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Rooting and carbohydrate availability in *Vitis* 140 Ruggeri stem cuttings

by

G. Bartolini[1]), P. Pestelli[1]), M. A. Toponi[2]) and G. Di Monte[2])

[1]) Institute for the Propagation of Woody Plants, CNR, Scandicci, Firenze, Italia
[2]) Institute of Plant Biochemistry and Ecophysiology, CNR, Monterotondo Scalo, Roma, Italia

Summary: Rooting in *Vitis* 140 Ruggeri rootstock appears to be correlated to the availability of soluble carbohydrates in the cuttings. This relationship was verified using stem cuttings collected from December to April by comparing those propagated after cold storage (C) with those collected directly in the field (F). The basal end of some of the cuttings of both groups (C and F) were dipped into deionized water before propagation (CW and FW). The mobilization of soluble carbohydrates paralleled with the pattern of the rooting process. In the cold stored material, rooting took place earlier than in the field material. The mobilization of soluble carbohydrates occurred at two stages: during the storage period at 2 °C (50 % if compared with the cuttings collected in the field) and during the first 20 d of rooting. On day 20, the carbohydrate content was reduced by 80 %. Rooting and carbohydrate availability appear to be associated.

Key words: carbohydrates, rooting, cold, Vitis, rootstock 140 Ruggeri.

Introduction

The formation of adventitious roots has been studied since the beginning of this century. However, there is still much controversy with regard to the nutritional requirements of the root (Veierskov 1988; Haissig and Davis 1994).

Rooting ability depends on genetic characteristics of the plant (Haissig and Riemenschneider 1988), environmental conditions (Levitt 1980; Sakai and Larcher 1987; Kurkela *et al.* 1988; Moe and Andersen 1988; Pearce *et al.* 1990), the exogenous supply of hormones and endogenous hormonal variations (Bartolini *et al.* 1986). In some cases, the ability to root can change over the years in the same plant and in the same species (Haissig and Davis 1994).

It appears that morphogenetic effects must be accompanied by chemical and physical cofactors, which also interfere with the initial hormonal induction (Bartolini *et al.* 1991).

Many experiments have indicated that the source of carbohydrates is critical for rooting giving that, after auxin induction in tissue cultures, there is very reduced photosynthesis (Gautheret 1966; Thorpe 1974).

Veierskov *et al.* (1982) and Veierskov (1988) studied the rooting response in *Pisum* cuttings by changing the light cycle or by supplying carbohydrates to the stock plants.

Altman and Wareing (1975) and Okoro and Grace (1976) showed the influence of leaves on the rooting process as a result of their interference with carbohydrate metabolism.

This paper reports the influence of different experimental approaches on both the rooting ability and the availability of soluble carbohydrate of *Vitis* rootstock 140 Ruggeri cuttings.

Material and methods

Material and experimental conditions: Cuttings from the rootstock 140 Ruggeri (*V. berlandieri* x *V. rupestris*), a difficult-to-root cultivar, cloned from mother plants, were studied. Four groups of cuttings were compared:

Group I (cold-stored - C): cuttings of canes were collected in October and cold-stored at 2 °C in a cold chamber (polythene container) and treated with a fungicide (Benomil).

Group II (in the field - F): cuttings were taken monthly directly from the field.

Group III (C + dipping - CW) and IV (F + dipping - FW): cuttings were taken from the two different groups C and F. Before rooting the basal end (2 cm) of each sample was placed in deionized water (10 ml per cutting for 24 h) at 18 °C, according to Kawase (1971).

The cuttings (100 per treatment; 5 replicates) were kept in a greenhouse and rooted in a perlite bed with basal heating (18-20 °C) and moistened by mist (5 s every 2 h) until day 60.

The percentage of the rooted cuttings was recorded for all four groups and samples for carbohydrate analysis were collected on days 20, 40 and 60 after rooting.

