Grapevine Response to Soil Temperature: Xylem Cytokinins and Carbohydrate Reserve Mobilization from Budbreak to Anthesis

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Abstract: Potted Shiraz grapevines, in a glasshouse, were exposed to two different soil temperatures (13°C and 23°C) to evaluate the effects on vegetative growth and floral development from dormancy to anthesis. Soil temperature had no effect on the time of budbreak, anthesis, or the number of flowers per inflorescence. At anthesis total biomass was similar for both treatments, whereas shoot biomass was greater in the warm soil. From dormancy to anthesis, both root and trunk biomass decreased in the cool soil and only root biomass decreased in the warmer soil, but by twice as much as that in the cool soil. During dormancy to anthesis decreases in total nonstructural carbohydrate accounted for most of the decrease in root biomass. At budbreak, 14 cytokinins representing four recognized classes were present in bleeding sap, with *trans*-zeatin riboside and isopentenyl adenosine as the dominant forms. Total and active free base cytokinin concentrations were similar for both treatments, while sap from vines in the warm soil had significantly lower concentrations of nucleotide cytokinins. However, delivery of cytokinins was significantly greater in the warm soil treatment. By anthesis, cytokinin concentrations were similar for both treatments, but total cytokinin concentrations in xylem sap had decreased by almost 90% from budbreak. Root-generated cytokinins appear to be associated with the mobilization of the carbohydrate reserves at the end of dormancy and the ensuing shoot growth. Comparison of results with those of previous studies reveals that, because of apical dominance and correlative inhibition, the response to soil temperature in terms of number of buds to break and time of budbreak is conditioned by the number of nodes per cane.

Key words: biomass, nonstructural carbohydrate, cytokinin, rootzone temperature

Using a hydroponic system, Woodham and Alexander (1966) showed that the growth and development of grapevines varied greatly depending on root temperature. Since then, further studies have provided evidence of a key role for soil temperature in the control of whole-plant growth and physiological development of grapevines (Skene and Kerridge 1967, Kliewer 1975, Zelleke and Kliewer 1979, 1980, Graham et al. 2002). However, the physiological processes by which soil temperature acts and the associated response characteristics remain unclear.

The seasonal reestablishment of both vegetative and reproductive growth of grapevines depends on the mobilization of a range of metabolites, including amino acids, mineral nutrients, and nonstructural carbohydrates from the perennial organs (roots, trunk and shoots) (Winkler 1958, Yang and Hori 1979, Cheng et al. 2004, Zapata et al. 2004). Researchers have highlighted the particular significance of roots as the main source of more than 80% of carbohydrates for seasonal reestablishment in grapevines (Loescher et al. 1990, Bates et al. 2002, Zapata et al. 2004). However, the impact of soil temperature on the mobilization of carbohydrate reserves following budbreak has received little attention.

Hormones are strong candidates as regulators of the growth responses of *Vitis* to root temperature, and Woodham and Alexander (1966) suggested that production by the grapevine roots of a plant hormone of the cytokinin group might be involved. Other researchers subsequently found that cytokinin levels in root exudates of Sultana vines grown in nutrient solution at root temperatures of 20°C and 30°C were different (Skene and Kerridge 1967). However, analytical methods at the time did not allow for identification of the types of cytokinin present in the sap. Many types of cytokinin have since been identified and are now known to exist in biochemically active and inactive forms (Sakakibara 2006). More recent advances in mass

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spectrometric characterization of cytokinins have allowed for more rigorous examination.

The objective of this study was to advance our understanding of the regulation of early seasonal growth and development of grapevines by examining the impact of soil temperature on the distribution of biomass during root carbohydrate-dependent growth and development up to anthesis and changes in cytokinin composition of the xylem sap between budbreak and anthesis.

Materials and Methods

Approximately three weeks before budbreak in late winter 2003, 30 potted, 3-yr-old grapevines, *Vitis vinifera* cv. Shiraz, were transferred to a glasshouse at the National Wine and Grape Industry Centre, Wagga Wagga, Australia. These were previously grown outdoors in 26-L white PVC pots (595 mm height × 245 mm diam) and pruned to three 5-bud spurs (the basal section of the previous season's shoots) to allow subsequent selection of three similar, strong, fruitful shoots. Two weeks after budbreak all but the selected shoots (usually one per spur) were removed to reduce variation among plants by directing growth and development toward the same number of well-exposed, inflorescence-bearing shoots. All shoots were trained vertically.

At the time of transfer, the roots of all vines appeared fully dormant, that is, all were lignified and there were no recently formed root tips. From that time through to anthesis, the rootzone soil of half the vines (i.e., 15 replicates) was maintained at a temperature of 13°C and the other half at 23°C. This was achieved by heat exchange with water in a recirculating system from temperature-controlled tanks through small-diameter plastic pipes installed in the pots 12 months before the start of the experiment. The pots were insulated, on the top and sides, to exclude solar radiation and minimize heat exchange between the soil and air surrounding the vines. Average minimum and maximum air temperatures for the study period were 11°C and 24°C, respectively, with an overall range of 6 to 30°C. Light was not controlled and was dependent on solar radiation.

