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## **A cost-effective system for injecting pure CO<sub>2</sub> into open top chambers: design and performance**

Mahabubur Mollah<sup>AD</sup>, Everard Edwards<sup>C</sup>, Dale Unwin<sup>B</sup>, Glenn Fitzgerald<sup>A</sup>, Rachel Kilmister<sup>B</sup>

<sup>A</sup> Department of Environment and Primary Industries – Horsham, 110 Natimuk Road, Horsham, Victoria 3401, Australia

<sup>B</sup> Department of Environment and Primary Industries – Irymple, crn Koorlong Ave & Eleventh Street, Irymple, Victoria 3498, Australia

<sup>C</sup> CSIRO Plant Industry - Waite Campus Laboratory, PO Box 350, Glen Osmond, SA 5064, Australia

<sup>D</sup> Corresponding author, email: [Mahabubur.Mollah@depi.vic.gov.au](mailto:Mahabubur.Mollah@depi.vic.gov.au)

### **Abstract**

Global average temperature is expected to rise between 0.2°C and 4.8°C by the end of the century, and globally atmospheric carbon dioxide concentration is expected to increase from a mean of 400  $\mu\text{mol mol}^{-1}$  in 2013 to 550-650  $\mu\text{mol mol}^{-1}$  within the next 50-80 years. Studies suggest there has already been an effect of warmer temperatures on grape growth and development, such as grape phenology, with harvest dates advancing and vintages becoming shorter over the past 30 years in Australia. Such changes create logistical problems for wineries and diminish grape quality. In addition, increasing carbon dioxide concentrations ( $[\text{CO}_2]$ ) are likely to alter grape vine growth and development. Understanding the combined effect of elevated carbon dioxide concentration ( $e[\text{CO}_2]$ ) and temperature together with their interactions on grape and wine qualities is necessary for industry adaption to future climate change. Therefore a system was developed to elevate  $[\text{CO}_2]$  to 650  $\mu\text{mol mol}^{-1}$  and increase temperature by 2°C around the grapevines in open top chambers (OTC) to simulate climate warming. The temporal and spatial distribution of CO<sub>2</sub> gas at the center of the OTC was maintained to within 72  $\mu\text{mol mol}^{-1}$  standard deviation of the target (650  $\mu\text{mol mol}^{-1}$ ), which is comparable to the distribution reported in Free Air CO<sub>2</sub> Enrichment (FACE) systems. The injection system described in this article consumed 60  $\text{g m}^{-3} \text{h}^{-1}$  of CO<sub>2</sub> which is less than one-fifth of the CO<sub>2</sub> consumed by the Australian Grains Free Air CO<sub>2</sub> Enrichment system (318  $\text{g m}^{-3} \text{h}^{-1}$ ) and a circular OTC (316  $\text{g m}^{-3} \text{h}^{-1}$ ) used in a previous study as well as very similar to the consumption of the ForestFACE (50  $\text{g m}^{-3} \text{h}^{-1}$ ). The cost-effective CO<sub>2</sub> injection system proposed here is therefore recommended for use inside OTCs in evaluating the effects of  $e[\text{CO}_2]$  in combination with elevated temperature in woody perennial crops.

**Keywords:** Grapevines, elevated CO<sub>2</sub>, temperature, open top chamber, Australia.

### **1 Introduction**

Analyses of vintage records suggest that climate warming is already having an effect on the phenology of grapevines (*Vitis vinifera* L.), with harvest dates advancing by about eight days per decade over the last 30 years in Australia (Webb et al. 2011), with similar trends in Europe (Jones et al. 2005). The changes in global and local temperatures during the periods represented by these studies were small compared to the predictions made in the Intergovernmental Panel on Climate Change (IPCC) Fifth Assessment Report, which predicts global average temperature to rise between 0.3°C and 4.8°C by the end of the century, with >2°C being likely in the majority of emission scenarios considered (Rusticucci et al. 2013). The largest driver of this increase in temperature is atmospheric carbon dioxide concentration ( $[\text{CO}_2]$ ), currently 401  $\mu\text{mol mol}^{-1}$  (Scripps Institution of Oceanography 2014). For example, the A1B emission scenario of the IPCC Fourth Assessment Report predicts

that  $[CO_2]$  will reach  $550 \mu\text{mol mol}^{-1}$  by 2050 (Carter et al. 2007). Consequently, agronomical-agronomically important traits such as yield, water use efficiency, phenology and pest and disease incidence on crops are likely to be impacted by climate change (e.g. high  $CO_2$  level) over the next 20 to 50 years.

