Reductions in biomass accumulation, photosynthesis in situ and net carbon balance are the costs of protecting Vitis vinifera ‘Semillon’ grapevines from heat stress with shade covering

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Abstract

Background and aims Covering whole vines with shade cloth is used to protect the vines from heat stress, but may have costs on vine productivity through reduced light availability. Our aim was to assess the carbon balance of vines growing with and without shade to quantify the impact of the covering.

Methodology Whole vines were covered with 70% shade cloth, and shoot leaf area and leaf, stem and bunch growth were followed over two growing seasons. Photosynthesis was measured in situ in all leaves along selected shoots over the growing season. A carbon balance was constructed from the difference in acquisition of carbon and the sequestration of carbon as biomass across the growing seasons.

Principal results Shade covering had no initial impact on shoot growth but later reduced leaf growth and later still bunch growth. Stem growth was unaffected. Photosynthetic properties were characteristic of shade leaves, with lower rates and lower light saturation compared with well-exposed leaves. Overall, net photosynthesis was reduced by 40% by the shade covering and was attributed to the reduced photon flux densities. From the carbon balance, vines were reliant on carbon reserves over 6 weeks after budbreak until current photosynthate increased sufficiently to supply the growth. Shade covering impacted most on biomass accumulation to leaves and bunches at the stage when the vines became autotrophic, consistent with the reduction in carbon acquisition. The markedly high carbon demand by bunches caused a mid-season negative carbon balance, implying that shoots had to draw further on reserves to supply the carbon.

Conclusions Shade covering over whole grapevines exacerbated the imbalance between the supply of and demand for carbon and greatly reduced vine biomass, especially reproductive allocation. Covering vines with shade cloth to protect the vines from heat events, therefore, had major costs in the carbon economy.

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Introduction

The need to protect grapevines from the many heat events that occur with a relatively high frequency in most parts of the grape-growing regions of Australia has been a major focus of recent research. The use of shade covering over whole panels of Semillon vines has been demonstrated to reduce canopy temperatures (Greer et al. 2010) and protect the berry-ripening process (Greer and Weston 2010). However, shade cloth reduced photon flux densities (PFDs) and this has affected vine performance. In particular, Greer et al. (2010) have shown that the shade delayed shoot development, but leaf expansion rates and leaf sizes increased and appeared to be a compensatory mechanism for the low-light regime. Another major response to the shade was a marked shift in shoot architecture towards a lower frequency of long shoots and a higher frequency of medium shoots compared with fully exposed vines. Greer et al. (2010) postulated that reduced carbohydrate availability may have accounted for the reduced frequency of long shoots.

Many studies have used shade covering over a variety of plant species and most, if not all, have demonstrated changes in biomass allocation or yield reduction (Cartechini and Palliotti 1995; Grassi and Minotta 2000; Cohen et al. 2005). The question then arises as to whether these reductions in biomass were photosynthetically driven. Certainly, photosynthetic rates of Sangiovese vines under several different levels of shading were significantly reduced (Cartechini and Palliotti 1995), as was also observed with Picea abies shoots under shade (Grassi and Minotta 2000). Similarly, some Mediterranean shrubs had higher rates in the sun than in the shade (Valladares et al. 2008). By contrast, with grapefruit shading, photosynthesis was increased in the shade because of reductions in temperature offsetting the reduction in irradiance (Cohen et al. 2005). However, it is not the photosynthetic rates per se that drive growth but the total carbon gained over the growing season, and this now needs to be assessed to determine the impact of shade on vine performance.

Previously, Greer and Sicard (2009) developed a carbon budget for potted Semillon vines growing in controlled-environment conditions and demonstrated that the vines had a negative carbon balance straight after budbreak for up to 6 weeks. This has been shown for a number of deciduous woody plants (Johnson and Lakso 1986; Maillard et al. 1994; Greer 1996; Radoglou and Teskey 1997). Thereafter, in the Semillon study (Greer and Sicard 2009) the vines produced more carbon than was sequestered into biomass. A net surplus was also achieved even though bunch development commenced at the same time as the shoots became autotrophic. Considerably more biomass was allocated to the bunches than the vegetative components, but the capacity of the vines to carry the crop load was not in question from the analysis of the carbon budget. In field-grown Riesling grapevines (Downton and Grant 1992), the total carbon fixed by spur-pruned vines was also more than adequate to supply the demand by stems, leaves and bunches, and a small surplus of carbon was reached across the growing season. Similarly, in a study of Actinidia delicosa vines (Greer et al. 2003), fruit also impacted on vegetative growth rates and surplus carbon was achieved, but the effect on net carbon balance was more marked than in these other studies.

As is evident in these studies of the carbon budget of deciduous woody plants, carbon reserves are required in spring to develop shoots from dormant buds (Loescher et al. 1990; Bennett et al. 2005; Holzapfel et al. 2010). It is, therefore, essential that such species have an adequate carbon acquisition capacity during the growing season, not only to supply all the vegetative and reproductive growth demands but also to replenish the reserves in the post-harvest period (Holzapfel et al. 2006). However, for grapevines at least, little is known of the carbon demands during the growing season in relation to supply to provide an adequate understanding of the dynamics of carbon flow between these competing needs. Downton and Grant (1992), however, assessed the carbon budget of Riesling vines and showed that 60–70 % of carbon gain was sequestered in vegetative and reproductive biomass. In the variety Semillon, Greer and Sicard (2009) showed, with potted vines in controlled-growth conditions, that 50 % of the fixed carbon was used in growth of the shoot. It remains now to assess the carbon budget of Semillon vines growing in vineyard conditions.

