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A custom-built simple system for conditioning and measurement of *in situ* whole-cluster transpiration

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Summary

A custom-built, low-cost gas exchange system designed for conditioning of the cluster microclimate and for fully automated measurements of *in situ* whole-cluster transpiration is presented. Measurements were carried out on potted Sangiovese grapevines at the onset of veraison. To increase the range of variability in cluster transpiration, air streams of different vapor pressure deficits (VPD) were created by conditioning the temperature of the incoming flow. Heating was created and maintained for 10 d (26 June - 5 July) by air flow through a metal segment equipped with three 75 W (warm) or 100 W (hot) light bulbs.

The cluster transpiration rates recorded for the unheated (control) clusters throughout the conditioning period varied from 0.18 to 0.28 mmol m^{-2} s^{-1}. While the daily transpiration rates of clusters supplied with warm air were similar to those of the control, water loss began to decrease significantly in clusters treated with hot air from day 4 onward and stayed lower throughout the remaining conditioning period. The gas exchange system presented here proved sensitive enough to detect the typically low transpiration rates of berries during ripening; effects due to air heating could be separated from fluctuations caused by daily variation of weather.

K e y w o r d s : grape berry, water loss, relative humidity, vapor pressure deficit, berry cuticle.

Introduction

Transpiration accounts for the majority of total grape berry water loss regardless of the stage of development (Düring *et al*. 1987; Lang and Thorpe 1989). Rates of berry transpiration are typically much lower than those recorded on leaves of grapevine due to both, reduced stomatal density and deposition of epicuticular, hydrophobic wax layers (Swift *et al.* 1973; Blanke and Leyhe 1987; Rosenquist and Morrison 1988). Rates of berry water loss calculated on a surface basis decrease sharply after veraison as a result of partial stomatal inactivation, although pre- and post-veraison rates do not differ very much if expressed per berry (Blanke and Leyhe 1987; Greenspan *et al.* 1994).

Estimates of berry transpiration are usually derived from weight or volume changes after a given time following excision (Greenspan *et al.* 1994). This method is fairly straightforward but typically destructive. To minimize the error due to excision, the cut pedicels are immediately sealed, whereas detached berries should be preferably repositioned to their original location in the cluster to retain a similar microclimate. The major limitations of this method are that other components of water exchange (xylem in- and outflow, phloem inflow) are excluded as potential contributors to the berry water budget and that, in sequential experiments, destructive berry sampling can differentially affect ripening of the retained berries.

The drawbacks of single-berry transpiration measurements may be overcome by a direct assessment of water loss from attached clusters. Besides not being destructive, such a measurement would take into account the differential contribution of cluster organs (rachis and berries). Moreover, it is expected that the transpiration rate of single berries varies as a function of their position in the cluster. External berries are subjected to a high radiation load and a low boundary layer and are likely to transpire more than internal ones. Variability in water loss among the berries of a cluster is also influenced by compactness; relatively loose clusters with some air circulation around the berries are expected to have transpiration rates on a surface basis higher than those of compact clusters.

It has been suggested that the flow of assimilates into the grape berry after veraison may be promoted by a water potential gradient between stem and cluster (Lang *et al*. 1986; Lang and Düring 1991). Since transpiration is known to be a controlling factor of water potential (Jones *et al.* 1985), reliable measurements of water loss may be more frequently needed in future research aimed to investigate the relationship between berry water status and ripening pattern.

To our knowledge a system to measure transpiration of attached clusters under controlled conditions has been reported only by Blanke and Leyhe (1987). This was based on a dew-point hygrometer control system cooling off the excess water vapor by transpiration to maintain an equilibrium between in- and outflow of air streams. The present study describes and evaluates a custom-built, low-cost gas exchange system designed for outdoor conditioning of the

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microclimate around the cluster and for *in situ* measurements of whole-cluster transpiration.

Material and Methods

Plant material: Five-year-old, own-rooted Sangiovese (clone 12 T) grapevines (*Vitis vinifera* L.) grown outdoor in 45 l pots filled with sand, peat and soil (1:1.5:1 v/v) were used. Four vines were chosen for uniformity at full bloom (first decade of June) and thinned to 12 single-clustered shoots per vine. Two weeks after fruit set each cluster was thinned by taking off the 'shot' or the very small berries to reduce cluster density and to improve the efficiency of air circulation around the berries. Thinning led to an average of 80 berries per cluster, which were judged as relatively loose. At veraison, two vines were placed in a shelter (85 % light transmission) for protection against bird damage.

