustainability of vineyards  
29 since no similar and efficacious sprays are presently available to reduce the impact of GTDs.  
30 Research efforts were devoted to identify other active ingredients and biological control  
31 agents but they remained limited in term of efficacy. New solutions might follow from a  
32 better understanding of the modes of action of sodium arsenite which are currently lacking,  
33 specially its impact on grapevine physiology. For this study, grafted plants cv. Tempranillo  
34 were sprayed by sodium arsenite at the end of the winter. During the vegetative period, the  
35 impact on plant physiology was studied by measurement of the photosynthetic activity, the  
36 vine growth and development, and some defense responses. Our results showed that arsenic  
37 was translocated throughout the vine with an increasing gradient from the leaves to the root  
38 system, that photosynthesis was firstly reduced and then stimulated, and that plant tolerance  
39 responses were induced especially antioxidant system. The activation of grapevine defense  
40 responses by sodium arsenite could be a complementary action to fight fungal pathogens in  
41 addition to the fungicide effect.

42

43 Keywords: sodium arsenite, Tempranillo, photosynthesis, plant **physiology**, plant response,  
44 translocation

45

46

## 47 **1. Introduction**

48 In agriculture, arsenic-based compounds were used firstly as insecticides in the 18th century.  
49 Such treatments were applied to control *Leptinotarsa decemlineata* in potatoes (Riley, 1876),  
50 *Aclypea opaca* in sugar beet (Grosjean, 1896) and *Anthonomus grandis* in cotton (Robinson,  
51 1926) and *Anthonomus quadrigibus* in apple tree (Quaintance and Scott, 1912) as examples.  
52 In the 19th century, arsenic compounds were then used as herbicides in either their inorganic  
53 forms, like sodium arsenite, or their organic forms (Schultz and Thompson, 1925; Hood,  
54 1985). In viticulture, they were also employed as insecticides against *Sparganothis pilleriana*  
55 (Marchal, 1919), *Eupoecilia ambiguella* (Capus and Feytaud, 1909) and *Lobesia botrana*  
56 (Capus and Feytaud, 1909). More recently, their use as fungicides was tested at the beginning  
57 of the 20th century, especially to manage grapevine trunk diseases (GTDs) (Ravaz, 1919,  
58 1924; Viala, 1926, Desaché et al., 1995), anthracnose (Vergnes, 1957) and Phomopsis cane  
59 and leaf spot (Hewitt, 1951). Other sporadic applications to limit the attacks by snails was  
60 reported by Vidal (1947).

61 Due to the high risks of sodium arsenite for humans and the environment (Spinosi et al.,  
62 2009), its use in agriculture was banned in Europe, especially in 2003 for viticulture. In  
63 viticulture, this withdrawal was not without consequences since no other active chemical  
64 spray treatment was available to reduce the impact of GTDs. These diseases are due to a  
65 complex of fungal pathogens which **are inhabit wood** (Mondello et al., 2018a). For GTDs,  
66 fungi infect the vine especially by pruning wounds which then colonize the trunk and other  
67 woody organs (Mugnai et al., 1999; Bertsch et al., 2013). The pathogens may live inside the  
68 vine as asymptomatic endophytes and after some period of latency that is typically interrupted  
69 by stress to the vine, the vine might express foliar symptoms and be thus considered as a  
70 symptomatic vine. **GTD foliar symptoms can be characterized by neither a sudden wilting of**  
71 **the whole plant or part of the plant, namely apoplectic symptoms or apoplexy, or**  
72 **discoloration with a typical tiger-stripe pattern or chlorosis in the case of the chronic form**  
73 **(Mugnai et al., 1999; Mondello et al., 2018a).**

74 A recent International Organisation of Vine and Wine (OIV) publication reported that in  
75 European countries such as France, Spain and Italy, the incidence of GTDs varied from 8 to  
76 19% (Fontaine et al., 2016) and 13% of unproductive vineyard represented a loss estimated of  
77 €1 billion in 2014. In USA, especially California, and in Australia, losses were US \$ 260  
78 million and AUS \$8.3 billion per year. Until 2003 and especially in Europe, the sodium  
79 arsenite was the sole product available to inhibit foliar symptoms of GTDs, especially Esca  
80 diseases. The effect was less against *Eutypa lata*, causal agent of eutypa dieback, and on

81 Botryosphaeriaceae spp. close to this reported on esca pathogens (Larignon et al., 2008).  
82 Since its ban, their incidence has increased because no product as efficient as sodium arsenite  
83 was available for application. Researches were conducted to control Esca disease and they  
84 were based especially on pruning wound protection (for review Mondello et al., 2018a), the  
85 main way for the causal agents to infect the grapevine, by trunk renewal (Mondello et al.,  
86 2018b), and by chemicals or biocontrol agents, prophylactic methods or the use of  
87 biostimulant/elicitors (for review Mondello et al., 2018a,b).  
88  
89 The limits to develop a new product with similar effects induced by sodium arsenite to control  
90 Esca disease are the gaps of knowledge on its mode of action. To date, no definitive  
91 mechanism has clearly been identified to explain how sodium arsenite acts in suppressing  
92 foliar symptoms. An explanation could be the effect of arsenic on plant physiology whose  
93 resulting perturbations could induce the appearance of symptoms (Magnin-Robert et al., 2011,  
94 Magnin-Robert et al., 2017, for review Fontaine et al., 2015). On this basis, it seems relevant  
95 to take an interest in the effects of sodium arsenite on the grapevine physiology. Nevertheless,  
96 no scientific publication deals specifically with the incidence of arsenic compounds on  
97 grapevine physiology. After the vineyard treatment with sodium arsenite, Carbonell-  
98 Barrachina et al. (1997a) observed a decrease of sodium arsenite concentration throughout the  
99 vegetative cycle and no accumulation of arsenic in leaves and fruits after three-years of  
100 treatment, due to a possible exudation into the soil from roots. Similarly, Larignon et al. (2008)  
101 conducted sodium arsenite treatment in vineyard and measured a greater concentration of  
102 arsenic in the trunk than in the leaves and the fruits, especially in the wood necrosis areas.  
103 They also reported no change in the contents of proanthocyanidic tannins and total soluble  
104 phenolic compounds in the healthy wood and in the periphery of the white rot. Finally, Santos  
105 et al. (2006) noticed that *in vitro* grapevine plants, treated by sodium arsenite, developed leaf  
106 senescence symptoms like a decrease of chlorophyll content, of leaf fluorescence and of  
107 growth in fresh weight, as well as an increase of lipid peroxidation.  
108 For other crops, few papers reported that this compound can penetrate into woody tissues and  
109 move inside the plant from roots to shoots and berries, and can be excreted in the soil (for  
110 review Li et al., 2016, Latowski et al., 2018). Regarding its impact on plant physiology,  
111 numerous disorders are triggered by arsenic, leading to plant growth and productivity decline,  
112 the alteration of ATP synthesis and photosynthesis, and the impact on membrane integrity and  
113 nutrient acquisition (for reviews, see Abbas et al., 2018, Chandrakar, 2018, Kofroňova et al.,  
114 2018). Due to the interactions of As(III) with sulfhydryl groups (-SH) of enzymes and the

115 ROS generation, plant metabolism is severely altered, notably the carbohydrate, lipid and  
116 protein metabolisms, and the DNA structure (for review Finnegan and Chen, 2012, Abbas et  
117 al., 2018). Detoxification mechanisms were also induced in plants but not studied intensively  
118 (Srivastava et al., 2007; Kumar et al., 2015).

119 There was interest to develop a mimic product to sodium arsenite to optimize the control of  
120 GTDs, especially Esca disease but also both Eutypa and Botryosphaeria diebacks, and other  
121 diseases managed in the past by the use of sodium arsenite. This involved the understanding  
122 of the relationship between the application, its penetration and translocation within the plant,  
123 and the impact on growth and physiology all may help to find a mimic product. The main  
124 goal of this study is thus to intensively study the impact of sodium arsenite on grapevine  
125 growth distinguishing both aerial and root systems, and on physiology focusing on the  
126 carbohydrate metabolism and plant defense response. For this reason, photosynthetic activity  
127 was measured and the expressions of targeted genes especially involved in defense response  
128 and stress tolerance were monitored.

129

## 130 **2. Material and Methods**

### 131 *2.1. Plant material, treatment with sodium arsenite and sampling*

132 In 2013, 1-year-old *Vitis vinifera* cv. Aragonez (= "Tempranillo"), defined as susceptible  
133 cultivar to GTDs, grafted on rootstock 1103P grapevine were potted individually in a 1 liter  
134 pot containing a sandy soil mixture (one-third sand, one-third soil, and one-third organic  
135 matter) and they grew under natural conditions (Figures 1S, 2S). In 2015 (March 5<sup>th</sup>) and in  
136 2016 (March 10<sup>th</sup>), dormant vines were sprayed with 1250 g/hl of sodium arsenite (PyrAlEsca),  
137 i.e. the vineyard concentration normally applied. The solution was sprayed using a manual  
138 sprayer until runoff. The experimentation was conducted twice and according to a completely  
139 randomized design: in 2015, 10 control plants and 10 plants treated with sodium arsenite  
140 according to the vineyard concentration (1250 g/hl); and in 2016, 20 control plants, 20 treated  
141 plants at 1250 g/hl and 20 treated plants at 312.5 g/hl of sodium arsenite, i.e. one quarter of  
142 the prior application rate.

143 Samples were collected at flowering (half of the plants) and at berry maturity (half of the  
144 plants), respectively approximately 3 months (T+90 days in 2015 and T+87 in 2016) and 6  
145 months (T+183 days in 2015 and T+178 days in 2016) after the treatment date (T). The  
146 collected plants were used to measure morphological parameters and quantify total arsenic  
147 concentration. Then, samples of leaves in the intermediate zone, green stems and root for each  
148 treatment were frozen in liquid nitrogen and then stored at -80°C until molecular analysis.

149 Flowering and berry maturity were selected since they are 2 significant phenological stages in  
150 the grapevine reproductive development and because of important sap flows that could  
151 promote the translocation of arsenic within the whole plant.

