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1 **Influence of ethylene on sucrose accumulation in grape berry**

2

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9

10 **Abstract:** Grape ripening is thought to be ethylene independent. However, Cabernet Sauvignon
11 berries that were treated with 1-methylcyclopropene (1-MCP), a specific inhibitor of ethylene
12 receptors, accumulated less sucrose over the following three weeks than did controls. This was
13 associated with a decreased RNA accumulation of two sucrose transporters (SUC11 and SUC12),
14 whose expression is triggered at the veraison stage when grape berries start to accumulate sugars.
15 These observations were performed over two consecutive years. This preliminary study suggests
16 that the role of ethylene in grape ripening needs to be reconsidered and that it could be related to
17 sugar accumulation.

18

19 **Key words:** grapevine, *Vitis vinifera*, ethylene signaling, sucrose transport, ripening

20

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22 Ville de Toulouse) for the grapes, and INRA and INPT for financial support. We also thank Dr C.
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25

1 **Introduction**

2

3 Grape ripening is thought to be ethylene independent since its classification as a non-climacteric
4 fruit (Coombe and Hale 1973, Abeles et al. 1992). However, the grape industry has been using
5 the 2-chloroethylphosphonic acid, an ethylene precursor also named ethephon, with some success
6 to enhance berry anthocyanin accumulation and acidity drop (Weaver and Montgomery 1974,
7 Shulman et al. 1985). Moreover, it is now well established that ethylene is present during the
8 ripening of non climacteric fruits such as grape or strawberry (Alleweldt and Koch 1977;
9 Trainotti et al. 2005). Recently, it was shown that ethylene synthesis is active just before the
10 inception of berry ripening and that treatments with 1-MCP (1-methylcyclopropene), a specific
11 inhibitor of ethylene receptors (Blankenship and Dole 2003), partially blocked berry growth,
12 acidity drop and anthocyanin accumulation (Chervin et al. 2004). Beyond these results, nothing is
13 known either on the potent role of ethylene during berry ripening or on what could be the
14 molecular target of ethylene in the grape berries.

15 On other plant species, there are some indications in the literature that sugar transport could be
16 one of the targets of ethylene action (Ishizawa and Esashi 1988 on rice; Saftner 1986 on sugar
17 beet). Interestingly, it was recently shown that sucrose, (and neither glucose nor fructose), was a
18 key signal responsible for the triggering of anthocyanin synthesis in plant tissues (Solfanelli et
19 al., 2006). These results can be combined to formulate the hypothesis that ethylene could alter
20 anthocyanin accumulation in grape berries through an effect on sucrose accumulation and/or
21 transport. In line with this hypothesis, we found the presence of four ethylene cis-elements in the
22 promoter of the *VvSUT1* (Chervin et al, unpublished), which is a functionally validated sucrose
23 transporter of the grape berry (Ageorges et al. 2000).

1 In this report, we focused on the role of 1-MCP on the sucrose accumulation as well as on the
2 relative abundance of transcripts coding for two sucrose transporters: *VvSUC11* (another name
3 given to *VvSUT1*) and *VvSUC12* that show increased expression around the veraison (Davies et
4 al. 1999) and have been functionally validated as sucrose transporters (Manning et al., 2001).
5 In a previous report (Chervin *et al.* 2004), we showed that 1-MCP was very unlikely to have
6 unspecific toxic effect since 1-MCP treatment before the inception of ripening did not produce
7 any effect on several variables such as berry diameter, skin anthocyanin accumulation and
8 decrease of juice acidity decrease.

9

10 **Material and Methods**

11

12 The grapevines, cv. Cabernet Sauvignon (*Vitis vinifera*, L.) were grown in a local vineyard in
13 Toulouse (Domaine de Candie) and 1-MCP treatments were performed as described previously
14 (Chervin et al. 2004). Briefly, the clusters were wrapped in polyethylene bags for 24 hours in
15 which 1-MCP ($4 \mu\text{l.l}^{-1}$) was injected or not. This treatment was performed around veraison,
16 approximately 9 weeks after full bloom, and when 50% berries per cluster had switched from
17 green to purple, started to soften and accumulate sugars (Terrier *et al.*, 2005). The bags were
18 removed after 24 hours and the clusters were either sampled for RNA extraction or left on the
19 vines until sampling for sugar concentrations (5 and 19 days after treatment). The experiments
20 were run for two consecutive years. For each extraction process, 20 berries were randomly
21 selected on each cluster before processing.