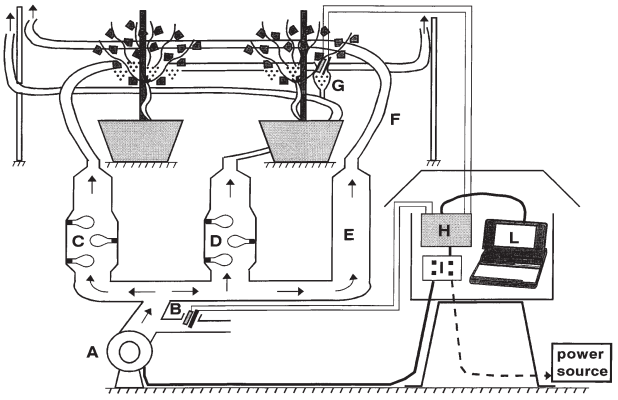

Fig. 1: Custom-built open gas exchange system for cluster microclimate conditioning and whole-cluster transpiration measurements. (**A**) centrifugal blower; (**B**) inlet air temperature and RH; (**C**) "warm" air stream equipped with 75 W light bulbs; (**D**) "hot" air stream equipped with 100 W light bulbs; (**E**) unheated control stream; (**F**) flexible, PVC pipe; (**G**) outflow air temperature and RH (only a single chamber outflow is represented for the sake of clarity); (**H**) CR 10 Campbell program and control module; (**I**) voltage transducer; (**L**) portable computer. Arrows indicate direction of air flow.

System description and cluster conditioning: The apparatus (Fig. 1) was set up under a shelter as a flow-through system with an air stream generated by an alternating current, centrifugal blower. The main flow was split into sub-streams and directed to the vines through a 80 cm diameter rigid, white plastic pipe which was connected to a flexible, internally smoothed PVC pipe (50 cm diameter) to facilitate access to the clusters at various canopy positions.

To increase the variability range of cluster transpiration, air streams of different vapor pressure deficits (VPD) were established by conditioning the temperature of the inflow. Two clusters per vine were randomly assigned either to a "warm" or "hot" treatment or used as unheated control. Heating (10 d, 26 June - 5 July) was achieved by feeding air through a metal segment inserted between the blower and the flexible ducts (Fig. 1) and equipped with three 75 W ("warm") or 100 W ("hot") light bulbs. Close to each cluster, a small hole was bored into the flexible pipe segment and fitted with a fine plastic pipe (3 mm internal diameter) to feed the air to the cluster enclosed in an inflated, transparent 0.025 mm thick polyethylene bag. Airflow through the conveying system was regulated by a single butterfly valve located upstream of the flow splitting. The airflow through each chamber was set at about 6 $l \cdot min^{-1}$; this rate was chosen to prevent water condensation inside the bag during daytime, to allow adequate bag inflation and to detect the typically low cluster transpiration rates. The chamber flow was repeatedly checked throughout the experimental period by a soap-film flow meter.

Each chamber included a relative humidity (RH) sensor (Philips ELCOMA, 2322/691/90001, thin-film capacitance type) and a 0.51 mm diameter, PVC-insulated copper-constantan thermocouple (type T). The RH sensors were placed in 7 cm x 2 cm (length by width) aluminum caps, which were tied and sealed to the bag at the bunch peduncle and protruded for about half of their length into the chamber. Several holes were drilled at both ends of the cap to allow ventilation of the sensor and to let the cluster chamber operate as an open system. The humidity sensors and the thermocouples were placed in close proximity to the cluster without touching it. An additional humidity sensor and thermocouple were inserted downstream of the butterfly valve into the rigid pipe to record inlet (reference) RH and air temperature.