152

### 153 *2.2. Quantification of total arsenic*

154 Total arsenic was quantified at flowering and at berry maturity, i.e. 3 and 6 months after the  
155 treatment respectively, in the grafted plant and soil. For the plant, the analysis was performed  
156 on leaves, on the trunk below the graft/bud union and on roots. Ten (5 controls and 5 treated)  
157 and 15 (5 controls, 5 As treated, 5 As  $\frac{1}{4}$  treated) samples per treatment were analyzed in 2015  
158 and 2016 respectively. Each plant sample was cut in small pieces of 1 cm<sup>3</sup>. Plant and soil  
159 samples were then lyophilized.

160 All grapevine organs (roots, trunk and leaves) and soil (0.25 g) samples were digested with  
161 3.0 mL of 69% nitric acid (trace analytical grade, Acros organic) and 1.0 mL of ultrapure  
162 water for 35 min at 200°C after 10 min of temperature increase in a microwave digester  
163 (Anton Paar, ECO). After mineralization, the volume is made up to 20 mL with ultrapure  
164 water. Blanks were prepared in the same way, omitting the sample. The obtained digested  
165 solutions are 20 times diluted and the concentrations of total arsenic in the grapevine and soil  
166 samples were determined using inductively coupled plasma-mass spectrometry (ICP-MS).  
167 ICP-MS (Agilent 7500ce) is equipped with a Peltier cooled Scott spray chamber, a pneumatic  
168 nebulizer (1 mL min<sup>-1</sup>, Glass Expansion), and an inert PFA sample introduction system. The  
169 7500ce instrument uses a collision reaction cell with Helium gas technology to remove  
170 polyatomic interferences (mainly ArCl<sup>+</sup>). The optimized operating conditions are summarized  
171 in Table 1S. The monitored isotope was <sup>75</sup>As and <sup>121</sup>Sb at 10ppb was used as internal standard  
172 (prepared from 1000 mg L<sup>-1</sup> As and Sb certified standard solutions (CPI International) diluted  
173 in 1% v/v HNO<sub>3</sub> solution). All the grapevine samples were analyzed in triplicates.

174

### 175 *2.3. Measurement of plant growth*

176 On each plant, the fresh weight and the volume of the root, and the number, the length and the  
177 diameter of the shoots were measured according to the laboratory protocol in order to evaluate  
178 the effect of the treatment on the plant development. The root system volume was estimated  
179 by water displacement. To avoid bias linked to the smaller shoots lastly grown, the mean  
180 diameters and lengths of the shoots corresponds to the means of the first three thicker and  
181 longer shoots. The shoots diameter corresponds to the diameter at the base of the first  
182 internode. The measurements were realized at both kinetic points, flowering and berry

183 maturity, and each year. Five biological replicates per condition and per kinetic point were  
184 used in 2015 and ten in 2016.

185

#### 186 *2.4. Measurement of photosynthetic activity*

187 Net photosynthesis ( $A_n$ ), stomatal conductance ( $g_s$ ), internal leaf  $CO_2$  concentration ( $C_i$ ) and  
188 transpiration ( $E$ ) were determined simultaneously on leaves with a portable infrared gas  
189 analysis system (Li-Cor Model 6400-XT, Lincoln, NE) using a standard leaf. Water use  
190 efficiency (WUE) was determined by the ratio of ( $A_n/g_s$ ) which provides the loss of water  
191 relative to the  $CO_2$  assimilation (Epron and Dreyer, 1993). Measurements were conducted  
192 between 8:00 a.m. and 12:00 a.m. before the “midday depression” (i.e., the minimal  $A_n$  linked  
193 mainly to stomata closure) (Chaumont et al., 1994). The infrared gas analysis system was  
194 equipped with a clamp-on leaf cuvette that exposed  $6\text{ cm}^2$  of leaf area. Environmental  
195 conditions during photosynthetic measurements were the following: photosynthetic active  
196 radiation =  $1,000\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ , relative humidity  $\approx 50\%$ , and chamber temperature of  $24^\circ\text{C}$ .  
197  $CO_2$  was maintained at a constant level ( $380\ \mu\text{mol.L}^{-1}$ ) using a LI-6400-01  $CO_2$  injector with  
198 a high-pressure liquid  $CO_2$  cartridge source. At the same time and only in 2015, the  
199 photosystem II activity ( $\Phi PSII$ ) was evaluated using a FMS2 chlorophyll fluorometer  
200 (Hansatech Instruments, United Kingdom). The photosynthesis measurements of each plant  
201 correspond to the mean of 3 measures made on 3 leaves located at the bottom, middle and top  
202 of the canopy. They were conducted on 5 biological replicates per condition in 2015 and on  
203 10 biological replicates per condition in 2016. Measurements were performed on the same  
204 leaves from flowering (i.e. 3 months after treatment) until the berry maturity (i.e. 6 months  
205 after treatment) and every 2 weeks.

206

#### 207 *2.5. Molecular analysis by qRT-PCR*

208 The analysis was performed on leaves, green stems and roots of control and treated plants  
209 collected at timepoints flowering and berry maturity, as for other analyses. For each plant,  
210 after **grinding** of the different samples, total RNA of leaves, green stems and roots were  
211 extracted from 50 mg, 100 mg and 150 mg of powder respectively. Total RNA was isolated  
212 using a  $\beta$ -mercaptoethanol and sodium acid-based protocol according to Spagnolo et al. (2012)  
213 for green stem and Magnin-Robert et al. (2014) for leaves and roots. In total 150 ng of total  
214 RNA was reverse-transcribed using Verso SYBR 2-step QRT ROX enzyme (ABgene, Surrey,  
215 UK) according to the manufacturer’s protocol. PCR conditions were those described by  
216 Bézier et al. (2002). The expression of selected genes according to the organ, summarized in

217 **Table 1**, was tracked by quantitative RT-PCR. The data were analyzed using CFX Manager  
218 software, and the relative levels of gene expression were determined following the method of  
219 Hellemans et al. (2007) with either EF1 $\alpha$  (leaf, green stem and root), 60SRP (leaf) or UBE2  
220 (green stem and root) used as internal reference genes according to the organ (**Table 1**).  
221 Results correspond to the relative gene expression in organs of treated plants at flowering and  
222 at berry maturity compared to the controls at corresponding timepoints. The value obtained  
223 for one treated plant correspond to the mean of the values of the relative gene expression of  
224 the treated plant compared to each individual control. In 2015, five biological replicates were  
225 used per condition and ten in 2016. The analyzed genes were considered up- or down-  
226 regulated when the value of relative expression was >2-fold or <0.5-fold compared to the  
227 controls, respectively.

228

229

## 230 *2.6. Statistical analysis*

231 Statistical tests were performed on R 3.3.3 software. For the arsenic content analysis, a  
232 Shapiro-Wilk test was done to check the normality of data. As the normality was not assumed  
233 in all cases, a nonparametric test of bivariate Wilcoxon was performed when the mean of  
234 arsenic and control were compared. The meaning of different symbols is ns ( $p > 0.05$ ), \*  
235 ( $p < 0.05$ ), \*\* ( $p < 0.01$ ) and \*\*\* ( $p < 0.001$ ). While the mean of three modalities were compared  
236 (As, As  $\frac{1}{4}$  and **Control**), a Kruskal-Wallis non-parametric test were led and significant  
237 differences between groups were indicated with letter (when letters are different, groups are  
238 significantly different with a p value < 0.05).

239 For the morphological and photosynthetic parameters, the statistical differences between the  
240 value of the treated plants, either with the full concentration of sodium arsenite or one quarter  
241 of it, and the one of the control plants were analyzed. According to the normality of the data  
242 and the equality of the variances, the comparisons of the means two by two were performed  
243 by Student's t-test if both normality and equality of variances were assumed, Aspin-Welch 's  
244 test if there was no equality of variances and Mann-Whitney's test if the normality was not  
245 assumed. Statistical tests were realized using R 3.3.3 software. Significant differences  
246 between each treated condition and the corresponding controls are marked by \*at  $p < 0.05$ , \*\*  
247 at  $p < 0.01$  and \*\*\*  $p < 0.001$ .

248

## 249 **3. Results**

### 250 *3.1. Concentration in total arsenic on grafted-plants*

251 Similar trends are reported in 2015 and 2016 (Figure 1, Table 2) for all the studied organs, i.e.  
252 leaves, trunk and roots, and in the soil. Globally, the concentration of total arsenic was  
253 significantly higher in treated grafted-plant than in controls (Figure 1). We noted that  
254 naturally a small quantity of arsenic was detected in all the plant organs of the controls for  
255 both sampling times, less than 1 mg/kg of DW (less than 3 mg/kg of DW in the soil). From  
256 flowering to berry maturity, we observed a significant increase of total arsenic content  
257 especially in leaves ( $p < 0.001$ ) in 2015 and 2016. The average concentrations of arsenic  
258 increased from the leaves to the root system, with close to 10 mg/kg DW in leaves and 18  
259 mg/kg DW in roots at timepoint 6 months. In the soil, the average concentration of arsenic  
260 was higher than inside the plant and could reach 50 mg/kg DW 6 months after treatment.  
261 Similar trend was noted for the plants treated with  $\frac{1}{4}$  rate of sodium arsenite, and the  
262 concentrations were intermediate between control and full sodium arsenite treated plants.

263

### 264 3.2. Growth of grafted plants

265 Control and treated plants expressed neither GTD foliar symptoms, nor of any other pests or  
266 diseases. In 2015, the treatment with sodium arsenite significantly affected the growth of the  
267 plants by decreasing the number, the length and the diameter of the green shoots especially at  
268 berry maturity (Table 3). Both the fresh weight and the volume of the root system tend to be  
269 lower for treated plants than for controls (Table 3). Similar trends were observed for plants  
270 sampled either at flowering or at berry maturity. In 2016, the treated plants showed also a  
271 global reduction of their growth but these differences were not significant at both flowering  
272 and berry maturity time (Table 2S). No specific alterations were observed on the growth of  
273 plants treated with  $\frac{1}{4}$  rate of sodium arsenite.