22

23 The sugar contents were assayed enzymatically with an EnzyPlus kit (Diffchamb, Västra
24 Frölunda, Sweden), using clear juice samples obtained from freshly harvested berries. Briefly,

1 whole berries (skin, pulp and seeds) were blended using a blender (Model 854501; SEB, Ecully,
2 France), then 10 mL sub-samples were centrifuged twice at 3,000 g for 3 min, pumping the juice
3 under the flocculating slurry before the second centrifugation. The determination of NADH
4 content was performed with a spectrophotometer at 340 nm.

5

6 RNA extraction was performed according to Boss *et al.* (1996) on berry tissues frozen
7 immediately after the 24 h period of 1-MCP treatment using liquid nitrogen. RNAs were treated
8 with DNase and checked for DNA contamination, using primers for *UFGT* genomic DNA
9 (forward 5'-CTGCAGGGCCTAACTCACTC-3' and reverse 5'-
10 TAGGTAGCACTTGGCCCATC-3'). The accumulation of RNAs of specific genes was then
11 performed by semi-quantitative RT-PCR. The primers for *SUC11*, were forward 5'-
12 TGCCTTGATATCCACACGAA-3' and reverse 5'- GGACCCTGGATTTATCAGCA-3', for
13 *SUC12*, forward 5'-CCTCTCAGCTGCTACAAGAACA-3' and reverse 5'-
14 AAAGCACAAGGCATCAAAGC-3' and for β -tubulin used as standard for result normalization
15 forward, 5'-TGCCACCTTTCAGATGAGTG.-3' and reverse 5'-
16 TTTTCAATACAAGCCCATTATGA-3'. The reactions were performed with 6 replicates from 3
17 different clusters, each picked on a different vine x 2 different years. The PCR reactions were
18 stopped after 25 cycles, as the signal intensities were approximately at 50% of the saturation. The
19 signal intensities were obtained by scanning images of gels stained with ethidium bromide, and
20 the scans were analyzed using SigmaScan Image (SPSS Inc., Chicago, IL).

21 In order to determine the LSDs at the 0.05 level, analyses of variance were performed with
22 SigmaStat (SPSS Inc., Chicago, IL). For each parameter, we checked there was no statistical
23 difference between the data of year one and year two (checked with t-tests; $P > 0.05$).

1

2 **Results and Discussion**

3

4 The 1-MCP, an inhibitor of ethylene receptors, reduced the sucrose content in grape berries in
5 comparison to control grapes at both sampling dates, 5 and 19 days after the treatment (Fig. 1).
6 At the same time, glucose and fructose accumulated at much higher levels than sucrose (around
7 60 g.L^{-1} and 80 g.L^{-1} , 5 and 19 days after veraison, respectively), similar levels were reported in
8 another study (Diakou *et al.*, 1997), and both hexose levels were not affected by 1-MCP. It can be
9 thus concluded that either 1-MCP specifically affected sucrose accumulation and transport, or it
10 stimulated the conversion of sucrose to glucose and fructose. However this accelerated
11 conversion could not be visible as the hexose concentrations are much higher than sucrose
12 concentrations. The difference in sucrose accumulation was not due to a difference in berry size
13 as no significant difference was found between berry sizes at both dates, although 1-MCP was
14 shown to have a longer term effect on berry size (Chervin *et al.* 2004).

