PHOTOSYNTHATES: MAINLY STORED AND YET LIMITING IN PROPAGATION OF ROSE CUTTINGS

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Abstract

Leaves are essential in the propagation of roses by cuttings. However, about the underlying principles, there is no unequivocal opinion in the literature. We investigated the hypothesis that current photosynthesis would represent the dominating factor. To validate this hypothesis variations in photosynthesis were created by reducing leaf area, covering of leaves and by reducing CO_2 air concentration. The consequences in terms of survival percentage, root formation, and carbohydrate concentrations were followed throughout the first 3 weeks of propagation. Moreover, the known promoting effect of auxins was studied in a comparative experiment to investigate potential auxin deficiencies created by the leaf treatments.
Leaf removal, leaf covering or CO_2 depletion had a quantitative negative effect on survival and growth of cuttings. Auxins did not promote rooting when leaves were covered or removed. Dry matter distributed uniformly by the leaf and stem of cuttings after 21 days of propagation. Unexpected, and perhaps contradictory, was that, although current leaf photosynthesis seemed the limiting factor for propagation, the majority of photosynthates was stored as starch. This apparent ineffective use of photosynthates could be explained in terms of competition within different sinks. The weak sink of starch accumulating organelles becomes the major sink relatively to the modest sink activity of the new roots and new shoots. In fact, the new formed organs accounted for less than 15% of the total dry weight of cuttings.
It is concluded that photosynthetic capacity of cuttings is to be considered an important determinant for quality in rose propagation. Further research should confirm the relationship between photosynthesis and its value as criterion for quality in rose propagation.

1. Introduction

Rose production in the Netherlands occupies an area of approximately 950 ha and generated in 1999 a total turnover of about 390 million U.S. Dollars. About 80% of this area is made in substrate, like rockwool or coconut peat. The Dutch rose propagation industry is based on the activity of 8 major propagators who, in total, are responsible for propagating every year 20 million young plants, for both local and external markets. The annual turnover of the propagation sector is around 28.5-30.4 million U.S. Dollars (Zuurbier, personal communication). The most usual propagation techniques are "stenting" (Van de Pol and Breukelaar, 1982) and softwood leafy cuttings.

During the last few years high levels of success have been achieved in the sector. Still, losses occur, and non-homogeneous material is produced. More than only clean and variety certified planting material, growers aim for homogeneity and fast establishment to

Proc. III IS Rose Research
Eds. N. Zieslin & H. Agbaria
Acta Hort. 547, ISHS 2001

minimise initial losses.

Leaves have a very important role on the growth potential of cuttings. Leaves influenced rooting and further growth of rose cuttings (Moe, 1973; Dubois and De Vries, 1985, 1991) as well of other species (Reuvani and Raviv, 1981; Leakey and Coutts, 1989; Newton *et al*., 1992).

Current photosynthates were a limiting factor for rooting of pea cuttings (Davis and Potter, 1981) and current photosynthesis during propagation sustains a positive carbohydrate balance that favours survival and rooting of cuttings (Yue and Margolis, 1993; Hoad and Leakey, 1996).

In this paper the role of leaves, and more specifically of photosynthesis and carbohydrates, on rooting and further growth of rose leafy softwood cuttings is described. The effect of photosynthesis on rooting and growth of cuttings was tested by suppressing photosynthesis either by leaf removal, light exclusion (leaf covering) or low CO_2 concentration. Leaf removal and covering were also combined with auxin application to test the relevance of hormonal treatments, under limited supply of photosynthates. Photosynthesis was measured at different moments of propagation. Results should increase the knowledge on the role of photosynthesis in rose propagation and can be used to better control and predict success in rose propagation.

2. Material and methods

2.1. General

Mother plants of *Rosa hybrida* Madelon® were grown in substrate in greenhouse and climate chamber facilities. Day/night temperature was set at 20/18°C in both cases. In the greenhouse, supplementary light was applied using high pressure sodium lamps (Philips SON-T plus 400W, 36.5 µmol m^{-2} s^{-1}) when global radiation outside the greenhouse dropped below 100 Wm^{-2}. Day length was 18 hours. In the climate chamber high pressure sodium lamps (Philips SON-T Agro 400W) and mercury lamps (Philips HPI-T 400W) provided a light intensity of 35-40 Wm^{-2} (170-190µmol $m^{-2}s^{-1}$) PAR at the top of the canopy during 16 hours. Relative humidity was set at 65-70% in the climate room.

