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Article ID: 1671-8224(2009)01-0017-08

To cite this article: Stefania PRINO, Federico SPANNA, Claudio CASSARDO. Verification of the stomatal conductance of Nebbiolo grapevine [J]. J Chongqing Univ: Eng Ed [ISSN 1671-8224], 2009, 8(1): 17-24.

Verification of the stomatal conductance of Nebbiolo grapevine

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Received 30 December 2008; received in revised form 17 March 2009

Abstract: Wine is one of the most important Italian export products, and Nebbiolo is one of the most respected Italian grapes. In the summer of 2007, a measurement campaign was carried out in a Nebbiolo vineyard located in Vezza d'Alba, near Cuneo, Italy. Using a gauge of trade gases and some other instruments, we recorded the stomatal conductance and also some physiological parameters useful for estimating the dependence of stomatal conductance on environmental variables. The goal of this experiment was improving the parameterization of grapevine evapotranspiration through the assessment of the stomatal conductance and, in particular, of the functional dependence of the stomatal conductance on the following variables: the photosynthetically active radiation, the atmospheric temperature, the atmospheric moisture deficit, and the carbon dioxide concentration. The observations allowed us to check and, in some cases, to adapt the existing general parameterizations found in literature. The results showed some significant differences with the existing parameterizations concerning the atmospheric temperature, the atmospheric moisture deficit, and the carbon dioxide concentration. The observations allowed us to check and, in some cases, to adapt the existing general parameterizations found in literature. The results showed some significant differences with the existing parameterizations obtained in this experiment, although referring to a specific plant and site (namely the Nebbiolo at Vezza d'Alba), could allow assessment of the best environmental conditions under which the Nebbiolo grapevine production is the best, and in future could be tested for other grapevines or climates. **Keywords:** grapevine; evapotranspiration; stomatal conductance; photosynthetically active radiation

CLC number: S313 Document code: A

1 Introduction

Nebbiolo (*Vitis vinifera* L.) is a late-ripening grape variety that enjoys moderate summers and long autumns, typical of the Italian Piedmont region, requiring as much ripening time as possible to balance its naturally high acidity. The benchmarks for Nebbiolo wines are the Barolo and Barbaresco wines, which possess a combination of muscular tannins and high acidity. In particular, Nebbiolo wines contain lower values of active lime, potassium, boron, iron and manganese [1] with respect to the most famous Barolo and Barbaresco.

Although Nebbiolo is one of the major representatives of Italian viticulture in the world, it currently is cultivated mostly in a few privileged areas of the northern Italy (namely 88.2% in the native regions Piedmont and Lombardy regions), as well as in some minor areas distributed mainly in Argentina, USA, Mexico and Australia. The limited spread of this vine outside its native area mainly is due to its weak adaptability to climates and soils different from the original ones, and also is retarded by the long vegetative cycle and its winemaking difficulty.

The great importance of wine to the Italian economy has been the subject of many studies aimed at developing tools to manage vineyards and improve wine quality. Specifically, to obtain information on the

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crop productivity, one of the primary objectives is the monitoring of physical and physiological processes related to environmental conditions. In fact, there is a close relationship between water relations and wine composition [2]. It is not surprising that in the typical Mediterranean climate, high temperatures and lack of rainfall during summer are the most important factors determining productivity of tree crops [3]. According to IPCC (2007) report [4], in the future climate, summer precipitation will be lower, and the frequency of extreme climatic phenomena, like heat waves, will increase. The combination of these two factors will impose higher evapotranspiration losses, and thus affect the production quality.

Therefore, the study of grapevine transpiration is useful to characterize optimal environmental conditions for the grape productivity. The entire process is a journey of water involving its absorption by roots in soil, insertion into the lymphatic system, transport from the root vascular system along the stem up to the leaves and final transpiration through the stomatal guard cells. To evaluate transpiration accurately, measurement of leaf stomatal conductance is needed. Stomatal conductance is a critical parameter for multiple aspects of viticulture research. It regulates carbon dioxide assimilation and plant respiration, and can be used to determine grapevine water use, water status, responses to climatic factors, and responses to chemical and insect injuries [5], as done, for instance, by Mannini et al. [6] who assessed the productivity of Nebbiolo wine by evaluating the leaf stomatal conductance of each plant.

