Ethanol sprays to release grapevine bud dormancy: a potential alternative to cyanamides

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Ethanol sprays to release grapevine bud dormancy: a potential alternative to cyanamides

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ABSTRACT

Aim: Grape growers sometimes use cyanamides (calcium or hydrogen) to release bud dormancy in warm climate regions, where the chilling requirement has not been met during winter. However, these products can cause damage to plants and are dangerous to handle, so alternatives would be welcomed by growers. Connections between metabolisms of ethanol, ethylene and cyanide revealed by previous studies led us to test the potential of ethanol sprays on bud break and early shoot growth.
Methods and results: Trials were performed over three years on *Vitis vinifera* grapevines trained in Guyot or cordon, and on cuttings in growth chambers. Cultivars used in the studies included Cabernet-Sauvignon, Syrah and Ugni blanc. The results show that ethanol can advance bud break of all three cultivars at concentrations ranging from 2.5 to 10 % ethanol in water. Ethanol stimulates bud development in both Guyot and cordon training systems. However, the timing of ethanol application is crucial, and late spring season applications reduce the effectiveness of the treatment.
Conclusions: Observations were performed over three different seasons. The trials revealed that ethanol sprays can advance bud break of different *Vitis vinifera* vines, trained with cane or spur systems.
Significance and impact of the study: Climate change impacts dormancy release, making it an increasingly important issue over the next few decades. An alternative to the dangerous use of cyanamides to promote bud break would greatly help growers. These preliminary results with ethanol are promising but should lead to trials in various growing areas and with various cultivars in order to confirm their potential for viticulture.

KEYWORDS

Bud break, grapevine, *Vitis vinifera*, ethanol, hydrogen cyanamide

INTRODUCTION

Sufficient grapevine chilling following leaf drop at temperatures between 0 °C and 10 °C results in dormancy release and uniform bud break with increasing temperatures in spring (Dokoozlian, 1999; Andreini *et al.*, 2009; Mathiason *et al.*, 2009; Mohamed and El-Sese, 2009; Avenant and Avenant, 2014; Alvarez *et al.*, 2018; Anzanello, *et al.*, 2018). Chilling requirements for grapevine bud break are genotype-specific within species, varying from 250 to 2250 hours (Londo and Johnson, 2014). A minimum of 200 hours chilling was necessary for homogeneous bud break of *V. vinifera* 'Perlette', and other *V. vinifera* cultivars require 50–400 hours of chilling for uniform bud break (Dookoozlian, 1999; Londo and Johnson, 2014; Anzanello *et al.*, 2018). Insufficient chilling hour accumulation contributes to extended dormancy and uneven or prolonged bud break, which impacts flowering time (Mathiason *et al.*, 2009; Keller, 2015; Melke, 2015).

In subtropical areas, dormancy release is a problem for several perennial crops, including grapevines (Sudawan *et al.*, 2016). Under such climates, dormancy release is uneven due to the lack of cold nights that are known to promote dormancy release in grapevines (Dokoozlian, 1999). To boost bud break in warm climates, growers sometimes use hydrogen cyanamide (CH_2N_2) to promote uniform grapevine bud dormancy release (Shulman *et al.*, 1983; Or *et al.*, 1999). The positive effect of cyanamide on hastening bud break was observed in a temperate region of south-west France on three local cultivars: Ugni blanc, Tannat and Cabernet Franc (Durquety *et al.*, 1988). However, cyanamides are toxic to humans and accidents can occur when handling them during application (Inamdar *et al.*, 2015). When looking for alternatives to cyanamides, Tohbe *et al.* (1998) showed that aminocyclopropane carboxylic acid (ACC) helped to induce bud break in grapevine. ACC is the precursor of ethylene in plants, and its conversion to ethylene is known to be associated with the production of hydrogen cyanamide (Lin *et al.*, 2009). Shi *et al.* (2018) showed recently that ethylene production is involved in grapevine bud break. In an earlier study, we showed that spraying ethanol on grapevines generated various responses, including the stimulation of ethylene production (Chervin *et al.*, 2001). Thus the objective of this short study was to test whether ethanol sprays could have an impact on bud break and early shoot growth.