Development of each bud was recorded daily. Time of budbreak was determined as the date on which the first green leaf became visible through the bud scales (Eichhorn–Lorenz [E-L] stage 4) (Pearce and Coombe 2005). Time of anthesis was determined as the date on which one bunch reached 50% cap-fall (E-L stage 23), which was estimated visually.

At budbreak, bleeding sap, induced by recutting the end of each spur and allowing a steady flow rate to be reached over the following two days, was collected from each vine over a 9-hr period. There was no bleeding from vines of either treatment before recutting. The volume of sap was recorded before it was stored at -80°C for cytokinin analysis. Fifteen individual cytokinins were analyzed according to a published method (Quesnelle and Emery 2007): *trans*-zeatin, *cis*-zeatin, dihydro-zeatin, isopentenyl adenine, *trans*-zeatin riboside, *cis*-zeatin riboside, dihydro-zeatin riboside, isopentenyl adenosine, zeatin *O*-glucoside, zeatin riboside *O*-glucoside, dihydro-zeatin *O*-glucoside, *trans*-zeatin nucleotide, *cis*-zeatin nucleotide, dihydro-zeatin nucleotide, and isopentenyl nucleotide.

The length of each shoot was measured with decreasing frequency of 3 to 20 days as necessary to characterize the course of shoot elongation. Leaf area was determined by monitoring leaf lamina length from the base to the tip on each leaf on one randomly selected shoot per vine. Leaf lamina length was converted to leaf area using a predetermined calibration ($y = 0.018x^{1.892}$). At anthesis, xylem sap was collected nondestructively using the root pressure chamber method (Yong et al. 2000) and adapted for use with large potted grapevines (Smith 2004). Briefly, this method involved pressurizing the root system for ~2 hr and collecting xylem sap from the cut petiole of the youngest fully expanded leaf of one shoot from each vine. The chamber pressure was varied during the collection period to maintain the collection rate close to the transpiration-driven flow rate as determined by gas exchange measurements of an adjacent leaf (LCA-4; ADC BioScientific, Hoddeson, UK). Pot weight was recorded before and after xylem sap collection to determine whole-vine transpiration loss over the collection period. Two pressure chambers were used to collect xylem sap concurrently from a vine in each treatment, while the pots were maintained within the treatment temperature range.

Flower number was determined by enclosing each inflorescence in a fine mesh bag just before first cap-fall. Before the start of the temperature treatments and again at anthesis, the roots, trunk, and shoots of five vines were separated and oven-dried at 70°C for dry biomass determination. For nonstructural carbohydrate analysis the entire root system and trunk of each vine was ground to 5 mm using a heavy duty cutting mill (Retsch ZM2000, Haan, Germany), and then a smaller portion ground to 0.12 mm (Retsch ZM100). Following three extractions from a 20 mg subsample with 80% aqueous ethanol, sucrose, D-glucose, and D-fructose concentrations were determined by enzymatic assay (Megazyme International, Bray, Ireland). For starch determination the residual sample was resuspended in dimethylsulfoxide and incubated with α-amylase and amyloglucosidase. Starch content of the orginal sample was calculated from the concentration of released glucose.

Results

Soil temperature had no significant effect on either the time of budbreak, which occurred 21 days after the beginning of the experiment (referred to here as "dormancy"), or the number of buds that opened (mean number, 13 per vine) (Table 1). Total shoot length per vine did not differ significantly between temperature treatments at any time from budbreak to anthesis (Figure 1). There was no lateral shoot growth observed in either treatment. At anthesis, the total leaf area of vines grown at 23°C was significantly greater than that of those at 13°C, a difference reflecting the greater area of leaves at nodes two to six of vines grown in the warmer soil (Figure 2). There was no treatment effect on leaf area at other node positions.

Table 1 Time (n, number) and proportion (%) of budbreak of Shiraz grapevines grown at different soil temperatures.

Soil temp	Days to budbreak (n)	Days to budbreak at distal node (n)	Buds that broke (n)	Buds that broke (%)
13°C	21.8[a]	21.3	14.5	96.7
23°C	20.7	20.9	14.7	98.3
Signif	ns[b]	ns	ns	ns

[a]Values are means (n > 15).
[b]ns indicates no significant difference using Tukey's HSD ($p < 0.05$).

Between dormancy and anthesis there was no significant difference in total dry biomass gain between the two soil treatments (Figure 3a). Notably, root dry biomass of vines grown at 23°C decreased by twice as much as that of vines grown at 13°C (Figure 3b). Losses in root nonstructural carbohydrate accounted for most of the loss in root dry biomass (Figure 4a, b), with total residual vine dry biomass (estimated as total dry biomass less nonstructural carbohydrate biomass) not significantly different (Figure 5a). Shoot dry biomass (including leaves and inflorescences) of vines grown at 23°C was significantly greater (8 g/vine, on average) at anthesis than those at 13°C (Figure 3c). Trunk dry biomass was also greater in vines grown at 23°C (Figure 3d). There was no difference in trunk nonstructural carbohydrate content between soil treatments (Figure 4c, d), with the difference in trunk dry biomass largely associated with an increase in trunk residual dry biomass (Figure 5b).

