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Novel, technical advance: A new grapevine transpiration prototype for grape berries and whole bunch based on relative humidity sensors

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ABSTRACT

Grape berry transpiration is considered an important process during maturation, but scientific evidence is scarce. In the literature, there is only one report showing reduced maturation when bunch transpiration is artificially slowed down. Traditionally, grape berry transpiration has been measured by weighing grape berries on scale for a given time, correctly assuming that the weight reduction is due to water lost. Commercially available instruments adequate to measure gas exchange in small fruits are not suitable for whole grape berry bunch.

Here, we present an open differential chamber system that can be used with isolated grape berries or alternatively with a whole grape berry bunch for measuring grape berry/bunch transpiration based on the use of relative humidity sensors from Vaisala.

When used with isolated grape berries, open differential chamber system validation was made by using Tempranillo grape berries collected at different phenological stages. For the whole bunch transpiration prototype, two different validations were made. Firstly, measurements were made inserting inside the chamber an increasing number of Eppendorf tubes filled with water. Secondly, transpiration was measured in whole Tempranillo bunches sampled at different phenological stages. An important output of this work is that the fact of detaching the bunch from the plant did not change the bunch gas exchange rates at least for several hours.

For validations, transpiration values obtained with our prototype were compared with water losses inferred from grape berry weighing on scale for a given time, obtaining highly significant correlations. We tested the system applying to the bunch an anti-transpirant, confirming that the anti-transpirant application reduced bunch transpiration and delayed maturity.

1. Introduction

Fruit transpiration is a key component of its water relations. Evidence comes from species such as peach (Morandi et al., 2010), kiwifruit (Clearwater et al., 2012) and grapevine (Zhang et al., 2017). Despite its importance, grape berry transpiration is an aspect of its physiology poorly investigated. It has been reported, for instance, that transpiration contributes to weight loss in the grape berry (Rogiers et al., 2004), and exerts effects on grape berry solute accumulation and ripening (Rebucci et al., 1997).

When compared with leaf transpiration, grape berry transpiration is much lower (Rogiers et al., 2004; Keller, 2015). As previously reported, grape berries transpiration is in the order of 0.1–0.2 mmol H_2O m^{-2} s^{-1} (Rebucci et al., 1997), whereas grapevine leaf transpiration is one order of magnitude higher, 1–3 mmol H_2O m^{-2} s^{-1} (Arrizabalaga-Arriazu et al., 2021). Grape berry surface stomata number is less than 1 mm^{-2} (Blanke and Leyhe, 1988), whereas in the leaf surface it may reach up to 180–200 (our unpublished results). In addition, wax occludes stomata or they are converted into lenticels after the phenological stage of fruit set (Blanke and Lenz, 1989; Hardie et al., 1996). This is why the internal factor that dominates grape berry transpiration is cuticular conductance (Possingham et al., 1967). External, environmental factors, such as

Abbreviations: FM, Fresh mass; PPFD, Photosynthetic photon flux density; RH, Relative humidity; T, Temperature; VPD, Vapor pressure deficit.

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temperature (T) and relative humidity (RH) that determine vapor pressure deficit (VPD), markedly influence grape berry transpiration (Zhang et al., 2017). Grape berry transpiration was reported to decrease along with grape berry development and ripening (Rogiers et al., 2004), likely caused by changes in the cuticular conductance (Zhang et al., 2017).

During the last 30 years, grape berry harvest in the vineyards occurs earlier (Martínez-Lüscher et al., 2016) because the fruits ripen faster (Martínez-Lüscher et al., 2015), and maturation takes place under heat stress that cause a decrease in the fruit quality (Kizildeniz et al., 2018). It is known that climate change is causing an imbalance between the accumulation of sugars and anthocyanins (responsible for the color and part of the stability of the wines in barrels) in the grape berry (Arrizabalaga et al., 2018). If grape berries are harvested with an optimal level of sugars, they have not reached the adequate concentration of anthocyanins that they should, and if they are harvested when achieved the optimal concentration of anthocyanins, the grape berries are overripened and accumulate high concentrations of sugars, which produce wines of a high alcoholic degree. This advanced maturity is thought to be related in some way to greater bunch transpiration (Keller, 2015). Berry transpiration might help sugar unloading because of the needs to discharge water after sugar left the phloem in the sink tissues (Zhang and Keller, 2017). For example, there is one report claiming that treatments that reduce bunch transpiration slow-down ripening (Rebucci et al., 1997). Although Castellarin et al. (2015) did not change berry transpiration intentionally, they found boxed berries (transpiration inhibited) had delayed ripening as well. More recently, Zhang et al. (2017) reported that the artificial reduction of grape berry transpiration decreased the solute accumulation rate and delayed color change. However, there is little evidence of the relationship, if any, between grape berry transpiration and maturation.

The simplest way of monitoring grape berry transpiration is measuring weight loss (water loss) as a function of time in a scale. Currently, there is no commercial equipment capable of carrying out transpiration measurements on grape berry bunches. Very scarce cases of studies that establish the background of this report can be found in the bibliography. Specifically, Blanke and Leyhe (1987) studied the cuticular transpiration of grape berries using an open system in which transpiration was calculated by the difference in humidity between the inlet and outlet gas of the cuvette where the bunch was placed. Later, Rebucci et al. (1997) used a large laboratory benchtop system to verify that the cluster's maturation speed depends at least in part on the transpiration rate. The equipment was based on the measurement of RH (or water vapor content in the air) and temperature with psychrometers and thermocouples respectively and a data storage system. In 2001, Palliotti and Cartechini studied gas exchange during cluster development, using commercial equipment from Analytical Development Co., ADC (LCA-3, UK) and a fruit chamber. The LCA-3 model (which is no longer sold) had the CO_2 and H_2O sensors in the console, not in the measuring clamp, which allowed different measuring devices to be attached to the equipment without the equipment being able to detect the absence of the sensors.

