**Extended abstract template for SSO only**

**MODELING THE IMPACTS OF WEATHER AND CULTURAL FACTORS ON ROTUNDONE CONCENTRATION IN A COOL CLIMATE**

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**Introduction**

**(Briefly review the pertinent literature and indicate the need for the research. Maximum 500 words)**

Aroma impact compounds and their interactions are an essential component of wine quality as they can contribute to pleasant and unpleasant wine sensory attributes. The recent aroma impact compound identified in grapes in the sesquiterpene rotundone (C12H22O), which is responsible for the key “black pepper” aroma of Shiraz (*Vitis vinifera*) wines (Wood et al., 2008). Rotundone is a strong aroma compound with a sensory detection threshold of 16 ng/L in red wine (Wood et al., 2008). It accumulates mainly within the berry exocarp beginning at the onset of fruit ripening (i.e., veraison) until harvest (Zhang et al., 2016). Since its first extraction from Shiraz grapes and wine, it has been identified in other red-fruited *Vitis vinifera* varieties across many wine-producing regions (Wood et al., 2008; Caputi et al., 2011; Geffroy et al., 2014) and to a lesser extent in white-fruited V. vinifera varieties (Caputi et al., 2011). Most recently, rotundone was extracted from grapes and wine of a red-fruited Vitis interspecific hybrid variety, Noiret (Homich et al., 2017).

It is still unclear why some grapevine varieties produce rotundone either at low or high concentration while others do not. To date, studies mainly focused on identifying environmental factors responsible for rotundone accumulation in the fruit which will ultimately influence the ‘peppery’ intensity of the wine (Geffroy et al., 2014; Zhang et al., 2015; Bramley et al., 2017). Rotundone concentration in *Vitis vinifera* and hybrid varieties was positively associated with cooler temperatures (i.e., cool vintages or cool sites; Homich et al., 2017). Grapes grown in shade, whether due to vineyard row orientation, cluster position within the vine canopy, or berry position within an individual cluster, had higher concentrations of rotundone when compared to grapes grown with higher solar exposure (. The increased rotundone concentrations were attributed to the negative, direct effects of solar radiation, to the increased temperatures of berries with high sun exposure, or their combination.

Less clear is the influence of cultural practices on rotundone accumulation. Fruiting zone leaf removal, a popular canopy management strategy, has been the most studied because it influences fruit sun exposure and temperature, but contrasting effects on rotundone accumulation were reported depending on timing and severity of its application (Geffroy et al., 2014; Homich et al., 2017). Environmental and viticultural factors might operate in tandem to determine rotundone concentration in the fruit and ‘peppery’ intensity of the wine. Understanding the relative importance of these variables on rotundone concentration may help clarify which sites or viticultural management methods are more conducive to producing wines with a desired level of pepperiness.

**Research Objectives**

**(Clearly state the purpose of the study. Maximum 150 words)**

The objectives of this two-year study were to identify the key climatic and viticultural variables that influence rotundone concentration in Noiret grapes using seven vineyards with varying weather conditions; and to investigate the relationships between fruit sunlight exposure, berry temperature, and rotundone accumulation in Noiret grapes at harvest. More broadly, this work provides insights into how multiple regression statistical methods can be used within a horticultural context to assess relationships between factors associated with both plants and the environment to identify which factors may be of the utmost importance for the topic at hand.

**Material and methods**

**(Briefly state the methodology with sufficient references. Use subtitles if necessary. Maximum 750 words)**

*Description of experimental sites*

The study was conducted in 2016 and 2017 at seven Noiret (*Vitis* hybrid cross of NY65.0467.08 and Steuben) vineyards located in the U.S. states of Pennsylvania and New York. At each vineyard, two panels (i.e., two sections of two-post spaces) of three or four contiguous vines (1.83-2.70 m long row each) were selected for data collection. The two experimental units were randomly assigned to either a control (C; fruiting zone non-defoliated) or fruiting zone leaf removal treatment (LR). Fruiting zone leaf removal was used to maximize the range of temperatures and cluster sun exposure across sites to better assess relationships between rotundone concentration and these micrometeorological factors, rather than to assess differences between C and LR treatments, as the treatments were not replicated at any site.

*Site-specific weather conditions*

Vineyard air temperature, rainfall, and photosynthetically active radiation (PAR) were recorded at 15-minute intervals with HOBO® weather sensors and dataloggers. As the weather stations only measured solar radiation from 400 to 700 nm (PAR), linear regression was used to develop a model to estimate solar radiation from PAR measurements. Several mesoclimatic (i.e., site specific) parameters were calculated for each site. Growing degree days (GDD) and total full-sun hours (SH) were calculated for each site.