Vines of each treatment produced five or six inflorescences. Differences in mean flower number per inflorescence were not statistically significant. Soil temperature had no effect on the time to anthesis, which occurred 50 days after budbreak for both treatments. At anthesis, soil temperature had no significant effect on single leaf midday assimilation rate, transpiration rate, and stomatal conductance among treatments (Table 2).

In bleeding sap collected at budbreak, 14 cytokinins representing four recognized classes (free base, riboside, *O*-glucoside, and nucleotide) were present (Table 3). *O*-Glucoside cytokinins were consistently present, but at such trace amounts the signal to noise ratio was not within the linear response of the LC-MSMS and they could not be accurately quantified. Total, riboside, and active free base cytokinin concentrations were similar in the sap from vines of both treatments. However, vines in the warm soil had significantly lower concentrations of nucleotide cytokinins and higher concentrations of dihydro-zeatin riboside. Two riboside cytokinins, *trans*-zeatin riboside and isopentenyl adenosine, were the prevailing cytokinin forms. At budbreak the flow rate of bleeding sap at 23°C (0.9 mL/hr) was significantly greater than that at 13°C (0.4 mL/hr), indicating that root generation and potential shoot delivery of the free base and riboside cytokinins was two- to three-fold greater in the warmer roots (Table 3). These also represent the most active cytokinins of those measured.

In pressure-extracted xylem sap, collected at anthesis, *trans*-zeatin riboside was the dominant cytokinin form (Table 4). No significant differences in cytokinin concentrations or generation rates were observed between treatments. Moreover, at anthesis no nucleotide or *O*-glucoside cytokinin forms were detected in either treatment.

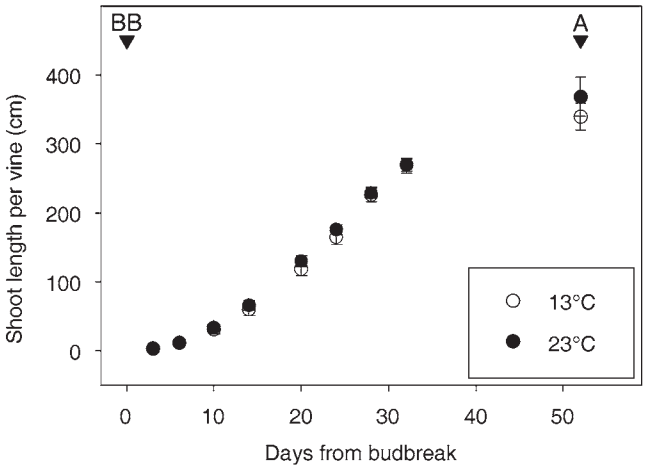

Figure 1 Total shoot length of Shiraz grapevines with three shoots per vine grown in soil at 13°C or 23°C. BB, budbreak; A, anthesis. Values are means ± se (n = 15).

Discussion

In previous studies, researchers found that time of budbreak was several days earlier at high root temperatures (Kliewer 1975, Zellecke and Kliewer 1980, Graham et al. 2002). These researchers also reported, as did Zelleke and Kliewer (1979), that a greater proportion of buds broke at higher soil temperatures. In our study, although temperature treatments and relative stages of their imposition were similar to other studies (Kliewer 1975, Zelleke and Kliewer 1979, 1980), there was little no difference in time of budbreak or in the proportion of buds that broke.

Differences in the proportion of buds that broke may be attributed to several notable differences in experimental protocol. Previous studies used a greater number of nodes per cane (10 versus 5 in our study), which introduced apical dominance and correlative inhibition of subapical buds as a strong, soil temperature-related determinant of the number of buds that broke (Kliewer 1975, Zelleke and Kliewer 1979, 1980). Apical dominance was barely evident in our study, where over 96% of all the retained buds in both treatments broke within 14 days of the mean budbreak date. Similarly, a study using 5 nodes per cane and a soil temperature range of ~7.2 to 22.5°C found that by the time at least 50% of the buds had broken on each vine (within 14 days of onset of treatment), the overall percent of budbreak was 75% and 85% for the coolest and warmest treatments, respectively

