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Research Article

# Soil Temperature Prior to Veraison Alters Grapevine Carbon Partitioning, Xylem Sap Hormones, and Fruit Set

Stewart K. Field,[1,2]* Jason. P. Smith,[1] Erin N. Morrison,[3] R. J. Neil Emery,[3] and Bruno P. Holzapfel[1,4]

[1]National Wine and Grape Industry Centre, Charles Sturt University, Locked Bag 588, Wagga Wagga, New South Wales, Australia 2678; [2]Current address: Viticulture and Wine Department, Nelson Marlborough Institute of Technology, 85 Budge Street, Blenheim 7240, New Zealand; [3]Biology Department, Trent University, Peterborough, ON K9J 7B8, Canada; and [4]New South Wales Department of Primary Industries, Wagga Wagga, Australia.

*Corresponding author (stewart.field@nmit.ac.nz)

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**Abstract:** To gain a better understanding of environmental impacts on grapevines and the physiological regulation of acclimation we determined the effects of soil temperature (14ºC or 24ºC) between anthesis and veraison on growth, non-structural carbohydrates, cytokinins, abscisic acid and leaf function of potted *Vitis vinifera* cv. Shiraz. Plants of each regime were selected from two groups that had been grown in a glasshouse from three weeks prior to budbreak at an average soil temperature of either 13ºC or 23ºC. Soil temperature between anthesis and veraison affected utilization and restoration of root and trunk non-structural carbohydrates and changes in biomass of major plant organs. Soil warming promoted shoot growth via utilization of starch reserves, while soil cooling promoted starch storage in both the root and wood and shifted overall biomass

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partitioning to the roots. A change in soil temperature from warm to cool through flowering was also associated with reduced fruitset. Diurnal courses of photosynthesis, transpiration and stomatal conductance after fruitset were significantly affected by soil temperature. Phytohormones (cytokinin and abscisic acid) were measured in the xylem sap and leaves at fruitset and veraison. Differences between these two sample types during grapevine development highlight a phytohormone shift likely involved in post veraison fruit ripening. We conclude that soil temperature significantly affects grapevine growth and that the responses are mediated largely by an influence of temperature on mobilization of non-structural carbohydrates from the roots.

**Key words:** abscisic acid, cytokinin, non-structural carbohydrate, root temperature, *trans*-zeatin, xylem sap

## Introduction

Temperature is a key environmental factor that influences grapevine phenology, growth and berry development. Air temperature has been primarily used to define climatic suitability for vineyard site location. However, temperature will have increasing importance on management decisions and varietal selection as climate of existing grape producing regions is expected to warm through the coming decades (Jones et al. 2005). The effects of soil temperature on grapevine growth and physiology are of practical interest because of the potential to modify root-zone temperatures independently of air temperature. Soil temperatures can be manipulated in the short to medium term through mulching with organic material or plastic sheeting (Van Der Westhuizen 1980), or may vary in response to cultivation or cover cropping practices (Pradel and Pieri 2000). In an experimental trial intended to maximize the soil temperature differential between treatments,

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difference of up to 8$^{o}$C (at 10 and 30cm depth) were achieved between heavy straw mulching and plastic applied under the vine row (Holzapfel et al. 2016). In establishment of vineyards, row orientation, row spacing and trellis design provides an opportunity for longer term modification of soil temperatures by varying the shading of solar radiation to the vineyard floor. A better understanding of the impact of root-zone temperature on grapevine physiology may therefore improve the management of vineyards in a warming climate.

Earlier studies of root-zone temperature effects on grapevine growth and development have largely relied on the use of potted plants in controlled environments. These studies have ranged from 11 to 30$^{o}$C, and either covered shorter periods from three to eight weeks after budbreak (Woodham and Alexander 1966, Skene and Kerridge 1967), or a longer period from dormancy to harvest (Zelleke and Kliewer 1979). The general finding across these experiments was the highest biomass production and shoot growth rates were observed with the warmest treatment regimes. These studies did not use root-zone temperature treatments above 35$^{o}$C which is the temperature that may impact root survival (Huang et al 2005). However, observations made by Woodham and Alexander (1966) suggest that shoot growth can be restricted or stopped if the differential between soil and air temperature is too great. Grapevine responses to soil temperature may therefore vary according to the environmental conditions experienced by the above ground parts of the plant.

Previously we have shown that soil temperature between dormancy and anthesis greatly affects the rate of carbohydrate reserve utilization for re-establishment of the canopy (Field et al. 2009). This suggested a direct impact of soil temperature on the mobilization of reserve carbohydrates in the root. The subsequent period from anthesis to veraison, [i.e. E-L stages 23 and 35 respectively

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(Pearce and Coombe 2005)], is particularly important from a commercial perspective as the fruitset and the initial stages of berry development contribute to yield every season.

The importance of phytohormones, particularly cytokinins (CKs) have been examined in relation to their role in crop yield and influence on plant cell division and differentiation (Mok and Mok 2001) and maintenance of source-sink strength through the mobilization of nutrients. Biotic and abiotic factors can greatly impact CK concentrations within plants (Mauch-Mani and Mauch 2005). Soil temperature was found to influence the concentration of specific CKs in the xylem sap of Shiraz grapes (Field et al. 2009). For example, increased levels of dihydrozeatin riboside (DHZR) and *trans*-zeatin riboside (*trans*ZR) were detected in the xylem sap of grapevines grown in warmer soil temperatures and it was suggested that this may stimulate shoot development in grapevines (Field et al. 2009). The dominance of CK types can vary among plant species as well as the location in the plant (Schäfer et al. 2015). Isopentenyl (iP) CKs have been found to accumulate during fruit ripening in the berries of Shiraz and other grapevine cultivars, likely due to tissue specific CK production (Böttcher et al. 2015). Cytokinins can also be transported throughout the plant with *trans* zeatin (*trans*Z) type CKs found mainly in the xylem sap (root to shoot transport) whereas iP types are mainly found in the phloem or leaf exudates, suggesting that *trans*Z CKs are important in root to shoot signalling and iP are mainly transported from source to sink organs (Matsumoto-Kitano et al. 2008).

The phytohormone abscisic acid (ABA) plays a role in seed maturation and dormancy, regulation of stomatal aperture as well as plant stress response and adaptation to environmental changes (Mauch-Mani and Mauch 2005, Bakht et al. 2013). ABA levels have been found to increase and

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aid in cold tolerance of some species (Bakht et al. 2013). Understanding the role of ABA with soil temperature as well as the potential interaction between phytohormone groups is important.

In this study we show that soil temperature between anthesis and veraison has a strong influence on grapevine growth and biomass distribution, and that a change in soil temperature prior to anthesis has a marked interactive effect on these responses; including the seasonal restoration of root carbohydrate reserves and the percentage of fruitset. These responses are interpreted in terms of carbohydrate reserve dynamics and their impact on photoassimilation and water use. Associated fluxes in xylem CKs and ABA, some of which have been shown to respond to soil temperature early in the season (Field et al. 2009), are examined to further elucidate their role in mediating grapevine responses to the soil environment.