*Fruiting zone weather conditions*

At each site wireless temperature data loggers (iButton Fob, Model DS9093Fl, Embedded Data Systems, Lawrenceburg, KY) were used to record air temperature at 20-minute intervals. Berry temperature was measured during fruit ripening at site 1 from September 16 to October 5, 2017, on two randomly chosen clusters from each experimental unit; linear regression was used to fit berry flesh temperature data to the air temperature data for both the LR and C treatments so that regression equations were used to estimate berry temperature for all sites for both seasons. Berry temperatures were used to calculate degree hour (DH) indexes, defined here as the percentage of hours the fruit temperature was within pre-defined intervals from veraison-to-harvest (Zhang et al., 2015).

Enhanced point quadrat analysis (EPQA; Meyers and Vanden Heuvel, 2008) was performed to assess canopy density and fruiting zone sunlight penetration. Characteristics related to canopy density and fruit sunlight exposure were then analyzed using Canopy Exposure Mapping Tools (v. 1.7, freeware from J.M. Meyers, Cornell University, Ithaca, NY; Meyers and Vanden Heuvel, 2008). The software was used to calculate leaf and cluster flux availability (LEFA and CEFA, respectively), which represents the percentage of the above-canopy photo flux that reaches a leaf or cluster, respectively.

*Vine characteristics*

At harvest, total number of clusters per experimental unit was counted and weighed. Twenty clusters were randomly collected from each experimental unit, stored at -20 °C, and later used for berry weight, chemical composition, carbon isotopic composition, and rotundone quantification analyses.

Grapevine nutrient status was determined by leaf petiole analysis at veraison 2016 and 2017.

A 200-berry sample was randomly taken from the frozen clusters collected at harvest for each experimental unit to assess vine water status via carbon isotope composition (δ13C) analysis (Gaudillère et al., 2002).

*Fruit chemistry and rotundone analysis*

In both years and for each experimental unit, fruit chemical composition data (total soluble solids [TSS], pH, and titratable acidity [TA]) were measured on a randomly selected 100-berry sample selected from the frozen clusters collected at harvest. Berry processing for rotundone extraction and analysis followed the protocol used by Homich et al. (2017). Analysis of rotundone was conducted via solid phase microextraction multidimensional gas chromatography-mass spectrometry (SPME-MDGC-MS) at the Australian Wine Research Institute (AWRI, Glen Osmond, SA) according to the protocol outlined in Geffroy et al. (2014).

*Statistical analysis*

Data analysis was performed using SAS statistical software (v. 9.4, SAS Institute, Cary, NC). Relationships between all measured variables were evaluated visually using PROC GPLOT, and PROC CORR was used to assess linear correlations between rotundone concentration and the 21 variables presented in Tables S1, S2, S3, S4, and S5. PROC REG was used to develop a series of multiple linear regression models and identify a subset of variables to be used for a predictive model. The same statistical approach was used to identify the fruiting zone weather variables that had the greatest influence on rotundone concentrations at harvest.

**Results**

**(Results should be explicitly described and illustrated. Subheadings can be used. Supporting tables/figures can be placed at the end of the document (no more than two tables or figures). Maximum 600 words)**

*Multiple linear regression analysis and selection of a rotundone mesoclimatic model*

Several candidate regression models were evaluated using different selection options, but they did not provide the optimal model with any given predictor variables, as they were prone to overfitting the data. Therefore, the RSQUARE option was used with PROC REG to better fit the data and aid in model selection. The best three models out of all models generated for one-, two-, three-, four-, five-, and six-variable models with the RSQUARE option are reported in Table 1, including values for various statistical parameters used for model selection. Analysis of *r*2, adjusted *r*2, *C*p, AIC, BIC, and MSE valuessuggested that a six-variable model may be overfitted and that a lower-variable model may be better-suited for predictive purposes (Table 1).

As more variables were added to the models, less additional variation was explained by each additional variable; this is reflected in the 0.064 increase in adjusted *r*2 when a fourth variable is added to the model, for example, when compared to the 0.017 increase when a fifth variable is added (Table 1). A slight decrease in mean square error (MSE) between the best fourth- and fifth-variable model indicated that each new variable added again explained a diminishing proportion of variation, and that models with fewer variables may be better suited for predictive purposes. Based on this, while also considering multicollinearity diagnostic statistics, *F*-values, adjusted *r*2 values, and other model-selection statistical criteria, the four-variable model including GDD*v*, Ca, crop load, and P emerged as the strongest candidate for use as a predictive model. Compared to other candidate models analyzed, this four-variable model was the best due to its increased predictive power (Table 1).