It is possible to artificially elevate  $[CO_2]$  around growing plants using various methodologies. Open top chambers (OTCs) have often been used as one of the methods for conducting experiments with  $e[CO_2]$  in the field, particularly when studying woody perennial plants (e.g. Barton et al. 2010, Langley et al. 2013). Field based studies of the effects of elevated  $[CO_2]$  ( $e[CO_2]$ ) on grapevines have been conducted (Bindi et al. 2001a, Gonçalves et al. 2009, Moutinho-Pereira 2009, Moutinho-Pereira 2010), but have been fairly short-term or limited in scope and are yet to be combined with control of temperature.

We have previously developed an OTC system (Edwards et al. 2012), using active heating, to elevate temperature around mature grapevines to study the effect of climate warming on grapevine development and grape quality parameters. Here, we describe the addition and testing of a prototype  $CO_2$  injection system for use in OTCs ( $CO_2InOTC$ ) to increase  $[CO_2]$  around the grapevines and compare the resource use and efficacy of our system with existing methodologies to study  $e[CO_2]$  effects in the field.

## 2 Materials and methods

### *System for injecting pure $CO_2$ into OTCs ( $CO_2InOTC$ )*

In the  $CO_2InOTC$  system, pure  $CO_2$  was injected (jets pointed downwards at  $45^\circ$  angle) into the atmosphere just below the canopy from both sides of the trellis, through 0.30 mm diameter holes at supply line pressures below 40 kPa. The intention of the design was for the  $CO_2$  to quickly mix with the chamber air and be transported in and around the grapevines by air movement (with or without the heating system fan running).

A  $CO_2$  sensor with relay facility (SEN51101S01, ETM Pacific Pty Ltd, North Sydney, NSW, Australia) was placed near the top shoot of the grapevine at the centre of the OTC and was used to switch the  $CO_2$  supply on or off, using a solenoid valve, in order to maintain the target  $[CO_2]$  of  $550 \mu\text{mol mol}^{-1}$ . Both the  $CO_2$  sensor and the solenoid valve were powered by 24-V DC (direct current). The major components of the  $CO_2InOTC$  system are shown in a schematic diagram (Fig. 1).

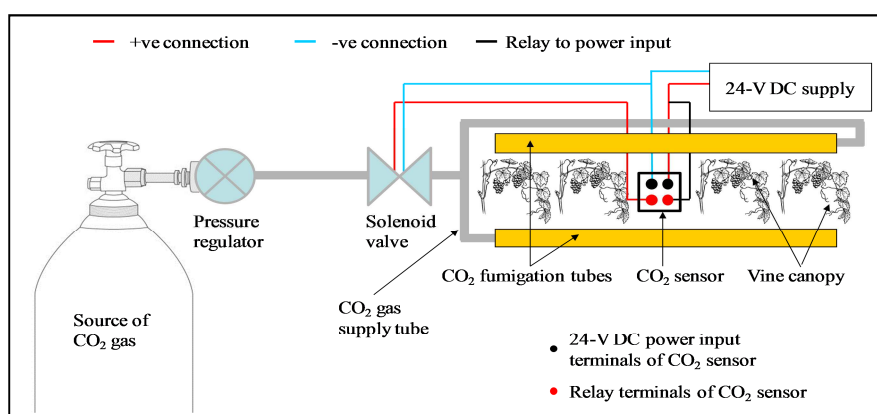


Figure 1: The major components of the prototype  $CO_2InOTC$  system.

