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Assessment of morphological changes and determination of best cane collection time for 140 RU and 5 BB

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Abstract

This study was carried out to assess morphological changes in the canes of 140 Ru and 5 BB and to determine best time for cane collection. The first part of the study included the morphological assessment of the rootstock canes during the winters of 2003–2004 and 2004–2005. Canes collected in the second part of the study in 2005–2006 were grown in pots to relate the changes with the vegetative growth characteristics.

Cane collection commenced at leaf fall and they were collected three more times at 15-day intervals. Morphological changes were determined from the transverse internodal cuts and included: width of pith, xylem, phloem plus bark tissues, ratios of cane width/pith, cane width/bark + phloem, xylem/pith and xylem/bark + phloem, and cane water content. Findings showed that lignification was best 30 and 45 days after leaf fall for 140 Ru and 15, 30 and 45 days after leaf fall for 5 BB.

Canes collected in 2005–2006 were used to relate the changes observed in the previous 2 years with the vegetative development. Two-bud cuttings were grown in pots and viable plant and rooting ratios, root fresh weight, shoot number and length, and node number were determined. Data obtained were found in agreement with the morphological findings. A 140 Ru had the best vegetative growth 30 and 45 days after leaf fall, and 5 BB grew well at each collection period.

Results showed that it is best not to collect canes from rootstock nurseries at or right after leaf fall due to poor lignification and subsequent poor growth.

Keywords: Grape rootstock; Cane; Morphology; Lignification; Vegetative growth

1. Introduction

Phylloxera (*Daktulosphaira vitifoliae* Fitch.) is one of the most significant limiting factors to grape-growing. The only proven method for long term control is to graft susceptible *Vitis vinifera* onto phylloxera tolerant vines, rootstocks, developed from native American grapevine species (Granett et al., 2001).

Cuttings well lignified and rich with reserves are better at rooting and growth than poor ones (Gautheret, 1966; Thorpe, 1974; Kısmalı, 1981; Bartolini et al., 1996; Hunter et al., 2004). In addition, callusing is higher when cuttings are highly lignified (Kozma et al., 1972; Kısmalı, 1978). Due to the high work load in large nurseries during winter, rootstock cuttings are usually started collecting right after leaf fall (end of November) and stored in cold rooms until grafting. Collecting rootstock canes at leaf fall is however not advised since translocation, conversion and storage of reserves continue after defoliation in the over wintering parts of vines (Coombe and Dry, 1992; Çelik, 1998) even if only at a very low level.

Wood fibers, wood parenchyma and ray regions are important sugar and starch storage organs (Eifert et al., 1961). Conversion from sugar to starch or vice versa takes place depending on winter air temperature (Winkler et al., 1974). Starch is at maximum and sugars are at minimum level in December and January. Conversion of sugars to starch starts in spring, but the overall reserve carbohydrate level declines due to consumption by the breaking buds (Eifert et al., 1961; Coombe and Dry, 1992). Harvesting of dormant cuttings is therefore advised for mid winter and not at the beginning of winter or when vines start taking up water in the spring (Kocamaz, 1995; Çelik, 1998).

The degree of lignification is best assessed by using the ratios of cane width/pith and phloem + xylem/pith values

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(Oraman, 1963; Dardeniz, 2001). There are different factors affecting the cane width/pith ratio. Dardeniz (2001) studied the effect of cluster thinning on cane lignification. He found that 30–60% cluster thinning increased cane width/pith ratio in Cardinal and Amasya compared to the controls. Dardeniz and Kısmalı (2001) found that the cane width/pith ratio of 140 Ru decreased with increasing shoot number. Dardeniz and Şahin (2005) examined cane hardening on the canes of grape cultivars Yalova İncisi (*Vitis vinifera* L.) grafted onto different rootstocks. Yalova İncisi had the best hardening on 1103 P, followed by 5 BB, 41 B and 140 Ru. Lignification level (cane width/pith) was determined on rootstock 140 Ru and 1103 P with different shoot loads (4, 8, 12 shoots per vine) (Dardeniz and Kısmalı, 2001). 140 Ru had the highest levels at four and eight shoot/vine. Best cane width/pith ratio was obtained from four shoots per vine for 1103 P. The same shoots were used as 2-bud cuttings in the winter and were grown in pots. It was found that shoot weight, shoot length and callus weight increased significantly as the cane matured.