## Materials and Methods

*Experimental system and plant material.*

From dormancy to anthesis own-rooted 3-year-old vines, *cv* Shiraz, were grown in a glasshouse in 26 L (60cm high) insulated pots with soil maintained at an average temperature of either 13ºC or 23ºC with a cooled or heated recirculating water system (Figure 1, Field et al. 2009). At anthesis two additional treatments were added by changing the soil temperature of half the vines in each treatment to the other soil temperature treatment [i.e. half of the vines that were previously grown at a cool soil temperature of 13°C prior to flowering were switched to the warm soil temperature of 23°C] (Figure 1). From now on the treatments are referred in the text as cool/cool (13ºC budbreak to anthesis / 13ºC anthesis to veraison), cool/warm (13ºC/23ºC), warm/warm (23ºC/23ºC) and warm/cool (23ºC/13ºC) respectively.

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Soil temperature was recorded at 5 min intervals, at a depth of 20cm in the center of each pot, using an automated logging system connected to digital temperature sensors (Maxim Integrated Products, California, USA). Soil temperatures did not differ by more than 0.5ºC between pots. During the period from anthesis to veraison the average soil temperature was increased by 2ºC in all treatments to avoid large differences between the increasing air temperature and the 13°C tank temperature (Figure 2). Air temperature, which were moderated by evaporative air-conditioning but not specifically controlled, was maintained between mean daily temperatures of 12°C to 27°C (Figure 2). Light was provided only by natural solar radiation.

All pots were filled with a 4:4:2 mixture of sandy loam, gravel, and peat when plants were initially planted. This allowed for a water holding capacity of approximately of 4L of plant available water per pot. Plants were watered daily to the point that excess water drained freely from the pots. Throughout the experiment, vines were fertilised monthly with 200mL of 20:1 diluted complete liquid fertiliser (Megamix plus, Rutec, Tamworth, Australia). The vines were sprayed with wettable sulphur and copper sulphate throughout the season to prevent mite and fungus infections.

*Measurements.*

Each vine was trained to three vertical shoots with two inflorescences per shoot when possible. This resulted in five to six inflorescences per vine. Flower and berry number per inflorescence were determined at anthesis and veraison respectively. The number of flowers was determined by enclosing each inflorescence in a nylon mesh bag just before flower opening and subsequently counting abscised flower caps and unopened flowers. Fruitset was determined when abscission of

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the undeveloped flowers and unexpanded ovaries ceased. Fruitset was quantified as the relationship between the number of berries and number of flowers expressed in percentage.

At three days prior to the start of cap-fall and at the completion of the study (approximately 50% veraison) 5 vines per treatment were destructively harvested, separated into root, trunk, shoot, leaf and fruit components, washed and oven dried at 70°C for dry biomass measurement. Non-structural carbohydrates (starch, sucrose, D-glucose, and D-fructose) in the roots and trunk were determined by the method described by Field et al. (2009). Total N concentration was determined by combustion analysis on a 50mg subsample using a VarioMAX combustion analyzer (Elementar, Hanau, Germany). At veraison, 100 berry samples for each potted vine were randomly collected, squashed, and the homogenized juice used for determination of total soluble solids (°Brix) using a digital refractometer (ATAGO PR-101, Tokyo, Japan). The remainder of the 100 berry sample was incorporated into the fruit component for dry weight biomass measurement.

The diurnal pattern of leaf photosynthesis, transpiration, stomatal conductance and water potential were determined on a clear day (3rd November) during the season between fruitset and veraison. Leaf photosynthesis, transpiration and stomatal conductance measurements were made using an LCA4 gas analyser (ADC Bioscientific, Hoddeson, UK), and immediately followed by the measurement of leaf water potential using a Scholander-type pressure chamber. Each measurement was made on the most recently fully expanded leaf on two shoots of each plant.

*Xylem sap collection and phytohormone analysis.*

Xylem sap was collected from three plants of each treatment through a 10 day period spanning the fruitset period, and then again through a 5 day period shortly prior to veraison using the root

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pressure chamber method described by Field et al. (2009). At the time of xylem sap collection, one leaf was excised and immediately frozen at -80°C. The xylem sap and leaf concentrations of abscisic acid (ABA), *trans*-zeatin (*trans*Z), *cis*-zeatin (*cis*Z), dihydrozeatin (DHZ), isopentenyl adenine (iP), *trans*-zeatin riboside (*trans*ZR), *cis*-zeatin riboside (*cis*ZR), dihydrozeatin riboside (DHZR), isopentenyl adenosine (iPA), zeatin *O*-glucoside (ZOG), zeatin riboside *O*-glucoside (ZROG), dihydrozeatin *O*-glucoside (DHZOG), *trans*-zeatin nucleotide (*trans*ZRP), *cis*-zeatin nucleotide (*cis*ZRP), dihydrozeatin nucleotide (DHZRP), and isopentenyl nucleotide (iPRP) were determined according to the method of Ross et al. (2004) for ABA and Quesnelle and Emery (2007) for CKs.

*Statistical analysis.*

For statistical analysis of reproductive development, growth, biomass components, and non-structural carbohydrates in the anthesis-veraison period a 2 x 2 factorial design with preconditioning and current soil temperature, with 5 replicates, was used (Genstat, Rothhamsted Experimental Station, Harpenden, Herts, United Kingdom). Within grapevine inflorescences, % fruitset varies inversely with flower number per inflorescence (Keller 2015). With a differences in flower numbers between treatments observed in our study, linear regression was used to examine the overall relationship between flower numbers and berry numbers, and then extended to each treatment to test for differences in slope and intercept. Means ± standard errors are presented for the cytokinin and ABA concentrations.

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## Results

*Shoot growth and leaf number.*

Soil temperature before anthesis had no effect on the total length of primary shoots, total leaf number or leaf area. However, soil warming between anthesis and veraison significantly ($p < 0.01$) increased average shoot length relative to the vines growing in cooled soil (Figure 3a). Within the cooled treatment, the reduction in growth was greater for the vines grown in warm soil between budbreak and anthesis. Leaf number per shoot was not significantly different (Figure 3b).

*Biomass partitioning, non-structural carbohydrates and total nitrogen.*

At veraison, fruit biomass of pre-anthesis warmed vines was significantly lower (Table 1). However, there was no significant difference in the other total dry biomass components of plants that had been warmed or cooled in both time periods (Table 1). Although, the ratio of shoot to root mass was 40% greater in post-anthesis warmed plants. The increase in the shoot:root ratio under the warm soil regime was caused by an increase in shoot and lateral growth and a decrease in root biomass (Table 1). The decrease in root biomass was associated with significantly lower storage of starch in the roots of warmed (19.5gDW/vine) compared to the cooled vines (41.1gDW/vine).