### *Assessment of $[CO_2]$ spatial distribution*

A multi-port IRGA was used to measure the spatial distribution of  $[CO_2]$  within the OTC. The details of design, operation and port selection are published elsewhere (Mollah et al. 2011).

## CO<sub>2</sub>InOTC trial I – winter 2011

In May 2011, after leaf fall, but before the vines were pruned, the CO<sub>2</sub>InOTC system was assembled inside a single OTC (Fig. 2). The objective of this trial was to determine the feasibility of the CO<sub>2</sub>InOTC system and if so, estimate the usage of CO<sub>2</sub> gas to calculate the overall cost of the CO<sub>2</sub>InOTC system. Data were collected for about 10 hours (the duration of sunlight) during each day on 3 and 4 May 2011.

Thirty two sampling locations were pre-determined representing the entire volume of space contained by the chamber; these were arranged in horizontal and vertical planes. Samples were collected at 0.75-m, 1.3-m and 1.7-m above the soil surface. The coordinates (x, y, z) of each location were recorded relative to the centre of the OTC (x, y, z = 0, 0, 0).

The multi-port IRGA was placed inside the OTC (see Fig. 2) and set to automatically log [CO<sub>2</sub>] at each of the 32 sampling points, with a 5.33 min interval between each full set of measurements. Date, time, wind speed, and wind direction were logged with every individual [CO<sub>2</sub>] measurements. The height of the CO<sub>2</sub> controller sensor and the height and spacing of fumigation tubes were adjusted until the data indicated that the desired spatial distribution of [CO<sub>2</sub>] inside the OTC was achieved.

## CO<sub>2</sub>InOTC trial II – summer 2011/12

The CO<sub>2</sub>InOTC system and multi-port IRGA were set up as in Trial I, in the same vineyard, but utilising a different panel of vines, and run between December 13<sup>th</sup> and 16<sup>th</sup> 2011 with fully grown vines (Fig. 3). As with Trial I, 32 sampling points in both horizontal and vertical planes inside the OTC were pre-determined and their coordinates (x, y, z) recorded. Samples were collected at 0.75-m, 1.35-m and 1.75-m above the soil surface, with an additional sampling point located 2.56-m above the soil surface, at the tip of the highest shoot. Nine different fumigation scenarios were trialled, comprising of two CO<sub>2</sub> fumigation tube heights; two angles of fumigation jets, two heights of CO<sub>2</sub> controller sensor and with the circulating fan component of the heating system on or off (Table 1).

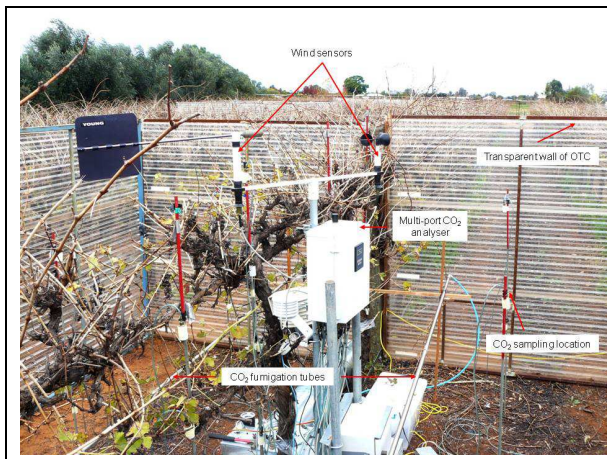


Figure 2: Set up for CO<sub>2</sub> injection, control and monitoring inside an OTC for Trial I (winter)

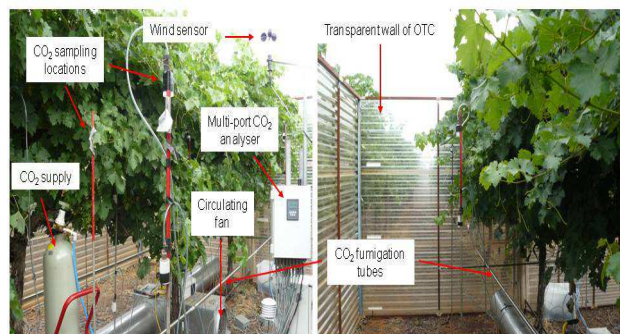


Figure 3: Set up for CO<sub>2</sub> injection, control and monitoring inside an OTC for Trial II (summer).

