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Department of Viticulture, University of Stellenbosch, South Africa

Effect of hot-water treatments on vine cuttings and one-year-old grafts

by

P. G. Goussard

Einfluß der Warmwasserbehandlung auf Rebenstecklinge und einjährige Pfropfreben

Zusammenfassung. — Unbewurzelte Stecklinge aus einjährigem Holz von *Vitis vinifera* cv. Cabernet Sauvignon wurden in 45, 50, 55, 60 und 65 °C heißes Wasser getaucht. Die Behandlungsdauer betrug jeweils 1, 5, 10, 30 und 60 min. Die Kallusbildung wurde im Temperaturbereich von 55 °C gehemmt, wenn die Einwirkungsdauer 10 min überstieg. Eine optimale Kallusbildung erfolgte bei 50 °C — 30 min und 60 °C — 1 min. Der Knospenaustrieb wurde bei höheren Temperaturen und längeren Behandlungszeiten (über 55 °C und mehr als 1 min) ebenfalls gehemmt. Einjährige, bewurzelte Pfropfreben von Cabernet Sauvignon/99 R. überlebten Temperaturen über 60 °C nicht, wenn sie länger als 1 min eingetaucht wurden.

Introduction

Baker (1962) stated that according to Jensen (1887 and 1888), hot-water treatment was the first type of thermotherapy used on plants. Birchfield and van Pelt (1958) include a list of references to such treatments. Today this procedure is being used widely in freeing many kinds of plants and seeds from fungi, bacteria, nematodes and phylloxera (Baker 1962, Coombe 1963, Hartmann and Kester 1975).

During hot-water treatments the plant is subjected to temperatures high enough and of a long enough duration (Baker 1962, Hartmann and Kester 1975) to destroy the pathogen, but not so high as to kill the plant.

In California, grapes were probably first subjected to hot-water treatments from as early as 1910, to be freed of phylloxera (Lear and Lider 1959). The Californian State Department of Agriculture approved hot-water treatments of phylloxera-infested vines as an alternative to the quarantining of such vines. These regulations called for treatment at temperatures of 125—130 °F (51.7—54.4 °C), for a period not less than 3 min or more than 5 min. In Australia, Coombe (1963) recommended submerging of entire vines in hot water at the same ranges as above. It was further stated that this treatment is considered to be feasible under commercial conditions, but that the water bath must be of sufficient size to maintain temperatures within the specified range, while the vines are immersed. Pre-heating the vines by immersion at 110 °F (43.3 °C) for 5 min aids in maintaining a constant temperature. This method has also been described by Moller and Fisher (1961).

Besides killing phylloxera, hot-water treatments have been found to be effective in eradicating root-knot nematodes from rootlings (Lear and Lider 1959). Results show that complete eradication of root-knot nematodes was obtained with the fol-

lowing temperatures and exposure times: 118 °F for 30 min, 120 °F for 10 min, 122 °F for 10 min, 125 °F for 5 min, 127 °F for 3 min, 130 °F for 2 min and 135 °F for 2 min.

Grapevines were freed of the causal agent of Pierce's disease by immersion of the entire plant in water at a temperature of 45 °C for 180 min, 50 °C for 20 min or 55 °C for 10 min (GOHEEN *et al.* 1973). According to BAKER (1962) parasitic *Phytopthora* spp. have low heat tolerance. MARAIS[1]) (personal communication) noted that unrooted grape cuttings were freed of *Phytopthora* spp. by immersion in hot water for 20 min at 50 °C, and rooted cuttings for 15 min at 50 °C.

The tolerance of plant material to hot-water treatments is an important aspect. Vines tolerated immersion in hot water at 45 °C for 24 h, 50 °C for 2.5 h and 55 °C for 10 min (GOHEEN *et al.* 1973). No injury to either tops or roots (LEAR and LIDER 1959) was observed at temperatures below 130 °F. Although old roots were killed at exposure of 2 min at 140 °F, new roots were produced on the trunk. At 145 °F most of the vines died.

In recent years *Phytopthora* spp. have become a severe problem in vineyards of the Republic of South Africa. In spite of evidence that material could be freed of *Phytopthora* spp. by hot-water treatments (BAKER 1962, MARAIS[1]), personal communication) little work has been done locally to determine the effects of such treatments on certain physiological processes in vine cuttings, for instance callusing.

Experiments were carried out to investigate the effect of hot-water treatments on callusing and bud-burst of unrooted cuttings and on sprouting and growth of rooted, one-year-old grafts. A wide range of temperatures and exposure times were involved.

Material and methods

For callusing and bud-burst investigations (experiment 1), one-year unrooted cuttings of Cabernet Sauvignon were used. Single bud cuttings with an average length of 10 cm were taken from the central parts of the canes. Apical cuts on these cuttings were about 2 cm above the bud-position and basal cuts about 7 cm below the bud-position. Immediately before treatments, all material was submerged in hot water at a temperature of 40—43 °C for 5 min.

