

1 **Ethanol vapours limit Botrytis development over the postharvest life of**
2 **table grapes**

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10 **Abstract:**

11 The application of ethanol vapours has been optimised over two seasons in order to prevent
12 rot development, caused by *Botrytis cinerea*, and stem browning in 'Chasselas' table grapes.

13 At a dose rate of 2 ml per kg of grapes, ethanol vapour was as effective as sulphur dioxide
14 pads. Consumer panels detected no significant difference in sensory perception between
15 controls and treated grapes. The ethanol vapour treatment could be easily implemented by the
16 table grape industry since the technology is similar to sulphur dioxide treatment.

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18

19 **Introduction**

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21 Table grapes are routinely treated with sulphur dioxide (SO₂) to reduce the incidence of
22 postharvest decay, largely caused by *Botrytis cinerea* (Lichter et al.,2002), during storage and
23 transportation. By limiting rot development, the SO₂ treatments allow to keep the grapes at
24 high humidity thus limiting stem browning, that is mainly due to desiccation. However SO₂
25 treatment may cause damage to the grape berries (discolouration, off-flavours), and sulphite
26 residues are not acceptable to some consumers.

27 Ethanol is known to influence ripening and senescence in some fruit plant tissues (Podd and
28 Staden, 1998), to reduce postharvest fungal decay (Gabler and Smilanick, 2001) and to kill
29 insect pests (Dentener et al., 1998). Application of ethanol to table grapes by dipping has
30 been shown to effectively improve storage life, mainly by limiting postharvest rot
31 development (Lichter et al., 2002; Karabulut et al., 2003). We have already investigated the
32 efficacy of ethanol vapours to control rots. Preliminary results indicated that the optimal
33 ethanol dose for effective disease control was less than 5 ml.kg⁻¹ of fruit (Chervin et al.,
34 2003). Since stem browning was higher at 5 ml ethanol.kg⁻¹ compared to SO₂ treatments, we
35 conducted new experiments in order to find a lower dose of ethanol that would still control rot
36 development without too high stem browning as in commercial SO₂ treatments,. In addition to
37 the optimisation of the ethanol dose, we also report on the use of a simple system to generate
38 ethanol vapours with pre-soaked paper pads.

39

40 **Material and methods**

41 'Chasselas' table grapes (*Vitis vinifera*, L.) were picked in a local vineyard (Montauban,
42 France) the second fortnight of September in 2002 and 2003 (at 20% Brix and 3.5 g.l⁻¹ tartaric
43 acid), and packed in wooden boxes (dimensions 40 x 28 x 12 cm) each containing 4 kg of
44 fruit. In the first year of experimentation (2002) there were five treatments: an untreated
45 control, one SO₂ pad per box (7 g Na₂S₂O₅), ethanol 1.25 ml.kg⁻¹, ethanol 3.75 ml.kg⁻¹ and
46 ethanol 7.5 ml.kg⁻¹. Each box was a replicate and there were three replicates per treatment. All
47 boxes were wrapped with individual polyethylene bags, then stored at 0°C for four weeks. In
48 2003 there were four treatments: an untreated control, one SO₂ pad per box, ethanol 2 ml.kg⁻¹
49 and ethanol 4 ml.kg⁻¹. All boxes were wrapped with individual polyethylene bags. The boxes
50 were sealed and stored as described above then assessed after four and seven weeks.

51 The ethanol vapours were generated by pre-soaking newspaper sheets (40 x 28 cm) in various
52 quantities of ethanol in order to reach the rate in ml.kg⁻¹. The sheets were left in contact with

53 ethanol in a sealed plastic bag for two hours to allow equilibrium of the ethanol into the paper
54 sheet. During fruit packing operations, a macro-perforated plastic sheet was placed between
55 the paper sheet and the grapes to prevent direct contact. Ethanol concentration in the box
56 headspace was measured with a Dräger pump (Dräger Sicherheitstechnik, Lübeck,
57 Germany) fitted with specific glass tubes (Chip Dräger Ethanol 100 - 2500 ppm ref.
58 6406370).

59 At the end of each storage period, the bags were removed and boxes were left at 8°C for half
60 an hour to limit condensation on the fruit, then transferred to ambient temperature (20°C) for
61 three days. Botrytis incidence was visually assessed by counting the number of affected
62 berries per bunch on all the bunches in each box. In the first experiment, when the average
63 number of rotten berries per bunch exceeded 5, the bunch was considered as "rejected", i.e.
64 not suitable for sale. During the second year experiment, the rotten berries of each box were
65 weighed and the result expressed as a percentage of the grape total weight in the box.