274

### 275 3.3. Photosynthesis measurements

276 Gas exchange data for year 2015 are shown in Figure 2 and presented according to the  
277 number of days after the treatment date (T).  $A_n$  was higher in control than treated plants from  
278 flowering, T+67 days ( $7.9 \mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$  in controls and  $5 \mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$  in treated) to  
279 bunch closure, T+119 days ( $7.8 \mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$  in controls and  $3.4 \mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$  in  
280 treated), and then,  $A_n$  was higher in treated plants than in control until berry ripening T+167  
281 days ( $5.6 \mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$  in controls and  $8.7 \mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$  in treated). Similar trend was  
282 observed for both  $g_s$  and  $E$ .  $C_i$  was either similar (at timepoints T+67, T+74, T+104, T+119  
283 days) or higher (at timepoints T+92, T+133, T+167 days) in treated plants compared to  
284 controls. For  $\phi\text{PSII}$ , the activity was lower in treated plants than in controls until bunch

285 closure (T+133 days) and then, similar to the controls to reach 0.6 at timepoint T+167 days.  
286 Compared to the experiment in 2015, similar trends were observed in 2016 but the differences  
287 were less huge the for full treated plants compared to the controls (Table 3S). For the grafted  
288 plants treated by ¼ sodium arsenite concentration, no significant differences during the  
289 measurement series were reported compared to the controls except for A<sub>n</sub>, Ci and WUE at  
290 T+130 days (Table 3S).  $\phi$ PSII was not measured in 2016 since no relevant changes were  
291 noted in 2015.

292

### 293 3.4. Molecular analysis

294 In 2015 and at flowering, most of changes in treated plants consisted of the upregulation of  
295 *SOD* in leaves, *CHV5* and *STS* in green stems and *GST1*, *HSP $\alpha$* , *CHV5* and *STS* in the root  
296 system (Table 4). In the green shoot, *TIP1* encoding for an aquaporin was down-regulated. At  
297 berry maturity, i.e. 6 months after the treatment with sodium arsenite, the pattern of  
298 expression is different with a repression of *HSP $\alpha$*  and an induction of *TIP1* in leaves (Table 4).  
299 In both green stem and roots, *TIP1* was also up-regulated. In the green stems only, *GST1* and  
300 *CHV5* were up-regulated and *HSP $\alpha$*  strongly down-regulated (Table 4). Compared to 2015,  
301 most of the induction of genes reported have been observed again in 2016 (Table 4S). We  
302 could note that for *HSP $\alpha$* , an inverse response in root at flowering and in leaves, green stems  
303 and root system at berry maturity was obtained. For the plants treated by ¼ rate of sodium  
304 arsenite, the pattern of the studied targeted genes is intermediate between the controls and  
305 plants full treated by sodium arsenite at both phenological stages (Table 4S).

306

## 307 4. Discussion

308 Our results confirmed that arsenic is translocated to all grapevine organs and accumulated in  
309 the soil. It is important to acknowledge that the treatment was applied in winter, at the end of  
310 rest period, when the plant was dormant. The spray was applied until runoff and invariably  
311 penetrated the soil. In our experiment, arsenic in the grafted Tempranillo has penetrated the  
312 bark and accumulated in all organs from roots to leaves. The detection in leaves demonstrates  
313 that arsenic moves through the plant via the vascular vessels. Many studies are concerned  
314 with the translocation of arsenic from roots to both shoots and leaves and it appears that the  
315 main form of arsenic found in xylem sap is inorganic arsenite As(III) (see Zhao et al., 2009  
316 for review). Except for leaves, total arsenic concentration *in planta* was similar at both  
317 flowering and berry maturity stage probably because the grafted plants were in pots, a closed

318 system with limited drainage. For comparison, Carbonell-Barrachina et al. (1997a) reported  
319 that low total arsenic quantity was detected in leaves and also in fruits and their quantity  
320 decreased during the vegetative cycle in both Aledo and Italia cultivars. In another crop such  
321 as rice, total arsenic concentrations decrease also markedly in the order of roots > stems and  
322 leaves > fruit (Abedin et al., 2002; Liu et al., 2006; Xu et al., 2008). All the authors suggest  
323 that remobilization of arsenic from stems and leaves to other organs, if any, may be limited.  
324 Indeed, As(III) can form complexes with thiols like phytochelatins, which are sequestered in  
325 vacuoles, decreasing the As translocation (Raab et al., 2005). Moreover, efflux of arsenic to  
326 the soil from the root and vice versa could be involved. The high level of arsenic in the soil  
327 could result from the sodium arsenite treatment but also from the root exudation (Xu et al.,  
328 2007; Zhao et al., 2009 for review). Arsenic uptake pathway is well documented solely in rice  
329 by Ma et al. (2008). They reported that both Lsi1 and Lsi2 transporters were involved in  
330 influx and efflux of arsenic respectively. By the xylem vessels, a low quantity of arsenic is  
331 translocated in above-ground tissues and could be accumulated (Zhao et al., 2009;  
332 Quaghebeur and Rengel, 2004; Kofroňova et al., 2018).

333  
334 The accumulation of arsenic alters the growth of the grafted plants especially the aerial organs.  
335 Compared to the controls, the **grapevines** treated with sodium arsenite showed a reduced  
336 growth in terms of the number, the length and the diameter of the green shoots especially at  
337 berry maturity. Amazingly, the development of the root system was affected by the treatment  
338 but not significantly. The root system inhibition could be correlated to the high concentration  
339 of arsenic detected in both the soil and the root system. The weak alteration of the root system  
340 could nevertheless have an impact on the development of the above-ground organs. **Such**  
341 **reduction of the plant growth was reported in field crops (Jacobs et al., 1970), especially in**  
342 **tomato (Carbonell-Barrachina et al., 1994; Carbonell-Barrachina et al., 1995), in bean**  
343 **(Carbonel-Barrachina et al., 1997b) and in rice (Marin et al., 1992, Zhao et al., 2009, Rahman**  
344 **et al., 2007).**

345  
346 The reduction of the plant growth could be correlated to an alteration of the primary  
347 metabolism, especially the photosynthesis activity, due to the arsenic toxicity as reported by  
348 Kofroňova et al. (2018). In the present study and especially in 2015 experiment,  $A_n$  decreased  
349 significantly in sodium arsenite treated grafted vines during the 4 months after treatment  
350 (T+119 days). This decrease is correlated to a closure of stomata since  $g_s$  is low.  
351 Consequently,  $E$  is also limited. In opposite,  $C_i$  remains either stable or increased suggesting a

352 weak assimilation of the CO<sub>2</sub> by the RuBisCo enzyme. Srivastava et al. (2013) reported also  
353 that arsenic stress affects negatively the photosynthesis activity in *Hydrilla verticillate*, an  
354 aquatic weed, 96h after the treatment. The decrease of photosynthesis could be correlated to a  
355 decrease of photosynthetic pigments, chlorophyll-a and -b, as observed in *Oryza sativa* L.  
356 (Rahman et al., 2007). Six months after the sodium arsenite treatment, we observed inversely  
357 a significant increase of A<sub>n</sub>, g<sub>s</sub> and E with a later increase of Ci. φPSII was firstly reduced in  
358 treated vines until T+119 days suggesting a stress and then it was similar between treated and  
359 control plants as described for the gas exchanges parameters. In the meantime, the quantity of  
360 arsenic increased in leaves from flowering to berry ripening suggesting that the accumulation  
361 of arsenic may stimulate the photosynthesis over time. This induction of the photosynthesis  
362 appeared too late to stimulate the plant growth since at berry maturity the plants treated by  
363 sodium arsenite were always less developed compared to controls. The decrease of  
364 photoassimilates due to a low photosynthesis the first four months and the probable low level  
365 of sugar storage inside the young grafted plants used (3-year-old) could explained the  
366 limitation of the growth. Differences noted at 3 months between sodium arsenite treated and  
367 control plants for growth were maintained at 6 months. In grapevine, an alteration of the  
368 photosynthetic mechanism was already reported after treatments with chemicals such as  
369 fludioxonil and fenhexamid (Petit et al., 2009, 2012) and by copper or sulfur (Palmer et al.,  
370 2003; Xia et al., 2006). The photosynthesis thus appears to be a crucial hub point to regulate  
371 toxicity responses under chemical stress including As. In our study, the impact of As could  
372 also result in the activation of some processes of detoxification and stress tolerance that may  
373 induce its accumulation in vacuole (Zhao et al., 2009, Gusman et al., 2013). To illustrate, the  
374 lettuce plants are able to accumulate sodium arsenite in leaves but also to protect the  
375 photosynthetic apparatus against As, probably through the activation of tolerance mechanisms  
376 (Gusman et al., 2013).

377

378 In complement to the alteration of both growth and photosynthetic activity, some processes of  
379 detoxification could be induced in sodium arsenite treated plants compared to controls, as  
380 suggested by Farooq et al. (2015, 2016) and reviewed by Kofroňova et al. (2018). In both  
381 leaves and green stems, the antioxidant system was altered as suggested by the **up-regulation**  
382 firstly of *SOD* at flowering, and then of *GSTI* at berry maturity. In the meantime, *CHV5*  
383 encoding for a chitinase was up-regulated but only in green stem. For the root system, *GSTI*,  
384 *CHV5* and *STS* were highly induced. All these strong inductions could be correlated to the  
385 higher level of arsenic content in roots and imply a reinforcement of stress tolerance

386 mechanisms and detoxification in presence of arsenic. Moreover, an oxidative stress was  
387 perceived by the vine since *GSTI* were mainly up-regulated and *SOD* up- or down-regulated  
388 according to the organs and the timepoint. For the antioxidant system, the enhanced activities  
389 of isoenzymes of superoxide dismutase, ascorbate peroxidase and glutathione reductase  
390 indicated that arsenic stress generates oxidative stress (Shri et al., 2009; Srivastava et al.,  
391 2013; Kumar et al., 2015 for review). Such plant responses to sodium arsenite were coupled  
392 with an antifungal growth activity correlated to both *STS* (Mazzullo et al., 2000; Coutos-  
393 Thévenot et al., 2001) and *CHV5* (Mauch et al., 1988) inductions. In the roots, the aquaporine  
394 TIP1 was also activated but only at berry maturity. Aquaporins belonging to NIPs (noduling-  
395 like intrinsic proteins) have the ability to transport multiple uncharged solutes including boric  
396 acid, salicylic acid (Wallace et al., 2006) as well as arsenic (Biernert et al., 2008, Ma et al.,  
397 2008). To date, it is supposed that some TIP channels may be permeable to arsenic and  
398 contribute to its transport in vacuole (Zhao et al., 2009). For the expression of *HSP $\alpha$* ,  
399 encoding for a heat shock protein, it was inversely up- and down-regulated in the plants at  
400 flowering and berry maturity, respectively. **At now**, it is known that arsenic may induce  
401 specific proteins expression in response to abiotic stresses such as a heat shock. Saltveit et al.  
402 (2004) observed a similar proteomic pattern between a heat shock and an arsenic stress in  
403 cucumber radicles. All these perturbations under arsenic stress have also been reported for  
404 other heavy metals or fungicides (for review Petit et al., 2012).