MATERIALS AND METHODS

Three experiments conducted to assess the effects of ethanol application on bud break are described below.

1. Experiment 1

Dormant Cabernet-Sauvignon cuttings were collected from a commercial vineyard in Toulouse, in south-west France. The vines were 20 years old, grafted onto 110 Richter rootstock, and pruned to a Guyot system. One-node cuttings were taken at node position 5 (starting from the base of mature canes). Sampling was performed in mid-January in 2003 during winter dormancy. Twenty 'one-node cuttings' were collected per treatment. The cuttings were placed in a house custom growth chamber at the Inra campus, Castanet-Tolosan: the day/night light cycle was 16 h/8 h; the light intensity was 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$; the day/night temperatures were 25°C/20°C and the relative humidity was 80 %. The bases of the cuttings were placed vertically into water, being careful not to submerge the bud in water. Treatments included ethanol application at 0 % (100 % water as control), 2.5 %, 5 %, and 10 %. Treatments were applied one day after transfer to the growth chamber. Application was made with a 1 L hand spray bottle, approximately 1 mL of solution per bud. Bud break was assessed visually three weeks after spraying. Ethanol sprays as described above were also performed on eight Cabernet-Sauvignon vines randomly chosen in the vineyard. The development stage was woolly bud (E-L stage 3) as detailed in Coombe (1995). In detail, five-bud canes were sprayed with ethanol 0 % (100 % water as control), 2.5 %, 5 % and 10 %, with spraying performed on eight different canes, each on different vines. The shoot lengths were measured one month after the spray.

2. Experiment 2

Ethanol sprays 0 % (100 % water as control), 2.5 %, 5 % and 10 %, were performed on 5-year-old cordon-trained Cabernet-Sauvignon vines in 2004, grafted on 3309 Couderc, growing at the INRA campus 15 km south of Toulouse; spraying was performed as described in Experiment 1 (E-L stage 3, and 1 ml per bud). The ethanol sprays were performed early March

at bud swell, the shoot lengths were measured one month after the spray.

3. Experiment 3

Dormant Cabernet-Sauvignon, Syrah and Ugni blanc cuttings were collected from the Inra campus at two different dates in mid-January and mid-February, 2007. One-node cuttings (4–5 cm) were taken using node 5 from the base of mature canes placed in water in the growth chamber, as described in Experiment 1. Ethanol sprays 0 % (100 % water as control), 2.5 %, 5 % and 10 %, were performed one day after transfer to the chamber, 1 mL total spray per bud, on five individual nodes per cultivar and per treatment. Bud break was assessed three weeks after treatment. The Eichhorn and Lorenz (E-L) system (Coombe, 1995) was used to define the following ordinal scale: 0 = dormant bud; 1 = bud swell (E-L 2); 2 = woolly bud (E-L 3); 3 = rosette of leaf tips visible (E-L 5); 4 = one or two leaves separated (E-L 7–9). Pictures of the bud stages are shown in Supplementary Figure 1.

4. Statistical analysis

t-tests were performed with Microsoft Excel (2016), ANOVA and Fisher's LSD calculations were performed using the DSAASTAT macro (v. 1.022) by Andrea Onofri.

