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(Z)-3-Hexenyl Butyrate Induces Stomata Closure and Ripening in *Vitis vinifera*

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Abstract: Agronomy solutions for modifying pre-harvest grape ripening are needed for a more sustainable viticulture. Field experiments were performed in *Vitis vinifera* L. vines to study the effect of the previously described stomata-closing compound (*Z*)-3-hexenyl butyrate (HB). Exogenous treatments at different doses were periodically carried out using a randomized block design. Firstly, we observed that HB was able to induce stomatal closure in grapevine plants. Under field conditions, the application of HB around veraison induced a higher color intensity in berries, and vines treated at higher doses reached this stage earlier than the un-treated controls. There was also a clear increase in both grape anthocyanin concentration and total soluble solids without having a negative impact on total yield. We therefore, confirm the role of HB as a universal natural stomatal closure compound and propose a new use for HB in viticulture as a ripening inducer, by accelerating anthocyanin accumulation.

Keywords: (*Z*)-3-hexenyl butyrate; stomata; ripening; *Vitis vinifera*

1. Introduction

Grapevine (*Vitis vinifera* L.) is one of the major crops worldwide, based on the number of cultivated hectares and its socio-economic value. Grape is a non-climacteric fruit that can be used as a table fruit, a dried raisin, and also for vinification and distillation. The onset of ripening, or veraison, is a key phenological stage in grapevine's lifecycle that involves a series of biochemical, physiological and organoleptic changes. Generally, this stage is characterized by berry sugar accumulation, organic acid level reduction, synthesis of anthocyanins and changes in texture, flavor compounds and aroma volatiles [1–3].

The grape berry is probably one of the fruits whose composition is most sensitive to the natural environment. In recent years, several environmental cues influencing berry ripening have been studied, such as light, water status, temperature and pathogens. High temperatures, shade or pathogenic attack impair ripening-associated mechanisms, while moderate water deficit, UV-B radiation or low temperatures positively promote veraison [4,5]. For this reason, winegrowers search for different viticultural practices to modulate ripening. Particularly in warm climates viticulture is nowadays required to better couple berry sugar accumulation with the phenolic maturity [6]. Consumers are also demanding wines with lower alcohol content. There are numerous researchers investigating how to modulate grape ripening with field practices [7]. These new agricultural practices could embrace a general strategic response or could be focused on specific threats, aiming for the short-term optimization

of grapevine development and growth, known as short-term strategies [8,9]. In this sense, a pre- and post-harvest application of different treatments has been effective in managing grapevine stress responses and modulating ripening. For example, exogenous treatments with different phytohormones such as abscisic acid (ABA), auxins, salicylic acid or gibberellins and specifically ethylene, promote changes in total phenol content and berry sugar, acidity and color [10–14]. Other types of treatments have also been studied, such as melatonin application, that could benefit phenolic content and antioxidant activity in the "Merlot" variety [15], or the application of humic acid or polyamines to increase leaf and berry pigments [16,17]. Regarding post-harvest treatments, ethylene is the most studied compound involved in ripening [18]. For instance, in Aleatico grape berries, post-harvest treatments with ethylene affect phenols, anthocyanins and the aromatic quality of both the grapes and the wine [19,20].

Vitis species are well adapted to drought conditions since they are able to survive even under severe soil water deficit because of their deep root system, capacity to carry out some tissue osmotic adjustment, efficient control of stomatal aperture and regulation of canopy growth [21,22]. Particularly in the Mediterranean Sea basin, grapevines are cultivated under conditions of water scarcity because of climate aridity and restricted water availability for irrigation. Vine stress tolerance depends on the vine cultivar [23]. Although this genotype-related tolerance involves different aspects, they are highly linked to differences in stomatal responses to abiotic stress [24]. Regulation of stomata closure is one of the best characterized plant physiological responses against different stresses. Stomata are in charge of controlling two main plant processes: photosynthesis and transpiration. Plants modulate stomatal closure to regulate CO_2 and water evaporation in response to many environmental and biochemical stimuli such as light, humidity or pathogenic attack. Across the diversity of plant species, stomata closure leads to lower stomatal conductance, reducing CO_2 uptake and subsequently limiting photosynthesis [25,26].