2 Theory of evapotranspiration

The saturation temperature of the leaf stomatal cavities (stomata) is the leaf temperature; water transpires from the stomata due to the pressure gradient, just as happens in the process of evaporation from a surface. The main difference between evaporation and transpiration is that, in the latter, the plant can exert some physiological controls by modifying the size of the stomata gate. The major factors that influence the opening and closing of stomata are [7-9]:

- 1) photosynthetically active radiation (PAR);
- 2) atmospheric humidity;
- water contained in stomata, which is dependent on soil water content;
- 4) air temperature; and
- 5) atmospheric carbon dioxide concentration. The transpiration rate can be represented with a

relation equivalent to that used for the evaporation process, using the flux-gradient relationship for the parameterization of turbulent processes and expressing the evapotranspiration flux by the method of resistances [9]. This methodology allows obtaining an equation formally similar to the Ohm law for electric circuits. The total evapotranspiration λE_v from the canopy leaves can be represented as follows [10].

$$\lambda E_{\rm v} = -\rho_{\rm atm} \lambda \left[q_{\rm s} - q_*(T_{\rm v}) \right] \left[f_{\rm wet}(\frac{L}{r_{\rm b}}) + (1 - f_{\rm wet})(\frac{L}{r_{\rm b} + r_{\rm s}}) \right],\tag{1}$$

where λ is the latent heat of evaporation and/or fusion; $\rho_{\rm atm}$ is the air density; $q_{\rm s}$ the specific humidity beneath vegetation; q_* the saturated specific humidity beneath vegetation dependent on the leave temperature $T_{\rm y}$; $r_{\rm b}$ the laminar resistance of the canopy referring to the layer of air around the leaves that is several millimeters deep; $r_{\rm s}$ the stomatal resistance; $f_{\rm wet}$ the wet fraction of canopy; and L the leaf area index. In this formula, there are two contributions to the evapotranspiration. The fraction multiplied by $(1 - f_{wet})$ refers to the dry part of the plant, in which evapotranspiration totally is given by the transpiration from stomata thus $r_{\rm b}$ and $r_{\rm s}$ act in series. The fraction multiplied by f_{wet} refers instead to the wet part of the plant (wetness due to a precipitation event or simply to dew). In this case, the evaporation does not involve the stomata, as it occurs directly from the water present on the leaves or other parts of the plant; thus only $r_{\rm h}$ is involved.

3 Theory of stomatal resistance

Conventionally it is more common to consider the stomatal conductance *G* (defined as the inverse of stomatal resistance r_s , i.e., $G = r_s^{-1}$) parameterized as the product of certain factors as in the following equation [11-12].

$$G = G_{\max} F_1 F_2 F_3 F_4 F_5 , \qquad (2)$$

where G_{max} is the maximum conductance, varying in accord with the vegetation type (in Ref. [13], it ranges from 0.11 for pines to 0.83 for Douglas fir). The functions F_1 , F_2 , F_3 and, F_4 , normally ranging between 0 and 1, make explicit the dependence on: (i) PAR or the short-wave radiation flux, (ii) the soil moisture content, (iii) the atmospheric water vapor deficit (i.e. the difference between the saturated and the actual humidity of the air), and (iv) the air temperature, respectively. The function F_5 , not included in Ref. [12], depends on the carbon dioxide concentration.

The dependence on PAR can be expressed by [7]

$$F_1 = \frac{\frac{r_{\min}}{5\,000} + f}{1+f},\tag{3}$$

where $r_{\min} = G_{\max}^{-1}$, and for the grape, is assumed equal

to 40 s m⁻¹; $f = \frac{1.1}{R_{GL}} \left(\frac{100}{r_{min}}\right) PAR$ is a function of PAR,

and R_{GL} is the Noilhan parameter [14], assumed equal to 100 W m⁻² for the grape. Considering that in the experimental measurements the instrument furnished all variables expressed in molar units, it was necessary to convert the values into metric units by using the following conversion formula [15]:

$$PAR/(\mu mol m^{-2}s^{-1}) = 4.5 PAR/(W m^{-2}).$$
(4)

The function of the soil moisture content F_2 is given by [12]

$$F_2 = 1 - 0.00119 \exp[0.81 \ (\theta_{s1} - \theta_1)], \tag{5}$$

where and θ_1 and θ_{s1} are the actual and saturated volumetric soil water contents, respectively.

The function of the atmospheric humidity F_3 is given by [7]:

$$F_{3} = 1 - 60 \left[q_{\text{sat}} \left(T_{\text{a}} \right) - q_{\text{a}} \right], \tag{6}$$

where q_a and q_{sat} are the atmospheric specific humidity and the saturated specific humidity, respectively; and T_a is the atmospheric temperature.