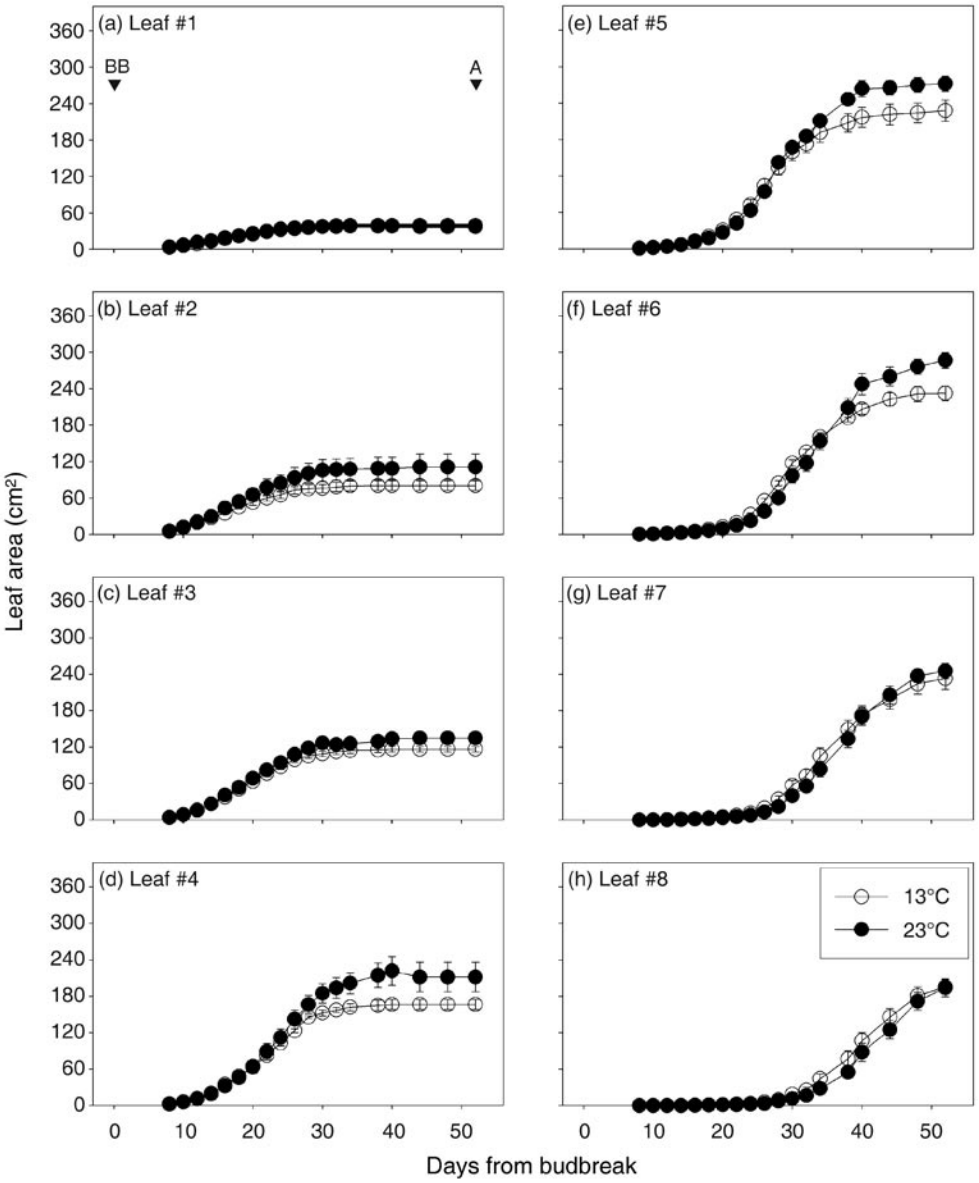

Figure 2 Leaf area at nodes 1 through 8 (**a**–**h**) of Shiraz grapevines grown in soil at 13°C or 23°C. BB, budbreak; A, anthesis. Values are means ± se (n = 15).

(Graham et al. 2002). In contrast, Kliewer (1975) found only 22% and 32% of all the retained buds broke at 11 to 15°C (13°C avg.) and 20 to 25°C (22.5°C avg.) soil temperatures, respectively. Similarly Zelleke and Kliewer (1980) reported budbreak as 33% and 79% at 12°C and 25°C, respectively. Had it not been for the strong effect of apical dominance preventing or delaying budbreak in nonterminal node positions, particularly at the lower soil temperatures, a soil temperature response in days to budbreak and proportion of buds to break may not have been as evident in both of these studies. The imprecision of mean time to budbreak as a measure of bud dormancy release when many buds fail to burst has been noted previously (Lavee and May 1997).

Regarding days to budbreak, when compared on the basis of budbreak at the terminal node, the data of Kliewer (1975), based on 10-node canes, reveals a 4-day delay for vines grown at 11 to 15°C (13°C avg.) compared with those grown at 20 to 25°C (22.5°C avg.). However, in a study using five-node canes (as in our study), budbreak was only one day earlier at 22°C than at 7.5°C soil temperature (Graham et al. 2002). This comparison indicates that time of budbreak response to soil temperature becomes greater as node numbers per cane increase. However, differences in grapevine cane temperature as small as 1°C can modify time to budbreak (Antcliff and May 1961), and differences have been reported in cane temperature of up to 2°C between and within treatments despite the use of fans to normalize potential differences in air temperature associated with noninsulated water baths (Kliewer 1975). In the study of Zellecke and Kliewer (1980), apical dominance is likely to have magnified any time of budbreak response because all buds (not just terminal buds) appear to have been counted in that determination.

Other differences in experimental conditions—such as cultivar, gradients between root zone and ambient glasshouse air temperatures, and, possibly, internal plant temperature gradients—make further comparative interpretation problematic. However, comparison of our results with those of preceding studies does reveal an impact of node number per cane in grapevine responses to soil temperature.