As stomata are major players in plant water use, the regulation of their closure is a potential target to optimize carbon assimilation efficiency and plant productivity. Exogenous treatments using inducers of stomatal closure may constitute an alternative way for the development of new phytosanitary products that improve not only the plant tolerance against both biotic and abiotic stresses but also the plant development. In this context, the volatile compound (*Z*)-3-hexenyl butyrate (HB) was identified in tomato plants as inducer of resistance against bacterial infection [27], and was patented for its ability to produce stomatal closure (P201730685), therefore, emerging as an excellent candidate for the sustainable control of stresses in agriculture. Moreover, the efficacy of HB has also been demonstrated in several plant species belonging to the *Arabidopsis*, *Medicago*, *Zea*, *Citrus* and *Nicotiana* genus, suggesting its role as possible universal natural stomatal closure agent [28].

In this work the role of the HB as an inducer of grape ripening that may act mimicking a moderate water deficit has been studied. For that purpose, open field experiments in grapevine crops were performed, using different HB concentrations and evaluating the vine's phenotype and different agronomic traits over time such as yield, fruit weight, color assessment or sugar and acid content.

2. Materials and Methods

2.1. Experimental Site Description, Plant Material and Maintenance

The trial performed to determine the effect of HB on the stomata aperture ratios was carried on potted Bobal grapevines (*Vitis vinifera* L. cv Bobal grafted onto 110R) at the IVIA (Instituto Valenciano de Investigaciones Agrarias, Valencia, Spain, 39°32′ N, 0°23′ W) experimental farm in July 2019. Both, un-fruited newly grafted vines (young) and 8 years old mature grapevines (old) were tested. Pots used for the young vines were of 9 L (0.23 m height and 0.25 m width). Mature vines were grown in seventy L pots (0.45 m height and 0.55 m width). In both cases, pots were filled with a mix of coco fiber substrate and compost.

An additional field trial was carried out in a commercial vineyard located in Alpera (Albacete, Spain, 38°57′ N, 1°13′ W). The experiment was conducted during August to September 2019 and the grapevine variety used was Garnacha tintorera (*Vitis vinifera* L. cv Alicante Bouschet grafted onto 161-49R). During the trial, average air temperature was 22 °C with a total precipitation of 80 mm and these conditions were similar to the historical data, except the precipitation in September which resulted to be higher (Table S1). The vineyard soil was sandy loam with flooding irrigation applied every 2 weeks. Neither pesticides nor fertilizers were used during the trial. Test site information and maintenance are shown in Tables S2 and S3.

2.2. Experimental Design and Treatment Application

In the potted cv. Bobal vines, three young and three mature vine plants were sprayed with 5 mM HB or 50 mM HB under open field conditions in order to determine the effect of HB on stomata aperture. In addition, three untreated plants were used as negative controls. Leaves were collected 24 h after treatments and stomata aperture was measured.

The second field trial was performed in a commercial vineyard and the experimental design used was a randomized block (27 m^2) designed with 6 plots with 6 plants in each one, with a 1.5 m of distance in the row and 3 m between the row. Six treatments were performed: four plots were treated with HB at 0.5, 5, 10 and 50 mM; one plot was treated with a commercial ripening inducer FRUITEL (48% Ethephon) and served as positive control; and finally, one control, untreated plot was used as a negative control. Four HB and FRUITEL applications were carried out using a motorized knapsack sprayer. The first application was done just before veraison (application A), and subsequent applications (B, C and D applications) were sprayed with a 7-day interval, since the effect of the compound has been described to persists from 7 to 10 days after the treatment in tomato plants [28]. Time units are referred as follows: X, DA, Y, being X day (0, 7, 14); DA Days After; Y day of treatment (A, B, C and D).

In this field trial, berry samples were randomly collected weekly after the second application (0 days after application B; 0 DA-B) to determine sugar content, pH, must acidity and color. Specifically, the anthocyanin, catechin and epicatechin content as well as the glucose, fructose, malic and tartaric concentrations were evaluated at 7 DA-D. At harvest (20 days after application D, that corresponds to a crop stage scale BBCH 89, the total yield and the number of bunches per plot were also determined.