Each RH sensor was calibrated using a condensation technique based on a column constructed from copper tubing immersed in an insulated flask containing water of a given dew-point temperature, which was measured with a thermocouple placed at the outlet below the water level. Different dew points were obtained by recirculating water of different temperatures inside the copper tubing. The air around the RH sensors was stirred by a fan and temperature was regulated by on-off light bulb switching. All sensors responded linearly ($r = 0.97 - 0.98$) from 25 to 70 % RH corresponding to a frequency output of 6.500-10.800 kHz. Sensor response to RH higher than 70 % was unsatisfactory due to increasing hysteresis.

Data collection and calculation of transpiration rates: System programming and automated data recording employed a CR10 data logger and control module (Campbell Sci., Leicestershire, UK) connected to a portable computer by an RS232 interface. A control program was downloaded to the logger for automated recording and storage of inlet and outlet temperature and RH values at 10 min intervals throughout the 10 d experimental period. The absolute humidities (H) were calculated using the equation reported by the ASAE (1982).

All clusters were harvested on 19 July and their volume measured by water displacement. Given the spherical shape of Sangiovese berries, the equatorial diameter of each berry on each cluster was recorded by a digital caliper and used to calculate mean berry surface. The volume of the rachis was taken at harvest by water displacement and its surface was

estimated using the same proportion which relates surface to volume in berries. The rachis surface accounted for about 4 % of the total bunch surface. Whole-cluster transpiration rates were calculated from air flow and the inlet-outlet differences in H and are expressed on an area basis.

Results and Discussion

Heating by the 100 W light bulbs induced a VPD significantly higher than that calculated for the unheated chambers over the 10 d conditioning period. The mean temperature increased by 1.7 °C for the hot treatment, whereas RH decreased by 8 % as compared to the unheated cluster chambers (Table). In spite of the large day-to-day fluctuations in temperature and RH values, these gradients were rather constant (not shown) suggesting that heating acted in a manner more or less independent of the ambient climatic variations.

T a b l e

Effects of air conditioning on the microclimate of bagged Sangiovese clusters. Daily means averaged from 9:00 to 20:00

Treatment	Temperature (°C)	Relative humidity (%)	VPD (kPa)
Unheated	34.6 a	59.0 a	2.43 b
Warm	35.3 a	55.3 ab	2.68 b
Hot	36.3 a	51.0 b	3.16 a

Mean separation within columns by Duncan Multiple Range Test (DMRT), 5 % level. VDP: vapour pressure deficit.

The time course change of VPD essentially followed that of air temperature (Fig. 2, top) and daily mean VPD of the hot treatment was always significantly higher than control values. The effect of cluster microclimate induced by the warm treatment was less marked and daily VPD was more erratically affected, exceeding those for control chambers only at times (Fig. 2, top).

The cluster transpiration rates (area basis) reported in Fig. 2 (bottom) are daily values averaged from 9:00 to 20:00 h (solar time). The rates recorded on the control (unheated) clusters throughout the conditioning period varied from 0.18 to 0.28 mmol m^{-2} s^{-1}. These values are higher than those found by Blanke and Leyhe (1987), which ranged from 80 to 150 mg dm^{-2} h^{-1} (corresponding to 0.122 - 0.231 mmol m^{-2} s^{-1}) at equivalent berry maturity stages for several grapevine cultivars. Besides differences due to genotype, this discrepancy can also be explained by the fact that Blanke and Leyhe worked under a controlled laboratory environment (20 °C, 50 % RH) with an evaporative demand (VPD = ca. 1.2 kPa) and a photon flux density (800 µmol m^{-2} s^{-1}) considerably lower than those recorded in our outdoor experiment.

The daily transpiration rates of clusters fed with warm air were similar to those of the control, whereas water loss