The function of the air temperature F_4 is given by [7]:

$$F_4 = 1 - 0.0016 \ (T_{\text{opt}} - T_{\text{a}})^2, \tag{7}$$

where T_{opt} is the optimum temperature of the plant, and generally is assumed equal to 298 K for all plants even if, in principle, it should depend on the vegetation type. This value agrees with other grape estimates (see Ref. [16]).

This approach is very simple and for this reason is widely used in meteorological models, but shows some limitations during extreme drought conditions, as exemplified in Ref. [17]. Moreover, the parameters in different parameterizations must be checked for each vegetation type.

4 Instrumentation and methods

The samples were gathered at the Azienda Sandrone, a farm located in Vezza d'Alba, Cuneo Province (more specifically, in a sub-region called Langhe), in Piedmont, Italy, and the stomatal leaf conductance and some meteorological variables were measured on 8 days during a period from June 21 to October 3, 2007. The main instrument used was the LCpro+, a gas exchange gauge for measuring and controlling the environment of a leaf contained in its chamber.

The dependence of stomatal conductance on the atmospheric variables was evaluated by pinching a leaf during each measurement (Fig. 1) and creating an artificial microclimate in the instrument chamber. Several measurements were carried out by changing one factor compatibly with the external climate and fixing all other variables. Each measurement was carried out manually and a period of 5 min was taken to allow the leaf to adapt to the new microclimate. For the dependence on the carbon dioxide concentration, a greater period (10 min) was used because the reaction time of the leaf was visibly larger.



Fig. 1 A representation of the broad leaf chamber window with an area of 6.25 cm^2 , for the leaf pinching [18]

The Mini-Irga sensor of LCPRO+ housed inside the plant leaf chamber provided gas exchange for photosynthesis experimentation. The gas analyzer was installed directly in the leaf chamber head, and thus the response delays in either gas exchange measurements or environmental control were very limited. The CO₂ measurements automatically compensated for atmospheric pressure and temperature. To provide full photosynthesis data, the LCPRO+ plant leaf chamber was fitted with environmental sensors, including two laser-trimmed water vapor sensors for transpiration data, and calibrated sensors for PAR and chamber temperature.

In each measurement, environmental factors were changed. The only factor excluded from measurement was the soil moisture content.

As the measurements were carried out on different days spanning different seasons and under different environmental conditions, it was necessary to develop a method to standardize all measurements to make them comparable between different experiments.

Therefore, the measured values were standardized into g_i , and then normalized into F_1 , F_3 , F_4 and F_5 of Eq. 2 using observed values of PAR, humidity, temperature and carbon dioxide concentration. Then, each measured value of G_s was normalized in F_i (*i*=1,3,4,5) with the corresponding value g_i as follows.

$$F_i = G_s g_i^{-1}, \ i = 1, 3, 4, 5.$$
 (8)

With the atmospheric vapor pressure e and the atmospheric pressure p_a provided by LCPRO+ measurement, the atmospheric specific humidity q_a and the saturated vapor pressure e_s were obtained using the following conversion equations:

$$q_{\rm a} = \frac{0.622e}{p_{\rm a} - 0.378e}; \tag{9}$$

$$e_{\rm s}(T_{\rm a}) = 6.107 8 \exp\left[\frac{17.269 (T_{\rm a} - 273.15)}{T_{\rm a} - 35.86}\right].$$
 (10)

In the above equations, the unit is kg kg⁻¹ for q_a , hPa for e_s and p_a , and K for T_a .

The maximum conductance G_{max} the LCPRO+ could work with was 1 mol m⁻² s⁻¹, i.e. 0.025 m s⁻¹ converted by Eq. 4.

5 Results

5.1 PAR

Table 1 shows the results of measured PAR and the stomatal conductance (G_s) , its standardization value (g_1) , and the dependence of normalized conductance (F_1) on PAR. Comparison of the thus obtained values of F_1 with those calculated with Eq. 3 are shown in Fig. 2.

From Fig. 2, the best fit regression line of the measured values (F_1) against those $(F_{1,estim})$ evaluated by Eq. 3 using the observed PAR is

$$F_{1,\text{estim}} = 1.02F_1 - 0.02$$

with a correlation coefficient of 0.99. It then can be concluded that the calculation of the empirical formula agrees well with the measured data, and Eq. 3 represents the measurements well.