In both roots and trunks, the concentration of starch decreased between dormancy and anthesis (Figure 4). When the soil temperatures were switched, the shift from cool to warm caused a decrease in root starch to comparable levels in vines that had been grown continuously in the warm soil. Conversely, a switch from warm to cool resulted in an increase of root starch concentrations to levels similar to that of vines grown continuously in the cool soil (Figure 4a). Trunk starch initially responded to the soil temperature treatments in a similar manner to root starch, with

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concentrations decreasing more in the warm than cool soil treatment after bud-break. However, the response was less pronounced during the second treatment period, and starch concentrations at veraison mainly reflected the differences established at anthesis (Figure 4b). No statistically significant differences were observed for soluble sugar concentrations in both the wood and root.

Tissue nitrogen concentrations (%DW) and total content (g/vine) were in most cases not responsive to soil temperature treatment. A significant increase was observed (*ca.* 14%) in root nitrogen concentration in response to soil warming between bud-break and anthesis. However, this increase in concentration was not associated with an increase in uptake with no significant difference in root N content (g/vine) observed.

*Reproductive development.*

Soil temperature prior to anthesis had no effect on flower number per inflorescence, with an average of 247 flowers per inflorescence in the cool treatment and 273 in the warm treatment. However, there was a significant interaction across the four treatment combinations, but as flower numbers were already established before the four temperature regimes were commenced at anthesis, this was attributed to pre-existing variation between vines. For a given number of flowers per inflorescence, the number of berries were reduced significantly by cooling the root-zone of previously warmed vines (Table 2). There was no difference between the other three treatments. This reduction was reflected at fruitset, although not significantly, with pre-warmed and then cooled vines having lower berry numbers per bunch (Table 2) and reduced fruit weight (Table 1) at veraison. Soil temperature had no significant effect on berry sugar concentrations at the time of veraison (Table 2).

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*Leaf water potential and gas exchange.*

Three weeks prior to veraison the diurnal courses of photoassimilation, transpiration and stomatal conductance, of single, newly matured leaves of plants of warmed and cooled plants were significantly different (Figure 5). Photoassimilation, transpiration and stomatal conductance of plants grown continuously in the warmed soil were generally higher throughout the day than those of plants grown continuously in the cooler soil. By mid-morning the leaf water potentials of the plants in cool soil were lower than those in the warm soil, despite their lower stomatal conductance and leaf area, but the difference had diminished by midday at which time the leaf water potential of both warmed and cooled plants was about -1.2MPa (Figure 5).

*Cytokinins and abscisic acid.*

Cytokinin ribosides were the main CKs detected in xylem sap at fruitset, with the predominant CK detected across all treatments being *trans*ZR (Table 3). Within the precursor nucleotide fraction DHZRP was the predominant CK detected and *trans*Z was the dominant CK in the freebase fraction. *Trans*-CK types were present in all treatment types where as *cis*CK types (with the exception of *cis*ZRP) and O-glucosides were not detected with in the xylem sap at fruitset (Table 3). In the corresponding leaf samples, all 15 CKs analyzed for were detected with a greater contribution from the nucleotide forms. The most abundant CKs detected were DHZR and ZROG followed by iPRP and *trans*ZRP. *Cis*-CK types and *O*-glucosides were detected in all leaf tissue sampled, highlighting the difference in CK profiles between xylem sap and leaves at fruitset.

At veraison, the type of CKs identified in the xylem sap was similar to that observed at fruitset (Table 4) with DHZRP remaining the dominant form in the nucleotide fraction. The main

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difference between the two dates was the concentration of *trans*Z and *trans*ZR which decreased by a factor of 10 and 8 respectively between fruitset and veraison, and *trans*Z was no longer detected in cool/warm and warm/cool treatments. For the leaf samples at veraison, the CK profile was similar to the leaves collected at fruitset. The most notable difference in terms of contribution to the overall leaf CK pool was an increase in the average concentration of iPRP from 82 to 289pmol g/DW. However, DHZR and ZROG remained amongst the more abundant CKs detected.

At veraison, the xylem sap concentrations of *trans*ZR, DHZR and iPA in post-anthesis warmed plants were lower than the cooled vines. For all xylem sap and leaf samples collected at fruitset, and the leaf samples from veraison, there was no apparent effect of soil temperature treatment on the type or concentration of CKs.

No differences in ABA concentrations were observed in the xylem sap at fruitset (Table 3). However, veraison xylem sap ABA concentrations in continuously warmed vines were higher than continuously cooled vines (Table 4). Interestingly, veraison xylem sap ABA concentrations in vines that had been switched at anthesis were between the two extremes of the continuously treated plants.

## Discussion

*Biomass partitioning and non-structural carbohydrates.*

Altering soil temperature from budbreak was previously shown to have little effect on the total dry biomass of grapevines at anthesis, but temperature differences caused dry biomass to be partitioned differently (Field et al. 2009). Soil warming between budbreak and anthesis promoted shoot biomass accumulation and leaf area development at the expense of root starch reserves (Field et

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al. 2009, Rogiers et al. 2011). Conversely, when a cooler soil temperature regime was maintained across the same period, shoot biomass was reduced but the rate of mobilization or utilization of root starch was also decreased. The effects of soil temperature regime in the period between anthesis and veraison were consistent with these vine responses. That is, there was no overall difference in whole vine biomass, but a significantly lower root:shoot ratio with warmer soil. Soil warming caused a decline in root starch concentrations of previously cooled vines, while starch concentrations of the continuously warmed plants remained low. Under cooled soil conditions, all vines had significantly higher starch in the roots at veraison. This was particularly pronounced for the previously warmed vines which more than doubled the amount of stored starch from anthesis to veraison. These results illustrate that soil temperature can exert considerable influence the balance between starch accumulation (storage) and utilization (annual growth). The enhanced above-ground growth of grapevines in sites where the soil warms rapidly in spring has been noted previously, particularly in cool sites (Jackson 2001). It is now clear that this response is attributable to accelerated utilization of carbohydrate reserves.

Another interesting finding was that fruit biomass, at the time of veraison, was significantly higher in vines that had previously been cooled prior to anthesis. This is possibly attributed to these plants having significantly greater root reserves, at anthesis, to support early berry growth. Many studies have reported that mobilization of carbohydrate reserves are used to help negate the effects of reduced assimilation (i.e. through leaf defoliation) on fruit development (see review by Holzapfel et al. 2010).