15 The relative reduction of sucrose accumulation by 1-MCP was stronger by day 5 ($P = 0.002$) than
16 by day 19 after treatment ($P = 0.079$). This could be due to *de novo* synthesis of ethylene
17 receptors, counter-acting the blocking of ethylene receptors by 1-MCP that is nearly irreversible
18 (Blankenship and Dole 2003). This could also be due to a less active role of ethylene on sucrose
19 transport as the grapes reach full ripeness. In general the more ripe the berries, the less the
20 applications of ethephon in commercial vineyards are effective in triggering reactions in grapes
21 (T. Desordons, pers. comm. 2005). Further experiments with 1-MCP applications at different
22 dates are needed to clarify this point, particularly at earlier dates than mid-veraison.

23

1 Preliminary observations using micro-array (data not shown) had suggested that 1-MCP
2 treatments reduced the expression of a sucrose transporter (*SUC11*) that contains few *cis*-
3 elements linked with ethylene signaling in its promoter region and that is up-regulated at veraison
4 (Davies et al., 1999; Ageorges *et al.*, 2000). Figure 2 shows that the RNA accumulation for both
5 *SUC11* and *SUC12* was down-regulated by 1-MCP although the reduction was significant only in
6 the case of *SUC12*. These results therefore partly suggest that ethylene mediated sugar
7 accumulation could be triggered by one or both sucrose transporters. In line with this hypothesis,
8 it will be interesting to check for the presence of ethylene response *cis*-element in the promoter
9 region of *VvSUC12*, not yet sequenced (C. Davies pers. comm., 2005).
10 Beyond a possible role of ethylene on sugar transporters, it is also possible that other mechanisms
11 are involved, such as those linked to vascular fluxes, that vary over berry development (Tyerman
12 et al. 2004 ; Rogiers et al. 2001) and are likely to modulate sugar transport.

13

14 **Conclusion**

15

16 The present work is preliminary but it provides some arguments in favor of a possible role of
17 ethylene during the ripening of grape berries through a modulation of sucrose accumulation and
18 transport. Further work is needed to address the temporal pattern of ethylene responsiveness as
19 well as the relationship between ethylene mediated sugar accumulation and anthocyanin
20 accumulation.

21

22 **Literature Cited**

23

1 Abeles, F.B., P.W. Morgan, and M.E. Salveit. 1992. Ethylene in Plant Biology. Academic Press,
2 New York.

3 Ageorges, A., N. Issaly, S. Picaud, S. Delrot, and C. Romieu. 2000. Identification and functional
4 expression in yeast of a grape berry sucrose carrier. *Plant Physiol. Biochem.* 38:177-185.

5 Alleweldt, G., and R. Koch. 1977. Ethylene content in ripening grape berries. *Vitis* 16:263-271.

6 Blankenship, S.M., and J.M. Dole. 2003. 1-Methylcyclopropene: a review. *Postharvest Biol.*
7 *Technol.* 28:1-25.

8 Boss, P.K., C. Davies, and S.P. Robinson. 1996. Analysis of the expression of anthocyanin
9 pathway genes in developing *Vitis vinifera* L. cv. Shiraz grape berries and the implication for
10 pathway regulation. *Plant Physiol.* 111:1059-1066.

11 Chervin, C., A. El-Kereamy, J.P. Roustan, A. Latche, J. Lamon, and M. Bouzayen. 2004.
12 Ethylene seems required for the berry development and ripening in grape, a non-climacteric
13 fruit. *Plant Sci.* 167:1301-1305.

14 Coombe, B.G., and C.R. Hale. 1973. The hormone content of ripening grape berries and the
15 effect of growth substance treatments. *Plant Physiol.* 51:629-634.

16 Davies, C., T. Wolf, and S. Robinson. 1999. Three putative sucrose transporters are differentially
17 expressed in grapevine tissues. *Plant Sci.* 147:93-100.

18 Diakou, P., A. Moing, L. Svanella, N. Ollat, D.B. Rolin, M. Gaudillère and J.P. Gaudillère. 1997.
19 Biochemical comparison of two grape varieties differing in juice acidity. *Aust. J. Grape Wine*
20 *Res.*, 3:117-126.

21 Ishizawa, K., and Y. Esashi. 1988. Action mechanism of ethylene in the control of sugar
22 translocation in relation to rice coleoptile growth. I. Sucrose metabolism. *Plant Cell Physiol.*
23 29:131-141.

