Ethanol reduces grapevine water consumption by limiting transpiration

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Abbreviations

EtOH – Ethanol
FTSW – Fraction of Transpirable Soil Water
FTSWt – Fraction of Transpirable Soil Water threshold
HSPs – Heat Shock Proteins
NTR – Normalised Transpiration Rate
sHSPs – small Heat Shock Proteins
WS – Water Stress
WW – Well Watered

Abstract

Studies suggest that ethanol (EtOH), triggers plant adaptation to various stresses at low concentrations (10 µM to 10 mM). This study investigates whether EtOH induces drought

acclimation in grapevine, as demonstrated previously in *Arabidopsis*, rice, and wheat. Preliminary results with bare root Gamay cuttings showed that those pre-treated with 10 µM EtOH aqueous solutions lost fewer leaves when deprived of water compared to controls. Subsequently, we ran a potted-cutting experiment with progressive soil water deficit. Plants pre-treated with EtOH solutions (0.4 and 250 mM) exhibited slower depletion of the fraction of transpirable soil water (FTSW), compared to controls. While 0.4 and 250 mM EtOH tended to decrease transpiration in early days, these EtOH pre-treated plants maintained higher leaf transpiration than controls after 10 days of soil water depletion. The transpiration response to FTSW was affected by EtOH application. EtOH pre-treatments limited plant leaf expansion without increasing leaf senescence, and increased root dry mass. The grapevine responses to EtOH priming followed typical hormesis curves. RNA-seq data revealed transcripts related to this EtOH priming effect. These results suggest that EtOH improves grapevine acclimation to drought, leading to potential water-savings in wine growing regions prone to high water shortages, linked to climate change.

Introduction

Many recent studies show that ethanol at physiological concentrations can have positive roles in plant development (Diot *et al*., 2024a). In particular, the work of Bashir *et al*. (2022) demonstrated that ethanol could induce drought acclimation in several plant species, including Arabidopsis, rice, and wheat. This induced acclimation is particularly interesting in the context of climate change, which is expected to increase water stress in certain geographical areas, such as the Mediterranean region (Cramer *et al*., 2018; IPCC, 2021). Given that viticulture is particularly developed in this region (OIV, 2022), we sought to test the effect of ethanol on grapevine under controlled conditions of water stress, to verify if the observations of Bashir *et al.* (2022) are applicable to this species.

Initially, experiments were conducted on grapevine cuttings with bare roots in water, with the main observation being the percentage of dried leaves one week after rehydration. Then, trials were carried out on potted grapevine cuttings, monitoring the weight of the pots to estimate plant transpiration, as this is a commonly monitored parameter in water stress experiments (Rodrigues *et al*., 2019). This gravimetric method enabled the estimation of the fraction of transpirable soil water (FTSW), a variable widely used as an indicator of the soil

water deficit imposed on the plant (Sinclair, 2005). Thus, the response of plant transpiration to soil drying can be characterised by the FTSW threshold, i.e. the soil water status above which the plant ecophysiological processes are sensitive to soil drying. According to the FTSWt, the eco-physiological processes showed differences in sensitivity to water deficit: leaf expansion exhibits the highest sensitivity, followed by plant transpiration, and leaf senescence (Kang *et al.*, 2024). Our innovative results about ethanol altering grapevine transpiration lead us to propose a companion paper to our RNA-seq data and results obtained regarding grapevine resistance to heat stress (Diot *et al.*, 2024b). We hope to quickly stimulate further research to find the appropriate conditions of EtOH application for the various varieties and geographical situations encountered in vineyards affected by water stress episodes.

Material and Methods

Plant material

Grapevine cuttings were made from one-year-old dormant Gamay shoots, clone 787, from a collection of genetic material whose plant material is tested to be safe for the main vine viruses (IFV Sud-ouest, Peyrole, France). The cuttings were prepared as described by Mullins (1966), with the following modifications. Single-node cuttings, 5-cm long with two cutter scars at the bottom to facilitate rooting, were disinfected with active chlorine (1.5% v/v) for one minute and rinsed three times with running water. Then, 12 cuttings were placed in 250 ml plastic crates filled with 150 ml water and covered with aluminium foil to hold the cuttings up, the lower end being immersed in water, the bud ending up upwards (Fig. 1A). The cuttings were left for one month in a growing chamber at 25°C/20°C 16h/8h (day/night) with light set to 250 $\mu mol.m^{-2}.s^{-1}$. The water level was maintained to the initial filling mark.

Two experiments were conducted, one with bare root cuttings (Exp. 1), one with potted cuttings (Exp. 2).

Ethanol treatments applied to bare-root cuttings

In Exp. 1, the water was removed and 150 ml of freshly prepared ethanol solutions at various concentrations were poured into the crates and left for 24 h to allow ethanol priming. Then, all solutions were removed and the cuttings were left without water for 38h (duration determined by preliminary trials). After this drying period, 150 ml of water were poured into

each crate, and the cuttings were left to recover in the growing chamber. The percentage of dry leaves was assessed one week later.

Ethanol treatments applied to potted cuttings

In Exp. 2, the one-month cuttings (Fig. 1B) were transferred into 105 ml nursery plastic pots, filled with P.A.M.2 Proveen→ substrate (Proveen, Netherlands), and water saturated. Plants were left to settle down and develop roots for one week in a growing chamber 25°C/20°C (day/night) with a 16 h photoperiod providing 250 $\mu mol.m^{-2}.s^{-1}$ photosynthetically active radiation at the top of the plants. The plants were maintained at maximum Fraction of Transpirable Soil Water (FTSW = 1) over the week. The pots were covered with aluminium foil on the top, and an additional 3 mm polystyrene sheet (Depron→, Germany) was added onto the top to limit soil evaporation (Fig. 1D, E, F, G, J). Evaporation did not exceed 10% of the FTSW after 30 days (Supplementary Fig. S1). Then, the day prior to the beginning of the water deficit, 52.5 ml of ethanol solutions at various concentrations were poured onto the pots, after removing temporarily the bottom aluminium foil to allow run-off of the solution into a saucer. The ethanol concentrations tested in this study were 0, 0.4, 2, 10, 50 and 250 mM. These concentrations correspond to ethanol/water ratios of 0, 0.002, 0.012, 0.06, 0.29, and 1.46% (v/v), respectively. The range was chosen with 5-fold increments to minimise errors from soil variability. Pairs of plants one well-watered (WW) and one water-stressed (WS) were established for all EtOH doses. A total of eight blocks were set-up, in each block we added one pot without any plant, to measure the evaporation of the pot.

Soil water status measurements

The day following the ethanol treatment (Exp. 2), the watering of Water Stress “WS” pots was stopped, while the Well Watered “WW” pots were maintained at FTSW = 1. FTSW was calculated from pot weights, as described previously (Lebon *et al*., 2006). The daily FTSW was calculated from pot weight on d day (pot weight d), the pot weight at full soil water capacity (pot weight at fc), and the pot weight when transpiration of the non-irrigated pot was less than 10% of WW controls of the same EtOH treatment (pot weight 10%). Prior to water deficit treatment, pots were irrigated to saturation, and after the excess water had drained, the pots were weighed to estimate the pot mass at full soil water capacity. Each pot was weighed daily, twice for the WW, before and after re-watering. $FTSW_d$ was then determined as follow (eq.1):

$FTSW_d$ = (pot weight d- pot weight 10%) / (pot weight fc - pot weight) (eq. 1)

Grapevine transpiration and leaf area measurements

Water loss for individual plants (T) over 24h (Exp. 2) was calculated from the pot weights difference, considering pot weight after irrigation (d-1 day) and before irrigation (d day) for WW. Then, transpiration rate per leaf area (Tr) was determined by dividing the daily pot weights difference by the total leaf area values measured as close as possible to the transpiration date (eq.2).

Tr (g. $cm^{-2}.d^{-1}$) = [pot weight d – (pot weight d-1)]/ total leaf area (eq. 2)

The leaf area of all plants was estimated using a regression between the longest leaf vein and the leaf area of 230 leaves (Supplementary Fig. S2). The leaf area was determined with the "easy leaf area" application (https://www.quantitative-plant.org/software/easy-leaf-area). For this purpose, leaf main vein lengths were measured twice, once around day 3 and once around day 8. The calculation of plant transpiration of days 2, 3, 4 and 5 was performed using the first set of leaf area estimates, and the transpiration of days 6, 7, 8, 9 and 10 was calculated using the second set of leaf estimates.

Normalised transpiration rate (NTR)

Daily plant transpiration values (T in $g.pot^{-1}.d^{-1}$) were normalised as described by King and Purcell (2017), according to equation 1. Briefly, a correction factor (CF) was calculated to adjust for differences in transpiration among plants within an EtOH treatment under WW conditions. The correction factor was calculated while all plants were WW (before Day 0) by dividing the water loss of individual plants by the average water loss of the WW plants at the same EtOH dose. A second correction was made to account for differences in transpiration that occurred among days after initiation of water deficit treatment. Water loss for individual plants was divided by the average water loss for WW plants of the same EtOH treatment (T_WW average). Normalised transpiration for each plant for a given weighing interval was:

NTR = [T /(T_WW average)]/CF (eq. 3)

Modelling the response of normalised transpiration rate to progressive soil water deficit

The response of NTR at the whole plant level to FTSW was fitted according to equation 2 (Muchow and Sinclair, 1991), in which "a" is the model parameter describing the response

163 of NTR to FTSW, "a" characterise the curvature of the fit. As the "a" parameter increases, the
164 sensitivity of the NTR strengthens at a given same FTSW value. The FTSW threshold (FTSWt)
165 represents the FTSW at which NTR (modelled by equation 4) starts to be influenced by soil
166 water deficit. FTSWt was reached when the NTR maximum was reduced by 5%, as proposed
167 for nonlinear models by Sadras and Milroy (1996). A higher FTSWt indicates a higher sensitivity
168 to water deficit (Andrianasolo *et al.*, 2016).

