THE ROLE OF THE LEAF IN GROWTH DYNAMICS AND ROOTING OF LEAFY STEM CUTTINGS OF ROSE

Joaquim Miguel Rangel da Cunha Costa

Promotoren:

Prof. dr. ir. H. Challa

Hoogleraar in de Tuinbouwbedrijfstechnologie

Prof. dr. M.T.M. Willemse

Hoogleraar in de Plantkunde

Co -promoters

Dr. ir. U. Van Meeteren, Universitair docent, Leerstoelgroep Tuinbouwproductieketens

Dr. ir. P.A. van de Pol, senior onderzoeker, PRO Plant Research Overberg

Dr. ir. C. J. Keijzer, Universitair docent, Leerstoelgroep Plantenfysiologie

Leden van de promotiecommissie:

Prof.dr. R. Moe, Landbouwuniversiteit van Noorwegen

Prof. dr. J. L. Van Went , Wageningen Universiteit

Mw. Dr. ir M.C. van Labeke, Proefcentrum voor Sierteelt, België

Dr. ir. H.J. van Telgen, Plant Pratijk Onderzoek Aalsmeer

THE ROLE OF THE LEAF IN GROWTH DYNAMICS AND ROOTING OF LEAFY STEM CUTTINGS OF ROSE

Joaquim Miguel Rangel da Cunha Costa

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. dr. ir. L. Speeleman
in het openbaar te verdedigen
op maandag 16 September 2002
des namidags te vier uur in de Aula

Costa JMRC, 2002
The role of the leaf in growth dynamics and rooting of leafy stem cuttings of rose

Thesis of Wageningen University – With Ref. - With summaries in English, Dutch, Portuguese and Spanish
ISBN 5808-687-9

Abstract

Costa, JMRC (2002) The role of the leaf in growth dynamics and rooting of leafy stem cuttings of rose, PhD thesis, Plant Sciences Group, The Netherlands. With English, Dutch, Portuguese and Spanish summaries.

The present study aims at better understanding the relation between photosynthesis of the original leaf, carbohydrates, rooting and growth of single node leafy stem cuttings of rose. This knowledge can be used to improve success and efficiency in propagation and improving the uniformity of the planting material of cut roses derived form cuttings.

The effects of the original leaf area on the growth of cuttings of *Rosa hybrida* Madelon® were investigated during the first 10 weeks after severance. Total plant dry weight, and dry weight of the roots in particular, were proportionally related with the original leaf area of cuttings. When leaf area was modified, leaf area duration was linearly related to the rooting and growth of cuttings during the first 21 days of propagation. The presence of the leaf during the first week of propagation was critical for survival and its removal caused stem rot. This was caused by low carbohydrate concentrations.

Cuttings remained photosyntheticaly active after severance. Photosynthetic rates decreased immediately after severance, but recovered up to 70% of the rates measured on leaves on mother plants and remained constant during propagation. The PSII efficiency decreased during propagation with a simultaneous increase in its heterogeneity across the leaflets (patchiness) which may be attributed to decreased sink activity rather then to water stress. The root and shoot tissues accounted for about 70% of the increase in total fresh weight after 21 days of propagation, whereas the remaining 30% increase was due to dry weight accumulation in the leaf and stem. About 55% of the dry weight accumulated consisted of carbohydrates, in particular starch, which accumulated mainly in the first 14 days in leaves and stem tissues (pith and medullar rays). This accumulation may be explained by reduced meristematic sink activity following severance. In fact, the newly formed roots and primary shoot after 21 days of propagation only represented 10% of the total dry weight of cuttings.

Reduced light integrals and low CO_2 concentrations resulted in reduced rooting and growth of cuttings and decreased carbohydrate levels. Number of roots, and particularly, dry weight of roots, were linearly related with total dry weight accumulation during the 21 days of propagation showing that photosynthetic activity of cuttings during propagation influences both root initiation and growth. The effects of low light, low CO_2 concentration, and leaf area reduction on rooting and growth of cuttings were similar indicating that these effects could be explained to a great extent by photosynthesis. Growth in general depended on the length of the period cuttings were photosynthetically active during propagation. An exception was the growth of the axillary primary shoot, which was more negatively affected by reduced photosynthetic activity in the first 11 days of propagation. Root initiation was also more negatively affected by low photosynthetic activity in the first 11 days of propagation whereas root growth responded to the integral of photosynthesis. Cuttings were able to efficiently use reserves for growth. Optimal rooting and further growth of cuttings rely on the synthesis of new photosynthates because storage is limited in single node stem cuttings.

Key words: *Rosa hybrida*, cut-rose, propagation, cuttings, leaf, rooting, root initiation, root growth, axillary primary shoot, severance, photosynthesis, carbohydrates, reduced sink activity, planting material, quality

Voorword/Preface

This book is the result of several years of living in Wageningen, a small Dutch town of the Gelderland province and is inevitably, the sum of "goede en slechte tijden". For the "goed tijden", and to overcome the "slechte tijden", many people have directly or indirectly contributed to. Here I mention them.

Firstly, I thank my co-promoter Dr. Peter Van de Pol for bringing me into contact with the world of cut-rose propagation and opening me the door for this Ph.D. Thanks also for interesting discussions about the rose sector and the relaxed afternoons in Overberg. My gratitude goes also to my other supervisors Dr. Uulke Van Meeteren, Dr. Coos Keijzer and Prof. Michael Willemse for their scientific guidance, corrections and advise. Special thanks, to my promotor Prof. Hugo Challa, who since the beginning put great effort on my project, and helped me a lot in the last decisive months, behaving many times more like a daily supervisor than a promoter. Thanks Hugo for your interest, criticism and for listening to the mistakes with patience and with always good mood!

I also thank Oege Borsboom for his valuable help during the first years of the project, his good mood, and support on the practical things related or not related (e.g. dinners, football in TV, "fitsen", the red Golf!) with my experiments. I am very greatfull to Dr. Ep Heuvelink for his guidance on the statistics and advice on various scientific matters. Thanks Ep for your support, enthusiasm and co-operation for the Almeria (Spain) study trip and its outcome, the book edition. Also propagating perennials in Vieracker was tiring, but pleasant.

My great gratitude extends to the people of the Tuinbouwproductieketens Groep, who showed great kindness to me. Thanks also to my "older" Ph.D. colleagues (Dr. Ximing Hu, Mr Lee, Loay Arafat, Dr. Jacomyn, Oliver, Susana and Dr. Jaap) and "recent" (Milza, Anke) for several good times passed together. Ximing thanks for your advice and encouragement. To the others, thanks for the dinners, the karaoke sessions, "tuinfeesten", and for those who went to Spain, "muchas gracias" for the nice week. Thanks Diedtzia for your careful corrections and willingness to help. Thanks Arjen and Sonja for the help with the hundreds of samples of carbohydrates (sorry for the delay in washing the bottles!!!).

I thank my "tough" and confident student from Slovakia, Jana Marcakova, who has done a great job during her study period in Wageningen. British regards, indeed, to Dr. Jeremy Harbinson, for his almost spontaneous and bright advise on matters related to photosynthesis and plant physiology, or more simple matters, like English corrections.

Bedankt ook te de mensen van Haagsteeg/Unifarm: Cees, Alex, Martin, Ruth Lambers, Teo Damen, Wim Scholten, Pete and Teus for their help when it was needed. Bedankt Wim for having "alles onder controle" in the quarantine building and for the opportunity to fully test my "nederlandse taal".

"Bedankt ook " to my WAHO volleyball team, who helped me to "decompress" from the work stress in these last years. To the members of the Ph.D. Students Platform, thanks for interesting discussions and the opportunity given to learn more about organisational matters of our Research School.

Thanks to Henriette van de Put for all support and concern in these last years, and very special thanks to the "little" lady from Orient, Onumpai who with her artistic skills helped me a lot with the overall lay out of the book. To my cousin António "obrigado" for your friendship. To the other Portuguese and non-Portuguese people with whom I had the opportunity to share good moments in Wageningen should say: muito obrigado, valeu, muchas gracias, grazie, bedankt, shokran, merci beacoup, danke schön, akujem vám to all.

To my beloved parents and family "MUITO OBRIGADO" for your continuous encouragement and support during these last years and also to my friends in Portugal who although marrying still did not forget me!!

MUITO OBRIGADO A TODOS!

Joaquim Miguel Rangel da Cunha Costa

AOS MEUS

List of abbreviations

ABBREVIATIONS

C_i - intercellular CO_2 mole fraction

DICA - sodium dichloroisocianuric acid

Rd - dark respiration

EC - electro conductivity

IBA - indole butyric acid

LAD - Leaf area duration

TC - total carbohydrates (sugars + starch)

PSII - photosystem II

Pn - net photosynthesis

Pg -gross photosynthesis

PPF- photosynthetic photon flux

PAR - photosynthetic active radiation

RH -relative humidity

Φ_{PSII} - photochemical yield of the photosystem II

Qa - primary stable electron acceptor of photosystem II

Fm - relative fluorescence yield achieved when all the Qa is reduced during a saturating pulse of light

Fss - relative fluorescence yield at steady state

CHAPTER 1

GENERAL INTRODUCTION

The cut-rose propagation industry in the Netherlands

Rose is the most important flower crop in the world in economical terms. Since the 90's roses lead over the cut flowers mostly due to the following factors: genetical improvement in the rose varieties, the emergence of new, out of season producing countries (Botden, 2001) and the emergence of new segment markets (Pertwee, 1995).

World-wide the floricultural industry with respect to crops like chrysanthemums, carnations and poinsettias relies on asexual propagation by rooting of cuttings (Davies et al., 1994). Vegetative propagation is also very important for roses. Roses can be vegetatively propagated "*in vivo*" by two major methods 1) by cuttings or 2) by grafting (e.g. layering, budding, bench grafting, root grafting and stenting). Plants produced by these methods fall in two main categories: 1) plants grown on own roots (cuttings) or 2) grafted plants. Large-scale propagation in the Netherlands is almost exclusively done by means of cutting, stenting (for clonal rootstocks) or bench grafting (for seedling rootstocks) (Hu, 2001).

Presently, rooted cuttings represent about 70% of the total planting material used in the Netherlands. Three main reasons accounted to the increased importance of cuttings in the Netherlands: 1) cultivation in substrate (rockwool and cocos peat) has been progressively substituting cultivation in soil; 2) the market life time of new cultivars has progressively being decreased (from 7 to 4 years); 3) grafted plants are more expensive (1.12 Euro compared to the 0.7 Euro paid for a rooted cutting) and imply higher initial investment, while specific rootstocks did not improve production

or quality in comparison to own rooted plants of certain cultivars. However, this panorama may change as soon as a new rootstock comes to the market offering clear advantages in terms of production and quality (Van de Pol, personal communication).

In the Netherlands, every year about 20 million plants are propagated by eight major rose propagators (Costa et al., 2001). On average, and considering a total area of 921 ha (data from 2001) and an average plant density of 7 plants m^{-2}, the total amount of plants needed in the Netherlands just to renew old crops is about 13 millions, on an annual basis. The remaining 7 million plants are exported to other countries, mainly within Europe, and in less extent to East Africa, Latin America or Asia. The majority of the plants produced for the Dutch market are rooted cuttings, whereas about 80% of the exported plants are stentings, winter grafts or year bushes. An excellent know-how, the use of modern facilities, the good logistics, a good image and a relatively big internal market characterise the rose "propagation industry" in the Netherlands. However, competition between propagators (in the Netherlands and abroad e.g. Ecuador, Kenya) has increased to very high levels. Expansion of cut-rose cultivation to Africa and South America in the 90's increased demand for planting material, but the increase in export was for the short term as propagators started to propagate in those countries. Moreover, another actual trend in cut-rose propagation is that big growers start to produce their own planting material with permission from the breeders.

As a result, although the market became larger it also became more competitive. One way to compete effectively is producing high quality planting material. Therefore, this subject will be considered into more detail.

Quality in cut-rose propagation and factors influencing it

To clarify the context of quality within the rose propagation sector, a small questionnaire on quality definition and on the main problems influencing quality was sent to several propagators and growers in the Netherlands. The results, and also the opinion of advisors were considered and combined with information from literature (Van der Meer, 1993; Schrama, 1996; De Hoog, 1998; Van Ruiten, 1999; Bos, 1999; Guikig, 1999; Van Telgen, 1999; Neefjes, 2001; Anonymus, 2001). Three major

objective quality parameters were mentioned both by propagators and growers: certified disease-free material, certified cultivar and required morphological characteristics (good root system as well as primary shoot, always with a high degree of uniformity). Morphological aspects like the presence of an intact and healthy original leaf, the size of the root system or the length of the new primary shoot were mentioned. Visible white and healthy roots were also characteristics mentioned for the planting material.

Uniformity was another aspect referred to, as non-uniform planting material causes great differences among plants in greenhouses and substituting slowly growing plants is always costly and does not solve for hundred percent the heterogeneity problem. In fact, differences in production within a crop are commonly attributed to heterogeneous planting material (Van de Pol and Pierik, 1995; Kuiper and Van de Pol, 1997; De Hoog, 1998). Moreover, the tendency of using lower plant density (plants/m^2) in greenhouses also increases the relevance of good quality planting material.

Considering that the material is cultivar certified and clean, the quality (here considered as the ability to grow) of rose planting material will depend on the characteristics of the roots (number, length, branching, age), the axillary primary shoot (length and leaf area), the condition of the original leaf and the uniformity of those characteristics (Fig. 1.1).

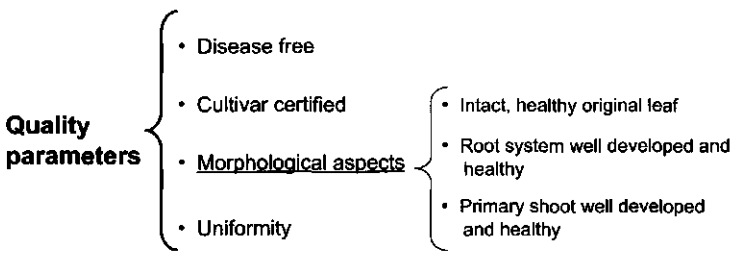

Figure 1.1 Quality of rose planting material derived from single node stem cuttings. The morphological aspects are one of the major determinants of the growth potential of cuttings and young plant material.

The relevant characteristics of roots and the primary shoot of rose planting material depend on environmental and technical conditions. Some of the potential factors influencing quality of cuttings and planting material are presented in Table 1.1, whereas Figure 1.2 illustrates the characteristics of the plant material as well as the facilities used nowadays in rose propagation in two of the most important countries for cut-rose cultivation and propagation: the Netherlands and Ecuador.

Why focus this study on leaves, with special attention to photosynthesis and carbohydrates dynamics?

Although success in rose propagation has increased to very high levels, losses and lack of uniformity in rooting and growth of cuttings are still observed. However, there is a need to increase efficiency in propagation and to produce good quality plant material. Moreover, faster turnover of rose cultivars gives less time to propagators to learn about the rooting characteristics of the new cultivars because rooting ability is also genetically determined.

Leaves are essential for rooting and growth of leafy stem cuttings. Leaves are a source of carbohydrates, mineral nutrients, hormones (e.g auxins) (Reuveni and Raviv, 1981). Leaves, via photosynthesis and transpiration activate movement of solutes, and water as well as of hormones (e.g. auxins and cytokins), within the cutting and influence the temperature regulation of the cutting.

Leaves, however, are also the most exposed and sensitive part of cuttings to environmental or mechanical disturbances from the moment they are growing on the mother plants till the moment of transplantation of planting material (rooted cuttings) into the greenhouse (see Table 1.1). Thus, a study on quality of rose cuttings or rose planting material derived from cuttings should address the role of leaves.

In softwood and semi-hardwood cuttings, the amount of reserves at severance is usually a limiting factor for survival and further growth (Okoro and Grace, 1976; Hartmann et al., 1997). In rose, single node leafy softwood or semi-hardwood stem cuttings, have also limited storage capacity due to the small size of the stem. Thus, the opportunity of cuttings to photosynthesise during propagation should be the main regulatory factor of survival and growth (rooting, growth of the axillary bud into

primary shoot and dry weight accumulation), and thus the quality of the planting material.

Table 1.1 Potential factors influencing the ability of single node softwood cuttings of rose to form roots and grow during propagation as well as after transplantation to the greenhouse (rooted cuttings with 3-4 weeks) at different moments of the propagation chain: on the mother plants, at severance, during propagation and after planting in the greenhouse.

Mother Plant	**Harvest and Handling**	***Propagation***	**Handling and Transport**	***Establishment* after Transplantation**
Growth conditions (light, nutrients, temperature, CO_2, watering)	Developmental stage	Aerial environment (light, RH,CO_2, temperature)	Environment: (RH, light, temperature)	Planting material morphology and growth potential: size of the root system, leaf area of the primary shoot
Pests and diseases	Transport conditions (e.g. number of shoots per vase, water levels in the vase)	Watering Substrate characteristics: water content, porosity, Nutrients	Mechanical damage of the original leaf and to the leaves of the new primary shoot	Environment: (RH, light, watering, temperature)
Pesticides	Mechanical damage imposed on the leaves of the flower shoots	Diseases (e.g. *Botrytis*, *Spharoteca pannosa*, soil born-diseases)	Desiccation of the original leaf and the leaves of the new primary shoot	Desiccation of the leaves of the primary shoot
	Desiccation of the leaves of the flower shoot	Auxin/fungicide treatments (too high concentration can cause leaf yellowing and leaf drop)		
	Storage duration	Duration of the "hardening-off" period for rooted cuttings (too long causes darkening and death of root tips)	Storage duration	
PROPAGATOR GROWER*	→	PROPAGATOR	→	GROWER

* In case the propagator has not enough plant material and needs to buy it from a commercial grower

General aims, approach and outline of the thesis

The general aim of this study is to analyse and to quantify the importance of the original leaf of single node (softwood/semi-hardwood) stem cuttings of cut-rose for

rooting and further growth (dry weight accumulation and partitioning). Based on the assumption that carbohydrates play a key-role in this relation, the dynamics of leaf photosynthesis, carbohydrates and carbon balance during propagation are investigated and related to the rooting ability and growth of cuttings.

The practical aim is to obtain knowledge about criteria for quality of planting material and related to this to improve the propagation process as such with respect to the role of the original leaf of cuttings. Derived from this practical aim, is an underlying scientific aim to assess the contribution of leaf photosynthesis and carbohydrates dynamics of cuttings to the success of propagation.

In **Chapter 2** a literature review is presented on adventitious root formation in leafy stem cuttings with emphasis on the regulatory role of leaves via photosynthesis and photosynthate supply. A conceptual model is established to describe the dynamics of carbon and growth in single node leafy stem cuttings of rose during the first 21 days of propagation as related to leaf characteristics.

Chapter 3 shows the quantitative effect of the area of the original leaf and its persistence in time (leaf area duration concept) on survival, rooting and growth of cuttings either on short (3 weeks after severance) or long term (10 weeks after severance). The relation between leaf area and carbohydrate content at the rooting zone (the most basal 15 mm of the stem) of cuttings and their susceptibility to stem black rot is also studied.

Chapter 4 describes the dynamics of the main anatomical events evolved in adventitious rooting (initiation and growth) as well as the dynamics of fresh and dry matter accumulation and partitioning and the dynamics of sugars (glucose, fructose and sucrose) and starch under standard (non-limiting) photosynthesis conditions during the first 21 days of propagation. The performance of the photosynthetic apparatus during propagation and its response to severance are assessed via quantification of CO_2 fixation and by measuring photochemical efficiency of the PSII (Φ_{PSII}).

Comparison of rooting and growth of cuttings with different leaf areas, and propagated under different light integrals and CO_2 air concentrations presented in **Chapter 5**, should provide evidence about the role of photosynthesis in explaining the effect of leaf area on rooting and growth of the primary shoot of cuttings. The effect of photosynthesis and carbohydrates in the different phases of propagation (root

initiation and root growth) is also reported.

In the general discussion, **Chapter 6**, the regulatory effect of photosynthesis and carbohydrates on rooting and growth is given and discussed on the basis of the present results. Several points needing attention for further research are also highlighted and the practical implications of this study are presented.