Table 1 Measurement results of photosynthetically active radiation PAR, stomatal conductance G_s and its standardized value g_1 , and normalized stomatal value F_1 dependent on PAR

Date	PAR /(W m ⁻²)	$G_{\rm s}$ /(mol m ⁻² s ⁻¹) /(mo	g_1 l m ⁻² s ⁻¹)	F_1
21 Jun. 2007	0.0	0.01	0.92	0.01
21 Jun. 2007	3.8	0.12	0.92	0.13
21 Jun. 2007	20.5	0.38	0.92	0.41
21 Jun. 2007	58.0	0.56	0.92	0.61
21 Jun. 2007	251.3	0.81	0.92	0.88
21 Jun. 2007	348.0	0.84	0.92	0.91
11 Jul. 2007	0.0	0.00	0.43	0.00
11 Jul. 2007	3.8	0.08	0.43	0.18
11 Jul. 2007	20.5	0.14	0.43	0.32
11 Jul. 2007	58.0	0.27	0.43	0.63
11 Jul. 2007	251.3	0.38	0.43	0.88
11 Jul. 2007	348.0	0.39	0.43	0.91
19 Jul. 2007	11.6	0.09	0.34	0.26
19 Jul. 2007	38.7	0.20	0.34	0.58
19 Jul. 2007	158.4	0.26	0.34	0.76
19 Jul. 2007	295.8	0.29	0.34	0.85
19 Jul. 2007	367.3	0.31	0.34	0.91
25 Jul. 2007	38.7	0.36	0.72	0.50
25 Jul. 2007	116.0	0.57	0.72	0.79
25 Jul. 2007	212.7	0.62	0.72	0.86
25 Jul. 2007	367.3	0.66	0.72	0.91
5 Sep. 2007	88.9	0.41	0.60	0.68
5 Sep. 2007	162.4	0.49	0.60	0.81
5 Sep. 2007	290.0	0.54	0.60	0.90

5.2 Atmospheric humidity

Fig. 3 reports the measurements carried out with varied atmospheric humidity, as well as the estimations given by Eq. 6. It is obvious that Eq. 6 underestimates the conductance with an increase of the atmospheric

humidity. The use of the formula proposed by Stewart and Gay [19]

$$F_{3} = 1 - 24[q_{\text{sat}}(T_{a}) - q_{a}] \text{ for } 0 < q_{\text{sat}}(T_{a}) - q_{a} < \frac{1}{24} \quad (11)$$

improves the agreement with the observations considerably. Both Eqs. 6 and 11 have the same form; only the numeric coefficient is different. From a theoretical point of view, the dependence of the stomatal conductance on moisture deficit is well explained by Lynn and Carlson [20]: an increased humidity deficit restrict the leaf water potential which, in turn, is responsible for the increased stomatal resistance.

The best fit regression line of the measured values (F_3) against those $(F_{3,estim})$ evaluated by Eq. 11 using the observed q_a leads to the relation

$$F_{3 \text{ estim}} = 0.99F_3 + 0.01$$

with a correlation coefficient of 0.99.

5.3 Atmospheric temperature

As it can be seen in Fig. 4, Eq. 7 (the upper curve) does not represent a good estimate of the experimental data: for temperatures different from the optimum value of 25 °C, Eq. 7 underestimates the conductance, and thus the transpiration. A modified equation with the maximum stomatal conductance still at 25 °C, but the coefficient equal to 0.003 6 rather than 0.001 6, i.e.



Fig. 2 Normalized stomatal conductance F_1 evaluated from the experimental measurements in function of photosynthetically active radiation PAR, compared with the results given by Eq. 3. [7]



Fig. 3 Normalized stomatal conductance F_3 evaluated from the experimental measurements in function of the atmospheric moisture deficit $(q_{sat} - q_a)$, compared with the function given by Eq. 6 [7] and that given by Eq. 11 [19].

$$F_4 = 1 - 0.003 \ 6(298 - T_a)^2 \tag{12}$$

shows quite better agreement with the measurements (dashed curve in Fig. 4).



Fig. 4 Normalized stomatal conductance F_4 evaluated from the experimental measurements in function of the atmospheric temperature *T*, compared with the function given by Eq. 7 [7] and that given by Eq. 12

The best fit regression line of the measured values (F_4) against those $(F_{4,estim})$ evaluated by Eq. 12 using the observed T_a leads to the relationship

 $F_{4,\text{estim}} = 0.99F_4 + 0.01$

with a correlation coefficient of 0.99.