1 Kühn, C. 2003. Identification and functional expression in yeast of a grape berry sucrose carrier.
2 Plant Biol. 5:215-232.

3 Lescot, M., P. Déhais, G. Thijs, K. Marchal, Y. Moreau, Y. Van de Peer, P. Rouzé, and S.
4 Rombauts. 2002. PlantCARE, a database of plant *cis*-acting regulatory elements and a portal
5 to tools for *in silico* analysis of promoter sequences. Nucleic Acids Res. 30:325-327.

6 Manning, K., C. Davies, H.C. Bowen, and P.J. White 2001. Functional characterization of two
7 ripening-related sucrose transporters from grape berries. Ann. Bot. 87:125-129.

8 Rogiers, S.Y., J.A. Smith, R. White, M. Keller, B. Holzapfel, and J.M. Virgona 2001. Vascular
9 function in berries of *Vitis vinifera* (L) cv. Shiraz. Aust. J. Grape Wine Res. 7:47-51.

10 Saftner, R.A. 1986. Effect of ethylene on sucrose uptake in root discs of sugar beet. Plant Cell
11 Physiol. 27:853-860.

12 Shulman, Y., S. Cohen, and C. Loinger. 1985. Improved maturation and wine quality of
13 Carignane grapes by ethephon treatment. Amer. J. Enol. Vitic. 36:264-267.

14 Solfanelli, C., A. Poggi, E. Loreti, A. Alpi, and P. Perata. 2006. Sucrose-specific induction of the
15 anthocyanin biosynthetic pathway in *Arabidopsis*. Plant Physiol. 140:637-646.

16 Terrier, N., D.Glissant, J. Grimplet, F. Barrieu, P. Abbal, C. Couture, A. Ageorges, R.
17 Atanassova, C. Léon, J.P. Renaudin, F. Dédaldéchamp, C. Romieu, S. Delrot and S.
18 Hamdi. 2005. Isogene specific oligo arrays reveal multifaceted changes in gene
19 expression during grape berry (*Vitis vinifera* L.) development. Planta.222:832-847.

20 Trainotti, L., A. Pavanello and G. Casadoro. 2005. Different ethylene receptors show an
21 increased expression during the ripening of strawberries: does such an increment imply a role
22 for ethylene in the ripening of these non-climacteric fruits? J. Exp. Bot. 56:2037-2046.

- 1 Tyerman, S.D., J. Tilbrook, C. Pardo, L. Kotula, W. Sullivan, and E. Steudle. 2004. Direct
2 measurement of hydraulic properties in developing berries of *Vitis vinifera* L. cv Shiraz and
3 Chardonnay. *Aust. J. Grape Wine Res.* 10:170-187.
- 4 Weaver, R.J., and R. Montgomery. 1974. Effect of ethephon on coloration and maturation of
5 wine grapes. *Amer. J. Enol. Vitic.* 25:39-41.
- 6

1 **Figure legends**

2 Figure 1: Sucrose accumulation in the juice of Cabernet Sauvignon berries sampled 5 and 19 days
3 following 1-MCP treatment, this latter being performed for 24h on whole bunches with 50%
4 colored berries wrapped in plastic bags \pm 1-MCP; n = 6, error bars show SE, the *P* value is the
5 probability that both means are equal (t-test).

6 .
7

8 Figure 2: Transcript accumulation of two sucrose transporters, *SUC11* and *SUC12* (named as in
9 Davies et al. 1999), in Cabernet Sauvignon berries after wrapping the clusters in plastic bags for
10 24h \pm 1-MCP; at the time of the treatment the clusters had 50% colored berries; n = 6, error bars
11 show SE, data collected over two seasons, normalized on control means for each gene data set.
12 The percentages under the arrows represent the drop of signal means, the *P* value is the
13 probability that both means are equal (t-test).