Pith in 1-year-old canes has short-thin walled cells with amorphous shapes and it contains chlorophyll pigments (Oraman, 1972; Çelik, 1998; Ağaoğlu, 1999). Properly grown and matured canes are well lignified when their piths are narrow and xylem tissues are wide (Çelik, 1998). Pith is much wider in young shoots, decreasing in width as they develop during the season (Ağaoğlu, 1999).

The morphological changes that occur in shoots of American grape species rootstocks at or right after leaf fall have not been fully studied. These kinds of detailed studies will enable both researchers and nurserymen to determine when the pith is narrow or the ratios of cane width/pith and xylem/pith are high to select the appropriate time for harvesting canes. The objective of this study was to periodically determine morphological changes in the canes of the rootstocks and correlate this data to vegetative development of the rootstocks.

2. Material and method

This study consisted of two parts. The first part comprised observations on the morphological changes in the canes of grapevine rootstocks, 140 Ru *(Vitis berlandieri x V. rupestris)* and 5 BB *(V. berlandieri x V. riparia)*. Canes were collected in the winters of 2003–2004 and 2004–2005 from the Fruit Propagation Station, located in Umurbey, Çanakkale, Turkey. In the second part (winter season of 2005–2006), rootstock canes were grown in pots to collect data on vegetative growth characteristics and relate these with the findings obtained in the first part.

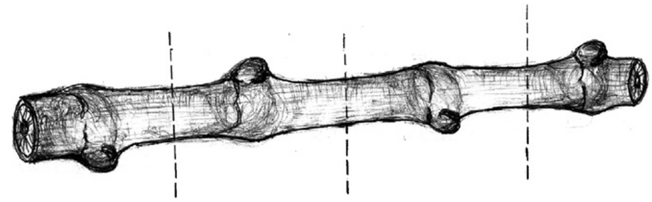

Fig. 1. Cut places on the canes of the rootstocks.

Rootstocks in the foundation block were 14-year-old, growing on the ground with 2 m × 2 m spacing. They were grown under dry land conditions with normal cultivation and fertilization. No shoot management techniques were applied during the study. Table 1 presents the soil characteristics of the block.

The trial was designed in randomized plots comprising four replicates and three vines per replicate. Canes were collected four times, leaf fall (20–25 November, 80% defoliation), 15 days later (5–10 December), 30 days later (20–25 December), and 45 days later (5–10 January). Canes were randomly chosen on the basis of equal thickness of the canes at each and every collection time. Two canes per vine were selected and cut into 4-bud pieces (1–4, 5–8, 9–12, 13–16, 17–20) to facilitate morphological measurements. The pieces were cut in the middle of the internodes (Fig. 1). The number of the transverse cuts per rootstock per year was 1440 (3 vines × 2 canes × 15 cuts × 4 replicates × 4 times). Characteristics listed below were measured with the aid of digital compass at the cut surfaces as shown in Fig. 2.

Cane width (mm): the arithmetic mean of the thinnest (CW_1) and the thickest (CW_2) points of the cane, xylem width (mm); the arithmetic mean of four corresponding point measurements at cut surface (X_1, X_2, X_3, X_4), pith width (mm); the arithmetic mean of the thinnest (P_1) and thickest (P_2) points of the cane, bark + phloem width (mm); the arithmetic mean of the visually determined thinnest (BP_1) and thickest (BP_2) points of the cane, water content (%) as described by (Kacar, 1972); [(fresh weight − dry weight) × 100/fresh weight]. The ratios of cane width/pith and xylem/pith were calculated to determine the lignification levels. Cane width/bark + phloem and xylem/bark + phloem ratios were also calculated for wood hardening determination.