References

Anonymous (2001) Algemene voorwaarden voor de vermeerdering van teeltmateriaal voor de teelt van rozen, opgesteld door de Gewasgroep roos van Plantum NL. http://www.plantum.nl/

Botden N (2001) Quality scan rose production by Edesa Group in East Africa on farm and chain level. Thesis Report, Horticultural Production Chains Group, Wageningen University, the Netherlands, 98pp

Bos R (1999) Quality of plant material a worthwhile investment. FlowerTech, Vol. 2, 4: 61-63

Costa JM, Challa H, Van Meeteren U, Van de Pol P A (2001) Photosynthates: mainly stored and yet limiting in propagation of rose cuttings. Acta Hort 547: 167-173

Davies FT, Davis TD, Kester DE (1994) Commercial importance of adventitious rooting to horticulture, In: TD Davis, B Haissig (eds) Biology of adventitious root formation. Basic Life Sciences. Plenum Press, NY, Vol. 62, pp 53-69

De Hoog J (1998) Teelt van kasrozen. Proefstation voor Bloemisterij en Glasgroente. The Netherlands, 218 pp

Guikig W (1999) De bewuste keuze beter te willen, daar gaat het om. Oogstplus Sierteelt. February

Hartmann HT, Kester DE, Davies FT, Geneve RL (1997) Plant Propagation, Principles and Practices. 6th ed. Prentice Hall Inc., Englewood Clifs, New Jersey, 770 pp

Hu X (2001) Growth and productivity of cut rose as related to the rootstock. PhD thesis, Wageningen University, the Netherlands, 108 pp

Kuiper D, Van de Pol PA (1997) Kwaliteit in the rozenketen: een product van vermeerdering en teelt. In: LFM Marcelis, AJ Haverkort (eds). Kwaliteit en millieu in de glastuinbouw: stimulans tot verniewing. AB-DLO Thema's 4, pp 17-28

Neefjes H (2001) Select Plant roos: een kind dat nog in de wieg ligt. Vakblad voor de Bloemisterij. 45: 56-57

Okoro OO, Grace J (1976) The physiology of rooting *Populus* cuttings. I. Carbohydrates and photosynthesis. Physiol Plant 36: 133-138

Pertwee J (1995) Production and marketing of roses 2nd ed. Pathfast Publishing, Frinton-on-Sea, Essex, England, 127 pp

Reuveni O, Raviv M (1981) Importance of leaf retention to rooting of avocado cuttings. J Am Soc Hort Sci 106: 127-130

Schrama P (1996) Rozentelers op substraat kiezen voor stek. Vakblad voor de Bloemisterij 17: 43

Van Telgen HJ (1999) Uitgangsmateriaal in de 21 eeuw. Kwestie van kwaliteit. Vakblad voor de Bloemisterij 45: 56-57

Van de Pol PA, Pierik RLM (1995) Newest developments in rose (*Rosa hybrida*) propagation. Rev Chapingo Hort 3: 15-22

Van Ruiten JEM (1999) Kan kwaliteit uitgangsmateriaal roos beter? Vakblad voor de Bloemisterij, 4

Van der Meer M (1993) Van optimaal uitgangsmateriaal naar ideale plantopbouw Vakblad voor de Bloemisterij 20: 34-35

Figure 1.2 A single node leafy stem cutting of cut-rose **(a)** propagation of cut-roses in greenhouse in the Netherlands **(b,c)** and in Ecuador (courtesy Walter Barentzen) **(d)**; propagation of cut-rose in climate chamber in the Netherlands **(e,f)**; recently rooted cuttings before hardening **(g)**; greenhouse facilities for hardening rooted cuttings **(h)** rooted cuttings in cocos fibre ready for transplanting **(i)**; young rooted plant one week after plantation in greenhouse **(j)**.

CHAPTER 2

ADVENTITIOUS ROOT FORMATION IN LEAFY CUTTINGS: a literature review on the role of leaves and carbohydrates and a conceptual model for growth of single node leafy stem cuttings of rose

The effect of leaves on propagation and rooting of leafy stem cuttings

Leaves play a very important role in propagation of leafy stem cuttings of rose and of a wide range of plant species (Table 2.1). One of the prime roles of leaves during propagation of leafy (softwood) stem cuttings is to guarantee their survival. Leaves further influence rooting and growth of the primary shoot (Table 2.1).

According to Reuveni and Raviv (1981) leaves exert their effect on rooting and growth of leafy stem cuttings via three major ways: as source of carbohydrates, as source of hormones and as source of different nutritional constituents other than carbohydrates (e.g. phenolic compounds like anthocianins (Hess, 1968; Jarvis, 1986;Wilson and Van Staden, 1990)).

Leaves may also influence rooting of cuttings by regulating their water relations during propagation (Hess and Snyder, 1957; Gay and Loach, 1977; Loach, 1988; Mudge et al., 1995; Aminah et al., 1997; Harrison-Murray and Howard, 1998). Leaves influence the water balance of cuttings to a great extent due to the water loss via leaf transpiration (Grange and Loach, 1983; Loach, 1988; Ofori et al., 1996; Aminah et al., 1997) and the negative effect of water stress on rooting is mainly due to reduced photosynthesis (Haissig, 1986; Davis and Potter, 1989; Harrison-Murray and Howard, 1998) because one of the most evident effects of water stress is closure of stomata (Hsiao, 1973).

Table 2.1 Effects of leaves (area, presence) on the propagation of leafy cuttings or leafy stem cuttings of different plant species (X: means positive effect; X (-): negative effect; X (+/-): positive and negative effect.

Species	Survival	Rooting			Shoot growth	References
		% rooting	N. roots	Root growth		
Ornamentals						
Ficus			X	X		Poole and Conover, 1984
Hedera helix	X			X		Gregory and Samantarai, 1950
Hibiscus		X	X	X		Van Overbeek et al., 1946
Coleus	X					Calma and Richey, 1930
Rosa	X	X		X	X (-/+)	Moe, 1973; Dubois and De Vries, 1985;1991; Ypema et al., 1987; Gillman and Zlesak,2000
Acer rubrum				X	X	Wilkins et al, 1995
Humulus lupulus L			X			Howard 1965
Epipremnum aureum					X	Wang, 1987
Fruit						
Prunnus		X	X			Breen and Muraoka,1974
Avocado	X	X				Reuveni and Raviv, 1981
Olea europea (olive)			X	X		Porlingis and Therios, 1976; Avidan and Lavee, 1978;
Cacao *(Persea americana Mill)*	X	X				Evans, 1952
Irvingia gabonensis	X	X	X			Shiembo et al., 1996
Vitis vinifera L. (in vitro)				X	X	Thomas, 2000
Vitis vinifera L. (in vivo)			X	X	X	Fournioux, 1997
Forest						
Eucalyptus	X	X			X	Wilson, 1994a; Hoad and Leakey, 1996
Triplochyton scleroxylon	X	X	X			Leakey and Coutts, 1989
Milicia excelsa	X	X	X		X(-)	Ofori et al., 1996
Terminalia spinosa	X				X	Newton et al., 1992
Leucaena leucocephala	X	X			X(-)	Dick et al., 1999
Calliandra calothyrsus	X				X	Dick et al., 1996

As a consequence it may be expected that a certain optimum leaf area for rooting exists based on the optimal balance between photosynthesis and transpiration (Leakey and Coutts, 1989; Newton et al., 1992; Ofori et al., 1996). Water stress may also induce changes in the hormonal metabolism in leaves (e.g. synthesis of abcissic acid) (Haissig, 1986).

Environmental factors such as light, temperature and relative air humidity determine the vapour pressure gradient (the driving force of transpiration) and will consequently influence the transpiration rate of the cutting (Rosenberg et al., 1992). High temperatures and light intensities increase leaf temperature and thus the leaf-to-air vapour pressure difference. This increases the transpiration rate causing water deficits which decrease rooting (Grange and Loach, 1983; Loach, 1988; Aminah et al., 1997). On the contrary, low irradiance (Evans, 1952; Loach and Whalley, 1978), high humidity (close to saturation) and leaf wetting (Hess and Snyder, 1957;

Harrison-Murray and Howard, 1998) decrease transpiration and prevent leaf wilting.

The roles of carbohydrates during propagation

Among the different ways that leaves may influence rooting and growth of cuttings during propagation their role as source of carbohydrates is probably one of the most relevant. To guarantee survival, carbohydrates are needed to support maintenance respiration. Concerning the effect of carbohydrates on rooting, some literature reports no effect (Ali and Westwood, 1966; Veierskov et al., 1982) or even a negative effect on rooting (Treeby and Cosidine, 1982). However, there is strong evidence demonstrating that carbohydrates do influence rooting of cuttings positively (Van Overbeek et al., 1946; Evans, 1952; Hess and Snyder, 1957; Howard and Sykes, 1966; Molnar and Lacroix, 1972; Nanda et al., 1971; Hansen et al., 1978; Haissig, 1984; 1986; Leakey and Coutts, 1989; Tshaplinski and Blake, 1989; Wiesman and Lavee, 1995; Druege et al., 2000; Pellicer et al., 2000).

Leaves may also influence growth of the axillary primary shoot via the supply of carbohydrates as suggested for *Eucalyptus* (Wilson, 1994a), *Acer* (Wilkins et al., 1995) and *Eppiperunum* (Wang, 1987). Also in rose plants, growth of a bud into a primary shoot was shown to depend on carbohydrate supply from the subtending leaves (Marcelis Van-Acker, 1994).

Besides the effect of carbohydrates on the percentage of rooted cuttings or on the number of roots formed (see references above), carbohydrates have been observed to influence root localisation in cuttings. The accumulation of assimilates in the rooting zone of cuttings has been suggested to be a necessary triggering factor for root initiation in woody and non-woody species (Lovell et al., 1972; Veierskov et al., 1982; Haissig, 1984; Rodriguez et al., 1988), although the critical concentration required to induce root initiation is apparently difficult to establish (Welander, 1994).

Friend et al. (1994) report that a common characteristic of species with pre-formed root initials and those ones which form "*de novo*" primordia, is the close proximity of the new root (sink) to the existent vascular tissue (source). The same authors suggest that the ease of rooting may be related with the proximity of leaf traces and with the ease of establishing early vascular connections between source and sink that are needed to provide carbohydrates and hormones required for root

formation. Root initiation seems to preferably occur in locations where carbohydrates are easily available like in tissues associated with the vascular tissue (Gregory and Samantarai, 1950; Davies et al., 1982; Lovell and White, 1986; Attfield and Evans, 1991; Hartmann et al., 1997; Hamann, 1998) or in tissues rich in starch (Doud and Carlson, 1977; Li and Leung, 2000). Supporting this is the fact that the hydrolysis of starch in soluble sugars has been, for several times, associated with rooting activity in cuttings (Nanda and Anand, 1970; Breen and Muraoka, 1974; Molnar and Lacroix, 1972; Gislerød, 1983; Grange and Loach, 1984; Jásik and De Klerk, 1997).

The role of carbohydrates at different moments of propagation (root initiation, root growth and primary shoot growth)

When analysing the effect of leaves and carbohydrates on rooting one should take in account that adventitious root formation is a multi-step process which may have different requirements in carbohydrates or other rooting factors (Haissig, 1986; De Klerk et al., 1999). Adventitious root formation may be described and classified in different ways (Jarvis, 1986; Gaspar et al., 1997; Hartmann et al., 1997; De Klerk et al., 1999;). A simple and generally accepted way is to distinguish two main phases: **root initiation** and **root growth** (Eriksen, 1973; Lovell and White, 1986). We will follow this approach.

Root initiation is characterised by the appearance of cells with large, centrally located nucleus and small vacuoles, which are capable of division. By active cell division, these cells give rise to the **root initials** (Stangler, 1956; Lovell and White, 1986) which at a later stage, and together with the surrounding cells, will be incorporated in an organised mass of meristematic tissue with a certain level of differentiation called **root primordium** (Stangler, 1956; Lovell and White, 1986). Subsequently, the root primordium establishes vascular connections with the existing vascular tissue and a **functional root** emerges (Lovell and White, 1986). Root initiation includes the processes of induction, cell dedifferentiation, division and differentiation, whereas root growth should include the processes of cell division, expansion and differentiation (Taylor, 1997) which gives rise to the root primordia through the cortex till emergence and further elongation of the functional root.

Root initiation has been commonly reported to be less dependent on

carbohydrates than root growth. Middelton et al. (1980) showed that root initiation (occurring on the first day following severance) in stem cuttings of *Phaseaolus aureaus* was not limited by carbohydrates whereas further root primordia growth depended on sugar supply. Likewise, Veierskov et al. (1982) found no relation between carbohydrate concentrations and the number of roots formed, but they suggested that carbohydrates could affect root growth. Moreover, an indirect evidence that root initiation depends less on the supply of current carbohydrates than root growth is the fact that leaf removal decreased more the dry weight than the number of roots (Fournioux, 1997). Furthermore, cuttings are able to initiate roots in darkness (Van Overbeek et al., 1946; Davis and Potter, 1981; Van de Pol, 1988) or under very low irradiance (93% shading) (Zaczek et al., 1999), although higher light levels after roots were formed promoted root growth (Zaczek et al., 1999). This, however, does not exclude the possibility that the content in reserves by the time of severance was enough to support survival and root initiation. Moreover, and in contrast with the view that carbohydrates do not influence root initiation, Lovell et al. (1972) showed that current photosynthesis and the accumulation of photosynthates in the lower petiole are pre-requisites for root initiation in *Synapsis* cotyledons. Welander (1994) and Haissig (1984) described the same for woody cuttings.

Low availability of carbohydrates may have a negative regulatory effect on mitotic activity (Moritz and Sundberg, 1996; Borisjuk et al., 1998; Muller et al., 1998). The mitotic activity of the cambium was shown to be influenced by sugars and hormones in *Pinus sylvestris* (Moritz and Sundberg, 1996). Also Muller et al. (1998) found that both cell division and elongation were limited by low contents in glucose and fructose. This implies that low carbohydrates, e.g. as consequence of reduced leaf photosynthesis, can decrease rooting, especially when root initiation occurs within the callus tissue.

The positive effect of carbohydrates on root growth is less controversial than the effect of carbohydrates on root initiation and it has been observed either in adult plants (Bingham and Stevenson, 1993; Muller et al., 1998; Pritchard and Rogers, 2000), seedlings (Van den Driessche, 1987;1991; Tinus et al., 2000) and in cuttings (Eliasson, 1968). Muller et al. (1998) found a linear relation between root elongation and the cumulative intercepted light in wheat plants. In CO_2 enriched environments the carbohydrates content increased in the roots and stimulated root growth either by promoting cell division or influencing cell elongation or both (Pritchard and Rogers,

2000) and current photosynthates marked with ^{14}C were shown to be incorporated in the new roots of conifer seedlings (Van den Driessche, 1987).

However, rooting of leafy cuttings can be optimized (larger number and dry weight of roots) when cuttings are propagated in environments favoring photosynthesis (Davis, 1988). In fact, cuttings to root require a critical minimum light level and light levels were closely related with rooting behavior of cuttings of different species like *Populus* and *Salix* (Elliasson and Brunnes, 1980), *Hedera helix* (Gregory and Samantarai, 1950), *Humulus lupulus* (Howard and Sykes, 1966), *Pinus* (Hansen et al., 1978) and *Shorea L.* (Aminah et al., 1997).

The positive regulating effect of photosynthesis on rooting of leafy cuttings has also been demonstrated by the positive effect of CO_2 enrichment (larger number of roots and faster rooting) with several species (Molnar and Cumming, 1968; Davis and Potter, 1981; 1983; French and Lin, 1984; Grant et al., 1992; Kunnenman and Ruesink, 1997) and the reduction of rooting of cuttings under low CO_2 concentrations (Davis and Potter, 1981). Survival, rooting and further growth of cuttings depends on keeping a positive balance between photosynthesis and respiration (Hess and Snyder, 1957; Howard and Sykes 1966; Howard, 1968; Yue and Margolis, 1993; Howard, 1994; Hoad and Leakey, 1996, Pellicer et al., 2000).

Conclusions and a conceptual model to relate leaves, carbohydrates and rooting of single node leafy stem cuttings of rose

From the foregoing literature review it has been shown that leaves have a strong regulatory effect on rooting and growth of cuttings and that photosynthesis and carbohydrates may be two of the most important leaf factors influencing rooting and growth of cuttings.

It has also been shown that severance from the mother plant disturbs those controls by interrupting or disturbing the physiological processes behind them. Severance disrupts water and nutrients supply from roots (Harrison-Murray and Howard, 1998) and may reduce leaf photosynthesis due to water stress and stomata closure (Loach, 1988; Smalley et al., 1991; Fordham et al., 2001). The typical response of cutting photosynthesis to severance is a strong reduction until the moment roots emerge (Howard and Sykes, 1966; Breen and Muraoka, 1974; Cameron and

Rook, 1974; Okoro and Grace, 1976; Gay and Loach, 1977; Loach and Whalley, 1978; Eliasson and Brunnes, 1980; Feldman et al., 1989; Smalley et al., 1991; Wiesman and Lavee, 1995). Simultaneously, respiration rates may increase in response to the higher temperature during propagation compared to the mother plants, and also due to the processes related with growth at the basal part of the cuttings (Dick et al., 1994).

Therefore, a cutting, after severance from the mother plant, is a structure out of balance. To re-achieve balance, the cutting needs to regain the capacity for water uptake by forming adventitious roots and re-establish its photosynthetic capacity.

The focus on photosynthesis and carbohydrates can be even more justified if we consider that single node leafy stem cuttings are plant material with limited storage capacity due to the type of the wood (semi-hardwood/softwood) (Okoro and Grace, 1976; Hartmann et al., 1997) and to the small size (volume) for storage (a 4 to 6 cm length single node stem). Moreover, photosynthesis is probably one of the most negatively affected processes by severance or by other stresses occurring during propagation.

Based on the previous literature review and considerations, and taking as major assumption that carbohydrate dynamics plays a key role in explaining the effect of the original leaf on the performance (survival, rooting, growth) of rose leafy stem cuttings a conceptual model is proposed to describe the most relevant interactions between the different parts of single node leafy stem cuttings of rose the original leaf, the stem, the adventitious roots and the axillary bud (future primary shoot), as well as the major processes (photosynthesis, respiration, carbon partitioning, rooting) occurring during the first 21 days of propagation. This period is considered in practice, the most critical for success and for uniformity of plant growth.

The conceptual model provides a basis for further analysis and quantification of all its components and processes: 1) post-severance photosynthesis; 2) respirational losses; 3) carbohydrate pools (starch and soluble sugars); 4) carbohydrate allocation and partitioning; 5) rooting. The model points out paths or relations where carbohydrates (content and partitioning) can be a limiting factor for rooting and growth of cuttings. This theoretical approach should help to clarify the relations between carbohydrates (stored or current photosynthates), rooting and growth of single node leafy stem cuttings of rose during the first 21 days of propagation and to

study to what extent photosynthesis rate and carbohydrate balance could explain rooting and growth behaviour of cuttings during propagation.

Components and carbon pools considered in the conceptual model

After severance, a single node leafy (softwood/semi-hardwood) stem cutting consists of three different components: the original leaf, the stem and the axillary bud (future primary shoot). The adventitious roots formed in response to severance/wounding, will become the fourth component. Two major pools of carbohydrates may be involved in the process of root formation (Wiesman and Lavee, 1995): the pool of new formed soluble carbohydrates (current photosynthates) and the pool of stored carbohydrates (sugars and starch). Both pools are very important during the first days of propagation to sustain maintenance respiration and guarantee survival and further support the energy costs with the initial growth related to the first anatomical events preceding root regeneration (e.g. wound healing and callus formation).

In this conceptual model the original leaf is the main source of carbohydrates due to photosynthesis. Due to transpiration, the original leaf can also influence the transport of water as well as the fluxes of nutrients or hormones (e.g. cytokinins) in the cuttings (Van Staden and Davey, 1979). The stem, due to its respiratory activity (maintenance or growth), storage capacity and little photosynthetic activity, acts as a sink. This sink activity can be beneficial because it allows photosynthesis to proceed despite the reduction of the sink activity following severance (Wilson, 1994b). The stem can also act as a source by remobilizing stored carbohydrates.

In our conceptual model a subdivision of the stem is considered: the carbohydrate status in the rooting zone (the basal 8-15mm of the stem segment) is assumed to have a specific significance for root formation and root growth (Fig. 2.1). One of the triggering points for rooting may be the accumulation of carbohydrates at the rooting zone during the early stages of root formation (Veierskov et al., 1982; Haissig, 1984; Welander, 1994). The stem can also be a source of energy or a storage buffer, through the reserves accumulated before severance, contributing to maintenance and growth of the roots or the primary shoot.

The stem is the physical link between the original leaf of cuttings and the adventitious roots and it allows the movement of carbohydrates, water, nutrients and

hormones between leaves and roots of the cutting.

The axillary bud, future primary shoot, acts during propagation as a sink. Growth of the axillary bud and primary shoot of cuttings depends on the supply of assimilates by the subtending leaf (Wang, 1987; Wilson, 1994a; Wilkins et al., 1995). In rose plants, the axillary bud outgrowth depends on the supply of carbohydrates by the parent leaf (Jiao et al., 1989; Marcelis van-Acker, 1994; Van Labeke et al., 2001).

Roots are the fourth component of the model and they are considered to be mainly a sink during the first 21 days of propagation.

The carbon relations between the 4 components of the model and the underlying regulating processes (see Figure 2.1.) can be described in the following way: there is a centrally carbohydrate pool consisting of mobile and stored $(CH_2O)_n$ and this pool is filled by photosynthesis, which may be limited by a lack of sinks, or by decreased stomatal conductance. This central pool of carbohydrates is used for maintenance, growth and growth respiration. Growth will correspond mainly to stem growth, in particular at the stem rooting zone where cambial activity is greater and callus is formed. The central pool of carbohydrates also supports growth of the new-formed organs: the axillary shoot and the adventitious roots.

Internal and external factors or processes influencing photosynthesis and carbon relations in cuttings during propagation

The cutting photosynthesis is affected by external environmental factors (light intensity and CO_2 air concentration) as well as by the area of the original leaf and light interception which have a direct effect on the photosynthetic capacity of cuttings (Fig. 2.1).

