Author: S. Y. Rogiers, J. P. Smith, B. P. Holzapfel and W. J. Hardie
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Author Address: suzy.rogiers@industry.nsw.gov.au
jasmith@csu.edu.au
bholzapfel@csu.edu.au
jhardie@csu.edu.au

Soil temperature moderates grapevine carbohydrate reserves after bud-break and conditions fruit set responses to photoassimilatory stress

Suzy Y Rogiers, Jason P Smith, Bruno P Holzapfel, W James Hardie

National Wine and Grape Industry Centre, Charles Sturt University, Wagga Wagga, NSW, 2678, Australia

Corresponding author:
Suzy Rogiers
National Wine and Grape Industry Centre
Charles Sturt University
Locked Bag 588
Wagga Wagga NSW
2678
Australia

Telephone: 61 2 6933 2436
Fax: 61 2 6933 2107
Email: suzy.rogiers@industry.nsw.gov.au

Jason P Smith
jasmith@csu.edu.au
address: as above

Bruno P Holzapfel
bruno.holzapfel@industry.nsw.gov.au
address: as above

W James Hardie
jhardie@csu.edu.au
address: as above

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Abstract

In cultivated grapevines (*Vitis vinifera* L.), sub-optimal photoassimilatory conditions during flowering can lead to inflorescence necrosis and shedding of flowers and young ovaries and consequently, poor fruit set. However before this study it was not known if carbohydrate reserves augment fruit set when concurrent photoassimilation is limited. Carbohydrate reserves are most abundant in grapevine roots and soil temperature moderates their mobilisation. Accordingly, we grew potted Chardonnay grapevines in soil at 15°C (cool) or 26°C (warm) from bud-break to the onset of flowering to manipulate root carbohydrate reserve status. Then to induce photoassimilatory responses we subjected the plants to low (94 μmol mol^{-1}) CO_2 or ambient (336 μmol mol^{-1}) CO_2 atmospheres during fruit setting. Analyses of photoassimilation, and biomass and carbohydrate reserve distribution confirmed that fruit set was limited by concurrent photoassimilation. Furthermore, the availability of current photoassimilates for inflorescence development and fruit set was conditioned by the simultaneous demands for shoot and root growth, as well as the restoration of root carbohydrate reserves. The study indicates that great seasonal variability in grapevine fruit set is a likely response of cultivated grapevines to photoassimilatory stresses, such as shading, defoliation and air temperature, and to variations in carbohydrate reserve status prior to flowering.

Key words: biomass partitioning, carbon dioxide, carbohydrates, flowering, grapevine, root growth, soil temperature

Introduction

Fruit setting, the transformation of a flower to a nascent berry, in grapevine (*Vitis* spp.), involves a sequence of physiological development that includes capfall, pollination, pollen tube growth, fertilisation of ovules, induction of cell division and enlargement of the ovary. Among *Vitis vinifera* L. grapevine cultivars the number of flowers on each inflorescence that set and develop into mature berries typically ranges between 20% and 30% (Mullins *et al.* 1992), but can be as low as 15%, or as high as 60% (May 2004). Variance in berry number per cluster accounts for up to 30% of the inter-seasonal variance in yield in warmer Australian grape growing regions (Krstic *et al.* 2005). In cooler regions, inter-seasonal variance in fruit set can be more extreme (Ebadi *et al*. 1995). Understanding the factors that

underlie variability in fruit set is essential to managing unwanted seasonal variations in winegrape production.

Photoassimilate distribution between developing grapevine inflorescences and shoots was first explored using labelled CO_2 as a marker of photoassimilate translocation by Hale and Weaver (1962) and later by others, notably by Koblet (1969). Those studies revealed the relatively low movement of photoassimilate into the inflorescence during fruit setting. Subsequently, using defoliation and/or shading Keller and Koblet (1994;1995) showed that photoassimilatory stress impaired fruit set and suggested that excessive ammonia, associated with necrotic disorders of inflorescences and young bunch stems, was a consequence of sub-optimal availability of carbon, rather than the cause. This view of a grape inflorescence that is highly sensitive to concurrent photoassimilation is entirely consistent with findings from many subsequent studies (e.g. Candolfi-Vasconcelos *et al*. 1994; Caspari *et al*. 1998; Lebon *et al*. 2008). Furthermore, it is not surprising that impaired fruit set, and associated physiological disorders *viz*. inflorescence necrosis and bunch stem necrosis, are a consequence of limited photoassimilation and common in viticulture given long standing evidence that pruning - an underlying practice - generally delays the completion of seasonal re-foliation and induces fruit set to take place in the presence of active shoot growth (Winkler 1929; Downton and Grant 1992) accompanied by strong, acropetal flow of current photoassimilates (Hale and Weaver 1962).

Increased fruit set in response to minimal pruning, shoot tipping and application of shoot growth retardants all point to fruit set in grapevines being limited by the supply of current photoassimilate due to their concurrent utilisation for shoot growth. However that shoot growth also requires carbohydrates stored in all perennial parts, but most abundantly in the roots (see review by Holzapfel et al. 2010 and references cited therein). On the other hand, increases in fruit set in pruned grapevines due to trunk or stem girdling (Coombe 1959, Weaver and McCune 1959, Caspari *et al*. 1998) indicate a simultaneous demand for carbohydrate between the developing ovaries and perennial organs (e.g. for root growth or reserve restoration). In other words, fruit set in cultivated grapevines is accompanied by several simultaneous carbon dependent processes whereas in unpruned grapevines, leaf development and fruit set at least, occur sequentially. Accordingly we are left with a scenario in which the carbon nutrition of developing ovaries appears to be conditioned by the availability of both current photoassimilates and carbohydrate reserves. This study confirms

that scenario by analysis of biomass distribution and reveals an important effect of soil temperature prior to cap-fall on fruit set.

In the first stage of this experiment, carbohydrate reserve status of potted grapevines at commencement of fruit set was varied by root temperature treatments imposed from bud-break until the first flower appeared. Then, in the second stage of the experiment, the plants were transferred to controlled environment chambers with either ambient or low CO_2 atmospheres. The aim of the low CO_2 treatment was to impose photoassimilatory stress independently of light intensity, temperature or defoliation, and confirm that reduced concurrent photoassimilation impairs fruit set. Then facilitated by the pre-treatments, we aimed to determine if carbohydrate reserves condition the impact of photoassimilatory stress on fruit set.

Materials and Methods

At dormancy, 40, three-year-old potted Chardonnay grapevines (*Vitis vinifera*, L., clone V5) on their own roots were pruned to two spurs with four buds per spur. The plants were then removed from the pots, washed to remove soil from the roots, and replanted in commercial potting mix in 40 L pots with a 1m long coil of 13 mm polyethylene irrigation tubing buried within the root-zone. The pots were placed in an opened glasshouse, where air temperature ranged between an average minimum of 10.3°C and maximum of 28.5°C. Daily maximum PAR averaged at 1000 μmol m^{-2} s^{-1}. The plants were irrigated daily and excess water was allowed to drain from the bottom of the pot.

Soil temperature treatments

Root warming depletes carbohydrate reserves by increasing starch catabolism (Field *et al*. 2009) and respiration (Huang *et al*. 2005). Hence in order to induce two cohorts of plants with either low or high carbohydrate reserves we grew the plants in soil maintained at 26°C (warm) or 15°C (cool) from bud-break (E-L Stage 4) (Pearce and Coombe 2005) to the beginning of anthesis (E-L stage 19).

