UNCOVERING THE EFFECTIVENESS OF VINEYARD TECHNIQUES USED TO DELAY RIPENING THROUGH META-ANALYSIS

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Abstract:

Context and purpose of the study – One of the most concerning trends associated with increasing heat and water stress is advanced ripening of grapes, which leads to harvesting fruit at higher sugar concentrations but lacking optimal phenolic (i.e. color and mouthfeel) and aromatic maturity. Mitigation techniques for this phenomenon have been studied for many years and practices to delay sugar accumulation have been identified, including antitranspirants, delayed pruning and late-source-limitation techniques. Evaluation of the efficacy of these vineyard practices has occurred across a wide range of environments, vintages, varieties and growing conditions. To assess the broader efficacy of these three vineyard practices, which are easy-to-implement and cost-effective, a meta-analytic approach was adopted using data retrieved from 43 original studies.

Material and methods – A systematic review of published studies on techniques to delay ripening was conducted searching several databases (Web of Science, Pub Med and Google Scholar). This initial database was further screened, and inclusion/exclusion criteria were applied, keeping only studies that met the prerequisites for meta-analysis. Effect size values were calculated as the difference between total soluble solids (TSS) in the control and treated groups to numerically express advanced ripening (negative effect size), delayed ripening (positive effect size) as well as the intensity of the delay. Standard errors were calculated for effect size values and used as a proxy for study accuracy. Forest plots were employed to calculate the average effect of each treatment and associated confidence intervals. Meta-regression models were built to identify relationships between effect size values and important viticultural parameters, including environmental and growing conditions.

Results – Data curation returned 242 effect size values for three practices proposed to delay ripening: antitranspirants (n = 102), delayed pruning (n = 45) and late source limitation (n = 56). Average effects for all treatments were significant, confirming the stability of treatment effects for the vineyard practices investigated. Factors impacting the effectiveness of the treatments were identified through meta-regression. For antitranspirants, the intensity of the delay was dependent on the active compound utilized as well as the timing of spraying. Late pruning was more effective when applied at later stages of apical bud development, and the ability to delay ripening adopting this approach was dependent on vine yield. Similarly, yield was an important parameter impacting the efficacy of late source limitation practices, which also resulted in larger delays when grapes were harvested at higher sugar concentrations. This study shows the usefulness of meta-analysis to demonstrate broader applicability of specific studies through a comprehensive, quantitative and fully data-driven analysis.

Keywords: antitranspirants; delayed ripening; late pruning; meta-analysis; source limitation; sugar accumulation.

1. Introduction

A changing climate is impacting grapevines growth and development, with consequences from the physiological level to yield and fruit quality (Jones and Webb, 2010). One of the traits that has been consistently associated with elevated temperature, increasing CO_2 levels and severe water stress is advanced ripening (Jones *et al.*, 2005). This phenomenon is not only the result of an earlier onset of the vegetative cycle, which may translate in early harvest, but also occurs due to more favorable conditions for sugar accumulation, a key feature of grape ripening (Cameron *et al.*, 2020). Faster sugar accumulation is not necessarily detrimental *per se*, although it translates into higher potential alcohol that may need to be addressed with remediation techniques but is also accentuated by a decoupling between sugars and other metabolites important for wine quality, such as organic acids, phenolic and aroma compounds (Previtali *et al.*, 2021; Sadras and Moran, 2012; Sweetman *et al.*, 2014).

Over the past two decades, mitigation techniques for rapid sugar accumulation have been thoroughly investigated, and recent qualitative reviews have attempted to summarize a large number of experimental trials conducted worldwide (Gutiérrez-Gamboa *et al.*, 2021; Palliotti *et al.*, 2014). Due to the unique environmental and growing conditions in which individual studies were executed, it is difficult to provide an estimate of treatment effects, which is a point of failure in qualitative reviews. Techniques of meta-analysis have been used in other fields of research to address this problem by applying sets of statistical procedures to quantitative summarize results of primary studies (Cuijpers, 2016; Philibert *et al.*, 2012). In our study (Previtali *et al.*, 2022), we applied meta-analysis to all publicly available experiments on vineyard techniques to delay ripening in an attempt to (1) establish average effects and (2) draw links between environmental and growing conditions and the intensity of treatment effects.