The water relations in cuttings are included in the concept because they may affect stomatal resistance and consequently leaf photosynthesis. A satisfactory water content, resultant of the balance between water uptake and water loss, is a pre-requisite for success in propagation of leafy stem cuttings (Loach, 1988). This balance is affected negatively by the removal of roots by severance and by excessive leaf transpiration (Loach, 1988; Grange and Loach, 1983; Aminah et al., 1997). Other factors may also disturb the normal water relations of cuttings such as the poor stem

transport function (Ikeda and Susaki, 1985) due to damage caused by severance or air emboli, although this effect will be not considered.

The environment (temperature) as well as the cuttings' morphology (volume of the stem, which is considered not to change during the first 21 days of propagation) influences the maintenance respiration of the different components of the cutting.

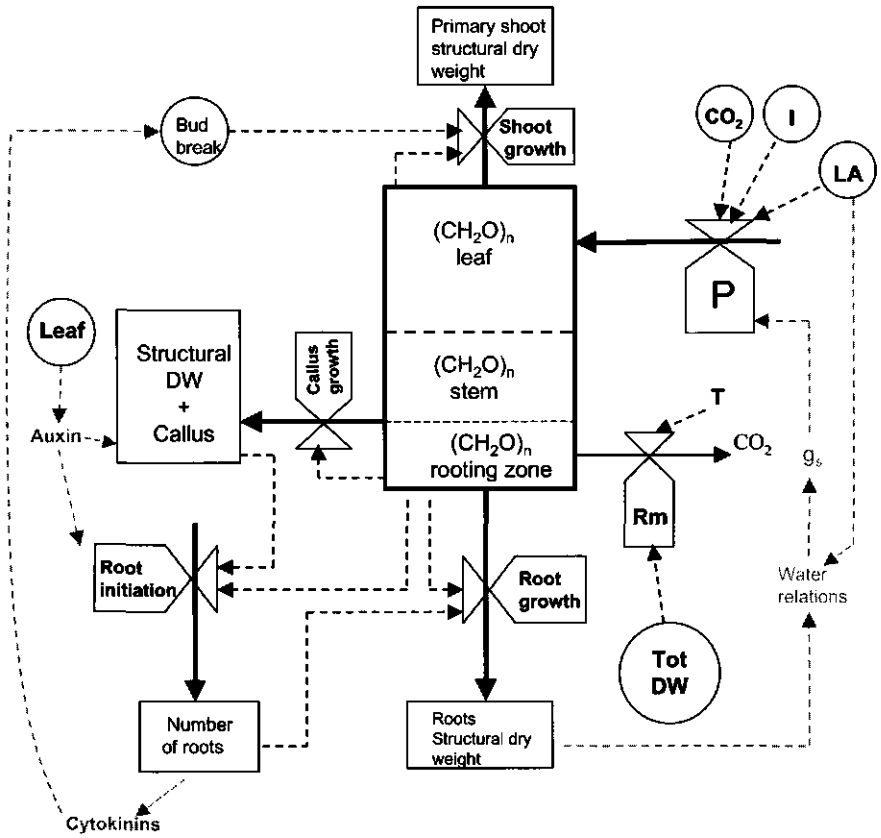

Figure 2.1 Relational diagram describing the carbohydrate dynamics, rooting and growth of single node leafy stem cuttings of rose, during the first 21 days of propagation, including some of the morphological and environmental factors (leaf area, light intensity and CO_2 air concentration) which influence photosynthesis and carbohydrate dynamics. Abbreviations: (La) leaf area; (I) light intensity; (P) photosynthesis; (R_m) maintenance respiration; (g_s) stomatal conductance; (T) air/substrate temperature.

References

Aminah H, Dick JMcP, Grace J (1997) Influence of irradiance on water relations and carbon flux during rooting of *Shorea leprosula* leafy stem cuttings. Tree Physiol 17: 445-452

Ali N, Westwood MN (1966) Rooting of Pear cuttings as related to carbohydrates, nitrogen and rest period. Proc Am Soc Hort Sci 88: 145-150

Attfield EM, Evans PK (1991) Developmental pattern of root and shoot organogenesis in cultured leaf explants of *Nicotiana tabacum* cv Xanthi nc. J Exp Bot 234: 51-57

Avidan B, Lavee S (1978) Physiological aspects of the rooting ability of olive cuttings. Acta Hort 79: 93-101

Bingham IJ, Stevenson (1993) Control of root growth: effects of carbohydrates on the extension, branching and rate of respiration of different fractions of wheat roots. Physiol Plant 88: 149-158

Borisjuk L, Walenta S, Weber H, Mueller-Klieser W, Wobus U (1998) High-resolution histographical mapping of glucose concentrations in developing cotyledons of *Vicia faba* in relation to mitotic activity and storage processes: glucose as possible developmental trigger. Plant J 15: 583-591

Breen PJ, Muraoka T (1974) Effect of leaves on carbohydrate content and movement of ^{14}C assimilate in plum cuttings. J Am Soc Hort Sci 99: 326-332

Calma VC, Richey HW (1930) Influence of the amount of foliage on rooting of *Coleus* cuttings. Proc Am Soc Hort Sci 27: 457-462

Cameron RJ, Rook, DA (1974) Rooting stem cuttings of Radiata pine: environmental and physiological aspects. New Zeal J For Sci 4: 291-298

Davies FT, Lazarte JE, Joiner JN (1982) Initiation and development of roots in juvenile and mature leaf bud cuttings of *Ficus pumila* L. Am J Bot 69: 804-811

Davis TD (1988) Photosynthesis during adventitious rooting. In: Adventitious root formation in cuttings. TD Davis, BE Haissig and N Sankhla (eds) Discorides Press, Portland Oregon, pp 79-87

Davis TD, Potter JR (1981) Current photosynthate as a limiting factor in adventitious root formation on leafy pea cuttings. J Am Soc Hort Sci 106: 278-282

Davis TD, Potter JR (1983) High CO_2 applied to cuttings: effects on rooting and subsequent growth in ornamental species. HortScience 18: 194-196

Davis TD, Potter JR (1989) Relations between carbohydrate, water status and adventitious root formation in leafy pea cuttings rooted under various levels of atmospheric CO_2 and relative humidity. Physiol Plant 77: 185-190

De Klerk G, Van der Krieken W, De Jong J (1999) Review the formation of adventitious roots: new concepts and possibilities. In Vitro Cell Dev Biol Plant 35: 189-1999

Dick, J McP, Dewar RC (1992) A mechanistic model of carbohydrates dynamics during adventitious root development in leafy cuttings. Ann Bot 70: 371-377

Dick J McP, Blackburn DG, McBeath C (1994) Stem respiration in leafy cuttings of *Prosopis juliflora* during the rooting process. New Forest 8: 179-184

Dick J McP, Bisset H, McBeath C (1996) Provenance variation in rooting ability of *Calliandra calothyrsus*. Forest Ecol Manag 87: 175-184

Dick J, Magingo F, Smith RI, McBeath C (1999) Rooting ability of *Leucaena leucocephala* stem cuttings. Agroforestry Syst 42: 149-157

Doud S, Carlson R (1977) Effects of etiolation, stem anatomy and starch reserves on root initiation of layered *Mallus* Clones. J Am Soc Hort Sci 102: 487-491

Druege U, Zerche S, Kadner RD, Ernst M (2000) Relation between nitrogen status, carbohydrate distribution and subsequent rooting of Chrysanthemum cuttings as affected by pre-harvest nitrogen supply and cold-storage. Ann Bot 85: 687-701

Dubois LAM, De Vries DP (1985) Onderzoek met stekken van 'Sonia'. Vakblad voor de Bloemisterij 45: 26-31

Dubois LAM, De Vries DP (1991) Variation in adventitious root formation of softwood cuttings of *Rosa chinensis* minima (Sims) Voss cultivars. Sci Hortic 47: 345-349

Eliasson L (1968) Dependence of root growth on photosynthesis in *Populus tremula.* Physiol Plant 21: 806-810

Eliasson L, Brunnes L (1980) Light effects on root formation in aspen and willow cuttings. Physiol Plant 48: 261-265

Eriksen EN (1973) Root formation in pea cuttings. I. Effects of decapitation and disbudding at different developmental stages. Physiol Plant 28: 503-506

Evans H (1952) Physiological aspects of the propagation of cacao from cuttings. J Hort Sci 58: 9-17

Feldman WR, Palzkill DA, Dobrenz AK, Hogan L (1989) Carbon dioxide exchange during mist propagation of jojoba cuttings. Agron J 81: 233-236

Fordham MC, Harrison-Murray RS, Knight L, Clay CM (2001) Decline in stomatal response to leaf water deficit in *Corylus maxima* cuttings. Tree Physiol 21: 489-496

Fournioux JC (1997) Adult leaves of grapevine cuttings stimulate rhizogenesis. Vitis 36: 49-50 Research note

French CJ, Lin WC (1984) Seasonal variations in the effects of CO_2, mist and supplementary lighting from high pressure sodium lamps on rooting of English Holly cuttings. HortScience 19: 519-521

Friend AL, Coleman, MD, Isebrands JG (1994) Carbon allocation to root and shoot systems of woody plants. In: TD Davis, BE Haissig (eds.) Biology of Adventitious Root Formation, Basic Life Sciences Vol. 62. Plenum Press, New York, pp 245-274

Gaspar T, Kevers C, Hausman, JF (1997) Indissociable chief factors in the inductive phase of adventitious rooting. In: A Altman, Y Waisel (eds), Biology of root formation and development. Plenum Press, New York, pp 55-63

Gay AP, Loach K (1977) Leaf conductance changes on leafy cuttings of *Cornus* and *Rhododendron* during rooting. J Hort Sci 52: 509-516

Gillman JH, Zlesak DC (2000) Mist applications of sodium silicate to Rose (*Rosa* L.x 'Nearly Wild') cuttings decrease leaflet drop and increase rooting. HortScience 35: 773

Gislerød HR (1983) Physiological conditions of propagation media and their influence on the rooting of cuttings. III. The effect of air content and temperature in different propagation media on rooting of cuttings. Plant Soil 75: 1-14

Grant WJR, Fan HM, Downton WJS, Loveys BR (1992) Effects of CO_2 enrichment on the physiology and propagation of two australian ornamental plants, *Chamelaucium unicanatum* (Schauer) x *Chamelaucium floriferum* (MS) and *Correa schlechtendalli* (Beher). Sci Hort 52: 337-342

Grange, RI, Loach K (1983) Environmental factors affecting water loss from leafy cuttings in different propagation systems. J Hort Sci 58: 1-7

Grange RI and Loach K (1984) Comparative rooting of eighty-one species of leafy cuttings in open and polyethylene-enclosed mist systems. J Hort Sci 59: 15-22

Gregory FG , Samantarai B (1950) Factors concerned in the rooting responses of isolated leaves. J Exp Bot 1: 159-193

Haissig BE (1984) Carbohydrate accumulation and partitioning in *Pinus banksiana* seedlings and seedlings cuttings. Physiol Plant 61: 13-19

Haissig BE (1986) Metabolic processes in the adventitious rooting of cuttings. In: MB Jackson (ed) New root formation in plants and cuttings. Long Ashton Research Station University of Bristol, UK, pp 141-190

Hamann A (1998) Adventitious root formation in cuttings of loblolly pine (*Pinus taeda* L.) developmental sequence and effects of maturation. Trees 12: 175-180

Hansen J, Strömquist L, Ericsson A (1978) Influence of the irradiance on carbohydrate and rooting of pine seedlings (*Pinus sylvestris* L.). Plant Physiol 61: 975-979

Harrison-Murray RS, Howard BH (1998) Environmental requirements as determined by rooting potential in leafy cuttings. In: KE Cockshull, D Gray, GB Seymour, B Thomas (eds). Genetical and

environmental manipulation of horticultural crops. CAB International 1998, pp 75-94

Hartmann HT, Kester DE, Davies FT, Geneve RL (1997) Plant Propagation, Principles and Practices. 6th ed. Prentice Hall Inc., Englewood Clifs, New Jersey, 770 pp

Hess CE (1968) Internal and external factors regulating root initiation. In: WJ Whittington (ed) Root Growth, Proceedings 15th Easter School, Nottingham. Butterworths, London, pp 42-53

Hess CE, Snyder WE (1957) A physiological comparison of the use of the mist with other propagation techniques procedures used in rooting of cuttings. Rep XIVth Int Hort Cong Vol. 2, 1133-1139

Hoad SP, Leakey RRB (1996) Effects of pre-severance light quality on the vegetative propagation of *Eucalyptus grandis* W. Hill ex. Maiden. Cutting morphology, gas exchange and carbohydrate status during rooting. Trees 10: 317-324

Howard BH (1965) Regeneration of the hop plant (*Humulus lupulus* L.) from softwood cuttings. I.The cutting and its rooting environment. J Hort Sci 40: 181-191

Howard BH, Sykes JT (1966) Regeneration of the hop plant (*Humulus lupulus* L.) from softwood cuttings. II. Modification of the carbohydrate resources within the cutting. J Hort Sci 41: 155-163

Howard BH (1968) The influence of 4 (indolyl-3) butyric acid and basal temperature on the rooting of apple rootstock hardwood cuttings. J Hort Sci 43: 23-31

Howard BH (1994) Manipulating rooting potential in stock plants before collecting cuttings. In: TD Davis and BE Haissig (eds.) Biology of Adventitious Root Formation, Basic Life Sciences Vol. 62. Plenum Press, New York, pp 123-142

Howard BH, Harrison-Murray RS (1995) Responses of dark-pre conditioned and normal light grown cuttings of *Syringa vulgaris* 'Madame Lemoine' to light and wetness gradients in the propagation environment. J Hort Sci 70: 989-1001

Hsiao TC (1973) Plant responses to water stress. Ann Rev Plant Physiol 24: 519-570

Humphries EC, Thorne GN (1964) The effect of root formation on photosynthesis of detached leaves. Ann Bot 28: 391-400

Ikeda T, Susaki T (1985) Influence of hydraulic condutance of xylem on water status in cuttings. Can J For Res 16: 98-102

Jarvis BC (1986) Endogenous control of adventitious rooting in non-woody cuttings. In: MB Jackson (ed.), New root formation in plants and cuttings. Long Ashton Research Station University of Bristol, UK, pp 191-222

Jásik J, De Klerk J (1997) Anatomical and ultrastructural examination of adventitious root formation in stem slices of aple. Biol Plant 39: 79-90

Jiao J, Gilmour M, Tsujita, MJ, Grodzinski B (1989) Photosynthesis and carbon partitioning in Samantha roses. Can J Plant Sci 69: 577-584

Kunneman BPAM, Ruesink JB (1997) Interactions between light, temperature and CO_2 in rooting of conifer cuttings. Acta Hort 418: 97-101

Leakey RRB, Coutts MP (1989). The dynamics of rooting *Triplochiton scleroxylon* cuttings: their relation with leaf area, node position, dry weight accumulation, leaf water potential and carbohydrate composition. Tree Physiol 5: 135-146

Li M, Leung DWM (2000) Starch accumulation is associated with adventitious root formation in hypocotyl cuttings of *Pinus radiata* . J Plant Growth Regul 19: 423-428

Loach K (1988) Water relations and adventitious rooting. In: TD Davis, BE Haissig, N Sankla (eds), Adventitious root formation in cuttings. Discorides Press, Portland Oregon, pp 102-116

Loach K, Walley DN (1978) Water and carbohydrate relationships during rooting of cuttings. Acta Hort 79: 161-167

Lovell PH, Illsley A, Moore KG, Moore (1972) The effect of light intensity and sucrose on root formation, photosynthetic ability, and senescence in detached cotyledons of *Sinapsis alba* L. and *Raphanus sativa* L. Ann Bot 36: 123-134

Lovell PH, White J (1986) Anatomical changes during adventitious root formation. In: MB Jackson

(ed), New root formation in plants and cuttings. Long Ashton Research Station University of Bristol, UK, pp 141-190

Marcelis-van Acker CAM (1994) Effect of assimilate supply on development and growth potential of axillary buds in roses. Ann Bot 73: 415-420

Middleton W, Jarvis BC, Booth A (1980) The role of leaves in auxin and boron-dependent rooting of stem cuttings of *Phaseolus aureaus* Roxb. New Phytol 84: 251-259

Moe R (1973) Propagation, growth and flowering of potted roses. Acta Hortic 31: 35-51

Molnar JM, Cumming WA (1968) Effect of carbon dioxide on propagation of softwood conifer and herbaceous cuttings. Can J Plant Sci 48: 595-599

Molnar JM Lacroix LJ (1972) Studies of the rooting of cuttings of *Hydrangea macrophylla*: enzyme changes. Can J Botany 50: 315-322

Moritz T Sundberg B (1996) Identification and quantification of cytokinins in active and dormant cambial region tissues from *Pinus sylvestris* (L.) stems. Physiol Plant 98: 693-698

Mudge KW, Mwaja VN, Itulya FM, Ochieng J (1995) Comparison of four moisture management systems for cutting propagation of Bougainvillea, Hibiscus and Kei Apple. J Am Soc Hort Sci 120: 366-373

Muller B, Stosser M, Tardieu F (1998) Spatial distributions of tissue expansion and cell division rates are related to irradiance and to sugar content in the growing zone of maize roots. Plant Cell Environ 21: 149-158

Nanda KK, Anand VK (1970) Seasonal changes in auxin effects on rooting of stem cuttings of *Populus nigra* and its relationship with mobilization of starch. Physiol Plant 23: 99-107

Nanda KK,Jain MK, Malhotra S (1971) Effect of glucose and auxins in rooting etiolated stem segments of *Populus nigra*. Physiol Plant 24: 387-391

Newton, AC, Muthoka PN, Dick J McP (1992) The influence of leaf area on the rooting physiology of leafy stem cuttings of *Terminalia spinosa Eng.* Trees 6: 210-215

Okoro OO, Grace J (1976) The physiology of rooting *Populus* cuttings. I.Carbohydrates and photosynthesis. Physiol Plant 36: 133- 138

Ofori DA, Newton AC, Leakey RRB, Grace J (1996) Vegetative propagation of *Milicia excelsa* by leafy stem cuttings: effects of auxin concentration, leaf area and rooting medium. For Ecol Manag 84: 39-48

Pellicer V, Guehl, JM, Daudet, FA, Cazet M, Riviere LM, Maillard P (2000) Carbon and nitrogen mobilization in *Larix x eurolepis* leafy stem cuttings assessed by dual ^{13}C and ^{15}N labeling: relationships with rooting. Tree Physiol 20: 807-814

Poole RT, Conover CA (1984) Propagation of ornamental *Ficus* by cuttings. HortScience 19: 120-121

Porlingis IC, Therios I (1976) Rooting response of juvenile and adult leafy olive cuttings to various factors. J Hort Sci 51: 31-39

Pritchard SG, Rogers HH (2000) Spatial and temporal deployment of crop roots in CO_2-enriched environments. New Phytol 147: 55-71

Reuveni O, Raviv M (1981) Importance of leaf retention to rooting of avocado cuttings. J Am Soc Hort Sci 106: 127-130

Rodriguez A, Albuerne M, Sánchez Tams, R (1988). Rooting ability of *Corylus avellana* L. macromorphological and histological study. Sci Hort 35: 131-142

Rosenberg D, Altman A, Bravdo B (1992) A physiological analysis of the differential rooting performance of Calamondin and Pelargonium cuttings under mist and high humidity. Acta Hort 314: 257-269

Stangler BB (1956) Origin and development of adventitious roots in stem cuttings of Chrysanthemum, Carnation and Rose. Cornell Experiment Station Memoir 342 pp

Shiembo PN, Newton AC, Leakey RRB (1996) Vegetative propagation of *Irvingia gabonensis*, a west African fruit tree. For Ecol Manag 87: 185-192

Smalley TJ, Dirr AM, Armitage AM, Wood BW, Teskey RO, Severson RF (1991) Photosynthesis

and leaf water, carbohydrate and hormonal status during rooting of stem cuttings of *Acer rubrum*. J Am Soc Hort Sci 116: 1052-1057

Taylor CB (1997) Plant vegetative development: from seed and embryo to shoot and root. Plant Cell 9: 981-988

Thomas P (2000) Microcutting leaf area weight and position on the stock shoot influence root vigour, shoot growth and incidence of shoot tip necrosis in grape plantelets in vitro. Plant Cell Tissue Org 61: 189-198

Tinus RW, Burr KE, Atzmon N, Riov J (2000) Relationship between carbohydrate concentration and root growth potential in coniferous seedlings from three climates during cold hardening and dehardening. Tree Physiol 20: 1097-1101

Treeby MT, Considine JA (1982) Propagation of *Vitis champini* planchon cv Ramsey: relationship btween carbohydrate metabolism during storage and cutting performance. Am J Enol Vit 33: 53-56

Tschaplinski TJ, Blake TJ (1989) Correlation between early root production, carbohydrate metabolism, and subsequent biomass production in hybrid poplar. Can J Botany 67: 2168-2174

Van de Pol, PA (1988) Partial replacement of the rooting procedure of *Chrysanthemum morifolium* cuttings by pre-rooting storage in the dark. Acta Hort 226: 519-524