405 Comparing 2015 and 2016 experiments, the impact of a sodium arsenite treatment on plant  
406 physiology was globally the same. The alterations of the plant growth, the photosynthesis and  
407 the plant defense response in terms of detoxification process and stress tolerance were  
408 significantly higher in 2015 compared to 2016. Since the experiments were conducted under  
409 natural conditions, the weak differences between 2015 and 2016 could be explained by  
410 climatic conditions, slightly different between both years. The temperatures were thus  
411 globally **lower** in 2016 compared to 2015 while **rainfall was** higher (Figures 1S, 2S).  
412 Independently to the soil flushing that could result to the rainfalls, the evolution of the arsenic  
413 content in the soil from flowering to berry maturity are close in 2015 and 2016.

414

## 415 **5. Conclusion**

416 Until 2003 in Europe, sodium arsenite treatments were applied to control fungal diseases  
417 especially against pathogens associated to trunk diebacks in grapevine. Since the ban of the  
418 use of As, no similar efficient treatment is available to manage GTDs. The present study  
419 concludes that arsenic was **translocated to leaves**, the photosynthesis was firstly reduced and

420 then stimulated, and plant tolerance responses were induced including the antioxidant system.  
421 More in depth experiments focusing on arsenic stress on the plant microbiome may be  
422 required to know its global impact in the tripartite vine-GTDs pathogens-plant microbiome.  
423 The last step will be to identify a similar product for both plant and human health with the  
424 same efficacy as sodium arsenite to control GTDs by acting as a fungicide (Larignon et al.,  
425 2008) and plant elicitor as described herein.

426

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432

### 433 **References**

- 434 Abbas, G., Murtaza, B., Bibi, I., Shahid, M., Niazi, N. K., Khan, M. I., Amjad, M., Hussain,  
435 M., Natasha, 2018. Arsenic Uptake, Toxicity, Detoxification, and Speciation in Plants:  
436 Physiological, Biochemical, and Molecular Aspects. *Int. J. Environ. Res. Public Health*  
437 15, 59-104. <https://doi.org/10.3390/ijerph15010059>.
- 438 Abedin, M.J., Feldmann, J., Meharg, A.A., 2002. Uptake kinetics of arsenic species in rice  
439 plants. *Plant Physiol.* 128, 1120–1128. <https://doi.org/10.1104/pp.010733>.
- 440 Bertsch, C., Ramírez-Suero, M., M., M. R., Larignon, P., Chong, J., Abou-Mansour, E.,  
441 Fontaine, F., 2013. Grapevine trunk diseases: complex and still poorly understood. *Plant*  
442 *Pathology*, 62(2), 243-265. <https://doi.org/10.1111/j.1365-3059.2012.02674>
- 443 Bézier, A., Lambert, B., Baillieul, F., 2002. Study of defence-related gene expression in  
444 grapevine leaves and berries infected with *Botrytis cinerea*. *Eur. J. Plant Pathol.* 108:  
445 111-120. <https://doi.org/10.1023/A:1015061108045>.
- 446 Bienert, G.P., Thorsen, M., Schüssler, M.D., Nilsson, H.R., Wagner, A., Tamás, M.J., Jahn,  
447 T.P., 2008. A subgroup of plant aquaporins facilitate the bi-directional diffusion of  
448 As(OH)<sub>3</sub> and Sb(OH)<sub>3</sub> across membranes. *BMC Biol.* 6, 26.  
449 <https://doi.org/10.1186/1741-7007-6-26>
- 450 Capus, J., Feytaud, J., 1909. L'eudémis et la cochylis en 1909. In: *La Vigne Américaine et la*  
451 *Viticulture en Europe*. 32<sup>ème</sup> année, n°9, septembre 1909, 276-278.

452 Carbonell-Barrachina, A., Burlo Carbonell, F., Mataix Beneyto, J., 1994. Effect of arsenite on  
453 the concentrations of micro-nutrients in tomato plants grown in hydroponic culture. J.  
454 Plant Nut., 17, 1887-1903. <https://doi.org/10.1080/01904169409364853>.

455 Carbonell-Barrachina, A., Burlo-Carbonell, F., Mataix-Beneyto, J., 1995. Arsenic uptake,  
456 distribution, and accumulation in tomato plants: effect of arsenite on plant growth and  
457 yield. J. Plant. Nut., 18, 1237-1250. <https://doi.org/10.1080/01904169509364975>.

458 Carbonell-Barrachina, A., Burlo-Carbonell, F., Mataix-Beneyto, J., 1997a. Effect of sodium  
459 arsenite on arsenic accumulation and distribution in leaves and fruit of *Vitis vinifera*. J.  
460 Plant. Nut., 20, 379-387. <https://doi.org/10.1080/01904169709365258>.

461 Carbonell-Barrachina, A., Burlo-Carbonell, F., Mataix-Beneyto, J., 1997b. Effect of sodium  
462 arsenite and sodium chloride on bean plant nutrition (macronutrients). J. Plant. Nut., 20,  
463 1617-1633. <https://doi.org/10.1080/01904169709365361>.

464 Chandrakar, V., Pandey, N., Keshavkant, S., 2018. Plant responses to arsenic toxicity:  
465 morphology and physiology, in: Hasanuzzaman, M., Nahar, K., Fujita, M. (Eds),  
466 Mechanisms of arsenic toxicity and tolerance in plants. Springer, Singapore, 27-  
467 48. Chaumont, M., Morot-Gaudry, J. F., Foyer, C. H., 1994. Seasonal and diurnal  
468 changes in photosynthesis and carbon partitioning in *Vitis vinifera* leaves in vines with  
469 and without fruit. J. Exp. Bot. 45, 1235-1243. <https://doi.org/10.1093/jxb/45.9.1235>.

470 Coutos-Thévenot, P., Poinssot, B., Bonomelli, A., Yean, H., Breda, C., Buffard, D., Esnault,  
471 R., Hain, R., Boulay, M., 2001. In vitro tolerance to *Botrytis cinerea* of grapevine 41B  
472 rootstock in transgenic plants expressing the stilbene synthase *Vst1* gene under the  
473 control of a pathogen-inducible PR 10 promoter. J. Exp. Bot. 52, 901–910.  
474 <https://doi.org/10.1093/jexbot/52.358.901>.

475 Desaché, F., Courlit, Y., Ménard, E., 1995. Optimiser la lutte chimique contre l'Esca.  
476 Phytoma. 470, 29-31.

477 Epron, D., Dreyer, E., 1993. Long-term effects of drought on photosynthesis of adult oak  
478 trees (*Quercus petraea* (Matt.) Liebl. and *Quercus robur* L.) in a natural stand. New  
479 Phyt. 125, 381–389. <https://doi.org/10.1111/j.1469-8137.1993.tb03890.x>.

480 Farooq, M.A., Islam, F., Ali, B., Najeeb, U., Mao, B., Gill, R.A., Yan, G., Siddique, K.H.M.,  
481 Zhou, W., 2016. Arsenic toxicity in plants: cellular and molecular mechanisms of its  
482 transport and metabolism. Environ. Exp. Bot. 132, 42-52.  
483 <https://doi.org/10.1016/j.envexpbot.2016.08.004>.

484 Farooq, M.A., Li, L., Ali, B., Gill, R.A., Wang, J., Ali, S, Gill, M.B., Zhou, W.J., 2015.  
485 Oxidative injury and antioxidant enzymes regulation in arsenic-exposed seedlings of

486 four *Brassica napus* L. cultivars. Environ. Sci. Pollut. Res. 22, 10699–10712.  
487 <https://doi.org/10.1007/s11356-015-4269-1>.

488 Finnegan, P. M., Chen, W., 2012. Arsenic toxicity: the effects on plant metabolism. Front.  
489 Physiol. 3, 182. <https://doi.org/10.3389/fphys.2012.00182>.

490 Fontaine, F., Pinto, C., Vallet, J., Clément, C., Gomes, A.C., Spagnolo, A., 2015. The effects  
491 of grapevine trunk diseases (GTDs) on vine physiology. Eur J Plant Pathol 144:707-721.  
492 <https://doi.org/10.1007/s10658-015-0770-0>

493 Fontaine, F., Gramaje, D., Armengol, J., Smart, R., Nagy, Z. A., Borgo, M., Rego, C., Corio-  
494 Costet, M. F., 2016. Grapevine Trunk Diseases. A review. ©OIV publications, 1st  
495 Edition (ISBN : 979-10-91799-60-7). Paris.

496 Grosjean, H., 1896. Rapport sur la destruction du silphe opaque par le vert de Scheele  
497 (arsénite de cuivre) en 1896. Bulletin du ministère de l'Agriculture. 346-351.

498 Gusman, G.S., Oliveira, J.A., Farnese, F.S., Cambraia, J., 2013. Arsenate and arsenite: the  
499 toxic effects on photosynthesis and growth of lettuce plants. Acta Physiol. Plant 35,  
500 1201–1209. <https://doi.org/10.1007/s11738-012-1159-8>.

501 Hellemans, J., Mortier, G., De Paepe, A., Speleman, F., Vandensompele, J., 2007. QBase  
502 relative quantification framework and software for management and automated analysis  
503 of real-time quantitative PCR data. Genome Biol. 8, R19. [https://doi.org/10.1186/gb-](https://doi.org/10.1186/gb-2007-8-2-r19)  
504 [2007-8-2-r19](https://doi.org/10.1186/gb-2007-8-2-r19).

505 Hewitt, W.B., 1951. Grape dead-arm control. Plant Dis. Rep. 35, 142-143.

506 Hood, R.D., 1985. Cacodylic acid: agricultural uses, biologic effects, and environmental fate.  
507 Veterans administration central office department of medicine and surgery agent orange  
508 projects office washington, D.C. 171 pages.

509 Jacobs, L.W., Keeny, D.R., Walsh, L.M., 1970. Arsenic residue toxicity to vegetables crops  
510 grown on plain field sand. Agron. J. 62, 588–591.  
511 <https://doi:10.2134/agronj1970.00021962006200050011x>.