The objective of the second part of study was to compare and relate the first part findings with the vegetative growth of the canes. The canes were collected from the same vines in the foundation block. Canes were also cut into 4-bud segments (1–4, 5–8, 9–12, 13–16, 17–20) to ease handling. They were treated with a fungicide (Captan, Southern Agricultural

Table 1
Soil profile features of the rootstock foundation block

Depth (cm)	Texture	Salinity (%)	pH (1:2.5)	Calcareous ($CaCO_3$%)	P (kg day^{-1} P_2O_5)	K (kg day^{-1} K_2O)	Organic matter (%)
0–30	Loamy	None	7.5–8.0	Low	High	Adequate	Low
30–60	Loamy	None	7.5–8.0	High	Low	Adequate	Low
60–90	Loamy	None	7.5–8.0	High	Low	Adequate	Very low

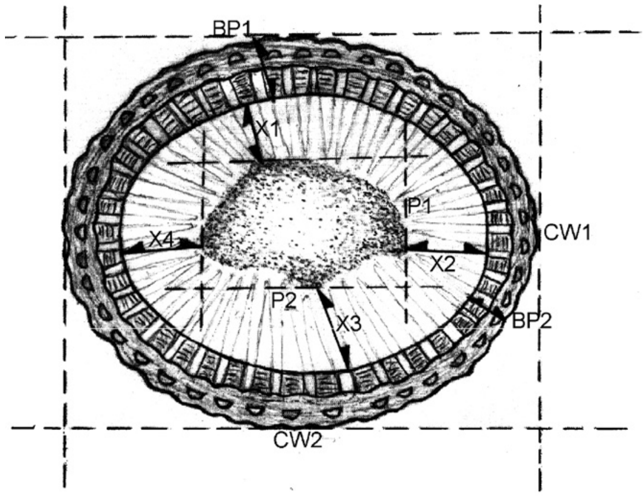

Fig. 2. Diagram showing how the measurements were taken CW: cane width, P: pith, X: xylem, and BP: bark + phloem.

Insecticides, Inc. Fla, USA) and stored at 4 °C and 80–90% relative humidity until 13–14 April 2006. Before planting, canes were soaked in water for 24 h (Saraswat, 1973). Canes were cut into 2-bud cuttings with lower bud disbudded. Equal sized cuttings were chosen as much as possible for every collection time.

The trial was designed in randomized blocks comprising four replicates and 75 cuttings (15 cuttings per 4-bud segment) per replicate. The total number of the cuttings planted for each rootstock was 1200 (75 cuttings × 4 replicates × 4 times). Plastic pots (13 cm × 20 cm × 7 cm) were filled with 158 g perlite and cuttings were planted in a 4 cm × 4 cm spacing. Every pot was fertilized with ammonium nitrate 33% (10 kg day^{-1}), di-ammonium phosphate 18–46% (4 kg day^{-1} P_2O_5) and potassium sulphate 50% (15 kg day^{-1} K_2O). Water (1000 ml) was added and pots were weighed. Following irrigations were based on this weight. Pots were weighed every week and irrigated to the initial weight. The experiment was completed approximately 8 weeks later when plants stopped growing.

The following characteristics were determined: viable plant ratio (%) – cuttings with healthy root and shoot/total cutting number; shoot ratio (%) number of cuttings with at least 10 cm shoot/number of total cuttings; rooting ratio (%) – number of cuttings with a least one healthy root/number of total cuttings; root–shoot fresh weight (g/plant) – total root–shoot weight/ number of total cuttings; cutting weight (g/cutting) – cutting was weighed after removal of shoot and root and the weight was divided by the total number of cuttings; node and shoot number per plant – shoot number/total number of cuttings; shoot length (cm/plant).

Data obtained both from the first and second parts of the study were evaluated with Minitab (version 13.1) statistical package program.

3. Results

The widest pith in 140 Ru was at leaf fall (3.53 mm) and the narrowest 30 days later (3.17 m). Rootstock 5 BB had its widest