The company ADC currently markets 1 L capacity fruit chambers. The current ADC equipments LCi and LCPro have the CO_2 and H_2O sensors in the clamp, which do not allow their use to measure with devices other than the original measuring clamp. However, the LCi leaf chamber can be disassembled, enabling gas exchange measurements in small fruits after assembling the fruit chamber as an accessory of the LCi leaf clamp. The 1 L fruit chamber is tube-shaped, with an approximate diameter of 10 cm and a height of 10 cm. This design is not adapted to the needs of the grape berry cluster (that can reach a length up to 15–20 cm × 10–15 cm), and is used for isolated fruit (typically for apples).

Prototypes have been recently developed using the approach of low-cost devices for monitoring gas exchange in fruits and vegetables. For instance, González-Buesa and Salvador (2019) used an Arduino-based instrument for the measurement of respiration. The prototype is a closed system, modular device based on open source software. Authors report as advantages simplicity, adaptability and low cost. The respirometer they developed allowed the continuous measurement of CO_2 concentration, barometric and differential pressures and temperature. With the data obtained, González-Buesa and Salvador calculated CO_2 production rates and predicted the O_2 consumption rates.

Taking into account this background, the aim of this work was to develop and validate a low-cost system/prototype capable of measuring grape berry/bunch transpiration, and to test whether transpiration can be used as an indicator of the speed with which grape berries are ripening. Although transpiration might be related to sugar unloading during ripening, it is not clear if transpiration can be an indicator for berry ripening rate. Especially, the surplus water from phloem unloading could be dissipated through xylem. This could make transpiration rate irrelevant to sugar unloading rate. The development and implementation of this prototype would help to scientists/growers to optimize vineyard management in order to mitigate the adverse future climate effects.

2. Materials and methods

2.1. Description of a new grapevine transpiration prototype for grape berries and whole bunch

Our first attempts to measure grape berry transpiration with berry/bunch chambers coupled to an infrared gas analyzer available in the laboratory were unsuccessful. This was in part due to the large air volume necessary to move when using the whole bunch chamber. We therefore decided to work with our own custom-built system, much cheaper, designed *ad hoc* for the variable sizes of chambers and plant material to be used, with better measurement range, precision and sensitivity than other more expensive devices and adapted/appropriate to the measurement concerned, i.e., grape berry/bunch transpiration. Our aim was to design and construct a prototype for measuring grape berry transpiration that could also be used with a whole grape berry cluster. This was achieved by using two different interchangeable chambers (using one or the another depending if measurements have to be carried out in grape berries or a whole bunch) with a capacitive thin-film polymer sensor based (Vaisala, HMP155) (Figs. 1 and 2). This sensor has a measurement range of 0–100% RH and precision of ±1–1.8% (in the range 1–100% RH and at between −20 and +40 °C). This improves the range and precision of other commercial instruments (i.e. ADC PLC-2A leaf chamber and LCA-2 portable analyzers: range 0–75%; precision ±3%).

Briefly, the air was taken from outdoor (crossing the plastic tube the wall of the laboratory) and, through humidification up to saturation, passing the air in an Erlenmeyer flask with water using a pump (Mumisuto) controlled by voltage regulation, and a Peltier system (set at 15 °C) (Fig. 1A and 2), the humidity of the water saturated air was adjusted to a constant and stable value of 42% (reference air) at a controlled flow (0.4 and 1 L min^{-1}, grape berries and whole bunch chamber, respectively) using a rotameter. These flows were chosen after carrying out different system optimization tests, to adjust it to a non-saturating RH measurement range (between 42% and 90%) and to avoid condensation. A 3-way valve system made possible to measure the humidity value in the reference air (without entering the grape berries or whole bunch chamber) and, by changing the valve position, the humidity value of the air after passing through the chamber where the grape berries or the whole bunch were/was located. The humidity value of the air increased after passing through the chamber, due to the transpiration of the grape berries/bunch. Reference air and air leaving the chamber (open differential system) were measured with a HMP155 Vaisala RH sensor (with a custom-made housing) and the value or evolution of the humidity was recorded with a datalogger. Measurements were made inside a small plant growth chamber (Conviron, EF7H, USA), allowing temperature to be fixed as desired (24 °C by default) and

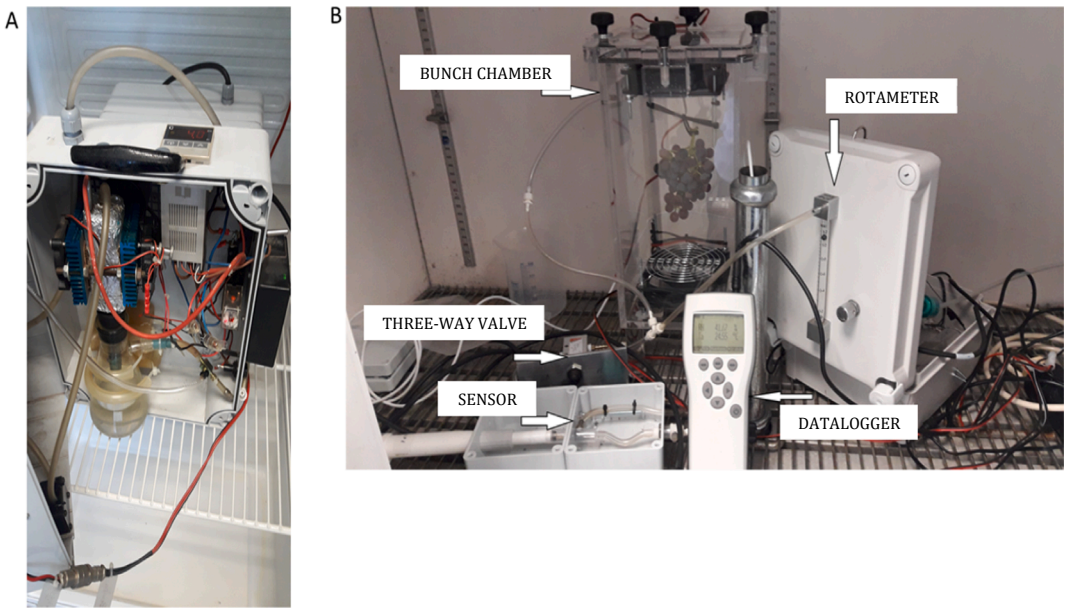

Fig. 1. Pictures of the prototype for measuring grape berry/whole bunch transpiration rates. (A) Detail of the system used to saturate of humidity and of the Peltier system used to control and set constant and stable the humidity of the reference, inlet air, and (B) rest of the prototype consisting mainly of a rotameter, a grape berries/whole bunch measuring chamber, a 3-way valve, a humidity sensor and a datalogger.