Van den Driessche R (1987) Importance of the current photosynthate to new root growth in planted conifer seedlings. Can J Forest Res 17: 776-782

Van den Driessche R (1991) New root growth of Douglas fir seedlings at low carbon dioxide concentration. Tree Physiol 8: 289-295

Van Labeke MC, Dambre P, Bodson M, Pien H (2001) Developmental changes in carbohydrate content in young rose shoots (*Rosa hybrida* 'Frisco'). Acta Hort 547: 193- 201

Van Overbeek J, Gordon SA, Gregory LE (1946) An analysis of the influence of the leaf in the process of root formation in cuttings. Am J Bot 33: 100-107

Van Staden J, Davey, JE (1979) The synthesis, transport and metabolism of endogenous cytokinins. Plant Cell Environ 2: 93-106

Veierskov B, Andersen AS, Stummann, BM, Henningsen KW (1982) Dynamics of extractable carbohydrates in *Pisum sativum*. I. Carbohydrate content and photosynthesis of pea cuttings in relation to irradiance and stock plant temperatures and genotype. Physiol Plant 55: 174-178

Wang, YT (1987) Effect of warm medium,light intensity, BA, and parent leaf on propagation of Golden Pothos. HortScience 22: 597-599

Welander M (1994) Influence of environment, fertilizer and genotype on shoot morphology and subsequent rooting of birch cuttings. Tree Physiol 15: 11-18

Wiesman Z, Lavee S (1995) Relationship of carbohydrate source and Indole-3-butyric-acid in olive cuttings. Aust J Plant Physiol 22: 811-816

Wilkins LC, Graves WR, Townsend AM (1995) Development of plants from single node cuttings differs among cultivars of Red Maple and Freeman Maple. HortScience 30: 360-362

Wilson PJ, Van Staden J (1990) Rhizocaline, Co-factors and the Concept of promoters and inhibitors of adventitious rooting- a review. Ann Bot 66: 479-490

Wilson PJ (1994a) Contributions of the leaves and axillary shoots to rooting in *Eucalyptus grandis* Hill ex.Maid. stem cuttings. J Hort Sci 69: 999-1007

Wilson PJ (1994b) Contributions of the stem to rooting in *Eucalyptus grandis* Hill ex. Maid. stem cuttings. J Hort Sci 69: 1009-1017

Ypema HL, Pol PA, Bollen, GJ (1987) Black rot of stentlings of roses: a disease caused by various soil fungi. Sci Hort 33: 269-280

Yue D, Margolis HA (1993) Photosynthesis and dark respiration of black spruce cuttings during rooting in response to light and temperature. Can J For Res 23: 1150-1155

Zaczek JJ, Heuser CW, Steiner KC (1999) Low irradiance during rooting improves propagation of oak and maple taxa. J Environ Hort 17: 130-133

CHAPTER 3

EFFECT OF THE LEAF AREA OF ROSE CUTTINGS

3.1 EFFECT OF THE ORIGINAL LEAF AREA ON GROWTH OF LEAFY STEM CUTTINGS AND PLANTING MATERIAL

Abstract

Leaf area influences root formation and growth of single-node softwood stem cuttings of rose. However, a complete assessment of the quantitative effect of the area of the original leaf on growth of cuttings and on derived planting material (rooted cuttings) is still lacking. Therefore, the aim of this study was to quantify the effect of the area of the original leaf of single node softwood cuttings on their growth until 10 weeks after severance. The concept of leaf area duration (LAD) which accounts for the effects of leaf area and its persistence in time was used. In two experiments the area of the original leaf of cuttings of *Rosa hybrida* Madelon® was reduced to varying dimensions during the first 3 weeks of propagation. After ten weeks, total plant dry weight, total leaf area and shoot length were proportional to the area of the original leaf but were not affected significantly by small reductions of leaf area. However, dry weight of roots was linearly related to the area of the original leaf, irrespective of the level of reduction. Three weeks after severance, total plant dry weight was linearly related with LAD indicating a direct relationship between the integral of photosynthesis and growth after the first 3 weeks. The moment of treatment had only a relevant effect on growth after 10 weeks when the original leaf area was reduced by more than 70%. Leaf removal during the first seven days of propagation was the most critical for survival and growth because of stem black rot. We conclude that under our conditions the original leaf area of cuttings is a good indicator for growth of roots from cuttings and planting material which can determine establishment after transplantation. Leaf area is also a good indicator of growth potential of cuttings and planting material in case of severe reductions.

Key words: Rosa hybrida, propagation, rooting, primary shoot, LAD, planting material quality

Part of this chapter has been published as :
Costa JM, Challa H 2002 *Scientia Horticulturae 95:111-121*

Introduction

It is well known that the original leaf of single-node softwood stem cuttings of rose has a strong effect on survival and rooting of cuttings (Moe, 1973; Dubois and De Vries, 1985 and 1991). Leaves may also directly influence growth of the primary shoot because initial growth of shoots in rose plants depends on assimilate supply by mature leaves (Zieslin and Mor, 1981, Marcelis-Van Acker, 1994; Van Labeke et al., 2001).

Although the relationship between leaf area and growth of cuttings in general terms is well documented, their quantitative relation is less well described and understood.

Growth analysis provides a well-established theoretical framework to understand and quantify growth of seedlings (Hunt, 1990; Margolis and Brand, 1990). With this method it is possible to determine the contributions of photosynthesis and of plant morphology to growth response to the environment.

For cuttings a similar approach would be possible, although the interpretation would be less straightforward and the assumption of source limitation may not be valid (Costa et al., 2001). After severance of a cutting the rooting process plays an important role before the axillary bud will produce a shoot with its own photosynthetic capacity. Nevertheless, the theoretical framework of classical growth analysis should be useful to increase insight into the role of the area of the original leaf on growth of cuttings during and after propagation.

Once established, such a quantitative relationship could be applied in practice. Though propagation of rose by leafy stem cuttings has reached high rates of success and efficiency, there are still quality problems related to non-uniform rooting of cuttings or slow establishment of planting material (rooted cuttings). A reliable quantitative method to assess growth potential (quality) of cuttings and planting material of rose based on external characteristics is still lacking. Such a method could be useful to establish quality standards for planting material and further, decrease variability of planting material by grading.

Variations in the area of the original leaf of cuttings may result from variations in growing conditions of the mother plants, but also from damage to the original leaf, by handling during propagation, or during transport and planting. We were interested

in assessing the effect of the area of the original leaf on growth potential of cuttings during propagation, e.g. the effect on the rooted cuttings (planting material), and the effect on the growth of the planting material after transplanting in the greenhouse. Our aim was to quantify to what extent growth of cuttings and planting material was influenced by reducing the area of the original leaf of cuttings during the first 3 weeks of propagation. We included also the moment of defoliation as a factor, to test the simple assumption that photosynthesis of the original leaf could explain the resulting variations in growth. Therefore, the leaf area duration (LAD) concept (Hunt, 1978; 1990) was applied. LAD accounts for the effects of leaf area and its persistence in time and represents the integrated photo-assimilation capacity of plants (Hunt, 1978; 1990), in this case of cuttings, during propagation.

Materials and methods

Effect of the area of the original leaf on plant growth 10 weeks after severance (Experiment 1)

Plant material and propagation

Mother plants of *Rosa hybrida* Madelon® were grown in a greenhouse (Wageningen, the Netherlands, 52°N) on rockwool slabs with controlled nutrients based on the standard solution for roses from Sonneveld and Voogt (1994) (pH=5.5, EC=180 mS m^{-1}), under a day/night temperature set point of 18/16°C and a photoperiod of 18 hours (5-23 h). Supplementary light was applied using high-pressure sodium lamps (Philips SON/T plus 400W, 36.5 µmol m^{-2} s^{-1} photosynthetic photon flux / PPF) when global radiation outside the greenhouse dropped below 100-150 Wm^{-2} and switched off when radiation was above 200Wm^{-2}. Cuttings were collected from the middle part of shoots bearing flower buds with a visible flower colour and sepals reflexing and consisted of an internode (55mm length and 4.7mm diameter on average), with an axillary dormant bud and an intact leaf of five leaflets. Cuttings were taken on 7 November 1995 and propagated in a greenhouse using propagation benches (200x250x35cm) (l, w, h) covered with a double layer of rigid acrylic sheet (about

50% light transmission). The rooting substrate was a mixture of peat and river sand 1:1 (v/v) with a temperature set-point of 23°C. The set point for day/night air temperature in the propagation room was 21/20°C. Humidity inside the benches was kept high by periodical manual misting. The natural daily global radiation during the experiment was about 2.3 MJm^{-2} and the photoperiod was extended to 18 hours by high pressure sodium lamps (Phillips SON/T plus, 70 W) providing a minimum light intensity of approximately 17.5 $\mu mol\ m^{-2}\ s^{-1}$ (PPF) at cutting level. The base of cuttings was treated with a talcum preparation of indolebutyric acid 0.4% (w/v) (Rhizopon, the Netherlands).

Treatments: Cuttings were defoliated to different degrees by removing from the original leaf: no leaflet (control), one leaflet, three leaflets or the entire leaf at 0, 7, 14 and 21 days after severance. Leaflets were removed in a basipetal order, starting at the terminal leaflet. The treatments resulted in average leaf areas of 58, 39 and 14 cm^2 for cuttings with 5, 4, and 2 leaflets respectively. Fifteen days after severance, cuttings were potted in 10-cm diameter plastic pots containing a commercial potting mixture (pH=6.0; EC=70 $mS\ m^{-1}$) and placed inside the propagation benches. Plants were hardened-off for 3 weeks before being moved to another greenhouse compartment with a day/night temperature set at 18°C/16°C respectively. The photoperiod was extended to 18 hours with high-pressure sodium lamps (Phillips SON/T Agro, 400 W) providing a minimum light intensity of about 50 $\mu mol\ m^{-2}\ s^{-1}$ (PPF) at plant level. Plants were placed on aluminium benches and grown till flower break and lateral buds of the primary shoot started to sprout. Plant density on the benches was 51 plants/m^2 till week 7 and 32 plants/m^2 afterwards.

Measurements: Number of roots, number of leaves and total leaf area was determined. The dry weight of roots, of the primary shoot (leaves and stem), and of the original stem segment of cuttings was determined after drying the material in a ventilated oven for 16 hours (6 hours at 70°C and 10 hours at 105°C). The number of days to axillary bud break (axillary bud length > 1.5 cm) was recorded. The length of the primary shoot was measured every week starting at week 4. Leaf area was determined with a LI-3100 Area meter (Li-Cor Inc., Lincoln, NE, USA).

Effect of leaf area duration on growth 3 weeks after severance (Experiment 2)

Plant material and propagation

Mother plants, grown from cuttings, were grown in two climate chambers (320 x 225 x 220 cm) (l, w, h), on cocos fibre slabs (Dutch Plantin Kokos, Boekel, the Netherlands) supplied with controlled nutrients (Sonneveld and Vogt, 1994) (pH=5.5; EC=150 mS m^{-1}). Day/night temperature was set at 20/18°C. A combination of high pressure sodium lamps (Philips SON/T Agro 400W) and metal halide vapour arc lamps (Philips HPI/T 400W) provided a mean light intensity of about 180 µmol m^{-2} s^{-1} (PPF) at the top of the canopy. The photoperiod was 16 hours. Relative humidity was set at 65-70%. Cuttings were harvested on 20 January 1999 and propagated in a climate chamber (265 x 225 x 220 cm) (l, w, h), with a temperature set point of 25°C. Mean PPF was 85µmol m^{-2} s^{-1} at cutting level (photoperiod and light sources were as for the mother plants). Cuttings were planted in 11 propagator boxes (57x37x23cm) with a plastic cover to maintain the relative humidity over 90-95%. Rooting substrate was a mixture of peat and sand 1:1 (v/v) with a temperature similar to air temperature. No auxin was applied to cuttings.

Treatments: Different leaf area duration (LAD) treatments were applied by removing no leaflets (control), one leaflet, three leaflets or the entire leaf from cuttings on day 0, 3, 7 or 11 of propagation. Cuttings in this experiment had larger leaves than cuttings from Experiment 1, 93, 61 and 22 cm^2 for leaves with 5, 4 and 2 leaflets respectively, probably due to different (light) growing conditions of the mother plants.

Measurements: Number and dry weight of roots, of the axillary bud, of the original stem segment and leaves were recorded after 21 days. Leaf area and dry weight were determined as described for Experiment 1. Growth of callus at the base of the stem segment was also scored according to a 4 point scale regarding the amount of callus formed: 0-no callus; 1-small and irregular callus; 2–medium and regular callus; 3- big regular amount of callus.

Experimental design and statistical analysis

A randomised complete block design with 4 (Experiment 1) and 11 blocks (Experiment 2) was used. Blocks in both experiments were divided into 13 plots to accommodate 13 treatments (control plus the different combination of remaining leaf area (3) x number of days after severance (4)) (Experiment1) or the 13 different LAD values including control (Experiment 2). Each plot contained five cuttings (Experiment 1) or a single cutting (Experiment 2). For statistical analysis we used the average per plot and data were considered normally distributed. Data from Experiment 1 were subjected to general analysis of variance ($P<0.05$). The effect of treatments on elongation of the primary shoot was analysed by analysis of variance, performed for each week of observation. The relation between the remaining leaf area and total plant dry weight, total leaf area, shoot dry weight and dry weight of roots 10 weeks after severance was tested by linear models. The effect of treatments on the time until axillary bud break was analysed by general analysis of variance. In Experiment 2, the effect of LAD and of leaf area on the measured variables was analysed by linear models. In both experiments treatments resulting in no survival were excluded from statistical analysis. Data were analysed using the statistical package GENSTAT 5 (IACR, Rothamsted, UK).

Results

Total dry weight of the plant after 10 weeks was not, or was only marginally, affected by removing about 30% of the original leaf area, but larger reductions in leaf area had a much stronger negative effect (Fig. 3.1.1). The same pattern was observed for total leaf area (responsible for the formation of dry weight), and also for shoot length. Root dry weight, in contrast, was proportional to the remaining area of the original leaf (Fig. 3.1.1).

The development of shoot length over time (Fig. 3.1.2) could help to understand the growth response observed after 10 weeks. Length of the primary shoot of the control and of plants with about 70% of the original leaf area followed the

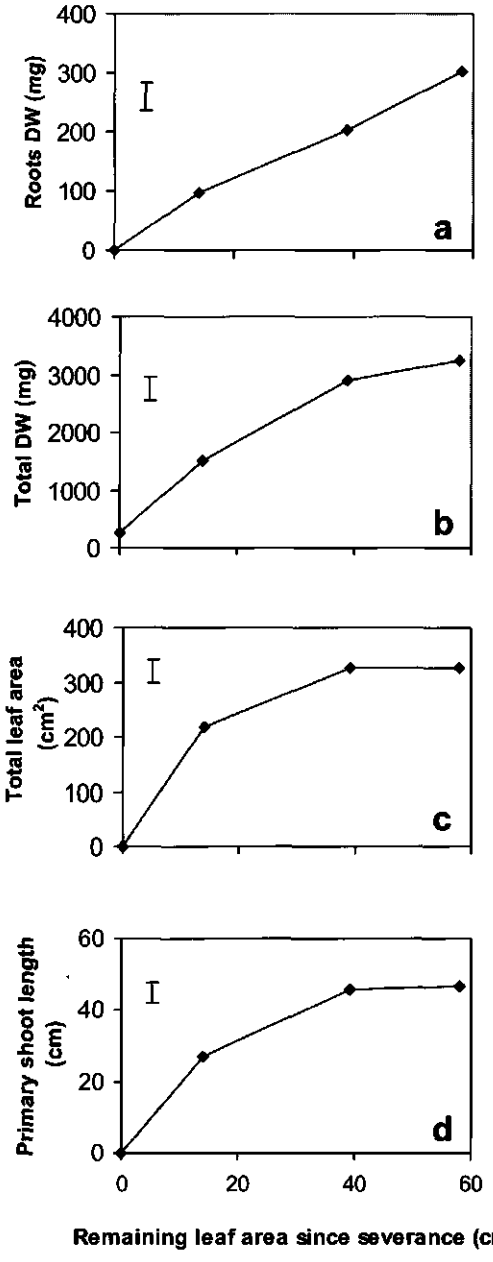

Figure 3.1.1 Effect of the remaining area of the original leaf (58 cm^2 (control), 39 cm^2, 14 cm^2 and 0 cm^2) of single node leafy stem cuttings of *Rosa hybrida* Madelon® on roots dry weight **(a)**, total plant dry weight **(b)**, total leaf area **(c)** and length of the primary shoot **(d)** of plants 10 weeks after severance (Experiment 1). Symbols represent the overall means from the four blocks. Vertical bar indicates the LSD of the treatments at 5% level of confidence. Total dry weight of cuttings with 0 cm^2 was estimated (200 mg) as these cuttings died before the 10 weeks.

characteristic s-pattern, but in the treatment with only 25% of the original leaf area shoot length was still increasing linearly on week 10 (Fig. 3.1.2).

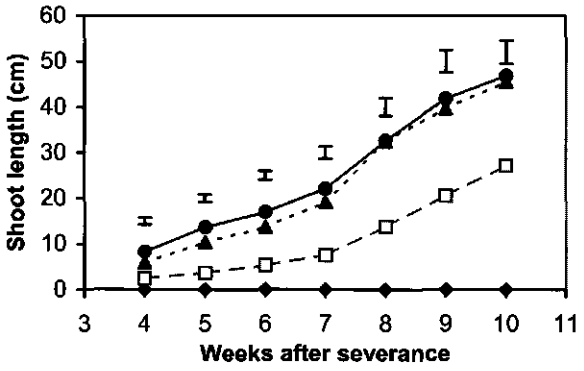

Figure 3.1.2 Length of the primary shoot between week 4 and 10 of Experiment 1 from plants of *Rosa hybrida* Madelon® propagated from leafy stem cuttings with an original leaf with an area of 58 cm^2 (control) (—●—), 39 cm^2 (---▲---), 14 cm^2 (- -□- -), 0 cm^2 (leafless) (—♦—) since severance. Vertical bars indicate least significant differences at 5% level (Student's t-test).

When the original leaf was removed the axillary shoot did not grow at all, because cuttings died. There was no clear effect of the treatments on axillary bud break, although it was slightly delayed when all or 70% of the leaf area was removed on or before day 7.

Three weeks after severance, total dry weight responded linearly to variations in LAD (Fig. 3.1.3), demonstrating that integrated cutting photosynthesis could fully account for the observed variations in dry weight. This was, however, not true for number and, in particular, for dry weight of roots, which seemed to respond primarily to the remaining leaf area, irrespective of the moment of leaf area reduction (Fig. 3.1.3 and 3.1.4). Larger LAD (Fig. 3.1.5) also positively influenced callus formation and no callus was formed if the original leaf was removed during the first 3 days of propagation.

It is interesting to note that the growth response 3 weeks after severance was different from that after 10 weeks, demonstrating that the new leaf area in the last 7 weeks has an important effect on dry matter production.

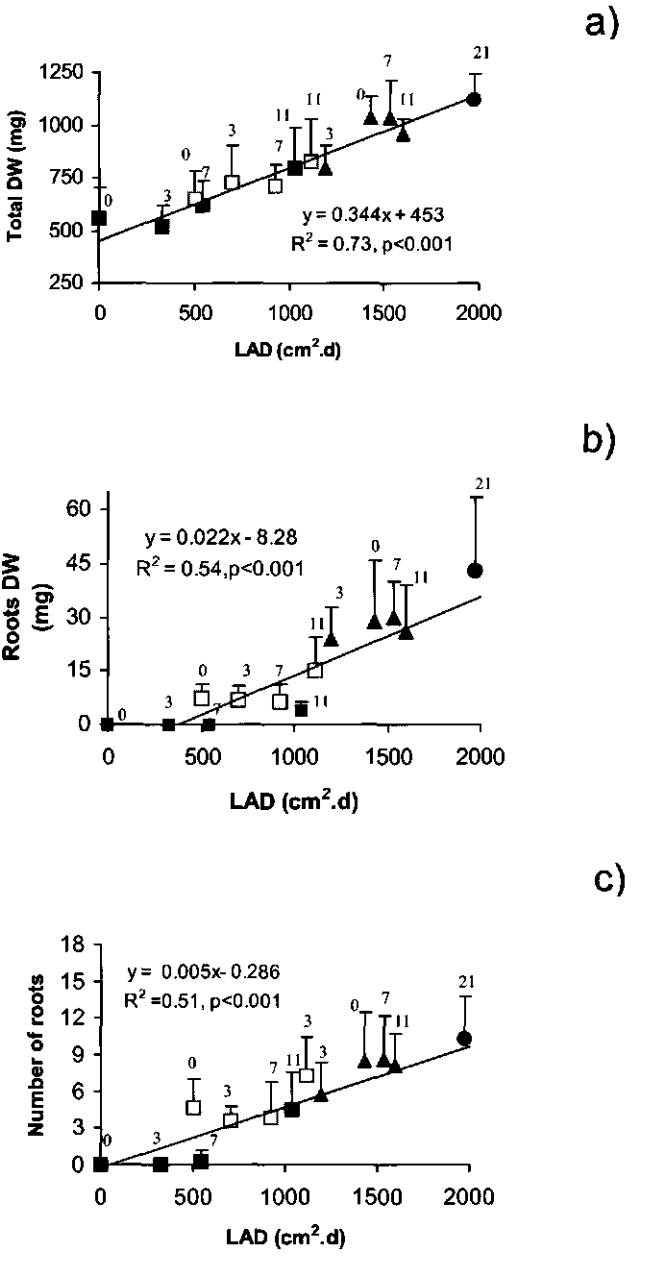

Figure 3.1.3 Effect of leaf area duration (LAD) on total dry weight **(a)**, dry weight of roots **(b)** and number of roots **(c)** measured 3 weeks after severance from single node leafy stem cuttings of *Rosa hybrida* Madelon® with a leaf area of 93 cm^2 (control) (●), 61 cm^2 (▲), 22 cm^2 (□) and 0 cm^2 (leafless) (■) after reduction of the original leaf on days 0, 3, 7 and 11 (Experiment 2). Symbols represent the overall means from the 11 blocks and vertical bars are the standard deviation of the mean. The solid line is a regression line through all data. Numbers over the standard deviation bars indicate the time of the treatment.