Carbohydrate analysis: The last 2 cm of the basal end of cuttings of the 4 groups were used. Samples were freeze-dried at -50 °C, stored at -20 °C and sub-

Correspondence to: Dr. G. Bartolini, Institute for the Propagation of Woody Plants, CNR, Via Ponte di Formicola, 76, I-50018 Scandicci (Firenze), Italy. Fax: 39-55-755121.

sequently ground and extracted with ethanol (75 %) at pH 7 at room temperature, 3 times over a 24 h period. The extract was filtered through a BIO-REX 5. The carbohydrate content was analyzed 3 times using HPLC, with a Waters Sugar-Pak 1 column, water as mobile phase. The flow rate was 0.5 ml/min and the temperature was 90 °C. A refractive index detector was employed. Sucrose, glucose and fructose were identified according to retention times of sugar standards (ROMANI *et al.* 1994).

Rooting and carbohydrate values were calculated using Tukey's test ($P<0.05$). The amount of carbohydrates was expressed as mg g^{-1} dry weight. All measurements were repeated 3 times.

Results

Observations during the period September-February 1991

Percentage of rooting: Fig. 1 a shows the rooting ability of cuttings collected from November to April. The rooting values ranged from 80 to 95 % on day 60; there were no significant differences among the groups. On day 40, the cuttings of all groups collected in January had not rooted. Those collected in February and March rooted earlier than the cold-treated cuttings.

Variations in soluble carbohydrates: Fig. 1 b shows the amount of glucose + fructose present in the basal end of cuttings at the time when cuttings were prepared from the samples collected from November to April. The material collected directly in the field (F and FW) from December to February showed values which were 25 % higher than those of the cold-stored (C and CW) samples. Dipping (CW and FW) did not affect this pattern.

Fig. 1 c shows the amount of sucrose in cuttings collected from November to April. The pattern appeared to be the same as shown in Fig. 1 b. However, the highest sucrose values were noted in January/February for both types of cuttings, i.e. those collected directly in the field and those collected from cold-stored material, but the values of the stored material were 25 % higher.

In Figs. 1 b and c, the values of C and CW decreased from December to March. This can possibly be explained by the earlier onset and end of the dormancy period of the cold-stored cuttings.