being constant and stable during the measurement (Fig. 1B and 2; see Fig. S1 for stability of RH and temperature). Temperature was also recorded by the HMP155 Vaisala sensor, which therefore simultaneously monitored both RH and temperature.

The grape berries chamber was a simple, 50 cm^3 syringe provided with a small mixing fan in its terminal (Sunon MEC25101V1-000V-A99, 25 × 25 × 10 mm, 13000 rpm), where the chamber volume can be adjusted by moving forward or backward the plunger. Air inlet was in the middle of the syringe cylindrical part, while air outlet was in the narrow part (Fig. 2).

The whole bunch chamber was a 15 × 15 × 30 cm (width × depth × height) transparent methacrylate cube with two mixing fans (Sunon MEC0251V1-000 V-G99, 120 × 120 × 25 mm, 3100 rpm) at the bottom and the uppermost parts with opposed flow, enabling the circulation and complete homogenization of the air inside the chamber. The chamber was hermetically sealed with 4 screws and an O-ring. A detached, whole bunch (or a bunch still attached to the plant, see below) can be placed inside with the help of a bunch bracket. Air inlet was placed at a side and outlet at the bottom of the chamber (Fig. 1B and 2).

2.1.1. Protocol for transpiration measurements

First, the reference air is passed through the sensor, with the 3-way valve in position B and with the tube coming from the chamber manually disconnected (Fig. 2). The reference RH value is recorded. Then, the tube coming from the bunch chamber is reconnected. The bunch is enclosed into the chamber. Reference air is passed through the chamber with the 3-way valve in position A. The bunch remains in the chamber for 30 min and RH values are recorded every 5 s. After 30 min, the RH of the reference air is recorded again as described previously. After checking its stability with the initial reference value, it is used for the calculations. Fig. S1 shows the high stability of the temperature and RH of the reference air over a period of one hour.

2.1.2. Calculations

Grape berries/bunch transpiration was expressed on a fresh mass (FM) basis. To calculate transpiration rates in μmol H_2O 100 g^{-1} FM s^{-1} from the difference in RH between the reference air and the air passing through the chamber, the following formula was used:

$$E = \frac{\Delta dv \cdot u}{FM} \quad (1)$$

where Δdv was the difference in absolute humidity (dv) between the reference air and the air into the chamber (expressed in mmol H_2O dm^{-3}), u was the air flow (in dm^3 s^{-1}) and FM is the bunch fresh mass (g). From Equation (1), dv can be calculated (as g H_2O dm^{-3}) from RH values as follows:

$$d_v = \frac{P_v}{R \cdot (T + 273)} \quad (2)$$

where P_v (atm) is the water vapor pressure of the reference air or the air into the chamber, T (°C) is the temperature into the chamber and R is the specific gas constant for water vapor (461.52 Pa m^3 kg^{-1} K^{-1}) expressed in atm m^3 kg^{-1} K^{-1}. From Equation (2), P_v (in mBar) can be calculated as:

$$P_v = P_s \cdot \frac{RH}{100} \quad (3)$$

where P_S is the saturation water vapor pressure of moist air and RH is the RH (%) in either the reference air or the air into the chamber. From Equation (3), P_s (mBar) can be calculated according to the World Meteorological Organization (WMO, 2018) as:

$$P_s = f(p) \cdot 6.112 \cdot e^{\frac{(17.62 \cdot T)}{(243.12+T)}} \quad (4)$$

where $f(p)$ is the pressure function, which adjusts for the barometric pressure dependency of the saturation vapor pressure (WMO, 2018):

$$f(p) = 1.0016 + 3.15 \cdot 10^{-6} \cdot p - 0.0074 \cdot p^{-1} \quad (5)$$

where p is the barometric pressure (mBar).

2.2. Experimental procedure

2.2.1. Plan material and growth conditions

Dormant cuttings of *Vitis vinifera* L. cv. Tempranillo (clone RJ-43) were obtained from the germplasm bank of "Estación de Viticultura y Enología de Navarra" (EVENA, Navarra, Spain).