## Statistical methods and contour mapping

There were 3,408 data points for the winter trial and 10,371 data points for the summer trial available for analysis. The data consisted of [CO<sub>2</sub>], wind speed and wind direction readings collected by the multi-port IRGA. All data collected were summarised using means, medians and standard deviations. The data collected for the summer trial were categorised according to the scenarios (Table 1). One sample sign tests and Student's t-tests were performed to identify the scenario with the best spatial distribution. The Kriging method of spatial interpolation (point type), employing a linear variogram model, was used to draw the contour

maps of [CO<sub>2</sub>] for the selected scenarios using Surfer 7 software (Golden Software Inc, CO, USA).

Table 1: Set-up scenarios tested during Trial II, summer 2011. The fumigation tubes were set to a spacing of 1.3 m and the jets were facing inwards, towards the trellis, in all cases.

Scenario	Fumigation tube height (m)	Jet orientation	CO <sub>2</sub> sensor height (m)	Circulating fan
1a	1.3	Parallel to ground.	1.95	Off
1b	1.3	Parallel to ground.	1.95	On
2a	1.3	Parallel to ground.	2.3	Off
2b	1.3	Parallel to ground.	2.3	On
3a	1.3	45° downwards.	2.3	Off
3b	1.3	45° downwards.	2.3	On
4a	0.75	Parallel to ground.	2.3	Off
4b	0.75	Parallel to ground.	2.3	On
5	0.75	45° downwards.	2.3	Off

### 3 Results

#### *Optimal positioning of system components*

Manually relocating the fumigation tubes in Trial I, whilst monitoring the [CO<sub>2</sub>] at each sampling location, resulted in the most uniform spatial distribution around the vines being achieved by setting the fumigation tubes at a distance of 1.4-m apart from each other, with the trellis in the centre, and 1.5 m above the soil surface (data not shown). The CO<sub>2</sub> sensor was placed at the centre of the OTC at a height of 1.75-m, just above the top cordon wire.

In Trial II, a total of nine ‘scenarios’ (four with and without the circulation fan running, one only without the fan, see Table 1) were tested with a full canopy on the vines, using various combinations of fumigation tube height, jet angle, CO<sub>2</sub> sensor position and with the circulating fan on or off. Analysing the results with a ‘one sample sign test’ showed that the mean [CO<sub>2</sub>] at different heights inside the grapevine canopy were significantly different from the target 550 μmol mol<sup>-1</sup> for scenarios 1a, 1b, 2b, 4a and 5, hence scenarios 1 & 5 were rejected. Scenario 4b exhibited a relatively high variance (data not shown), so scenario 4 was also rejected. The standard deviation (SD) of [CO<sub>2</sub>] for each of the remaining scenarios (2 and 3) was relatively small and consistent, but comparing the mean [CO<sub>2</sub>] across all heights, it was scenario 3 that had the most consistent result, combined with the lowest SD (Table 2). Therefore, scenario 3 was selected as the best set up for CO<sub>2</sub> injection inside OTCs.

Table 2: Mean [CO<sub>2</sub>] and SD around the grapevine canopy at three heights for the two scenarios where mean [CO<sub>2</sub>] did not differ significantly from set point.