2.3. Stomatal Aperture Measurement

Grapevine leaf impressions were obtained by coating the abaxial part of five randomly selected leaves from three independent grapevines, with a thin layer of nitrocellulose-based glue (Imedio, Bolton Group, Madrid, Spain) and peeling off the dried layer carefully. Epidermal strips were mounted on glass slides and observed under a Leica DC5000 microscope (Leica Microsystems S.L.U.) [28] Pictures of different leaf regions were analyzed using the NIH's *Image*J software. Stomatal aperture ratio was calculated as stomata width/stomata length in at least 50 stomata for each experimental vine. A value of 1 was considered for a totally opened stomata.

2.4. Color and Total Anthocyanin Content Assessment

To determine color differences, a total of 200 samples per plot at the same height from the ground were analyzed by using a visual scale from 0 to 10, being 0 light green berry, 10 totally purple berry and intermediate degrees within green and purple: yellow, red, light purple. For anthocyanin quantification, 100 g of frozen berry tissue was homogenized (100 mL of water were added to facilitate homogenization) filtered and centrifuged in order to obtain aqueous grape extracts. Total anthocyanin quantification was determined using a previously described method [29] using a modified pH differential method. Absorbance of 6 independent grape extracts (100 µL) was measured with a spectrophotometer at 510 and 700 nm, adding 900 µL of 0.025 M KCl at pH 1.0 and 0.4 M CH_3COONa at pH 4.5, respectively.

Absorbance readings were converted to total milligrams of cyanidin 3-glucoside per 100 g berry fresh weight using its molar extinction coefficient ($\varepsilon = 26900$ L mol^{-1} cm^{-1}).

2.5. Sugar Content Determination

Total soluble solids (° Brix) was determined by measuring the refraction index in the grape juice coming from 200 samples. Glucose and fructose content from 6 independent grape extracts was quantified by high-performance liquid chromatography (HPLC), using a Transgenomic ICSep ION-300 ion exclusion column (300 × 7.8 mm) coupled to a refractive index detector (Waters 2414) converting the refractive index units (RIUs) into an electrical signal (mV) [30]. The must was centrifuged, and the supernatant was isocratically eluted at 60 °C with 5 mM H_2SO_4 at 0.4 mL min^{-1}, over a total run of 30 min. The compounds were quantified with the Waters Breeze software using external standard curves converting mV into g/100 mL grape juice.

2.6. Grape Juice Acidity Analysis

The total acidity content was determined by titrating 5 mL of 200 independent grape extracts containing phenolphthalein, with a sodium hydroxide solution in continuously stirring until a specific pH end point was reached, indicated by pink color change. Malic and tartaric acids determinations from 6 independent grape extracts were quantified by HPLC as previously described (Section 2.5). The pH value was obtained directly from the extracted must using a pH-meter.

2.7. (+)-Catechin and (−)-Epicatechin Analysis

The determination of (+)-catechin and (−)-epicatechin was performed by mass spectrometry using a 1515 Waters HPLC binary pump, a 996 Waters photodiode detector (range of maxplot 240–400 nm, spectral resolution of 1.2 nm) and a ZMD Waters single quadrupole mass spectrometer equipped with an electrospray ionization ion source. Samples (20 µL) from six independent grape extracts were injected at room temperature into a reverse-phase Sun Fire 5-µm C18 (4.6 by 150 mm; Waters) column. A 20-min linear gradient of 1% (vol/vol) acetic acid (J. T. Baker) in Milli Q water to 100% methanol at a flow rate of 1 mL/min was applied. (+)-Catechin and (−)-epicatechin were quantified with Masslynx Waters software using authentic standards.

2.8. Statistical Analysis

To study the effect of the applied treatments for each assessment data, means were compared using Student–Newman–Keuls test ($p = 0.05$). Statistical analyses of two variables were performed by using Student's *t*-test. Statistical procedures were applied using the software ARM Revision 2019.

3. Results

3.1. Evalutation of HB Efficacy in Grapevine Plants under Open-Field Conditions

The capacity of HB as a natural stomatal closure compound was previously tested in different crops including tomato, tobacco, maize, citrus or alfalfa [28]. However, HB efficacy in the genus *Vitis* had not been tested yet. To study the effect of HB on stomata closure of grapevine plants, exogenous treatments at different concentrations were performed on vines at two different developmental stages: young grapevines and old grapevines.