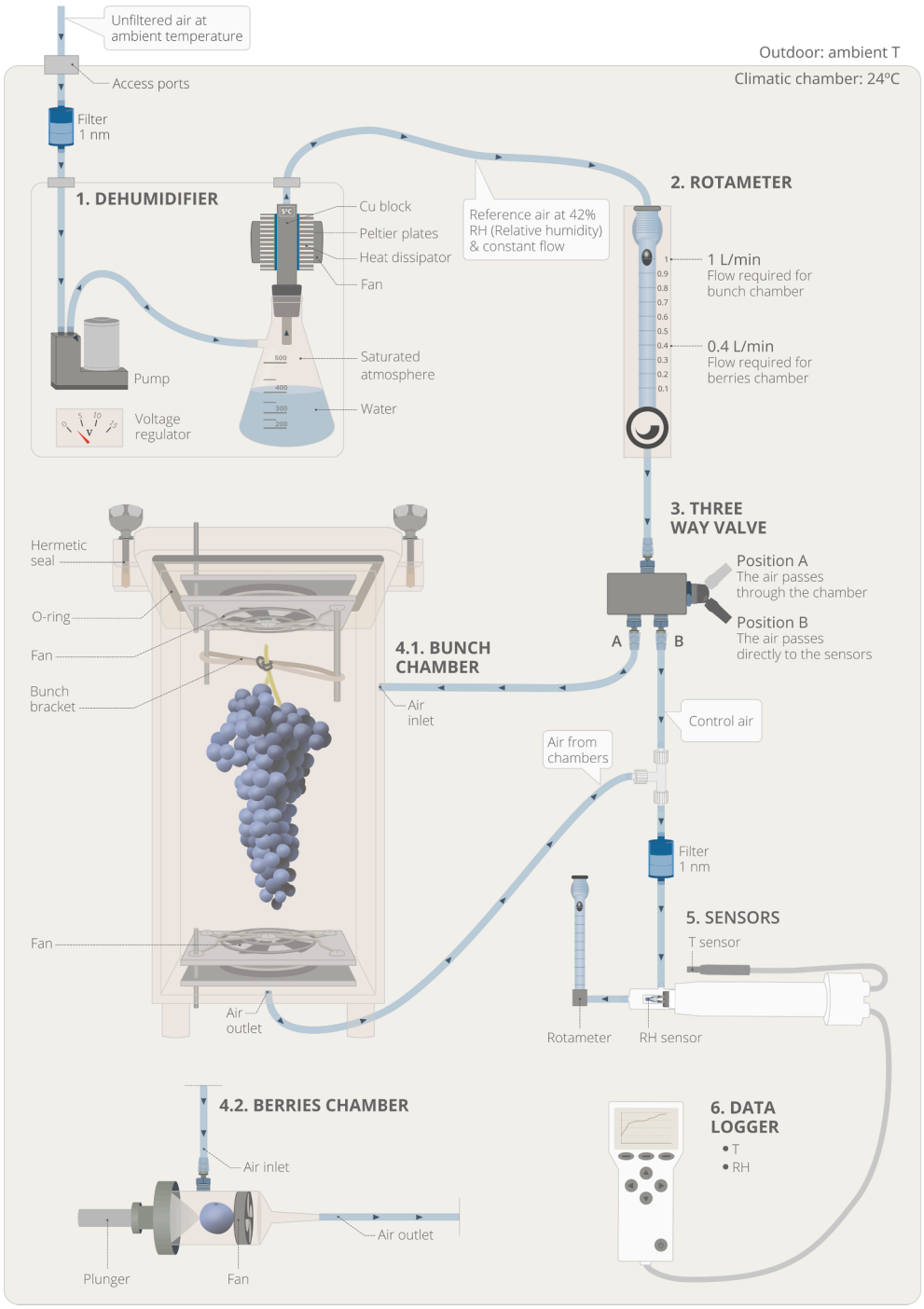

Fig. 2. Scheme of operation of the custom-built system for transpiration measurements in a whole bunch, and its variant for grape berries. Drawings have been made by Vega Asensio (www.norarte.es).

Cuttings were chosen to obtain fruit-bearing cuttings according to Mullins (1966) and Ollat et al. (1998), as described in detail in Morales et al. (2016). Rooting was induced using indole butyric acid (300 mg L^{-1}) in a rock-wool heat-bed (27 °C) kept in a cool room (4 °C). After one month, the rooted-cuttings were planted in 0.8 L plastic pots that contained a mixture of sand, perlite and vermiculite (1:1:1, in volume) and then transferred to a Growth Chamber Greenhouse (GCG; Morales et al., 2014) settled at 24 °C/14 °C and 50%/70% RH (day/night) and photoperiod of 15 h with natural daylight supplemented with high-pressure metal halide lamps (OSRAM®, Augsburg, Germany), providing a photosynthetic photon flux density (PPFD) of 500 μmol m^{-2} s^{-1} at inflorescence level that was triggered when natural daylight dropped below 900 μmol m^{-2} s^{-1}. Nutrient solution was applied as proposed by Ollat et al. (1998): NH_4NO_3 (64.5 mg L^{-1}), $(NH_4)_2HPO_4$ (75 mg L^{-1}), KNO_3 (129 mg L^{-1}), $MgSO_4{\cdot}7H_2O$ (125 mg L^{-1}), $Ca(NO_3)_2{\cdot}4H_2O$ (248 mg L^{-1}), $(NH_4)_2SO_4$ (66 mg L^{-1}), Fe-EDDHA (280 mg L^{-1}), H_3BO_3 (2.86 mg L^{-1}), $MnCl_2{\cdot}4H_2O$ (1.81 mg L^{-1}), $ZnSO_4{\cdot}7H_2O$ (0.22 mg L^{-1}), $CuSO_4{\cdot}5H_2O$ (0.08 mg L^{-1}) and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (0.016 mg L^{-1}). Leaves and shoot tip were removed during flowering stem growth until fruit set (only 4 leaves per plant were allowed to grow) and only one shoot per plant and a single flowering stem was allowed to develop on each plant, in order to get only one grape berry bunch per plant. At fruit-set, the cuttings were transplanted to 7.5 L plastic pots containing a peat and perlite (2:1, v/v) mixture.

2.2.2. Grape berry transpiration experiment

In a first experiment, plants grew up from fruit set to maturity in the GCG in the same conditions as described in section 2.2.1. Five grape berries per plant were sampled in five phenological stages: pea size, chickpea size, mid-veraison (50% of red grape berries in the bunch), 15 days after veraison and maturity (around 23 °Brix). At veraison, we sampled berries with half of the berry green and half red. Grape berry transpiration was measured as described in section 2.1.