Table 2
Measurements taken on transverse cuts of 140 Ru and 5 BB graft material

Rootstock	Collection time	Pith (mm)			Xylem (mm)			Bark + phloem (mm)			Cane width/pith		
		year 1	year 2	Mean	year 1	year 2	Mean	year 1	year 2	Mean	year 1	year 2	Mean
140 Ru	Leaf fall	3.24 a	3.77 a	3.53 a	2.15 b	1.95 a	2.05 b	0.864 a	0.863 a	0.864 a	2.85 c	2.22 d	2.50 c
	15 days later	3.01 b	3.72 a	3.40 b	2.19 b	2.04 ab	2.11 b	0.792 b	0.810 b	0.801 b	3.08 b	2.39 c	2.70 b
	30 days later	2.90 b	3.39 c	3.17 d	2.53 a	2.17 bc	2.32 a	0.799 b	0.734 c	0.763 c	3.38 a	2.70 a	3.00 a
	45 days later	2.91 b	3.57 b	3.28 c	2.58 a	2.16 c	2.34 a	0.866 a	0.796 b	0.826 b	3.32 a	2.57 b	2.89 a
	LSD	1%	1%	1%	1%	1%	1%	1%	1%	1%	1%	1%	1%
5 BB	Leaf fall	2.74 a	3.51 a	3.16 a	2.26 a	2.21	2.23	0.989 a	1.054 a	1.025 a	3.33 b	2.57 c	2.91 b
	15 days later	2.44 b	3.36 b	2.93 b	2.00 b	2.23	2.12	0.898 bc	1.000 b	0.953 b	3.60 a	2.76 b	3.15 a
	30 days later	2.70 a	3.10 c	2.92 b	2.15 a	2.18	2.17	0.873 c	0.893 c	0.884 c	3.36 b	2.94 a	3.13 a
	45 days later	2.75 a	3.07 c	2.93 b	2.36 a	2.11	2.22	0.934 b	0.921 c	0.927 b	3.27 b	2.93 a	3.08 a
	LSD	1%	1%	1%	1%	ns	ns	1%	1%	1%	1%	1%	1%

Rootstock	Collection time	Cane width/bark + phloem			Xylem/pith			Cane moisture (%)			Xylem/Bark + phloem		
		year 1	year 2	Mean	year 1	year 2	Mean	year 1	year 2	Mean	year 1	year 2	Mean
140 Ru	Leaf fall	10.68 d	9.75 d	10.17 c	0.682 b	0.524 c	0.596 c	51.05 b	54.57	52.81 ab	2.42 d	2.25 d	2.33 d
	15 days later	11.62 b	11.04 c	11.30 b	0.758 b	0.552 c	0.645 b	53.62 a	54.26	54.26 a	2.71 c	2.50 c	2.60 c
	30 days later	12.17 a	12.57 a	12.39 a	0.899 a	0.649 a	0.761 a	47.80 c	54.85	51.33 b	3.10 a	2.98 a	3.03 a
	45 days later	11.05 c	11.63 b	11.38 b	0.906 a	0.606 b	0.736 a	48.07 c	54.33	51.20 b	2.91 b	2.70 b	2.79 b
	LSD	1%	1%	1%	1%	1%	1%	1%	ns	1%	1%	1%	1%
5 BB	Leaf fall	9.12 c	8.61 d	8.84 d	0.873	0.637 c	0.744 b	48.18 b	63.58 a	55.88 a	2.29 a	2.09 c	2.18 c
	15 days later	9.63 b	9.26 c	9.43 c	0.854	0.676 bc	0.758 ab	52.98 a	61.55 ab	57.27 a	2.21 a	2.23 b	2.22 c
	30 days later	10.24 a	10.24 a	10.24 a	0.835	0.722 a	0.773 ab	46.74 b	61.18 ab	53.96 ab	2.45 b	2.45 a	2.45 a
	45 days later	9.52 b	9.71 b	9.63 b	0.879	0.705 ab	0.781 a	46.67 b	51.86 b	49.27 b	2.50 b	2.29 b	2.38 b
	LSD	1%	1%	1%	ns	1%	5%	1%	5%	5%	1%	1%	1%

Table 3
Characteristics determined for growing plants of 140 Ru and 5 BB

Rootstock	Collection time	No. of cutting	Whole plant (%)	Shoot growth (%)	Rooting (%)	Node number (n/plant)	Root fresh weight (g/plant)	Shoot fresh weight (g/plant)	Cutting fresh weight (g/cutting)	Shoot number (n/plant)	Shoot length (cm/plant)
140 Ru	Leaf fall	300	70.0 b	92.9	76.1 b	5.9	1.16 b	1.12 c	11.9	1.51	10.6 b
	15 days later	300	82.3 ab	96.3	85.3 ab	5.9	1.42 ab	1.15 bc	11.9	1.51	12.0 b
	30 days later	300	85.4 a	96.4	89.5 a	6.8	1.57 a	1.54 ab	12.2	1.54	16.2 a
	45 days later	300	85.8 a	97.3	88.5 a	6.6	1.83 a	1.40 a	12.3	1.65	15.2 a
	LSD		1%	ns	5%	ns	1%	1%	ns	ns	1%
5 BB	Leaf fall	300	58.4 b	95.2	76.1	6.2	0.53 b	1.26 b	11.8 ab	1.54 b	14.0 b
	15 days later	300	72.8 ab	93.7	87.3	7.2	0.76 a	1.68 a	11.6 b	1.79 a	18.5 a
	30 days later	300	74.3 a	97.0	85.6	6.6	0.83 a	1.64 a	12.9 ab	1.68 ab	17.4 ab
	45 days later	300	69.6 ab	93.6	81.3	7.0	0.78 a	1.80 a	13.2 a	1.58 b	18.5 a
	LSD		5%	ns	ns	ns	1%	1%	5%	1%	1%