As shown in Figure 1, in both young and old grapevines, the application of HB at a dose of 50 mM resulted in a statistically significant stomata closure at a. However, 5 mM HB treatments only resulted to induce statistical differences on the young grapevines.

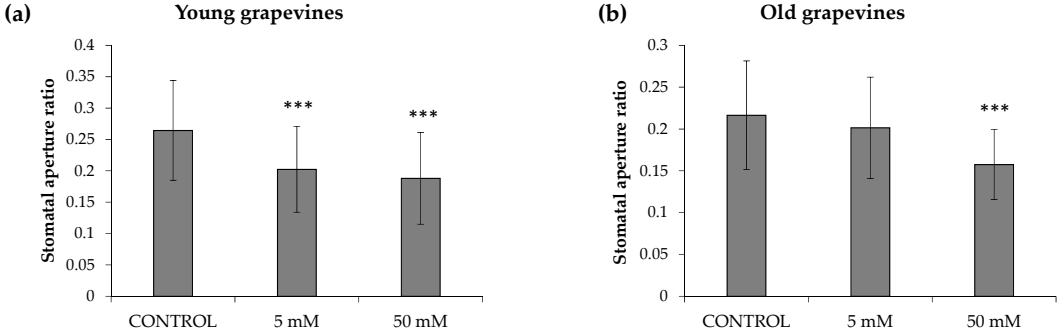

Figure 1. Effect of (*Z*)-3-hexenyl butyrate (HB) on stomatal aperture ratio of *Vitis vinifera* L. cv Bobal leaves. Stomatal aperture mean values ± SE are shown of one representative experiment. Leaves from young grapevines (**a**) and old grapevines (**b**) were collected 24 h after treatments at different HB concentrations. Asterisks (***) indicate statistically significant differences between control and HB-treated grapevine plants at each concentration with $p < 0.001$.

3.2. Study of the Effect of HB in Ripening and Color Assessment

During ripening, a change of berry color is produced as a consequence of anthocyanins accumulation [31,32]. To study the phenotypical effect of HB-mediated stomata closure on grapevine plants, the color development of berries was firstly analyzed, by using a color scale adapted from Fernández-López et al. [32] (Figure 2a). Differences in berry color in HB-treated grapevine plants with regard to non-treated plants appeared since 0 DA-C, when almost all HB-treated plots showed a higher color intensity in berries. The positive effect of HB on berry color increased along the experiment, even surpassing the color change induced by FRUITEL which was used as the positive control. This was particularly noticeable at 7 DA-D, when statistically significant differences were observed using 5 mM HB. At this time, the increase in the color scale for HB treatments ranged within 11% to 29%, for 0.5 mM and 5 mM treatments, respectively. These results were in line with the number of days at which each treated plot reached veraison. Most of the treatments with the volatile compound hastened this process (Figure 2b). Interestingly, both 10 mM and 50 mM HB-treatments reached effects comparable to those produced by the commercial compound FRUITEL.