Scenario	Sampling height above the ground					
	0.75-m		1.35-m		1.75-m	
	Mean [CO <sub>2</sub> ] (μmol/mol)	SD [CO <sub>2</sub> ] (μmol/mol)	Mean [CO <sub>2</sub> ] (μmol/mol)	SD [CO <sub>2</sub> ] (μmol/mol)	Mean [CO <sub>2</sub> ] (μmol/mol)	SD [CO <sub>2</sub> ] (μmol/mol)
2a	508	58	603	89	621	113
2b	573	96	594	43	587	123
3a	547	67	603	104	591	83
3b	511	58	582	73	593	73

### *Spatial distribution of [CO<sub>2</sub>]*

The CO<sub>2</sub>InOTC system encloses three vines along a vine row (one panel), but the central vine is used for physiological measurements and sample collection wherever possible to minimise edge effects. Consequently, whilst maintaining as even a spatial distribution of [CO<sub>2</sub>] as possible is desirable, it is particularly important that the central vine has the minimum possible temporal variation in elevation of [CO<sub>2</sub>] above background levels, which averaged approximately 375 µmol mol<sup>-1</sup> during the day, increasing by about 20 µmol mol<sup>-1</sup> during the night to 395 µmol mol<sup>-1</sup>.

As this was an open top system, temporal variation in [CO<sub>2</sub>] was likely to be driven by variation in wind speed and direction. The former ranged between 0 and 3.4 m s<sup>-1</sup> during the second test period, but averaged only 0.7 m s<sup>-1</sup>. In fact, the middle vine received the desired amount of CO<sub>2</sub> regardless of wind direction, because injection occurred all around that vine and the CO<sub>2</sub> addition was controlled based on the [CO<sub>2</sub>] above it. So, the system was largely independent of wind direction.

The system could also be fitted with a sensor to shut off the CO<sub>2</sub> supply if wind speed exceeds the set target, thus minimising the loss of CO<sub>2</sub> at wind speeds that prevent effective control. However, this was not implemented in our trials as such speeds were not encountered during our testing.

During Trial 1, where there were no leaves on the vines to influence air movement and the CO<sub>2</sub> sensor was positioned at 1.75 m, the influence of wind on the spatial distribution was evident with the highest [CO<sub>2</sub>] measured inside the OTC on the windward side (results not shown). During Trial 2, where the jet angle and CO<sub>2</sub> sensor height were optimised and a full canopy was present, the best spatial distribution of [CO<sub>2</sub>] was observed with scenario 3 (see above) and exhibited a 2\*SD (95% confidence level) of approximately 25% of set-point horizontal (Fig. 4) and vertical (Fig. 5) planes, with or without the circulating fan in use. This was comparable to the spatial distribution of [CO<sub>2</sub>] achieved in wheat fields during the AGFACE experiments (Mollah et al. 2010, Mollah et al. 2011) and as good as or better than that reported for an area distributed FACE (Bunce 2011), a system with which the CO<sub>2</sub>InOTC system is somewhat similar.

The results presented here for the CO<sub>2</sub>InOTC system are most likely to be influenced by the artefacts of the OTC, such as restricted airflow, altered temperature and RH, reduced interception of radiation and precipitation on and around the crop inside the OTC. Although the system was designed to work within the OTC, due to the need to control air temperature and minimise CO<sub>2</sub> use, a similar system could be established in a free-air situation. If successful, it would provide both a cost-effective and portable solution, not only for grapevines but potentially for other perennial orchard crops like apples, pears, etc. with shallow canopies.

FACE systems that inject pure CO<sub>2</sub> in the atmosphere generate a large and highly variable [CO<sub>2</sub>] around the fumigation tubes (Okada et al.2001; Mollah et al.2009; Mollah et al.2011). However, the CO<sub>2</sub>InOTC system uses very low injection pressure i.e. 40 kPa compared with 500 kPa for AGFACE. Consequently, it was expected that there would be a smaller and less variable [CO<sub>2</sub>] near the fumigation tubes for our system. This was demonstrated by the results, e.g., a mean of 582 µmol mol<sup>-1</sup> inside the grape canopy at 65 cm (tube spacing 1.3 m) from the fumigation tube with a SD of 73 µmol mol<sup>-1</sup> (Fig. 5b) compared with a mean of about 800 µmol mol<sup>-1</sup> and a SD of 450 µmol mol<sup>-1</sup> for a similar distance in AGFACE (Mollah et al.2011). The CO<sub>2</sub>InOTC system injects CO<sub>2</sub> downward (not targeting canopy) at a 45° angle which will also minimise the biological impacts, if any, of pulses of high CO<sub>2</sub>.