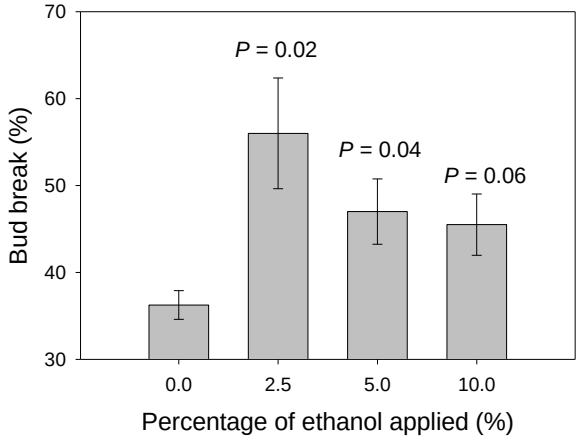

FIGURE 1. Percentage bud break as a function of the ethanol concentration (aqueous solutions). The one-node cuttings of Cabernet-Sauvignon were sprayed at bud swell stage (Eichhorn and Lorenz (E-L) stage 3), after transfer into the growth chamber. N = 4 replicates of 20 buds each; error bars show SE; *P* values are the probabilities that the treatment mean differs from the control mean (t-test).

RESULTS

The first trials in Experiment 1 were performed on Cabernet-Sauvignon cuttings. The ethanol sprays stimulated greater percent bud break than the water control treatments (Figure 1). The results were significant for 2.5 and 5 % ethanol. The second trials in year 1 also showed a stimulation of bud burst, and shoot length after ethanol spray (Figure 2A) on a Guyot cane. The significant results were observed in bud 5, counting from the base of the cane. The increase in the apical shoot growth treated with 2.5 and 5 % ethanol was on average +200 % compared to controls.

Experiment 2 used cordon-trained Cabernet-Sauvignon, and a significant increase in shoot

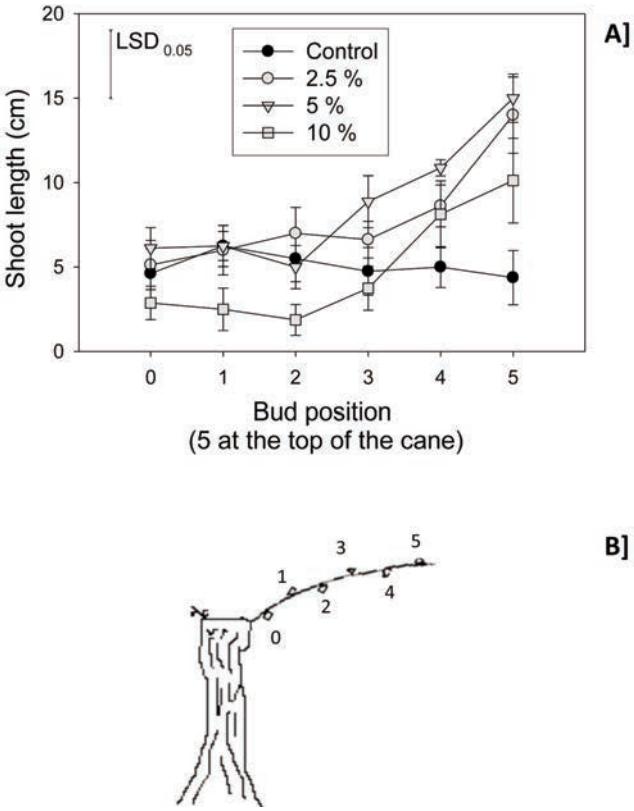

FIGURE 2. (A) Shoot length as a function of the concentration of ethanol sprayed (aqueous solutions) and the node position on the cane of Cabernet-Sauvignon, pruned to a Guyot training system. All canes were sprayed at woolly bud stage (E-L stage 3) in the vineyard. N = 8 different vines, one cane per vine; error bars show SE; the Fisher LSD value was calculated at $P < 0.05$ level to compare between ethanol applications. (B) Bud position on Guyot-trained vines prior to treatments.