Soil temperature was regulated according to the method of Field *et al*. (2009) where water was recirculated from two temperature-controlled water tanks through the coiled irrigation tubing buried within each pot. The pots were insulated on the sides with 10 mm dense foam

to minimize heat exchange between the soil and air. A disc of foam insulation was also placed on the soil surface, however this was not sealed so as to allow air circulation. Soil temperature, recorded at 30 minute intervals at approximately the centre of the root mass (DS18B20, Maxim Integrated Products, Sunnyvale, CA), and fluctuated diurnally on average ± 3.5°C from the mean in both plant groups. With the dehiscence of the first flower cap (onset of cap-fall, 32 days after bud-break) sample plants of both groups (n=5) were destructively harvested, dried at 80°C, weighed and analysed for carbohydrates as described below.

CO_2 Conditions

The remaining plants were assigned to controlled environment chambers (TPG-6000-TH, Thermoline Scientific, Smithfield, Australia) and subjected to either ambient or low CO_2 atmospheres. To reduce photosynthesis in one chamber, a low CO_2 environment was created by recirculating air through purpose-built scrubbers filled with a CO_2 absorbent (Sodasorb, Grace Sodasorb, Sydney). In the second chamber, CO_2 concentration was not controlled, and is referred to as the 'ambient' treatment. In subsequent tables and figures these treatments are referred to as cool/ambient (n=8), cool/low (n=7), warm/ambient (n=7) and warm/low (n=8). In both chambers CO_2 concentrations were monitored on a daily basis by manual readings with an LCA-2 gas analyser (ADC Bioscientific, Hoddeson, UK). The low CO_2 concentration averaged 94 μmol mol^{-1} and the ambient CO_2 336 μmol mol^{-1}. The growth chambers were held at 22°C/8°C day/night temperatures, and a 12 hour photoperiod of 1100 μmol m^{-2} s^{-1} PAR at mid-leaf canopy height. Soil temperature was not regulated. The plants remained in those chambers until fruit set was visually assessed as complete. Figure 1 provides a timeline of when the treatments were imposed. The plants were then destructively harvested and assessed for dry weight and carbohydrates as described below. Structural biomass was determined from the difference between total dry biomass of each plant part and the corresponding weight of total non-structural carbohydrates (TNC). Structural biomass, thus determined, includes a small mineral nutrient reserve component of less than 5% (Mengel and Kirkby, 1982).

Plant measurements

Twelve days after bud-break, all but four shoots, each with two inflorescences, were removed from each plant. The length of each shoot and inflorescence was measured thereafter one to three times per week. The length of the inflorescence was measured from

the first lateral branch to the most distal flower. The width of four leaves on one shoot of each plant was also measured. The leaves, at nodes 2-5, comprised the leaf below the proximal inflorescence, those opposite each inflorescence, and the leaf above the distal inflorescence. Total flower number was determined by enclosing each inflorescence in nylon mesh bags just before any flowers opened and subsequently counting abscised flower caps and unopened flowers. The course of flowering in each treatment was assessed at approximately weekly intervals by visual estimates of percent cap-fall until inflorescences had reached E-L stage 26 (100% cap-fall). Fruit set was regarded as the stage when abscission of undeveloped flowers and unexpanded ovaries stopped; leaving only nascent berries (ovaries that had begun to swell). Fruit set was quantified as the number of nascent berries as a proportion of the total number of flowers.

Photosynthesis and leaf intercellular CO_2 concentrations were measured prior to cap-fall and during anthesis (31 and 40 days after bud-break) using a portable photosynthesis system (LI-6400, LI-COR Biosciences Inc., Lincoln NE). Measurements were carried out in the late morning with the chamber at 1000 μmol m^{-2} s^{-1} PAR and 25°C block temperature on four fully developed leaves (at nodes 2 to 5) on one shoot of four plants per treatment.

Carbohydrate analysis

Plant roots, trunks, shoots and inflorescences were separated and oven dried at 80°C until constant weight. The plant parts were first ground through a heavy duty cutting mill (Retsch SM2000, Hann, Germany) to 5 mm, and then a sub-sample was ground to 0.12 mm in a centrifugal mill (Retsch ZM200). Soluble sugars of a 20 mg subsample were extracted twice with 1 ml of 80% ethanol at 80°C for 10 min, and a single 1 ml wash of 80% ethanol at room temperature. After centrifuging between each wash the three aliquots were combined and the concentration of sucrose, D-fructose and D-glucose determined using commercial enzyme assays (K-SUFRG, Megazyme International, Bray, Ireland). Briefly, this involved the conversion of each sugar to glucose-6-phosphate (G6P), and quantification of NADPH following oxidation in the presence of NADP+ and G6P-dehydrogenase. Starch in the remaining wood sample was solubilized in pure dimethylsulfoxide at 98°C and then hydrolyzed with α-amylase and amyloglucosidase. Released D-glucose concentrations were determined using a glucose oxidase/peroxidase based enzyme assay (K-GLUC, Megazyme International).

Statistical analysis

A one-way ANOVA was used for comparison of treatment effects following the soil temperature pre-treatments while a two-way ANOVA was used following the CO_2 treatments by means of GenStat release 13.0 (VSN, Hertfordshire, UK). Changes over time in shoot and inflorescence parameters were tested using generalised linear models with SAS Ver. 9.13 (SAS Institute, Cary, NC, USA) and least squares means and standard errors determined. Sigmaplot graphing and statistics software (SPSS Inc., Chicago, IL) was used for simple linear regression analyses between fruit set and vine TNC.

Results

A. Soil temperature responses: bud-break to cap-fall

Shoot growth and leaf area

Soil warming from bud-break significantly increased the number of shoots that emerged and their development. When the plants were thinned to four shoots 12 days after bud-break an average of 6.9 shoots with a mean fresh weight of 21 g per plant were removed from plants in warm soil whereas an average of 4.5 shoots with a mean fresh weight of 12 g per plant were removed from plants in cool soil. Shoot length and elongation rate was highest in plants in warm soil (Fig. 2a,b) and by day 28, the average shoot length of plants in warm soil was 69 ± 2 cm and in cool soil, 54 ± 2 cm. Leaf number and leaf width of plants in warm soil were also greater by 7 and 14%, respectively (data not presented).

Inflorescence development to cap-fall

By 28 days from bud-break, and shortly before the first flowers emerged, average inflorescence rachis length (Fig. 2) and dry weight ($P<0.01$) was greatest in plants in warm soil (62.0 mg compared to 43.4 mg in the cool treatment). Both rachis sugar content and concentration of plants in warm soil were more than double that of those in cool soil (Fig. 3). Flower number per inflorescence was also 19% higher in the warm soil (Table 3). Flower weight was not significantly affected by soil temperature (1.08 mg, $P=0.8$), but flower sugar concentrations were greatest in plants in warm soil (Fig. 3).

Photosynthesis

The average photosynthetic rate of the first four emergent leaves (the reference leaves) was not significantly affected by soil temperature and averaged at 9.6 ± 0.3 μmol CO_2 m^{-2} s^{-1} at 32 days after the soil temperature treatments commenced (Table 4). Notably this was despite the stomatal density of the reference leaves formed concurrently with soil warming being *ca* 16% lower than that of leaves of plants from cooled soil as we have previously described (Rogiers *et al*. 2011).