2.2.3. Bunch transpiration experiment

In a second experiment, plants with similar phenological stage and bunch size characteristics were divided homogeneously into two groups and placed, at fruit set, in two different GCGs. The GCGs were settled at different temperature and RH (T/RH) regimes: 24 °C/14 °C (day/night) and RH of 55%/70% (day/night) vs. 28 °C/18 °C (day/night) and RH of 43%/58% (day/night) in order to enlarge the range of transpiration values. All plants were fertilized with the solution proposed by Ollat et al. (1998) (see above for nutrient solution composition) and well irrigated (no water stress symptoms were observed). Plants grew under these conditions up to maturity (ripening of berries at ca. 23 °Brix). Whole bunches were sampled at five developmental stages: pea size, chickpea size, onset of veraison (2–3 red grape berries per bunch), 100% veraison and maturity (5–12 bunches per stage and environmental condition). Bunch transpiration was measured as described in section 2.1. With an exploratory approach of using other sensors with the prototype, a CO_2 sensor (Vaisala CARBOCAP® Carbon Dioxide Probe GMP343) from Vaisala was tested, in order to record bunch respiration simultaneously to transpiration. Since phenological stages are affected by temperature in Tempranillo (Arrizabalaga et al., 2018), all plants were tagged and the phenology of each individual plant was monitored as described in section 2.2.5.

2.2.4. Detaching the cluster from the plant

In viticulture studies, it is common, upon sampling in the vineyard, transferring clusters immediately to the laboratory in sealed plastic bags inside a cooler for further analyses. In order to investigate whether detaching the cluster from the plant has effects on grape berry transpiration, a new series of measurements were performed. At the beginning of these measurements, the grape berry bunch was kept attached to the plant, and after around 35 min later it was cut. Eight plants were used for these measurements, and the effect of cutting was evaluated comparing grape berry transpiration before and after the cutting. For these measurements, bunches with green berries (pea or chickpea size) were used.

2.2.5. Artificially-reduced grape berry transpiration

In order to investigate the role of grape berry transpiration on ripening, grape berry transpiration was artificially reduced using different solutions of the commercial anti-transpirant VaporGard®: D0, D1, D5 and D10, dose 0, 1, 5 and 10% v:v, respectively (8 plants per treatment). The anti-transpirant was applied at the onset of veraison, spraying whole bunches, making sure that the grape berries inside the bunch received solution and plants were grown in the greenhouse at 24 °C/14 °C (day/night) and RH of 45%/63% (day/night) up to maturity. Bunch transpiration was measured at maturity as described in section 2.1. The phenology of each individual plant was monitored by measuring the elapsed time in days from the beginning of veraison until berries maturity in the bunch (ripening of berries at ca. 23 °Brix).

2.2.6. Statistical analyses

Data in plots are represented as individual measurements or as mean ± standard error (SE). For the validation of the transpiration prototype using grape berries and bunches, and for the validation of the bunch transpiration prototype using Eppendorf tubes filled with water, the regression line and the coefficient of determination (R^2), on the basis of the Pearson's correlation coefficient, were calculated. One-way ANOVA and Fisher's Least Significant Difference (LSD) post-hoc tests were used for mean values comparison. The statistical package XLStat 7.5.2 Pro® was used.

3. Results and discussion

3.1. Validation of the grape berry transpiration prototype

Transpiration rates were measured in detached Tempranillo grape berries during the development of the grape berry by gravimetric technique (weighing, and recording the weight loss in a given time with a scale), compared to measurements made using the Vaisala-based equipment, and used to validate the prototype. Five grape berries were enclosed into the syringe-type chamber for each measurement. When grape berries of all plants at the five different phenological stages of this study were included in the analyses, the regression between grape berry transpiration measured by weighing and using the prototype returned a positive and highly significant relationship ($R^2 = 0.97$; $P < 0.0001$) (Fig. 3A). This result validates the transpiration measurements of the prototype when using the grape berries chamber.

Fig. 3B shows that grape berry transpiration significantly decreased as phenology advanced. Due to grape berry inability to regulate its transpiration rate (Possingham et al., 1967), the decline of transpiration during ripening is most likely due to changes in wax composition (Possingham et al., 1967; Riederer and Schreiber, 2001). Different research groups point out to an enrichment in long-chain aliphatic compounds that constitute the major barrier to water evaporation along with grape berry phenology development (Grncarevic and Radler, 1971; Yamamura and Naito, 1983).

3.2. Validation of the bunch transpiration prototype

For the validation of the prototype using the whole bunch chamber,

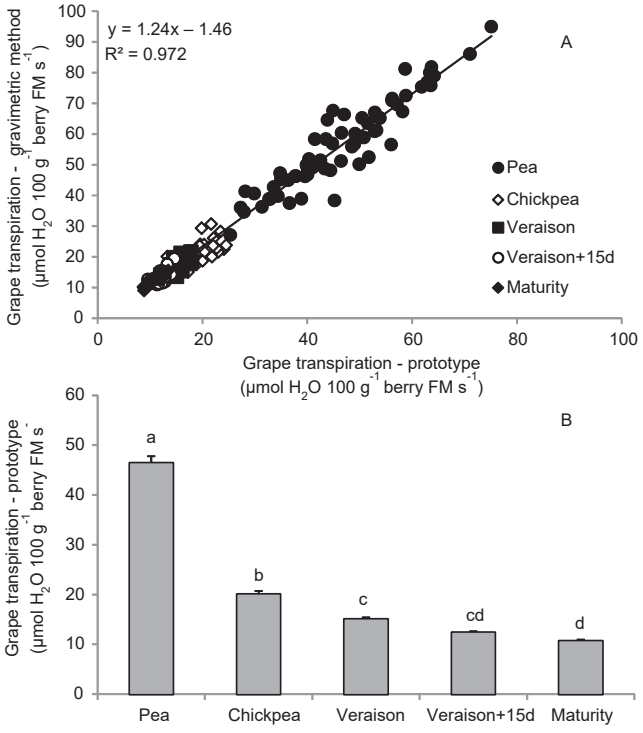

Fig. 3. Transpiration rates (µmol H_2O 100 g^{-1} berry FM s^{-1}) measured in detached grape berries throughout the development of the grape berry by gravimetric technique (by weighing in a scale) compared to measurements made using the prototype based on the Vaisala RH sensor (A) and evolution throughout grape berry development (B). Regression line and coefficient of determination (R^2) were calculated in Fig. 3A. FM = fresh mass.