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*Flowering and fruitset.*

In grapevines the transition from flower to berry relies on photoassimilates; normally from basal leaves (Keller 2015). Concurrent demand for those assimilates from growing shoots and restoration of carbohydrate reserves limit their availability for fruitset; as inferred from treatments that enhance fruitset such as trunk girdling or shoot tipping, or those that diminish it e.g. severe pruning, defoliation or leaf shading (see review by Holzapfel et al. 2010). Our study revealed that soil temperature-induced tensions in carbohydrate demand during flowering influence the degree of fruitset. Lower fruitset percentage was induced by cooling previously warmed roots. Tabing et al. (2013) also found that reducing the root temperature from 20ºC to 10ºC for a two week period over flowering reduced fruitset in *cv* Chardonnay vines. Interestingly, in this study, the marked reduction in fruitset was associated with the increase in root carbohydrates. Poor fruitset has been attributed to competition with rapid shoot growth (May, 2004). However, after removing potential effects of different flower numbers at anthesis (by regression analysis), plants with the lowest shoot growth rate (elongation or dry biomass) between anthesis and veraison had significantly less fruitset than all other treatments. This is attributable to the fact that restoration of root carbohydrate reserves was also occurring during this period to a greater extent (ca 200% greater) than all other treatments. This demonstrates a strong acclimation that favors reserve accumulation over maximal fruitset; at least when demand by both functions is simultaneous. It also suggests that viticultural practices such as shoot tipping and application of growth retardants to improve fruitset are not likely to be effective when root carbohydrate reserves are low and restoration is favored by cool soil temperature.

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Fruitset remained unaffected when the cool soil temperature was maintained post-anthesis. This can be largely attributed to their higher reserve status at the onset of flowering. Continuously warmed plants also had unimpaired fruitset. Accelerated foliar development, including a high proportion of fully autotrophic proximal leaves possibly accounted for this. Rogiers et al. (2013) also showed that constant soil temperature up until fruitset (warm, ambient or cool) had no effect on % fruitset. Previously cooled with subsequent warming, with greater mobilization of reserves to the shoot, interesting had no effect on fruitset. Biomass analysis suggests these reserves were utilized solely in shoot growth.

Poor fruitset is sometimes attributed to restricted nutrient uptake from cold soil. However, no significant differences in nitrogen content (g/vine) between treatments and the normal fruitset in the continuously cooled plants indicates that nutrient uptake was not a factor in this regard. Furthermore, improved nutrient uptake seen in vines grown in a warmer soil had no effect on % fruitset in a similar experimental set-up (Rogiers et al. 2013; Clarke et al. 2015).

*Cytokinins and ABA.*

*Trans*-zeatin riboside was the major CK form in the xylem sap at the times of flowering and fruitset and is consistent with the composition of xylem sap in many other plants (Emery and Atkins 2002). However, nucleotide forms that were not detected at flowering (Field et al. 2009) were apparent both at fruitset and veraison, when DHZRP was one of the main dominant CK forms along with *trans*ZR. Thus, marked changes in the xylem sap CK profile occurred across the season. Notably, at the time of veraison, *trans*ZR concentrations were considerably higher in vines subjected to cool soil temperature post anthesis.

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Soil temperature treatment had little or no effect on stomatal conductance, photoassimilation rate, or transpiration of the most recently fully expanded leaves at anthesis (Field et al. 2009). However, by the completion of fruitset, 40 days later, those functions of similar stage of development leaves were significantly lower in vines grown in cooler soil. It is well known that low soil temperatures decrease transpiration by reducing absorption of water directly by decreasing the permeability and hydraulic conductivity of roots to water and indirectly by increasing the viscosity of water (Kozlowski 1987). Those effects may have contributed to both the lower transpiration rate and the lower mid-morning leaf water potential of the cooled plants. As leaf area increased markedly between the time of flowering and veraison in the present study for vines in all treatments, the total canopy transpiration would have increased concomitantly. If low root conductance had occurred in cooled vines, then supply of water to the vines would have been impeded in this treatment. Leaf transpiration rates in cooled vines were indeed significantly reduced compared to vines at warmer soil, consistent with a restricted supply of water. Reductions in stomatal conductance apparently mediated the low transpiration rates of the cooled vines to maintain the vines water potential.

ABA has been shown in numerous studies to reduce transpiration by closing stomata in response to water stress (Keller 2015). However, our study did not convey this with warmed vines, with assumed higher stomatal conductance, having higher ABA concentrations in the xylem sap at veraison. Veselova et al. (2005) found that differences in stomatal opening caused by different root temperatures were not caused by ABA. Furthermore, their work revealed that decreased levels of CK in the xylem sap closed stomata of wheat seedlings when roots were cooled. CKs are known to be involved in regulating stomatal conductance, with application of synthetic CK generally opening the stomata and reversing the effect of ABA on stomata under water stress (Stoll et al.

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2000). However, application of BAP at low concentrations to leaves of sugar maple (Reeves et al. 2007) reduced stomatal conductance by up to 40%. Furthermore, when ZR and iPA were introduced into the transpiration stream of well watered *Arubutus unedo* plants, there was no stimulatory effect on transpiration, with ZR even reducing transpiration compared to control plants (Burschka et al. 1985). Therefore, the higher concentrations of riboside CK's in the xylem sap of vines exposed to cool soil conditions at veraison relative to those exposed to warm conditions could possibly be a root/shoot signal that closed the stomata in response to low temperature-induced reductions in root hydro-conductivity.

Leaf CK concentrations did not differ greatly nor was there a consistent pattern between the soil treatments. However, the CK profile in leaf tissue differed from the xylem sap, with DHZR, ZROG, and *trans*ZRP and iPRP being the predominant CK forms at both sample times. These differences in CK profiles between xylem sap and leaf tissue highlight the different and changing roles of CK's from fruitset to veraison. The dominance of *trans*Z CK types, particularity *trans*ZR relative to iP types in the xylem sap highlight the biased distribution of these CKs within the plant. Root derived *trans*Z CKs are typically found in xylem and iP type CKs are typically found in phloem (Hirose et al. 2008). This compartmentalization of CKs suggests selective transport systems for *trans*Z and iP types and their role in acropetal and systemic long distance signals (Hirose et al. 2008). Grafting experiments using multiple isopentenyltransferase (a key gene in CK biosynthesis) *Arabidopsis thaliana* mutants highlighted the importance of root derived *trans*Z CKs and their transport from root to shoot being necessary in shoot development (Matsumoto-Kitano et al. 2008). In the current study no *cis*Z CKs or *O*-glucosides were detected in the xylem sap further supporting the role of CK compartmentalization and dominance of CK transport in the

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xylem and not CK processing or local synthesis during this time. At veraison, xylem sap *trans*ZR was reduced relative to fruitset; this may reflect a switch from CK long distance signalling from root-shoot CKs in the xylem towards a preference for local CK production in the leaves and fruit. This switch in CK dominance would reflect the developmental changes which occur post veraison, i.e. fruit ripening. Böttcher et al. (2015) noted a decreased contribution of *trans*Z CKs and an increased concentration of iP in grape berries during fruit ripening in four grapevine cultivars. Böttcher et al. (2015) also identified 38 CK related genes and examined their expression across 16 weeks post flowering in developing Shiraz grape berries. Developmental changes in the expression of genes related to CK biosynthesis, processing etc., suggested that local CK biosynthesis more likely contributes to the post veraison accumulation of iP within the examined grapevine berries (Böttcher et al. 2015).