Biomass and non-structural carbohydrates at cap-fall

Perennial parts: roots and trunks

At cap-fall the total dry biomass of the plants of both temperature treatments was similar; as was the total structural dry biomass (Table 1). Root dry biomass was however 16% lower in warmed plants and since there were no significant differences in root structural biomass this can be attribute to variation in root TNC *viz.* 20 g compared to 33 g for those in the warm and cool soil, respectively. In plants in warm soil, starch accounted for 17 g of the total perennial reserves and represented 13.1% of total root dry biomass. Whereas in plants in cool soil starch accounted for 30 g of the total reserves in the perennial parts and represented 19.5% of total root dry biomass (Table 2). The concentration of soluble sugar was higher in the roots of the plants in warm soil as compared with the cool soil (Table 2). There was no effect of soil temperature on carbohydrate concentrations in the trunks (Table 2).

Shoots

By cap-fall, neither the total nor structural dry biomass of the shoots was significantly different (Table 1). The TNC content of shoots at that time, however, was 3.9 g and 1.9 g following the warm and cool soil treatments respectively (Table 1). Sugar concentrations were also higher in the shoots following the warm soil treatment (Table 2).

B Effects of CO_2 stress and pre-flowering soil temperature during fruit setting

Flowering, flower abscission and fruit set

Soil temperature prior to cap-fall significantly affected the rate of cap-fall. Eight days after cap-fall, in ambient CO_2 the average cap-fall per inflorescence was 75% and 60% for pre-warmed and pre-cooled plants respectively; indicating that pre-warming slightly accelerated

the onset of flowering perhaps by enhanced carbon nutrition from accelerated foliar development and/or from carbohydrate reserve mobilisation.

Soil temperature prior to cap-fall and CO_2 during flowering significantly affected flower abscission, remaining intact over and percentage fruit set. Flower abscission was nearly double in the low CO_2 plants as compared with high CO_2 plants with the highest abscission rates in pre-warmed plants grown under low CO_2 (Table 3). Furthermore, in ambient CO_2 the number of remaining intact ovaries and fruit set were significantly higher in pre-cooled plants than those that were pre-warmed (58% and 43%, respectively). In low CO_2, an average of 30 ovaries remained on each cluster for pre-warmed plants but only 20 ovaries in pre-cooled plants. Because of the higher flower number in the prewarmed plants, however, there was no effect of soil temperature pre-treatment on fruit set in low CO_2. Fruit set was linearly correlated to shoot % TNC and root total TNC (Fig. 4), however the variance accounted for was slightly greater in the roots.

Ovary growth

CO_2 stress delayed ovary growth (Fig. 5a). In ambient CO_2 ovary expansion was evident in some inflorescences within 15 days of cap-fall but in low CO_2 little growth was evident at that time. Thereafter ovaries in both groups grew measurably but growth of those in low CO_2 was significantly delayed. Soil warming prior to flowering further delayed ovary growth, regardless of CO_2 level.

Inflorescence stem necrosis

As fruit setting proceeded a number of inflorescences or parts of inflorescences of plants in low CO_2 became necrotic. The effect was accentuated in pre-warmed plants. Fig. 5b shows the proportion of inflorescences that were either unaffected or only partly affected and thus supported expanding ovaries. By fruit set all inflorescences of plants in ambient CO_2 supported expanding ovaries whereas of those of plants in low CO_2, only *ca* 80% that were pre-cooled and *ca* 30% that were pre-warmed did so.

Clearly ovary growth, the abscission of undeveloped flowers and ovaries and the survival of inflorescence stems during fruit setting in grapevines is conditioned not only by concurrent photoassimilation but also by soil temperature which, based on the low carbohydrate

reserves of the pre-warmed plants, almost certainly impacts through its moderation of the availability of an internal carbohydrate source.

Shoot growth and leaf area

Whereas before cap-fall, shoot elongation rate of plants in warmed soil exceeded that of plants in cooled soil, as flowering proceeded, the relative courses of shoot elongation rate generally switched (Fig 2b). The shoot elongation rate of (low reserve) plants in warm soil continued to decrease and their growth had almost stopped before completion of fruit set. In contrast the shoot elongation rate of those plants in cool soil initially increased and generally remained above that of the others. The switch almost certainly reflected the availability of carbohydrate reserves; both the residual amounts resulting from the pre-treatments and their mobilisation after the change in soil temperature conditions at cap-fall.

There was a strong interaction between CO_2 stress and prior soil temperature on the rate of shoot elongation during fruit set. Notably shortly after the imposition of CO_2 stress the rate of shoot elongation of plants in cooled soil was markedly greater than that of those in warmed soil. This finding indicates that the higher carbohydrate reserves of plants in cool soil were providing an important buffer against effects of photoassimilatory stress on shoot growth. The initial enhancement of shoot elongation under extremely low CO_2 was accompanied by a significant depression in inflorescence growth (Fig 2d) and thus appears to be at the expense of carbon that otherwise would have been directed to inflorescences.

Photosynthesis

CO_2 stress significantly affected the average photosynthetic rate of the reference leaves (Table 4). The average photosynthetic rate was 6.7 and 1.7 μmol CO_2 m^{-2} s^{-1} in ambient and low CO_2 respectively; a reduction of 75%. Leaf intercellular CO_2 concentration was 40% lower in the low CO_2 compared with that in ambient CO_2. However as we noted earlier the stomatal density of the reference leaves formed concurrently with soil warming was lower than that of leaves of soil-cooled plants. Furthermore both prior soil temperature and CO_2 stress induced significant differences in stomatal density of leaves at higher nodes. Accordingly those responses and treatment related differences in leaf /sink ratios preclude further interpretation of photoassimilation based on the reference leaves alone. However comparative photoassimilatory responses were alternatively measurable in terms of net changes in biomass.

Dry biomass and carbohydrate reserves

Total biomass

Although at first flower soil temperature treatments had not affected total dry biomass, by fruit set CO_2 stress had reduced total vegetative dry biomass significantly by *ca* 22% (Table 1). Structural and non-structural carbohydrate dry biomass were reduced *ca* 16% and 48% respectively; thus indicating a relatively higher impact on carbohydrate reserves.

Structural biomass

By fruit set CO_2 stress had reduced both average total perennial structural dry biomass and structural shoot dry biomass by *ca* 16% compared with non-CO_2 stressed plants (Table 1). The reduction in perennial biomass was due primarily to less root growth. Trunk dry biomass, which comprised only *ca* 21% of the perennial biomass, showed a smaller response to CO_2 stress. Prior warming increased total plant structural dry biomass by *ca* 6%.

Total non-structural carbohydrates

At fruit set, significant effects of CO_2 stress on TNC were evident (Table 1). Overall, low CO_2 reduced the TNCs in the perennial parts of the plants by 45%. Prior warming decreased root TNC by 22% and total vine TNC by 19% with only minor changes in the shoots and no significant differences in the trunk.