two different approaches were used. First, measurements were made by inserting inside the transpiration chamber an increasing number (from 1 up to 13) of Eppendorf tubes filled with water for 30 min. Values were compared with those obtained by weighing the Eppendorf tubes before inserting them into the chamber and after the 30 min of measurement, and calculating the weight difference. In other words, an "artificial" validation was performed by measuring the increase in humidity in the chamber in relation to the weight loss of the tubes (measurements made with different number of tubes). This first calibration was chosen because it is independent of grapevine variety, phenological stage or whatever related to plant material. It could be said that it is a technical calibration of the chamber. This "technical" calibration was carried out under two conditions of temperature and base RH: 24 °C and 55% RH vs. 28 °C and 43% RH. Data supported a relationship between both types of measurements ($R^2 = 0.95$; $P < 0.0001$ for 24 °C and 55% RH, Fig. 4B; $R^2 = 0.95$; $P < 0.0001$ for 28 °C and 43% RH, Fig. 4C; $R^2 = 0.93$; $P < 0.0001$ for the pooled data, Fig. 4A).

A second validation of the whole bunch chamber prototype was made with whole Tempranillo bunches sampled at five phenological stages, and comparing these measurements with the traditional water loss on a scale. In this second case, it is a calibration adapted to the plant material to be used. This validation gave very good results. Temperatures and RH during the measurements were identical to those used with the "technical" validation. The regression returned a positive relationship between transpiration recorded with the prototype and by weighing ($R^2 = 0.95$; $P < 0.0001$, Fig. 5A).

Bunch transpiration values found with our prototype were compared to those reported in the literature working with other devices, varieties and experimental conditions. Comparisons are not easy because data are

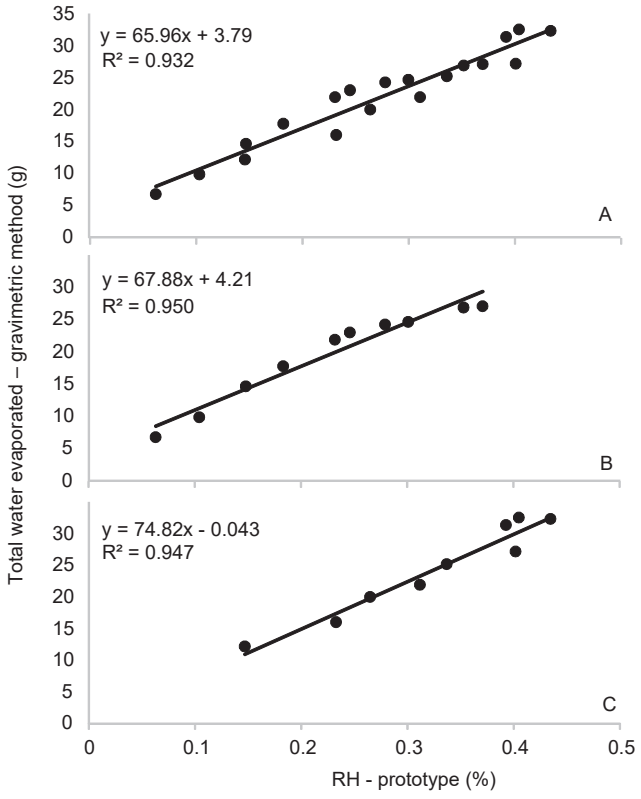

Fig. 4. Measurements made with the transpiration whole bunch chamber (RH change) by inserting inside it an increasing number (from 1 up to 13) of Eppendorf tubes filled with water compared with the values obtained by weighing on a scale such different number of Eppendorf tubes and calculating the water loss in a given time (g of water). (A) pooled data, (B) measurements made at 24 °C and 55% RH, (C) measurements made at 28 °C and 43% RH. Regression lines and coefficients of determination (R^2) were calculated.

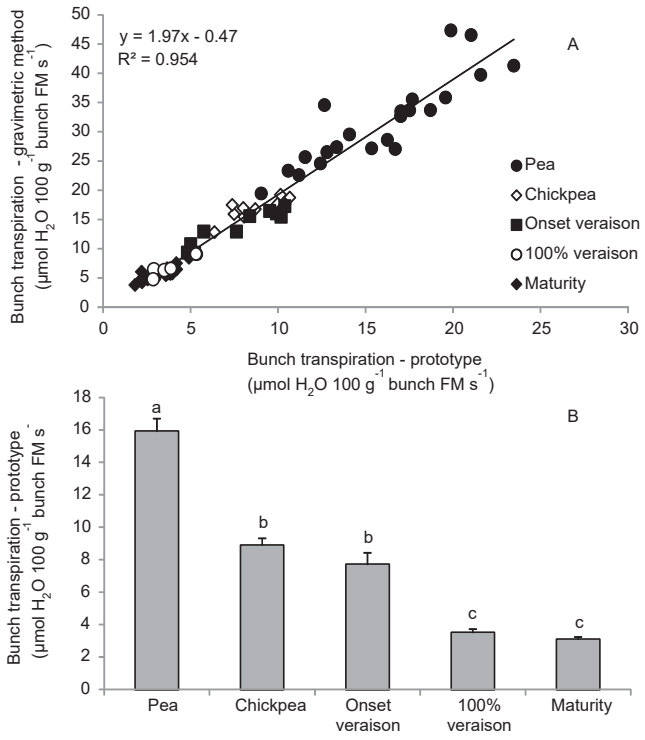

Fig. 5. Transpiration rates (µmol H_2O 100 g^{-1} bunch FM s^{-1}) measured in whole bunches throughout the development of the grape berry by gravimetric technique (by weighing in a scale) compared to measurements made using the prototype based on the Vaisala RH sensor (A) and evolution throughout grape berry development (B). In order to enlarge the range of values, data include bunches from plants grown at 24 °C/14 °C (day/night) and RH of 55%/70% (day/night), and at 28 °C/18 °C (day/night) and RH of 43%/58% (day/night). Regression line and coefficient of determination (R^2) were calculated in Fig. 5A. FM = fresh mass.