512 Kofroňova, M., Masková, P., Lipavská, H., 2018. Two facets of world arsenic problem  
513 solution: crop poisoning restriction and enforcement of phytoremediation. Planta 248:  
514 19-35. <https://doi.org/10.1007/s00425-018-2906-x>

515 Kumar, S., Dubey, R.S., Tripathi, R.D., Chakrabarty, D., Trivedi, P.K., 2015. Omics and  
516 biotechnology of arsenic stress and detoxification in plants: current updates and  
517 prospective. Environ. Int. 74, 221-230. <https://doi.org/10.1016/j.envint.2014.10.019>.

518 Larignon, P., Darne, G., Menard, E., Desache, F., Dubos, B., 2008. Comment agissait  
519 l'arsénite de sodium sur l'Esca de la vigne ? Progr. Agric. Vitic., 125, 642-651.

520 Latowski, D., Kowalczyk, A., Nawiesniał, K., Listwan, S., 2018. Arsenic uptake and  
521 transportation in plants, in: Hasanuzzaman, M., Nahar, K., Fujita, M. (Eds),  
522 Mechanisms of arsenic toxicity and tolerance in plants. Springer, Singapore, 1-26.

523 Li, N., Wang, J., Song, W.-Y., 2016. Arsenic uptake and translocation in plants. *Plant Cell*  
524 *Physiol.* 57, 4-13. <https://doi.org/10.1093/pcp/pcv143>.

525 Liu, W.J., Zhu, Y.G., Hu, Y., Williams, P.N., Gault, A.G., Meharg, A.A., Charnock, J.M.,  
526 Smith, F.A., 2006. Arsenic sequestration in iron plaque, its accumulation and speciation  
527 in mature rice plants (*Oryza sativa* L.). *Environ. Sci. Technol.* 40, 5730–5736.  
528 <https://doi.org/10.1021/es060800v>.

529 Ma, J.F., Yamaji, N., Mitani, N., Xu, X.Y., Su, Y.H., McGrath, S.P., et al., 2008. Transporters  
530 of arsenite in rice and their role in arsenic accumulation in rice grain. *Proc. Natl. Acad.*  
531 *Sci. U.S.A.* 105, 9931-9935. <https://doi.org/10.1073/pnas.0802361105>.

532 Magnin-Robert, M., Letousey, P., Spagnolo, A., Rabenoelina, F., Jacquens, L., Mercier, L.,  
533 Clément, C., Fontaine, F., 2011. Leaf strip of Esca induces alteration of photosynthesis  
534 and defence reactions in pre-symptomatic leaves. *Funct. Plant Biol.* 38, 856-866.

535 Magnin-Robert, M., Spagnolo, A., Dilezitoko, A., Cilindre, C., Mercier, L., Schaeffer-Reiss,  
536 C., Van Dorsselaer, A., Clément, C., Fontaine, F., 2014. Proteomic insights into  
537 changes in grapevine wood in response to Esca proper and apoplexy. *Phytopathol.*  
538 *Mediterr.* 53, 168–187. [http://dx.doi.org/10.14601/Phytopathol\\_Mediterr-12913](http://dx.doi.org/10.14601/Phytopathol_Mediterr-12913).

539 Magnin-Robert, M., Adrian, M., Trouvelot, S., Spagnolo A., Jacquens, L., Letousey, P.,  
540 Rabenoelina, F., Harir, M., Roullier-Gall, C., Clément, C., Schmitt-Kopplin, P., Vallat,  
541 A., Abou-Mansour, E., Fontaine, F., 2017. Alterations in grapevine leaf metabolism  
542 occur prior to Esca apoplexy appearance. *Mol. Plant-Microbe Interact.* 12, 946-959.  
543 <https://doi.org/10.1094/MPMI-02-17-0036-R>.

544 Marchal, P., 1919. La lutte hivernale contre la pyrale de la vigne par l'emploi des arsénicaux.  
545 *Progrès Agricole et Viticole* 72, 296-302.

546 Marin, A.R., Masscheleyn, P.H., Patrick Jr., W.H., 1992. The influence of chemical form and  
547 concentration of arsenic on rice growth and tissue arsenic concentration. *Plant Soil* 139,  
548 175–183. <https://doi.org/10.1007/BF00009308>.

549 Mauch, F., Mauch-Mani, B., Boller, T., 1988. Antifungal hydrolases in pea tissue II.  
550 Inhibition of fungal growth by combinations of chitinase and beta-1, 3-glucanase. *Plant*  
551 *Physiol.* 88, 936-942. <https://doi.org/10.1104/pp.88.3.936>.

552 Mazzullo, A., Di Marco, S., Osti, F., Cesari, A., 2000. Bioassays on the activity of resveratrol,  
553 pterostilbene and phosphorous acid towards fungi associated with Esca of grapevine.

554 Phytopathol. Mediterr. 39, 357-365. [http://dx.doi.org/10.14601/Phytopathol\\_Mediterr-](http://dx.doi.org/10.14601/Phytopathol_Mediterr-1574)  
555 [1574](http://dx.doi.org/10.14601/Phytopathol_Mediterr-1574).

556 Mondello, V., Songy, A., Battiston, E., Pinto, C., Coppin, C., Trotel-Aziz, P., Clément, C.,  
557 Mugnai, L., Fontaine, F., 2018a. Grapevine trunk diseases: a review of fifteen years of  
558 trials for their control with chemicals and biocontrol agents. Plant Disease. 102, 1189-  
559 1217. <https://doi.org/10.1094/PDIS-08-17-1181-FE>.

560 Mondello, M., Larignon, P., Armengol, J., Kortekamp, A., Vaczy, K., Prezman, F., Serrano,  
561 E., Rego, C., Mugnai, L., Fontaine, F., 2018b. Management of Grapevine Trunk  
562 Diseases: knowledge transfer, current strategies and innovative attempts adopted in  
563 Europe. Phytopathol. Mediterr. 57(3), 369-383.  
564 [http://dx.doi.org/10.14601/Phytopathol\\_Mediterr-23942](http://dx.doi.org/10.14601/Phytopathol_Mediterr-23942)

565 Mugnai, L., Graniti, A., Surico, G., 1999. Esca (black measles) and brown wood-streaking:  
566 two old and elusive diseases of grapevines. Plant Dis. 83, 404-417.  
567 <https://doi.org/10.1094/PDIS.1999.83.5.404>

568 Palmer, J.W., Davies, S.B., Shaw, P.W., Wünsche, J.N., 2003. Growth and fruit quality of  
569 'Braeburn' apple (*Malus domestica*) trees as influenced by fungicide programmes  
570 suitable for organic production. New Zeal J Crop Hort Sci 31, 169-177.  
571 <https://doi.org/10.1080/01140671.2003.9514249>.

572 Petit, A.N., Fontaine, F., Clément, C., Vaillant-Gaveau, N., 2009. Gating in grapevine:  
573 relationship between application of the fungicide fludioxonil and circadian rhythm on  
574 photosynthesis. Environ. Pollut. 157, 130-134.  
575 <https://doi.org/10.1016/j.envpol.2008.07.017>.

576 Petit, A.N., Fontaine, F., Vasta, P., Clément, C., Vaillant-Gaveau, N., 2012. Fungicide  
577 impacts on photosynthesis in crops plants. Photosynthesis Research 111, 315-326.  
578 <https://doi.org/10.1007/s11120-012-9719-8>.

579 Quaghebeur, M., Rengel, Z., 2004. Arsenic uptake, translocation and speciation in *pho1* and  
580 *pho2* mutants of *Arabidopsis thaliana*. Physiol. Plant. 120, 280-286.  
581 <https://doi.org/10.1111/j.0031-9317.2004.0240.x>.

582 Quaintance, A.L., Scott, W.M., 1912. The most important insect and fungous enemies of the  
583 fruit and foliage of the apple. Farmer's Bulletin. N°492. 48 pages.

584 Raab, A., Schat, H., Meharg, A.A., Feldmann, J., 2005. Uptake, translocation and  
585 transformation of arsenate and arsenite in sunflower (*Helianthus annuus*): formation of  
586 arsenic phytochelatin complexes during exposure to high arsenic concentrations. New  
587 Phytol. 168, 551-558. <https://doi.org/10.1111/j.1469-8137.2005.01519.x>.

- 588 Rahman, M.A., Hasegawa, H., Rahman, M.M., Islam, M.N., Miah, M.A.M., Tasmen, A.,  
589 2007. Effect of arsenic on photosynthesis, growth and yield of five widely cultivated  
590 rice (*Oryza sativa* L.) varieties in Bangladesh. *Chemosphere* 67, 1072-1079.  
591 <https://doi.org/10.1016/j.chemosphere.2006.11.061>.
- 592 Ravaz, L., 1919. Encore l'apoplexie de la vigne. *Prog. Agric. Vitic.* 52, 601-603.
- 593 Ravaz, L., 1924. L'apoplexie de la vigne. *Prog. Agric. Vitic.* 81, 109-110.
- 594 Riley, C.V., 1876. *Potato pests*. Ed. New York Orange Judd Company. 108 pages.
- 595 Robinson, J.M., 1926. Dusting cotton with calcium arsenate for boll weevil control.  
596 Agricultural Experiment Station of the Alabama Polytechnic Institute, Auburn. Circular  
597 51, May 1926.
- 598 Saltveit, M.E., Peiser, G., Rab, A., 2004. Effect of acetaldehyde, arsenite, ethanol, and heat  
599 shock on protein synthesis and chilling sensitivity of cucumber radicles. *Physiol. Plant.*  
600 120, 556-562. <https://doi.org/10.1111/j.1399-3054.2004.0280.x>.
- 601 Santos, C., Fragoeiro, S., Oliveira, H., Phillips, A., 2006. Response of *Vitis vinifera* L. plant  
602 inoculated with *Phaeoacremonium angustius* and *Phaeoconiella chlamydospora* to  
603 thiabendazole, resveratrol and sodium arsenite. *Sci. Hortic.* 107, 131–136.  
604 <https://doi.org/10.1016/j.scienta.2005.04.015>.
- 605 Schultz, E.R., Thompson, N.F., 1925. Some effects of sodium arsenite when used to kill the  
606 common barberry. United States Department of Agriculture. In Cooperation with the  
607 University of Wisconsin and the Wisconsin Department of Agriculture. Department  
608 Bulletin N°1316, 19 pages.
- 609 Shri, M., Kumar, S., Chakrabarty, D., Trivedi, P.K., Mallick, S., Misra, P., Shukla, D.,  
610 Srivatsava, S., Tripathi, R.D., Tuli, R., 2009. Effect of arsenic on growth, oxidative  
611 stress, and antioxidant system in rice seedlings. *Ecotoxicol. Environ. Saf.* 72, 1102-  
612 1110. <https://doi.org/10.1016/j.ecoenv.2008.09.022>.
- 613 Spagnolo, A., Magnin-Robert, M., Alayi, T.D., Cilindre, C., Mercier, L., Schaeffer-Reiss, C.,  
614 Van Dorselaer, A., Clément, C., Fontaine, F., 2012. Physiological changes in green  
615 stems of *Vitis vinifera* L. cv. Chardonnay in response to Esca proper and apoplexy  
616 revealed by proteomic and transcriptomic analyses. *J. Proteome Res.* 11, 461-475.  
617 <https://doi.org/10.1021/pr200892g>.
- 618 Spinosi, J., Févotte, J., Vial, G., 2009. *Eléments techniques sur l'exposition professionnelle*  
619 *aux pesticides arsenicaux. Matrice cultures – expositions aux pesticides arsenicaux.*  
620 Institut de veille sanitaire. Saint-Maurice, France, 19 pages.