CO_2 stress also affected the proportions of TNC components *viz.* starch and soluble sugar, in the major plant parts at fruit set (Table 2). Overall low CO_2 reduced starch concentrations in the roots, trunks and shoots by 31%, 33%, and 63% respectively. However, CO_2 stressed plants that had been previously warmed had 35%, 38%, and 63% lower starch concentrations in the roots, trunks and shoots by respectively than those that had been cooled. Sugar concentration in the roots and trunks was not significantly reduced by low CO_2 but in the shoots it was reduced by 53%. That striking reduction may be attributed to both the relatively low photoassimilation rate and starch reserves in the CO_2-stressed shoots, and the depleted levels of starch in the trunks and roots.

There was no effect of soil temperature prior to cap-fall on total vegetative biomass, or the biomass of shoots or roots at the end of the study. However, at that time, structural biomass

made up a greater proportion of total biomass of plants that were carbohydrate depleted during flowering compared with those that were not. This indicates that reserves mobilised by warming had accelerated structural growth but the restoration of those carbohydrate reserves had been delayed.

Grapevine biomass distribution during fruit set

Figure 6 shows the distribution of carbon between the various plant parts between cap-fall and fruit set in response to CO_2 stress and prior soil temperature. The average total dry biomass increase of plants grown in low CO_2 was only 19% of those grown in ambient CO_2. That smaller gain in biomass was partly the result of less shoot structural dry biomass, and less total dry biomass of perennial parts. Plants in low CO_2 that had been pre-treated in warm soil had greater gains in both root structural biomass and TNC, than those from cooled soil in which both structural biomass and TNC of the roots decreased. As we have noted earlier our measures of structural biomass included non-carbohydrate reserves, so it is likely that the loss of structural biomass partly reflects their depletion. Whether or not necrosis of roots and/or inherent variation between the plants sampled at capfall and those sampled at fruit set account for part of the loss is open to speculation. Similarly in all but the pre-cooled plants in ambient CO_2 there was a loss of dry biomass in the trunks which we have attributed to structural sources. The losses ranged from 11% to 16%. Losses of non-carbohydrate reserves from trunks and variance in the measured parameters may partly account for those losses but secondary development of mature trunk tissues following seasonal distribution of reserves may have also contributed.

In both low CO_2 treatments structural biomass gains of the fruit clusters were on average only 2% of those in ambient CO_2. In pre-cooled plants there was a loss in cluster structural biomass. This accords with observations of stem necrosis and points to a dominant contribution of concurrent photoassimilation to fruit set. Evidently the high TNC reserve in the perennial parts at the onset of flowering could not compensate for the low availability of current photoassimilates. In low CO_2 the shoots acquired the greater proportion of biomass gained between first flower and fruit set whereas in ambient CO_2 the greater proportion of biomass was acquired by perennial parts; thus indicating that the cultivated grapevine responds to CO_2 stress at this time by favouring foliar development.

Discussion

Our study supports previous findings that fruit set in cultivated grapevines may be greatly limited by concurrent photoassimilation (Keller and Koblet 1994; Caspari *et al*. 1998). The fruit-set of photosynthetically stressed vines was more than halved compared with vines grown at the normal carbon dioxide level. Previous attempts to demonstrate the effects of concurrent photoassimilatory stress on grapevine fruit set have relied on leaf removal and/or alteration of the light environment (e.g. Keller and Koblet 1994; Caspari *et al*. 1998; Aziz 2003; Grechi *et al*. 2007). However, those approaches potentially confound effects of photoassimilatory capacity with other influences, for example leaves are an important source of hormonal regulators of a number of plant functions, and shading potentially confounds influences of temperature and light quality with photosynthetic capacity alone. In this study we avoided those possible influences by the use of CO_2 depletion. Notably, in severe carbon stress, largely imposed by CO_2 depletion, we observed necrosis of inflorescences stems as has been reported from studies where carbon stress was imposed by low light (Keller and Koblet 1994).

Of course, in vineyards, photosynthetic stress is not caused by low atmospheric CO_2 levels but it can be caused by environmental factors such as low light intensity, low temperature and drought stress (Kriedemann and Smart 1971). Leaf shading and leaf removal also cause photosynthetic stress. While some of those factors can be avoided others, which if severe enough, represent a seasonal risk that we have now shown cannot be mitigated by the amount of previously stored carbohydrate at cap-fall; under such photosynthetic stress most available carbohydrate went into shoot growth. The initial enhancement of shoot elongation under extremely low CO_2 that was accompanied by a significant depression in inflorescence growth provides a clear illustration of preferential shoot growth under those conditions.

Based on the prior moderation of non-structural carbohydrates by soil temperature, this study also reveals that the availability of current photoassimilates for inflorescence development and fruit set is conditioned by the demand of shoots, roots and depleted carbohydrate stores in other parts of the plant- most notably the roots. Fruit set as defined is a function of flower number and the number of intact ovaries remaining after abscission ceases. Thus while fruit set under photoassimilatory stress was reduced in both pre-cooled and pre-warmed plants, our results show that both the number of intact ovaries and fruit set were conditioned by

significant interactions between CO_2 stress and pre-flowering soil temperature. Strong general correlations between fruit set and non-structural carbohydrates, particularly in the roots, across the full range of carbohydrate perturbing treatments indicate a strong influence of stored carbohydrates on fruit set even though at the lowest TNC levels, in our study, the lack of CO_2 evidently precluded a fruit set response.

Flower number per inflorescence in grapevine is determined after bud-break and has been shown previously to be enhanced by high carbohydrate reserves (Scholefield *et al*. 1977; Goffinet 2004; Bennett *et al*. 2005; Smith and Holzapfel 2009). Accordingly, in the present study, the increased flower number accompanied by the temperature-enhanced depletion of the carbohydrate reserves in perennial parts, indicates that greater reserve mobilisation elicited the response. While reserve mobilisation almost certainly accounted largely for the higher sugar concentrations in the shoot stems, the inflorescence rachises and flowers during soil warming, the greater photosynthetic rates of the earlier maturing leaves and possibly the inflorescences themselves (Vaillant-Gaveau *et al*. 2011) are also likely to have contributed. Although the warmed vines had earlier cap-fall this did not result in earlier or greater fruit set. On the contrary, ovary expansion was delayed. It appears that the higher sugar concentrations were transitory, and by the time of ovule fertilisation, were not high enough to result in successful ovule development.

In our study new fine root growth had commenced by the onset of flowering so it appears that grapevine root, shoot and inflorescence growth were simultaneously dependent on carbohydrates during fruit set. In cultivated grapevines the periodicity of fine root growth can vary between seasons, but generally begins just before flowering and then continues through fruit set to the onset of ripening (Van Zyl 1984; Comas *et al*. 2005). However, the greater increase in root structural biomass of the pre-warmed plants compared with the pre-cooled plants in ambient CO_2 during fruit set indicates that soil temperature between bud-break and flowering influences seasonal variation in the periodicity of root growth and hence the extent of concurrent demand for carbohydrates between roots, shoots and setting fruit.