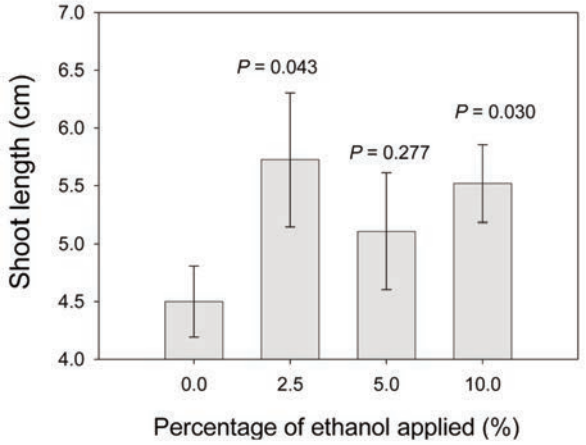

FIGURE 3. Shoot length of Cabernet-Sauvignon one month after spraying, with various ethanol concentrations (aqueous solutions), at woolly bud stage (E-L stage 3) in the vineyard.
The vines were cordon-trained. The number of buds treated were N = 64, 40, 37 and 79, for 0, 2.5, 5 and 10 % ethanol, respectively. Error bars show SE. P values indicate whether the treatment means differ significantly from the control mean (t-test).

length was observed following the ethanol sprays (Figure 3). On average, the increase in shoot growth was 20 % greater than the control treatments.

Experiment 3 was performed on one-node cuttings of three different cultivars (Figure 4). When the dormant cuttings were sampled from the vines in mid-January (Figure 4 left panel), ethanol stimulated the rate of bud break in all three cultivars. The scoring is related to the modified Eichhorn-Lorenz (E-L) grapevine growth stages as indicated in the figure caption (Coombe, 1995). The higher the number, the more advanced the bud break. However, when the cuttings were sampled one month later, after a series of 11 days with an average daily temperature below 5°C (200–250 additional chilling hours, as shown in Supplementary Fig.2), the ethanol sprays showed no significant effects on bud break (Figure 4 right panel), with control samples showing faster bud development compared to controls of the earlier sampling date (Figure 4, left panel).

DISCUSSION

Our results show that ethanol promotes bud break and early shoot development when chilling fulfilment is incomplete. Relatively small ethanol concentrations (2.5 to 5 %) seem effective to promote bud dormancy release. During Experiment 1, the canes were not bent horizontally at the time of trial (Figure 2B), which may explain why the top buds developed faster, as there is apical dominance in grapevine (Keller, 2015). We did not measure physiological and molecular changes associated with increased chilling and bud break (Mathiason *et al.*, 2009), thus cannot speculate on the biochemical changes associated with the enhanced bud break. Our aim was to document the impact of ethanol on buds that had not received adequate chilling hours and stimulate more research regarding this treatment. Ethanol treatments could be useful to growers with increased global warming, which may affect winter chilling fulfilment in perennial fruit crop production regions (Luedeling, 2012).

The results obtained in Experiment 3 (Figure 4, left) showed that ethanol sprays can have a positive impact on bud break in buds that did not experience enough chilling to release dormancy rapidly. After a cold episode, resulting in an additional 250–260 hours of chilling (Supplementary Figure 2), ethanol application provided no enhancement of the rate of bud break as there was no difference between the control and ethanol treatments in any cultivar (Figure, 4 right). Thus ethanol sprays need to be applied when the chilling requirement has not been met.

Ethanol is less toxic than cyanamides, and therefore it may have advantages for growers if proven to be an efficient solution to release dormancy. Ethanol after chilling fulfilment does not present the risk of bud and crop damage that has been shown with mistiming applications of cyanamides at typical production concentrations for grapevine (580 mM) (Or *et al.*, 1999). In peaches, the optimal concentration of hydrogen cyanamide to induce bud break was 125 mM (Siller-Cepeda *et al.*, 1992). Both studies reported phytotoxic effects of hydrogen cyanamide application (bud break inhibition or delay, and bud or stem damage) when it is not timed correctly relative to chilling fulfilment and bud break induction or was used at higher concentrations. The ethanol application is potentially less phytotoxic as the high concentration of 10 %, corresponding to a 2 M concentration, caused no phytotoxic effects even in fully chilled bud in these studies.