Table 3.1.1 Combined effect of the remaining area (0,14, 39 and 58cm^2) of the original leaf of cuttings of *Rosa hybrida* Madelon® after removal of leaflets 0, 7, 14 and 21 days after severance on different morphological parameters measured 10 weeks after severance.

Leaf area remaining After removal (cm^2)	Time of removal (days after severance)	Survival (%)	Number roots	Total leaf area (cm^2)	Number leaves shoot	Shoot length (cm)	DW roots (mg)	DW cutting stem (mg)	DW leaves (mg)	DW primary shoot (mg)	DW total plant (mg)
0	0	0	-	-	-	-	-	-	-	-	-
0	7	45	9.8	78	3.8	11	29	221	245	300	734
0	14	100	15.5	167	8.1	21	70	269	531	762	1099
0	21	95	20.2	261	10.8	34.	150	339	949	1471	1959
14	0	100	9.8	219	9.2	27	96	285	800	1145	1503
14	7	95	18.4	242	9.8	31	106	308	879	1256	1657
14	14	95	17.5	249	10.5	33	130	315	929	1373	1749
14	21	100	18.7	273	10.7	37	130	351	1013	1593	2038
39	0	100	16.0	326	11.1	46	204	374	1329	2342	2913
39	7	100	23.2	292	11.4	43	189	380	1202	1982	2560
39	14	95	15.9	310	11.9	43	155	388	1147	1743	2258
39	21	95	19.3	339	12.4	46	204	438	1395	2152	2868
58 (control)	-	100	24.4	327	12.3	47	302	489	1388	2443	3235
LSD[a]		-	6.8	44	1.75	5.5	49	60	193	352	424

[a] Least Significant Difference (Student's t-test;P=0.05)

Leaf removal immediately after severance or on day 7 had a significant negative effect on survival and rooting compared to the other treatments as it caused, in both experiments, losses due to stem black rot (Table 3.1.1 and Fig. 3.1.3). The timing of the treatments had hardly any significant effect after 10 weeks on most of the morphological parameters when a small reduction (about 30%) of leaf area occurred (Table 3.1.1).

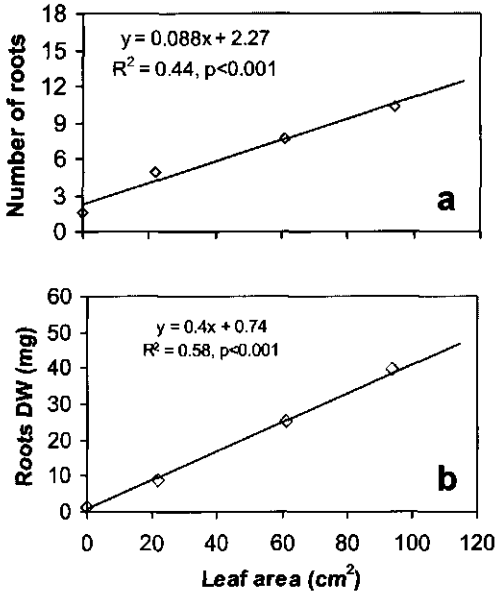

Figure 3.1.4 Effect of the remaining leaf area on number **(a)** and dry weight **(b)** of roots measured three weeks after severance from single node leafy stem cuttings of *Rosa hybrida* Madelon®. Symbols represent the overall mean from each group of cuttings with 93 cm^2 (control), 61 cm^2, 22 cm^2 and 0 cm^2 (leafless) (Experiment 2). The solid line is a regression line through all data.

Discussion

Dry weight accumulation, total plant leaf area and shoot length after 10 weeks were relatively insensitive to reductions in the area of the original leaf. Only severe reductions in leaf area (equal to or over 70%) had a significant, proportional effect on plant size after 10 weeks (Fig. 3.1.1 and Table 3.1.1). The mechanism can be explained as follows. When the original leaf is removed during the first week of

propagation the cutting cannot survive and will die as a result of stem black rot. Even when the cuttings survived growth stopped and neither were roots formed nor did the axillary bud grow. When leaf area is not totally reduced (e.g. when 30% of leaf area is maintained after severance), cuttings do not die but growth is strongly restricted (Table 3.1.1). The later the original leaf is removed or drastically reduced, the better the chance for the cutting to survive and to develop into a normal plant (Table 3.1.1).

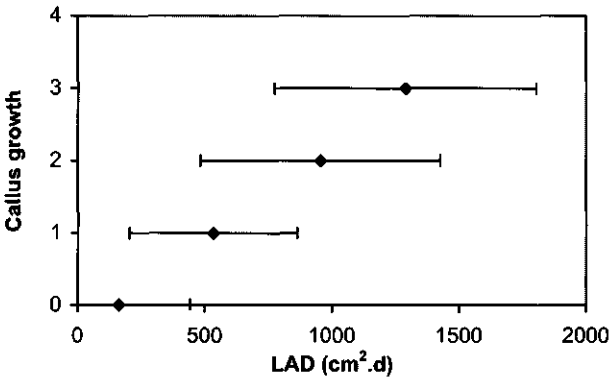

Figure 3.1.5 Effect of leaf area duration (LAD) on callus growth measured 3 weeks after severance from single node leafy stem cuttings of *Rosa hybrida* Madelon®. Symbols represent the mean LAD found for each class of callus growth. The horizontal bars are the standard deviation of the mean.

A strong reduction in the area of the original leaf lead to a proportional reduction in the rate of dry matter production after the first 3 weeks (Fig. 3.1.3), when new leaves are not yet formed, or are too small to contribute to plant photosynthesis. In general, the later leaf area is reduced, the less the total dry weight is affected (Fig. 3.1.3). The close relationship between LAD and total dry weight after 3 weeks demonstrates that the integrated photosynthetic capacity mainly determined dry weight increase in this case. The differences in dry weight of the cutting observed after 3 weeks, brought about by different moments of leaf removal, were not observed in root dry weight, showing that growth of roots is only affected by current photosynthesis and not by reserves formed previously. A possible explanation could be that because roots initiate during the first seven days of propagation (data not shown) root growth did not benefit from extra photosynthesis during the first week, and the last time interval (7 – 11 days after severance) was too short to develop any effect. Between weeks 3

and 10 after severance, growth is dominated by the contribution of the new shoot to plant photosynthesis; compared to the original leaf area of 58 cm^2 control plants after 10 weeks had an area of 327 cm^2 (Table 3.1.1). The new shoot forms a flower after a fixed number of leaves and then stops growing. The moment the new shoot is formed and the size of the leaves are factors that should determine growth of the plant between week 3 and 10 after severance. As there was no clear effect of the treatments on the number of days till bud break (data not presented), we conclude that the total leaf area formed after bud break should be the main factor causing variation in plant dry weight after 10 weeks. This is supported by the findings of Berninger (1994) and Bredmose (1997) who showed that the growth rate of young rose plants obtained from single node cuttings is strongly influenced by the PPF available between axillary bud break and the flower bud becoming visible.

Total leaf area, and the closely related length of the shoot (Pieters et al., 1999), was not affected by moderate reductions in area of the original leaf (Table 3.1.1 and Fig. 3.1.1), showing that growth of the axillary primary shoot was not source limited. However, more severe reduction in the area of the original leaf did negatively affect growth of the primary shoot (Table 3.1.1 and Fig. 3.1.2). Under those conditions, growth of the primary shoot was source limited in agreement with previous literature indicating the promotive effect of assimilates on growth of flower shoots of rose (Marcelis van-Acker, 1994; Van Labeke et al., 2001).

The size of the root system, in contrast with the total plant dry weight, was proportionally related to the area of the original leaf of cuttings. Root growth responded to leaf area after the first 3 weeks of propagation and after the period from week 3 till week 10, during which it responded even to moderate leaf area reduction. This suggests that sink strength of roots was affected by the treatments which may be also concluded from the number of roots observed 3 weeks after severance. Thus, the area of the original leaf of cuttings can be considered a good indicator of root growth of cuttings and of planting material which influences the establishment of planting material after transplantation as reported for roses (Fuchs, 1986) and other species (Bentz et al., 1985; Margolis and Band, 1990; Van Iersel, 1999). Leaf area is also a good indicator of growth potential of cuttings and planting material in the case of severe reductions of the area of the original leaf of cuttings.

Acknowledgements

The authors thank Dr. Ep Heuvelink , Dr. Uulke van Meeteren, and Dr. Peter van de Pol for major comments during the elaboration of this paper.

References

Bentz S, Stimart DP, McIntosh MS (1985) Root and shoot growth patterns of newly rooted woody plants. J Am Soc Hort Sci 110: 308-313

Berninger E (1994) Development rate of young greenhouse rose plants (*Rosa hybrida*) rooted from cuttings in relation to temperature and irradiance. Sci Hort 58: 235-251

Bredmose N (1997) Chronology of three physiological development phases of single stemmed rose (*Rosa hybrida* L.) plants in response to increment in light quantum integral. Sci Hort 69: 107-115

Costa JM, Challa H, Van Meeteren U, Van de Pol PA (2001) Photosynthates: mainly stored and yet limiting in propagation of rose cuttings. Acta Hort 547: 167-174

Dubois LAM, De Vries DP (1985) Onderzoek met stekken van 'Sonia'. Aanwijzingen voor opkweek goed kasrozenstek. Vakblad voor de Bloemisterij 45: 26-30

Dubois LAM, De Vries DP (1991) Variation in adventitious root formation of softwood cuttings of *Rosa chinensis minima* (Sims) Voss cultivars. Sci Hort 47: 345-349

Fuchs HWM (1986) Root regeneration of rose plants as influenced by applied auxins, Acta Hort 189: 101-107

Hunt R (1978) Plant growth analysis. Studies in Biology, n.96, Edward Arnold, London, UK

Hunt R (1990) Basic growth analysis, Unwin Hyman Lda, London, UK

Marcelis-van Acker CAM (1994) Effect of assimilate supply on development and growth potential of axillary buds in roses. Ann Bot 73: 415-420

Margolis HA, Band DG (1990) An eco-physiological basis for understanding plantation establishment. Can J Forest Res 20: 375-390

Moe R (1973) Propagation, growth and flowering of potted roses. Acta Hort 31: 35-51

Pieters GA, Van den Noort ME, Van Nijkerken JA (1999) Growth adaptation of leaves and internodes of poplar to irradiance, day length and temperature. Tree Physiol 19: 933-942

Sonneveld C, Voogt W (1994) Het berenken van voedingsopplosingen, voor plantenteelt zonder arde. PBG Serie: Voedingsopplosingen glastuinbouw, 10, 21 pp

Van Iersel M (1999) Auxin applications affect post-transplant CO_2 exchange rate and growth of bare-rooted Vinca [*Catharanthus roseus* (L.) G.Don] seedlings. J Am Soc Hort Sci 124: 234-238

Van Labeke MC, Dambre P, Bodson M, Pien H (2001) Developmental changes in carbohydrate content in young rose shoots (*Rosa hybrida* 'Frisco'). Acta Hort, 547: 193-201

Zieslin N, Mor Y (1981) Plant management of greenhouses. Formation of renewal canes. Sci Hort 15: 67-75

3.2 EFFECT OF LEAF AREA AND CARBOHYDRATE CONTENT ON STEM BLACK ROT DURING PROPAGATION

Abstract

The relation between carbohydrate content at the rooting zone of cuttings and black rot incidence was studied for single node leafy stem cuttings of rose. Black rot incidence, rooting and carbohydrate content at the rooting zone (basal 15mm of the stem) of cuttings were observed on days 0, 3, 7 and 11 of propagation for cuttings with a leaf area of 69 cm^2 (control), 18 cm^2, 0 cm^2 (leafless) and also for leafless cuttings treated with a 4-day apical pulse of glucose 4.5% and bactericide sodium dichloroisocianuric acid (DICA). In a second experiment, black rot incidence in leafless cuttings was compared with that of cuttings with an intact leaf or leafless cuttings treated with glucose or sucrose at 4.5% and DICA. Partial removal of the original leaf diminished dry weight and carbohydrate accumulation at the rooting zone and reduced rooting. Total leaf removal caused stem rot. Sugar pulse delayed the appearance of black rot symptoms, however, it did not prevent stem black rot nor enable leafless cuttings to root. DICA promoted the inhibiting effect of sugar application on black rot appearance. The type of rooting substrate used (new, steamed or used) had no effect on black rot incidence. We suggest that stem black rot in rose softwood cuttings is a physiological disorder caused by lack of carbohydrates at the rooting zone.

Key words: *Rosa hybrida*, carbohydrate, susceptibility to stem black rot, rooting

Introduction

In leafy stem cuttings of rose, leaf removal or drastic reduction of the original leaf area during the first 7 days of propagation caused loss of cuttings by stem black rot (Costa and Challa, 2002). Carbohydrate depletion due to insufficient photosynthetic activity might be one of the reasons as it has been suggested for other species where dry weight loss and rotting occurred (Howard and Harrison-Murray, 1995). The fact that softwood/semi hardwood material has a relatively small amount of reserves at the moment of severance (Okoro and Grace, 1976; Hartmann et al., 1997) is in line with this hypothesis. It has been suggested that the susceptibility of leafless cuttings of rose to stem black rot, a disease caused by various soil fungi, is related to the energetic status of cuttings (Ypema et al., 1987).

The present work addresses the question to what extent the leaf area of cuttings influences sensitivity of stem tissues to black rot via its effect on the carbohydrate content at the rooting zone. This problem was addressed by reducing the original leaf area and by supplying leafless cuttings with sugar solutions.

Materials and methods

Plant material and propagation

The growth of mother plants of *Rosa hybrida* Madelon® and the procedure of taking the cuttings and the rooting circumstances were the same as described in chapter 3.1 for Experiment 1.

Eight mL plastic vials were used to pulse cuttings with distilled water or with glucose and sucrose solutions at 4.5% (w/v). The vials were attached upside down, on the top of cuttings, above the axillary bud (Van de Pol and Marcelis, 1988), and sealed with parafilm to avoid leaking. The bactericide DICA (sodium dichloroisocianuric acid, SIGMA, USA) was applied in a concentration of 50 mgL^{-1} using destilled water (Jones and Hill, 1993). The pH of the solutions was adjusted to 6.7-7 using KOH and H_3PO_4 (1M). The sugar pulse had a duration of 4 days since cuttings were particularly sensitive to leaf removal during the first 3-7 days of propagation (Costa and Challa, 2002). Moreover, most of the vials were empty by then.

Experiment 1. Effect of leaf area reduction on rooting, stem black rot and carbohydrate content at the rooting zone of cuttings

Experiment 1 started on 26 February 1996 and had a daily light integral 4.99 $MJm^{-2}d^{-1}$. Cuttings with five leaflets (control) average total leaf area of 69 cm^2, that of the two most proximal leaflets (18 cm^2), leafless cuttings and leafless cuttings pulsed with glucose 4.5% and DICA, were observed on days 0, 3, 7 and 11 of propagation.

Measurements: Incidence of stem blackening, number and dry weight of roots and dry weight of the basal 1.5cm part of the stem (rooting zone) were recorded. Samples from the rooting zone of the stem were analysed for starch and sugars (glucose, fructose and sucrose). Cuttings were harvested about 6 h after the beginning of the photoperiod. The samples, a total of 20 per treatment, were cut, frozen in liquid nitrogen and stored at -21°C. Prior to carbohydrate analysis material was freeze dried for 5 days and then powdered in a ball mill. About 15 mg of the powdered plant material was weighed and extracted sequentially for soluble carbohydrates and starch. Soluble carbohydrates were extracted in 5 ml of 80% v/v aqueous ethanol containing melezitose (40 mg L^{-1}) as internal standard in a shaking water bath at 80°C for 20 min. The first centrifugation of samples was made at 5500g for 5 minutes. One ml of the supernatant from each sample was then transferred to a reaction vial and dried in a vacum centrifuge (SpeedVac, Savant, Farmingdale, NY, USA) (type AES 2000) for two hours. The centrifuge tube with the pellet was stored at -20°C and used separately for further starch determination. One mL of ultra-purified water (milli-Q gradient A10, Millipore, USA) was added to the dry residue in the reaction vials and cleared of phenolic compounds with polyvinylpolypirollidone (PVPP). Samples were centrifuged at 13200g for 15 minutes. Half a mL of the supernatant was transferred to HPLC tubes for determination of glucose, fructose and sucrose by using a high perfromance anion exchange-chromatograph (HPAEC) equipped with a Waters 600E pump, Spark Marathon plus autosampler, DIONEX PED detector and a DIONEX PA-1 column, using 100 mM NaOH as eluent at a flow rate of 1 mL min^{-1} at 25°C. Starch was extracted from the pellet remaining after the sugar extraction. The powder was washed three times with 80% ethanol to extract soluble carbohydrates and the residue was then dried in a Speedvac. Starch in the dried residue was then solubilized by adding 2 mL of a 1g L^{-1} thermostable alpha-amylase solution (SERVA 13452) and incubating at 90°C for 30

minutes. This was followed by hydrolysis to glucose by adding 1 mL of amyloglucosidase (Boehringer, Mannheim) from *Aspergillus niger* (1 gL^{-1} in citrate buffer 50mM pH=4.6) and placing in a shaking water bath at 60°C for 15 min. After centrifuging, the supernatants were analysed for glucose by HPAEC. The starch content was expressed as mg glucose per mg dry weight. Total carbohydrates (TC) were determined by summing the concentrations of sugars (glucose, fructose and sucrose) and starch.

Experiments 2 and 3. The effect of an external supply of glucose, sucrose and DICA on black rot and rooting of leafless cuttings

Experiment 2 started on 23 November 1995 and included four treatments: intact leaf cuttings (control), leafless cuttings, leafless cuttings pulsed with distilled water and DICA and leafless cuttings pulsed with glucose 4.5%. To test the effects of DICA and other sugar (sucrose), Experiment 3 started on 18 December 1995 by observing the following treatments: leafless cuttings (control), leafless cuttings pulsed with water and DICA, leafless cuttings pulsed only with glucose 4.5%, leafless cuttings pulsed with glucose 4.5% and DICA and leafless cuttings pulsed with sucrose 4.5% and DICA. Black rot incidence was recorded in Experiment 2 between days 13 and 39 and in Experiment 3 between days 15 and 41. The maximal duration of the pulses was 4 days in both cases.

Experiment 4. The effect of type of rooting substrate (steamed, used or new substrate) on black rot incidence in leafless cuttings

Cuttings with an intact leaf and leafless cuttings were harvested and propagated in the greenhouse on 12 November 1997, in three rooting substrates differing in the inoculum potential of pathogens: 1) steamed substrate, 2) fresh substrate and 3) used substrate. Nine propagators (57 x 37 x 23 cm) (*l, w, h*) with a plastic cover were used and placed in the greenhouse. Daily light integral was 2.5 $MJm^{-2}d^{-1}$ and natural day length was extended to 18 hours by using high pressure sodium lamps (SON-T 150 W) which provided a minimum light intensity of about 35 $\mu mol\ m^{-2}\ s^{-1}$ (PPF) at cuttings

level. No auxin or substrate heating were used. Black rot incidence was recorded 16 days after severance.

Experimental design and statistical analysis

In Experiments 1, 2 and 3 a randomised complete-block design was used with 4 blocks. In Experiment 1 there were 13 plots, with 5 cuttings per plot; Experiment 2 had 4 plots per block, and 5 cuttings per plot; and in Experiment 3 there were 5 plots per block and 6 cuttings per plot. In Experiment 4 a split-plot design was used with 3 blocks with substrates as main plots and groups of six cuttings with an intact leaf (control) or leafless as sub-plots. Black rot incidence was analysed by binomial regression ($P<0.05$) using a logit link function. For means separation, confidence intervals were calculated by multiplying the standard errors of the predicted means by the t value at 5% level considering the degrees of freedom of the residuals. Carbohydrates content, number and dry weight of roots and dry weight of the rooting zone of the stem were analysed by analysis of variance ($P<0.05$) by using the statistical package GENSTAT 5 (IACR, Rothamsted, UK).