169 $$NTR = 1/[1 + 9 \times \exp(a \times FTSW)] \qquad \text{(eq. 4)}$$

170

171 **Re-watering of WS plants and experiment ending (Exp.2)**

172 After preliminary tests, to find a parameter to decide the time at which the WS plants
173 will be re-watered, it was decided to choose the wilting of at least two top leaves in the canopy
174 for Exp. 2. We thought initially to choose the time when plants reached FTSW = 0, but some
175 plants showed severe wilting before reaching FTSW = 0. The plants were then re-watered with
176 30 ml the first day, then the amount of water was calculated daily to reach FTSW = 0.95. The
177 plants were harvested one week after re-watering. The WW plants and their corresponding
178 WS plants (being part of the same pair, as defined above) were harvested the same day. The
179 harvest period was spread over a week from day 28 to day 34 after the beginning of Exp. 2.
180 On the harvest day, all leaves and roots were collected and the leaf area was estimated directly
181 with Easy Leaf Area application. The percentage of dried leaves was calculated over the seven
182 oldest leaves, as at day 0 there was a minimum of seven leaves per plant (example of dry
183 leaves in Fig. 1I). Finally, the roots were dried at 60°C for at least 72h, and then weighed.

184

185 **RNA-seq analyses on grapevine cell cultures**

186 RNA-seq analyses have been performed on *Vitis vinifera* cv. Gamay Fréaux cell cultures. The
187 complete material and method is described in Diot *et al*. (2024b). However, the following
188 modifications in the formal analysis have been done: the relative estimated transcript counts
189 have been performed only with the two conditions used in this study (comparison between
190 6h_0mM and 6h_1mM) and for this analysis, the thresholds for Differentially Expressed Genes
191 were set at |Log2 Fold Change| > 0.5, and padj < 0.1. The raw data discussed in this publication
192 have been deposited in NCBI's Gene Expression Omnibus (Edgar *et al*., 2002) and are
193 accessible through GEO Series accession number GSE275842
194 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE275842).

195

196 **Statistical treatments**

197 Data normality and homoscedasticity hypotheses were first assessed using
198 respectively Shapiro-Wilk tests and Levene tests (SWL tests). When SWL tests were successful,
199 ANOVA were used to assess the effects of water treatment, ethanol treatments, their
200 respective interactions on transpiration, green leaf area at harvest, leaf senescence (dried
201 leaves), root dry weight, FTSW threshold, and "a" parameter describing the response of NTR
202 to FTSW; and multi-comparisons tests were assessed with Tukey HSD tests. When SWL tests
203 were not successful, we used rank-based nonparametric Kruskal-Wallis tests followed by
204 pairwise Wilcoxon tests. We also completed these analyses with pairwise tests: Student tests
205 when normality and homoscedasticity were respected, otherwise, we used pairwise Wilcoxon
206 tests. Differences were considered significant when $p < 0.05$. Fits of NTR to FTSW were
207 performed using the non-linear least squares (NLS) regression model. The goodness of fit was
208 assessed by calculating the Rsquare (R^2), root mean square error (RMSE), and mean squared
209 error (MSE). All fitting and statistical analyses were performed with Rstudio version 4.2.1,
210 using the packages 'car' and 'rstatix'.

211

212

213 ## Results

214

215 **Ethanol priming reduced the percentage of dried leaves in bare root cuttings subjected to a**
216 **temporary water depletion**

217 Figure 1 shows the various stages of the culture of Gamay single-node cuttings, from
218 the bare root initial one-month growing stages in water and plastic crates (Fig. 1A) then
219 potting stages (Fig. 1B, C) and the growing chamber in environmentally controlled conditions
220 (Fig. 1J). A preliminary experiment (Exp.1) was conducted with the bare root cuttings, by
221 priming them with EtOH at various concentrations, before removal of the aqueous solutions
222 for 38h, then re-filling the crates with water. After an additional week, we observed the
223 amount of dried leaves (Fig. 2). The priming with EtOH reduced the percentage of dried leaves
224 from 35.5% (controls at 0 mM) to 17.7% (EtOH at 0.01 mM), without being significant. But, as
225 observed with increasing EtOH doses, there was a clear trend of reduction of the dried leaves
226 compared to controls. Instead of running more bare root trials to reach significance levels,

227 with conditions which are not natural, we decided to run a larger trial, with cuttings in light
228 potting soil (Exp. 2), and to follow a larger set of parameters. At least, these conditions can be
229 met in some nurseries.
230
231 **Ethanol maintained higher Fractions of Transpirable Soil Water than controls both for well**
232 **watered and dried pots**
233 The changes in FTSW over a three-week period in potted single-node cuttings (Exp. 2)
234 were plotted for WW pots (Fig. 3A) and WS pots (Fig. 3B). FTSW of WW pots were brought up
235 daily to FTSW of 0.95. The results show the FTSW just before re-watering the pots. Most pots
236 remained above 0.7, regardless of the EtOH concentration used for priming, which was a one-
237 time-24 h-treatment preceding day 0. The FTSW of WS pots gradually decreased after their
238 last irrigation on day 0, and we observed that, in plants primed with 250 mM EtOH, the FTSW
239 decreased more slowly than in other conditions. Interestingly, plants primed with 250 mM
240 also kept a higher FTSW than controls for WW plants (Fig. 3A), even if it was less obvious than
241 in WS plants. We also noticed slight differences around day 5, between WS controls and WS
242 plants primed with 0.4 and 10 mM EtOH, with EtOH-primed cultures maintaining a higher
243 FTSW than controls. Finally, given that there was a negligible decrease in FTSW in pots without
244 plants due to soil evaporation (Supplementary Fig. S1), we assumed that changes in FTSW
245 were primarily due to plant transpiration and tested this hypothesis in a series of subsequent
246 analyses.
247
248 **Ethanol slowed down the grapevine transpiration**
249 Figure 4 shows the changes in transpiration per unit of leaf area from day 2 to day 10,
250 a period with the biggest FTSW differences between the various treatments (Fig. 3). The full
251 statistics regarding the Figure 4 graphs are given in Supplementary Table S1, to avoid
252 overloading the transpiration graphs. The transpiration of WS plants started to be significantly
253 lower than WW plants from day 6 (Fig. 4; Supplementary Table S1). It is noticeable that 250
254 mM EtOH reduced significantly plant transpiration, in comparison to controls, from day 4 to
255 day 5 in WS plants, and from day 3 to day 8 in WW plants. We also observed that, compared
256 to the controls, the transpiration of WS plants treated with EtOH showed opposite trends at
257 the beginning and at the end of the experiment. For example, cuttings treated with 250 mM
258 of EtOH had a lower rate of transpiration than controls at days 4 and 5, but displayed higher
259 transpiration than controls at days 9 and 10. Figure 4 also shows that WS plants which

transpired less than controls at the beginning of the experiment, were transpiring more than controls at the end, this was particularly noticeable in 0.4 and 250 mM treated plants, thus confirming trends of smaller FTSW drop observed in Figure 3. Indeed, when pooling the transpiration results from day 2 to day 6, there was a significant transpiration decrease in the plants treated with 0.4 mM EtOH compared to controls (data not shown). We also noticed that there was no significant difference between WS plant transpiration at days 8 to 10, when testing with multiple comparisons obtained after ANOVA (Supplementary Table S1). But when testing the results with pairwise tests, Student or Wilcoxon (Supplementary Table S2), significant differences were noticed at days 8 to 10, as pre-treating with 250 mM led to higher transpiration than in plants pre-treated with 0, 2 and 50 mM EtOH.

Finally, we observed a bell-shaped distribution in the transpiration rates according to increasing EtOH doses (not taking the controls into account), particularly noticeable in WW and WS at day 10 (Supplementary Fig. S3). This is classical of a hormesis effect, which will be discussed later.

Ethanol altered the plant transpiration response to FTSW

The normalised transpiration rate (NTR) response to FTSW is shown in Figure 5. According to the values of R^2, RMSE, MSE (Fig. 5), the performance of non-linear fitting was satisfactory. NTR started at 1 when the plants transpired at the same rate as the WW plants within the same EtOH treatment, then dropped to lower values when FTSW became a limiting factor for transpiration. The FTSW threshold (FTSWt) from which NTR dropped below 0.95 was determined. We observed that priming plants with 250 mM led the plants to switch the FTSWt to higher values (FTSWt = 0.59) than plants primed with lower doses (FTSWt < 0.46) (detailed values and stats are given in Supplementary Table S3). Regarding the "a" parameter, the curvature coefficient, pairwise comparisons showed significant differences between 0.4 mM and 50 mM, compared to 250 mM. Thus, priming plants with 250 mM EtOH showed earlier regulation of transpiration in response to a progressive soil drying.