The objective of the present study was, therefore, to measure the growth of vines growing with and without shade covering in vineyard conditions to determine the accumulation of carbon as biomass and also to measure the carbon fixation capacity of the shoots to estimate the net carbon balance over the growing season. A second objective was then to assess the relative contributions of stored and current photosynthetic supporting vine growth.

Materials and methods

This study was undertaken on a commercial vineyard in the Riverina, NSW, Australia, over two growing seasons from 2007–08 to 2008–09. The vines were planted in 2003 on own roots and in north–south-oriented rows.
Irrigation and fertilizers were supplied through dripper lines at a rate of 2.4 L h⁻¹ for 12 h per week. Budbreak occurred in late September and flowering in early November, and bunches were harvested in early February.

**Shade cloth treatment**
Neutral-density shade cloth (70%) was placed over whole panels of three vines just prior to budbreak and maintained over the vines until after harvest. A wooden T-shaped structure was used to support the shade cloth 2.4 m above the canopy. A panel of well-exposed vines was used as a control and both treatments were replicated in the vineyard. The shade cloth reduced the incident irradiance within the canopy by 70% and PFDs were typically < 400 µmol m⁻² s⁻¹. Canopy temperatures under the shade cloth were also lower by 3–5 °C compared with air temperatures. Photon flux density, screened air and canopy temperatures, and vapour pressure deficits in both the uncovered and covered vines were measured with a quantum sensor (LI190; Li-Cor Biosciences, Lincoln, NE, USA), a humitter (HPM50; Vaisala, Helsinki, Finland) and infrared radiometers (IRR-P; Apogee Instruments, Logan, UT, USA). All sensors were logged (CR1000; Campbell Scientific, Logan, UT, USA) and averaged over hourly intervals throughout the growing season from mid-October to just beyond harvest.

**Vine measurements**
In each growing season, six shoots were randomly selected from each vine of each treatment from both the eastern and western sides of the canopies. The lengths of each selected shoot were measured at about weekly intervals throughout the growing season. In the second season, the width and length dimensions of each leaf on two of the selected shoots per vine were also measured at regular intervals to enable leaf area development to be determined as the simple product of width and length. Allometric relationships between stem length and dry matter, and between leaf area and dry matter, were applied to determine dry matter accumulation of stems and leaves across the growing season. All data were informally adjusted to ensure that each stem or leaf dry weight estimation at the end of the growing season matched that actually measured at the destructive harvest by adjusting the ratio of measured to estimated values.

Measurements of the lengths of each bunch on each of the six selected shoots on each vine were also measured at regular intervals after flowering had occurred. Using an allometric relationship between bunch length and dry matter (Greer and Sicard 2009), the changes in dry matter accumulation for each bunch over the growing season were determined. Again informal adjustments occurred to ensure that calculated determinations match the measured values at harvest. All bunches on the selected shoots of each vine were harvested by about 12 February, taken to the laboratory and dried at 60 °C for 2 weeks to determine dry matter content.

Approximately 1 month later (mid-March), the selected shoots on each vine were destructively harvested and taken to the laboratory. Leaf areas were measured on all leaves using a leaf area meter (LI-3000; Li-Cor, Lincoln, NE, USA). Then all stems and leaves were oven-dried at 60 °C for 2 weeks to determine dry matter content.

**Gas exchange**
From about the time when individual leaves were ~35 mm in width, gas exchange was measured using an open gas exchange system (LCA4; Analytical Development Company, Hoddeson, UK). All leaves on each of two of the selected shoots in each panel were measured at about weekly intervals. In the first season, these measurements commenced late in the growing season (early January), but in the second growing season from early budbreak (9 October). All leaves on the selected shoots were measured on each occasion; thus an increasing number of measurements was required because the shoots developed up to 30 leaves as the season progressed. All measurements were recorded during daylight hours from 9 am to 5 pm in situ at the prevailing orientation of each leaf on the shoot, and the PFD and leaf temperatures reflected the leaf orientation in each case.

**Net carbon balance**
The total carbon fixed per shoot throughout the growing season was assessed by firstly integrating the net rate of photosynthesis over the day, assuming that the measured rates represented a daily value (cf. Cartechini and Palliotti 1995; Edson et al. 1995). Secondly, the net daily CO₂ fixation per shoot was determined as the product of the area of each leaf and its attendant photosynthetic rate summed over all the leaves present on the shoot of each day of the measurements. These summations were then summed over all measurement days over the whole growing season. The rates of dark respiration of these leaves were determined separately and used in the daily accumulation of CO₂. Net carbon acquisition was then determined by taking into account the molecular fraction of carbon in CO₂ (Wullschleger et al. 1997; Greer et al. 2003).
The carbon accumulated as biomass was determined from the total biomass accumulated over the growing season for each shoot firstly by assuming that 45% of the biomass was elemental carbon (Walton and Fowke 1995). The product was then mathematically differentiated using Microcal's Origin software to determine the rate of carbon accumulation per day over the whole growing season for each shoot in each treatment. The net daily carbon balance was then determined as the mathematical difference between the rates of carbon acquisition and the rates of carbon sequestered as biomass for each shoot.