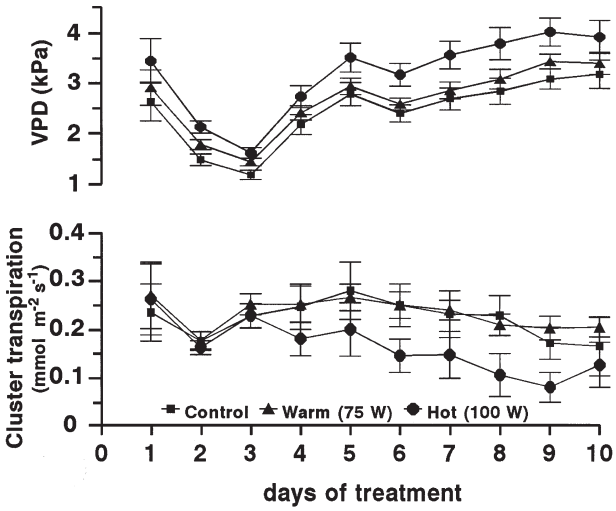

Fig. 2: Daily vapour pressure deficit (VDP) and transpiration rates calculated for the bagged Sangiovese clusters subjected to heat conditioning treatments. Diurnal values were obtained by averaging temperature and RH values recorded at 10 min intervals from 9.00 to 20:00. Vertical bars indicate 2 x standard error (SE).

began to decrease significantly in the hot-treated clusters from day 4 onward and stayed lower throughout the remaining period of conditioning (Fig. 2, bottom).

The correlation between the daily mean VPD and the cluster transpiration rate calculated for the data pooled over treatments fit a sigmoid model, indicating that water loss was constant in the range from 1.2 to 3 kPa and started to decline beyond this upper limit (Fig. 3). Thus, berries were unable to meet the highest evaporative demand and reacted with a water conservation mechanism. This response in consonant with other studies (Skoss 1955; Yamamura *et al.* 1986), which have demonstrated that cuticle and wax development on fruit is sensitive to changes in light, temperature and relative humidity. In grapevine, it has been shown that berries growing in full sun at high temperature have higher cuticle weight than berries developing in the shade (Rosenquist and Morrison 1989). Yet, the mechanism by which the hot-treated clusters showed an inhibited water

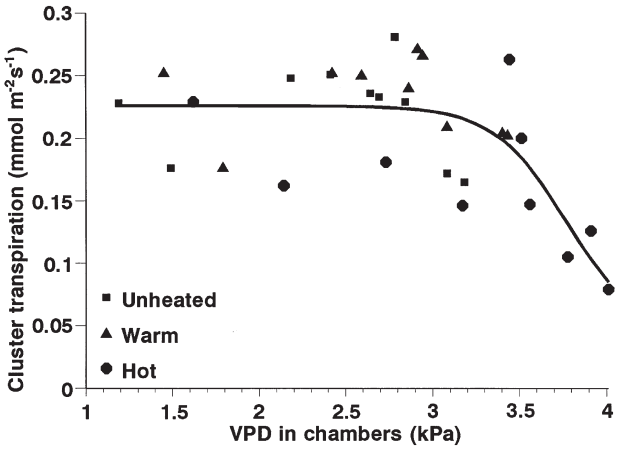

Fig. 3: Relationship between vapour pressure deficit (VDP) and cluster transpiration determined on daily mean values pooled over treatments. $y = 0.039 + 0.19/(1+\exp(-(x-3.77)/-0.21))$, $R^2 = 0.51$.

loss at high temperature and VPD remains unknown. It is well known that post-veraison berries regulate their water loss primarily through the multi-layered cuticle and the soft epicuticolar wax (Radler 1965). In fact, as the berry matures, number of stomata decreases to <1 mm^{-1} and they increasingly develop to corky lenticels (Hardie *et al.* 1996). This suggests that the biochemistry of the components of the soft wax, which have been reported as the main regulators of berry water loss at post-veraison (Grncarevic and Radler 1967; Possingham *et al*. 1967), is sensitive to increasing VPD (likely via a direct effect of temperature) and leads to a strengthening of the barrier to water flow.

The layout of our experiment did not enable a simultaneous comparison of transpiration rates calculated from whole-cluster records and estimated on excised single berries by weight loss. However, post-veraison estimates of the latter for Cabernet Sauvignon berries (Greenspan *et al*. 1994) and Pinot Noir (Rebucci 1995) under similar VPDs ranged from 0.267 to 0.250 mmol m^{-2} s^{-1}, an interval which is in good agreement with the control data reported in Fig. 2. It should be pointed out that the clusters tested here were relatively loose, hence those factors which in more compact clusters may limit the transpiration of berries (shading, high boundary layer resistance) had little impact in the present study. Under such circumstances, the averaged whole-cluster transpiration rates (surface basis) are expected not to differ significantly from rates determined by weight loss of individual berries.