Our findings that xylem sap flow, and by inference root pressure, at budbreak in 13°C soil was less than half that at 23°C and that both time of budbreak and proportion of buds that broke were similar suggest that root pressure does not directly determine either of those features. However, differences in flow or pressure at terminal node positions may become more important with greater cane length and node number.

The greater shoot biomass of vines grown in the warmer soil is consistent with previous results in which shoot biomass increased with temperature up to 30°C (Woodham and Alexander 1966, Skene and Kerridge 1967, Zelleke and Kliewer 1979, Graham et al. 2002). However, we found the response was due to greater stem diameter and leaf area and there was no effect on shoot length, which may be

attributed to the well-recognized, inverse relationship between shoot number and shoot growth rate (Winkler et al. 1974). In contrast to the earlier experiments with up to 19 shoots per vine, our experiment had 3 shoots per vine, and shoots of each treatment grew at close to the maximal rate of 25 mm per day (Winkler et al. 1974).

The increase in total aboveground dry biomass (trunk included) of vines in warmer soil was offset by a greater decrease in root dry biomass than that of vines in cooler soil. The overall result was a similar total biomass for both treatments at anthesis. This response is consistent with the hypothesis that mobilization and transport of plant growth substances and vital metabolites from the roots is enhanced by soil temperatures up to 30°C (Cooper 1973). In particular, promotion of shoot growth and reduced root growth is consistent with the effects of cytokinins (Werner et al. 2003).

In a study using potted vines, there was little change in root biomass between budbreak and anthesis (Conradie 1980, Araujo and Williams 1988). In a study using a trench culture system, the dry matter of roots (and perennial parts in total) declined progressively between budbreak and anthesis (Zapata et al. 2001). The authors reported cultivar-associated losses between 25% and 50% (dry weight) and later confirmed they were due to losses in starch and necrosis of the roots (Zapata et al. 2004). We did not observe root necrosis; however, there were losses of root biomass of 6% and 12% in cool and warm soils, respectively. Nonstructural carbohydrate losses of 25% at 13°C and 59% at 23°C similarly accounted for 87% and 97% (respectively) of the loss in root biomass.

Measurements of photoassimilation rate of individual leaves at anthesis confirmed that photosynthesis at that time was not affected by differences in soil temperature. However, based on the greater leaf biomass of the vines in the warmer soil at anthesis, it is likely that greater photoassimilation contributed to the total biomass of those vines.

Root respiration in support of metabolic functions not associated with growth may have contributed to the greater loss of nonstructural carbohydrate from the vines grown at the higher soil temperature. However, in Concord vines, root respiration initially declined but then gradually increased to the original rate when soil temperature was reduced from 30°C to 20°C (Huang et al. 2005), suggesting that root respiration may acclimatize to soil temperature.

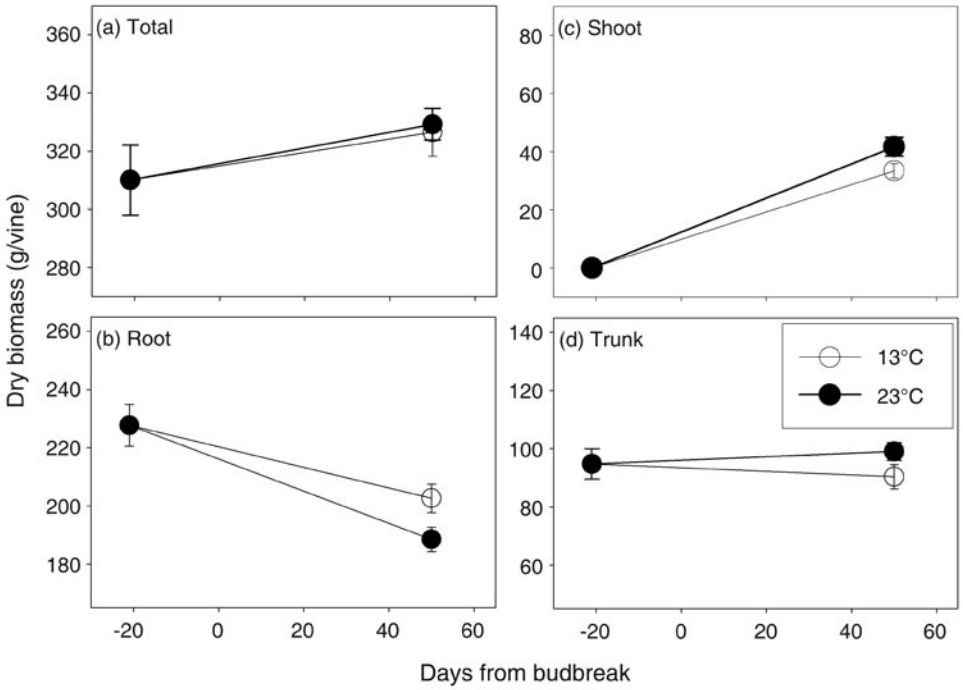

Figure 3 Changes in dry biomass of Shiraz grapevines grown in soil at 13°C or 23°C from dormancy to anthesis: (**a**) total vine, (**b**) root, (**c**) shoot, (**d**) trunk. Values are means ± se (n = 5).