66 The assessment of stem browning was performed visually using the following 0 to 5 scale: the
67 scores were 0, 1, 2, 3, 4, and 5 for stem browning being <10%, 10 to 30 %, 30 to 50 %, 50 to
68 70%, 70 to 90% and > 90%, respectively.

69 Sensory analyses were performed with consumer-type panellists, using a hedonic scale
70 derived from Poste et al. (1991). The scale for sensory appreciation was a continuous line of
71 10 cm and the extremities at each end of the scale were: "I dislike extremely" (equivalent to 0)
72 and "I like extremely" (equivalent to 10). The 20 to 25 panellists in each session were asked
73 to mark the scale line with a pencil tick to give an indication of their appreciation. The
74 advantage of the continuous scoring system is that it suits most of the parametric statistical
75 tests. The samples were presented to them in a given order and were coded with five digit
76 numbers. There were as many different tasting orders as possible, as we appreciate food as a
77 function of what we ate before. The tasting sessions were performed after lunch.

78 The differences between treatments were analysed by ANOVA and LSD using SigmaStat
79 3.0.1 (SPSS, Chicago, IL, USA).

80

81 **Results and Discussion**

82

83 Ethanol vapour at doses equal or higher than 3.75 ml.kg⁻¹ resulted in grapes to a similar
84 commercial standard as SO₂ treatment after the four week storage (Table 1). However rots
85 caused by *B. cinerea* were not effectively controlled after seven weeks of cold storage (data
86 not shown). There was no significant difference ($P > 0.05$) in stem browning between the
87 control, 1.25 ml.kg⁻¹, 3.75 ml.kg⁻¹ and SO₂ treatments after four weeks of storage, and ethanol
88 at 7.5 ml.kg⁻¹ tended to increase stem browning (data not shown), suggesting that high dose
89 rates may induce phytotoxicity. The dose rate of 3.75 ml ethanol per kg of fruit gave
90 headspace concentrations of 220 ± 80 ppm of ethanol, over four weeks of storage at 0°C, and
91 this dose was sufficient to ensure fruit quality and control rot development without increasing
92 stem browning. Co-workers at the experimental station in Montauban detected a slight
93 ethanol taint with grapes treated with ethanol at 3.75 and 7.5 ml.kg⁻¹, so further sensory
94 analyses were carried out on grapes treated with ethanol doses between 1.25 and 3.75 ml.kg⁻¹.

95

96 The following year, both ethanol doses (2 and 4 ml.kg⁻¹) and the SO₂ pad treatments
97 significantly ($P < 0.05$) reduced the rot development in comparison to the control and there
98 was no significant ($P > 0.05$) difference between SO₂ and the ethanol treatments (Figure 1a).
99 All three treatments significantly ($P < 0.05$) reduced stem browning (Figure 1b) compared to
100 the control, but SO₂ and ethanol at the low dose of 2 ml.kg⁻¹ were significantly ($P < 0.05$) more
101 effective at reducing this disorder compared to ethanol at the high dose of 4 ml.kg⁻¹.

102 The sensory analyses showed that no consumer would detect a difference between postharvest
103 treated berries by visually assessing the samples (Figure 1c). However SO₂ treated grapes

104 were significantly ($P < 0.05$) less appreciated than the controls when assessors tasted the grape
105 samples (Figure 1d). The results obtained after four weeks cold storage and three days at 20°C
106 followed a similar pattern (data not shown).

107

108 In the preliminary study (Chervin et al., 2003), we showed that ethanol vapours also had the
109 potential to reduce berry shatter. It would be worth checking this with other cultivars than
110 ‘Chasselas’.

111

112 Overall our results confirm that ethanol has a potential for improving postharvest shelf-life of
113 table grapes, whether it is applied during a dipping treatment (Lichter et al., 2002) or with a
114 pad generating vapours (*e.g.* a paper impregnated with liquid ethanol). Ethanol could be used
115 in conjunction to SO₂, and this may allow a reduction of the dose of this latter, however
116 further research is necessary to develop this combination. Further developments may include
117 silica gel imbibed with alcohol, as previously described by Suzuki et al. (2004).