Cuttings were collected from shoots bearing flower buds with visible colour and reflexing sepals. The cuttings were cut from the middle of the shoot and consisted of an internode plus a node with a dormant axilar bud and a leaf of five intact leaflets. Cuttings were propagated also in greenhouse and climate chamber facilities. In greenhouse we used rooting benches (200x250x35cm) (l,w,h) covered with a double layer acrylic cover, whereas in the climate chamber we used small plastic propagators (57x37x23cm) (l,w,h) with a plastic cover. Natural light in the greenhouse propagation compartment was supplemented by Philips SON-T 70W lamps that provided a minimum intensity of 3.5 Wm^{-2} (17.5 µmol $m^{-2}s^{-1}$) at cuttings level. Day length was 18 h. In the climate chamber, high pressure sodium lamps (Philips SON-T Agro 400W) and mercury lamps (Philips HPI-T 400W) provided about 17 Wm^{-2} (83 µmol $m^{-2}s^{-1}$) PAR at cuttings level during 16 hours. In the greenhouse, day/night air temperatures were set at 21°C and 20°C respectively. In the climate chamber, temperature was set at 25°C. Air humidity in the rooting benches or propagators was kept very high by periodic hand misting. Rooting substrate was a mixture of peat and sand 1:1 (v/v), bottom heated at temperatures ranging between 23 for the greenhouse facilities and 26°C for the climate chamber.

2.2. Experiments and measurements

2.2.1. Experiments 1a and 1b. Leaf removal and leaf covering in combination with auxin (IBA) treatments

Experiment 1a started on 3 September 1997 and consisted of 3 treatments: 1) five leaflets cuttings, 2) five leaflets cuttings with the leaf covered by aluminium foil and 3)

leafless cuttings. Cuttings were observed on days 4, 8 and 12 of propagation.

Experiment 1b started on 25 February 1999 and observed 6 treatments: 1) five leaflets cuttings, 2) five leaflets cuttings treated with auxin 3) covered leaf cuttings, 4) covered leaf cuttings treated with auxin, 5) leafless cuttings and 6) leafless cuttings treated with auxin. The auxin was applied to cuttings by dipping them in a commercial talcum powder preparation with 0.4% IBA (v/v) (Rhizopon, the Netherlands). Cuttings were observed on day 0, 7 and 14 of propagation.

Measurements: Number and dry weight of roots, dry weight of the axilar primary shoot, stem, leaves and total cutting dry weight were determined. Black rot incidence (percentage of cuttings with stem rot) was quantified.

Experimental design: Both experiments used a randomized complete block design. Experiment 1a had 4 blocks, each containing 9 plots of 5 cuttings. Experiment 1b had six blocks, each containing 18 plots with one cutting per plot. The treatments were distributed at random by the plots.

2.2.2. Experiments 2a and 2b. Carbohydrates dynamics under standard propagation conditions and after reducing leaf area

Experiment 2a started on 26 February 1996 and observed cuttings with five leaflets, two leaflets (most proximal), and leafless cuttings on days 0, 3, 7 and 11 of propagation. Experiment 2b started on 23 April 1998 and observed five leaflets cuttings on days 0, 7, 14 and 21 of propagation.

Measurements: Number and dry weight of roots, dry weight of the basal 1.5 cm part of the stem (stem rooting zone) and upper stem and leaves were, measured. Black rot incidence was recorded in Experiment 2a. Glucose, fructose, sucrose and starch were analysed from freeze dried leaves, the stem rooting zone, the upper part of the stem and from roots. Experiment 2a observed only the rooting zone. Soluble carbohydrates were determined by high performance liquid chromatography (HPLC) using a Dionex system equipped with a detector DIONEX PED and column DIONEX PA-1 using NaOH (100 mM) as eluente, at a flow rate of 1 ml min^{-1} at 25°C. The remaining insoluble pellet from extraction process was used for enzymatic determination of starch with a termostable alpha-amylase (Serva, Germany) and amyloglucosidase (Boehringer Mannheim, Germany).

Experimental design: Both experiments used a randomised complete block design. Experiment 2a had 4 blocks, each with 12 plots whereas Experiment 2b had 10 blocks each with 4 plots of one cutting. Treatments were applied at random to the plots.