After soil warming had depleted carbohydrate reserves in roots at cap-fall structural growth and carbohydrate restoration in those parts took place simultaneously with fruit set. This was particularly evident in ambient CO_2 but even occurred to some degree in low CO_2. We interpret that phenomenon and the concurrent increase in non-inflorescence associated

biomass of shoots as an adaptation that ensures survival of the plant by providing a buffer against subsequent seasonal environmental contingencies – albeit at the expense of part or all of the plant's reproductive capacity in the current season. That interpretation accords with the similar, proposition of Geiger and Servaites (1991) concerning the general dependence of perennial plants on carbohydrate reserves for survival under photoassimilatory stress; but our finding of simultaneous functions indicates that preserving the buffer capacity, where possible, is itself an imperative. For example, considering the changes in biomass and carbohydrate reserves in roots during fruit set, we see that in pre-cooled plants in low CO_2, where the initial reserves were high (19.5%DW) but photoassimilation was greatly reduced, there was a loss of root reserves and structural biomass Whereas in pre-warmed plants in low CO_2 where the initial reserves were lower (13.1%DW) there was a small increase in reserves and structural biomass presumably facilitated by their greater leaf area and stomatal density (see Rogiers *et al*. 2011), and smaller carbon distribution to shoot biomass.

We emphasize that our observations were of cultivated grapevines in which fruit set is accompanied by several simultaneous carbon dependent processes viz. foliar and seasonal fruit development and restoration of carbohydrate reserves and root growth, whereas in unpruned grapevines leaf development and fruit set at least, occur sequentially (Winkler 1929; Clingeleffer 1989). Pruning involves the seasonal removal of 80% or more of the previous season's shoot growth; a practice that the species in its wild state would not regularly encounter. However in the wild state herbivory and/or winter freezing may cause similar if not as severe destruction of seasonal growth of grapevines from time to time. The shoots of unpruned grapevines are much shorter and thinner than their cultivated counterparts (Downton and Grant 1992), and as Fisher and Ewers (1991) have shown, the natural vine form generally, with its lower stem to leaf ratio than self supporting trees and shrubs, does appear to have greater susceptibility to structural injury. Thus while some caution is required in attributing responses to an extreme human-induced perturbation *i.e.* pruning, in adaptive terms. It does seem likely that physiological adaptation to similar phenomena in the wild determines grapevine responses to carbon-related environmental impacts during fruit set

In cultivated grapevines the onset of restoration of carbohydrate reserves also varies somewhat around flowering (see Holzapfel *et al*. 2010 and references cited therein) and has been linked to susceptibility to flower abscission by several correlative studies, e.g. Winkler

(1929); Zapata *et al*. (2004). In those studies, delays in reserve restoration were attributed to delays in attainment of net positive total plant carbon balance related to delays in leaf canopy development, which in turn were related to increasing pruning severity. Considering the importance of concurrent photoassimilates, as we have demonstrated here, concurrent reserve restoration associated with pruning does lead to a greater susceptibility to floral abscission and this study does shed further light on the circumstances leading to sub-optimal fruit set so commonly encountered in viticulture. Whether or not the delaying effect of pruning on seasonal re-foliation is also a function of carbohydrate reserve status at bud break has not been determined but it does seem likely, and if so, introduces another source of seasonal variation in fruit set.

Finally, the depletion of carbohydrate reserves in roots by soil warming after bud-break is noteworthy insofar as it confirms and extends the application of an experimental technique developed by Field et al 2009. It also reveals a hitherto unrecognised influence on root carbohydrate reserves and indicates a plant response that has received little attention in relation to adaptation to global warming.

Conclusions

Fruit set in grapevines may be limited by concurrent photoassimilation however, based on the prior moderation of carbohydrate reserves by soil temperature, the availability of current photoassimilates for inflorescence development and fruit set appears to be conditioned by the simultaneous carbon demand for shoot growth, root growth and carbohydrate reserve restoration - most notably in roots. Noting that such simultaneous demand is likely induced by pruning, we conclude that fruit set in cultivated grapevines is particularly susceptible to both photoassimilatory stress and soil temperature-induced moderation of carbohydrate reserves after bud-break. Accordingly we reason that soil temperature in combination with other conditions, both cultural (e.g. pruning, defoliation, plant training) or environmental (e.g. drought, radiation, cloud, wind) that induce photoassimilatory stress, is the primary source of the significant season-to-season variation in grapevine fruit set and yield.

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Table 1. Biomass distribution between roots, trunks, and shoots at cap-fall and fruit set. The soil temperature pre-treatments (15°C or 26°C) were applied from bud-break until cap-fall (32 days after bud-break). The CO_2 treatments (336 µmol mol^{-1} or 94 µmol mol^{-1}) were applied from cap-fall to fruit set (60 to 67 days after bud-break).

	Root		Trunk		Shoot		Total	
Pre-treatment / CO_2	**cap-fall**	**fruit set**	**cap-fall**	**fruit set**	**cap-fall**	**fruit set**	**cap-fall**	**fruit set**
				Structural dry weight (g)				
cool/ambient	121.9	142.9	39.2	42.3	24	60	185	245
cool/low		121.5		34.8		47		204
warm/ambient	110.0	150.5	52	46.1	36	60	198	257
warm/low		120.9		43.4		54		219
Pre-treatment	ns[A]	ns	*	*	ns	ns	ns	*
CO_2 treatment	-	***	-	*	-	**	-	***
Interaction	-	ns	-	ns	-	ns	-	ns
				Total non-structural carbohydrates (g)				
cool/ambient	33.0	55.9	4.0	5.5	1.9	3.5	39	65
cool/low		31.7		3.2		1.2		36
warm/ambient	20.2	45.9	4.8	5.6	3.9	3.2	29	55
warm/low		22.9		3.4		1.2		27
Pre-treatment	*	***	ns	ns	*	*	ns	***
CO_2 treatment	-	***	-	***	-	***	-	***
Interaction	-	ns	-	ns	-	ns	-	ns
				Dry weight (g)				
cool/ambient	155.0	198.9	43.1	47.8	26	63	224	310
cool/low		153.3		38.0		48		240
warm/ambient	130.2	196.4	56.7	52.0	39	64	226	312
warm/low		143.8		46.8		56		246
Pre-treatment	**	ns	ns	*	ns	ns	ns	ns
CO_2 treatment	-	***	-	**	-	***	-	***
Interaction	-	ns	-	ns	-	ns	-	ns

[A]*,**,*** and ns indicate significance at p<0.05, <0.01, <0.001, and not significant, respectively.

Table 2. Root, trunk and shoot starch and sugar concentrations at cap-fall and fruit set. The soil temperature pre-treatments (15°C or 26°C) were applied from bud-break until the onset of cap-fall (32 days after bud-break). The CO_2 treatments (336 or 94 μmol mol^{-1}) were applied from cap-fall to fruit set (60 to 67 days after bud-break).

Pre-treatment / CO_2	Root		Trunk		Shoot	
	cap-fall	**fruit set**	**cap-fall**	**fruit set**	**cap-fall**	**fruit set**
			Starch concentration (%DW)			
cool/ambient	19.5	26.1	8.1	10.4	1.88	1.7
cool/low		19.0		7.4		0.6
warm/ambient	13.1	21.4	7.6	9.7	3.43	1.6
warm/low		13.9		6.0		0.6
Pre-treatment	* [A]	***	ns	**	ns	ns
CO_2 treatment	-	***	-	***	-	***
Interaction	-	ns	-	ns	-	ns
			Sugar concentration (%DW)			
cool/ambient	1.7	2.0	0.81	0.97	5.31	3.9
cool/low		1.7		0.96		1.8
warm/ambient	2.4	1.9	0.82	1.13	6.07	3.4
warm/low		1.9		1.08		1.6
Pre-treatment	*	ns	ns	***	*	***
CO_2 treatment	-	ns	-	ns	-	***
Interaction	-	ns	-	ns	-	ns

[A] *, **, *** and ns indicate significance at $p<0.05$, <0.01, <0.001, and not significant, respectively.