621 Srivastava, S., Mishra, S., Tripathi, R.D., Dwivedi, S., Trivedi, P.K., Tandon, P.K., 2007.  
622 Phytochelatins and antioxidant systems respond differentially during arsenite and  
623 arsenate stress in *Hydrilla verticillata* (Lf). Royle. Environ Sci Technol. 41, 2930–2936.  
624 <https://doi.org/10.1021/es062167j>.

625 Srivastava, S., Sharma, Y.K., 2013. Arsenic occurrence and accumulation in soil and water of  
626 eastern districts of Uttar Pradesh, India. Environ. Monit. Assess. 185, 4995–5002.  
627 <https://doi.org/10.1007/s10661-012-2920-6>.

628 Shri, M., Kumar, S., Chakrabarty, D., Trivedi, P.K., Mallick, S., Misra, P., Shukla, D., Mishra,  
629 S., Srivastava, Tripathi, R.D., Tuli, R., 2009. Effect of arsenic on growth, oxidative  
630 stress and antioxidant system in rice seedlings. Ecotox. Environ. Saf. 2, 1102-1110.  
631 <https://doi.org/10.1016/j.ecoenv.2008.09.022>.

632 Viala, P., 1926. Esca. Annales des Epiphyties. 180 pages.

633 Vidal, J.L., 1947. Les maladies de la vigne. Guy Le Prat Ed., Paris. 127 pages.

634 Vergnes, A., 1957. Sur les traitements de l’anthracnose maculée. Prog. Agric. Vitic. 147, 304-  
635 309.

636 Wallace, I.S., Choi, W.G., Roberts, D. M., 2006. The structure, function and regulation of the  
637 nodulin 26-like intrinsic protein family of plant aquaglyceroporins. Biochim. Biophys.  
638 Acta Biomembr. 1758, 1165-1175. <https://doi.org/10.1016/j.bbamem.2006.03.024>.

639 Xia, X.J.; Huang, Y.Y.; Wang, L.; Huang, L.F.; Yu, Y.L.; Zhou, Y.H.; Yu, J.Q., 2006.  
640 Pesticides-induced depression of photosynthesis was alleviated by 24-epibrassinolide  
641 pretreatment in *Cucumis sativus* L. Pestic. Biochem. Physiol. 86, 42–48.  
642 <https://doi.org/10.1016/j.pestbp.2006.01.005>.

643 Xu, X.Y., McGrath, S.P., Zhao, F.J., 2007. Rapid reduction of arsenate in the medium  
644 mediated by plant roots. New Phytol. 176, 590-599. <https://doi.org/10.1111/j.1469-8137.2007.02195.x>.

645

646 Xu, X.Y., McGrath, S.P., Meharg, A., Zhao, F.J., 2008. Growing rice aerobically markedly  
647 decreases arsenic accumulation. Environ. Sci. Technol. 42, 5574–5577.  
648 <https://doi.org/10.1021/es800324u>.

649 Zhao, F.J., Ma, J.F., Meharg, A.A., McGrath, S.P., 2009. Arsenic uptake and metabolism in  
650 plants. New Phytol. 181, 777-794. <https://doi.org/10.1111/j.1469-8137.2008.02716.x>.

651

652 **Figure captions**

653

654 Figure 1. Arsenic content in leaves, trunk below the graft/bud union, roots and soil on control  
655 (T, black bars) and treated (A, gray bars) grafted-plants cv Tempranillo in 2016. Bar plain  
656 corresponds to the samples collected at flowering and bar with white points to the samples  
657 collected at berry maturity. Values  $\pm$  SD are represented on the graph. Significant differences  
658 between control and treated plants for the different organs and for each collecting time are  
659 marked by \* at  $p < 0.05$ , \*\* at  $p < 0.01$  and \*\*\* at  $p < 0.001$ .

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661 Figure 2. Changes in A, net photosynthesis ( $A_n$ ); B, stomatal conductance ( $g_s$ ); C, internal leaf  
662  $CO_2$  concentration ( $C_i$ ); D, transpiration (E); E, photosystem II activity ( $\Phi PSII$ ) and F, water  
663 use efficiency (WUE) as  $A_n/g_s$ , measured in leaves of control (black squares) and treated  
664 (grey squares) plant cv Tempranillo by sodium arsenite in 2015. Measurements were  
665 performed at different dates from before the flowering, 67 days after the treatment with  
666 sodium arsenite, to the berry maturity, 167 days after the treatment. The mean  $\pm$  SE was  
667 calculated from 5 biological replicates. Significant differences between control and treated  
668 plants are marked by \* at  $p < 0.05$ , \*\* at  $p < 0.01$  and \*\*\* at  $p < 0.001$ .

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687 Table 1. Primers of genes analyzed by real-time reverse-transcription polymerase chain

688 reaction. \*TC TIGR: tentative consensus sequences

Function	Gene	Primer Sequences	GenBank or TC TIGR Accession Number
Housekeeping genes	<i>UBE2</i> (ubiquitin carrier protein E2)	5'-CATCCTTCAGGACAAGTGGT-3' 5'-GAGCTTTACAGATATACGAAACCG-3'	XM_002275879.1
	<i>EF1-<math>\alpha</math></i> (elongation factor 1- $\alpha$ )	5'-GAACTGGGTGCTTGATAGGC-3' 5'-AACCAAATATCCGGAGTAAAAGA-3'	GU585871
	<i>60SRP</i> (60S ribosomal protein L18)	5'-ATCTACCTCAAGTCCTAGTC-3' 5'-CAATCTTGTCCTCCTTCCT-3'	XM_002270599
	Phenylpropanoid metabolism	<i>STS</i> (stilbene synthase)	5'-AGGAAGCAGCATTGAAGGCTC-3' 5'-TGCACCAGGCATTTCTACACC-3'
Defense protein		<i>CHV5</i> (class V chitinase)	5'-CTACAACTATGGCGCTGCTG-3' 5'-CCAAAACCATAATGCGGTCT-3'
	Detoxification and stress tolerance	<i>PR1</i> (pathogenesis-related protein 1)	5'-GGAGTCCATTAGCACTCCTTTG-3' 5'-CATAATTCTGGGCGTAGGCAG-3'
<i>GST1</i> (glutathion-S-transferase 1)		5'-TGCATGGAGGAGGAGTTCGT-3' 5'-CAAGGCTATATCCCCATTTCTTC-3'	AY156048
		<i>HSP<math>\alpha</math></i> (alpha crystalline heat shock protein)	5'-TCGGTGGAGGATGACTTGCT-3' 5'-CGTGTGCTGTACGAGCTGAAG-3'
Water stress	<i>SOD</i> (superoxide dismutase)	5'-GTGGACCTAATGCAGTGATTGGA-3' 5'-TGCCAGTGGTAAGGCTAAGTTC-3'	AF056622
	<i>TIP1</i> (tonoplast intrinsic aquaporin)	5'-ATCACCAACCTCATTATATGC-3' 5'-GTTGTTGTCTCAACCCATTTCC-3'	AF271661

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693 **Table 2.** Arsenic contents (mean value  $\pm$  SD) in mg/kg DW measured in 2015 and 2016 in  
 694 leaves, trunk below the graft/bud union, roots and soil of the grafted grapevine cv.  
 695 Tempranillo untreated (C) and treated (full, As and one quarter of the prior application rate,  
 696 As 1/4). Significant differences between the three modalities (As, As 1/4 and C) for each area  
 697 are indicated by letters (if letters are different, the difference between groups are significant).  
 698 Nd: not determined.  
 699

		Leaves			Trunk			Roots			Soil		
		C	As 1/4	As	C	As 1/4	As	C	As 1/4	As	C	As 1/4	As
2016	Flowering (mean)	0.01 a	0.91 b	2.35 c	0.48 a	1.72 b	3.94 b	0.54 a	5.15 b	10.44 c	1.22 a	7.17 b	15.62 c
	Flowering SD	0.00	0.40	0.68	0.20	0.64	1.81	0.18	3.35	4.27	0.23	3.48	5.97
	Berry maturity (mean)	0.19 a	5.26 b	9.00 c	0.43 a	3.25 b	7.46 c	0.60 a	5.02 b	14.35 c	1.48 a	19.64 b	33.75 b
	Berry maturity SD	0.12	1.20	1.76	0.17	1.91	3.31	0.23	1.45	9.29	0.64	12.85	31.01
2015	Flowering (mean)	0.12 a	nd	3.42 b	nd	nd	nd	0.48 a	nd	9.80 b	nd	nd	nd
	Flowering SD	0.00	nd	0.64	nd	nd	nd	0.02	nd	1.27	nd	nd	nd

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 701

702 Table 3. Growth parameters of the grafted grapevine cv. Tempranillo untreated (Control) and  
 703 treated by sodium arsenite (Treated) measured at flowering and at berry maturity in 2015.  
 704 Values are means  $\pm$  SE of 5 biological replicates. Significant differences between each treated  
 705 **condition** and the controls are marked by \* at  $p < 0.05$ , \*\* at  $p < 0.01$ , \*\*\* at  $p < 0.001$ .