118

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130 **Literature cited**

- 131 Chervin, C., Westercamp, P., El-Kereamy, A., Rache, P., Tournaire, A., Roger, B., Goubran,
132 F., Salib, S. and Holmes, R., 2003. Ethanol vapours to complement or suppress sulfite
133 fumigation of table grapes. *Acta Hort.*, 628, 779-784.
- 134 Dentener, P. R. Alexander, S. M. Bennett, K. V. and McDonald, R. M., 1998. Postharvest
135 control of lightbrown apple moth using ethanol. *Acta Hort.*, 464, 279-284.
- 136 Gabler, F. M. and Smilanick, J. L. 2001. Postharvest control of table grape gray mold on
137 detached berries with carbonate and bicarbonate salts and disinfectants. *Amer. J. Enol.*
138 *Vitic.*, 52, 12-20.
- 139 Karabulut, O.A., Smilanick, J.L., Gabler, F.M., Mansour, M. and Droby, S., 2003. Near-
140 harvest applications of *Metschnikowia fructicola*, ethanol, and sodium bicarbonate to
141 control postharvest diseases of grape in central California. *Plant Dis.*, 87, 1384-1389.
- 142 Lichter, A., Zutkhy, Y., Sonogo L., Dvir O., Kaplunov T., Sarig P. and Ben-Arie R., 2002.
143 Ethanol controls postharvest decay of table grapes. *Postharvest Biol. Technol.*, 24,
144 301-308.
- 145 Podd, L. A. and Staden, J. van., 1998. The role of ethanol and acetaldehyde in lower
146 senescence and fruit ripening - a review. *Plant Growth Regul.*, 26, 183-189.
- 147 Poste, L.M., Mackie, D.A., Butler, G. and Larmond E., 1991. Hedonic scaling test.
148 *Laboratory Methods for Sensory Analysis of Food*, ed. Agriculture Canada,
149 publication 1864/E, pp. 64-67.
- 150 Suzuki, Y., Toshikazu, U. and Terai H. 2004. Inhibition of senescence in broccoli florets with
151 ethanol vapor from alcohol powder. *Postharvest Biol. Technol.*, 31, 177-182.

152 **Table and Figure captions**

153 Table 1: Percentage of weight of table grapes (cv. Chasselas), accepted for sale as a function
154 of Botrytis development. Grape bunches were visually assessed in 2002 after four
155 weeks of storage at 0°C plus three days at 20°C. The ethanol (EtOH) doses applied
156 over cold storage are in ml per kg of fruit and the SO₂ was applied using a
157 commercially available pad, LSD = least significant difference.

Treatment	Control	EtOH 1.25	EtOH 3.75	EtOH 7.5	SO ₂	LSD
% accepted	10.7	35.2	89.5	84.8	85.0	23.9