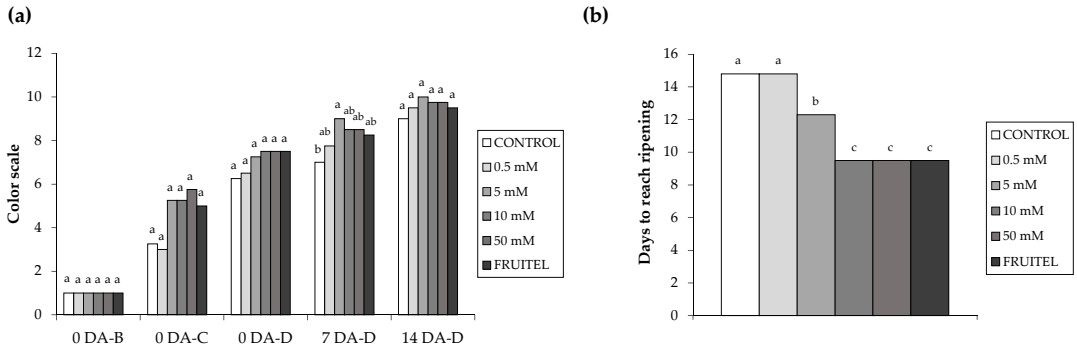

Figure 2. Color change of *Vitis vinifera* L. cv Alicante Bouschet berries of non-treated (CONTROL) plants, HB weekly-treated plants at different concentrations (0.5, 5, 10, 50 mM) and plants treated with the ripening inducer FRUITEL. (**a**) Evolution of color of non-treated, HB-treated and FRUITEL-treated plants. (**b**) Number of days until CONTROL, HB and FRUITEL treated plants reached ripening. An ANOVA test was performed, and different letters indicate statistical significances with a *p*-value < 0.05.

3.3. Study of Phenolic Compounds Accumulation Induced by HB Treatments

In grapevine, anthocyanins' accumulation initiates at veraison, and this is highly correlated with the change of berry color during ripening [31,32]. Total anthocyanin content was analyzed in samples corresponding to 7 DA-D and 10 and 50 mM treatments, in which we observed the greater effect in

berry ripening (Figure 2a,b). Total anthocyanin content (Figure 3) was significantly and much higher in treated plants, which also correlates with the advanced color development in berries of HB-treated plants previously observed (Figure 2a).

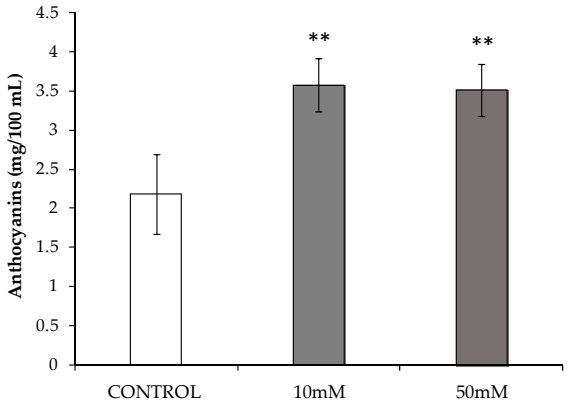

Figure 3. Total anthocyanin content was measured as mg of cyanidine-3-glucoside in 100 mL of grape juice from CONTROL and HB-treated *Vitis vinifera* L. cv Alicante Bouschet plants. Samples were collected at time 7 DA-D. Asterisks (**) indicate statistically significant differences between control and treated plants at each concentration with $p < 0.01$.

Different monomeric-3-flavonols, as (+)-catechin and (−)-epicatechin, are also involved in grapevine ripening, but in this case, these polyphenols decline rapidly at veraison [33]. As expected, (+)-catechin and (−)-epicatechin content was lower in berries of plants treated with a higher dose of HB, corroborating the effect of this compound on phenolics associated with the ripening phenomenon. However, no statistically significant differences were observed between control and treated plants (Figure S1).

3.4. Sugar Accumulation Determination

To further analyze the effect of HB in berry ripening, we evaluated sugar accumulation in berries, a well-described physiological process in ripening. Slight differences between treatments were observed during ripening, reaching levels comparable to those produced by FRUITEL. However, no significant differences were found in any treatment, including those performed with FRUITEL at any time studied (Figure 4a).