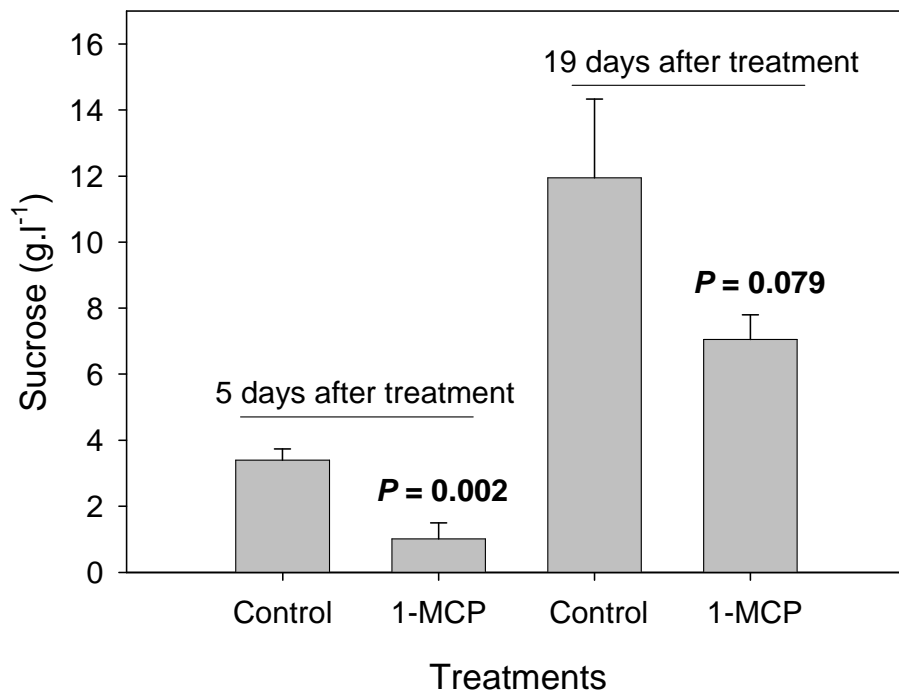


Figure 1

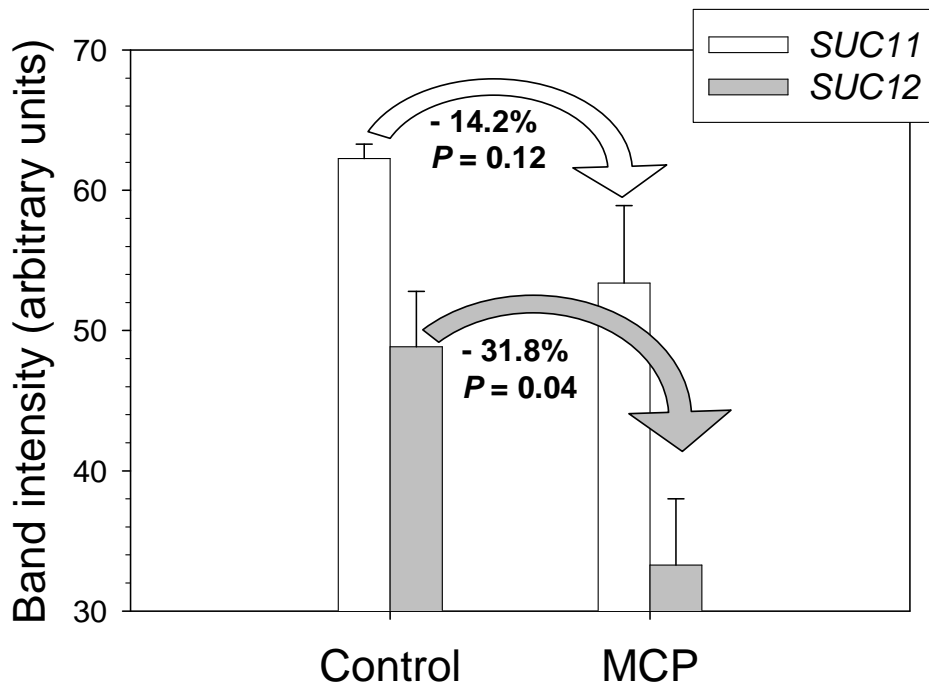


Figure 2