Results

Leaf removal inhibited dry matter and carbohydrate accumulation at the rooting zone (Fig. 3.2.1) and caused stem black rot (Table 3.2.1) whereas keeping part of or the entire original leaf allowed significant dry weight accumulation at the rooting zone (Fig. 3.2.1F), mostly related to callus formation and rooting (Table 3.2.2), and avoided stem rot (Table 3.2.1). Dry weight accumulation in control cuttings or in cuttings with 18cm^2 was paralled by an accumulation of total non-structural carbohydrates (starch and sugars), in particular starch (Fig. 3.2.1). After 11 days of propagation starch content increased about 7 times whereas sucrose or glucose contents doubled. The increase in sugars and starch started after day 3. Carbohydrate content at the rooting zone of leafless cuttings decreased slightly during the 11 days of propagation, in particular after day 3 (Fig. 3.2.1). The four days pulse of sugars did not avoid stem rot (Table 3.2.1) nor promoted rooting in leafless cuttings (Table 3.2.2), but delayed significantly the appearance of stem rot symptoms (Fig. 3.2.2 and Table 3.2.2). The glucose pulse had

also no effect on carbohydrates content in the rooting zone of leafless cuttings after 3 days (Fig. 3.2.1), but the decrease in carbohydrates was slightly delayed compared to leafless cuttings not treated with sugar. In fact by day 3, concentrations of sucrose,

Table 3.2.1 Black rot incidence, number and dry weight of roots and dry weight of the basal stem of single node leafy stem cuttings of *Rosa hybrida* Madelon®, 11 days after severance (Experiment 1).

Treatment	Black rot incidence (%)	Number of roots	DW roots (mg)
Five leaflets (control)	0a	12.6a	9.3a
Two leaflets	0a	5.6b	2.1b
Leafless (gluc 4.5% + DICA)	35b	0.0c	0.0b
Leafless	90c	0.0c	0.0b
LSD (P=0.05)		3.5	4.5

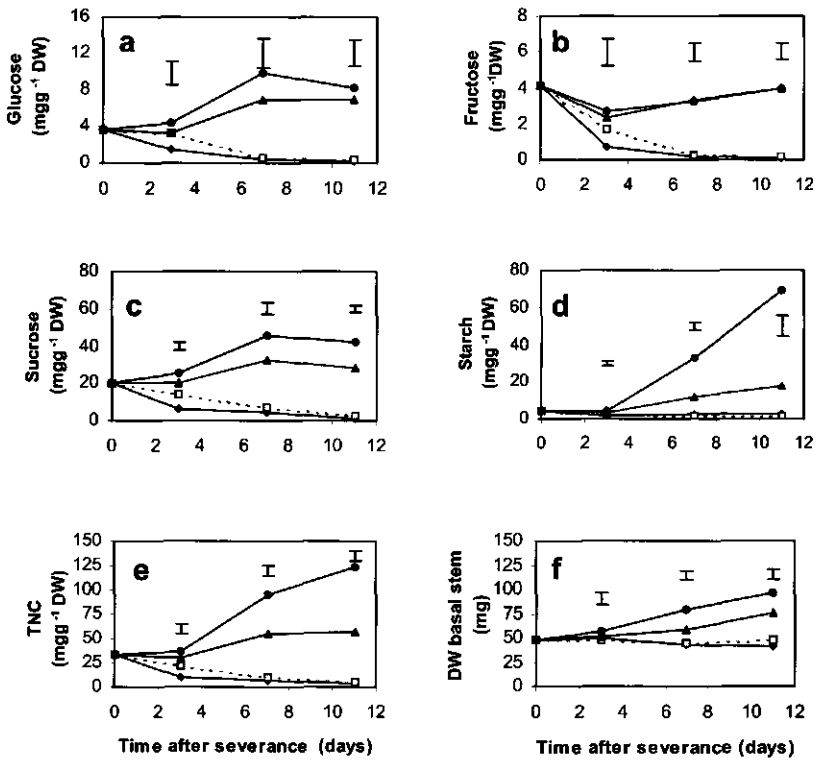

Figure 3.2.1 Concentrations (mg/gDW) of glucose **(a)**, fructose **(b)**, sucrose **(c)**, starch **(d)** and total carbohydrates (sugars + starch) **(e)** and dry weight (mg) **(f)** for the rooting zone of single node stem cuttings of *Rosa hybrida* Madelon® without the original leaf (leafless) (—♦—), leafless and treated with glucose 4.5 % and DICA (—□--), with two leaflets (—▲—) and with an intact leaf (five leaflets) (—●—) on days 0, 3, 7 and 11 of propagation in Experiment 1. Vertical bars indicate $LSD_{0.05}$ from Student's *t*-test.

Table 3.2.2 Black rot incidence in single node leafy stem cuttings of *Rosa hybrida* Madelon® as influenced by leaf removal and glucose and sucrose at 4.5% supply in Experiment 3. Values followed by different letters within each column are significantly different at 5% level (Student's t-test).

Treatment	Black rot incidence (%)				
	Day 15	Day 18	Day 21	Day 29	Day 41
Leafless (control)	92 a	92a	96a	100a	100a
Leafless (water+DICA)	96 a	96a	96a	96a	96a
Leafless (gluc)	50 b	71ab	96a	100a	100a
Leafless (gluc + DICA)	33 b	58b	67b	92a	92a
Leafless (suc + DICA)	50 b	54b	63ab	87a	92a

and glucose were significantly higher in the leafless cuttings treated with sugar than in the non treated cuttings. DICA improved the effect of sugars, but did not avoid stem blackening (Table 3.2.2). Black rot incidence was not influenced by the type of substrate used (steamed, used or new): leafless cuttings showed blackening symptoms in all the substrates (Table 3.2.3).

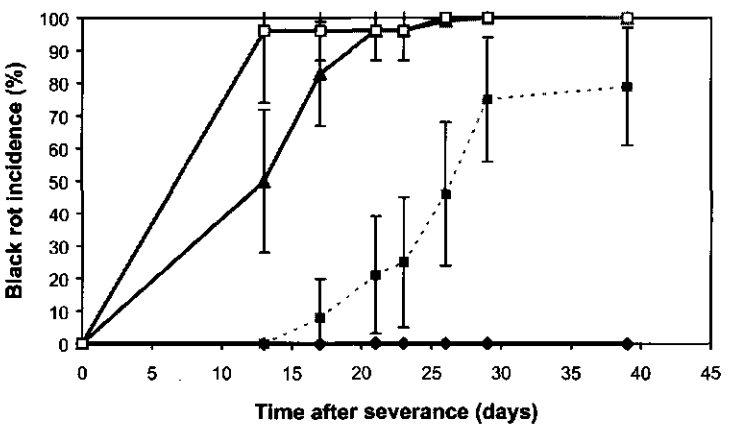

Figure 3.2.2 Black rot incidence in single node stem cuttings of *Rosa hybrida* Madelon® with an intact leaf (five leaflets) (—♦—), leafless (—□—), leafless treated with water and DICA (—▲—) and leafless treated with glucose 4.5% and DICA (---■---) in Experiment 2. Vertical bars indicate lower and upper confidence limits at 5% level (Student's *t*-test).

Table 3.2.3 Black rot incidence in single node leafy stem cuttings of *Rosa hybrida* Madelon® without (leafless) or with the original leaf (five leaflets) propagated in the different types of substrates (steamed, fresh, used), after 16 days of propagation in Experiment 4. Values are means per block (± SE) (n=3).

Treatment	Black rot incidence %		
	Steamed substrate	Fresh Substrate	Used Substrate
Five leaflets	0	0	0
Leafless	100	77 ± 10	94± 10

In one of the experiments (Experiment 2), 25% of cuttings without leaves and treated with glucose were able to form small roots after a long period (triple the time of the control; data not presented). Sugars also promoted axillary bud break (data not presented).

Discussion

The presence of leaflets (five or two) or the supply of carbohydrates to the rooting zone of cuttings seem to be pre-requisites to avoid stem black rot incidence in rose softwood cuttings. Keeping the original leaf or part of it enables accumulation of carbohydrates and dry weight at the rooting zone of cuttings which is known to promote rooting (Okoro and Grace, 1976; Haissig, 1984; Hoad and Leakey, 1996; Howard and Harrison-Murray, 1995; Pellicer et al., 2001).

Carbohydrate depletion observed in leafless cuttings (Fig. 3.2.1) can be expected as consequence of the absence of photosynthetic activity and continuos stem respiration (Buttrose, 1966; Cameron and Rook, 1974; Yue and Margolis, 1993; Howard, 1994).

When carbon resources are depleted the cuttings are not able to sustain maintenance and die or the tissues lose their resistance against soil born fungi, as suggested for rose cuttings (Ypema et al., 1987). For *Syringa* cuttings it has been suggested that stem rot was a non-pathogenic disorder primarily due to carbohydrate starvation (Howard and Harrison-Murray, 1995). The relation between carbohydrate content of tissues and their susceptibility to disease has been mentioned in literature

(Horsefall and Dimond, 1957; Sabet and Hassan, 1961; Schonenweiss, 1967; Patil and Dimond, 1968; Kiyomoto and Bruehel, 1977) and sugars, like glucose and sucrose, are known to induce the resistance of plants to pathogens (Cohen et al., 1996).

Feeding cuttings with sugar solutions delayed symptoms of stem blacking probably because the substrate for respiration was provided or because the establishment of the parasite-host relationship was delayed (Tousson et al., 1960). Thus, the small delay in carbohydrate decrease observed in the sugar-pulsed leafless cuttings could justify the delay in the appearance of black rot symptoms (Fig. 3.2.2). However, the external pulse of sugars could not substitute the effect of the original leaf on promoting rooting. The short duration and volume of the sugar pulse may also justify why it was not totally effective in avoiding stem black rot and promoting rooting but it is also possible that photosynthesis is needed or that other factors which originate in leaves (e.g. auxins) are influencing rooting.

We conclude that black rot incidence in softwood cuttings of rose is closely related to carbohydrate depletion at the rooting zone of cuttings during the first days of propagation due the absence of photosynthetic activity. This paper raises the possibility that losses occurring during propagation of cut roses can be erroneously attributed to soil born pathogens whereas the prime cause is the physiological status of the cuttings (e.g., too low carbon levels due to limited photosynthate supply).

References

Buttrose MS (1966) Use of carbohydrate reserves during growth from cuttings of grape vine. Austr J Biol Sci 19: 247-256

Cameron RJ, Rook DA (1974) Rooting stem cuttings of Radiata pine: environmental and physiological aspects. New Zeal J For Sci Vol 4(2): 291-298

Cohen R, Blaier B, Schaffer AA, Katan J (1996) Effect of acetochlor treatment on *Fusarium* wilt and sugar content in melon seedlings. Eur J Plant Pathol 102: 45-50

Costa JM, Challa H (2002) The effect of the original leaf of rooting and initial growth of planting material of rose. Sci Hort 95: 111-121

Haissig BE (1984) Carbohydrate accumulation and partitioning in *Pinus banksiana* seedlings and seedling cuttings. Physiol Plant 61: 13-19

Hartmann HT, Kester DE, Davies FT, Geneve RL (1997) Plant Propagation, Principles and Practices. 6th edition. Prentice Hall Inc., Simon & Schuster, Upper Sadle River, New Jersey, 770 pp

Hoad SP, Leakey RRB (1996) Effects of pre-severance light quality on the vegetative propagation of *Eucalyptus grandis* W. Hill ex. Maiden. Cutting morphology, gas exchange and carbohydrate status during rooting. Trees 10: 317-324

Horsfall JG, Dimond AE (1957) Interactions of tissue sugar, growth substances and disease susceptibility. Z PflanzenK 64: 415-421

Howard BH, Harrison-Murray RS (1995) Responses of dark-pre conditioned and normal light grown cuttings of *Syringa vulgaris* 'Madame Lemoine' to light and wetness gradients in the propagation environment. J Hort Sci 70: 989-1001

Howard BH (1994) Manipulating rooting potential in stock plants before collecting cuttings. In: TD Davis, B. Haissig (eds) Biology of adventitious root formation. Basic Life Sciences Vol. 62, pp 123-142

Jones RB, Hill M (1993) The effect of germicidas on the longevity of cut flowers. J Am Soc Hort Sci 118: 350-354

Kiyomoto RK, Bruehl GW (1977) Carbohydrate accumulation and depletion by winter cereals differing in resistance to *Typhhula idahoensis*. Phytopatology 67: 206-211

Okoro OO, Grace J (1976) The physiology of rooting *Populus* cuttings. Carbohydrates and photosynthesis. Physiol Plant 36: 133-138

Patil SS, Dimond AE (1968) Repression of the polygalacturonase synthesis in *Fusarium oxysporum f.sp. lycopersici* by sugars and its effect on symptom reduction in infected tomato plants. Phytopathology 58: 676-682

Pellicer V, Guehl, JM, Daudet, FA, Cazet M, Riviere LM, Maillard P (2000) Carbon and nitrogen mobilization in *Larix x eurolepis* leafy stem cuttings assessed by dual ^{13}C and ^{15}N labeling: relationships with rooting. Tree Physiol 20: 807-814

Sabet KA, Hassan HM (1961) Effect of ringing cotton stems on content of reducing sugars and insoluble nitrogen and on the degree of infection with *Xanthomonas malvacearum*. Nature 190: 934-935

Schonenweiss DF (1967) Susceptibility of weakened cottonwood stems to fungi associated with blackstem. Plant Dis Rep 51: 933-935

Tousson TA, Shirley MN, William CS (1960) The effect of the nitrogen sources and glucose on the pathogenesis of *Fusarium solani f. phaseoli*. Phytopathology 50: 137-140

Van de Pol PA, Marcelis LFM (1988) Apical aplication of aqueous solutions to roses via flower tubes - a technique with possibilities. Sci Hort 4: 123-129

Ypema HL, Van de Pol, PA, Bollen GJ (1987) Black rot of stentlings of roses: a disease caused by various soil fungi. Sci Hort 33: 269-280

Yue D, Margolis HA (1993) Photosynthesis and dark respiration of black spruce cuttings during rooting in response to light and temperature. Can J Forest Res 23: 1150-1155

CHAPTER 4

DYNAMICS OF ROOTING AND GROWTH OF ROSE CUTTINGS UNDER STANDARD PROPAGATION CONDITIONS

4.1 ANATOMICAL AND MORPHOLOGICAL DESCRIPTION OF ROOTING OF LEAFY STEM CUTTINGS OF *ROSA HYBRIDA* MADELON®

Abstract

The timing of the different rooting developmental phases (initiation and growth) of single node leafy stem cuttings of *Rosa hybrida* Madelon® was investigated. The rooting anatomy of cuttings propagated under controlled environment (23-25°C, 85 μmol m^{-2} s^{-1}, 16 h day^{-1}, without auxin treatment), was observed during the first 14 days of propagation by making cross sections from the basal 15 mm of the stem after 0, 3, 7, 10/11 and 14 days of propagation. Cambial activity was detected on day 3 by proliferation of undifferentiated cells between the cambium and the sclerenchyma. At day 7, meristematic centers and root primordia at the dome-shape phase or elongating were observed and roots emerged between days 11 and 14. Xylem vessels were formed by days 10 and 14 to repair xylem water transport, although in other cases, by day 14, the new xylem vessels could be observed with larger diameters and roots increased in length. We conclude that root initiation occurs between days 3 and 7 and can be extended till day 11 whereas the differentiation and initial growth of root primordia until emergence would mainly occur between days 7 and 11 and root growth. Root growth and vessel enlargement are the main anatomical events occurring after day14.

Key words: adventitious root formation, callus, root initiation, root growth

Introduction

Adventitious root formation is a process characterised by different developmental phases involving specific anatomical changes in specific tissues. A rather simplified, but still generally accepted way to characterise and describe the process of adventitious root formation is to distinguish two main phases: initiation and growth (Lovell and White, 1986). The initiation phase is characterised by the processes of induction and activation involving cell division and formation of the root initials, which at the later stage and after further cell divisions and the incorporation of neighbouring cells will originate in an organised structure, the root primordium. The root growth phase corresponds mainly to the growth of the formed root primordium till emergence and further root elongation.

Several authors (Carlson, 1933; Stangler, 1956; Fouda and Schmidt, 1994; Gerritsen, 1995) have previously studied adventitious root formation in rose. However, some discrepancies could be identified among their results concerning the timing of the different phases and processes, the location of the anatomical events or the tissues involved. This may probably be due to the differences in several aspects such as the species, the environmental conditions or the analysing technique. Consequently it has generated the need of analysing the dynamics of the anatomy of adventitious root formation under our specific experimental plant and environmental conditions. Therefore, the aims of this study are to establish the timing of the different developmental phases of rooting for cuttings of *Rosa hybrida* Madelon® and to identify the tissues involved in the process. This will enable to relate further the dynamics of physiological processes as CO_2 fixation and carbohydrate metabolism during rooting and help the understanding of the metabolism of adventitious root formation (Haissig, 1986).

Materials and methods

Mother plant material and propagation procedures

The growth of mother plants of *Rosa hybrida* Madelon® and the procedure of taking the cuttings and the rooting conditions were the same as described in chapter 3.1 for Experiment 2. Cuttings were inserted in 10 cm diameter plastic pots (one cutting per pot) containing a mixture of peat and sand 1:1 (v/v) and distributed by several

propagator boxes (blocks), 5 in Experiment 1 and 12 in Experiment 2. Air temperature was set at 23°C in Experiment 1 and at 25°C in Experiment 2. In both experiments, eight cuttings were used per sampling date for the anatomical observations.

Anatomical and histological investigations

The histology of the rooting process for the first 14 days of propagation was studied by using embedded and fresh sections from the basal 15mm of the stem of rose cuttings, the rooting zone, collected on day 0, 3, 7, 10 and 14 of propagation. Sections from the rooting zone were divided into smaller pieces, fixed in formalin-acetic acid-ethanol (FAA) (5:5:90) in vacuom for about 1 hour. Pith was excised for faster air removal. The material was left overnight in the fixative and then dehydrated in an alcohol series (half an hour in subsequently ethanol 70%, 80%, 85%, 90%, 96% and 100%). After dehydration the material was embedded in Technovit 7100 (hydroxy-ethyl-metacrylate). Cross and longitudinal sections were made at a thickness of 10 μm on a rotation microtome. Sections were mounted on slides, stained with toluidine blue O (96%), covered with euparal and a coverslip. Fresh sections were sectioned with a slide microtome at a thickness of 30 and 60μm and mounted in glycerine gelatine without staining.

In Experiment 2, stem pieces of the basal 8mm of the stem segment of cuttings were sampled on days 3, 7, 11 and 14 of propagation and placed in a fixative (FAA). Cross sections of the most basal 2.5-3mm mm were cut at 40-50μm using a microtome. Sections were mounted on slides and covered with glycerine - gelatine and a coverslip. In both experiments, observations were made using bright field illumination on a Zeiss microscope (CarlZeiss, Oberkochen, Germany). The pictures were made with a video system framegrabber Panasonic 3 Digital CCD and were read in a Photoshop computer program. Cell number was counted for Experiment 1.

Results

Stem anatomy at severance (day 0):

The epidermis was the first tissue encountered from the outside of the stem section. It consisted of a single cell layer and was covered with a cuticle (Fig. 4.1.1). The cortex was composed of collenchymatous and parenchymatous cells (Fig. 4.1.1). Next to the epidermis we found collenchyma in 4 to 5 cell layers, which was interrupted where stomata occurred (Fig. 4.1.1). Some layers of chlorenchyma bordered the collenchyma with starch. Next to this was the parenchyma with about 10-15 cell layers including the endodermis. The outmost parenchyma cells of the cortex were bigger and had thicker cell walls than the innermost ones. Below the cortex locally there are 5 to 6 rows of cells that become sclerenchymatous, regardless whether they belonged to the perycycle or phloem, and were localized facing the vascular bundles (Fig. 4.1.1). The primary medullary rays were multiseriate (3 to 6 cells wide) and with 18 to 23 cells layers including the cambium). The cambial zone consisted of 4-8 layers of initials (Fig. 4.1.1). Due to its thin cell walls the cambium tended to fracture during sectioning as shown in the Fig. 4.1.1. Vascular bundles are collateral with phloem and xylem separated by a cambial layer (Fig. 4.1.1). The xylem is composed of 5-9 tracheids (Fig. 4.1.1). Adjacent to the cambium layer is the (secondary) phloem. The number of phloem cells counted varied between 4 and 7.

Anatomy of wound healing and rooting (Experiment 1)

Meristematic activity of the cambium was visible 3 days after severance by proliferation of undifferentiated cells between the cambium and the sclerenchyma (Fig. 4.1.2a). Proliferation of parenchyma cells (callus) due to cambial activity increased the cortex width (Fig. 4.1.2b). By day 7, callus was also not only visible internally but also externally, as shown in the tangential section of the stem rooting zone (Fig. 4.1.3a). On day 7 several meristematic centers (groups of cells in division) were visible at the basal end of the stem segment (Fig. 4.1.3). It was also possible to visualise cells with meristematic characteristics and visible nucleus, and smaller cells due to less vacuolisation, in tissues close to vessels, most probably at the cambial

zone (Fig. 4.1.4). Ten days after severance, initiation of root primordia became visible within the parenchyma tissue by formation of meristematic centers (Fig. 4.1.2c) and formation of new xylem occurs (Fig. 4.1.2). By day 14 the new vessels were larger (Fig. 4.1.2d), primordia elongated through the cortex and roots became macroscopically visible.