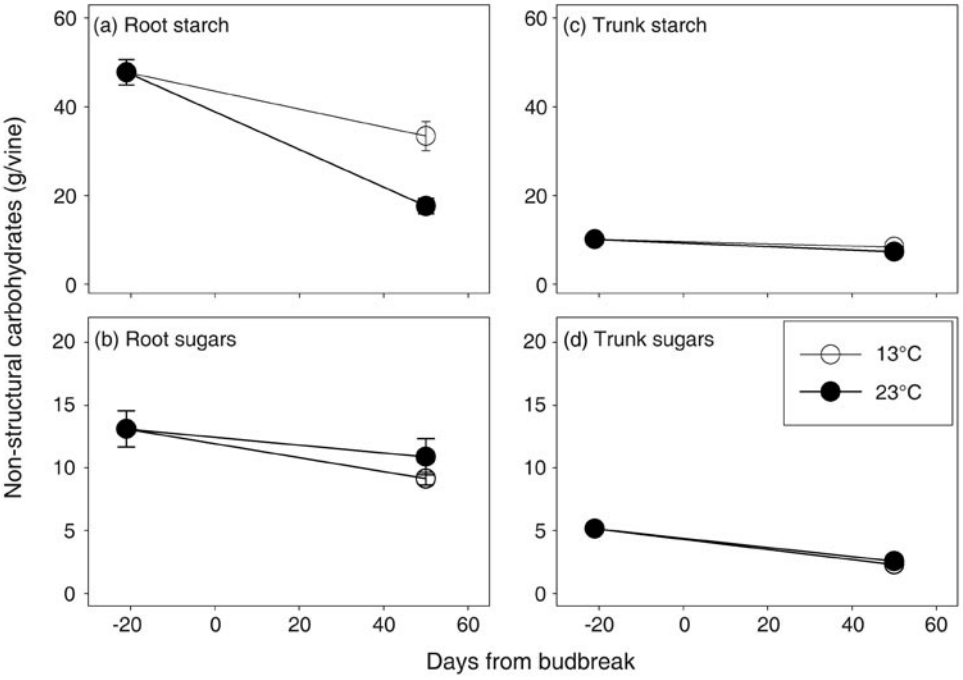

Figure 4 Changes in nonstructural carbohydrate content of Shiraz grapevines grown in soil at 13°C or 23°C from dormancy to anthesis: (**a**) root starch, (**b**) root total soluble sugars, (**c**) trunk starch, (**d**) trunk total soluble sugars. Values are means ± se (n = 5).

In this study flower number per inflorescence was not significantly influenced by soil temperature, which is consistent with Sultana grown in a hydroponic system (Woodham and Alexander 1966).

Following dormancy, the generation of root pressure reestablishes the xylem stream to supply water and an array of root-derived substances (including glucose, fructose, amino acids, minerals, cytokinins, and other plant-growth substances involved in the induction and maintenance of growth following budbreak) and for subsequent transpiration. Although sap bleeding as a result of late-winter pruning of cultivated vines is common, apart from guttation from leaf hydathodes, bleeding is not a natural function of intact grapevines. However, analysis of the sap does provide useful insights regarding the internal physiological functions. In our study, riboside cytokinins

Table 2 Midday photosynthesis (A), transpiration (E), and stomatal conductance (g_s) of single leaves at anthesis of Shiraz grapevines grown at two soil temperatures.

Soil temp	A (μmol CO_2/m² *s)	E (mmol H_2O/m² *s)	g_s (mol H_2O/m² *s)
13°C	9.7[a]	3.8	0.16
23°C	10.2	4.1	0.18
Signif	ns[b]	ns	ns

[a]Values are means (n > 15).
[b]ns indicates no significant difference using Tukey's HSD ($p < 0.05$).

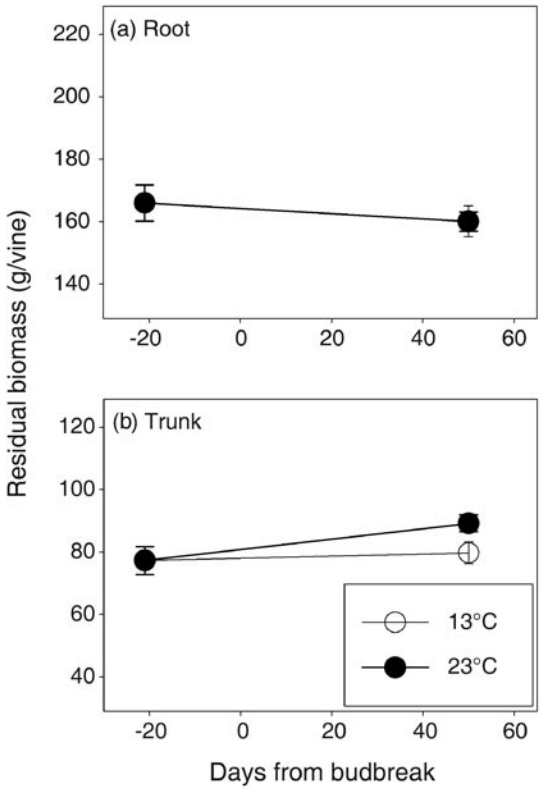

Figure 5 Changes in root and trunk residual dry biomass of Shiraz grapevines grown in soil at 13°C or 23°C from dormancy to anthesis: (**a**) root, (**b**) trunk. Values are means ± se (n = 5). Residual dry biomass estimated as total dry biomass less total nonstructural carbohydrate biomass.