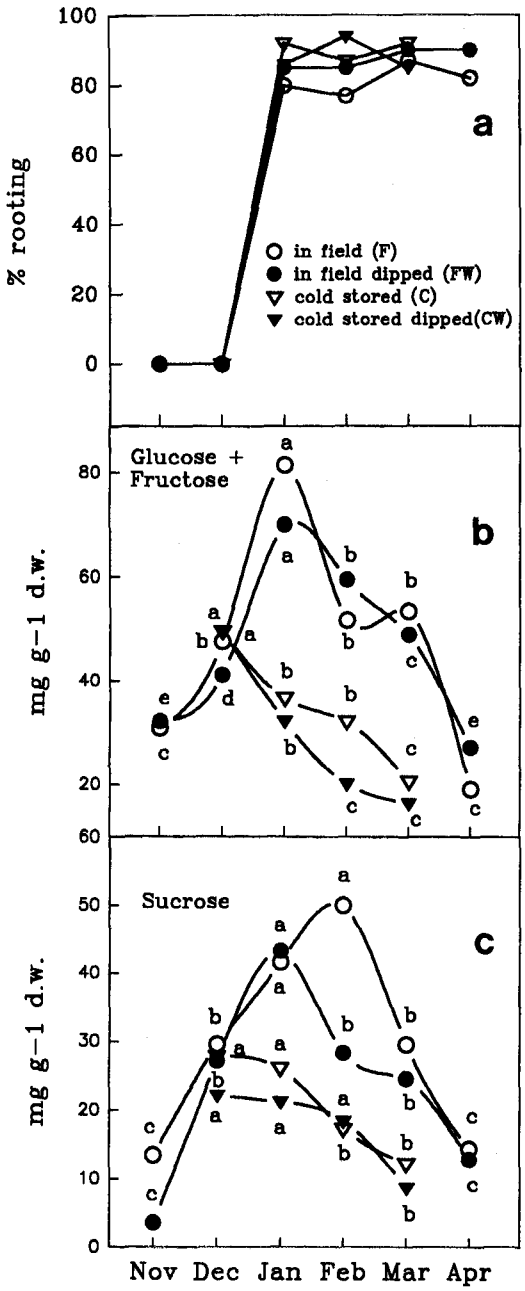


Fig. 1: Rooting ability and carbohydrates of cuttings. The data were recorded after 60 d of basal heating (data not significant among treatments). Treatments: collected in the field (F: ○), collected in the field and dipped (FW: ●), collected from cold-stored material (C: ▽), collected from cold-stored and dipped material (CW: ▼). **a**: rooting ability as percent of cuttings collected from November to April. **b**: Amount of glucose + fructose in cuttings collected from November to April. The letters correspond to $P<0.05$ for each treatment. **c**: Amount of sucrose in cuttings collected from November to April. The letters correspond to $P<0.05$ for each treatment.

Observations during the rooting period

To evaluate the relationship between rooting ability and variations in extractable carbohydrates (glucose + fructose and sucrose) the material collected in December, January and February was chosen given that it had undergone the period of dormancy and after-dormancy.

Percentage of rooting: Fig. 2 (F, C, FW and CW) shows the rooting of cuttings in December, January and February during a period of 60 d in a perlite bed. In December the material did not root at all. The cuttings prepared from cold-stored material started rooting in January. The dipping treatment appeared to enhance the process slightly.

In the material collected in December, the rooting took place after 110 d and only in a small number of samples. After day 40 only cold-stored cuttings showed an increase of the rooting ability.

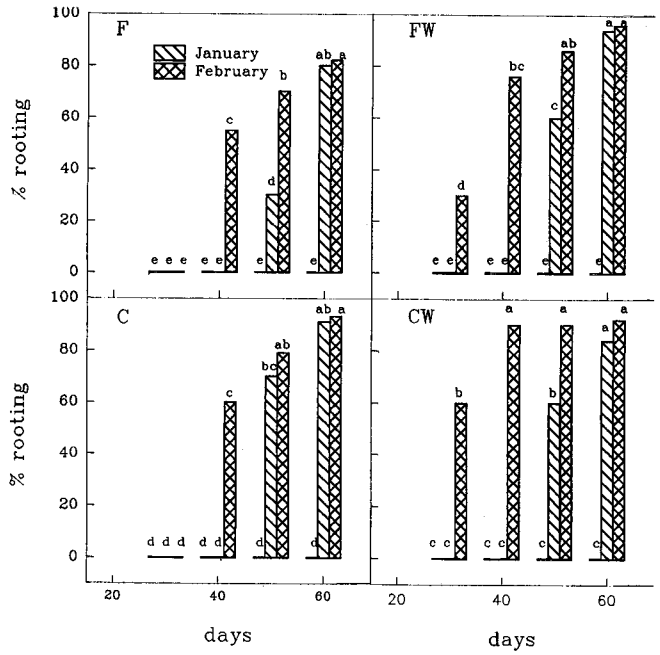
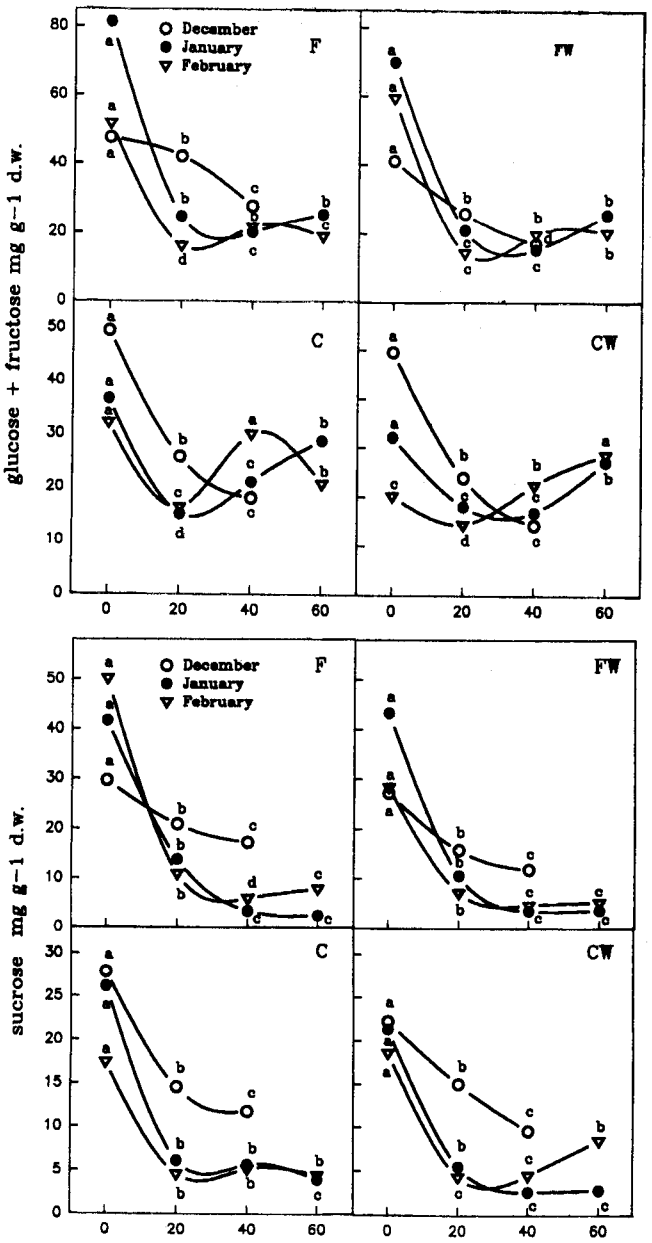