Data analysis

All data were analysed using generalized linear models with SAS V9.13 (SAS Institute, Cary, NC, USA), and least squares means and standard errors were determined. All data were analysed according to a randomized design, and statistical significance was assessed at the 5% level. The Boltzmann sigmoid curve was fitted using Origin V8.1 (OriginLab Corporation, Northampton, MA, USA) to biomass accumulation of the different vine components in accordance with Seleznyova and Greer (2001). SAS V9.13 was also used to extract photosynthetic data in relation to time of measurement to determine diurnal courses in photosynthesis. Similarly, data were extracted to determine photosynthetic light responses and a hyperbolic tangent fitted to these data according to Greer and Halligan (2001).

Results

Vine attributes

Across both growing seasons, there were no significant ($P > 0.05$) differences in either the number of shoots set on the vines or the number of bunches per vine (Table 1) between the exposed and shaded treatments. However, there was a marked and significant ($P < 0.05$) decrease in the number of shoots (20%) and bunches (30–40%) in the second growing season of the study compared with the first growing season. In both cases, an average of just over two bunches per shoot occurred in each treatment.

Biomass accumulation

Across the whole growing season, the stem and leaf biomass of each shoot accumulated in a curvilinear pattern (Fig. 1) and reached a maximum about 80 days after budbreak (DAB). For the stems, there was no statistical difference between exposed and shaded shoots in both seasons and the total stem biomass averaged 33 ± 2.8 g. By contrast, leaf biomass accumulation was significantly ($P < 0.01$) higher in exposed shoots compared with those in the shade (Table 2), and reached 28.5 ± 1.3 g compared with 20.1 ± 0.9 g in the 2007/08 season and similar amounts in the 2008/09...
leaves. Biomass to the stems was greater than that to the growing season. Thus, in both cases allocation of biomass to the stems was greater than that to the leaves.

The biomass of bunches (Fig. 1) greatly exceeded the allocation to stems and leaves, and over both seasons reached maximum dry weights of 130–140 g for the exposed vines and \(\sim 100 \pm 8\) g for the shaded vines, and these differences were statistically significant \((P < 0.01)\). It was noteworthy that the total biomass accumulated over the growing season in a somewhat double sigmoid pattern (Fig. 1). The first increase in total biomass occurred because of the biomass accumulation as leaves and stems, and subsided as these stopped growing. Then the total biomass increased again in accordance with the development and growth of the bunches in the later half of the growing season. Because of significantly higher leaf and bunch biomass allocation in the exposed compared with the shaded vines, the total biomass was also significantly \((P < 0.01)\) higher, at \(\sim 200 \pm 12\) g compared with 150 \(\pm\) 11 g (Table 2).

The dynamics of growth of the stems and leaves of exposed vines were generally similar (Table 3) in that timing of maximum growth rates occurred around 33 DAB but the canopy leaf area extended over a longer time interval (14 days) compared with the stem extension (10 days). By contrast, canopy leaf area expansion of shaded vines was delayed in reaching maximum rates by nearly 6 days compared with stem extension, but the duration of maximum growth was similar in each at about 10 days. Bunches reached their maximum growth some 70 DAB after the leaves and stems in both cases, but maximum growth rates of the bunches were maintained over 7 days, and hence markedly shorter than for stems and leaves. Shade covering thus had no effect on bunch development.

**Leaf area development**

Budbreak occurred close to 25 September in both seasons, and leaf measurements commenced within 15 days of budbreak. The pattern of leaf area along the shoot showed a regular quadratic shape (Fig. 2): the area of the first few leaves increased dramatically to reach a maximum at positions 6 and 7 and then, further along the shoot, leaf areas declined, in part because of recent leaf appearance. This same pattern was retained throughout the growing season but stretched out, with the maximum leaf areas occurring in a broad peak between positions 6 and 11, and again, along the shoot, leaf areas declined.

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**Table 2. Biomass accumulation of Semillon grapevines at harvest.** Components of the total biomass (mean ± SE, \(n = 12\)) per shoot at harvest of Semillon vines over two growing seasons and growing with and without shade covering as indicated. In all cases there was an average of 2.2 bunches per shoot.

| Year     | Treatment | Leaf biomass (g) | Stem biomass (g) | Bunch biomass (g) | Total biomass (g) |
|----------|-----------|------------------|------------------|-------------------|------------------|
| 2007/08  | Exposed   | 28.5 ± 1.3       | 35.5 ± 3.6       | 132.5 ± 7.3       | 196.4 ± 15.9     |
|          | Shaded    | 20.6 ± 0.9       | 30.1 ± 4.2       | 98.2 ± 10.8       | 148.3 ± 12.7     |
| 2008/09  | Exposed   | 28.1 ± 0.7       | 33.7 ± 3.5       | 150.1 ± 6.3       | 209.2 ± 8.8      |
|          | Shaded    | 19.1 ± 1.0       | 31.9 ± 2.9       | 107.6 ± 5.2       | 158.8 ± 6.5      |