Overall, the gas exchange system presented here proved sensitive enough to detect the typically low berry transpiration rates. The data example reported for the 10 d recording period also showed that the effects due to the superimposed treatments (e.g. air heating) could be separated from fluctuations caused by daily variation of climate. Besides the simple design and set up and the suitability for field applications, another positive features of the system is automation, which allows long unattended periods of recording and low cost. In fact, the system is primarily the outcome of the skilled assembly of various pieces of equipment (data logger, temperature and RH sensors, etc.) which are usually available to researchers in applied plant physiology.

References

American Society of Agricultural Engineers; 1982: Psychrometric Data. Amer. Soc. Agr. Eng. St. Joseph, Mich.

Blanke, M. M.; Leyhe, A.; 1987: Stomatal activity of the grape berry cv. Riesling, Müller-Thurgau and Ehrenfelser. J. Plant Physiol. **127**, 451-460.

Düring, H.; Lang, A.; Oggionni, F.; 1987: Patterns of water flow in Riesling berries in relation to developmental changes in their xylem morphology. Vitis **26**, 123-131.

Greenspan, M. D.; Shackel, K. A.; Matthews, M. A.; 1994: Developmental changes in the diurnal water budget of the grape berry exposed to water deficits. Plant Cell Environ. **17**, 811-820.

Grncarevic, M.; Radler, F.; 1967: The effect of wax components on cuticular transpiration-model experiments. Planta **75**, 23-27.

Hardie, W. J.; O'Brien, T. P.; Jaudzems, V. G.; 1996: Morphology, anatomy and development of the pericarp after anthesis in grape, *Vitis vinifera* L. Austr. J. Grape and Wine Res. **2**, 97-142.

Jones, H. G.; Lakso, A. N.; Syvertsen, J. P.; 1985: Physiological control of water status in temperate and subtropical fruit trees. Hort. Rev. **7**, 301-343.

Lang, A.; Düring, H.; 1991: Partitioning control by water potential gradient: Evidence for compartmentation breakdown in grape berries. J. Expt. Bot. **42**, 1117-1122.

- -; Thorpe, M. R.; 1989: Xylem, phloem and transpiration flows in a grape: Application of a technique for measuring the volume of attached fruits to a high resolution using Archimedes principle. J. Expt. Bot. **40**, 1069-1078.

- -; - -; Edwards, W. R. N.; 1986: Plant water potential and translocation. In: J. Cronshaw, W. J. Lucas, R. T. Giaquinta (Eds.): 'Phloem Transport', 193-194. Alan R. Liss, Inc., New York.

Possingham, J. V.; Chambers, T. C.; Radler, F.; Grncarevic, M.; 1967: Cuticular transpiration and wax structure and composition of leaves and fruit of *Vitis vinifera*. Aust. J. Biol. Sci. **20**, 1149-1153.

Radler, F.; 1965: The main constituents of the surface waxes of varieties and species of the genus *Vitis*. Amer. J. Enol. Vitic.**16**, 159-167.

Rebucci, B., 1995: Interventi esogeni di condizionamento dello stato idrico dell'acino: aspetti metodologici ed effetti sull'accumulo dei soluti. Tesi di Dottorato di Ricerca, Bologna.

Rosenquist, J. K.; Morrison, J. C.; 1988: The development of the cuticle and epicuticular wax of the grape berry. Vitis **27**, 63-70.

- -; - -; 1989: Some factors affecting cuticle and wax accumulation on grape berries. Amer. J. Enol. Vitic. **40**, 241-244.

Skoss, J. D.; 1955: Structure and composition of plant cuticle in relation to environmental factors and permeability. Bot. Gaz. **117**, 55-72.

Swift, J. G.; Buttrose, M. S.; Possingham, J. V.; 1973: Stomata and starch in grape berries. Vitis **12**, 38-45.

Yamamura, H.; Naito, R.; Tamura, H.; 1986: Effects of light intensity and humidity around the clusters on the formation of surface wax and the resistance to berry splitting in "Delaware" grapes. J. Jap. Soc. Hort. Sci. **55**, 138-144.

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