Table 3. Fruit set after ambient (336 µmol mol^{-1}) or low (94 µmol mol^{-1}) CO_2 exposure during flowering following soil temperature pre-conditioning (15°C or 26°C) from bud-break until the onset of cap-fall.

Pre-treatment/ CO_2	Flower number		Abscised flowers and undeveloped ovaries	Intact ovaries	Fruit set %
cool/ambient	117	113[A]	49	64	58
cool/low		121	101	20	19
warm/ambient	139	133	75	57	43
warm/low		144	114	30	20
Pre-treatment	*[B]		***	ns	***
CO_2 treatment	-		***	***	***
Interaction	-		***	*	***

[A] pre-CO_2 treatment population means
[B] *,**,*** and ns indicate significance at $p<0.05$, <0.01, <0.001, and not significant, respectively.

Table 4. Leaf photosynthesis in response to soil temperature and CO_2. The soil temperature pre-treatments (15°C or 26°C) were applied from bud-break until the onset of cap-fall (32 days after bud-break). The CO_2 treatments (336 µmol mol^{-1} or 94 µmol mol^{-1}) were applied from cap-fall to fruit set (60 to 67 days after bud-break). Photosynthesis was measure on the first four emergent leaves at 31 and 40 days from bud-break.

Pre-treatment / CO_2	**Photosynthesis (µmol CO_2 $m^{-2}s^{-1}$)**	
	31 days after bud-break	**40 days after bud-break**
cool/ambient	9.8[A]	6.9
cool/low		1.7
warm/ambient	9.3	6.5
warm/low		1.7
Pre-treatment	ns	ns
CO_2 treatment	-	***
Interaction	-	ns

[A] pre-CO_2 treatment population means
[B] *,**,*** and ns indicate significance at $p<0.05$, <0.01, <0.001, and not significant, respectively.

Fig. 1. Timeline of treatment applications. Cool (15°) and warm (26°C) soil pre-treatments were applied in the glasshouse from bud-break until cap-fall. At this point the pre-treatments were discontinued and plants were subjected to ambient (336 µmol mol^{-1}) or low (94 µmol mol^{-1}) CO_2 atmospheres. The CO_2 treatments were applied in controlled environment chambers (22°/8°C; day/night) until fruit set.

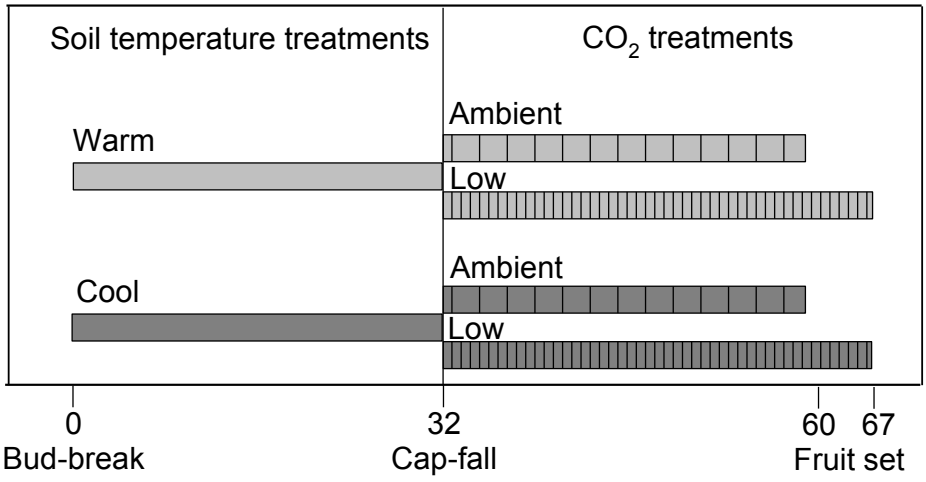

Fig. 2. Shoot (a) length and (b) growth rate and inflorescence (c) length and (d) growth rate between bud-break and fruit set (mean ± s.e., for the shoot data n=16 plants x 4 shoots/plant prior to cap-fall while n=8 plants x 4 shoots/plant after cap-fall); for the inflorescence data n=16 plants x 8 inflorescences/plant prior to cap-fall and n=8 plants x 8 inflorescences/plant after cap-fall.). The plants were exposed to a warm or cool soil pre-treatment for 32 days (indicated by vertical line). Subsequently the soil temperature treatments were discontinued and plants were subjected to ambient or low CO_2 atmospheres from cap-fall to fruit set. The soil temperature pre-treatment x time interaction was significant ($P<0.001$) on shoot length between bud-break and fruit set. The main effects of soil temperature pre-treatment ($P<0.001$) and time ($P<0.001$) were significant on shoot growth rate between bud-break and fruit set. The soil temperature pre-treatment x time interaction was significant ($P<0.05$) on inflorescence length between bud-break and cap-fall while the main effect of time ($P<0.01$) and the CO_2 x soil temperature pre-treatment interaction was significant ($P<0.05$) between cap-fall and fruit set. The main effect of the soil temperature pre-treatment was significant ($P<0.01$) on inflorescence growth rate between bud-break and fruit-set. CO_2 also had a significant effect on inflorescence growth rate ($P<0.01$) between cap-fall and fruit set.