Table 3 Concentrations (pmol/mL) and generation rates (pmol/hr) of cytokinin forms identified at budbreak in bleeding sap of Shiraz grapevines grown at two soil temperatures.

	Concn (pmol/mL)		Generation rate (pmol/hr)	
Cytokinin form	**13°C**	**23°C**	**13°C**	**23°C**
trans-Zeatin	3.2 ± 0.3[a]	3.2 ± 0.3	1.2 ± 0.1*	3.2 ± 0.4*
cis-Zeatin	d	d	d	d
Dihydro-zeatin	d	d	d	d
Isopentenyl adenine	1.4 ± 0.1	0.9 ± 0.1	0.5 ± 0.1*	0.9 ± 0.1*
Total free base	4.9 ± 0.4	4.2 ± 0.3	1.8 ± 0.2*	4.1 ± 0.4*
trans-Zeatin riboside	17.4 ± 2.0	24.2 ± 3.0	6.5 ± 0.9*	26.3 ± 4.9*
cis-Zeatin riboside	0.5 ± 0.1	0.4 ± 0.1	0.2 ± 0.1*	0.4 ± 0.1*
Dihydro-zeatin riboside	2.3 ± 0.1*	3.0 ± 0.3*	0.8 ± 0.1*	3.2 ± 0.4*
Isopentenyl adenosine	24.7 ± 1.3	22.3 ± 1.4	9.0 ± 0.6*	22.0 ± 1.8*
Total riboside	44.9 ± 2.8	49.9 ± 3.6	16.5 ± 1.5*	51.8 ± 6.7*
Zeatin *O*-glucoside	d	d	d	d
Zeatin riboside *O*-glucoside	d	nd	d	nd
Dihydro-zeatin *O*-glucoside	nd	nd	nd	nd
trans-Zeatin nucleotide	2.8 ± 0.7*	0.9 ± 0.2*	1.1 ± 0.3	1.0 ± 0.3
cis-Zeatin nucleotide	d	d	d	d
Dihydro-zeatin nucleotide	1.0 ± 0.1*	0.5 ± 0.1*	0.3 ± 0.1*	0.6 ± 0.1*
Isopentenyl nucleotide	2.8 ± 0.3*	0.5 ± 0.2*	1.0 ± 0.1*	0.4 ± 0.1*
Total nucleotide	6.7 ± 1.0*	2.0 ± 0.3*	2.5 ± 0.5	2.0 ± 0.4
Total cytokinin	56.6 ± 3.8	56.2 ± 3.6	20.9 ± 2.0*	58.0 ± 7.3*

[a]Values are means ± se (n = 15). * indicates significant differences using Tukey's HSD ($p < 0.05$). d indicates the compound was detected at trace levels; nd indicates not detected.

were predominant in the bleeding sap at budbreak or pressure-extracted sap at anthesis, as with cytokinins in the transport fluids of other plant species (Emery and Atkins 2002). Increased shoot growth and suppressed root growth is a well-documented effect of cytokinins. For example, transgenic tobacco plants with greatly reduced endogenous cytokinin levels had stunted shoots and increased root growth (Werner et al. 2003). In our study, the significantly greater shoot biomass, higher dihydro-zeatin riboside and *trans*-zeatin riboside, and lower concentrations of their precursor nucleotide forms in sap of the warmer temperature vines indicate that those cytokinins play a role in stimulating grapevine shoot growth. Lavee and May (1997) concluded that cytokinins may have in some cases been mistaken for dormancy-breaking agents as they enhance shoot elongation. Our finding that there was no effect on time of budbreak despite large differences in cytokinin composition and concentration supports that view.