Treatments were carried out at temperatures of 45, 50, 55, 60 and 65 °C. The exposure times for the respective temperatures were 1, 5, 10, 30, and 60 min. After this treatment all cuttings for callusing were placed in cold water so as to cool down; they were then cut at an angle of 45 ° at the basal and apical ends (see Fig. 1a) and

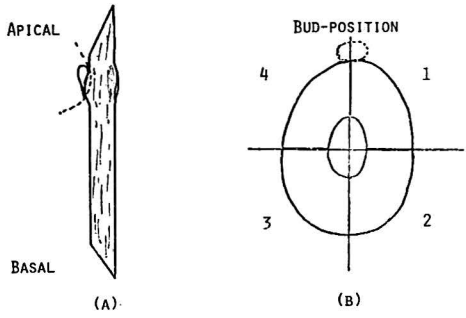


Fig. 1: a) Apical and basal cut-surfaces of cuttings. b) Quartering of apical and basal cut-surfaces.

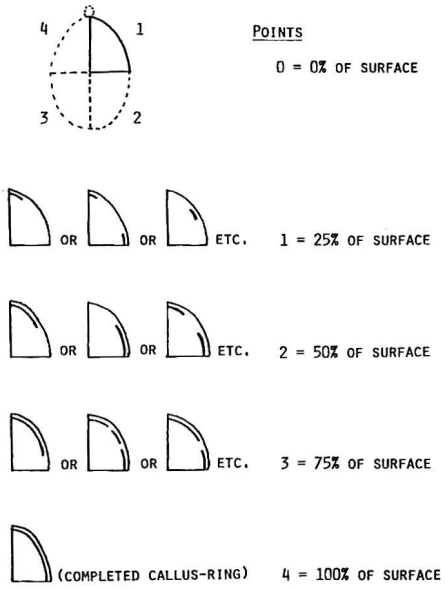
a) Schnittfläche von Trieb- und Wurzelpol der Stecklinge. b) Quadranten der Schnittfläche.

[1]) MARAIS, P. G., O.V.R.I., Private Bag X5026, Stellenbosch 7600, Republic of South Africa.

the buds were removed. All material was immersed in 1 % Captan for 2 min, to prevent fungal attacks. Callusing took place in wooden boxes filled with moist sawdust (wetted with 1 % Captan) at a temperature of 27—28 °C. Results were tabulated after 14 d. Callus formation was calculated numerically, using the following procedure:

The apical- and basal cut-surfaces of individual cuttings were divided into four quarters (Fig. 1b). The quarters on every cut-surface were numbered clockwise, with the bud-position in every case at the 12 o'clock mark. At every quarter, callus-tissue was visually registered in numeric values. The surface of callus at every quarter was recorded out of a maximum of 4 points (see Fig. 2). The amount of callus at every quarter was recorded out of a maximum of 3 points (see Fig. 3). The maximum points awarded to each quarter made 7, (4 + 3). Thus, a maximum of 28 points, (7 × 4), could be awarded to each cut-surface (4 quarters).

Cuttings for bud-burst experiments were freshly cut at basal ends after treatments, without removal of individual buds. Following this procedure, they were put in glass containers with 2 cm of water at the bottoms. Bud-burst experiments were carried out at an ambient temperature of 30 °C under continuous lightning conditions. Results were tabulated after 21 d.



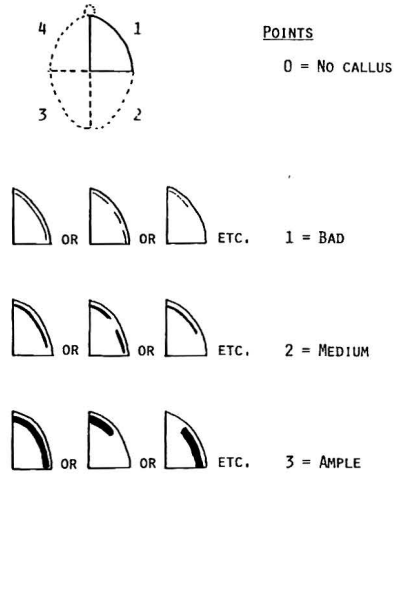


Fig. 2: Numeric calculation of surface of callus per quarter. Take quarter 1 for example. ▬ = callus.

Fig. 3: Numeric calculation of amount of callus per quarter. Take quarter 1 for example. —, ▬, ▬ = callus.

Abb. 2: Schema für die Bonitierung der Kallusoberfläche eines Quadranten am Beispiel von Quadrant 1. ▬ = Kallus.

Abb. 3: Schema für die Bonitierung der Kallusmenge eines Quadranten am Beispiel von Quadrant 1. —, ▬, ▬ = Kallus.