Long distance transport as well as local CK production in the Shiraz grapevine leaves during fruitset and veraison is reflected in the abundance of CK forms detected. Nucleotide CKs are precursors to active CKs and are considered the first product in CK biosynthesis, these are modified to semi-active ribosides which are further modified to the active freebase forms (Sakakibara 2006). This sequence of CK processing can be modified through direct activation of nucleotide forms to freebases etc. (further explanation Frébort et al. 2011). The detection of higher levels of nucleotides, particularly iPRP (at veraison) in the leaves indicate local CK production may become more important during this development change as well as reflect the potential shift from *trans*Z to iP CK type dominance.

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The shifts in CK concentration within xylem sap as well as leaf tissue may reflect the changing role of xylem derived root CKs towards local CK production. Thus, xylem sap CK involved in the mobilization of starch-derived solutes early in the season may possibly change roles to regulating leaf stomatal conductance later in the season when leaves are able to supply their CK needs.

*Viticultural implications.*

The impact of soil temperature and its seasonal consequences has previously received little attention compared with atmospheric temperature. Although soil temperature and atmospheric temperature correlate generally, grapevine root temperature is also influenced by vineyard soil features such as texture, color and moisture content (Jackson 2001), and may be modified greatly by viticultural floor management practices such a mulching inter-row swards, and cultivation (Walpole et al. 1993), and also by grapevine density and foliar management. Thus, the grapevine growth responses to soil temperature between anthesis and veraison have important practical implications, not only in terms of fruitset and consequent berry development, but also canopy management and the capacity of grapevines to deal with seasonal contingencies.

## Conclusion

We conclude that soil temperature from dormancy to veraison significantly affects the utilization and restoration of non-structural carbohydrates from roots and trunks and relative changes in biomass of major plant organs during that period. Enhanced mobilization of starch, from warming previously cooled roots, appears to support increased shoot and leaf growth. Carbohydrate reserve status is shown to condition the magnitude of growth responses to soil temperature between anthesis and veraison. Notably, the responses to warm soil conditions reveal an inherent

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preference, in grapevines under those conditions, for shoot and fruit development over carbohydrate reserve accumulation. However, in cool soil, carbohydrate reserve-depleted plants favor recovery over fruiting; at least up to veraison when seed becomes viable and fruit ripening commences. Consequently, soil temperature will alter the level of carbohydrate reserves with which grapevines enters the post-veraison phase; hence determining plant capacity to respond to seasonal carbon-related contingencies during fruit ripening and the restorative demand by leaf-fall.

An apparent shift from *trans*Z to iP CK type dominating in the leaf tissue later in the season suggests a shift towards local leaf production for supplying CK. Root derived xylem sap CK that appears to be important for mobilization of starch early in the season may possibly change role to regulating leaf stomatal conductance later in the season.

Finally, in view of the impacts of soil temperature on grapevines ranging from seasonal balances between shoot and root growth, floral development, plant water use, photosynthesis and the temporal availability of carbohydrate reserves, we conclude that soil temperature and the influence of cultural practices warrant much closer attention in viticultural systems.

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**Table 1** Biomass components and root:shoot ratio of potted Shiraz grapevines at veraison as influenced by root-zone temperature treatments between budbreak and veraison.

| Treatment | Shoot (g DW) | | Total biomass (g DW) | | | | | Root:shoot |
|---|---|---|---|---|---|---|---|---|
| | main | lateral | root | trunk | shoot | fruit | vine | ratio |
| **Budbreak-anthesis** | | | | | | | | |
| cool | 169 | 29.0 | 220 | 120 | 198 | 46.7 | 585 | 1.1 |
| warm | 156 | 21.5 | 224 | 124 | 177 | 37.0 | 562 | 1.3 |
| **Anthesis-veraison** | | | | | | | | |
| cool | 150 | 20.1 | 233 | 121 | 170 | 39.3 | 564 | 1.4 |
| warm | 174 | 30.4 | 211 | 123 | 205 | 44.3 | 583 | 1.0 |
| **Temperature regime** | | | | | | | | |
| cool/cool | 160 | 24.0 | 231 | 119 | 184 | 45.9 | 580 | 1.3 |
| cool/warm | 177 | 33.9 | 209 | 122 | 211 | 47.4 | 590 | 1.0 |
| warm/cool | 140 | 16.2 | 235 | 124 | 156 | 32.8 | 548 | 1.5 |
| warm/warm | 172 | 26.8 | 213 | 123 | 198 | 41.3 | 576 | 1.1 |
| **Significance** | | | | | | | | |
| Budbreak-anthesis | ns | ns | ns | ns | ns | * | ns | ns |
| Anthesis-veraison | ns | * | ns | ns | ns | ns | ns | ** |
| Interaction | ns | ns | ns | ns | ns | ns | ns | ns |

*, **, and ns indicate significance at $p \leq 0.05$, $p \leq 0.01$and not significant.

**Table 2** Effect of root-zone temperature treatments between budbreak and veraison on reproductive development parameters of potted Shiraz grapevines.

| Treatment | Flowers / inflorescence | Berries /cluster | % Fruitset | Berry / flower number relationship | Soluble Solids (ºbrix) |
|---|---|---|---|---|---|
| cool/cool | 230 a | 85 | 38.7 b | y = 0.17x + 44.8 b | 7.1 |
| cool/warm | 269 a | 88 | 34.2 b | y = 0.17x + 41.4 b | 6.8 |
| warm/cool | 304 b | 76 | 26.1 a | y = 0.17x + 23.6 a | 7.2 |
| warm/warm | 240 a | 81 | 35.3 b | y = 0.17x + 39.5 b | 6.3 |
| **Significance** | * | ns | * | * | ns |

*, and ns indicate significance at $p \leq 0.05$ and not significant.