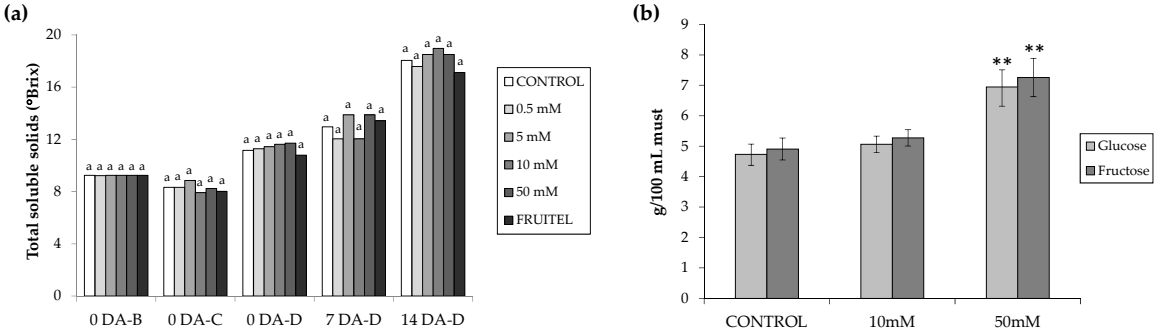

Figure 4. Effect of HB treatments on the sugar content of *Vitis vinifera* L. cv Alicante Bouschet berries. (**a**) Total soluble solids (° Brix) of non-treated (CONTROL) plants, HB weekly-treated plants at different concentrations (0.5, 5, 10, 50 mM) and plants treated with the ripening inducer FRUITEL. (**b**) Glucose and fructose content in must of berries from CONTROL and HB-treated plants at 7 DA-D. An ANOVA test was performed, and same letter indicates no statistical significances with a p-value < 0.05. Asterisks (**) indicate statistically significant differences between control and treated plants at each concentration with $p < 0.01$.

The must glucose and fructose concentrations were analyzed by high performance liquid chromatography. For this purpose, we evaluated 10 and 50 mM HB treatments, which seemed to display the most promising results regarding berry ripening. In this case, the must of the berries treated with HB at 50 mM showed significant differences in levels of glucose and fructose with regard to non-treated plants (Figure 4b).

3.5. Acidity Evaluation

Another key process in ripening is the reduction of organic acid concentration; therefore, both total acidity values and pH were evaluated. Regarding total acidity content, differences were observed from the first treatment, although no statistical differences between control and HB-treated plants were found (Figure 5a). In addition, no significant differences were observed in malic nor tartaric acid accumulation (Figure S2). However, HB-treated grapevine berries presented statistically higher pH values than non-treated plants at 7 DA-D, when HB treatments at higher concentrations reached similar pH values than FRUITEL (Figure 5b).

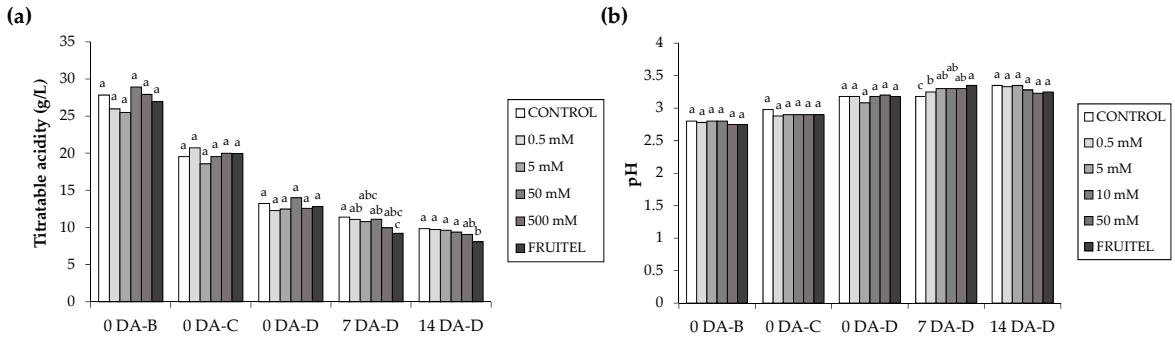

Figure 5. Acid content evolution of *Vitis vinifera* L. cv Alicante Bouschet berries of non-treated (CONTROL) plants, HB weekly-treated plants at different concentrations (0.5, 5, 10, 50 mM) and plants treated with FRUITEL. (**a**) Changes in total acidity content and (**b**) pH of berries of non-treated, HB-treated and FRUITEL-treated plants. An ANOVA test was performed, and different letters indicate statistical significances with a p-value < 0.05.

3.6. Effect of HB Treatments in Crop Level and Yield

The application of HB, even at the highest dose did not affect the grapevine yield performance (Table 1).

Table 1. Yield of *Vitis vinifera* L. cv Alicante Bouschet vines of non-treated (CONTROL) plants, HB weekly-treated plants at different concentrations (0.5, 5, 10, 50 mM) and plants treated with the ripening inducer FRUITEL. An ANOVA test was performed. The letter "a" indicates that no statistically significant differences were observed.