**Table 3. Kinetic parameters of the growth patterns of Semillon vines.** Parameters derived from fitting the Boltzmann function to the accumulation of biomass of each individual component of Semillon shoots averaged over two growing seasons with and without shade covering. The maximum mass is determined as the upper asymptote, timing of maximum growth was the inflexion point and duration was the window of the maximum growth rate (see Seleznyova and Greer (2001) for further details).

| Treatment | Component | Maximum mass (g) | Timing of maximum growth (days) | Duration of growth (days) |
|-----------|-----------|------------------|---------------------------------|--------------------------|
| Exposed   | Stems     | 36.1 ± 0.8       | 32.7 ± 0.8                      | 9.8 ± 0.9                |
|           | Leaves    | 28.3 ± 0.4       | 34.2 ± 1.5                      | 14.1 ± 1.2               |
|           | Bunches   | 119.8 ± 4.9      | 104.2 ± 1.0                     | 7.1 ± 0.4                |
| Shaded    | Stems     | 34.1 ± 0.5       | 29.6 ± 0.9                      | 9.9 ± 1.1                |
|           | Leaves    | 19.8 ± 0.1       | 36.3 ± 1.2                      | 11.4 ± 0.8               |
|           | Bunches   | 82.8 ± 6.2       | 104.3 ± 1.6                     | 7.3 ± 0.7                |
It was notable that the leaves along the shoots of shaded vines were generally larger in the peak positions at about 0.022 ± 0.001 m² compared with comparable positions on the shoots of exposed vines where the leaves were about 0.015 ± 0.007 m², and these differences were significant (P < 0.001). However, at shoot positions beyond about node 12, shaded leaves tended to be smaller than comparable exposed leaves, and this pattern persisted from mid to late season. Because all these leaves were relatively small, at least compared with those in peak nodal positions, they had little impact on the total shoot leaf area. For instance, for the shaded shoots, total leaf areas were 0.189 ± 0.009, 0.302 ± 0.016 and 0.312 ± 0.018 m² at 42, 83 and 104 DAB, whereas for the exposed vines, total leaf areas for the same times were 0.155 ± 0.024, 0.278 ± 0.036 and 0.289 ± 0.037 m².

**Photosynthesis**

**Along the shoot** The rates of photosynthesis along the shoots initially showed a similar pattern to the leaf areas at 42 DAB (Fig. 3), increasing from leaf 1 to a maximum at leaf 8, and thereafter the rates declined consistently between leaves 9 and 16. In the exposed vines, the maximum photosynthetic rate at this time was about 11.1 ± 0.4 μmol (CO₂) m⁻² s⁻¹ and in the shaded vines it was about 7.6 ± 0.7 μmol (CO₂) m⁻² s⁻¹, and this difference was significant (P < 0.01). Integrating the photosynthetic rates across all leaves and taking the shoot leaf area into account at the time indicated that the shaded leaves had 17% lower overall photosynthesis (12.8 ± 0.5 cf. 15.4 ± 0.7 μmol (CO₂) shoot⁻¹ s⁻¹) than the exposed leaves.

Later in the growing season (83 DAB), differences in the rates of photosynthesis between exposed and shaded leaves in the basal node positions (1–12) were not significant (P > 0.05) but thereafter they were. Further along the shoot, the rates of photosynthesis of shaded leaves remained relatively low at about 5–6 μmol (CO₂) m⁻² s⁻¹ at least up to node position 18. By contrast, not only did the rates of photosynthesis of leaves further along the shoots of the exposed vines continue to increase between node positions 9 and 16, but the rates also increased to a higher maximum of 13.3 ±
0.5 μmol (CO$_2$) m$^{-2}$ s$^{-1}$. Beyond these nodal positions, the leaves of the exposed vines retained higher rates of photosynthesis across the shoot compared with the shaded shoots. This resulted in a 2.7-fold higher overall photosynthesis for the exposed shoots ($58 \pm 0.5$ μmol (CO$_2$) shoot$^{-1}$ s$^{-1}$) compared with the shaded shoots ($21.8 \pm 0.2$ μmol (CO$_2$) shoot$^{-1}$ s$^{-1}$) when integrated over the whole shoot.

The pattern in photosynthetic rates along the shoots was repeated at 104 DAB except that differences in the rates between exposed and shaded leaves were small right up to nodal position 17, and only beyond this position were the rates of photosynthesis higher in the exposed than in the shaded leaves. However, the rates of photosynthesis at this time were no higher than $10$ μmol (CO$_2$) m$^{-2}$ s$^{-1}$ in all leaves of both treatments and markedly lower than earlier in the growing season. On this occasion, integration of photosynthesis across the shoots of the shaded vines ($29.3 \pm 0.3$ μmol (CO$_2$) shoot$^{-1}$ s$^{-1}$) was 32% lower than for the exposed vines ($43.1 \pm 0.5$ μmol (CO$_2$) shoot$^{-1}$ s$^{-1}$).