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**Table 3** Effect of root-zone temperature treatments between budbreak and fruitset on concentration of cytokinins and abscisic acid identified in xylem sap and leaves of Shiraz grapevines at the time of fruitset.

| | Fruitset xylem sap (pmol/ml) | | | | Fruitset leaf (pmol/g DW) | | | |
|---|---|---|---|---|---|---|---|---|
| **Cytokinin** | **cool/cool** | **cool/warm** | **warm/cool** | **warm/warm** | **cool/cool** | **cool/warm** | **warm/cool** | **warm/warm** |
| Free base | | | | | | | | |
| *trans*Z | 0.8 ± 0.2 | 1.2 ± 0.1 | 1.2 ± 0.4 | 0.9 ± 0.3 | 7.5 ± 3.3 | 7.5 ± 3.3 | 4.0 ± 1.5 | 5.4 ± 0.8 |
| *cis*Z | nd | nd | nd | nd | 9.3 ± 3.7 | 10.7 ± 3.6 | 17.3 ± 4.3 | 17.1 ± 2.0 |
| DHZ | d | nd | nd | nd | 2.1 ± 0.5 | 1.7 ± 0.6 | 1.9 ± 0.5 | 2.0 ± 0.2 |
| iP | d | 0.1 ± 0.0 | 0.1 ± 0.0 | d | 1.8 ± 0.0 | 2.8 ± 0.6 | 2.7 ± 0.3 | 2.5 ± 0.3 |
| Riboside | | | | | | | | |
| *trans*ZR | 10.2 ± 5.1 | 10.1 ± 2.0 | 10.7 ± 4.0 | 10.0 ± 3.1 | 5.9 ± 0.5 | 6.3 ± 0.2 | 5.0 ± 1.0 | 9.5 ± 1.7 |
| *cis*ZR | nd | nd | nd | nd | 1.1 ± 0.2 | 0.7 ± 0.0 | 1.0 ± 0.1 | 1.3 ± 0.1 |
| DHZR | 0.9 ± 0.3 | 0.9 ± 0.1 | 0.9 ± 0.2 | 0.8 ± 0.2 | 98.6 ± 17.3 | 87.2 ± 43.1 | 83.6 ± 19.5 | 83.1 ± 13.7 |
| iPA | 0.9 ± 0.3 | 0.7 ± 0.1 | 1.1 ± 0.3 | 1.1 ± 0.3 | 8.4 ± 0.3 | 10.1 ± 1.1 | 11.8 ± 0.6 | 16.3 ± 6.6 |
| *O*-glucosides | | | | | | | | |
| ZROG | nd | nd | nd | nd | 93.5 ± 23.8 | 62.2 ± 16.7 | 67.4 ± 8.3 | 56.3 ± 14.2 |
| DHZOG | nd | nd | nd | nd | 3.9 ± 0.6 | 5.1 ± 1.0 | 4.3 ± 0.7 | 4.1 ± 1.1 |
| ZOG | nd | nd | nd | nd | 7.0 ± 1.0 | 4.1 ± 0.7 | 5.3 ± 0.6 | 5.8 ± 1.3 |
| Nucleotides | | | | | | | | |
| *trans*ZRP | 0.9 ± 0.2 | 1.4 ± 0.5 | 1.3 ± 0.6 | 1.5 ± 0.2 | 71.8 ± 12.0 | 73.2 ± 2.0 | 72.8 ± 21.5 | 147.4 ± 26.4 |
| *cis*ZRP | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.2 ± 0.1 | 0.1 ± 0.1 | 12.3 ± 2.3 | 9.2 ± 3.5 | 12.7 ± 3.6 | 15.6 ± 0.8 |
| DHZRP | 1.2 ± 0.2 | 1.2 ± 0.0 | 1.2 ± 0.1 | 1.0 ± 0.0 | 14.6 ± 2.8 | 34.4 ± 0.3 | 19.1 ± 5.2 | 30.4 ± 3.6 |
| iPRP | 0.2 ± 0.2 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 61.0 ± 13.9 | 90.6 ± 24.0 | 93.4 ± 11.1 | 82.8 ± 21.4 |
| ABA | 149 ± 9 | 137 ± 25 | 211 ± 41 | 120 ± 12 | | | | |

Means ± SE mean (n = 3); nd = not detected; d = below limit of quantification (< 0.05 pmol $ml^{-1}$).

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**Table 4** Effect of root-zone temperature treatments between budbreak and veraison on concentration of cytokinins and abscisic acid identified in xylem sap and leaves of Shiraz grapevines at the time of veraison.

| | **Veraison xylem sap (pmol/ml)** | | | | **Veraison leaf (pmol/g DW)** | | | |
|---|---|---|---|---|---|---|---|---|
| **Cytokinin** | **cool/cool** | **cool/warm** | **warm/cool** | **warm/warm** | **cool/cool** | **cool/warm** | **warm/cool** | **warm/warm** |
| Free base | | | | | | | | |
| *trans*Z | 0.3 ± 0.3 | nd | nd | 0.1 ± 0.06 | 3.5 ± 0.5 | 3.1 ± 0.4 | 7.8 ± 3.2 | 9.9 ± 3.2 |
| *cis*Z | nd | nd | nd | 0.1 ± 0.05 | 25.0 ± 5.1 | 13.1 ± 2.5 | 13.8 ± 7.1 | 22.2 ± 8.9 |
| DHZ | nd | nd | nd | nd | 2.5 ± 0.7 | 2.4 ± 0.8 | 4.1 ± 0.9 | 3.1 ± 1.2 |
| iP | 0.1 ± 0.0 | d | d | 0.1 ± 0.01 | 5.0 ± 0.6 | 3.3 ± 0.6 | 6.8 ± 2.1 | 5.7 ± 0.5 |
| Riboside | | | | | | | | |
| *trans*ZR | 3.0 ± 1.6 | 0.2 ± 0.1 | 2.0 ± 0.8 | 0.2 ± 0.08 | 6.4 ± 0.2 | 5.1 ± 1.3 | 12.4 ± 2.7 | 11.0 ± 4.9 |
| *cis*ZR | nd | nd | d | nd | 0.8 ± 0.0 | 0.9 ± 0.0 | 0.9 ± 0.2 | 1.1 ± 0.3 |
| DHZR | 0.6 ± 0.1 | 0.3 ± 0.0 | 0.5 ± 0.1 | 0.2 ± 0.01 | 47.2 ± 6.6 | 87.1 ± 9.5 | 68.8 ± 5.9 | 57.0 ± 9.0 |
| iPA | 0.3 ± 0.0 | d | 0.3 ± 0.1 | 0.1 ± 0.03 | 37.7 ± 23.4 | 14.6 ± 4.6 | 29.6 ± 5.8 | 19.2 ± 4.0 |
| *O*-glucosides | | | | | | | | |
| ZROG | nd | nd | nd | nd | 67.1 ± 8.4 | 74.0 ± 21.8 | 97.6 ± 20.2 | 94.1 ± 15.2 |
| DHZOG | nd | nd | nd | nd | 6.4 ± 0.5 | 6.9 ± 0.8 | 6.2 ± 1.2 | 8.1 ± 1.1 |
| ZOG | nd | nd | nd | nd | 11.8 ± 2.1 | 18.6 ± 7.3 | 22.1 ± 7.6 | 17.1 ± 0.6 |
| Nucleotides | | | | | | | | |
| *trans*ZRP | 0.7 ± 0.3 | 0.2 ± 0.0 | 0.6 ± 0.1 | 0.4 ± 0.06 | 78.0 ± 26.9 | 65.5 ± 16.4 | 126.7 ± 19.5 | 81.2 ± 19.4 |
| *cis*ZRP | 0.1 ± 0.1 | nd | nd | 0.1 ± 0.07 | 11.4 ± 2.1 | 16.9 ± 1.6 | 19.5 ± 7.3 | 12.5 ± 2.4 |
| DHZRP | 2.5 ± 1.4 | 1.1 ± 0.1 | 0.7 ± 0.0 | 1.3 ± 0.10 | 14.6 ± 2.0 | 10.6 ± 1.1 | 15.0 ± 1.2 | 14.4 ± 4.9 |
| iPRP | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.01 | 263.8 ± 35.0 | 194.7 ± 40.5 | 442.4 ± 82.7 | 254.2 ± 54.0 |
| ABA | 116 ± 16 | 142 ± 24 | 151 ± 10 | 206 ± 8 | | | | |