	Flowering		Berry maturity	
	Control	Treated	Control	Treated
Number of green shoots	3.00 $\pm$ 0.45	2.00 $\pm$ 0.32	4.20 $\pm$ 0.37	2.40 $\pm$ 0.40*
Length of green shoots (cm)	35.60 $\pm$ 1.03	12.60 $\pm$ 3.86**	50.30 $\pm$ 4.31	26.00 $\pm$ 5.82*
Diameter of green shoots (cm)	0.81 $\pm$ 0.04	0.60 $\pm$ 0.04**	0.80 $\pm$ 0.05	0.51 $\pm$ 0.03**
Fresh weight of root system (g)	209.08 $\pm$ 43.54	168.67 $\pm$ 36.59	318.73 $\pm$ 50.01	286.86 $\pm$ 30.38
Volume of root system (cm <sup>3</sup> )	216.78 $\pm$ 42.10	163.58 $\pm$ 30.28	366.83 $\pm$ 45.86	278.70 $\pm$ 34.03

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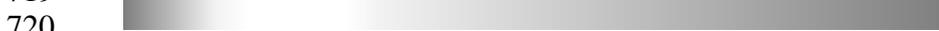
707

708 Table 4. Relative expression levels of the 6 selected genes recorded by RT-qPCR in leaves,  
 709 green stems and roots of grafted grapevines cv. Tempranillo treated with sodium arsenite at  
 710 flowering and at berry maturity in 2015, i.e. 90 and 183 after the treatment respectively.  
 711 Values correspond to the means  $\pm$  SE of 5 biological replicates. Gene expression of untreated  
 712 plant (control) was used as reference to calculate relative expression. A given gene was  
 713 considered up- or down-regulated when the value of relative expression was  $>2$ -fold or  $<0.5$ -  
 714 fold compared to the controls, respectively. Genes down- or over-expressed appear in shades  
 715 of grey, with expression levels lower or higher than 0.1 and 30, respectively, in dark grey. In  
 716 white, there is no change in gene expression compared to the controls. nd: not determined; ne:  
 717 not expressed.

	Flowering			Berry maturity		
	Leaves	Green stems	Roots	Leaves	Green stems	Roots
<i>GST1</i>	ne	ne	64.95 $\pm$ 19.81	21.03 $\pm$ 2.86	6.25 $\pm$ 2.72	6.90 $\pm$ 2.90
<i>SOD</i>	26.86 $\pm$ 2.04	2.7 $\pm$ 0.19	1.03 $\pm$ 0.06	0.63 $\pm$ 0.13	0.97 $\pm$ 0.05	1.09 $\pm$ 0.21
<i>HSP<math>\alpha</math></i>	2.63 $\pm$ 0.40	0.73 $\pm$ 0.10	3.28 $\pm$ 0.73	0.29 $\pm$ 0.00	0.08 $\pm$ 0.02	0.42 $\pm$ 0.12
<i>CHV5</i>	0.56 $\pm$ 0.16	4.18 $\pm$ 2.23	9.79 $\pm$ 3.95	0.60 $\pm$ 0.14	16.96 $\pm$ 7.23	6.32 $\pm$ 2.25
<i>STS</i>	nd	4.59 $\pm$ 1.27	5.47 $\pm$ 1.77	nd	1.3 $\pm$ 0.41	2.04 $\pm$ 0.69
<i>TIP1</i>	0.52 $\pm$ 0.10	0.28 $\pm$ 0.01	0.83 $\pm$ 0.11	2.43 $\pm$ 0.22	2.01 $\pm$ 0.14	7.26 $\pm$ 0.67

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719  $\leq 0.1$     0.5    2     $\geq 30$



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724 **Supplementary material**

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726 **Table 1S. ICP-MS operating parameters**

RF Power	1550 W
Carrier gas	0.9 Lmin <sup>-1</sup>
Make-up gas	0.21 L min <sup>-1</sup>
Collision gas	He (5 mL min <sup>-1</sup> )
Spray chamber temperature	2°C
Detector mode	Pulse Counting
Integration time	0.3 s

727

728 Table 2S. Growth parameters of the grafted grapevine cv. Tempranillo untreated (Control)  
 729 and treated by sodium arsenite (full, As and one quarter of the prior application rate, As ¼)  
 730 measured at flowering and at berry maturity in 2016. Values are means ± SE of 10 biological  
 731 replicates. Significant differences between each treated **condition** and the controls are marked  
 732 by \* at p<0.05, \*\* at p<0.01, \*\*\* at p<0.001.

	Flowering			Berry maturity		
	Control	As 1/4	As	Control	As 1/4	As
Number of green shoots	4.40 ± 0.48	2.90 ± 0.41*	2.90 ± 0.23*	3.20 ± 0.29	2.40 ± 0.22*	2.70 ± 0.21
Length of green shoots (cm)	37.31 ± 3.12	39.86 ± 3.25	30.27 ± 4.57	50.03 ± 5.56	47.13 ± 4.48	32.80 ± 5.25*
Diameter of green shoots (cm)	0.47 ± 0.02	0.47 ± 0.02	0.43 ± 0.03	0.55 ± 0.03	0.54 ± 0.02	0.47 ± 0.03
Fresh weight of root system (g)	203.53 ± 24.14	207.30 ± 24.07	199.36 ± 23.58	192.69 ± 22.57	179.87 ± 36.71	224.72 ± 29.08
Volume of root system (cm <sup>3</sup> )	181.63 ± 13.47	185.93 ± 19.82	192.95 ± 19.86	187.89 ± 27.92	168.70 ± 32.31	214.37 ± 27.44

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735 Table 3S. Changes in net photosynthesis ( $A_n$ ), stomatal conductance ( $g_s$ ), internal leaf  $CO_2$   
736 concentration ( $C_i$ ), transpiration (E) and water use efficiency (WUE) as  $A_n/g_s$  measured in  
737 leaves of grafted grapevine cv. Tempranillo untreated (Control) or treated by sodium arsenite  
738 in 2016 (full, As and one quarter of the prior application rate, As  $1/4$ ). The mean  $\pm$  SE was  
739 calculated from 10 biological replicates at different dates from before the flowering, 96 days  
740 after the treatment with sodium arsenite, to the berry maturity, 157 days after the treatment  
741 date. Significant differences between each treated conditions and the controls are marked by \*  
742 at  $p < 0.05$ , \*\* at  $p < 0.01$ , \*\*\* at  $p < 0.001$ .

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Days post treatment	Control	As 1/4	As
<b><math>A_n</math> (<math>\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}</math>)</b>			
96	6.34 $\pm$ 0.30	6.49 $\pm$ 0.24	5.69 $\pm$ 0.15 *
109	5.38 $\pm$ 0.23	5.66 $\pm$ 0.27	5.59 $\pm$ 0.38
130	2.86 $\pm$ 0.36	4.19 $\pm$ 0.55 *	2.99 $\pm$ 0.25
136	4.59 $\pm$ 0.49	4.54 $\pm$ 0.34	4.28 $\pm$ 0.43
157	3.45 $\pm$ 0.43	3.77 $\pm$ 0.43	3.09 $\pm$ 0.54
<b><math>g_s</math> (<math>\text{mol H}_2\text{O m}^{-2}\text{s}^{-1}</math>)</b>			
96	0.13 $\pm$ 0.01	0.14 $\pm$ 0.01	0.09 $\pm$ 0.01 ***
109	0.11 $\pm$ 0.02	0.14 $\pm$ 0.01	0.10 $\pm$ 0.01
130	0.05 $\pm$ 0.00	0.06 $\pm$ 0.01	0.03 $\pm$ 0.00 **
136	0.08 $\pm$ 0.01	0.10 $\pm$ 0.01	0.07 $\pm$ 0.01
157	0.08 $\pm$ 0.01	0.09 $\pm$ 0.01	0.07 $\pm$ 0.01
<b><math>C_i</math> (<math>\mu\text{mol CO}_2 \text{ mol}^{-1}</math>)</b>			
96	292.22 $\pm$ 2.11	291.58 $\pm$ 4.01	246.70 $\pm$ 17.03 *
109	261.18 $\pm$ 29.56	302.28 $\pm$ 3.96	275.06 $\pm$ 7.94
130	280.27 $\pm$ 11.37	246.54 $\pm$ 11.71 *	203.83 $\pm$ 17.31 **
136	279.36 $\pm$ 4.47	290.68 $\pm$ 6.11	264.85 $\pm$ 9.91
157	303.10 $\pm$ 9.93	294.94 $\pm$ 5.83	297.15 $\pm$ 7.28
<b>E (<math>\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}</math>)</b>			
96	1.64 $\pm$ 0.08	1.55 $\pm$ 0.09	1.18 $\pm$ 0.11 **
109	1.40 $\pm$ 0.14	1.65 $\pm$ 0.11	1.30 $\pm$ 0.08
130	0.97 $\pm$ 0.08	1.22 $\pm$ 0.13	0.73 $\pm$ 0.06 *
136	1.21 $\pm$ 0.11	1.50 $\pm$ 0.10 *	1.16 $\pm$ 0.12
157	1.32 $\pm$ 0.16	1.65 $\pm$ 0.14	1.23 $\pm$ 0.22
<b>WUE (<math>\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}</math>)</b>			
96	48.96 $\pm$ 1.28	48.83 $\pm$ 2.12	77.46 $\pm$ 10.83 **
109	52.29 $\pm$ 3.65	43.83 $\pm$ 2.49 *	60.56 $\pm$ 4.96
130	55.71 $\pm$ 7.16	76.45 $\pm$ 7.39 *	103.22 $\pm$ 10.97 **
136	57.37 $\pm$ 2.80	50.08 $\pm$ 3.87	66.12 $\pm$ 6.26
157	41.97 $\pm$ 6.17	46.04 $\pm$ 3.66	44.83 $\pm$ 4.81

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748 Table 4S. Relative expression levels of the 6 selected genes recorded by RT-qPCR in leaves,  
 749 green stems and roots of grafted grapevines cv. Tempranillo treated with sodium arsenite (full,  
 750 As and one quarter of the prior application rate, As ¼) at flowering and at berry maturity in  
 751 2016, i.e. 64 and 188 after the treatment respectively. Values correspond to the means ± SE of  
 752 10 biological replicates. Gene expression of untreated plant (control) was used as reference to  
 753 calculate relative gene expression for the treated plant (As and As¼). A given gene was  
 754 considered up- or down-regulated when the value of relative expression was >2-fold or <0.5-  
 755 fold compared to the control, respectively. Genes down- or over-expressed appear in shades  
 756 of grey, with expression levels lower or higher than 0.1 and 30, respectively, in dark grey. In  
 757 white, there is no change in gene expression compared to the control. ND: not determined.