2. Material and methods

Data collection and curation – Database searches were performed in the main scientific databases (Web of Science, PubMed, Google Scholar) to collect primary research reports between 2000 and 2020. The results were carefully screened by applying rigorous inclusion and exclusion criteria to validate the scientific soundness of primary studies as well as to meet the requirements for meta-analysis. Data curation led to 43 studies qualifying for meta-analysis. A new variable was created to represent the intensity of the treatment effect, called effect size (ES) and calculated as the difference in TSS between control and treated groups on the day of harvest. Using this new variable, the direction of the effect was represented (**Figure 1**), with negative and positive ES values signifying advanced and delayed ripening respectively and null ES values representing no effect. In addition, absolute values represented the intensity of the effect. Standard error values were also recorded as a proxy for study accuracy and other variable of interest (i.e., environmental conditions, growing practices) were added to the database. In total, 242 ES values were collected in the database.

Meta-analysis – Meta-analysis was applied to sets of data related to three treatments: antitranspirants (AT), delayed pruning (DP) and late source limitation techniques (LSL). Forest plots (Cuijpers, 2016) were used to estimate the average ES value by treatment, and confidence intervals (CI) were used to define treatment effectiveness to delay ripening.

Meta-regression – Techniques of meta-regression were applied for the three treatments. Models were created where the response variable was the effect size and experimental and environmental variables were used as explaining variables. Models were screened by combining mathematical approaches such as Akaike Information Criterion (AIC) and meaningfulness from a viticultural standpoint. Significance of random and fixed effects was assessed to obtain the final model and estimates for significant terms (quantitative and qualitative) were extracted.

Statistical analysis – Data were collected from primary studies using Microsoft Excel (2020). Statistical analysis, including forest plots and meta-regression, was performed using R (R, Foundation for Statistical Computing, Vienna, Austria) version 4.0.5 in RStudio (RStudio Inc., Boston, MA, USA).

3. Results and discussion

3.1. *Effectiveness of vineyard practices to delay ripening*

Meta-analysis techniques account for study-to-study variation, improving the quality of estimated treatment effects (Cuijpers, 2016). For this reason, meta-analytic tools are utilized to test treatment stability across different environments or other experimental conditions. In our meta-analysis, we utilized forest plots to estimate the average treatment effect for three techniques to delay ripening: antitranspirants, delayed pruning, and late source limitation. It must be noted that other techniques have proven successful in delaying sugar accumulation in grapevines, such auxin application, phloem-restriction by girdling cluster peduncles or late season irrigation (Gutiérrez-Gamboa *et al.*, 2021; Palliotti *et al.*, 2014). However, the three treatments selected for this study had the largest sample size (n ≥ 35) and were best suitable for applications at the commercial scale. The distribution of ES values by treatment were as follows: antitranspirants (n = 102), delayed pruning (n = 45) and late source limitation (n = 56). Significant estimates and CIs > 0 were found for all treatments, confirming the overall effectiveness of these practices to delay ripening (**Table 1**). Predicted average ripening delays were 0.74 °Brix (CI: 0.54-0.94) for AT and 1.57 °Brix (CI: 1.14-2.00) for DP. As for LSL techniques, which includes late defoliation and late shoot trimming, predictions were extracted both combined (1.16 °Brix, CI: 0.88-1.45) and for separated treatments: late defoliation (1.04 °Brix, CI: 0.80-1.29) and late shoot trimming (1.51 °Brix, CI: 0.57-2.44).

