Supplementary Materials S2. Standardisation of root, trunk and cane tissue sampling

Introduction

Previous researchers have reported on vine carbohydrate dynamics based on destructive sampling. Carbohydrate concentrations vary between different vine parts and positions in vines [2,9,10] and in the context of environment. Understanding of this variation in carbohydrate concentrations is critical to the design of a robust sampling strategy.

Objective

To standardise the sampling of root size, trunk core and cane from a standing vine.

Materials and methods

Cane or trunk core samples were collected from three, and root samples were collected from four, non- experimental vines of the same block just after pruning of vines (19 June 2014) i.e. approximately one month prior to budburst as storage tissues contain maximum carbohydrates reserves [10]. Samples were stored for 1–2 hours in a freezer (-20°C) on site before 3–4 hour transport to the laboratory in an insulated container with ice. Samples were then stored in an air-tight zipped bag in a freezer (-20°C) until processing. After sampling, one vine was fully excavated to estimate the total dry weight of a typical vine of a 14-year-old Menindee Seedless grafted on 5BB Kober in the subtropics.

Root sample

Root samples were collected by digging at 15–20 cm distance from the trunk at a depth of 5–30 cm depending on root availability. Roots up to 10 mm diameter were collected from four vines. Samples were soaked overnight in 1% w/v ground breaker® (active constituent 9.5 % buffered polylignosulfonates) solution for clay removal. After washing in tap water, roots were

cut into pieces and sorted based on three size categories; small (< 2 mm), medium (2–5 mm) and large (> 5 mm) diameter measured with a digital caliper when necessary (Figure SI). Analysis of variance (ANOVA) was made considering four vines as replications and three roots categories as treatment.

Figure SI. Root size standardised for carbohydrate assessment sampling.

Trunk core sample

Trunk core samples were collected from four vines to standardise the sampling location within a vine and the tissue health of the trunk core. Core samples having 5.15 mm diameter across the trunk were collected using Haglöf increment borers from 10, 20, 30, 40 and 50 cm above the graft union (Figure SII).

Figure SII. Stem core sampling from different height of the trunk using Haglöf increment borers.

Three different wood symptoms related to health status were visually apparent; healthy and moist, and necrotic (rotted white or black dead) status of stem core samples were observed. Tissues that visually confirmed healthy or necrotic states were processed to measure fresh weight, dry weight and non-structural carbohydrates (TNC). Twenty stem cores were collected from four vines; 15 samples of three vines were processed for carbohydrate assessments and 5 sample of one vine used for histological observations using PAS (periodic acid Schiff [32]). Starch in xylem ray cells stains with PAS was observed in healthy tissues only (Figure SIII). ANOVA was made considering individual vine as replicates and heights of trunk core sampling position as treatment.

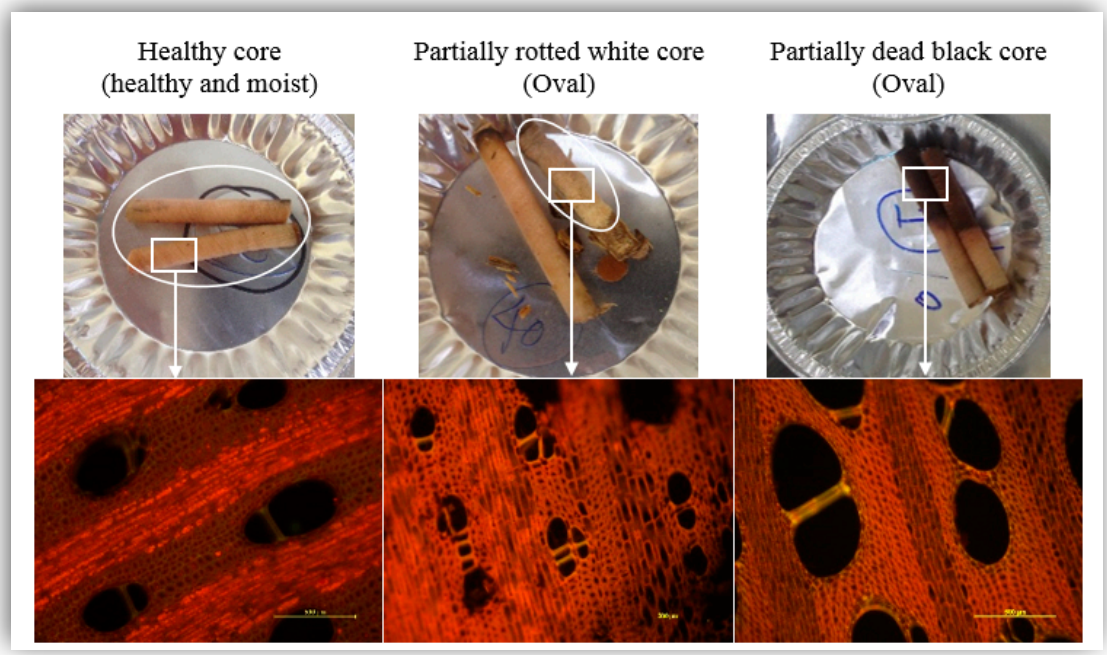

Figure SIII. Trunk core collected after pruning (dormant stage) showing different health status of tissues. Visually categorised healthy, rotted and dead core free-hand section stain with PAS viewed under Nikon Eclipse E600 fluorescence microscope filter V2A in respective column.