Soil water deficit and ethanol differentially impacted the leaf development and senescence

As shown in Figure 6 the soil water deficit limited the leaf expansion over the 4-week trial. At the end of the experiment, the average leaf area of the WW control plants was just above 200 cm² (Fig. 6A), when the one of the WS control plants was just above 100 cm² (Fig. 6B). The EOH priming also reduced the leaf expansion, this was particularly noticeable on the

293 plants primed with 250 mM EtOH which were harbouring an average leaf area twice smaller
294 than the controls (0 mM EtOH) in WW plants (Fig. 6A) and in WS plants (Fig. 6B).
295 The results of Figure 7A showed that, at harvest, WW plants harboured around 10% of dried
296 leaves, while WS plants harboured around 80% of dried leaves (Fig. 7B). In none of these two
297 series, WW and WS, the ethanol changed significantly the % of dried leaves.
298
299 **Soil water deficit reduced root development**
300 Regardless of the EtOH priming, all the root systems of WW plants reached dry weights
301 around 500 mg (Fig. 8A), while the ones of WS plants hardly exceeded 200 mg.
302 Similarly to the transpiration rates (Fig. 4), the WW plants displayed a trend of hormetic
303 response to EtOH, with a boost of root dry weight for EtOH doses around 2mM. The root
304 system at this latter dose presented an increase in dry weight by 20% compared to the WW
305 controls at 0 mM. This trend was also visible in WS plants (Fig. 8B), but its intensity was
306 lowered by the water deficit. However, none of these responses were significantly different
307 from controls.
308
309 **RNA-seq analysis highlights some metabolisms potentially involved**
310 As we ran RNA-seq experiments in parallel to this study, using a similar Gamay cultivar
311 (Diot *et al.*, 2024b), we analysed this RNA-seq data focussing on metabolisms described by
312 Bashir *et al*. (2022) as major causes of ethanol-induced resistance to drought in other plant
313 species they studied. The Figure 9 shows that some genes involved in metabolisms of abscisic
314 acid (ABA), aldehyde dehydrogenase (Ald DH), gamma-aminobutyric acid (GABA),
315 neoglucogenesis, starch and sucrose were differentially regulated 6 hours after priming
316 Gamay cell cultures with 1 mM ethanol. We also checked the responses of genes involved in
317 calcium related metabolisms and found many whose expression was either up- or down-
318 regulated by ethanol priming. But no strong trend was observed, either upward or downward
319 in any particular metabolism. The number of calcium related genes was standing out, but this
320 is also due to the fact that calcium related genes are more represented in Differentially
321 Expressed Genes (DEGs) than genes related to the metabolisms cited above. Finally, it is worth
322 noticing that the only three sucrose related DEGs were all down regulated.
323
324

325 ## Discussion

326

327 **Ethanol priming and grapevine bare root cuttings**

328 Water is becoming a scarce resource and the risk of excessive drought linked to climate
329 change is a serious concern for vineyard management (van Leeuwen *et al*., 2024). Our
330 observations may provide solutions to limit water use by the grapevine, possibly by limiting
331 transpiration, following an ethanol priming at very low doses, close to physiological values
332 obtained in Gamay cells, 100 µM to 2 mM (Diot *et al.*, 2024b); however we did not measure
333 endogenous EtOH in Gamay cuttings. In a preliminary experiment with bare root cuttings, we
334 observed a better acclimation to drought induced by ethanol priming (Fig. 2), but using a non-
335 physiological drought simulation, i.e. removing suddenly the water from bare-root cuttings
336 and leaving the plants without water for more than 30 hours. Nevertheless, the obvious
337 reduction in dry leaves observed in plants pre-treated with 10 µM EtOH, one week after re-
338 watering, was noteworthy; this latter concentration corresponds to 0.0006% of ethanol in
339 water, which is extremely low, but has been hypothesised to be in the range of detectable
340 level of EtOH for organisms to use as a signal to adapt to stressful environments, by eliciting
341 physiological changes (Diot *et al*. 2024a).

342

343 **Ethanol reduces water use by potted cuttings**

344 These preliminary results on bare root cuttings prompted us to carry out more
345 physiological tests with plants in soil, in order to apply water deficit kinetics on root systems
346 closer to vineyard conditions. The FTSW dropped below 0.1 between 10 and 15 days in most
347 water-deprived plants (Fig. 3B), which is consistent with the dynamics reported in other
348 studies on grapevine with different pot sizes and various climatic conditions (Lebon *et al.*,
349 2006). The most obvious effect of ethanol priming was the slower drop of FTSW after priming
350 with 250 mM (Fig. 3B), a limitation of soil water use that was also observable in well-watered
351 plants pre-treated with a similar EtOH dose (Fig. 3A).

352

353 **Ethanol reduces cuttings transpiration**

354 As pots without plants showed marginal FTSW decrease over the period of experiment
355 (Supplementary Fig. S1), we assumed that all water loss was due to transpiration. And as it
356 was demonstrated by Bashir *et al*. (2022) in three other plant species, the EtOH priming

357 reduced plant transpiration in grapevine too (Fig. 4). The strongest effect was obtained with
358 250 mM EtOH, which corresponds to a dilution of 1.4% EtOH/water (v/v). This EtOH
359 concentration reduced transpiration in both well-watered and water-deprived plants, as soon
360 as two days after the priming, even if the transpiration drop became significant only after
361 three days. Interestingly, the WS plants treated with this 250 mM concentration maintained
362 a higher transpiration rate than WS controls (pre-treated with water alone) after 8 days of
363 experiment This effect could be beneficial in cases of combined water deficit and heat stress,
364 as transpiration is critical to cool down leaf temperature (Costa *et al.*, 2012). Even more
365 interesting, we want to attract reader attention to a similar set of responses obtained with
366 the EtOH smallest dose, 0.4 mM which corresponds to 0.002% EtOH/water (v/v). Indeed, the
367 reduction of transpiration was also observed in the early days of experiment, but to a lesser
368 extent than 250 mM, and where the maintained transpiration was also observed at the end
369 of the 10 day period. Even if some of these effects are not significant in our experiment
370 conditions, the drop of transpiration induced by 0.4 mM, compared to water stress controls
371 (pre-treated with water alone), was noticeable from day 2 to day 4, and it was lower by up to
372 20%. This could generate big water savings at a vineyard scale, and definitely prompt us to
373 test these effects at a larger scale. As in the 250 mM conditions, the 0.4 mM EtOH also
374 maintained a higher transpiration rate than water stress controls after 8 to 10 days of
375 experiment.
376
377 **Transpiration variation follows a hormesis response**
378 These effects obtained at low and high EtOH are logical, as EtOH is known to generate
379 hormesis responses (Calabrese and Baldwin, 2003; Diot *et al.*, 2024a). These responses
380 harbour a bell shaped curve as a function of increasing concentration of the priming chemical.
381 This is quite obvious in all curves of the well-watered plants (Fig. 4). To illustrate this point, we
382 reproduced the plots of day 10 with bell-shaped curves in Supplementary Fig. S5. The green
383 curve highlights an increased transpiration at low ethanol doses in WW plants. Remarkedly,
384 the water stress led to inversing the hormesis response, highlighted by the red curve in WS
385 plants. Hormesis responses are known to be the initial parts of sigmoid curves (Kendig *et al.*,
386 2010), and it is known that environmental conditions can modulate hormesis responses in
387 plants (Belz and Cedergreen, 2010). These latter showed that environmental factors, such as
388 temperature and light, modulated the stimulation of lettuce growth by low doses of a
389 phytotoxin, parthenin. However, further experiments would be necessary to bring more

390 explanations about modulation of grapevine transpiration in response to a combination of
391 EtOH priming and water stress.

392 The progressive soil drying affects plant transpiration; the FTSWt on plants not
393 subjected to ethanol priming was equal to 0.42. This value is very close to 0.4, the value
394 reported for grapevines in previous studies using the two-segment plateau regression
395 procedure (Lebon *et al.*, 2003, Hofmann *et al.*, 2014). FTSWt values calculated using the two-
396 segment plateau regression procedure resulting in lower values than those obtained in our
397 study with the inverse exponential model (Kang *et al.*, 2024). The response of transpiration to
398 FTSW is employed in various dynamic crop models to monitor vineyards water status (Lebon
399 *et al.*, 2003; Valdés-Gomez *et al.,* 2009 ; Ramos *et al.*, 2014; Er-Raki *et al.,* 2021), and it is worth
400 noting that ethanol is modulating this response. According to the "a" parameter describing
401 the response of NTR to FTSW, priming with 250 mM EtOH showed a higher "a" than smaller
402 doses, which indicates an earlier regulation of transpiration in response to a progressive soil
403 drying (FTSWt = 0.42 vs 0.59 for 0 mM EtOH and 250 mM, respectively). This response is
404 associated with a reduction of transpiration and leaf growth even under non-limiting soil
405 water conditions. It should be concluded that high doses of ethanol reduce plant transpiration
406 regardless of the water status of the soil, with a greater response when the soil dries out.
407

408 **Ethanol effects on leaf and root developments**

409 The hormesis effect was also observed when looking at the green leaf area at harvest,
410 around four weeks after trial beginning (Fig. 6) with a slight stimulation of leaf area at 2 mM,
411 regardless of the water-deficit conditions. These results also showed that high doses of
412 ethanol (250 mM) limited the leaf development whatever the water deficit (in WW and in
413 WS), probably due the beginning of a toxic effect of ethanol at this high concentration, already
414 visible at 50 mM. This toxic response to exogenous ethanol has already been observed in
415 oilseed rape by Wu *et al.* (2019). However, the water deficit induced a stronger effect than
416 ethanol, clearly limiting leaf development in all WS cases compared to WW cases.