2.2.3. Experiment 3. Rooting and dry weight accumulation under low CO_2 air concentration

Experiment 3 started on 27 July 1999. Cuttings were propagated under normal (300-350 ppm) and very low (80-120 ppm) CO_2 concentration.

Measurements: Number and dry weight of roots and total dry weight of cuttings was measured during the first 21 days of propagation.

Experimental design: The experiment used a split plot design with two blocks, with different CO_2 concentrations as the main plots and the time of observation as subplots. Each subplot contained one cutting. Six cuttings were used per treatment.

2.3. Statistical analysis

Data were analysed by calculating the standard error (SE) of the mean from all the replicates. Analysis of variance ($P<0.05$) was performed using the statistical package GENSTAT, and mean separation was made calculating the least significance difference (*t*-test).

Proc. III IS Rose Research
Eds. N. Zieslin & H. Agbaria
Acta Hort. 547, ISHS 2001

3. Results

Leaf removal and leaf covering promoted stem rot incidence and inhibited rooting and dry weight accumulation in rose cuttings (Table 1). Lowering CO_2 concentration to 80-120 ppm reduced rooting and dry weight accumulation on day 21 by about 50% (data not presented). Auxin promoted rooting when leaves were present, but not, when leaves were covered or removed (Table 2). Moreover, auxin application fastened black rot incidence in cuttings with the leaf covered or removed (Table 3).

Dry weight and carbohydrates increased in leafy cuttings during propagation (Table 4). Leaves and stems had about the same dry weight after 21 days. Roots and the primary shoot represented less than 15% of the total cutting dry weight (Table 4), Dry weight increase was mostly attributed to the accumulation of carbohydrates (Table 4). In the upper stem, for example, about 71% of the dry weight increase was attributed to the increase of the TNC. The great majority of the photosynthates was stored as starch in both stem and leaves (Table 4). Carbohydrate accumulated mostly in the cuttings with higher number of leaflets (Figure 1). On the other hand, carbohydrates decreased strongly in leafless cuttings till day 11 (Figure 1). Leafless cuttings were affected by stem rot (data not presented).

4. Discussion and conclusions

The effect of leaves on rooting via photosynthesis and carbohydrate synthesis was studied for rose softwood cuttings. Suppression of current photosynthesis by either leaf covering, leaf removal or low CO_2 decreased rooting and dry weight accumulation. This indicates the strong regulating effect of current photosynthesis on propagation of rose softwood cuttings and is in accordance with results found for other species propagated by leafy cuttings (Davis and Potter, 1981; Leakey and Coutts, 1989; Newton *et al*, 1992; Hoad and Leakey, 1996). The fact that auxin had no promoting effect on rooting when leaves were absent or covered (Table 2) supports our hypothesis that photosynthesis and photosynthates are the prime factor regulating survival and rooting, and not the capacity of leaves to supply hormones (auxins).

Unexpected, and apparently contradicting to what was previously stated, was the fact that current photosynthesis was strongly driven to storage (Table 4). Contrary to the observation in intact plants, that photosynthesis is converted in growth, cuttings accumulate starch during the first 21 days of propagation. A possible explanation for that phenomenon in cuttings resides in limited sink activity as suggested in literature (Humphries and Thorne, 1964; Feldman *et al*., 1989). In fact, we may consider that an intact rose plant has 2 major sinks: the shoot and the root system. After severance, these sinks are removed and new sinks, new roots and a new axilar shoot, have to be formed. The remaining weak sinks, stem growth and starch storage, became major sinks, much stronger than the root initials or the primary shoot. This could explain that current photosynthesis is still needed for rooting, although the new formed roots and primary shoot represented less that 15% of the total cutting dry weight after 21 days of propagation.

Cuttings were photosynthetically active since the first days of propagation and contributed for dry weight (carbohydrate) accumulation. Net photosynthesis levels maintained constant during the 21 days of propagation. On average, those values were 0.09 mg CO_2 m^{-2} s^{-1} (unpublished own results).

Photosynthesis arises, as one of the most important limiting factors of rooting of rose softwood cuttings, and therefore, on the quality of rose starting material. Optimal control of photosynthesis during rose propagation would permit faster rooting and higher use efficiency of propagation facilities. Also, faster rooting, reduces the possibility for variation caused by external factors like diseases, temperature or light. To finalise, the recent developments in propagation towards more environmental friendly methods (e.g. excluding auxin treatments) (Kunneman, 1999) make the role of leaves in propagation

even more important, as rooting and growth potential depend strictly on them.