Treatment	Yield (t/ha)
CONTROL	19.72 [a]
0.5 mM	19.41 [a]
5 mM	20.53 [a]
10 mM	19.90 [a]
50 mM	19.91 [a]
FRUITEL	19.80 [a]

4. Discussion

The impacts of climate change are reducing the capacity of natural resources (biodiversity, soil and water) to sustain the food demand of the world's increasing population. Predictable increases in

temperature, changes in extreme weather events and precipitation patterns, as well as reduction in water availability may result in reduced agricultural productivity. This has prompted scientists to develop new crop varieties or manipulate potential targets in order to obtain more stress-resistant crops [34]. Stomatal closure is among the earliest of the plant responses to the changing environment, so stomata could be considered as potential targets for manipulation to improve plant productivity in stress conditions. We have previously identified a volatile compound emitted by infected tomato plants named as HB, which induces stomata closure in different plant species [27,28]. In the present research, HB treatments were effective in grapevines, confirming its role as a universal natural stomata closer agent (Figure 1). Interestingly, this volatile resulted to be more effective on young plants since stomatal conductance depends on leaf and plant age mainly due to differences in biochemical limitations [35,36]. Other exogenous treatments have also been reported to induce stomatal closure in *Vitis vinifera* L. plants. For instance, treatments with the well-described phytohormone involved in stomatal closure, ABA [37]; or exogenously applied sugars such as sucrose, maltose, trehalose and trehalose-6-phosphate induced a significant closure of stomata in a dose-dependent manner [38]. Moreover, the effects of pinolene as an anti-transpirant on a grapevine has also been described [39,40]. However, to our knowledge, this is the first time that a natural volatile compound is described to produce this effect on *Vitis vinifera* L. plants.

The obtained results might have implications for improving crop performance under soil–water deficit. In semi-arid climates, tools for improving water use efficiency (WUE) are needed and, for instance, deficit irrigation is often employed as a tool to increase the intrinsic leaf gas exchange (i.e., leaf photosynthesis by stomatal conductance) since normally the relationship between stomatal conductance and photosynthesis is curvilinear [41]. The exogenous application of HB could be used to stimulate stomatal closure reducing water use in those periods of time when soil water content might severely limit plant performance. However, it should be considered that a reduction in stomatal conductance could impair leaf photosynthesis rates. The potential effects of HB application on the overall vine source capacity and the carbon partitioning within the vines should be investigated in order to determine the long-term effects of HB applications on vine vegetative growth, crop water use and longevity and productivity.

Once the role of HB as a stomata closer was confirmed in the grapevine, we studied the impact of this effect on different processes, since it was also demonstrated that HB treatments in tomato plants induced resistance against *Pseudomonas syringae* [28]. Phenotypically, grapevine plots treated regularly with HB at different concentrations showed higher color intensity in berries, comparable to, or even higher than, those plots that were treated with the ripening inducer FRUITEL (Figure 2a). This change in color development also correlated with the number of days on reaching veraison (Figure 2b), proposing a new role for HB as a ripening inducer. Interestingly, effects produced by 10 mM and 50 mM treatments were very similar, thus indicating a saturation of the effect on the color, probably related with saturation on the stomata closure. Other ripening-associated phenomena, such as phenolic content, were also analyzed. Total anthocyanin content was significantly higher in HB-treated plots at 10 and 50 mM, with respect to non-treated plants at 7 DA-D (Figure 3), which properly correlates with the effect of the volatile in color development. Moreover, the decrease of monomers of 3-flavan-3-ol after veraison was also confirmed in 50 mM HB treated plants, which showed reduced content of (+)-catechin and (−)-epicatechin in berries, being the observed differences that were statistically no significant (Figure S1). The presence of these tannins also participates in wine organoleptic properties, influencing astringency and bitter perception. Higher amounts of (+)-catechin and (−)-epicatechin lead to higher intensity of oral astringency and bitterness [42]. Color development and tannin content are important in the maturity index of many fruits. To further study the role of HB in grapevine ripening, we analyzed other parameters involved in fruit ripening. It is well known that, at veraison, berry sugar contents rise while acid levels decrease. The balance between sugars and acids is considered one of the main aspects of grape and wine quality. HB treatments increased glucose and fructose content, especially at higher concentrations (Figure 4), whereas no significant differences were observed in acid contents (Figure 5 and Figure S2). However, the musts obtained from HB-treated grapevines at

7 DA-D showed statistically higher pH values than the musts of non-treated plants, reaching similar pH values than FRUITEL treatments when applied from 5 mM onwards. Several studies have described a relationship between the stomata aperture and the sugar accumulation in the grapevine. In this sense, Kaolin has been described to increase anthocyanin content without altering the sugar content, and the anti-transpirant pinolene reduced both the anthocyanin and sugar contents [39,43–45].