Across the whole growing season and averaged over all leaves, there were highly significant ($P < 0.001$) effects of shade in both seasons on overall rates of photosynthesis, with mean rates for the exposed vines ranging from $5.5 \pm 0.2$ to $6.4 \pm 0.1$ μmol (CO$_2$) m$^{-2}$ s$^{-1}$ in the two seasons, while comparable rates for the shaded vines were $3.3 \pm 0.2$ and $4.0 \pm 0.1$ μmol (CO$_2$) m$^{-2}$ s$^{-1}$. In large part, differences in the rates of photosynthesis between exposed and shaded leaves on each occasion of measurement were related to the PFD incident upon the leaves (Fig. 4). For the shaded leaves, PFDs were typically <500 μmol (photons) m$^{-2}$ s$^{-1}$, whereas the exposed leaves experienced PFDs well above 1000 μmol (photons) m$^{-2}$ s$^{-1}$ except in the basal positions (nodes 1–7) of the shoot. However, the decline in photosynthetic rates along the shoot, especially in the late emerging leaves, was not necessarily related to the incident PFD but additionally to development of the leaf and gaining of photosynthetic competence, at least up to 83 DAB. Late in the season, the incident PFD appeared to drive leaf photosynthesis in all cases.

**Leaf photosynthetic rates over time** The rates of photosynthesis of selected leaves throughout development are shown in Fig. 5A. For exposed vines, there were clear differences in the maximum
photosynthetic rates achieved by each leaf, with the maximum increasing successively between leaves 5 and 15: from $7.0 \pm 0.5$ to $\sim 10.3 \pm 0.8$ and then to $13.4 \pm 0.5 \text{ mol (CO}_2\text{)} \text{ m}^{-2} \text{s}^{-1}$ for leaves 5, 10 and 15, respectively. By contrast, leaf 20 had a lower maximum rate than leaf 15. In all cases, however, leaf photosynthesis followed a regular pattern of increasing rates over the first few weeks to a peak rate and then declined markedly to lower but somewhat steady rates throughout the remaining growing season. Again, the steady-state rate depended on leaf position: $2–3 \text{ mol (CO}_2\text{)} \text{ m}^{-2} \text{s}^{-1}$ for leaf 5 to $5–6 \text{ mol (CO}_2\text{)} \text{ m}^{-2} \text{s}^{-1}$ for leaves 15 and 20. A similar pattern of changes in photosynthesis was evident for each leaf in the shaded vines (Fig. 5B), except that differences in maximum rates between leaves 10 and 15 were not different while leaf 5 had the lowest maximum rate and leaf 20 had the highest maximum rate. However, in all cases, the maximum rates for the shaded vines ($4.5–7.2 \text{ mol (CO}_2\text{)} \text{ m}^{-2} \text{s}^{-1}$) were lower than those for the exposed vines ($7–14 \text{ mol (CO}_2\text{)} \text{ shoot}^{-1} \text{s}^{-1}$).

**Diurnal variation in photosynthesis** Across the day, photosynthesis measured on the leaves of exposed vines varied only slightly and generally within the range of $8 \pm 1 \text{ mol (CO}_2\text{)} \text{ m}^{-2} \text{s}^{-1}$, with a trend towards higher rates at midday (Fig. 6) and lower rates in late afternoon. This lack of a marked diurnal variation in photosynthesis was attributable to the PFD being $> 1200 \text{ mol (photons)} \text{ m}^{-2} \text{s}^{-1}$ throughout the day. By contrast, photosynthesis of leaves on shaded vines tended to increase through to early afternoon and then decline but was generally within the range of $5 \pm 1 \text{ mol (CO}_2\text{)} \text{ m}^{-2} \text{s}^{-1}$ and the PFDs were between 300 and $600 \text{ mol (photons)} \text{ m}^{-2} \text{s}^{-1}$. Integration of these data over the measurement period (0800–1700 h) indicated that $2.7 \text{ g C m}^{-2} \text{ d}^{-1}$ of carbon acquisition occurred for the exposed vines while $1.8 \text{ g C m}^{-2} \text{ d}^{-1}$ occurred for the shaded vines.

**Photosynthetic light responses** Light responses were determined from all the field measurements of photosynthesis and the PFD at each measurement (Fig. 7). There were significantly ($P < 0.05$) higher apparent photon yields ($0.0247 \pm 0.0018 \text{ mol (CO}_2\text{)} \text{ mol (photons)}^{-1}$) for the shaded leaves compared with the exposed leaves ($0.0166 \pm 0.0014 \text{ mol (CO}_2\text{)} \text{ mol (photons)}^{-1}$). The differences in maximum rates of photosynthesis were also significantly different ($P < 0.01$), with the exposed leaves averaging $8.03 \pm 0.25 \text{ mol (CO}_2\text{)} \text{ m}^{-2} \text{s}^{-1}$ and the shaded leaves averaging $5.44 \pm 0.15 \text{ mol (CO}_2\text{)} \text{ m}^{-2} \text{s}^{-1}$. Light saturation of photosynthesis occurred at a PFD of 580 (photons m$^{-2}$ s$^{-1}$) for the shaded leaves, while for the exposed leaves light saturation was more than twice as much at 1270 (photons m$^{-2}$ s$^{-1}$).