CONCLUSION

The ethanol sprays enhanced bud break and early shoot growth of different grape cultivars and in

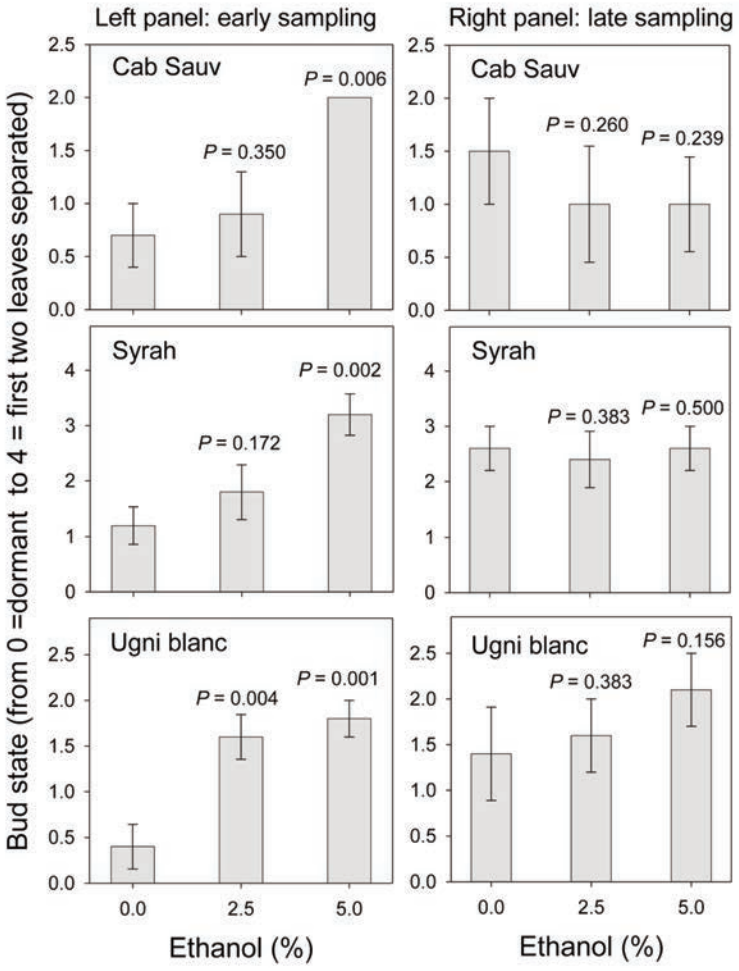

FIGURE 4. Bud stage as a function of the application rate of ethanol sprayed (aqueous solutions) on one-node cuttings of three *V. vinifera* cultivars.
Spraying was performed at the dormant stage, after transfer into the growth chamber. "Cab Sauv" stands for Cabernet-Sauvignon, the other cultivar names are not abbreviated. N = 5 individual node replicates. The Eichorn and Lorenz system was used to define the following ordinal scale: 0 = dormant bud; 1 = bud swell (E-L 2); 2 = woolly bud (E-L 3); 3 = rosette of leaf tips visible (E-L 5); 4 = one or two leaves separated (E-L 7–9). Pictures of bud stages are shown in Supplementary Figure 1.

different chilling conditions and training systems. These ethanol treatments may be a promising alternative to cyanamide bud break treatments. The means by which ethanol enhances bud break are not known; however, they may be related to ethylene and oxidative reactions (Shi *et al.*, 2018; Halaly *et al.*, 2008); but validation of these hypotheses requires functional genomic analyses. Further studies of cultivars under differing chilling fulfilment conditions in vineyards and controlled conditions should develop protocols for ethanol use to promote dormancy release in grapevines and other crops. The primary aim of this short communication is to stimulate future research.

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Two supplementary figures are available from the OenoOne website:
https://oeno-one.eu/article/view/2497

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