417 The observation of high percentages of dry leaves, mainly in WS plants (Fig. 7), was as
418 expected a classical plant response to water deficit (Fischer and Turner, 1978). The ethanol
419 was not significantly modulating this drought effect in WS plants, even if there was a slight
420 trend to increasing it at the highest dose. Because the choice of the harvest date was based
421 on the wilting of top leaves, and because the 250 mM treated plants showed nearly no wilting,
422 they were probably harvested too late. Nevertheless, the absence of ethanol effect on the

423 percentage of dry leaves in WW plants (Fig. 7), always below 10%, proved that we did not use
424 highly toxic ethanol concentrations.
425 The last phenotype we described in this experiment was the dry weight of the root
426 systems (Fig. 8). Once again, we observed a hormesis effect in the WW plants with an
427 increased root weight from 500 mg in controls to 600 mg in 2 mM EtOH treated plants, thus a
428 +20% increase in dry weight. This trend was not significant, with a t-test p-value = 0.15,
429 nevertheless this boosting effect of ethanol on root development has already been observed
430 in various plant species, such as mung bean (Bhattacharya *et al*., 1985) and oilseed rape (Wu
431 *et al.*, 2019). At higher EtOH doses, above 2 mM, the dry root weight decreased to control
432 levels.
433
434 **Regulations at the transcription level, potential roles for small heat shock proteins**
435 In an attempt to find explanations for the differences in transpiration, we used the
436 RNA-seq data from a companion paper (Diot *et al*. 2024b), performed on Gamay cells, to
437 screen transcript accumulation in response to an exogenous ethanol application. First, we
438 must highlight that Gamay Freaux is genetically very close to Gamay, as it is Gamay mutant
439 (see cultivars numbers 4377 and 4382 in https://vivc.de/). We searched particularly for
440 markers of the metabolisms revealed by Bashir *et al.* (2022) as major causes of ethanol-
441 induced resistance to drought: ABA, Ald DH, GABA, neoglucogenesis, starch and sucrose.
442 However, no marker stood out as a critical consequence of the priming by ethanol, even if a
443 slight trend for down regulation of sucrose related genes was observed. Nevertheless, the
444 RNA-seq data are available through the companion paper (Diot *et al.*, 2024b) and it may be a
445 resource for teams looking for a particular gene or sets of genes. We also checked for
446 regulation of calcium related gene transcripts, as calcium involvement has been described in
447 stomatal closure (Huang *et al.*, 2019), but there was no tendency for up or down regulation of
448 calcium related genes. Diot *et al.* (2024b) observed a strong induction of small heat shock
449 proteins (sHSPs) after ethanol priming of Gamay cells. The link between sHSPs and drought
450 stress is known (Rizhsky *et al*., 2002; Sarkar *et al.*, 2009) and Reddy *et al*. (2014) have observed
451 the induction of class I and II sHSPs following drought stress in barley. Finally, it is worth noting
452 that heat shock protein families, HSC70 and HSP90, are known to be involved in stomatal
453 closure (Clément *et al.*, 2011). Whether small HSPs are also involved in stomatal closure would
454 deserve further research, but our observations: EtOH priming reduced transpiration, as shown

455 in this article, and EtOH priming induced a strong sHSPs up-regulation, as shown in the RNA-
456 seq data (Diot *et al.*, 2024b), highlight a potential positive correlation.
457
458

459 Conclusion

460 We showed that ethanol priming induces a slowdown of the grapevine transpiration,
461 which was obvious with the 250 mM dose, but could also be effective at much lower dose
462 around 0.4 mM. This latter concentration corresponds to an ethanol dilution of 0.002% in
463 water, which would be easy and economical to implement; even though the ethanol dilution
464 of 1.4% in water, equivalent to 250 mM, is still relatively low. Confirming the promising effects
465 of such low doses would deserve further studies with older vines, either at greenhouse or
466 vineyard scales. Additionally, we also observed that grapevine responds to the range of
467 ethanol concentrations in a typical hormesis way. This clearly requires more studies to better
468 understand this type of biochemical responses, linked to chemical priming or hormesis, which
469 are now attracting more attention from the scientific community (Sako *et al*., 2020; Salinitro
470 *et al*. 2021). Finally, the links between sHSPs, ethanol priming and plant resistance to drought
471 also deserves more attention.
472
473

474 Supplementary data

475 The following supplementary data are available at the bottom of this file.
476 **Fig. S1**. Variations of FTWS with time in pots without plants
477 **Fig. S2.** Regression between the longest leaf vein and the leaf area
478 **Fig. S3.** Hormesis effects on transpiration, leading to bell-shape curves
479 **Table S1.** Multiple comparisons on Fig. 4 data
480 **Table S2.** Pairwise comparisons on Fig. 4 data
481 **Table S3.** Response parameters of water deficit for NTR
482

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487

488 **Author contributions**

489 NAK, AP, PM and CC conceptualised the project and designed the research. OY provided the
490 Gamay cuttings. NAK, BQ, PM and CC performed the cuttings experimentations. AD performed
491 RNA-seq experiments on Gamay cells. NAK, AD, PM and CC ran statistical tests. All co-authors
492 contributed to writing and editing the manuscript.

493

494 **Conflict of interest**

495 The authors declare they have no conflict of interest.

496

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504 Toulouse INP.

505

506 **Data availability**

507 The data underlying this article will be shared upon request to the corresponding authors.

508

509 **Companion article**

510 This article is one of two companion articles submitted to the same journal. The second article
511 by Diot et al. is entitled "Responses of grapevine cells to physiological doses of ethanol, among
512 which induced resistance to heat stress".

513

514

515

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Fig. 1. Various stages of the culture of Gamay cuttings: **A)** bare root cuttings in water; **B-C)** repotting after one month; **D-G)** variability of cuttings; **H)** measurement of leaf surface with Easy Leaf Area; **I)** example of dry leaves on the bottom at the end of the experiment; **J)** set-up in the growth chamber.

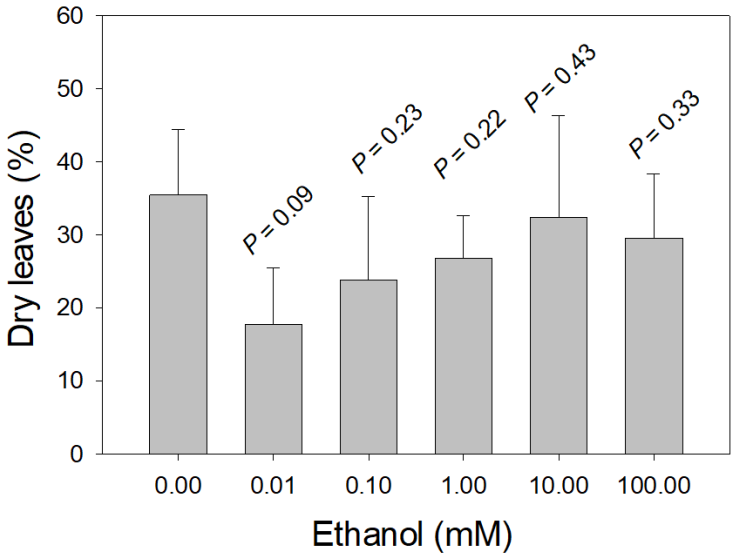


Fig. 2: Effects of ethanol (EtOH) priming on Gamay one-node cuttings with bare roots (preliminary study); One-month-old cuttings were primed with EtOH at various concentrations for 24h, then left without water for 38h, then assessed for dry leaves one week after rewatering; 4 replicates of 10 cuttings per concentration. Multiple comparisons with t-tests against controls (0).

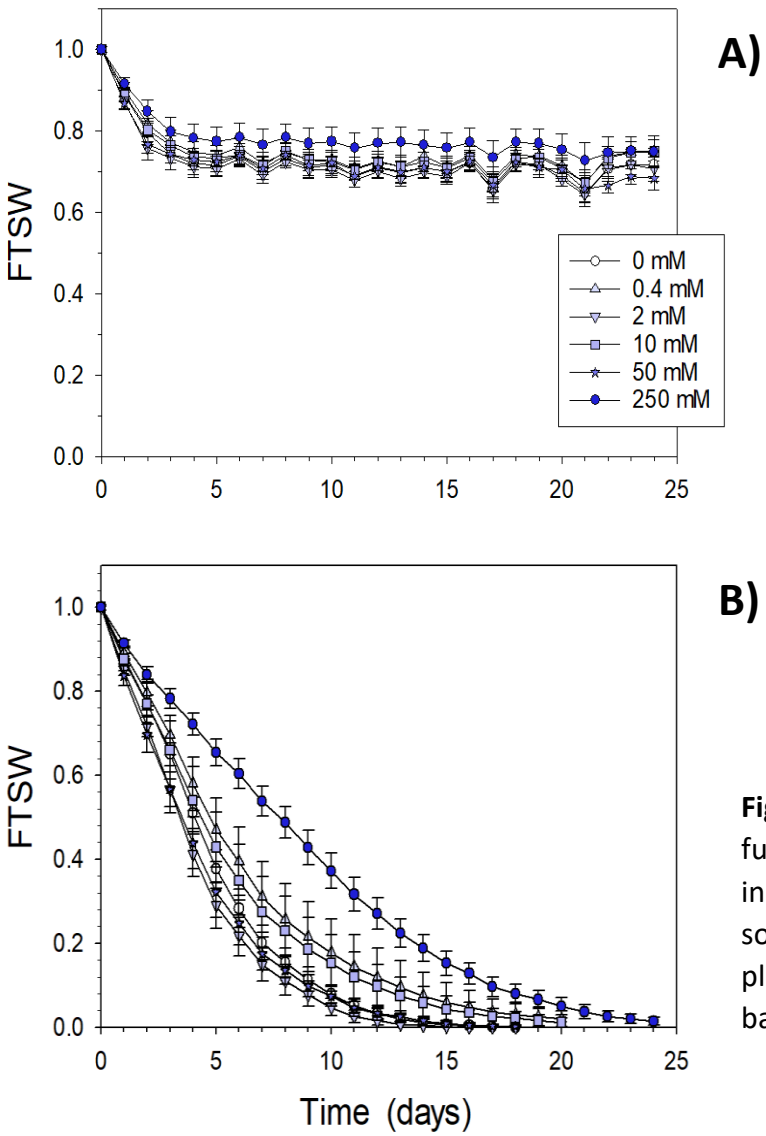


Fig. 3. Effects of ethanol (EtOH) priming on FTSW as a function of time after « soil water deficit » initiation in Gamay one-node cuttings growing on light potting soil. **A)** well-watered plants, WW, **B)** water stress plants, WS, irrigation stopped at day 1. n = 8, error bars show S.E.