Anatomy of wound healing and rooting (Experiment 2)

Meristematic cambial activity was visible by day 3 through proliferation of undifferentiated parenchymatous cells in the region between the cambium layer and the (secondary) phloem or sclerenchyma as observed in Experiment 1. Such meristematic activity increased thickness of this tissue and therefore, the region between the cambium and the xylem, visible by day 7 was larger. By day 7 meristematic centers were visible within the new parenchyma tissue (Fig. 4.1.5a). Those meristematic centers would result in either root primordia or new vessels, that would make contact with the primordia. By day 7 root primordia in an early phase, the dome-shape phase, or elongating through the cortex (almost emerging) could be observed (Fig. 4.1.5b). By day 11, the original anatomical structure of the stem was completely disrupted due to the meristematic cambial activity and parenchyma proliferation. Meristematic centers were visible within the parenchyma (Fig. 4.1.5c) and cortex width increased compared to day 7. Simultaneously, root primordia were visible elongating or emerging between the gaps of the sclerenchyma layer (Fig. 4.1.5d). On day 11, roots were visible macroscopically. The connection of the new roots to stem vessels was visible through the junction of the vessels of the new-root to stem vessels (Fig. 4.1.5d). On day 14, the new xylem vessels formed within the parenchyma tissue had larger diameters than vessels from day 11 (Figs. 4.1.5e and 4.1.5f). The medullary rays also were differentiating and increased in size (Figs. 4.1.5e and 4.1.5f). Our observations showed also that root initiation occurred mostly within the most basal 2mm of the rooting zone.

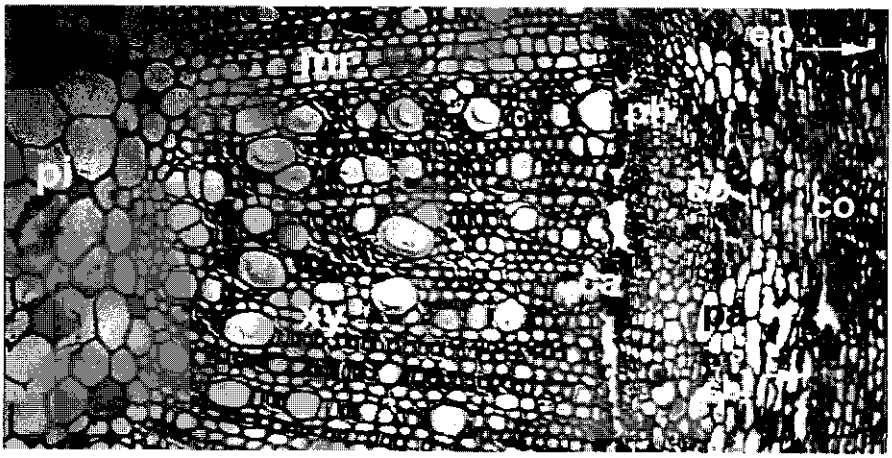

Figure 4.1.1 Survey of the stem cross section (10μm) from a single node leafy stem cutting of *Rosa hybrida* Madelon®, on day 0 of propagation, showing the epidermis (ep); collenchyma (co); chlorenchyma (chl), schlerenchyma (sc); phloem (ph), xylem vessels (xy), medular rays (mr), cambium (ca), parenchyma (pa), pith (pi) (150x).

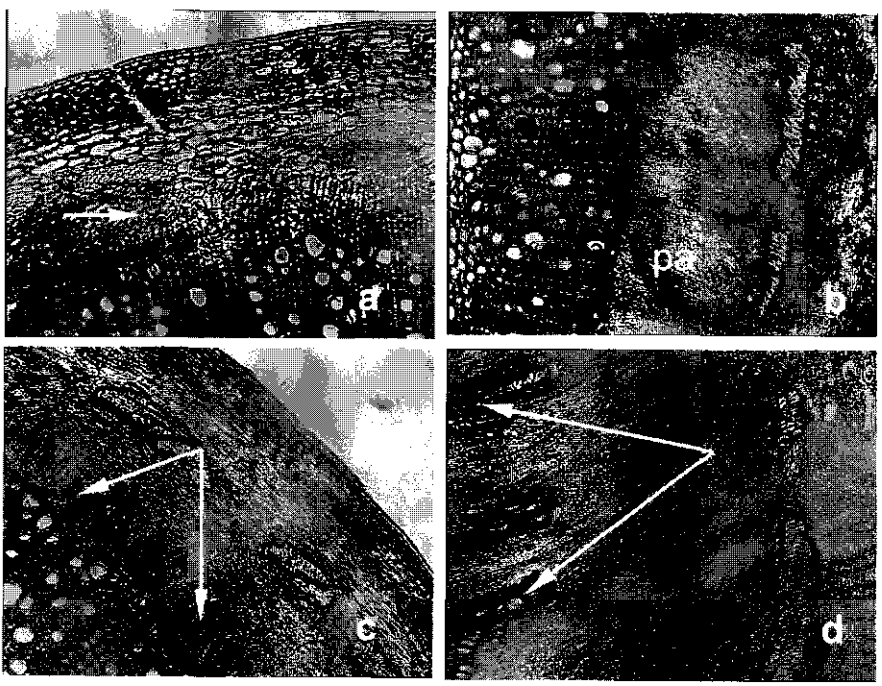

Figure 4.1.2a-d Non-stained cross sections (30-60 μm) from the rooting zone of the stem of leafy stem cuttings of *Rosa hybrida* Madelon® on day 3 **(a)** (30μm), day 7 **(b)** (60μm), day 10 **(c)** (60μm) and 14 **(d)** (60μm), in Experiment 1, showing meristematic activity and new callus tissue. Note cambium activity on day 3 (arrow) and the new parenchyma from that cambium (pa) on days 7 and 10. On days 10 (**c**) and 14 (**d**) newly formed tracheids are visible (arrows) (150x).

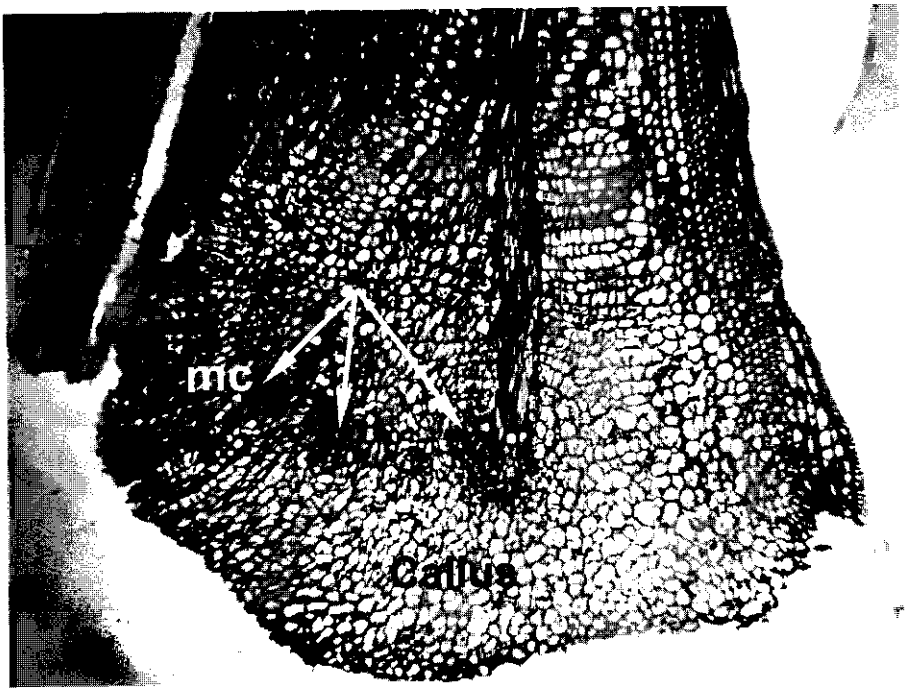

Figure 4.1.3 Radial section (10 µm) of the rooting zone of the *Rosa hybrida* Madelon® cuttings on day 7 of propagation. Note proliferation of callus as well as of meristematic centers (mc) (arrows) which will commonly differentiate into root primordia (80x).

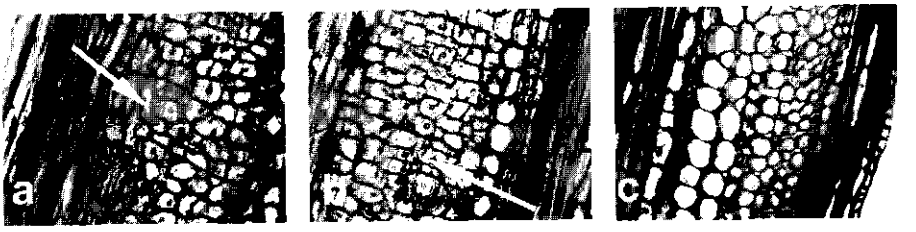

Figure 4.1.4a-c Radial sections (10 µm, 300x) from the basal part of the stem of cuttings of *Rosa hybrida* Madelon® with meristematic cells of the cambial zone **(a,b)** with clearly visible nuclei (arrow) near the xylem if compared with the cortex **(c)** (300x).

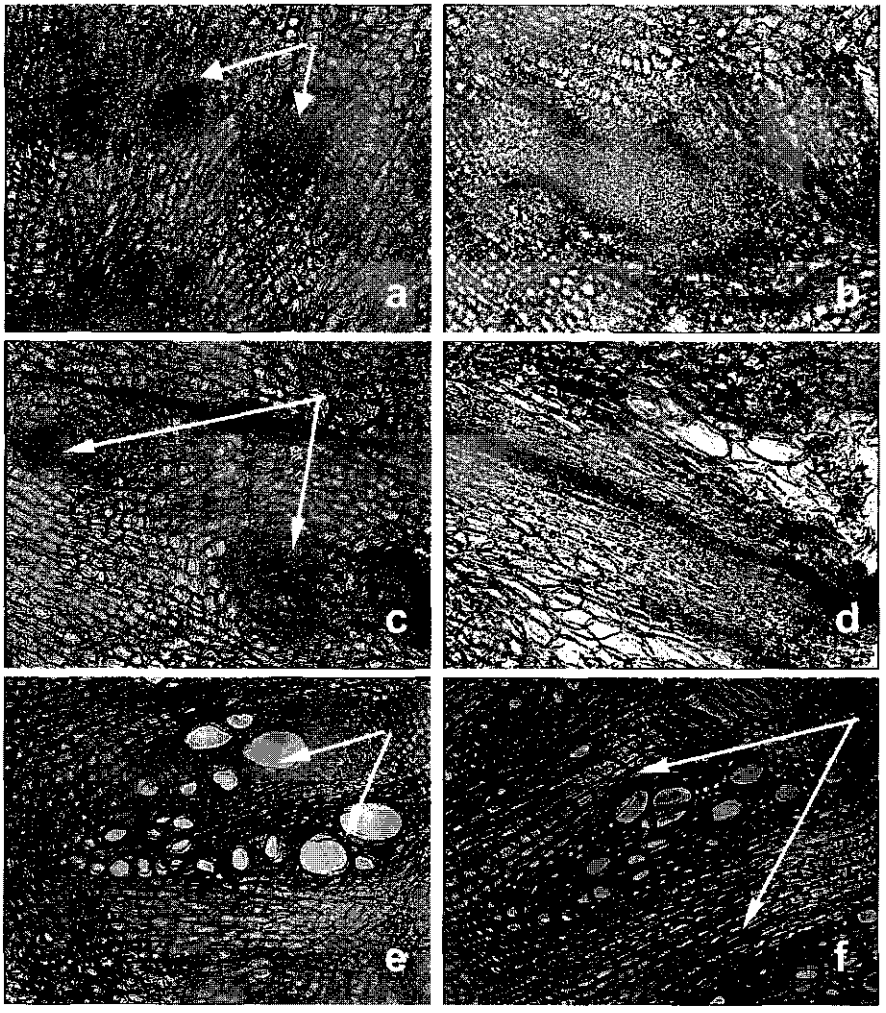

Figure 4.1.5a-f Cross-sections (50µm) of the rooting zone of cuttings of *Rosa hybrida* Madelon® on days 7, 11, and 14 of propagation in Experiment 2. **(a)** Meristematic centers, with newly differentiated vessels within the new callus tissue on day 7; **(b)** Primordia in the dome-shape phase on day 7; **(c)** Meristematic centers with vessels netwerk on day 11; **(d)** Note root primordia elongating through the cortex on day 11; **(e)** Note enlarged xylem vessels (arrows) and **(f)** medullar rays (arrows) elongating on day 14 (150x).

Discussion

The current study shows that active proliferation of callus and new parenchyma tissue, probably initiated due to cambial meristematic activity, precedes adventitious root formation in *Rosa hybrida* Madelon® cuttings. After 7 days of propagation, meristematic cells were visible near the xylem as well as the phloem and the cambial

zone seems to produce xylem as well as phloem. After 10 to 14 days xylem vessels are formed as a repair of the xylem water transport, but also some intra-phloem could be produced. The meristematic centers observed by day 7 to 10 within the new parenchyma tissue, might have thus correspondence with the spirally oriented nests wound tracheids found by Hamman (1998) in *Pinus taeda* cuttings.

Carlson (1933) mentioned that rooting of rose cuttings involved strong cambium activity by which a large amount of phloem tissue was produced. The present results confirm that roots initiate within that new parenchyma tissue, close to the secondary xylem in contrast with the observations of Stangler (1956) who found that root initials are initiated in the secondary phloem close to the cambium. Also Fouda and Schmidt (1994) found for *Rosa rugosa* cuttings that adventitious roots originated from the callus tissue at the basal cut surface, but considered the cambial zone as the main tissue where root primordia were formed. However, it must be mentioned that it is difficult to say whether or what parts of this new parenchyma belong to the cambium, phloem and xylem respectively.

Considering the timing of the rooting process for cuttings propagated under temperatures ranging between 23 and 25°C without auxin treatment, the first meristematic events from the cambium at the xylem zone should occur in the first 3 days of propagation. Root initiation would occur between days 3 and 7 and can even prolongate till day 11. Differentiation and growth of root primordia till emergence would mainly occur between days 7 and 11. Roots were macroscopically visible by day 11 in Experiment 2, which was faster than Experiment 1 and was most probably due to the fastening effect of higher temperatures on rooting (Moe, 1973; Borowski et al., 1987). After day 11, root primordia would elongate till emerge. Meanwhile the newly emerged roots would also elongate. The new xylem vessels formed within the parenchyma tissue would also increase in diameter. A schematic illustration of the phases and related anatomical events during the rooting process for single node leafy stem cuttings is presented in Fig. 4.1.6.

Our results show that roots in rose do not develop directly from pre-formed or dormant root primordia. Callus formation and formation of wound vascular tissue within the callus precedes root initiation. Thus, proliferation of parenchyma or phloem tissue (callus) seems a prerequisite for root formation in rose softwood cuttings as

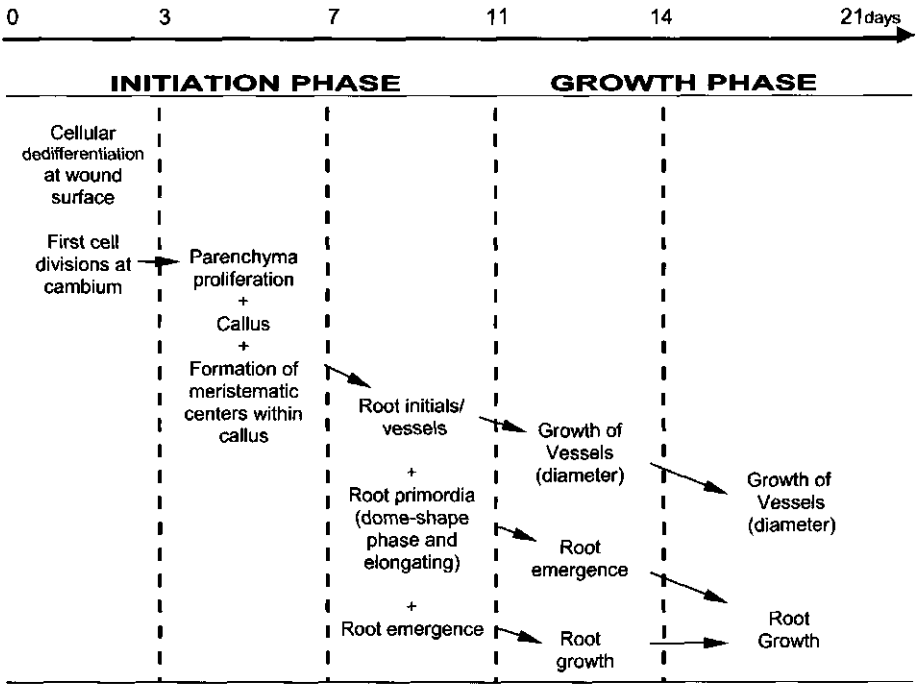

Figure 4.1.6 A schematic illustration of the phases and related anatomical events during the rooting process for single node leafy stem cuttings of *Rosa hybrida* Madelon® not treated with auxins and propagated at a temperature of 25°C, under a light intensity of 85 μmol m^{-2} s^{-1} and photoperiod of 16 h day^{-1}.

previously mentioned for other woody species like *Leucodendron* (Pérez-Francés et al., 2001), *Mallus* (Mackenzie et al., 1986) or *Pinus* (Hamann, 1998).

Besides providing the place where root initiation can occur, callus formation during healing prevents entry of pathogenic organisms at the wound (Cline and Neely, 1983) and decreases susceptibility of cuttings to stem rot (Howard and Harrison-Murray, 1995). Moreover, the fact that callus had differentiated xylem vessels before roots emerge suggests that callus might contribute to water transport before the new roots are functional. Supporting this view are the observations of Von Schaesberg et al. (1993) who justified increased assimilation activity of cuttings following formation of exogenous callus by increased water uptake and stomatal conductance.

Acknowledgements

The authors thank Wim van Veenendaal for his technical advice during the anatomical analysis and Dr. K. Keijzer and Prof. M. Willemse for major comments during the elaboration of this chapter.

References

Borowski E, Hagen P, Moe R (1987) Air and root medium temperature and the rooting of chrysanthemum and rose cuttings. Acta Agrobot 40: 1-2, 27-39

Carlson MC (1933) Comparative anatomical studies of Dorothy Perkins and American Pillar Roses. 1. Anatomy of canes. II.Origin and development of adventitious roots in cuttings. Contrib of Boyce Thompson Institute, Vol 5., pp 313 - 330

Cline MN, Neely D (1983) The histology and histochemistry of the wound-healing process in Geranium cuttings. J Am Soc Hort Sci 108: 496-502

Fouda RA, Schmidt G (1994) Histological changes in the stems of some *Rosa* species propagated by leafy cuttings as affected by IBA treatments. Acta Agron Hung 43: 265-275

Gerritsen J (1995) De invloed van hoge temperatuur op de verschillend fasen van de adventieve wortelvorming van *Rosa* cv Motrea. Thesis, Tuinbouplantenteelt, Wageningen University, 76 pp

Haissig BE (1986) Metabolic processes in the adventitious rooting of cuttings. In: MB Jackson (ed) New root formation in plants and cuttings. Long Ashton Research Station University of Bristol, UK. pp 141-190

Hamann A (1998) Adventitious root formation in cuttings of loblolly pine (*Pinus taeda* L.) developmental sequence and effects of maturation. Trees 12: 175-180

Howard BH, Harrison-Murray RS (1995) Responses of dark-pre conditioned and normal light grown cuttings of *Syringa vulgaris* 'Madame Lemoine' to light and wetness gradients in the propagation environment. J Hort Sci 70: 989-1001

Lovell PH, White J (1986) Anatomical changes during adventitious root formation. In: MB Jackson (ed) New root formation in plants and cuttings. Long Ashton Research Station University of Bristol, UK. pp 141-190

Mackenzie KAD, Howard BH, Harrison-Murray RS (1986) The anatomical relationship between cambial regeneration and root initiation in wounded winter cuttings of apple rootstocks M26. Ann Bot 58: 649- 661

Moe R (1973) Propagation, growth and flowering of potted roses. Acta Hortic 31: 35-51

Pérez-Francés JF, Melián-Capote MN, Martin-Pérez R, Rodriguez-Pérez JA (2001) An anatomical study of adventitious root development in wounded cuttings of *Leucadendron discolor* and *Leucadendron* 'Safari Sunset' (Proteaceae). Acta Hort 545: 191-193

Schwarz JL, Glocke PL, Sedgley M (1999) Adventitious root formation in *Acacia baileyana* F. Muell. J Hort Sci Biotech 74: 561-565

Stangler BB (1956) Origin and development of adventitious roots in stem cuttings of Chrysanthemum, Carnation and Rose. Cornell Experiment Station Memoir, 342 pp

Von Schaesberg N, Ebert G, Ludders P (1993) Leaf gas exchange of mango (*Mangifera indica* L.) cuttings during adventitious root formation. Angew Bot 67: 14-16

4.2 DYNAMICS OF DRY WEIGHT, PHOTOSYNTHESIS AND RESPIRATION DURING ROOTING OF LEAFY STEM CUTTINGS OF ROSE

Abstract

Gross and net photosynthesis of leaves of mother plant shoots, severed flower shoots and single node leafy stem cuttings of *Rosa hybrida* Madelon® were measured to quantify the effect of severance and rooting on photosynthesis. CO_2 fixation and total respiration of cuttings was also measured during the first 24 hours and the first 21 days of propagation and the daily carbon balance was estimated for days 0 (0.5 h after severance), 7, 11 and 21 of propagation. Severance of cuttings from the flower shoots decreased net photosynthesis by 75 to 95 %, although partial recovery occurred within a few hours. During propagation, net photosynthesis at 85µmol $m^{-2}s^{-1}$ (PPF) varied between 0.09mg CO_2 $m^{-2}s^{-1}$ and 0.17 mg CO_2 $m^{-2}s^{-1}$ and no positive effect of rooting on CO_2 fixation was found. Total cutting respiration increased until day 21 probably due to biomass. Daily carbon balance was positive since severance and carbon losses due to respiration represented about 30 to 55% of the daily gross CO_2 fixation. The root and shoot tissues accounted for about 70% of the increase in total fresh weight after 21 days whereas the remaining 30% increase was due to dry weight accumulation in the leaf and stem. Total fresh weight to dry weight ratio decreased until day 14 suggesting that carbohydrates were accumulating in the stem and leaves which could have limited photosynthetic activity and could partly explain why no positive effect of rooting on photosynthesis was observed.