Fig. 2: Rooting ability of cuttings collected in December (—), January and February, during a period of 40 d in a perlite bed. Note that the cold-stored (C) and cold-stored and dipped (CW) samples started rooting earlier than others. The dipping treatment appeared to induce negligible differences in the rooting process. For treatments: see Fig. 1. The letters correspond to P<0.05 for each treatment.

Variations in soluble carbohydrates: Fig. 3 and 4 shows that at the onset of the rooting period the amounts of glucose + fructose and sucrose in the cold-stored material were significantly lower than those in the cuttings collected monthly directly in the field. There were no significant differences between the two with regard to the dipping treatment in water.

During the first 20 d of the rooting period, there was a general reduction of soluble carbohydrates in all samples, subsequently reaching the same value (20 mg g^{-1} d.w.) (Figs. 3 and 4).



Figs. 3 and 4: Amount of glucose + fructose (above) and sucrose (below) of cuttings during the rooting period; collected in December (○), January (●) and February (▽) from material collected in the field (F and FW) and collected from cold-stored material (C and CW). The letters correspond to P<0.05 for each treatment.

Discussion

The response of the cuttings, collected from December to April, to the different treatments confirm that rooting is correlated to the large amount of soluble carbohydrates which suddenly decreases at the end of the dormancy period. During the first 20 d, the decrease of carbohydrates appears to correspond to the reactivation of the tissues leading to the formation of primordia.

Cold storage appeared to tigger the mobilization of carbohydrates. After day 20, the amount of carbohydrates was similar in cold-stored cuttings and in those collected in the field. The mobilization of soluble carbohydrates in the cold-stored cuttings appeared to occur in two periods: during the treatment of the material and during the first 20 d of the rooting period.

The first mobilization of the carbohydrates within the cold-stored material induced a reactivation in the tissues. Thus, an earlier onset of the rooting process was possible when compared to those collected directly in field. The formation of meristemoids took place around day 10 and root primordia formation continued until day 40.

The increases in carbohydrate availability after day 30 were probably related to shoot formation of the cuttings and to root growth. The availability of carbohydrates may induce some anomalies in the developmental pattern.

Therefore, we conclude that a large amount of carbohydrates is essential to initiate and accompany the rooting process. During this process, the carbohydrates act as a source of energy and as constitutive elements for the newly-formed cells.