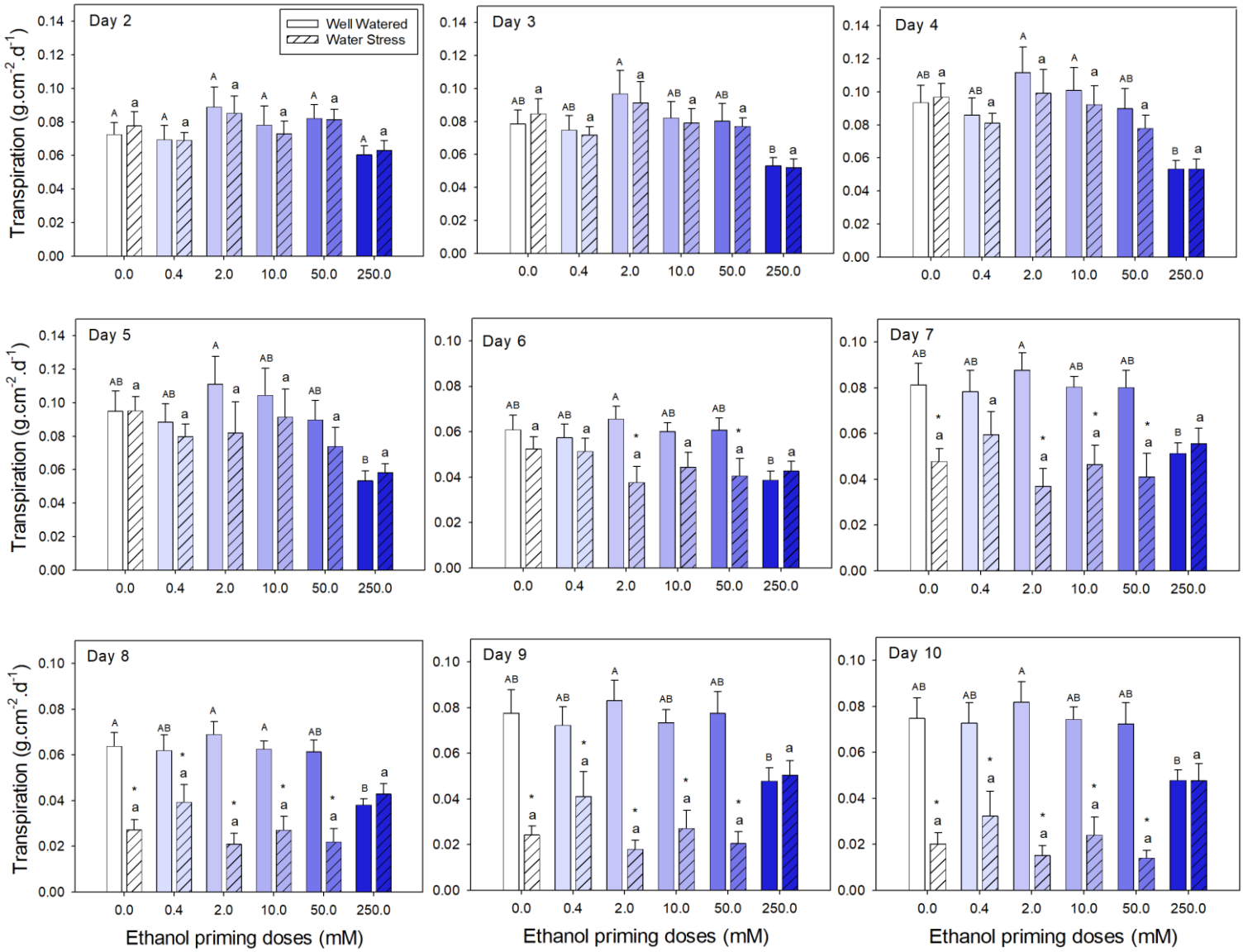


Fig. 4. Effects of ethanol (EtOH) priming on transpiration (Tr) of Gamay one-node cuttings in pots, from day 2 to day 10 after the initiation of soil water deficit n = 8, error bars show S.E.; all statistical details in Supp. Table S1; multiple comparisons: capital letters to compare within WW; small caps to compare within WS; * stands for significant differences at 0.05 between WW and WS within the same EtOH dose.

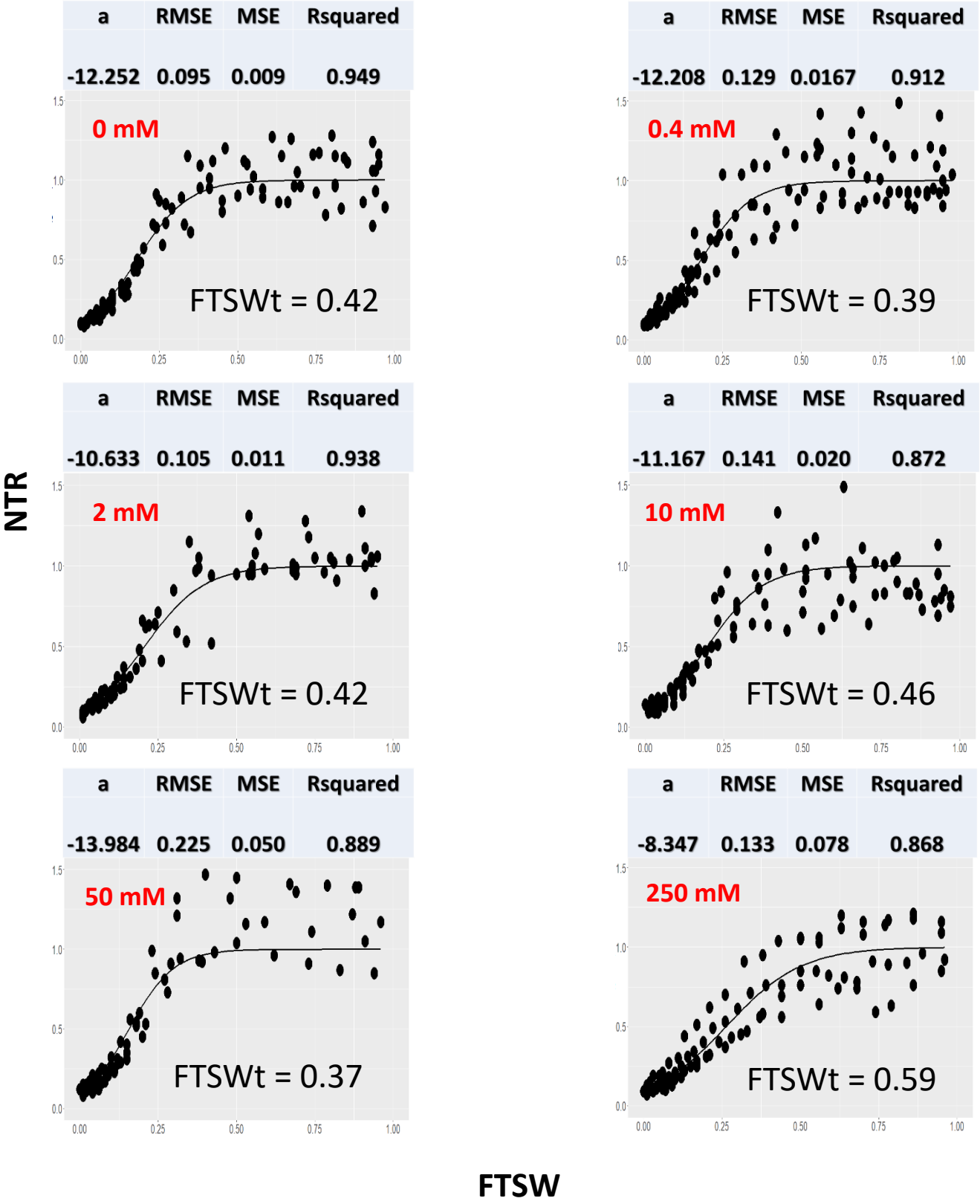


Fig. 5. Response of normalised transpiration rate (NTR) at the whole plant level to the fraction of transpirable soil water (FTSW) for six ethanol priming doses 0, 0.4, 2, 10, 50 and 250 mM. The curve fitting and calculations have been performed with the R software as described in material and methods. "a" represents the curvature of the fit; RMSE stands for root mean square error; MSE stands for mean square error; Rsquared is the goodness of fit; FTSWt stands for FTSW threshold.

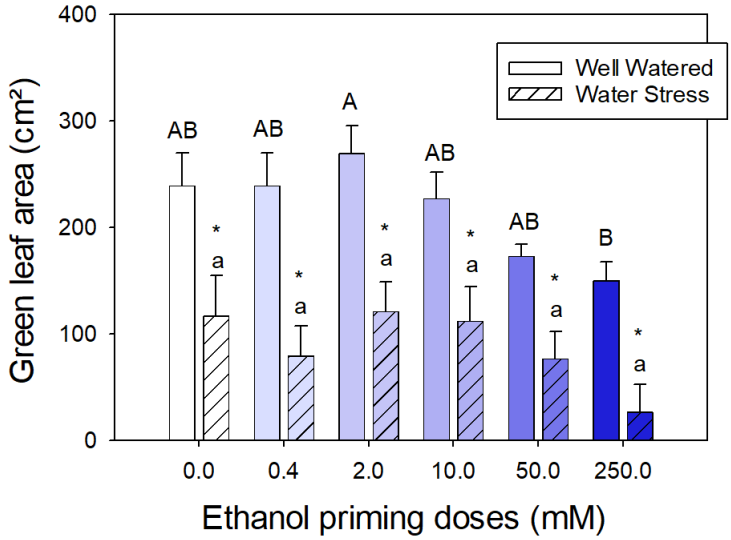


Fig. 6. Impact of ethanol (EtOH) priming on leaf area at harvest, n = 7 plants, error bars show SE. Multiple comparisons by Tukey HSD. Capital letters to compare within WW, small caps to compare within WS, and * to show $p < 0.05$ when comparing water deficit effect within a similar EtOH dose.