**Net carbon balance** The net daily carbon acquisition for whole shoots (Fig. 8A) increased relatively slowly just after budbreak, but then increased more rapidly in the exposed vines.
to reach a maximum rate at about 55 DAB but just after 40 DAB for the shaded vines. It was noteworthy that in both shaded and exposed vines, the maximum rate of carbon acquisition was sustained over 30–40 days but also the maximum rate differed significantly (P < 0.01) from 1.17 ± 0.04 g C shoot\(^{-1}\) d\(^{-1}\) in exposed vines to 0.82 ± 0.09 g C shoot\(^{-1}\) d\(^{-1}\) in shaded vines. The differences were maintained throughout the growing season. In both cases, the rate of carbon acquisition declined from about 90 DAB until about bunch harvest when the rates remained constant. Across the whole growing season, the exposed plants acquired 103.5 ± 5.8 g C shoot\(^{-1}\) and the shaded vines 69.2 ± 5.9 g C shoot\(^{-1}\), some 35% less carbon fixation.

The rates of carbon sequestered into biomass (Fig. 8B) reached a maximum rate within 35 DAB; there was little difference between shaded and exposed leaves, and mostly not significant. It was also apparent that the rate of carbon sequestration began declining from this point in the growing season and approached a zero rate at about 75 DAB, coincident with stem and leaf biomass stopping accumulating. Thereafter, the rate of carbon sequestration increased again, coincident with bunch growth until 120 DAB when the bunches reached their maximum growth. After harvest of the bunches, the rate of carbon sequestration declined dramatically to reach a negative rate of −0.9 to −1.3 ± 0.1 g C shoot\(^{-1}\) d\(^{-1}\), indicative of loss of carbon from the shoots. In total, the vines in exposed conditions sequestered 67.2 ± 7.3 g C shoot\(^{-1}\) over the whole growing season while shaded vines sequestered 45.4 ± 5.3 g C shoot\(^{-1}\), again ~32% less than the exposed shoots.

The net carbon balance (Fig. 8C) was negative in both exposed and shaded shoots when growth commenced after budbreak, in keeping with higher rates of carbon sequestration compared with rates of carbon acquisition. The net carbon balance remained negative until about 40 DAB, and integration of these data indicated that 10.2 ± 2.5 and 16.9 ± 3.5 g C shoot\(^{-1}\) for the exposed and shaded vines, respectively, were required from outside of the shoot to support their growth, presumably from reserves. After about 45 DAB, the net carbon balance increased and reached a maximum of ~1.14 ± 0.11 g C shoot\(^{-1}\) d\(^{-1}\) at 75 DAB for the exposed vines and 0.65 ± 0.30 g C shoot\(^{-1}\) d\(^{-1}\) though slightly earlier at 60 DAB. Throughout the bunch ripening period, there was a major demand for carbon with the bunches actively growing, such that a decline in the net carbon balance, which ultimately became slightly negative, occurred until the bunches stopped growing. After the bunches were removed at harvest, the net carbon balance again became strongly positive when carbon demand by the shoot ceased. During the mid-season, the net carbon balance was higher in the exposed compared with the shaded vines, but otherwise there were little to few differences between them for the rest of the growing season. However, the surplus carbon for the whole growing season differed markedly, with the exposed vines gaining 35.2 ± 4.3 g C shoot\(^{-1}\) surplus and the shaded vines gaining 16.6 ± 2.3 g C shoot\(^{-1}\), a 53% reduction.

Discussion
Shade covering over Semillon grapevines affected some of the components of biomass accumulation over the growing season. In particular, leaf and bunch biomass growth was significantly reduced but stem biomass was unaffected. The dynamic pattern of stem biomass accumulation in both treatments was also unaffected, reaching maximum rates at the same time. This suggested that shade covering had no effect on stem growth and development. By contrast, the dynamic pattern of leaf biomass accumulation was
significantly affected, with a marked delay in the shaded vines achieving the maximum rates compared with the exposed vines. Perhaps of more significance, however, was that leaf biomass accumulation in both treatments initially commenced at about the same rate at a time when the vines were heterotrophic and dependent on carbon reserves in the roots to support this growth (Holzapfel et al. 2010). This suggests that shade should have had no effect until the vines became fully autotrophic and dependent on current photosynthesis. That bunch biomass accumulation was markedly affected by the shade covering was certainly consistent with the impact of shade on photosynthetic light response, given that bunch growth occurred when the vines were fully autotrophic. Over both seasons, some 25–40 % lower biomass accumulation occurred in the shaded compared with the exposed vines. It was evident that by the time the bunches commenced growth, vegetative growth of the shoots had all but stopped. Thus, there appeared to be little competition for carbon between vegetative and reproductive growth, given the temporal separation in their development. Indeed, the rates of bunch biomass accumulation were also markedly higher than those that occurred with the leaves and stems (cf. Flore and Lakso 1989) and a clear indication of the strength of the bunches as a carbon sink (Petrie et al. 2000).