Researchers have pointed out that in vivo sap flow rates distort xylem hormone concentrations and the more relevant measure is potential delivery rate (Else et al. 1995). Therefore, we further analyzed the data using differences in the flow rate of bleeding sap to reflect differences in root pressure or the characteristics of the conducting vessels, a type of bleeding sap analysis used by others. For instance, stimulation of tomato leaf growth was consistently associated with increased concentration of the physiologically active forms of cytokinins, zeatin and zeatin riboside, in the xylem exudate (Rahayu et al. 2005), suggesting a major role for cytokinins as long-distance signals mediating the shoot response to perception in roots. Taking into account the sap flow and potential generation rates, our results revealed a much more marked temperature-related increase in the capacity of the vines to supply the xylem sap with the more active free base and riboside cytokinins. This response and the general inverse relationship between bleeding sap flow rate and nucleotide cytokinin concentrations suggests a possible causal relationship in which cytokinins are involved in the mobilization of solutes. First, it indicates that increasing temperature promotes the transformation of precursor nucleotide cytokinins to the more active riboside and free base forms (Sakakibara 2006), all of which increased. Zeatin riboside affects the mobilization of carbohydrate reserves from seeds of other dicotyledonous species (Munoz et al. 1990), and the finding that cytokinins are involved in the induction of an extracellular invertase and hexose transporter (Roitsch and Ehneb 2000) suggests that they may regulate the loading of carbohydrates into xylem tissues. More recently, this has been supported by the demonstration that cytokinin deficiency syndrome (via overexpressed cytokinin oxidase/dehydrogenase) leads to drastically weaker sink strength of shoot tissues (Werner et al. 2008). Moreover, this effect is attributable to lowered mobilization of soluble sugars from source tissues and decreased ability of sink tissues to utilize carbohydrates because of lower invertase activity.

Whether or not root pressure per se is attributable to carbohydrate loading remains to be determined. While at least some contribution of osmotically active sugars seems likely, amino and other organic acids could play a major role (Anderson and Brodbeck 1989).

Table 4 Concentrations (pmol/mL) and generation rates (pmol/hr) of cytokinin forms identified at anthesis in xylem sap of Shiraz grapevines grown in two soil temperatures.

	Concn (pmol/mL)		Generation rate (pmol/hr)	
Cytokinin form	**13°C**	**23°C**	**13°C**	**23°C**
trans-Zeatin	0.3 ± 0.3[a]	0.5 ± 0.2	8.7 ± 7.0	24.6 ± 13.9
cis-Zeatin	nd	nd	nd	nd
Dihydro-zeatin	nd	nd	nd	nd
Isopentenyl adenine	0.5 ± 0.2	0.3 ± 0.2	14.5 ± 5.5	17.2 ± 13.8
Total free base	0.8 ± 0.4	0.8 ± 0.4	23.2 ± 11.2	41.8 ± 26.6
trans-Zeatin riboside	6.3 ± 3.2	5.2 ± 1.7	184.2 ± 60.6	204.8 ± 44.5
cis-Zeatin riboside	nd	nd	nd	nd
Dihydro-zeatin riboside	0.7 ± 0.2	0.4 ± 0.2	21.8 ± 5.1	17.9 ± 9.0
Isopentenyl adenosine	0.4 ± 0.1	0.4 ± 0.1	13.8 ± 0.8	15.7 ± 5.3
Total riboside	7.4 ± 3.4	5.9 ± 2.0	219.9 ± 58.4	238.4 ± 57.1
Zeatin *O*-glucoside	nd	nd	nd	nd
Zeatin riboside *O*-glucoside	nd	nd	nd	nd
Dihydro-zeatin *O*-glucoside	nd	nd	nd	nd
trans-Zeatin nucleotide	nd	nd	nd	nd
cis-Zeatin nucleotide	nd	nd	nd	nd
Dihydro-zeatin nucleotide	nd	nd	nd	nd
Isopentenyl nucleotide	nd	nd	nd	nd
Total cytokinin	8.1 ± 3.8	6.7 ± 1.7	243.1 ± 67.6	280.2 ± 68.0

[a]Values are means ± s.e. (n = 3). nd indicates the compound was not detected.

This study has highlighted significant grapevine responses to soil temperature in the range of 13°C to 23°C. In regard to the relevance of these findings to field-grown vines, there is little published information regarding root-zone temperature profiles. Preliminary measures at Wagga Wagga show that seasonal soil temperature at 70 cm depth is between 13 and 28°C (S.K. Field and J.P. Smith, unpublished data, 2008), suggesting that similar responses to the study presented here are likely in vineyards. The scalability of these findings is the subject of further study by our group, with the prospect of adding an edaphic dimension to predictive models of grapevine responses to global climate change that currently rely on atmospheric temperature.

Conclusion

Soil temperature is an important determinant of both the rate of depletion and structural utilization of carbohydrate reserves from roots during growth of grapevines from budbreak to anthesis. It has a clear and positive relationship to shoot biomass and leaf area. Development of the current season inflorescences is not greatly influenced by soil temperature within the range of 13 to 23°C. At budbreak grapevine xylem sap contains cytokinins of four major classes, although *O*-glucoside cytokinins occur in only trace amounts. At that time both xylem sap flow and cytokinin composition of the sap are influenced greatly by soil temperature. Root-generated cytokinins, and riboside cytokinins in particular, appear to be associated with the mobilization of carbohydrates at the end of dormancy and the ensuing shoot development. The similarity of time of budbreak—regardless of large differences in soil temperature, bleeding sap flow rate (by inference, root pressure), nucleotide cytokinin concentration, and the capacity of the sap to increase free base and riboside cytokinins—indicates that release of buds from dormancy is not directly regulated by those soil temperature-associated features. Comparison of the results with previous studies indicates that due to apical dominance and correlative inhibition, grapevine responses to soil temperature, in terms of number of buds to break and time of budbreak, are conditioned by the number of nodes per cane.

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