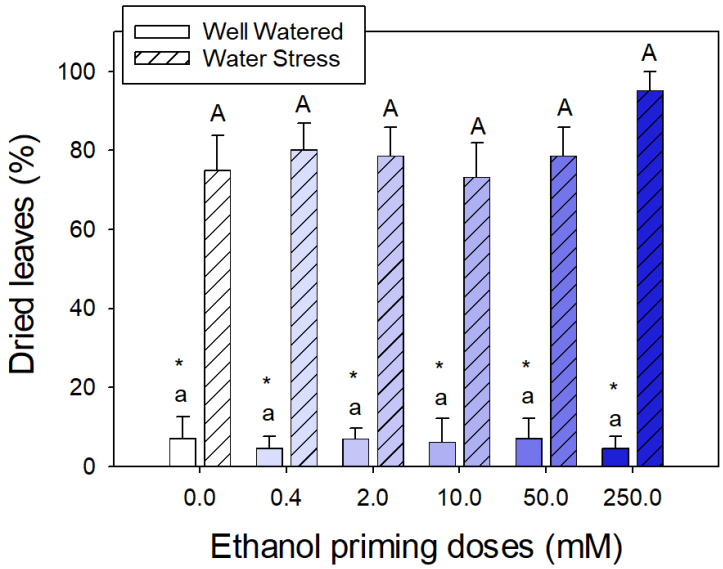


Fig. 7: Impact of ethanol (EtOH) priming on the % of dry leaves at harvest, n = 7 plants, error bars show SE. No significant difference due to EtOH within WW and WS by Tukey HSD, but * show a $p < 0.05$ in all comparisons of water deficit effect within each EtOH dose.

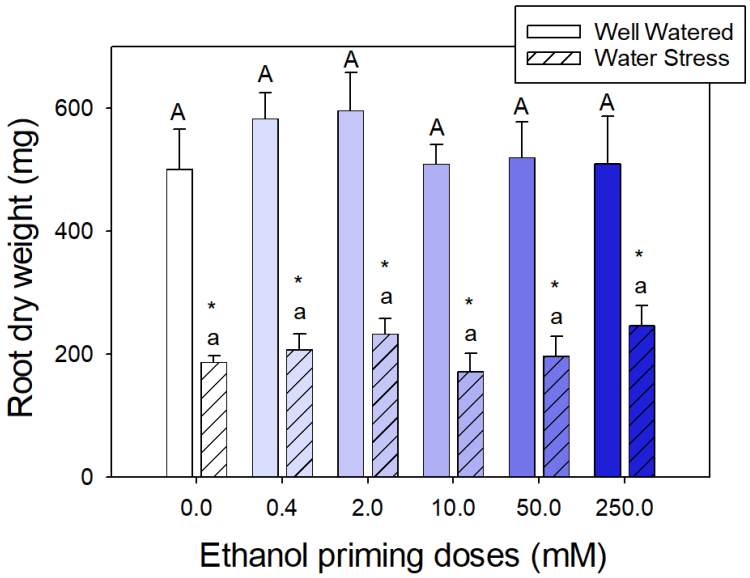


Fig. 8. Impact of ethanol (EtOH) priming on the root development at harvest, n = 7 plants, error bars show SE. No significant difference due to EtOH within WW and WS by Tukey HSD, but * show a $p < 0.05$ in all comparisons of water deficit effect within each EtOH dose.

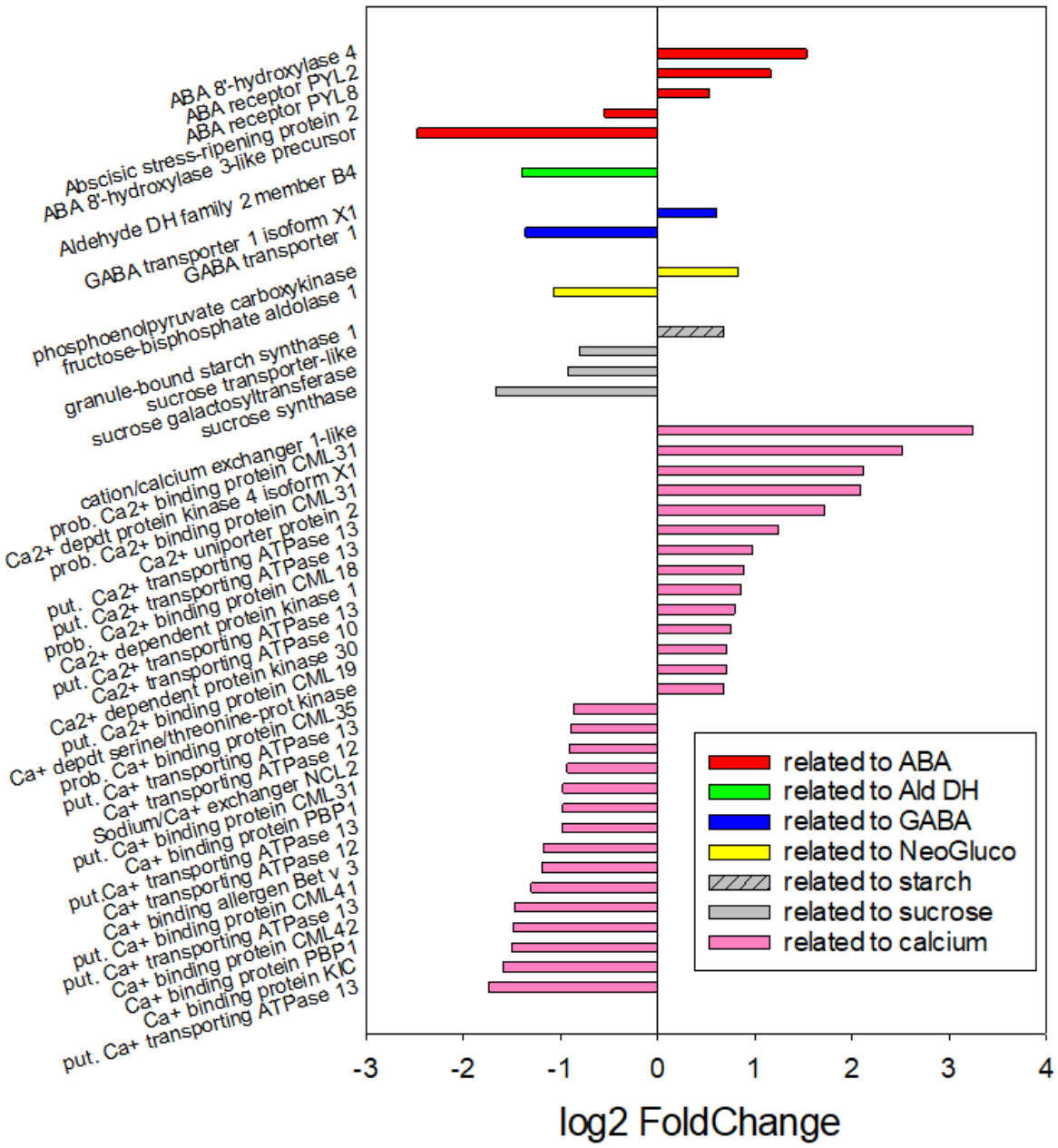
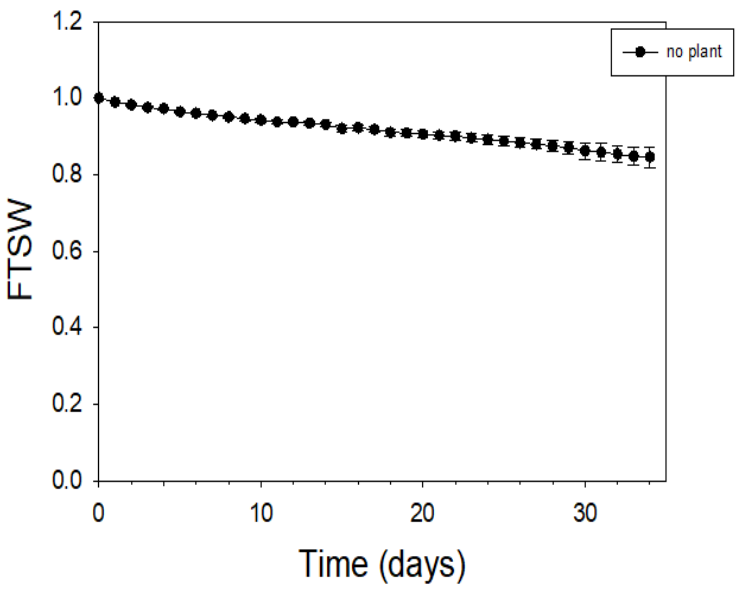
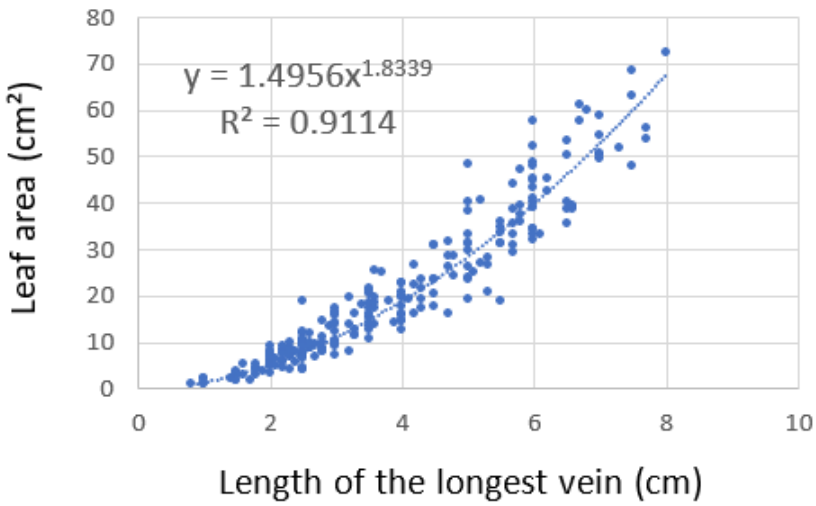


Fig. 9. Differentially Expressed Genes linked to metabolisms related to stomatal closure, for the comparison 0 vs. 1 mM EtOH priming of Gamay cell cultures, 6 hours after treatment (see companion paper Diot *et al*., 2024b).