pith size at leaf fall (3.16 mm), but had decreased to a constant level 15 days later. The xylem kept developing after leaf fall from 2.05 mm to 2.11, 2.32 and 2.34 at later dates, respectively. However, no statistically different results were obtained for 5 BB (Table 2).

The thickest bark + phloem value was noted at leaf fall for both rootstocks. Cane width/pith ratio, accepted as the best expression of cane lignification (Dardeniz, 2001) was higher at later dates. 140 Ru had the best ratio at 15 (2.70) and 30 days later (3.00) and it decreased after 45 days to 2.89. Statistically higher values of cane width/pith ratio were obtained for 5 BB after leaf fall. Values of cane width/bark + phloem followed similar pattern with those of cane width/pith for both stocks. As expected, xylem/pith ratios increased with time after leaf fall (Table 2).

Water content of the canes decreased over time. The water content of the 140 Ru canes was 52.81% at leaf fall, decreasing to 51.20% 45 days later. 5 BB canes had more water compared to 140 Ru canes, except at 45 days later (49.27%). The increase noted in water content 15 days after leaf drop was accounted for by rain that fell before this time period.

Xylem/bark + phloem ratio increased over time in 140 Ru, rising from 2.33 to 2.79. 5 BB had relatively lower ratios, ranging from 2.18 to 2.38 (Table 2).

The highest viable plant ratio was obtained 30 and 45 days later in 140 Ru, and 15 days later in 5 BB. It was the lowest when canes were taken at leaf fall for both rootstocks. Highest rooting was again 30 (89.5%) and 45 (88.5%) days after leaf fall for 140 Ru and 15 (87.3%) and 30 (85.6%) days after in 5 BB.

Cane collection time did not affect shoot growth ratio. Root fresh weight, shoot length and shoot numbers were lowest at leaf fall for both rootstocks (Table 3).

4. Discussion

The objective of this research was to study changes in the canes of 140 Ru and 5 BB in order to determine the best time to collect plant material for propagation purposes.

For both stocks, pith, bark + phloem and water content decreased, but the ratios of cane width/pith, cane width/bark + phloem and xylem/bark + phloem increased. The pith was widest at leaf fall for both rootstocks, and then reduced in size. The findings support the information that after leaf fall, anatomical changes continue within the shoots (Eifert et al., 1961; Çelik, 1998).

It was found that the best lignification for 140 Ru was 30 and 45 days after leaf fall. The ratio showed an increase after defoliation in 5 BB. The finding that pith, which is wide earlier in the season and gets smaller during the remainder of the season continued decreasing its size after leaf fall to a certain extent as xylem tissue developed, is in agreement with the results of Ağaoğlu (1999).

Increased cane hardening resulted in better vegetative growth characteristics such as higher rooting and shoots growth and supported findings by Dardeniz (2001) and Dardeniz and Kısmalı (2001). Because well lignified canes have better rooting and vegetative growth with good callus formation, which are key for graft success (Kozma et al., 1972; Kısmalı, 1978, 1981; Dardeniz, 2001), it was deduced from this study that rootstock canes should not be collected from the foundation blocks immediately after leaf fall.

5. Conclusion

Along with the decrease and increase in pith and xylem tissues, respectively, temporal changes in cane width/pith and xylem/pith ratios suggest that rootstock canes should be collected at least 30 or 45 days after leaf fall for 140 Ru and 15 days after for 5 BB rootstock. Their vegetative growth is closely related with the developmental and morphological condition of the canes. Therefore, it is advised not to collect 1-year-old canes from the rootstocks at or right after leaf fall when the cane hardening is worst.

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