3.2. *Unraveling factors affecting the efficacy of the treatments investigated*

Meta-regression helps to uncover important factors affecting the efficacy of a treatment. This statistical procedure was applied to the database of delayed ripening observations. Separate models were fitted for each treatment and similarly explanatory variables were treatment-specific and based on known or expected viticultural and environmental factors. Significant factors for each treatment are reported in **Table 2** and explained in more details below.

3.2.1. Antitranspirants

The rationale of using antitranspirants to delay sugar accumulation is the ability of certain chemical compounds to limit the rate of vine transpiration (Palliotti *et al.*, 2014). This may happen directly by decreasing photosynthetic activity, reducing sucrose available for transportation to the fruit, and/or indirectly by limiting berry transpiration which drives TSS increases by a concentration effect. It was shown that the choice of the active compound as well as the timing of spraying are key factors in defining the intensity of the ripening delay (**Table 2**). By applying meta-analysis techniques, it was possible to fully compare the efficacy of two different active compounds, namely kaolin and di-1-*p*-menthene (also called pinolene). The AT model showed that di-1-*p*-menthene was more effective compared to kaolin, leading to significantly larger ripening delays, on average 1.3 °Brix higher than kaolin. Additionally, the delaying power of AT was increased when these compounds were applied close to veraison (+0.5 °Brix) compared to early applications at fruitset, which can be alternatively achieved by combining an early and late application, such as flowering and veraison (+1 °Brix compared to single application).

2.3.2. Delayed pruning

Vineyard pruning is a necessary practice to regulate vine growth and achieve the targeted production goals. In contrast to the traditional pruning performed during vine winter dormancy, it has been shown that postponing pruning activities until after budburst can be used to delay fruit ripening (Palliotti *et al.*, 2014). The foundation of delayed (or late) pruning is vine acrotony, whereby apical buds are the first to develop on a cane (Bangerth, 1989). By pruning vines after the apical buds have developed, late pruning takes advantages of the growth inhibition on basal buds carrying the production for the current year. Meta-regression of DP data showed the effect of two main factors on the efficacy of this technique (**Table 2**), namely the pruning stage and vineyard yield potential. Pruning at later stages of apical bud development correlated to larger ripening delays (up to 4 °Brix when pruning was executed at BBCH 18), which was explained by more resources allocated for the development of apical buds and less for basal ones destined to production. Additionally, it was shown that late pruning was consistently more effective in low-yielding vineyards (< 2.5 kg/m of cordon).

2.3.3. Late source limitation

Another technique to slow down sugar accumulation that has been tested thoroughly aims to reduce the production of phtosynthates by the canopy. This can be achieved by late apical defoliation, where leaf removal is targeted to the portion of the canopy above the fruit zone, or late shoot trimming. In both cases, a portion of the apical-to-the-bunch leaves is removed, which includes younger leaves that display the highest rate of photosynthesis close to and during ripening (Poni and Intrieri, 2001). Meta-analysis of LSL data identified three significant factors (**Table 2**): grape maturity level at harvest, yield as well as the effect on yield. Late defoliation and late shoot trimming were found to be equally effective at slowing down sugar accumulation. Significant delays to ripening (i.e. ES values > 0) were achieved by late source limitation when grapes were harvested at 23 °Brix or higher, which suggests this technique is better suited for red grapes commonly harvested at or after this maturity level. Best results were also observed in high yielding vines with yield > 2.5 kg/m of cordon, possibly due to an additional effect of crop load on ripening. Positive ES values were also correlated with negative effects on yield, indicating that this technique can only delay ripening if the treatment does not affect yield or increases it.

4. Conclusions

While meta-analysis is gaining momentum in agronomic research, we have performed a fully data-driven meta-analysis of 53 studies and 242 observations of delayed ripening. This study exemplifies how the powerful tool of meta-analytic techniques can serve to further elucidate treatment effects alongside treatment stability across environments and growing conditions that are unique in each primary study. In addition, meta-regression can help to depict trends between treatment effects and variables of interest, the knowledge of which can aid growers' decisions to maximize the effectiveness of the management practices they implement.