5.4 Carbon dioxide concentration

The maximum stomatal conductance was observed near a CO_2 concentration of 400 μ L/L which also was the typical environmental value measured in the vineyard. This is supported by Bunce [21], who reported the maximum conductance value appeared when the CO_2 concentration was close to the environmental level.

The data were fitted given the following relations:

$$F_{5} = \begin{cases} \exp[0.002\ 7(\text{CO}_{2} - 400)] & \text{for } \text{CO}_{2} \le 400\ \mu\text{L/L}, \\ 1 - 0.001\ 3(\text{CO}_{2} - 400) & \text{for } \text{CO}_{2} > 400\ \mu\text{L/L}. \end{cases}$$
(13)

Fig. 5 shows the measurements of the measured normalized conductance (F_5) and the curves given by Eq. 13, which appear in good agreement with each other. The best fit regression line of the measured values (F_5) against those ($F_{5,\text{estim}}$) evaluated by Eq. 13 using the observed CO₂ concentration leads to the relationship

 $F_{5,\text{estim}} = 1.01F_5 - 0.01$

with a correlation coefficient of 0.99.

This result agrees with other research. For instance, Moutinho-Pereira et al. [22] found that stomatal conductance fell and net CO_2 assimilation rate significantly increased in elevated CO_2 conditions, leading to improvements in intrinsic water use efficiency; the same result was found by Tognetti et al. [23] for olive trees. Baldocchi and Wong [24] reported a peak in the stomatal conductance corresponding to CO_2 concentration values between 300 µL/L and 400 µL/L. Bunce [25] found the transpiration rate slowed and Wei et al. [26] observed the stomatal conductance decreased with higher CO_2 concentrations. On the contrary, Bunce [27] found that the stomatal conductance did not differ for temperate deciduous tree species at increased CO_2 levels.

Note that the decrease of stomatal conductance with increasing CO_2 concentrations does not mean that the quality will decrease: as Bindi et al. [28] have shown, elevated atmospheric CO_2 levels are correlated to a significant biomass gain and also to enhanced wine quality.



Fig. 5 Normalized stomatal conductance F_5 evaluated from the experimental measurements in function of the carbon dioxide concentration (CO₂), compared with the function given by Eq. 13.

6 Conclusions

An experiment was performed in a Nebbiolo vineyard located at Vezza d'Alba, near Cuneo, in Piedmont, northwestern Italy. The zone is one of the few areas where the Nebbiolo grape is autochthon.

During the experiment period from June to October, 2007, the stomatal conductance of some individual leaves in the vineyard was evaluated, together with some other meteorological parameters (atmospheric temperature and humidity, PAR and carbon dioxide concentration).

The measured data permitted checking the dependence of stomatal conductance on the abovementioned meteorological factors. Such parameterization of stomatal conductance is a crucial key needed to evaluate the evapotranspiration of a plant by using land surface schemes, which in turn is an essential parameter for knowing the physiological status of the plants.

The results indicate that existing parameterizations of stomatal conductance present in the scientific literature in function of some environmental parameters also work well for the Nebbiolo grape in some cases, but are not sufficiently accurate in other cases. In particular, the dependence on the atmospheric moisture and temperature require different coefficients to give good performance.

Concerning the dependence of the stomatal

conductance on the CO_2 concentration, this experiment shows that the maximum conductance was recorded at the typical CO_2 concentration of the considered vineyard, which in this case was about 400 µL/L. This result, even if necessary to be confirmed by a larger number of measurements carried out under different climate conditions, is particularly significant from the point of view of the climate. In fact, the decrease of the stomatal conductance for CO_2 concentrations higher than the environmental values seems to suggest that the Nebbiolo grapevine could decrease its production at increased CO_2 concentrations.

The results obtained in this experiment can be used for setting a land surface model to characterize the best environmental conditions for improving the quality of the Nebbiolo grapevine, and studying all physical, hydrological and physiological processes.

The values obtained in this study refer to only one year, one specific climatic zone, and one specific kind of grape. To assess some scenarios related to the future climate change, it would be particularly interesting to track the stomatal conductance variation in relation to the change of meteorological variables for longer periods of time and different plants, and over a wider area.

Acknowledgements

The authors are indebted to Mattia Sanna and Tiziana La Iacona (Phytosanitary Service, Piedmont Region, Turin, Italy) for the valuable help provided in the management of the experimental campaigns of measurements.

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Edited by LUO Min