Vines in the shade also had much larger leaves, at least in the basal half of the shoot, compared with those on exposed vines. This pattern of larger leaf sizes in the basal portion was apparent early in the growing season when the canopy leaf area was > 20 % larger than in exposed vines. Later, the advantage in leaf area declined to < 10 % in mid and late season, when a higher proportion of leaves on shoots in both treatments were similar in size. These data are consistent with the well-established leaf response to shade (Givnish 1988) in that generally leaf area increases while leaf thickness declines. The differences in leaf area occurring early in development are perhaps surprising as these Semillon leaves are largely preformed in the dormant bud (Greer et al. 2010) and their size ontogenetically determined (Greer and Jeffares 1998). However, from the current study, it would appear that expansion of preformed leaf primordia is perhaps more dependent on current photosynthetic carbon assimilation than previously considered and warrants investigation.

It is well established that shade plants have lower rates of light-saturated photosynthesis than well-exposed plants (Givnish 1988), and the photosynthetic light responses for the Semillon leaves conform with this conclusion. Also, the light saturation for shaded leaves was < 600 μmol (photons) m⁻² s⁻¹ whereas it was more than double this for exposed leaves. Further evidence for the impact of shade comes from the diurnal rates of photosynthesis. Similar photosynthetic responses between exposed and shaded plants have been reported for Sangiovese vines (Cartechini and Palliotti 1995) and P. abies seedlings (Grassi and Minotta 2000). These shade responses of the Semillon leaves thus conform well with the generalized response to shade.

The rates of photosynthesis measured in situ at the native orientation of each leaf in the exposed and shaded treatments initially mirrored the pattern of leaf size along the shoot (see also Downton and Grant 1992; Poni and Giachino 2000) in the early part of the growing season (42 DAB), except that rates were significantly higher in the exposed than in the shaded leaves. Most of these differences were clearly related to the incident PFDs within the canopy of each treatment (cf. Fig. 7). However, some differences also pertained to the leaves, especially those emerging late that were still actively expanding and, therefore, not fully competent for maximum photosynthesis (Ho and Shaw 1977; Wüllschleger and Oosterhuis 1990; Bertamini and Nedunchezian 2003). This lack of photosynthetic competency was most apparent in mid-season (83 DAB) when the rates of photosynthesis in these same leaves (nodes 10–17) increased dramatically to > 10 μmol (CO₂) m⁻² s⁻¹ in exposed vines and at least up to 5 μmol (CO₂) m⁻² s⁻¹ in the shaded vines. It was also noteworthy that in spite of higher leaf area in the shaded vines, the total CO₂ fixation per shoot, integrated over all leaves, was higher (15.4–58 μmol (CO₂) shoot⁻¹ s⁻¹ at 42 and 83 DAB, respectively) in exposed than in shaded vines (12.8–21.8 μmol (CO₂) shoot⁻¹ s⁻¹). A similar difference (43.1 cf. 29.3 μmol (CO₂) shoot⁻¹ s⁻¹ for exposed and shaded vines) in total CO₂ fixation occurred late in the growing season (104 DAB). Thus, the shade covering had a considerable impact on incident PFDs within the canopy and a flow-on effect on rates of photosynthesis, consistent with the photosynthetic light response of the shaded leaves. These results also conform well with the effect of shade on the photosynthesis of Sangiovese leaves (Cartechini and Palliotti 1995) and Riesling leaves (Schultz et al. 1996).

It was apparent from the estimated carbon acquisition in the first 40 days of growth and development that little net gain in carbon occurred in either treatment. After that, however, carbon gain increased rapidly, more so in the exposed than in the shaded vines, as the leaves became fully expanded and photosynthetic rates of the early-emerging leaves become maximal. It was thereafter that the penalty of shade covering on the vines’ carbon budget became most
apparent and, on average, 0.8 g C shoot\(^{-1}\) d\(^{-1}\) (4 g C m\(^{-2}\) d\(^{-1}\)) was gained throughout the main period of the growing season whereas the exposed vines gained 1.2 g C shoot\(^{-1}\) d\(^{-1}\) (7.7 g C m\(^{-2}\) d\(^{-1}\)), on average. A similar penalty of shade on carbon gain was also shown in Alocasia macrorrhiza (Sims et al. 1994). These estimates of carbon gain were, nevertheless, well within the ranges determined for other grapevine varieties, including Tempranillo (7 g m\(^{-2}\) d\(^{-1}\); Escalona et al. 2003) and Riesling vines (4.3 g m\(^{-2}\) d\(^{-1}\); Weyand and Schultz 2006). The seasonal pattern of change in the carbon acquisition also conformed to that in San-giovese vines (Palliotti et al. 2004). Differences in photosynthesis between the exposed and shaded Semillon leaves clearly accounted for the penalty in carbon acquisition rather than canopy leaf area of the shaded vines.

Later in the growing season, when no further increase in leaf area of the Semillon shoots had occurred and a reduction in the rates of photosynthesis had also occurred, carbon gain in both treatments started to decline progressively from about 80 DAB onwards to low rates. Over the whole growing season, the exposed shoots gained a total of 100 g C shoot\(^{-1}\) while shaded vines gained a total of 70 g C shoot\(^{-1}\). These data strongly confirm the conclusion that reduced PFDs from the shade covering caused a marked reduction in carbon gain. However, these total gains compare well with an estimated 59 g C shoot\(^{-1}\) for Riesling vines (Downton and Grant 1992) but contrast with \(\sim 280\) g C shoot\(^{-1}\) for fruiting A. delicosa vines (Greer et al. 2003).