*Model partial validation of predictive rotundone mesoclimatic model*

To validate the chosen model, the dataset was split into two randomized datasets (*n* = 20, *n* = 16) and multiple linear regression was performed on the first validation data set. The same four-variable model (GDD*v*, Ca, crop load, and P) was selected by FORWARD selection and the RSQUARE option as the optimal fit for the validation data set. The model equation of the four-variable model was then used to generate predicted rotundone concentrations for the second validation data subset (*n* = 16)*.* The strong linear relationship between predicted and actual rotundone concentrations support the use of the four-variable model use as a predictive model for determining rotundone concentrations (Figure 1).

*Multiple linear regression analysis and selection of a rotundone microclimatic model*

Different candidate models were evaluated and results indicated that a three-variable model (DH10, DH30, and CEFA*p*) was the best candidate with an *r*2 of 0.57 and an adjusted *r*2 of 0.51 (data not shown). Further diagnostic analyses of multicollinearity, model residuals, outliers, and influential observations reaffirmed the strength of the three-variable model as the best candidate model.

**Conclusion**

**(Conclusions should include generalizations inferred from the results, exceptions to these generalisations, implications of the work. Maximum 100 words.)**

We constructed a four-variable predictive model that explained ca. 83% of rotundone concentration variation in Noiret grapes at harvest using data from both study years. The multiple linear regression model indicated the rotundone concentration was positively related to GDDv, crop load and P, and negatively related to Ca concentration in leaf petiole. Analysis of model residuals and partial model validation supported the strength of the chosen model, though to increase confidence in the model it would be necessary to further validate it with an external data set.

**Acknowledgements**

**(Maximum 100 words)**

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**Literature Cited**

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**Tables and Figures**

**(Maximum of two)**

|  |
| --- |
| **Table 1**. The best multi-variable models evaluated during model selection for rotundone prediction. |
| **No. of Variables** | **Model variables** | ***r*2** | ***C*pa** | **AICb** | **BICc** | **MSEd** |
| 1 | SH800*v*2 | 0.567 | 29.0 | -95.6 | -95.9 | 0.034 |
| 1 | GDD*v* | 0.512 | 35.8 | -92.2 | -92.8 | 0.038 |
| 1 | SH800*v* | 0.433 | 45.7 | -87.8 | -88.9 | 0.045 |
| 2 | GDD*v*, Ca | 0.703 | 14.0 | -104.6 | -104.0 | 0.024 |
| 2 | SH800, SH800*v* | 0.651 | 20.5 | -99.9 | -100.1 | 0.028 |
| 2 | GDD*v*, TSS | 0.641 | 21.7 | -99.1 | -99.4 | 0.029 |
| 3 | GDD*v*, Ca, crop load | 0.789 | 5.23 | -112.6 | -109.8 | 0.018 |
| 3 | GDD*v*, Ca, pH | 0.764 | 8.38 | -109.3 | -107.4 | 0.020 |
| 3 | GDD*v*, Ca, pruning wt  | 0.761 | 8.82 | -108.9 | -107.1 | 0.020 |
| 4 | GDD*v*, Ca, crop load, P | 0.853 | -0.68 | -121.0 | -113.9 | 0.013 |
| 4 | GDD*v*, Ca, pH, pruning wt | 0.842 | 0.71 | -118.9 | -112.6 | 0.014 |
| 4 | GDD*v*, Ca, pruning wt, rainfall | 0.839 | 1.05 | -118.4 | -112.3 | 0.014 |
| 5 | GDD*v*, Ca, P, pruning wt, pH | 0.873 | -1.17 | -123.3 | -112.7 | 0.011 |
| 5 | GDD*v*, Ca, P, pruning wt, TA | 0.873 | -1.16 | -123.2 | -112.6 | 0.011 |
| 5 | GDD*v*, Ca, P, pruning wt, yield | 0.872 | -1.10 | -123.1 | -112.6 | 0.011 |
| 6 | GDD*v*, Ca, P, pruning wt, rainfall, cluster no. | 0.897 | -2.24 | -127.5 | -110.9 | 0.010 |
| 6 | GDD*v*, Ca, P, pruning wt, rainfall, yield | 0.896 | -2.11 | -127.2 | -110.8 | 0.010 |
| 6 | GDD*v*, Ca, P, pruning wt, pH, cluster no.  | 0.894 | -1.80 | -126.5 | -110.6 | 0.010 |
| **Best regression model equation to be used for rotundone prediction** |
| **Year** | **Model** |  |  |  | ***r*2** | **Adj. *r*2** |
| 2016 & 2017 | Rot. = -0.53 + 0.568 \* P – 0.336 \* Ca + 0.018 \* crop load + 0.003 \*GDDv  | 0.853 | 0.828 |



**Figure 1.** Relationship between predicted and observed rotundone concentrations (ng/kg) generated using SAS’ PROC SCORE. R2=0.753; p<0.05.