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Tables

1. Effect of leaf covering and leaf removal on survival (black rot incidence), root formation and fresh and dry weight accumulation in cuttings of *Rosa hybrida* 'Madelon' after 12 days of propagation in Experiment 1a. Data are means followed by the SE (n=19).

Treatment	Black rot incidence %	Number Roots (mg)	D.W. Roots (mg)	D.W. Primary shoot (mg)	Total cutting DW (mg)
Control (5 leaflets)	0	5.8 ± 0.9	7.8 ± 1.5	4.4 ± 2.2	817 ± 18
Covered	70	0	0	0	420 ± 16
Leafless	95	0	0	0	-[1]

[1] data not determined

2. Combined effect of leaf covering and auxin (IBA 0.4%) application on the number of roots on cuttings of *Rosa hybrida* Madelon ® during the first 14 days of propagation in Experiment 1b. Data are means followed by the SE (n=6).

Treatment	Number of roots			
	Day 0	Day 7	day 11	Day 14
Five leaflets	-	-	4.7 ± 2.3	6.0 ± 1.3
Leaf covered	-	-	-	-
Leafless	-	-	-	-
Five leaflets + IBA	-	0.8 ± 0.5	13.5 ± 2.4	16.8 ± 3.2
Leaf covered + IBA	-	-	-	-
Leafless + IBA	-	-	-	-

3. Combined effect of leaf covering or leaf removal and auxin (IBA 0.4%) application on black rot incidence on cuttings of *Rosa hybrida* Madelon ® during the first 14 days of propagation in Experiment 1b.

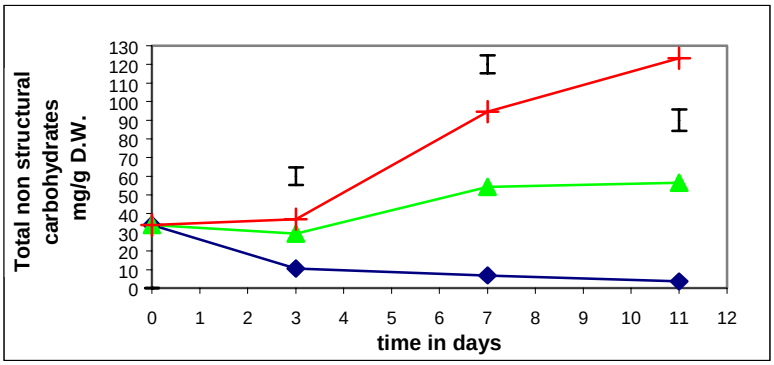
Treatment	Black rot incidence (%)			
	Day 0	Day 7	day 11	Day 14
Five leaflets	-	-	-	-
Leaf covered	-	-	-	-
Leafless	-	-	17	50
Five leaflets + IBA	-	-	-	-
Leaf covered + IBA	-	-	32	83
Leafless+IBA	-	50	50	100

4. Concentration of starch and total non-structural (TNC) carbohydrates (glucose, fructose, sucrose and starch) and dry weight from the leaves, basal 1.5. cm stem, upper stem and roots of cuttings of *Rosa hybrida* Madelon ®, after 21 days of propagation in Experiment 3b.

Cutting Part	Dry weight (mg)		TNC (mg/g D.W.)		Starch (mg/g D.W.)	
	Day 0	Day 21	Day 0	Day 21	Day 0	Day 21
Leaves	354	604	114	295	26	200
Upper stem	280	416	37	258	7	221
Basal 1.5 cm Stem	104	193	32	181	6	143
Root	0	93	-	90	-	5
Shoot	0	90	-	180	-	23

Proc. III IS Rose Research
Eds. N. Zieslin & H. Agbaria
Acta Hort. 547, ISHS 2001

Figures



1. Total concentration of non-structural carbohydrates at the stem rooting zone of leafless (—◆—), two leaflets (—▲—), and five leaflets (—✚—) cuttings of *Rosa hybrida* Madelon ® on days 3, 7, and 11 of propagation. Vertical bars indicate $LSD_{0.05}$.