In concert with the decreased acquisition of carbon by the shaded Semillon vines, sequestration of carbon in biomass was also affected by the shade treatment. However, only small differences in the rates of carbon accumulation occurred between vines of both treatments across the growing season. Of more significance, the rates of carbon accumulation in both cases increased to reach an initial peak of \(0.8\pm 1.1\) g C shoot\(^{-1}\) d\(^{-1}\) close to 40 DAB. This coincided with the rapid increase in shoot growth that occurred in the first 6 weeks after bud-break. After that, the rates of carbon accumulation declined steadily over a 50-day period to approach a rate close to negligible carbon sequestration in concert with vegetative growth ceasing. However, from about 90 DAB onwards, the rate of carbon accumulation then increased again to peak at 1.4 and 1.0 g C shoot\(^{-1}\) d\(^{-1}\) for exposed and shaded vines, respectively. Clearly, the flush of bunch growth accounted for this second peak of carbon accumulation. Thus, in vineyard-grown vines, carbon accumulation was bimodal, with an initial peak reflecting vegetative growth and a second peak resulting from reproductive growth. Consistent with this, fruiting A. delicosa vines also had a bimodal pattern of carbon accumulation (Greer et al. 2003). Across the whole growing season, the exposed Semillon vines sequestered a total of 67 g C shoot\(^{-1}\) and the shaded vines 45 g C shoot\(^{-1}\) as biomass compared with 42 g C shoot\(^{-1}\) for Reisling vines (Downton and Grant 1992) and 32.3 g C shoot\(^{-1}\) for potted Semillon vines (Greer and Sicard 2009), reasonably comparable given different grapevine varieties and growth conditions.

The relatively slow development of photosynthetic carbon acquisition and the high rates of carbon accumulation in the Semillon vines resulted in a negative net carbon balance over the first 40 days of growth (see also Johnson and Lakso 1986; Wibbe et al. 1993), indicative of carbon reserves being consumed to support growth (Goffinet 2004; Bennett et al. 2005; Field et al. 2009; Holzapfel et al. 2010). In total, the shoots of the exposed vines required some 10 g C shoot\(^{-1}\) while the shaded shoots required nearly 17 g C shoot\(^{-1}\) of imported carbon during this period of high growth rate. Consistent with this, some 30 g vine\(^{-1}\) of starch was lost from roots over a similar time frame in Shiraz vines (Field et al. 2009) and a similar amount in Pinot Noir and Merlot vines (Zapata et al. 2004), conforming with mobilization of reserve carbon to supply shoot growth. It was notable that the leaf growth rates of shaded and exposed vines differed little and were maintained at a high rate during this period of heterotrophic shoot growth, suggesting that the mobilization process was unaffected by the shade covering. But when the shoots shifted to an autotrophic state (beyond 40 DAB; see also Zapata et al. 2004), leaf growth of the shaded vines declined markedly in keeping with low rates of carbon acquisition.

The net carbon balance was essentially positive once the bunches had been harvested and a strong net gain in carbon ensued and was presumably exported from the shoot. In fact, after harvest some 26 and 21 g shoot\(^{-1}\) of surplus carbon were accumulated for the exposed and shaded vines, respectively, but these would be expected to increase up to leaf fall. Over the whole growing season, a net gain of 35 g C shoot\(^{-1}\) for the exposed vines and 16.5 g C shoot\(^{-1}\) for the shaded vines resulted, and these values compare very well with that estimated for controlled-environment-grown Semillon vines (37.2 g C shoot\(^{-1}\); Greer and Sicard 2009) and for spur-pruned Riesling vines (\(\sim 17\) g C shoot\(^{-1}\); Downton and Grant 1992).

Conclusions and forward look

Covering Semillon vines with shade cloth to reduce exposure to heat had major costs in terms of the carbon budget. Lower incident PFDs within the shaded canopy...
induced leaves with characteristic shade attributes: low light-saturated photosynthesis; low light saturation but large leaf areas. Together, these attributes caused a decrease in carbon acquisition which flowed on to reduced allocation to leaf biomass and later on to bunch biomass. Thus, shade had a synergistic effect on the carbon balance of these vines through reductions in carbon acquisition and biomass allocation, and therefore was a costly method of protecting vines from high-temperature events. It therefore behoves us to find an alternative means of protecting the vines. We are currently investigating whether hydrocooling vines will provide protection without the same carbon costs and may be more beneficial as no change in light interception is involved. The methods involve pulsed water misting applied at different temperature thresholds, and early results show that photosynthetic rates actually increased when temperatures were prevented from increasing during a heat event.

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Contribution by the authors
All authors contributed to the research, through participation in setting up the trial and conducting the measurements. The senior author was responsible for the data analysis and preparation of the manuscript. All authors reviewed and contributed comments to the manuscript.

Conflicts of interest statement
None declared.

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