Cane sample

Canes varied in colour, diameter and internodal length within a vine. Six cane samples (13th node including the internode towards the 14th node) from three vines were collected. Internode diameter at the distal end of each sample was measured using a digital caliper. Dry weights (% fresh weight) of canes within a vine are presented with standard errors.

Sample processing and carbohydrates assessment

All samples were oven dried, ground and subjected to carbohydrate analysis using enzymatic assay kit as described in chapter 5.2.5. ANOVA was performed using Genstat 16.1 (VSN International, UK) and mean separation was done by Tukey's hst ($\alpha = 0.05$).

Results and Discussion

Root size and carbohydrates

There were no significant differences in soluble sugar content in different size roots. Smaller to medium root sizes (≤ 5 mm) had significantly higher TNC and starch content than the larger category (> 5 mm) (Table SI).

Table SI. Effects of root size on carbohydrate concentration of Menindee Seedless, QLD, Australia during June 2014.

Root diameter class	Carbohydrates (% w/dw)		
	Soluble sugar	Starch	TNC
< 2 mm	1.06[a]	13.02[a]	14.08[a]
2–5 mm	1.20[a]	12.72[a]	13.92[a]
> 5 mm	1.12[a]	9.57[b]	10.68[b]

Means within the same column with the same letter are not significantly different Tukey's ($\alpha = 0.05$).
TNC Total non-structural carbohydrates, w/dw Dry weight.

Trunk core sampling position and carbohydrates

There was no significant difference in soluble sugars, starch and TNC (% w/dw) of healthy tissue with respect to the position of trunk core samples within a vine (Table SII). The dry weight (% fw) was also consistent between positions in healthy tissues (53.5 ± 0.76 sd, n = 15) but it was significantly different in necrotic tissues (56.1 ± 15.8 sd, n = 15) between treatments and vines (data not presented).

Table SII. The effects of trunk core position on carbohydrate concentration cv. Menindee Seedless, QLD, Australia during June, 2014.

Core above graft union (cm)	Carbohydrates (% w/dw) in healthy core			Dry weight of healthy core (% fw)
	Soluble sugars	Starch	TNC	
10	2.57	6.88	9.45	53.4
20	2.70	5.68	8.38	53.7
30	2.58	5.91	8.48	53.52
40	2.70	6.91	9.61	53.52
50	2.56	6.04	8.59	53.24
F-test (*P* value)	0.84^{ns}	0.45^{ns}	0.41^{ns}	0.96^{ns}

ns = non-significant. DW Dry weight, TNC Total non-structural carbohydrates.

The TNC content in the gross trunk core was greatly influenced by the proportion of necrotic tissue (Figure SIV). The necrotic tissue varied between vines and between sampling positions within a vine.

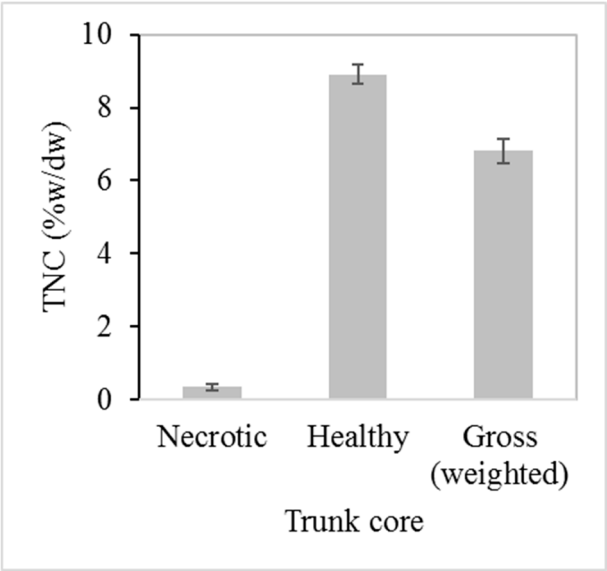

Figure SIV. Comparison of total non-structural carbohydrates in necrotic, healthy and gross (weighted) trunk core samples. Error bars ± se (n = 15).

Carbohydrate variation in canes

Starch concentration differed between canes on the same vine for the same node position (Table SIII). Dry weight (% fw), starch concentration and TNC concentration were significantly correlated to internodal diameter of the cane at 13th node (r^2 = 0.68, 0.69, 0.69; < 0.05, respectively).