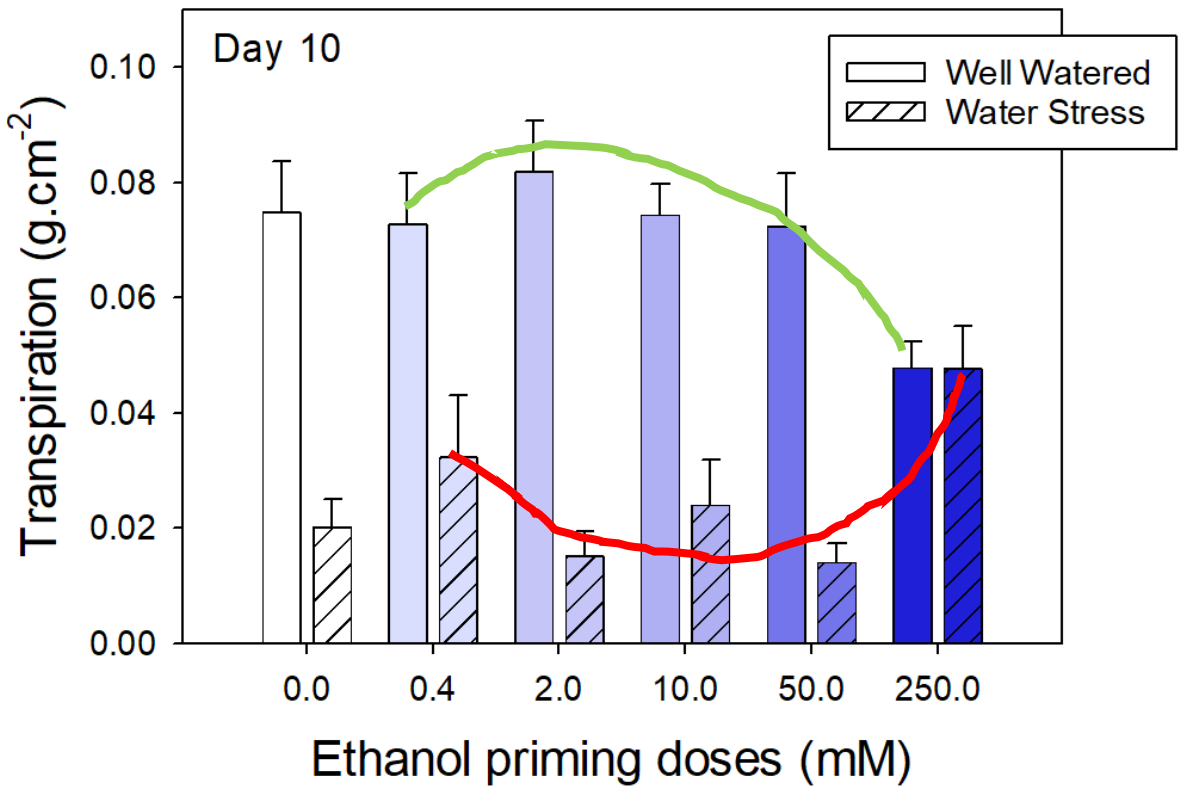
Supplementary Data



Supplementary Fig. S1. Variation of FTSW over time in pots without plants, n = 8 pots, error bars show SE.



Supplementary Fig. S2. Regression between the longest vein and the leaf area, n = 233 leaves measured in 30 different Gamay one-node cuttings, dedicated to this purpose, in the same experiment and similar growing conditions.



Supplementary Fig. S3. Hormesis effects on transpiration, leading to bell-shape curves (green for WW, red for WS) as a function of increasing ethanol doses. The water stress is changing the bell-shape curve, and such an environmental effect on the hormesis response has been described by Belz and Cedergreen (2010).

Supplementary Table S1. Post-hoc multiple comparisons issued from ANOVA on Tr values (data in Fig. 4) performed with R software.

Tab 1 - Water treatment effect on Tr with EtOH treatments pooled, and EtOH treatments effect on Tr with water treatments pooled, as a function of time (02 to 10 days)

		02 days		03 days		04 days		05 days		06 days		07 days		08 days		09 days		10 days	
		Mean Tr	Wilcoxon	Mean Tr	Wilcoxon	Mean Tr	Wilcoxon	Mean Tr	Wilcoxon	Mean Tr	Tukey HSD	Mean Tr	Tukey HSD	Mean Tr	Wilcoxon	Mean Tr	Tukey HSD	Mean Tr	Wilcoxon
Water	WW	0.0748	a	0.0775	a	0.0892	a	0.0904	a	0.0570	a	0.0762	a	0.0590	a	0.0715	a	0.0702	a
	WS	0.0753	a	0.0773	a	0.0853	a	0.0817	a	0.0454	b	0.0480	b	0.0297	b	0.0296	b	0.0248	b
EtOH	0 mM	0.0751	AB	0.0816	A	0.0951	A	0.0950	A	0.0567	A	0.0645	A	0.0455	A	0.0509	A	0.0475	A
	0,4 mM	0.0693	AB	0.0732	A	0.0834	A	0.0838	A	0.0541	A	0.0683	A	0.0499	A	0.0556	A	0.0512	A
	2 mM	0.0872	A	0.0942	A	0.1058	A	0.0975	A	0.0526	A	0.0640	A	0.0465	A	0.0527	A	0.0507	A
	10 mM	0.0758	AB	0.0808	A	0.0969	A	0.0984	A	0.0528	A	0.0645	A	0.0452	A	0.0518	A	0.0509	A
	50 mM	0.0818	A	0.0786	A	0.0839	A	0.0818	AB	0.0507	A	0.0606	A	0.0417	A	0.0490	A	0.0433	A
	250 mM	0.0604	B	0.0525	B	0.0532	B	0.0551	B	0.0399	A	0.0525	A	0.0399	A	0.0481	A	0.0467	A

Tab 2 - Interaction of water and EtOH treatment effects on Tr, as a function of time (02 to 10 days)

	02 days		03 days		04 days		05 days		06 days		07 days		08 days		09 days		10 days	
Water x Ethanol	p = 0.4		p = 0.0227		p < 0.01		p = 0.016		p = 0.1		p = 0.02		p < 0.01		p < 0.01		p < 0.01	
WW		**Wilcoxon**		**Wilcoxon**		**Wilcoxon**		**Wilcoxon**		**Tukey HSD**		**Tukey HSD**		**Tukey HSD**		**Tukey HSD**		**Tukey HSD**
0 mM	0.0726	A	0.0786	AB	0.0934	AB	0.0949	AB	0.0609	AB	0.0813	AB	0.0638	A	0.0775	AB	0.0748	A
0,4 mM	0.0695	A	0.0748	AB	0.0860	AB	0.0884	AB	0.0574	AB	0.0784	AB	0.0619	A	0.0722	AB	0.0727	A
2 mM	0.0890	A	0.0967	A	0.1116	A	0.1111	A	0.0656	A	0.0877	A	0.0689	A	0.0831	A	0.0818	A
10 mM	0.0782	A	0.0822	AB	0.1009	A	0.1044	AB	0.0601	AB	0.0803	AB	0.0610	A	0.0734	AB	0.0743	A
50 mM	0.0821	A	0.0803	AB	0.0898	AB	0.0898	AB	0.0608	AB	0.0802	AB	0.0614	A	0.0775	AB	0.0724	A
250 mM	0.0588	A	0.0527	B	0.0531	B	0.0531	B	0.0382	B	0.0505	B	0.0380	B	0.0466	B	0.0460	A
WS																		
0 mM	0.0777	a	0.0847	a	0.0968	a	0.0951	a	0.0525	a	0.0478	a	0.0273	a	0.0243	a	0.0202	a
0,4 mM	0.0692	a	0.0719	a	0.0811	a	0.0798	a	0.0513	a	0.0596	a	0.0393	a	0.0411	a	0.0323	a
2 mM	0.0853	a	0.0913	a	0.0992	a	0.0819	a	0.0377	a	0.0370	a	0.0210	a	0.0180	a	0.0152	a
10 mM	0.0730	a	0.0792	a	0.0924	a	0.0915	a	0.0444	a	0.0465	a	0.0271	a	0.0272	a	0.0240	a
50 mM	0.0814	a	0.0770	a	0.0780	a	0.0739	a	0.0405	a	0.0411	a	0.0219	a	0.0206	a	0.0141	a
250 mM	0.0630	a	0.0521	a	0.0533	b	0.0583	a	0.0427	a	0.0556	a	0.0429	a	0.0504	a	0.0476	a