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Tables and Figures

Table 1: Meta-analysis of vineyard practices to delay sugar accumulation in grapes.

	Sample size (n_{ES})	ES (°Brix)	CI-95%
Antitranspirants	102	0.74	0.54 - 0.94
Delayed pruning	45	1.57	1.14 - 2.00
Late source limitation	56	1.16	0.88 - 1.45
Late defoliation		1.04	0.80 - 1.29
Late shoot trimming		1.51	0.57 – 2.44

Abbreviations: ES, effect size; CI, confidence interval.

Table 2: Significant factors affecting the efficacy of management practices to delay sugar accumulation in grapes.

	Regression coefficient	*p*-value
Antitranspirants		
Chemical compounds (kaolin)[a]	-1.296	**< 0.0001**
Timing of spraying[b]		
Post-fruitset	0.284	0.347
Bunch closure	-0.010	0.982
Pre-veraison	0.508	**0.007**
Veraison	0.164	0.695
Second application	0.901	**< 0.0001**
Delayed pruning		
Pruning stage (BBCH)[c]		
05 (bud swelling)	1.624	0.081
09 (budburst)	1.543	**0.033**
13 (2-3 leaves expanded)	2.803	**< 0.001**
18 (7-8 leaves expanded)	4.265	**< 0.0001**
Yield[d]	-2.624	**< 0.0001**
Late source limitation		
$\mathbf{TSS_{Control}}$	0.279	**< 0.001**
$\mathbf{ES_{Yield}}$	-0.804	**< 0.0001**
$\mathbf{Yield_{Control}}$	0.729	**< 0.0001**

Abbreviations: ES, effect size

Notes:

[a] The reference category for chemical is di-1-*p*-menthene, with coefficient set to 0

[b] The reference category for timing of application is pre-flowering, with coefficient set to 0

[c] The reference category for pruning stage is BBCH 01 (late winter dormancy), with coefficient set to 0

[d] Yield was log transformed to fit an inverse relationship with ES

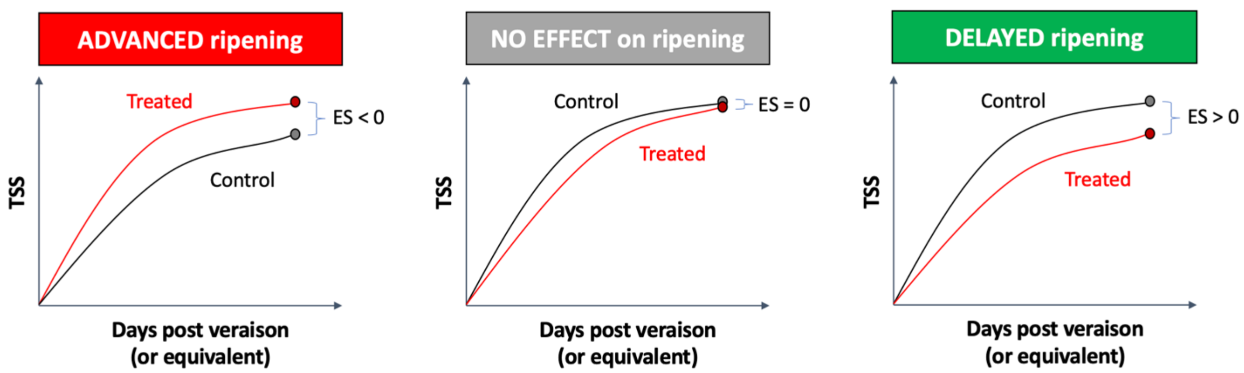

Figure 1: Creation of a new variable (effect size, ES) from Total Soluble Solids (TSS) values reported in original research on techniques to delay ripening. Positive, negative and null ES values represent delayed, advanced and

no effect respectively, and the absolute value reflect the intensity of the effect. ES values were calculated as $TSS_{Control}$ - $TSS_{Treated}$ on the harvest date.