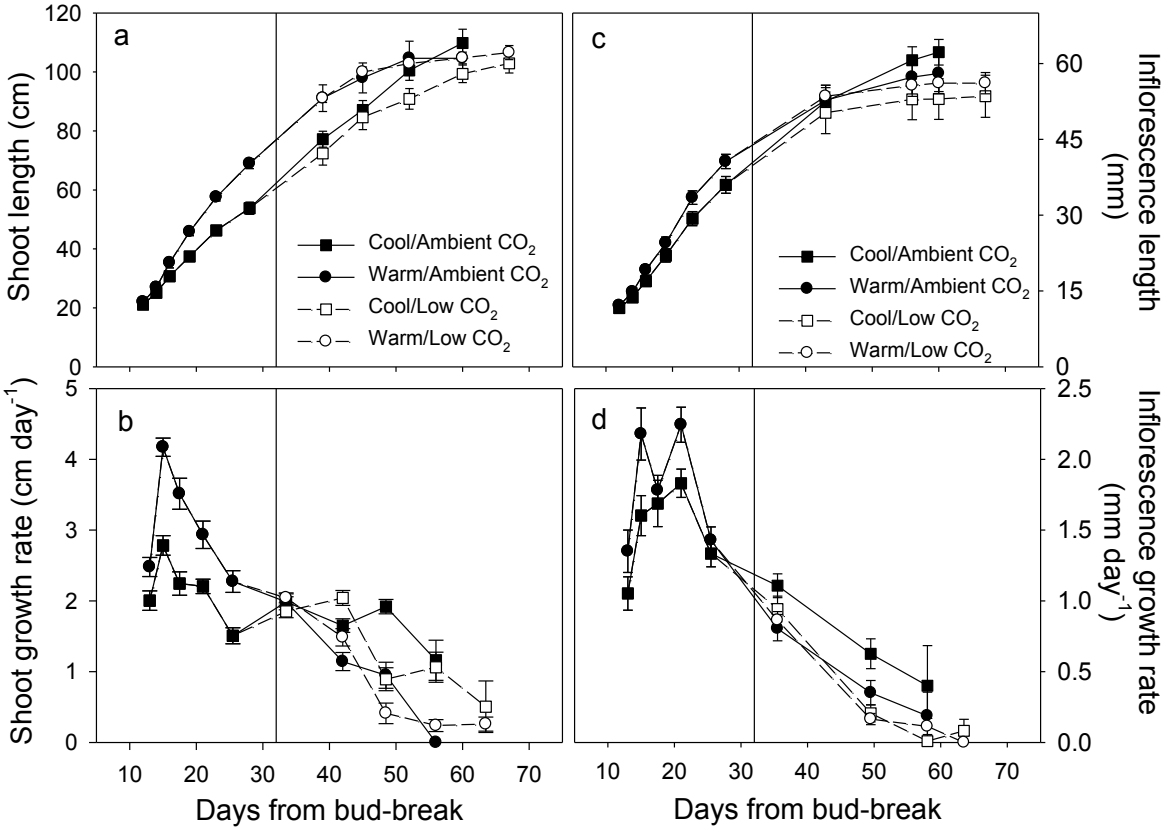

Fig. 3. Sugar concentration (% DW) and content (mg) of inflorescence rachises and flowers at cap-fall (mean ± s.e., n=5 plants x 4 inflorescences/plant). Sugar represents the combined glucose, fructose and sucrose fractions. Sugar content of flowers refers to that of all flowers of the inflorescence. The soil temperature treatment had a significant effect on flower sugar concentration ($P<0.05$) as well as rachis sugar concentration ($P<0.05$) and sugar content ($P<0.001$)

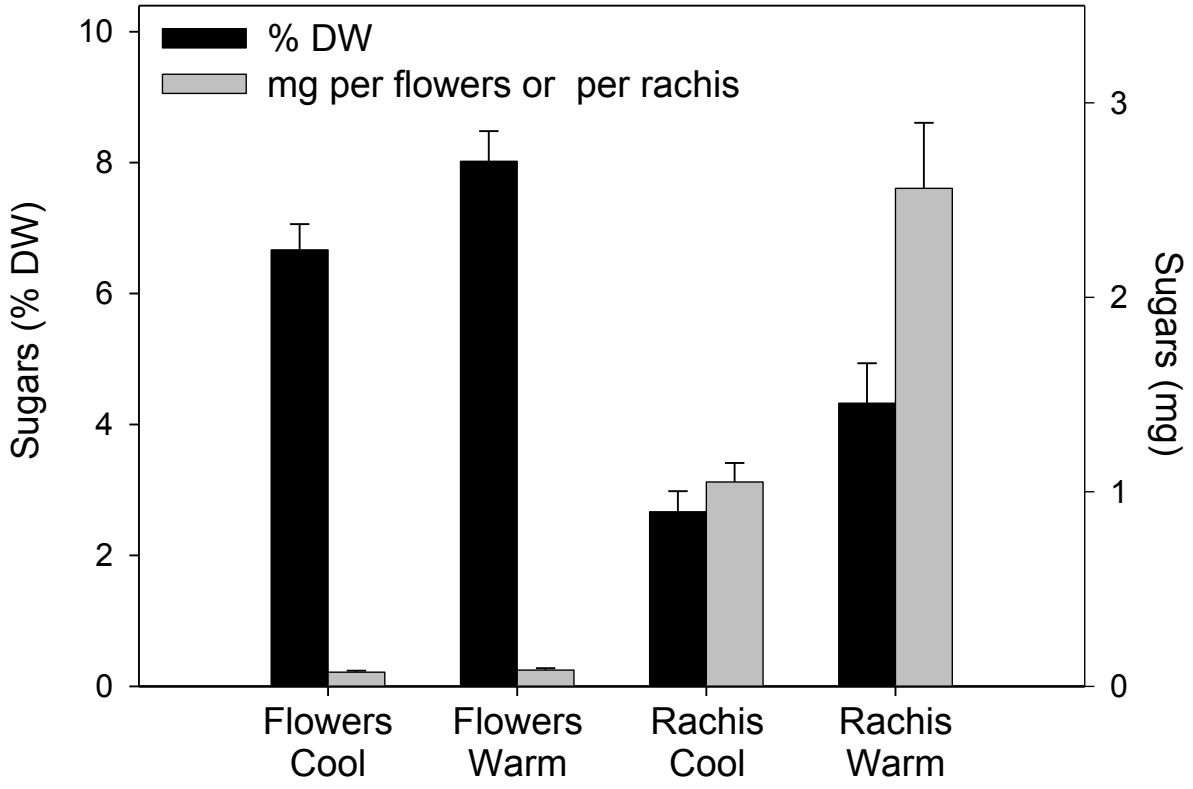

Fig. 4. Fruit set as a function of shoot (%) and root (g) total non-structural carbohydrates at cap-fall. Each data point is the average of eight inflorescences per vine. Both the regressions were highly significant (P<0.001)