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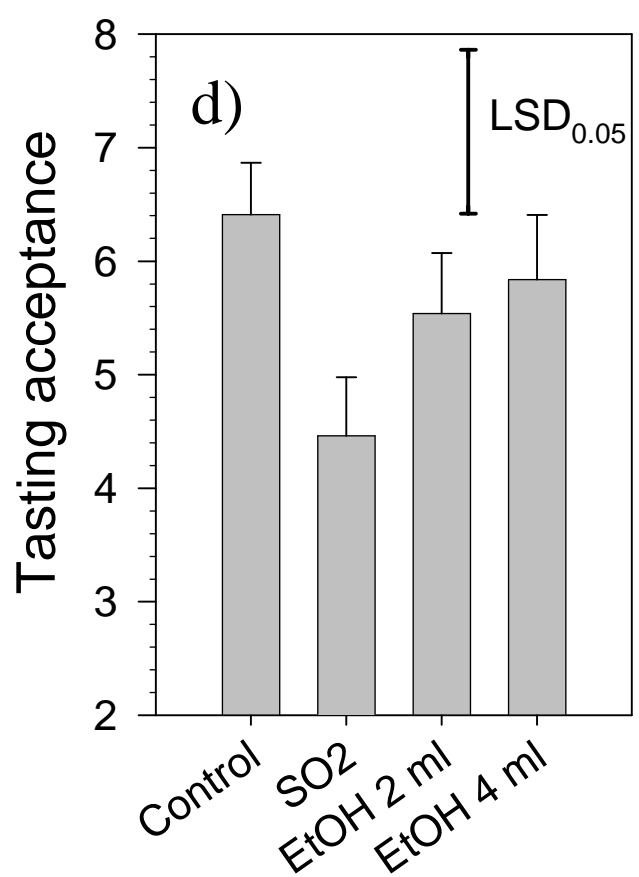
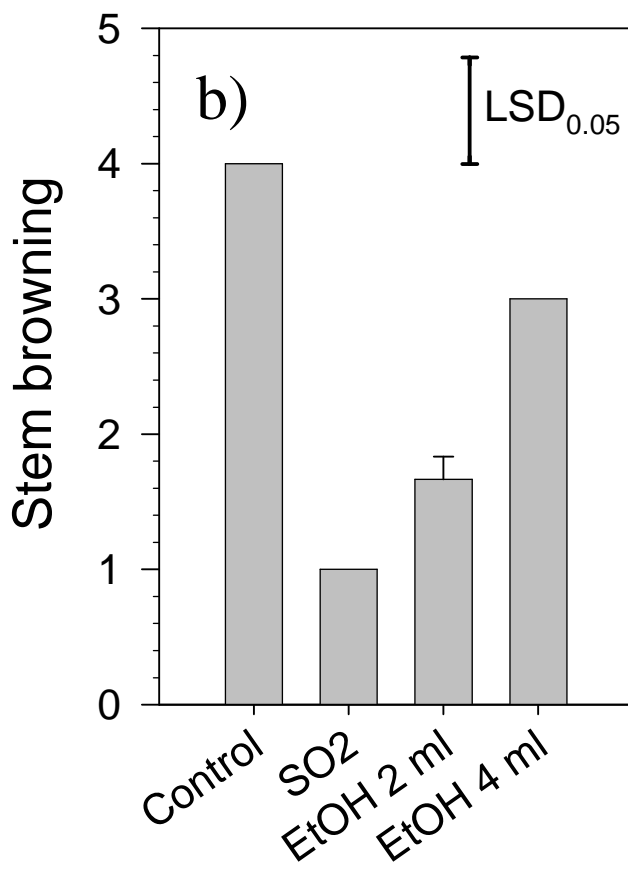
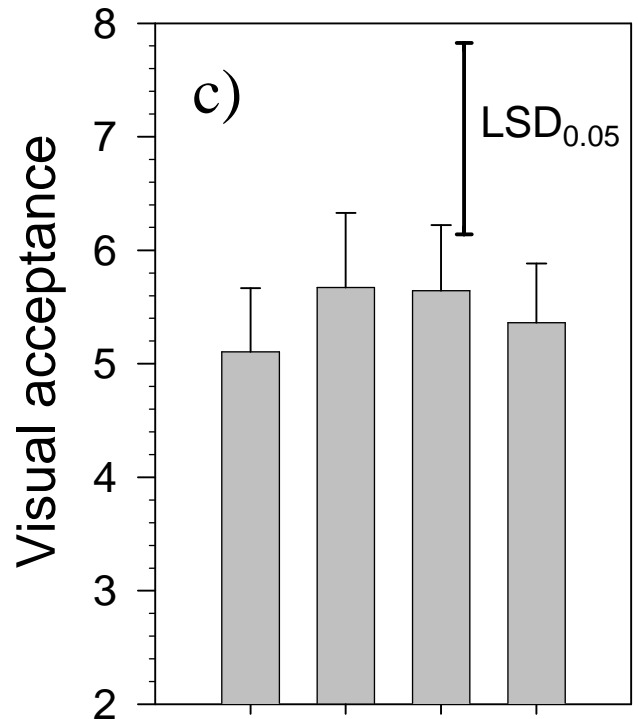
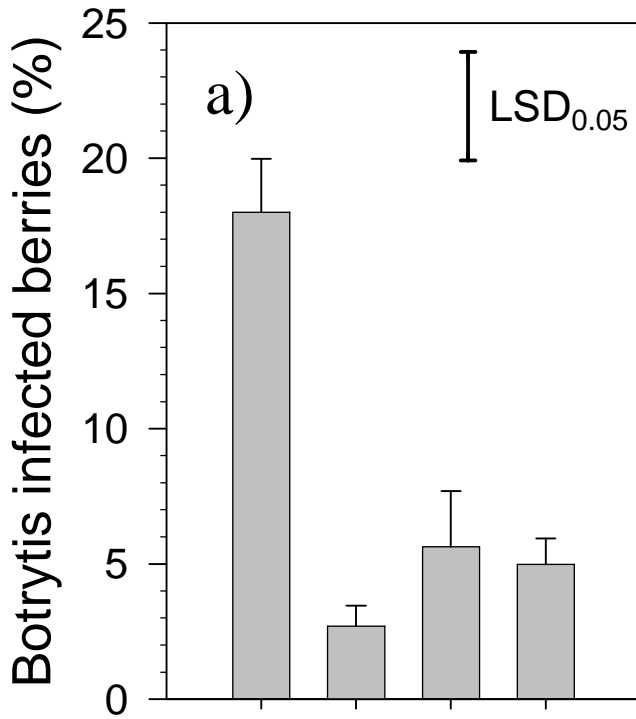
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161 Figure 1: Percentage of Botrytis infected berries (a) and severity of stem browning (b) as a
162 function of SO₂ or ethanol treatments (EtOH) after seven weeks at 0°C and three days
163 at 20°C in 2003. Sensory evaluation of treated grapes by visual assessment (c) and
164 tasting (d), using 0 to 10 scales. The ethanol doses are quantities in ml per kg of fruit
165 and SO₂ was applied using a commercially available pad. Error bars represent standard
166 error of the mean. LSD = least significant difference.

167

168



Treatments

Figure 1