Exogenous pre-harvest treatments with different compounds have been widely explored and similar results have been observed. For instance, nitrogen application in grapevines delays berry sugar accumulation during ripening, but increases anthocyanin content, enhancing wine color [46]. Phytohormonal control has also been suggested as another strategy since hormones such as ABA, ethylene, brassinosteroids or gibberellins provide many signals for the onset of ripening [4,47,48]. Other compounds known as elicitors such as benzothiadiazole, methyl jasmonate, chitosan and yeast extract, have demonstrated their efficacy for increasing grape phenolic content [49–51]. HB treatments could somehow be activating ethylene biosynthesis in grapevines, therefore promoting the anthocyanin accumulation in a similar manner to the positive control (FRUITEL; [52]). Future experiments to test the possible induction of genes involved in the ethylene biosynthesis, such as *ACS* or *ACO*, will result in great interest.

In this study, we present HB as a new natural product, whose application requires practically no equipment, which acts as a stomata closing agent promoting grapevine ripening under open-field conditions. The clear increase in grape anthocyanin content in response to the HB treatment without any clear effect on grape total soluble solids is considered a very positive result for wine making and particularly for wine aging purposes. For premium wine quality, grapes need to be harvested at high sugar concentration in order to reach full grape phenolic maturity. This results in wines with too high an alcohol content. Our results suggest that HB applications could be used as a field practices to clearly increase grape color potential. Further investigation is required in other grapevine varieties, in addition to the Alicante Bouschet tested here, which has the peculiarity to accumulate anthocyanin both in the berry skin and pulp tissues. It would be also interesting to further study the effect of HB application volatile against other biotic grapevine stresses related to stomata control, such as the downy mildew. Since *Plasmopara viticola* uses stomata to penetrate into leaves [53], HB treatments could limit the entrance of the pathogen. Finally, a metabolomics study of the volatile organic compound profiles of control and HB treated grapes would result also of great interest.

5. Conclusions

In this study, we confirm the role of HB as both a natural stomatal closure compound, and a ripening inducer which accelerates anthocyanin accumulation under open-field conditions without having, in the short-term, a negative impact on yield. Therefore, this volatile could be used to control the physiology of ripening in *Vitis vinifera* L. by promoting veraison. Broadly, HB could also act as a phytoprotector for the sustainable control of abiotic stresses in agriculture. The present research paves the way to further longer-term studies determining the potential effects of HB application during different periods of the vine development and as a possible tool to mitigate the effects of severe soil water deficit on vine performance.

6. Patents

The compound (*Z*)-3-hexenyl butyrate has been patented by the Spanish Patent and Trademark Office (P201730685) and licensed by Químicas Meristem SL (Moncada, Valencia, Spain).

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/8/1122/s1, Figure S1. Catechin and epicatechin content of must from Vitis vinifera berries of CONTROL and HB-treated plants Figure S2. Malic and tartaric acid content of must from Vitis vinifera berries of CONTROL and HB-treated plants Table S1. Conditions during the trial. Table S2: Test site information. Table S3. Maintenance.

Author Contributions: The work herein presented was carried out with the collaboration of all the authors. J.M.B. defined the research theme. C.P., D.S.I., I.R., M.P.L.-G. and P.L. carried out the laboratory experiments. M.P.L.-G.,

P.L. and I.R. contributed to the experimental design and interpreted the data. C.P. drafted the article. J.M.B., M.P.L.-G., D.S.I.; P.L. and I.R. participated in revising it critically for important intellectual content. All authors have read and agreed to the published version of the manuscript. Each author has participated sufficiently in the work to take public responsibility.

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