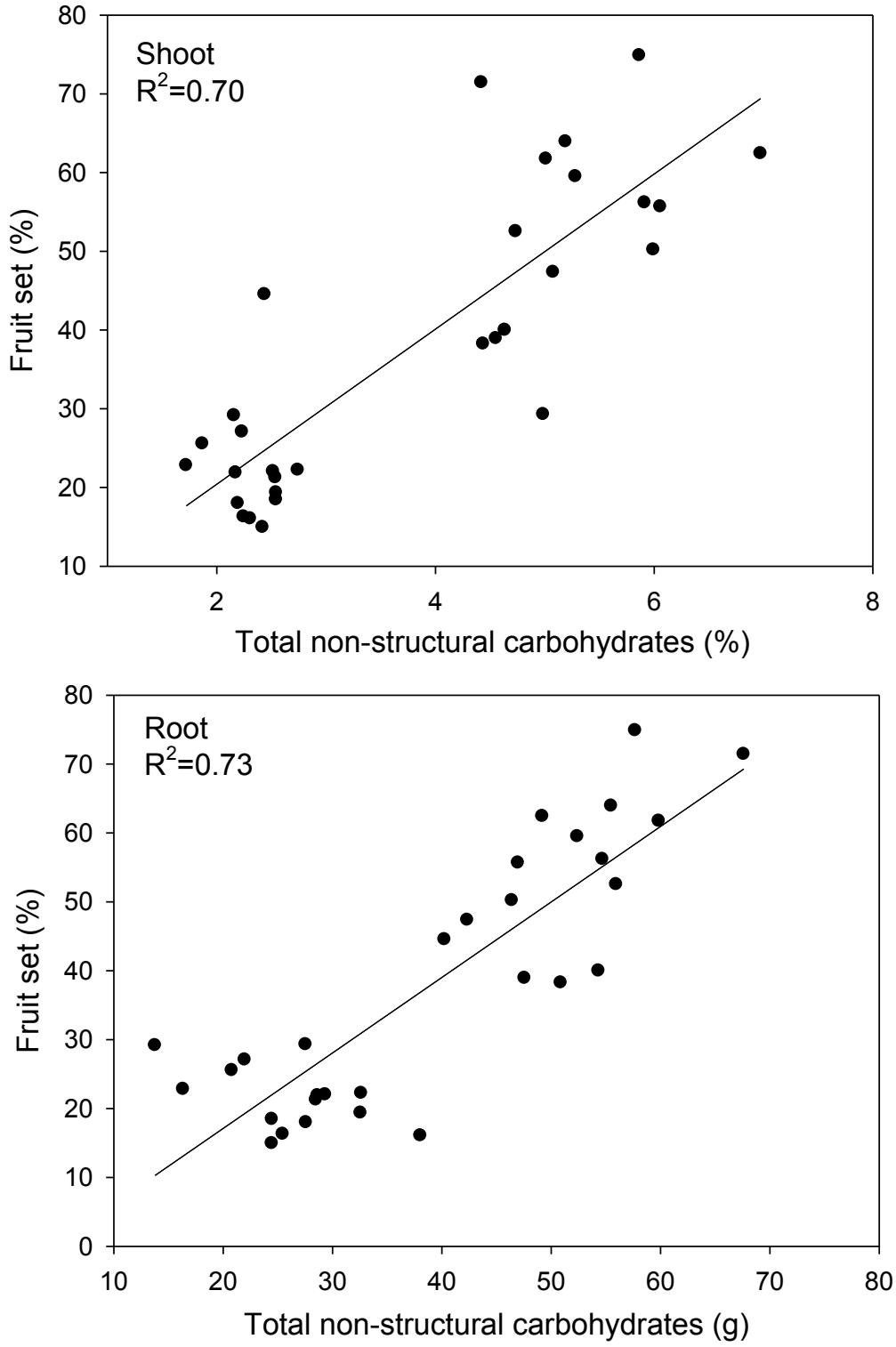

Fig. 5. Ovary expansion during ambient or low CO_2 and following soil temperature pre-treatments prior to flowering. (a) Ovary diameter (mean ± s.e., n= 8 plants x 8 inflorescences/plant x 10 ovaries/inflorescence). (b) Percentage of inflorescences with expanding ovaries that reached the post-flowering stage (mean ± s.e., n= 8 plants x 8 inflorescences/plant). The main effects of soil temperature pre-treatment ($P<0.01$) and CO_2 ($P<0.001$) on ovary diameter was significant at each sampling date. Likewise the main effects of soil temperature pre-treatment ($P<0.05$) and CO_2 ($P<0.001$) on percentage of inflorescences with expanding ovaries was significant at 50 days from bud-break until harvest. The soil temperature pre-treatment x CO_2 interaction on ovary diameter was significant ($P<0.001$) at 56 and 60 days after bud-break.

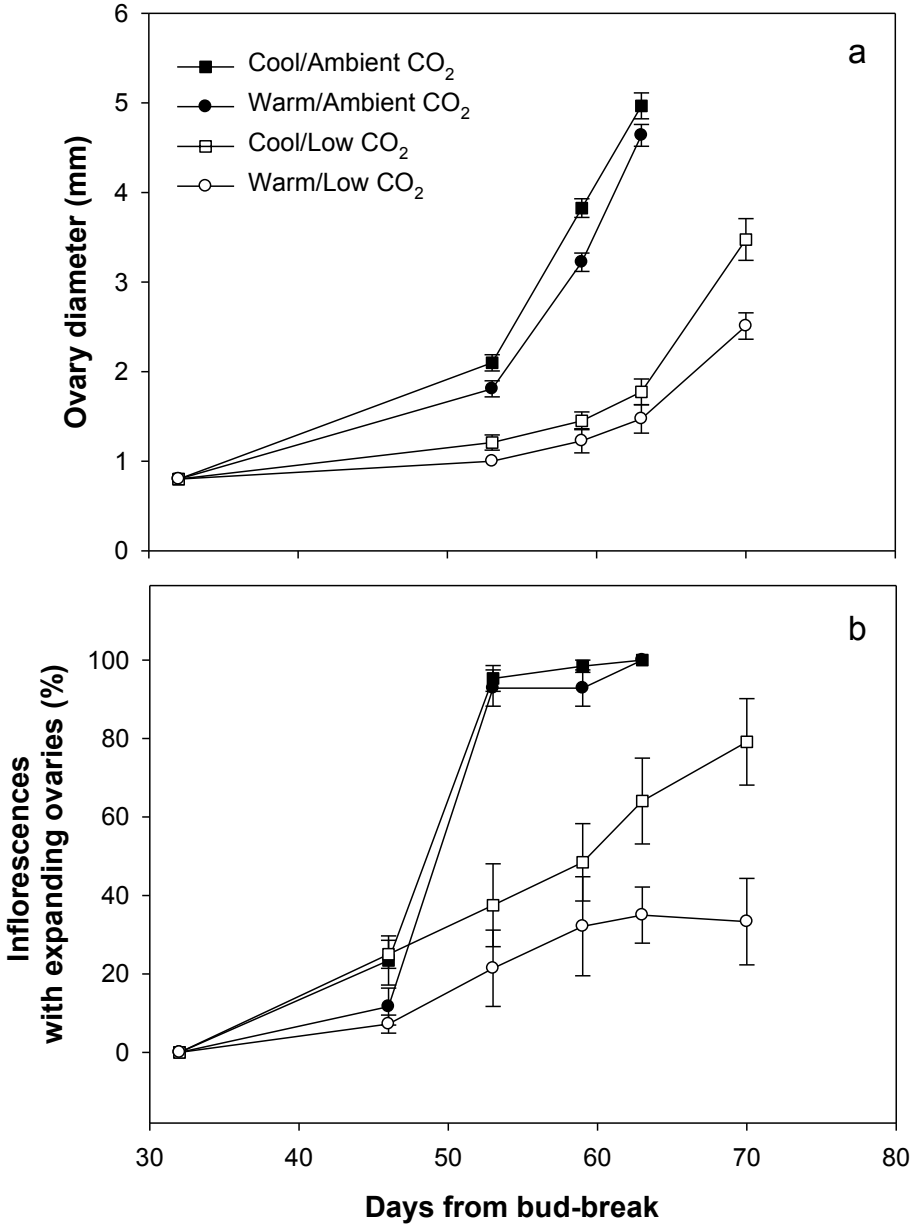

Fig. 6. Changes in carbon biomass of grapevine parts from cap-fall until fruit set as influenced by atmospheric CO_2 concentration (ambient or low) and prior soil temperature (cool or warm). Arrow size is proportional to the relative quantity of carbon biomass flow to the plant parts. Fruit comprises retained ovaries and stems. Net biomass gain represents photoassimilation less respiration and dry weight of shedded parts *viz.* undeveloped flowers and flower caps. The soil temperature pre-treatment was significant on changes in total non-structural carbohydrates (TNC) of trunk ($P<0.05$) and shoots ($P<0.001$) and changes in structural biomass of roots ($P<0.05$), trunk ($P<0.01$) and shoots ($P<0.01$). The CO_2 treatment was significant on changes in TNC of roots ($P<0.001$), trunk ($P<0.001$), shoot ($P<0.001$) and clusters ($P<0.001$) as well as changes in structural biomass of roots ($P<0.001$), trunk ($P<0.05$), shoot ($P<0.001$) and clusters ($P<0.001$). There were no significant interactive effects.