expressed in different bases. Grape berry transpiration in Cabernet Sauvignon 55 days after anthesis was 0.024 g H_2O g^{-1} fresh weight h^{-1} (measuring at mid-morning and between 26 °C and 33 °C) with a LCA-3 infrared gas analyzer device; Palliotti and Cartechini, 2001), in growth chamber-grown fruit-bearing Cabernet Sauvignon cuttings 100 days after anthesis 3.37 mg h^{-1} $berry^{-1}$ (Ollat et al., 2002), in Pinot Noir at lag-phase and Sangiovese 10 days after bunches showed initial color changes (obtained with a custom-built system) 0.250 and 0.169 mmol H_2O m^{-2} s^{-1}, respectively (Rebucci et al., 1997) and in Ehrenfelser, Müller-Thurgau and Riesling 13 weeks after full bloom (measuring with an hygrometer-based system at 20 °C and 52% RH) 112.5, 100 and 83.3 mg H_2O dm^{-2} h^{-1}, respectively (Blanke and Leyhe, 1987). Values found for Tempranillo transpiration measured with the prototype (from 5 to 45 µmol H_2O 100 g^{-1} bunch FM s^{-1}; Fig. 5A) are, after proper unit conversion of those reported in the literature (assuming a mean FM per berry of 1 g and 0.75 cm of mean radius), in the range of the few values previously reported (from the lowest value of 5.2 to the highest one of 37 µmol H_2O 100 g^{-1} bunch FM s^{-1} both reported in Cabernet Sauvignon; recalculated from Ollat et al., 2002; Palliotti and Cartechini, 2001, respectively). Our data from Tempranillo indicate that bunch transpiration decreased throughout grape berry development, from pea size to maturity (Fig. 5B). However, it has been reported that grape berry transpiration rate peaked at around veraison and then declined thereafter (Zhang et al., 2017). It can be concluded that trends are different when expressed in different units. We found a decreased trend from pea size to maturity when grape berry transpiration was expressed in µmol H_2O 100 g FM^{-1} s^{-1}, whereas Zhang et al. (2017) reported, when expressed in mg H_2O $berry^{-1}$ day^{-1}, an increased trend from green berries (green hard) to veraison (red/purple) decreasing thereafter.

Despite units and possible varietal effects, differences found in the literature may, therefore, arise from the phenological stage (Fig. 5B; Zhang et al., 2017) and/or the temperature and RH (i.e., the VPD) at which measurements were made (Zhang et al., 2017), the former as an internal factor and the latter as the main external condition driving grape berry transpiration.

Despite the good relationships found, absolute values recorded with the bunch prototype and by weighing differed (see axes X and Y in Fig. 5A). One possibility to explain this difference is the large volume of the chamber, necessary to allocate bunches of very different sizes, from very small to very large (depending on the phenological stage, variety, etc.). It was designed and constructed to fulfill such requirement. A clue for the volume of air necessary to move during the measurement as cause of the difference in absolute values was obtained when comparing measurements made with the bunch chamber with those of the grape berries chamber. Thus, differences in absolute values obtained with the grape berries chamber (Fig. 3A) were smaller than those obtained with the bunch chamber (Fig. 5A). Additionally, the transpiration rates per gram of FM of detached grape berries (values with the grape berry chamber ranging from 8 up to 80 μmol H_2O 100 g^{-1} berry FM s^{-1}) were considerably higher than those of the whole bunches (values with the bunch chamber ranging from 2 up to 25 μmol H_2O 100 g^{-1} bunch FM s^{-1}). These differences may be explained by the fact that when measuring the 5 detached grape berries the entire surface of the grape berries is highly exposed and the gas exchange is optimal. In the case of the whole bunch measurements, the gas exchange of the grape berries inside the bunch is probably not as good (especially if the bunch is somewhat compact), which is reflected in lower transpiration rates per gram of FM.

For comparative purposes between treatments, the direct result of the bunch prototype can be used but, in the case of absolute values were required, the following conversion formula can be used: $y = 1.97x - 0.47$, where y is the transpiration measured by weighing and x is the transpiration value obtained with the bunch prototype.

3.3. Testing the bunch transpiration prototype

Fig. 6 shows a representative example of one of the measurements recording continuously the transpiration of a bunch, in which the humidity has increased from 42% to 59% as a result of the transpiration of the bunch (red trace) (temperature during the measurements was maintained at around 24.5 °C, green trace). At the beginning of the measurement, the bunch was kept attached to the plant, and around 35 min later (see axis X and arrow), the peduncle was cut. It can be observed that the cutting of the cluster did not cause changes in transpiration on the measured time scale (consistently repetitive; n = 8), in line with a previous report (Zhang and Keller, 2015). These results open new possibilities for the future, because bunches could be sampled in vineyards in the field and could be taken to the laboratory to carry out measurements without the fact of cutting the cluster affecting the measurement. It was verified that the lack of effect of the cut of the grape cluster was maintained over time at least for 4 h (not shown), because from the field to the laboratory a certain time may elapse.

Also, Fig. 6 illustrates how this prototype can incorporate new sensors to convert it in a multi-purpose device. A CO_2 sensor (Vaisala CARBOCAP® Carbon Dioxide Probe GMP343) was tested (blue trace), which records bunch respiration. As a consequence of bunch respiration, the CO_2 concentration in the bunch chamber increased from 400 to 475 ppm. Other gas sensors can be envisaged as targets to be incorporated in an improved prototype version (ethylene, O_2, volatile organic compounds, etc.).