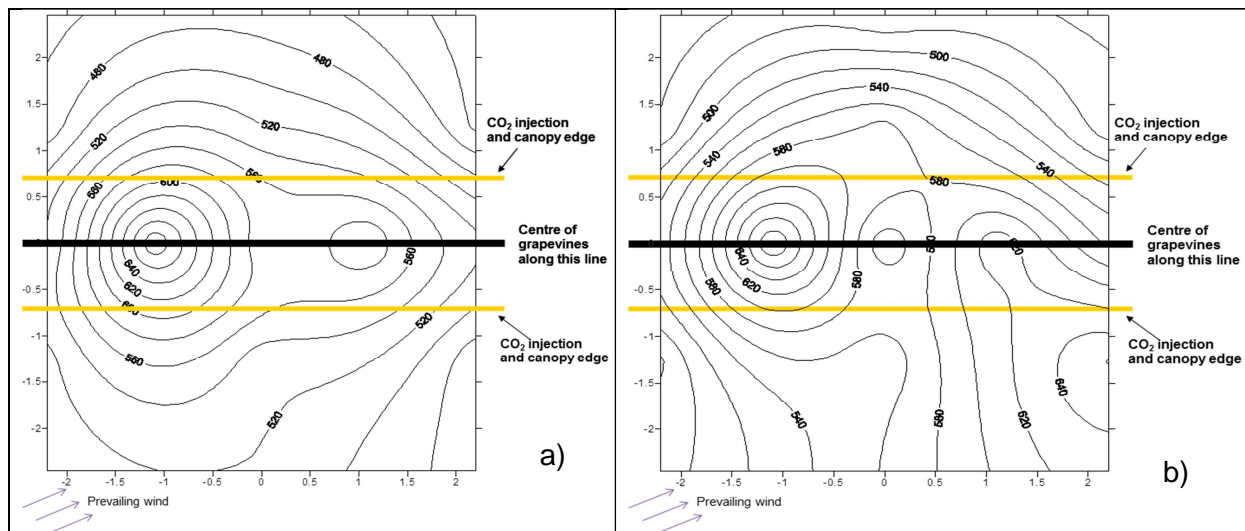


Figure 4. Top view of spatial distribution of [CO<sub>2</sub>] at a) 1.35 m and b) 1.75 m above the soil surface inside the OTC during Trial II (Summer), scenario 3b (heating system on).