Table SIII. Cane diameter, dry weight (% fw) and carbohydrates concentration of cv. Menindee Seedless, QLD, Australia during June 2014.

Vine	Cane diameter (mm)	dw (% fw)	Carbohydrates (% w/dw)		
			Soluble sugars	Starch	TNC
Vine 1	6.58 ± 0.63	45 ± 1.96	1.81 ± 0.12	5.00 ± 1.18	6.81 ± 1.22
Vine 2	7.09 ± 0.53	46 ± 1.71	1.87 ± 0.21	5.26 ± 1.40	7.13 ± 1.56
Vine 3	8.01 ± 0.45	52 ± 1.16	1.97 ± 0.03	8.46 ± 0.37	10.43 ± 0.36

Values are mean ± se (n = 6 canes)

Standardisation of sampling protocol

Factors such as rootstock, growing environment, variety, sampling season and management have a role in governing carbohydrate concentration and root size. Roots were generally categorised into classes based on root diameter; however, the categories are not similar in diameter classes. Higher TNC content in medium sized roots (2–5 mm diameter) was lower than in smaller (< 0.2 mm) and higher than in larger roots (> 10 mm), in agreement with previous studies. For example, Hunter [9] divided roots of Pinot Noir planted in spacing treatments into five classes; < 0.5, 0.5–2, 2–5, 5–10 and > 10 mm diameter and found the lowest carbohydrates (9–12 % w/dw) in larger roots (> 10 mm) with consistently higher concentrations (14–21% w/dw) observed in small to medium roots classes (0.5–2 and 2–5 mm). In an experiment with three classes of roots; small (< 3 mm diameter), medium (3–7 mm) and large (> 7 mm) for own rooted 12 years old Shiraz, Holzapfel et al. [10] also reported lower to higher concentration with larger to smaller root diameter. In contrast to these findings, Bennett [2]

reported that the small roots (3–5 mm) had lower TNC (8.8% w/dw) while medium (10–15 mm) roots and large roots (≥ 20 mm) had significantly higher TNC of 23.1 and 25.7% w/dw, respectively.

For the dynamics study of carbohydrates, frequent sampling over the same vine is mandatory. This study has confirmed that carbohydrate concentration was not affected by the position of core sampling within a trunk. Previous reports have also indicated consistency in carbohydrate concentration with respect to position of sampling within trunk. For example, TNC content in samples taken from bottom, middle and top of the trunk; and sun exposed and shade side of the trunk were statistically similar in Chardonnay grown in cool climate of New Zealand [2]. The proportion of cane/spur was only accounted < 5% of total estimated TNC (Table SIV).

Table SIV. Dry weight and estimated carbohydrate content in a typical vine representative of experimental vines, after pruning, 2014.

Vine parts	dw	Soluble sugars	Starch	TNC	[a]Estimated TNC
	(g)	(% w/dw)	(% w/dw)	(% w/dw)	(g/vine)
Cane/spur (1–3 y old)	549 (5.6)	1.89 ± 0.3	6.24 ± 3.0	8.13 ± 3.2	45 ± 17 (4.4)
Wood (trunk/cordon and above ground root stock)	6547 (66.7)	2.62 ± 0.2	6.28 ± 1	8.9 ± 1.0	582 ± 67 (57.9)
Roots (all below ground)	2725 (27.8)	1.20 ± 0.1	12.72 ± 0.7	13.92 ± 0.7	379 ± 19 (37.7)
Whole vine	9821				1006

[a]TNC content in vine or vine parts was calculated assuming the average TNC in cane (13th node), root (2–5 mm diameter) and trunk core sample represents the total biomass in the respective tissue in column 1. Values in parenthesis represent the % of their column total.

Total carbohydrates in a typical vine

A typical vine of 14 years Menindee Seedless grafted on 5BB Kober weighed approximately 10 kg (dry mass) when dormant (Table SIV). The estimated TNC (g/vine) was approximately 1 kg. During winter, cane and spurs accounted for 5.5% of total dry mass and 4.5% of total estimated TNC content respectively.

Conclusions and recommendation

The following recommendations are made for vine tissue sampling.

Root samples 2–5 mm diameter are sufficiently available for frequent sampling just outside 15 cm from trunk. Sampling position of trunk core in between graft union and trunk head did not differ in carbohydrates contents, hence the sample is independent of sampling position in terms of height. Necrotic tissue affected the concentration and, hence, removal of necrotic tissues is recommended.

Variation in carbohydrates was observed on the 13^{th} node position between canes. Carbohydrate content in canes was largely influenced by the diameter of the cane. The study of carbohydrate dynamics in root and trunk tissue planned with controlled number of nodes per vine after pruning, hence, the cane sampling was not performed for Chapter 5.