Tab 3 - Interaction of WW and WS treatment effects on Tr, as a function of time (02 to 10 days)

	02 days		03 days		04 days		05 days		06 days		07 days		08 days		09 days		10 days	
WW x WS																		
Ethanol	diff of means	p	diff of means	p	diff of means	p	diff of means	p	diff of means	p	diff of means	p	diff of means	p	diff of means	p	diff of means	p
0 mM	did not test*		0.0061	0.624	0.0034	0.818	0.0002	0.992	0.0084	0.282	0.0335	0.002	0.0365	<0.001	0.0533	<0.001	0.0546	<0.001
0,4 mM			0.0029	0.824	0.0049	0.745	0.0087	0.627	0.0061	0.449	0.0188	0.089	0.0226	0.004	0.0311	0.005	0.0404	<0.001
2 mM	*no factor showed		0.0055	0.674	0.0125	0.411	0.0292	0.104	0.0279	<0.001	0.0507	<0.001	0.0479	<0.001	0.0651	<0.001	0.0667	<0.001
10 mM	sig. effect by 2W ANOVA		0.0031	0.814	0.0085	0.574	0.0129	0.469	0.0157	0.053	0.0338	0..003	0.0353	<0.001	0.0463	<0.001	0.0503	<0.001
50 mM			0.0033	0.819	0.0119	0.483	0.0159	0.425	0.0202	0.026	0.0391	0.002	0.0394	<0.001	0.0569	<0.001	0.0583	<0.001
250 mM			0.0011	0.939	0.0001	0.996	0.0048	0.812	0.0040	0.66	0.0043	0.73	0.0048	0.581	0.0026	0.829	0.0002	0

Supplementary Table S2. Pairwise comparisons on Tr values (data in Fig. 4) performed with R software.

Day 2

WW						
Ethanol Doses	**0**	**0.4**	**2**	**10**	**50**	**250**
0	/	0.786	0.257	*0.959*	0.398	0.206
0.4		/	0.216	*0.867*	0.310	0.379
2			/	*0.574*	0.666	0.056
10				/	*0.414*	*0.281*
50					/	0.040
250						/

WS						
Ethanol Doses	**0**	**0.4**	**2**	**10**	**50**	**250**
0	/	0.395	0.576	0.691	0.745	0.241
0.4		/	0.157	0.660	0.127	0.421
2			/	0.351	0.763	0.122
10				/	0.411	0.347
50					/	0.059
250						/

Day 3

WW						
Ethanol Doses (mM)	**0**	**0.4**	**2**	**10**	**50**	**250**
0	/	0.759	0.286	0.780	0.897	0.024
0.4		/	0.226	0.582	0.693	0.054
2			/	0.414	0.400	0.017
10				/	0.898	0.025
50					/	0.032
250						/

WS						
Ethanol Doses (mM)	**0**	**0.4**	**2**	**10**	**50**	**250**
0	/	0.234	0.677	0.669	0.511	0.022
0.4		/	0.163	0.468	0.492	0.023
2			/	0.452	0.354	0.035
10				/	0.841	0.038
50					/	0.008
250						/

Day 4

WW						
Ethanol Doses	**0**	**0.4**	**2**	**10**	**50**	**250**
0	/	0.622	0.342	0.671	0.827	0.006
0.4		/	0.200	0.411	0.811	0.014
2			/	0.610	0.312	*0.001*
10				/	0.573	0.009
50					/	0.013
250						/

WS						
Ethanol Doses	**0**	**0.4**	**2**	**10**	**50**	**250**
0	/	0.013	*0.779*	0.755	0.133	0.003
0.4		/	*0.397*	0.376	0.750	0.009
2			/	*0.710*	*0.366*	*0.010*
10				/	0.334	0.022
50					/	0.037
250						/

Day 5

WW						
Ethanol Doses (mM)	**0**	**0.4**	**2**	**10**	**50**	**250**
0	/	0.698	0.440	0.642	0.769	0.011
0.4		/	0.287	0.439	0.934	0.016
2			/	0.775	0.343	0.008
10				/	0.502	0.014
50					/	0.014
250						/

WS						
Ethanol Doses (mM)	**0**	**0.4**	**2**	**10**	**50**	**250**
0	/	0.193	*0.121*	0.845	0.149	0.008
0.4		/	*0.613*	0.513	0.662	0.064
2			/	*0.383*	*0.836*	*0.432*
10				/	0.418	0.136
50					/	0.273
250						/

Day 6

WW						
Ethanol Doses (mM)	**0**	**0.4**	**2**	**10**	**50**	**250**
0	/	0.699	0.589	0.918	0.990	0.014
0.4		/	0.331	0.702	0.683	0.022
2			/	0.433	0.552	0.002
10				/	0.918	0.002
50					/	0.006
250						/

WS						
Ethanol Doses (mM)	**0**	**0.4**	**2**	**10**	**50**	**250**
0	/	0.880	0.114	0.351	0.213	0.224
0.4		/	0.159	0.444	0.279	0.316
2			/	0.501	0.789	0.597
10				/	0.711	0.849
50					/	*1.000*
250						/

Day 7

WW						
Ethanol Doses (mM)	**0**	**0.4**	**2**	**10**	**50**	**250**
0	/	0.829	0.603	0.929	0.931	0.017
0.4		/	0.440	0.844	0.884	0.021
2			/	0.418	0.498	0.002
10				/	0.984	0.001
50					/	0.005
250						/

WS						
Ethanol Doses (mM)	**0**	**0.4**	**2**	**10**	**50**	**250**
0	/	*0.328*	*0.336*	*0.955*	*0.950*	*0.284*
0.4		/	0.106	0.346	0.232	0.783
2			/	0.421	0.749	0.118
10				/	0.689	0.450
50					/	0.289
250						/

Day 8

WW						
Ethanol Doses (mM)	**0**	**0.4**	**2**	**10**	**50**	**250**
0	/	0.841	0.543	*0.798*	0.776	0.003
0.4		/	0.440	*0.779*	0.949	0.007
2			/	*0.279*	0.362	*0.000*
10				/	*0.950*	*0.001*
50					/	0.002
250						/

WS						
Ethanol Doses (mM)	**0**	**0.4**	**2**	**10**	**50**	**250**
0	/	0.192	0.352	0.987	0.471	0.041
0.4		/	0.069	0.238	0.111	0.740
2			/	0.433	0.896	0.009
10				/	0.546	0.080
50					/	0.021
250						/

Day 9

WW						
Ethanol Doses (mM)	**0**	**0.4**	**2**	**10**	**50**	**250**
0	/	0.696	0.686	0.729	0.997	*0.072*
0.4		/	0.387	0.904	0.679	0.032
2			/	0.373	0.674	0.006
10				/	0.703	0.008
50					/	0.018
250						/

WS						
Ethanol Doses (mM)	**0**	**0.4**	**2**	**10**	**50**	**250**
0	/	0.168	0.282	0.736	0.564	0.003
0.4		/	0.083	0.331	0.152	0.542
2			/	0.320	0.696	0.001
10				/	0.512	0.055
50					/	0.004
250						/

Day 10

WW						
Ethanol Doses (mM)	**0**	**0.4**	**2**	**10**	**50**	**250**
0	/	0.872	0.579	0.966	0.856	0.021
0.4		/	0.480	0.876	0.979	0.027
2			/	0.481	0.479	*0.014*
10				/	0.851	0.003
50					/	*0.073*
250						/

WS						
Ethanol Doses (mM)	**0**	**0.4**	**2**	**10**	**50**	**250**
0	/	*0.382*	*0.232*	*0.955*	*0.282*	*0.011*
0.4		/	*0.232*	*0.613*	*0.181*	*0.093*
2			/	*0.456*	*0.945*	*0.010*
10				/	0.291	0.060
50					/	0.002
250						/

numbers in standard font obtained by Student test

numbers in italic font obtained by Wilcoxon test

Supplementary Table S3. Parameters describing the NTR response to FTSW variations, modulated by six ethanol doses. All fitting and calculations details are given in Material and Methods

EtOH	a		FTSWt		R^2
	mean	group	mean	group	
0 mM	-12.25	A	0.42	*a*	0.95
0.4 mM	-12.21	A	0.39	*a*	0.92
2 mM	-10.63	A	0.42	*a*	0.96
10 mM	-11.17	A	0.46	*a*	0.87
50 mM	-13.98	A	0.37	*a*	0.89
250 mM	-8.35	A	0.59	*a*	0.86

Groups in standard font obtained by Tukey HSD test
Groups in italic font obtained by Wilcoxon test

After anova on the "a", the effect of the treatment was not significant (p = 0.1)
After anova on the "FTSWt", the effect of the treatment was not significant (p = 0.06)
Then, we ran pairwise comparisons with other tests (see below)

coefficient "a"						
	0 mM	**0.4 mM**	**2 mM**	**10 mM**	**50 mM**	**250 mM**
0 mM	/	*0.51*	*0.28*	*1.00*	*0.41*	*0.15*
0.4 mM		/	0.26	0.48	0.41	0.04
2 mM			/	0.80	0.089	0.23
10 mM				/	0.21	0.23
50 mM					/	0.02
250 mM						/

numbers in standard font obtained by student test
numbers in in italic font obtained by wilcoxon test

FTSWt						
	0 mM	**0.4 mM**	**2 mM**	**10 mM**	**50 mM**	**250 mM**
0 mM	/	*0.60*	*0.27*	*1.00*	*0.56*	*0.08*
0.4 mM		/	0.26	*0.82*	0.70	0.06
2 mM			/	*0.80*	0.11	0.17
10 mM				/	0.35	0.22
50 mM					/	0.09
250 mM						/

numbers in standard font obtained by student test
numbers in in italic font obtained by wilcoxon test