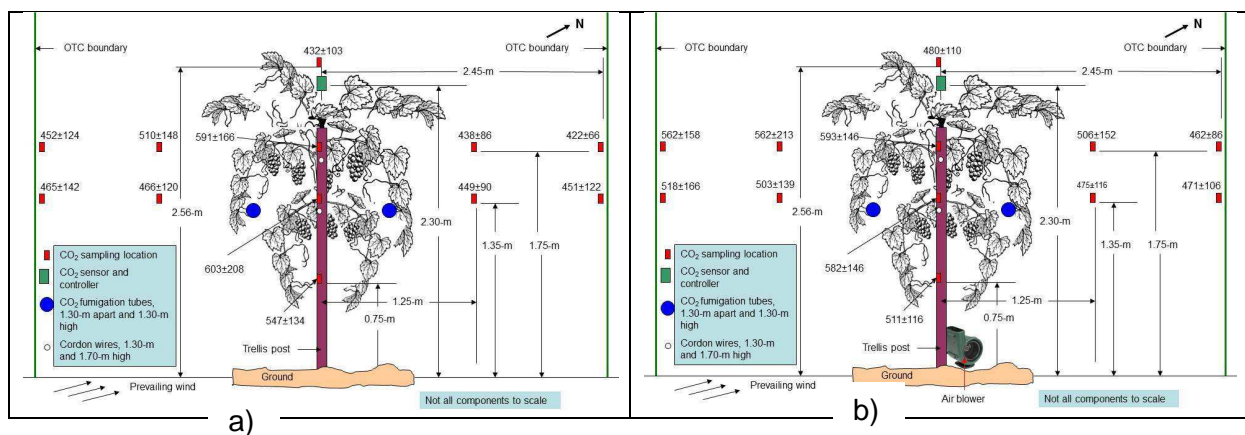


Figure 5. The mean [CO<sub>2</sub>] ± 2\*SD (for 95% confidence level) in and around grapevine with full canopy inside an OTC for a) scenario 3a (circulating fan off) and b) scenario 3b (circulating fan on).

### Cost-effectiveness of the CO<sub>2</sub>InOTC system

The data from the AGFACE experiment showed that for an average relative humidity (RH) of 44%, average temperature of 17°C and median wind speed of 0.7 m s<sup>-1</sup> the CO<sub>2</sub> consumption was 318 g m<sup>-3</sup> h<sup>-1</sup> (unpublished). On the other hand, the CO<sub>2</sub> consumption inside the OTC in the vineyard was 60 g m<sup>-3</sup> h<sup>-1</sup>, less than one-fifth of AGFACE, for similar conditions, e.g. average RH of 35%, average temperature of 20.7°C and median wind speed of 0.7 m s<sup>-1</sup>. In the early 1990s, OTCs were built in UK to expose plants in the field to elevated concentrations of CO<sub>2</sub> (Ashenden et al. 1992). The CO<sub>2</sub> consumption in those OTCs was estimated to be 316 g m<sup>-3</sup> h<sup>-1</sup>, also much higher than the consumption reported here and similar to AGFACE.

At the wind speeds occurring during testing, the CO<sub>2</sub>InOTC system on average used 4 kg of CO<sub>2</sub> per hour. This low consumption of CO<sub>2</sub> can be attributed to the low pressure used for injection of CO<sub>2</sub> (40 kPa vs 500 kPa for AGFACE) and the mode of injection. Inside the OTC, CO<sub>2</sub> was released underneath the canopy from both sides of the vine row and pointing towards the ground at 45° angle, so the jets of CO<sub>2</sub> from both sides could converge underneath the canopy. The mixture of CO<sub>2</sub> and air travelled up through the canopy and reached the CO<sub>2</sub> sensor and controller near the top of the canopy (2.3-m above the ground, Fig. 6). This mode of injection minimised the loss. In FACE rings, CO<sub>2</sub> is released above the canopy from the upwind side, so the prevailing winds can transport the mixture of CO<sub>2</sub> and

air to the downwind side of the ring. This requires high pressure release and large amount of CO<sub>2</sub> is lost through turbulence.

There have been very few experiments studying the impact of e[CO<sub>2</sub>] on grapevines. Recently, a FACE system was established at Geisenheim, Germany to test the effects of e[CO<sub>2</sub>] on grapevines, but this has not yet been switched on. In the late 1990s, a FACE system for grapevines was established in Italy (Bindi et al. 2001b), similar to the concept of ForestFACE (Hendrey et al. 1999). There are no published data on CO<sub>2</sub> consumption for either system and for the former at least, data are not available (pers. com. Bindi 2013). However, the ForestFACE study reported that CO<sub>2</sub> consumption was much higher than an OTC on an absolute basis but similar to that of the OTC on a per unit volume basis. The consumption averaged 50 g m<sup>-3</sup> h<sup>-1</sup> for average wind speed of 1.5 m s<sup>-1</sup> (Hendrey et al. 1999) and this may be indicative of the Italian system, since it used a similar design.

## 4 Conclusions

The CO<sub>2</sub>InOTC system is a cost-effective way to inject CO<sub>2</sub> inside an OTC to elevate [CO<sub>2</sub>] in and around grapevines or other woody perennial row crop. The spatial distribution of CO<sub>2</sub> gas by CO<sub>2</sub>InOTC with or without blower assisted air circulation was as good as or better than the distribution reported for several other FACE systems.

## 5 Acknowledgements

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