Means ± SE mean (n = 3); nd = not detected; d = below limit of quantification (< 0.05 pmol $ml^{-1}$).

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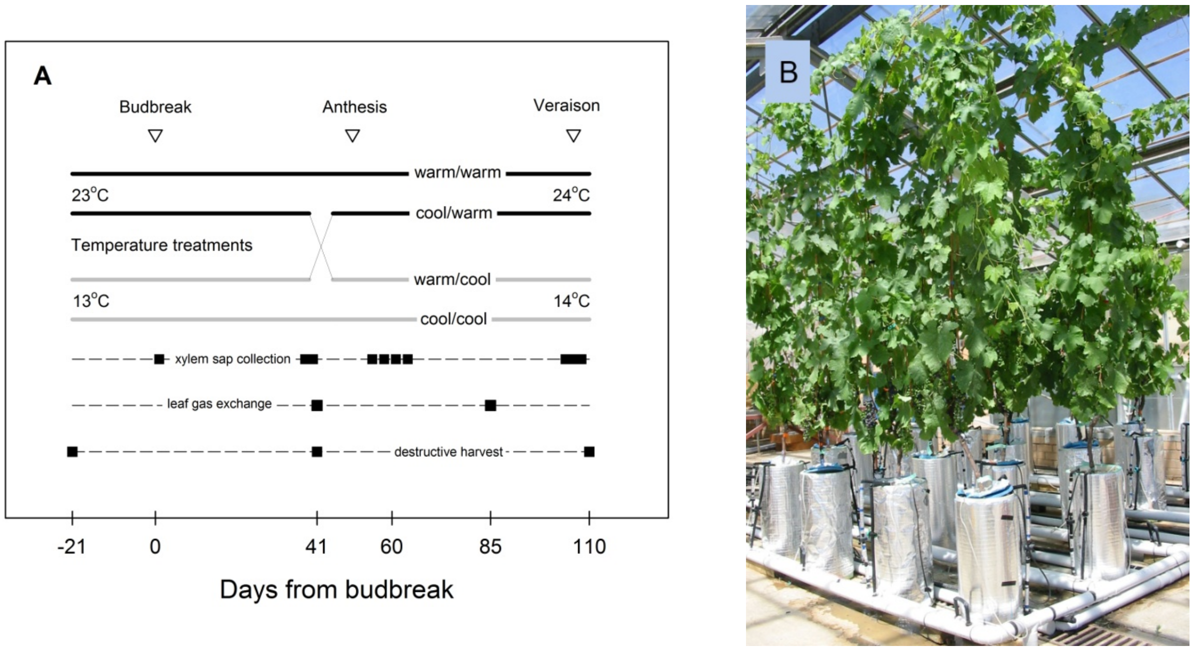


**Figure 1** Schematic diagram of root-zone temperature treatment schedule in relation to developmental stage and measurement schedule (**A**), and the root-zone temperature control system and remaining vines just prior to the final destructive harvest at veraison (**B**).

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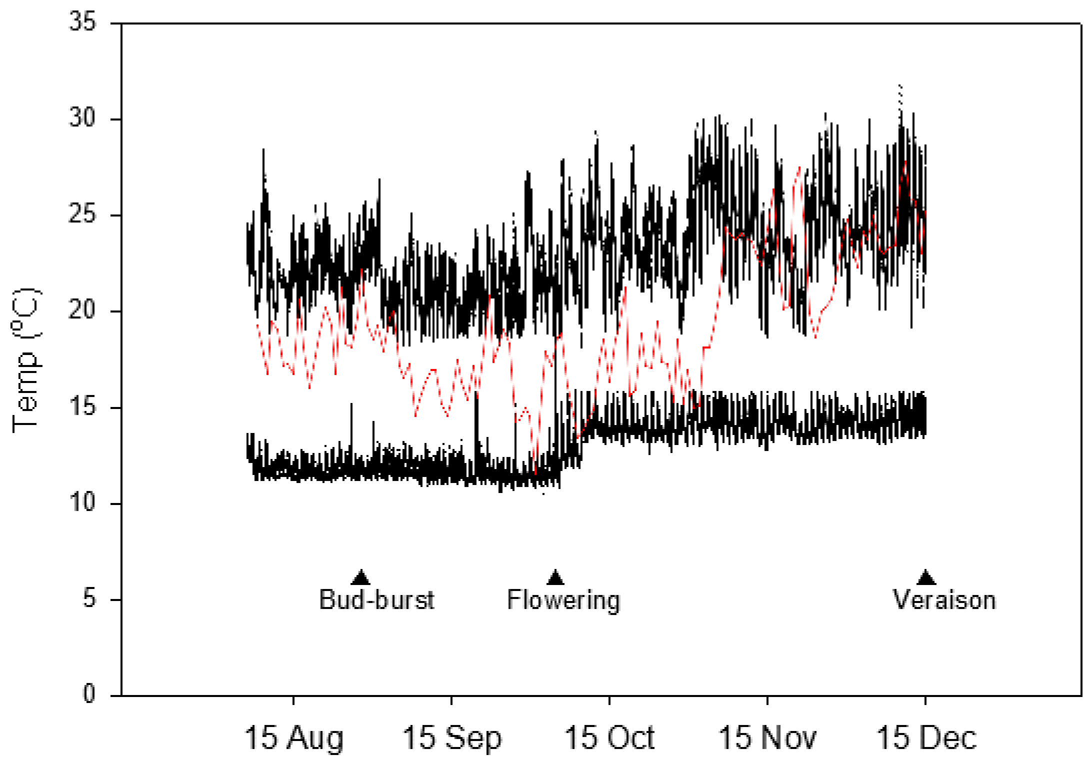


**Figure 2** Mean daily air temperature (red colored line) and mean temperature of pots at a soil depth of 20 cm (approximately center of root mass). Temperatures were logged every 5 minutes for the duration of the experiment.