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Literature

ALTMAN, A.; WAREING, P. F.; 1975: The effect of IAA on sugar accumulation and basipetal transport of ^{14}C-labelled assimilates in relation to root formation in *Phaseolous vulgaris* cuttings. Physiol. Plant. **3**, 32-38.

BARTOLINI, G.; TOPONI, M. A.; SANTINI, L.; 1986: Endogenous GA-like substances in dipping waters of cuttings of two cv. *Vitis* rootstocks. Amer. J. Enol. Viticult. **37**, 1-6.

- -; - -; - -; 1991: Propagation by cuttings of two *Vitis* rootstocks: Diffusion of endogenous phenolic compounds into the dipping waters. Phyton **52**, 9-15.

GAUTHERET, R. J.; 1966: Action des basses températures sur les phénomènes de rhizogenèse manifestés par les tissus de Topinambour cultivés *in vitro*. C. R. Acad. Sci., Paris **262**, 2153-2155.

HAISSIG, B. E.; DAVIS, T. D.; 1994: A historical evaluation of adventitous rooting research to 1993. In: DAVIS, T. D.; HAISSIG, B. E. (Eds.): Biology of Adventitous Root Formation, 275-331. Plenum Press, New York.

- -; RIEMENSCHNEIDER, D. E.; 1988: Genetic effects on adventitous rooting. In: DAVIS, T. D.; HAISSIG, B. E.; SANKHLA, N. (Eds): Adventitous Root Formation in Cuttings, 47-60. Discorides Press, Portland.

KAWASE, M.; 1971: Diffusible rooting substances in woody ornamentals. J. Amer. Soc. Hort. Sci. **96**, 116-119.

KURKELA, S.; FRANCK, M.; HEINO, P.; LANG, V.; PALVA, E. T.; 1988: Cold induced gene expression in *Arabidopsis thaliana* L. Plant Cell. Rep. **7**, 495-498.

LEVITT, J.; 1980: Responses of Plants to Environmental Stresses (2nd ed.), 185-191. Acad. Press, New York.

MOE, R.; ANDERSON, A. S.; 1988: Stock plant environmental and subsequent adventitious rooting. In: DAVIS, T. D.; HAISSIG, B. E.; SANKHLA, N. (Eds.): Adventitous Root Formation Cuttings, 214-234. Discorides Press, Portland.

OKORO, O. O.; GRACE, J.; 1976: The physiology of rooting Populus cuttings. I. Carbohydrate and photosynthesis. Physiol. Plant. **36**, 133-138.

PEARCE, R. S.; HAWTHORN, P. H.; RYLE, G. J. A.; 1990: A carbon 14 study of metabolism in tall fescue seedlings acclimating to growth at low temperatures. J. Exp. Bot. **41**, 1393-1404.

ROMANI, A.; BALDI, A.; TATTINI, M.; VINCIERI, F. P.; 1994: Extraction, purification procedures and HPLC-RI analysis of carbohydrates in olive (*Olea europaea* L.) plants. Chromatographia **39**, 35-39.

SAKAI, A.; LARCHER, W.; 1987: Frost Survival of Plants. Responses and Adaptations to Freezing Stress. Springer Verlag, Berlin.

THORPE, T. A.; 1974: Carbohydrate availability and shoot formation in tobacco callus cultures. Physiol. Plant. **30**, 77-81.

VEIERSKOV, B.; 1988: Relations between carbohydrates and adventitious root formation. In: DAVIS, T. D.; HAISSIG, B. E.; SANKHLA, N. (Eds.): Adventitious Root Formation in Cuttings, 70-78. Discorides Press, Portland.

- -; ANDERSEN, A. S.; STUMMANN, B. M.; HENNINGSEN, K. W.; 1982: Dynamics of extractable carbohydrates in *Pisum sativum*. II. Carbohydrate content and photosynthesis of pea cuttings in relation to irradiance and stock plant temperature and genotype. Physiol. Plant. **55**, 174-178.

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