In addition, we tested the effect of artificially reducing bunch transpiration, applying to the grape berry bunches different doses of the anti-transpirant VaporGard®. Measurements of transpiration made with the prototype at maturity revealed that whole bunches treated with the highest doses of anti-transpirant (D5 and D10) transpired significantly less water than the controls (D0) (Fig. 7). In addition, we found that the anti-transpirant applied to the cluster in the different doses significantly slowed down ripening, increasing the elapsed time from the beginning of veraison until maturity from 48 to 62 days (D0 and D10 respectively, p = 0.009) (data not shown). Data suggest that this slowdown in maturation may be related to some extent to lowered bunch transpiration.

4. Conclusions

In this paper, we report the design and construction of a prototype to measure transpiration in grape berries. Our prototype follows the approach of low-cost devices for monitoring fruits and vegetables (González-Buesa and Salvador, 2019). Two interchangeable chambers

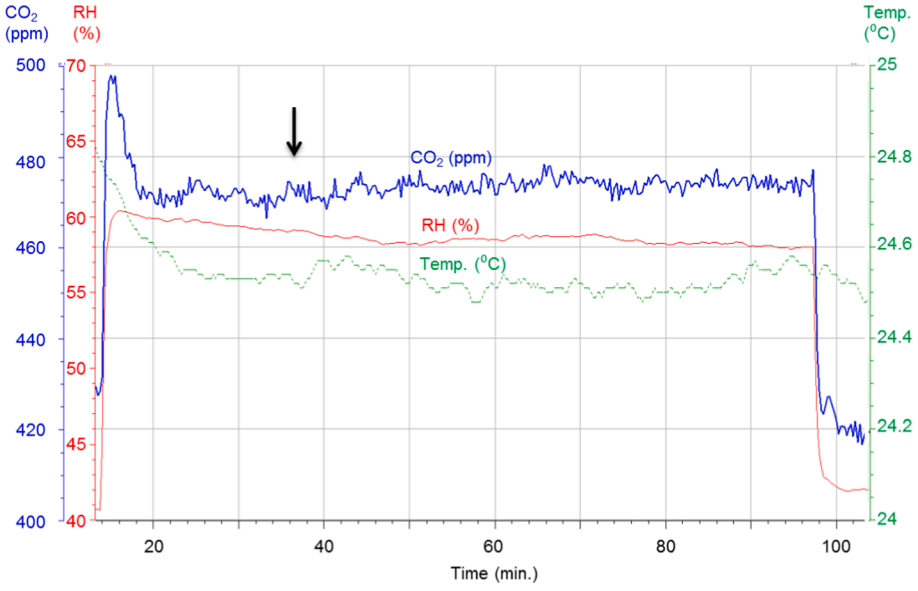

Fig. 6. Illustrative example of recordings of transpiration (red line) and respiration as CO_2 emission (blue line) of a whole grape berry bunch using the prototype. Green line is the recorded temperature during the measurements. The arrow indicates the moment when the grape berry bunch was detached from the plant (see text).

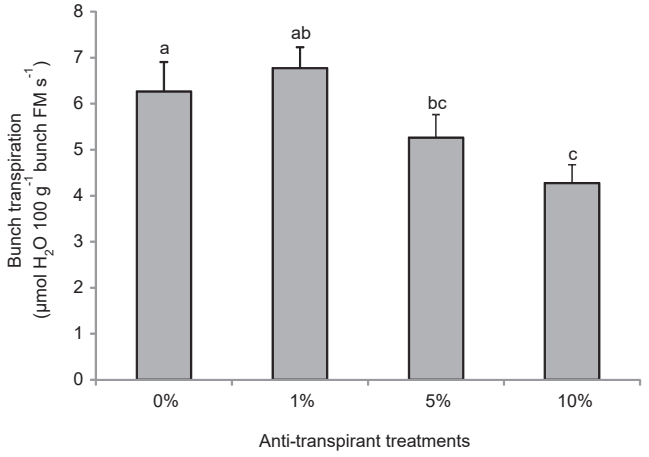

Fig. 7. Transpiration rates (μmol H_2O 100 g^{-1} bunch FM s^{-1}) of the grape berry bunches at maturity as a function of the dose of anti-transpirant (D0, D1, D5 and D10; dose 0, 1, 5 and 10%, respectively) applied at beginning of veraison. Data are mean ± S.E. (n = 8). Treatments with letters in common are not statistically different ($p > 0.05$). FM = fresh mass.

can be used as desired: (i) a syringe-based chamber to measure transpiration in detached grape berries, and (ii) a cube made with transparent methacrylate where a whole grape berry bunch (attached to the plant, or detached) can be enclosed. Measurements with the grape berry/bunch prototype were validated, using weight loss in scale as the traditional, standard technique to measure transpiration. Validations gave very good results. Prototype has the advantages of making transpiration measurements with a great level of environmental control, maintaining constant and stable parameters such as temperature and the RH of inlet air. Temperature and RH can be set as desired, matching values that simulate growing natural conditions. Air temperature and RH determines the air vapor pressure deficit, which has been previously reported to drive grape berry transpiration (Zhang and Keller, 2015). Other advantage is that measurements can be performed with the bunches attached to the plants, but however detaching the cluster did not alter the measurement, which open the possibility of transporting a large number of bunches to the laboratory to carry out the measurements. Finally, measurements made indicate that applying the anti-transpirant VaporGard® at the beginning of veraison delays maturation and suggest that may be related to some extent to lowered bunch transpiration. We plan to use the whole bunch prototype in experiments under climate change conditions (high temperature, elevated CO_2 and low RH), to evaluate whether transpiration and ripening times are somehow related. Since other sensors (CO_2, ethylene, O_2, volatile organic compounds, etc.) can be incorporated to the prototype, we are also working on the simultaneous measurement of bunch transpiration and respiration (CO_2 emission and O_2 consumption).

Author contributions

AU, FM and IP designed the prototype and the experiments, and interpreted the data. AU, IP, JJI, MCA, NG, HS, MO, JL and MB performed the experiments. FM and IP obtained funds, analyzed the data, drafted, proofread and finalized the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.compag.2022.106890.

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