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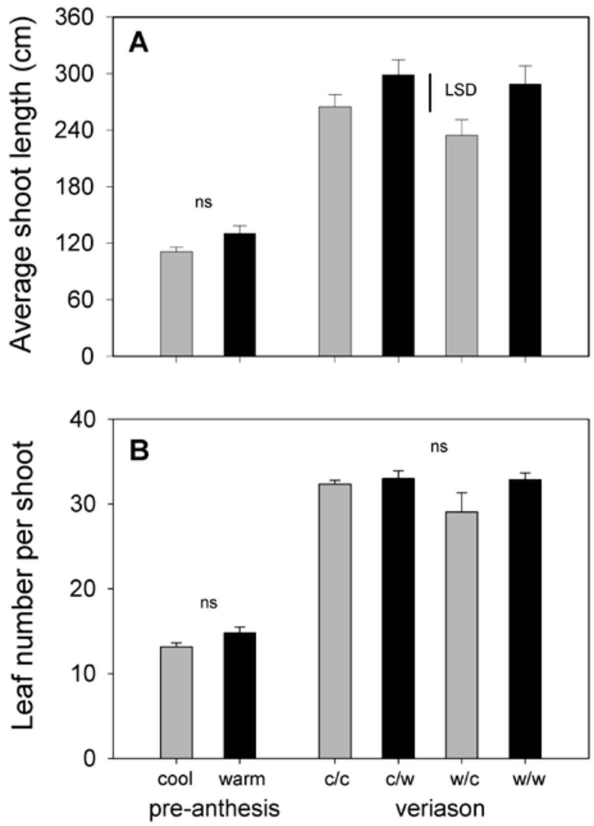


**Figure 3** Effect of root-zone temperature on average shoot length (**A**) and leaf number per shoot (**B**) as recorded prior to anthesis and at veraison. LSD indicates significant difference between treatments at $p \leq 0.05$. ns = not significant. Error bars ± SE mean (n = 15).

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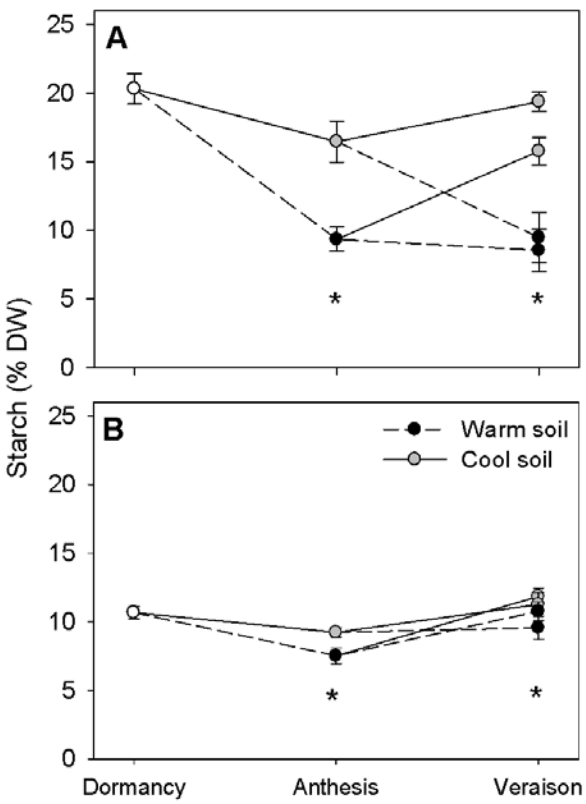


**Figure 4** Effect of root-zone temperature on starch concentrations in root (**A**) and trunk (**B**) tissue from dormancy to veraison. * indicates significant difference between treatments at $p \leq 0.05$. Error bars ± SE mean (n = 5).

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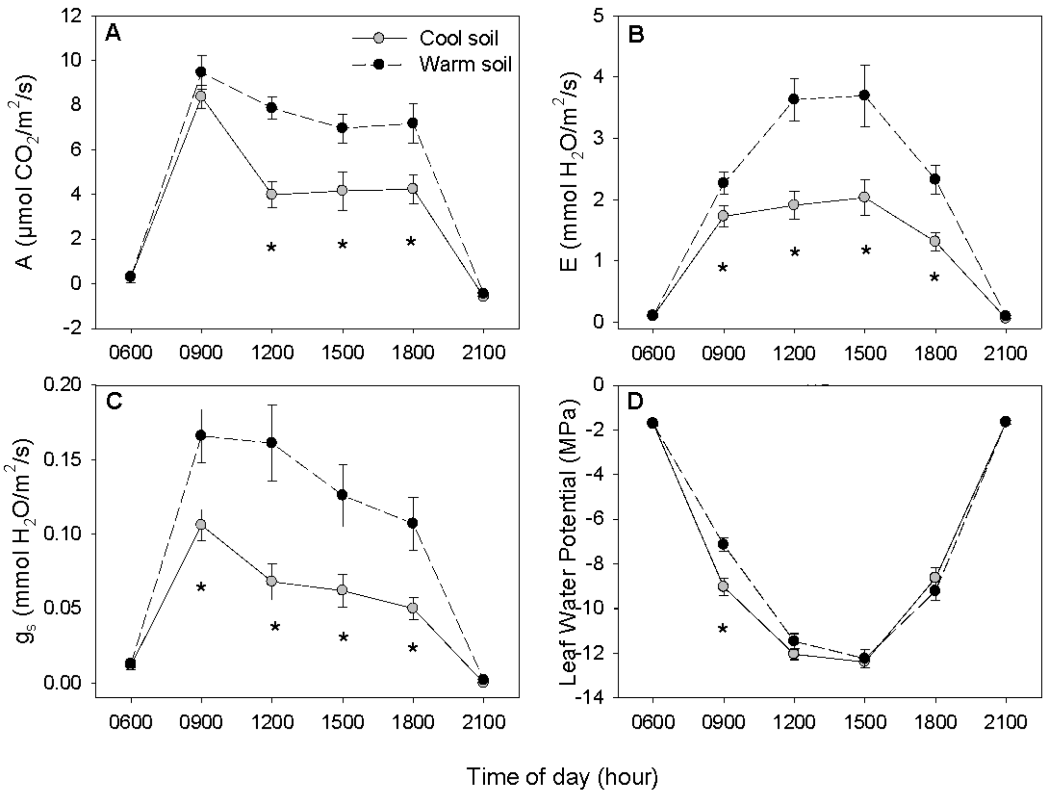


**Figure 5** Effect of root-zone temperature in the anthesis-veraison period on leaf diurnal photosynthesis (**A**), transpiration (**B**), stomatal conductance (**C**) and leaf water potential (**D**) as measured 85 days after budbreak (three weeks prior to veraison). * indicates significant difference between treatments at $p \leq 0.05$. Error bars ± SE mean (n = 10).