	Flowering						Berry maturity					
	Leaves		Green stems		Roots		Leaves		Green stems		Roots	
	As	As¼	As	As¼	As	As¼	As	As¼	As	As¼	As	As¼
<i>GST1</i>	1.53 ± 0.12	1.09 ± 0.37	3.38 ± 0.59	1.00 ± 0.17	8.60 ± 3.40	6.18 ± 2.04	6.22 ± 1.67	2.56 ± 0.45	0.35 ± 0.08	1.87 ± 0.55	0.81 ± 0.44	1.69 ± 0.33
<i>SOD</i>	0.90 ± 0.12	2.36 ± 0.21	1.39 ± 0.07	1.22 ± 0.08	0.77 ± 0.10	1.06 ± 0.22	1.11 ± 0.15	0.67 ± 0.08	1.05 ± 0.10	0.83 ± 0.04	0.57 ± 0.18	0.50 ± 0.02
<i>HSPα</i>	9.51 ± 3.19	2.91 ± 0.58	1.41 ± 0.10	1.73 ± 0.26	0.26 ± 0.10	0.45 ± 0.06	72.23 ± 8.46	1.16 ± 0.21	11.22 ± 4.39	1.45 ± 0.35	17.75 ± 5.15	40.73 ± 0.41
<i>CHV5</i>	0.73 ± 0.06	0.85 ± 0.05	1.11 ± 0.26	0.64 ± 0.09	3.53 ± 0.51	1.80 ± 0.41	2.17 ± 0.53	3.37 ± 0.47	0.75 ± 0.14	0.86 ± 0.18	2.94 ± 0.46	1.95 ± 0.45
<i>STS</i>	0.92 ± 0.07	0.58 ± 0.13	3.77 ± 0.72	0.55 ± 0.08	2.12 ± 0.62	1.26 ± 0.23	ND	ND	0.59 ± 0.13	0.78 ± 0.17	0.75 ± 0.13	2.62 ± 0.59
<i>PR1</i>	0.46 ± 0.10	1.30 ± 0.16	1.19 ± 0.32	2.43 ± 0.32	2.31 ± 1.17	2.95 ± 0.73	1.50 ± 0.19	2.68 ± 0.27	0.39 ± 0.12	0.48 ± 0.12	0.07 ± 0.02	0.28 ± 0.07

758 ≤ 0.1    0.5    2    ≥ 30  
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 761

Figure 1.

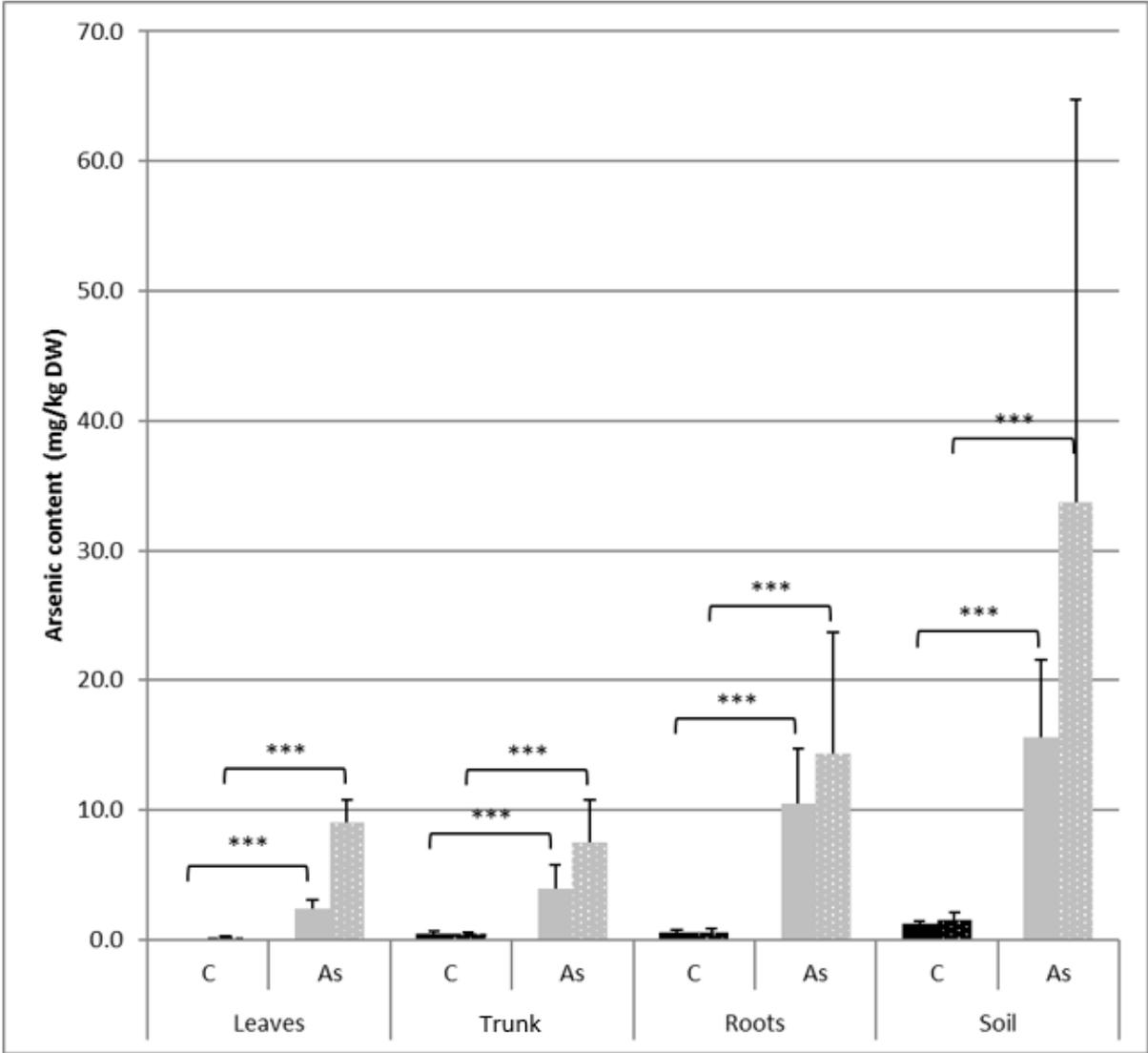
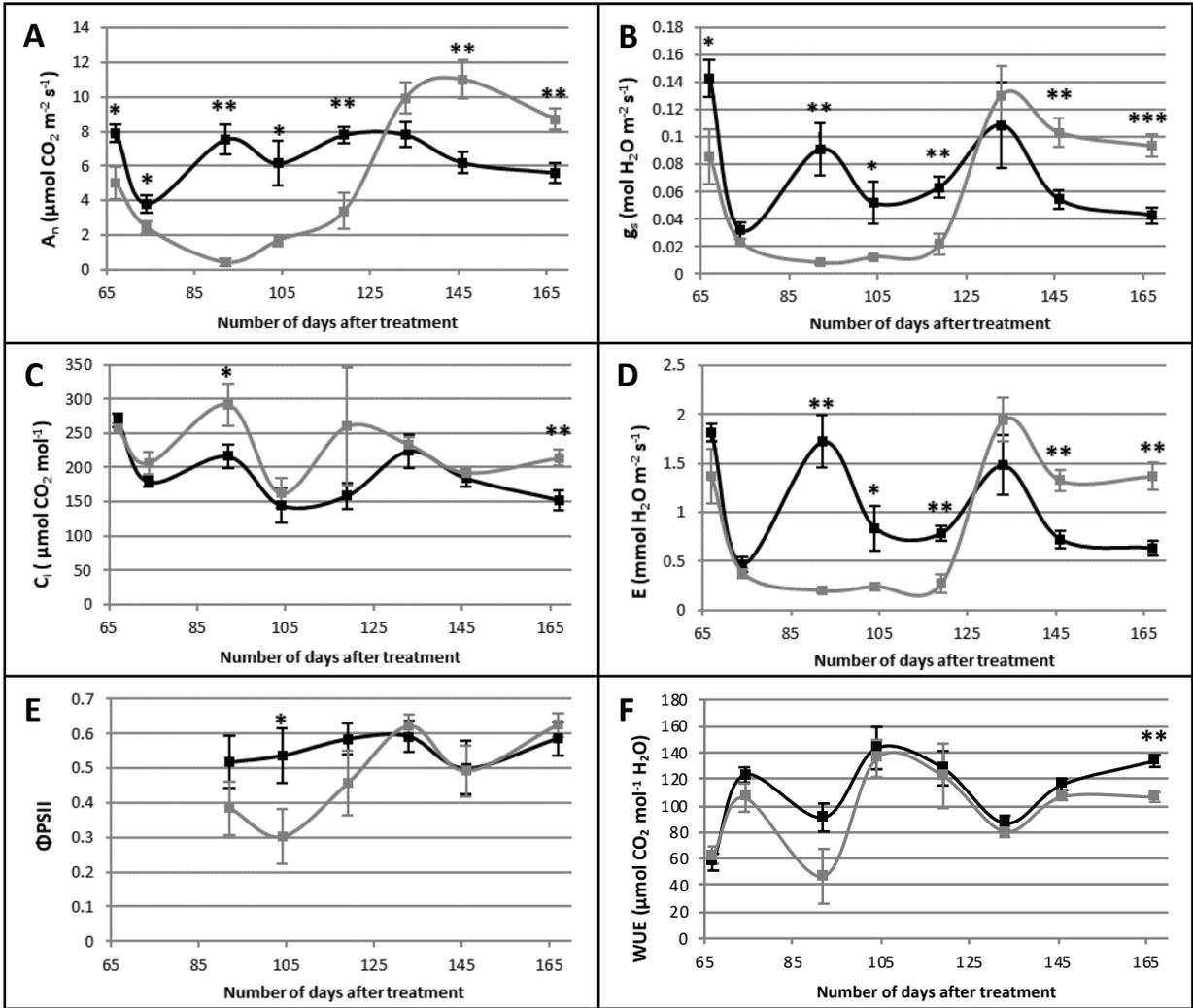


Figure 2.





GTD foliar  
Symptoms inhibition



Sodium arsenite  
treatment in winter



2 sampling periods:  
Flowering  
Berry maturity

Reduction of  
growth

Alteration of  
photosynthesis and  
plant defense  
responses

Quantification As