Table 1

Effect of hot-water treatments on callusing and bud-burst of one-year-old cuttings of Cabernet Sauvignon

Einfluß der Warmwasserbehandlung auf Kallusbildung und Austrieb einjähriger Stecklinge von Cabernet Sauvignon

Treatment		Bud-burst: Number out of 20 cuttings after 21 d	Callus formation: Average for 20 cuttings out of 28 possible points after 14 d	
Temperature °C	Interval min		Apical	Basal
Control		20	19.2	24.8
45	1	20	18.1	23.1
	5	20	18.4	23.9
	10	20	22.8	24.1
	30	20	20.5	24.4
	60	20	22.9	26.6
50	1	20	18.6	25.0
	5	20	19.5	23.9
	10	20	19.8	25.6
	30	20	23.8	26.8
	60	20	18.2	25.0
55	1	20	16.8	24.0
	5	20	20.7	24.6
	10	20	14.5	22.6
	30	18	7.0	16.3
	60	16	6.4	14.0
60	1	20	26.3	27.0
	5	18	14.0	15.5
	10	10	7.4	14.4
	30	0	0.0	0.0
	60	0	0.0	0.0
65	1	20	9.0	12.6
	5	0	0.0	0.0
	10	0	0.0	0.0
	30	0	0.0	0.0
	60	0	0.0	0.0

In the first experiment 20 cuttings were submerged for each treatment. The control received no hot-water treatments.

To investigate the effects of hot-water treatments on sprouting and growth (experiment 2), one-year-old grafts (Cabernet Sauvignon/99 R.) were removed from the nursery. Twenty grafts were included for each treatment. The temperatures and

Table 2

Effect of hot-water treatments on sprouting and growth of one-year-old grafts. Cabernet Sauvignon/99 R.

Einfluß der Warmwasserbehandlung auf Austrieb und Wachstum einjähriger Pfropfreben. Cabernet Sauvignon/99 R.

Treatment		Sprouting and growth:
Temperature °C	Interval min	Number out of 20 grafts after 60 d
Control		20
45	1	20
	5	20
	10	20
	30	20
	60	20
50	1	20
	5	20
	10	20
	30	20
	60	20
55	1	20
	5	20
	10	20
	30	18
	60	14
60	1	18
	5	10
	10	6
	30	0
	60	0
65	1	12
	5	0
	10	0
	30	0
	60	0

exposure times for these treatments were the same as for experiment 1. Entire vines were submerged for the required intervals. Following cooling in cold water after each treatment, the grafts were planted in the vineyard. After planting, they were pruned so as to leave at least 2 buds on each cane.

Results were tabulated after 60 d. Only those with a vigorous growth-rate were included in the results. The control received no hot-water treatments.

Results and discussion

The results (Table 1) show no inhibition of callus formation at water temperatures of 50 °C and below, up to exposure times of 60 min. At 55 °C callus formation was inhibited after exposure to hot water for longer than 10 min, in spite of not severe damage to bud-burst activities. Although immersion in hot water at a temperature of 60 °C for 1 min did not inhibit callusing, longer immersion seemed to be inhibiting. Higher temperatures (above 60 °C) showed inhibiting effects on callus formation. Although bud-burst (Table 1) was not inhibited and cut out with immersion for 1 min at 60 and 65 °C, longer intervals did show inhibiting effects. These results seemed to be in line with some other workers (Lear and Lider 1959, Goheen *et al.* 1973) in connection with heat tolerance of grape material.

Although no specific results were recorded in connection with promoting effects of hot-water treatments on callusing, it seemed that the process was hastened with certain treatments. Also, the amount of callus formed from certain treatments (for example 50 °C for 30 min and 60 °C for 1 min) showed a promoting effect, when compared with the control. Bud-burst was also hastened by nearly all treatments, except at 60 °C for 30 and 60 min and 65 °C for 5, 10, 30 and 60 min. It was found that the cuttings that showed no signs of callus and bud-burst were unable to survive the high temperatures.

In experiment 2 (Table 2) it was found that high temperatures and long exposure times, for instance 55 °C for longer than 10 min and 60 and 65 °C (all intervals), were inhibiting on sprouting and growth. Grafts that showed sprouting at these treatments were lacking in overall growth. All grafts that showed no bud-burst after 60 d were found to have died. It was further observed that, where grafts showed vigorous growth, the callus-tissue was unaffected by hot-water treatments. These results connected with those noted by other workers (Goheen *et al.* 1973).

Summary

One-year-old grape cuttings were submerged in hot water at temperatures of 45, 50, 55, 60 and 65 °C respectively. Intervals for each temperature range were 1, 5, 10, 30 and 60 min. Callusing was inhibited at temperatures of 55 °C for longer than 10 min. Optimum callusing was obtained at 50 °C for 30 min and 60 °C for 1 min. Bud-burst was also inhibited at higher temperatures and longer exposure times (above 55 °C with intervas longer than 1 min). One-year-old grafts could not survive temperatures above 60 °C at immersion times of longer than 1 min.

Acknowledgement

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P. G. GOUSSARD
Dept. of Viticulture
University of Stellenbosch
Stellenbosch 7600
Republic of South Africa