Key words: Severance, gross and net photosynthesis, leaf dark respiration, carbon balance, fresh and dry mass allocation

Introduction

To survive, root and successfully grow leafy stem cuttings should be able to photosynthesise to sustain a positive carbon balance (Yue and Margolis, 1993; Hoad and Leakey, 1996; Pellicer et al, 2000), specially if reserves at severance are limiting as it happens in general with softwood or semi-hardwood material (Hartmann et al., 1997).

Severance disrupts the normal supply of water, nutrients and hormones in cuttings which alone or in combination with the propagation environment can have a strong influence on photosynthesis (Fordham et al., 2001). Although cuttings may be photosynthetically active before roots are formed (Smalley et al., 1991; Svenson et al., 1995), photosynthesis rates of cuttings have been reported to remain very low until roots emerge (Cameron and Rook, 1974; Okoro and Grace, 1976; Eliasson and Brunes, 1980; Davis and Potter, 1981; Yue and Margolis, 1993; Svenson et al., 1995; Wiesman and Lavee, 1995). Simultaneously, respiration of cuttings may also increase during propagation (Cameron and Rook, 1974) in particular in the tissues of the stem rooting zone (Dick et al., 1994). Thus, carbon unbalance may become easily a limiting factor for survival and growth of leafy stem (softwood/ semi-hardwood) cuttings of rose during the first days of propagation.

However, the effect of severance and rooting on photosynthesis and respiration of rose cuttings was not quantified for the first hours and days of propagation considered the most critical for survival and growth. Therefore, our aim is to quantify changes in the carbon balance during propagation by quantifying changes in photosynthesis and respiration of cuttings in response to both severance and rooting. For that purpose we measured net photosynthesis and total respiration during the first hours or days of propagation. Fresh and dry weights from the different parts of cuttings were also determined to quantify the dynamics of fresh weight and dry weight accumulation and allocation in time.

Materials and methods

Plant material

The growth of mother plants of *Rosa hybrida* Madelon® and the procedure of taking the cuttings and the rooting conditions were the same as described in chapter 3.1 for

Experiment 2, with the only difference that cuttings were inserted in 10 cm diameter plastic pots (one cutting per pot) containing rooting substrate.

Dynamics of leaf net photosynthesis and dark respiration (Experiments 1,2 and 3):

The net photosynthesis from leaves on mother plant shoots, detached flower shoots and cuttings were determined during the first hours (0.5, 1, 3, 5, 9, 11 and 24h) (Experiment 1) or days (0, 3/4, 7, 11, 14 and 21) (Experiments 2 and 3) of propagation at a low PPF (50 to 85 μmol m^{-2} s^{-1}). These light intensities were achieved by covering the leaf chamber with different shadowing nets.

The net photosynthesis was measured from the terminal leaflet of leaves by using a portable infrared gas analyzer (LCA-2, Analytical Development Cor. Hoddesdon, UK), connected to a leaf chamber (PLC), an air supply unit (ASUM) and a data-logger (DL2). The leaf chamber had sensors for measuring temperature, relative humidity and irradiance and enclosed a leaf area of 6.25 cm^2 (transmittance of 85% for PAR). Air was drawn from outside of the building to avoid large fluctuations in CO_2 air concentrations, and pumped at an average rate of 360 (±20) cm^3 min^{-1} (6ml s^{-1}) through the leaf chamber into the analyzer. For each measurement part of the leaflet was enclosed for at least 50s, till CO_2 readings become stable. Dark respiration was measured by covering the leaf chamber with a black plastic cover and gross photosynthesis was estimated by summing net photosynthesis and leaf dark respiration. Measurements on mother plant shoots, detached shoots and cuttings followed the same leaf, but measurements on cuttings during propagation were only done once to avoid damage. Before each measurement for cuttings, the surface of the leaflet was cleaned with an absorbent paper to remove water droplets. Cuttings remained in the pots during the measurements. Measurements were made between 9-14h after the light period starts. A small ventilator was used to avoid extreme high temperatures close to the leaf chamber. Temperature in the cuvette (leaf chamber) varied between 22 and 26°C whereas RH varied between 40 and 60%. According to previous literature, this range of temperatures should not influence significantly photosynthesis rates (Bozarth et al., 1982; Pasian and Lieth, 1989; Yamaguchi and Hirata, 1998). Leaf respiration is however more sensitive. Pasian and Lieth (1989)

report an increase in leaf respiration by a factor of 1.4 when temperature increased from 20 to 30°C. This factor will be considered in future calculations.

Dynamics of the total respiration of cuttings (Experiments 4, 5 and 6):

The total respiration of cuttings was measured during the first hours (0.5 and 4h) (Experiment 4) and the first 0, 7, 11 and 21 days of propagation (Experiments 5 and 6). The entire cutting was enclosed in glass pots after being removed from the pots and washed with water. The glass pots had a volume of 670 mL. After enclosing cuttings in the pot air samples were collected using a 3 mL syringe through a septum in the cap of the pots. The pots were then immediately covered with a black plastic film and about one hour later new air samples were collected. Samples were analyzed for CO_2 concentration by injecting 1 mL of air into a gas chromatograph Micro GC CP 2002 (Chrompack, the Netherlands) equipped with a column module Haye Sep A. By calculating the difference between the amount of CO_2 at time 0 and samples collected one hour later, the respiration rate of the cutting was calculated.

Dynamics of rooting morphology, fresh and dry weight accumulation and allocation:

The initial fresh weight of all the cuttings was determined in Experiment 3 before inserting cuttings in the substrate. After photosynthesis and respiration measurements, number of roots, fresh and dry weight of roots, of the axillary bud, of the original leaf, of the basal 1.5cm of the stem (rooting zone) and of the total stem segment were determined. Stem length and diameter were determined with a digital caliper (Digimatic CD-15D, Mitutoyo) and leaf area was measured with a LI-3100 Area meter (Li-cor Inc., Lincoln, NE, USA). Dry weight was determined after drying the material in a ventilated oven for 16 hours (6 hours at 70°C and 10 hours at 105°C). The data presented on rooting morphology and fresh and dry weight accumulation is from a representative experiment.

An estimation of the daily carbohydrate balance

The carbon balance for cuttings (BC) during the first 21 days of propagation was estimated on the basis of the following formula: $BC=(Pg*L_{light}) - (R_{Total}* 24h)$, where Pg represents the gross photosynthesis of the cutting; L_{light} the length of the light period and R_{total} the total cutting respiration.

Experimental design and statistical analysis

The experimental design used in all the experiments was a completely randomised block design. Experiment 1 and 5 used 6 blocks, Experiments 4 and 6 used 8 blocks and Experiments 2 and 3 used 12 blocks. Each block corresponded to a propagator and was divided in different number of plots: 2 plots (Experiment 4), 3 plots (Experiments 5 and 6), 6 plots (Experiments 2 and 3) and 8 plots (Experiment 1). In all the experiments there was one cutting per plot. Data on fresh and dry weights against time was analysed by linear regression analysis by using the statistical package GENSTAT 5 (IACR, Rothamsted, UK). Data on root and shoot morphology and data on photosynthesis and dark respiration rates are presented by means (±SE) for each sampling date .

Results

Rooting morphology and dynamics of fresh and dry weight and dry weight allocation

Fresh and dry weight of cuttings increased significantly during the first 21 days of propagation (Fig. 4.2.1a). Fresh weight of the stem increased moderately and about 70% of that increase was due to fresh weight accumulation at the stem rooting zone motivated by callus formation at the basipetal end of the stem segment which was visible since day. Leaf fresh weight had no significant change during propagation (Fig. 4.2.1a). The total fresh weight increased in great extent due to the formation of adventitious roots, macroscopically visible by day 11 (Fig. 4.2.2), and to growth of

the axillary bud into a primary shoot also visible since day 11 (Fig. 4.2.2). Root and shoot elongation occurred mainly after day 11.

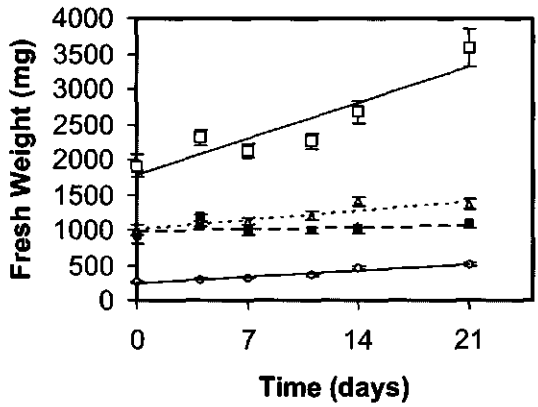

Figure 4.2.1a Fresh weight dynamics for the total cutting (—□—) (Y=1788+72.6T, R^2=0.41,p<0.001), the entire stem segment (----Δ----) (Y=1023.1+19.1T, R^2=0.22, p<0.001), the stem rooting zone (—o—) (Y=250.7+12.6T, R^2=0.62, p<0.001) and the original leaf (— -■- —) (Y=1003.9+3.15T, ns) of single node leafy stem cuttings of *Rosa hybrida* Madelon® during the first 21 days of propagation. Lines are regression lines estimated from the overall data. The error bars represent the standard error of the mean (n=12).

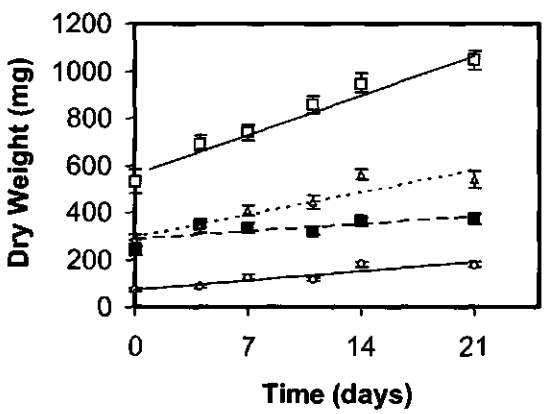

Figure 4.2.1b Dry weight dynamics for the total cutting (—□—) (Y=561+23.97T, R^2=0.58, p<0.001), the entire stem segment (----Δ----) (Y=299+13.6T, R^2=0.45, p<0.001), the stem rooting zone (—o—) (Y=74.5+5.5T, R^2=0.55, p<0.001) and the original leaf (— -■- —) (Y=290+4.4T, R^2=0.11, p<0.001) of single node leafy stem cuttings of *Rosa hybrida* Madelon® during the first 21 days of propagation. Lines are regression lines estimated from the overall data. The error bars represent the standard error of the mean (n=12).

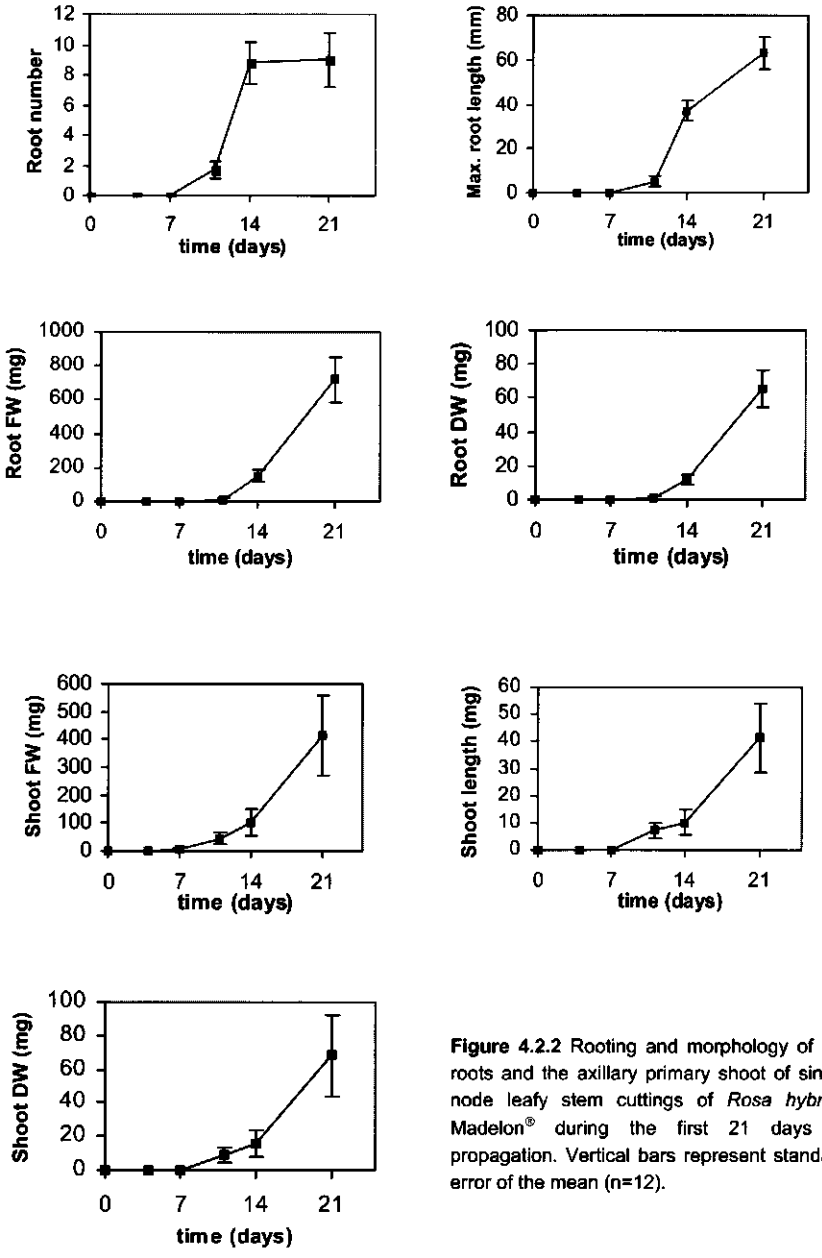

Figure 4.2.2 Rooting and morphology of the roots and the axillary primary shoot of single node leafy stem cuttings of *Rosa hybrida* Madelon® during the first 21 days of propagation. Vertical bars represent standard error of the mean (n=12).

Dry weight accumulation was more pronounced than fresh weight accumulation till day 14. By day 0 the total FW/total DW ratio was 3.7 whereas after 14 days of propagation the ratio was 2.8. At severance, dry weight was equally

partitioned between the leaf and the stem segment, but after 21 days of propagation, the percentage of dry weight due to leaves decreased slightly whereas the stem maintain its initial proportion (Figs. 4.2.3a and 4.2.3b).

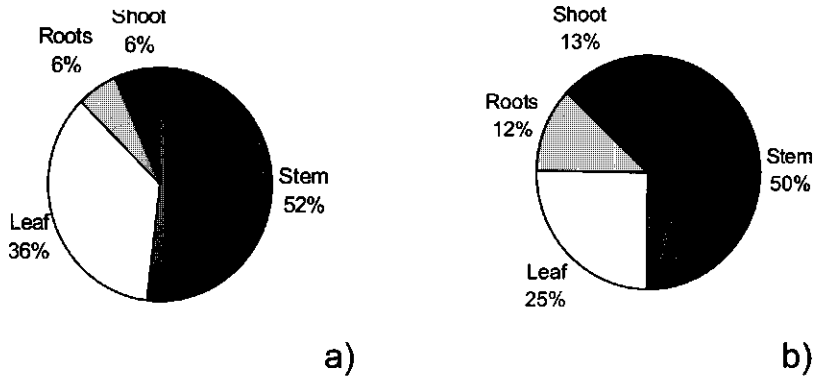

Figure 4.2.3 Dry matter allocation into the different organs (the stem segment, the leaf, roots and the primary shoot) of single node leafy stem cuttings of *Rosa hybrida* Madelon® as percentage of the DW weight accumulated **(a)** or as percentage of the total cuttings dry weight **(b)** after 21 days of propagation.

Roots and the axillary primary shoot represented together 12% of the total dry weight of cuttings after 21 days of propagation, although it corresponded to about 25% of the dry weight accumulated in the same period (Fig. 4.2.3b). Cuttings showed a net gain in the total fresh weight since the first days of propagation (Fig. 4.2.4).

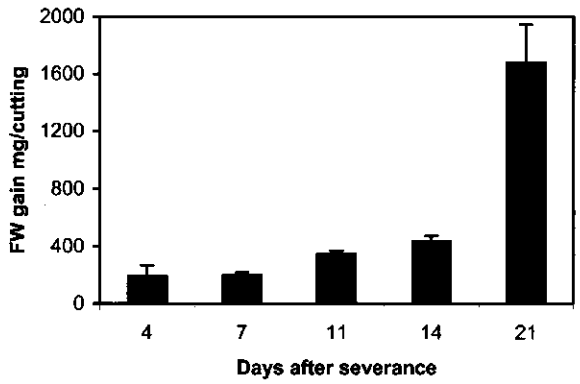

Figure 4.2.4 Fresh weight gain (mg) in single node leafy stem cuttings of *Rosa hybrida* Madelon®, including the fresh weight of the new roots and the primary shoot during the first 21 days of propagation. Error bars indicate the standard error of the mean (n=12).

Gross and net photosynthesis and total cutting respiration during propagation.

Detaching the flower shoot from the mother plant had no or moderate negative effect on the photosynthesis of the leaves located at the intermediate part of the shoot (Figs. 4.2.3 and 4.2.4), whereas detaching the cutting from the shoot decreased net photosynthesis by 75 to 95 % (Figs. 4.2.5 and 4.2.6). Net photosynthesis of cutting recovered from severance within a few hours (Fig. 4.2.5), but only partially because net photosynthetic rates of leaves on cuttings were lower during propagation than rates of leaves on the mother plants (Figs. 4.2.5 and 4.2.6). Net and gross photosynthesis rates were rather stable during propagation and did not respond to

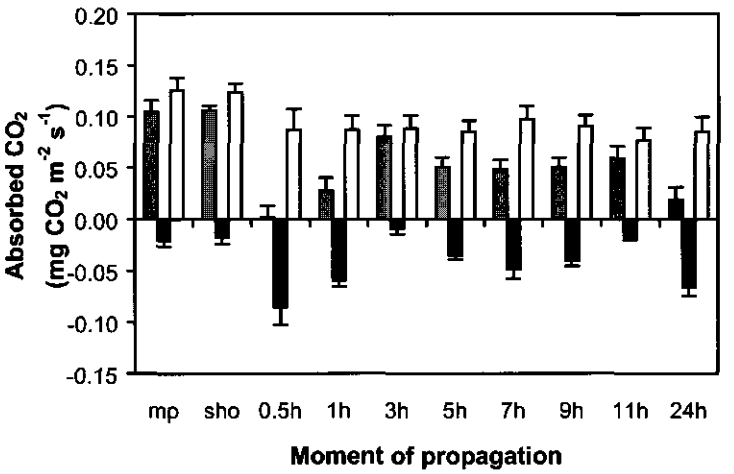

Figure 4.2.5 Net photosynthesis (▩), leaf dark respiration (■) and gross photosynthesis (□) for the mother plant leaves (mp), leaves on detached flower shoot (sho) and single node leafy stem cuttings of *Rosa hybrida* Madelon® during the first 24 hours of propagation measured at 50 $\mu molm^{-2}s^{-1}$ (PPF) and at T= 21.4-23.8°C. Error bars indicate SE of the mean (n=6).

callus nor to root formation (Figs. 4.2.6 and 4.2.7). In the different experiments, and possibly due to variation in leaves, the net photosynthesis rates at $85\mu molm^{-2}s^{-1}$ (PPF) varied between 0.09 $mgCO_2m^{-2}s^{-1}$ (Fig. 4.2.6) and 0.17 $mgCO_2m^{-2}s^{-1}$ (Fig. 4.2.7). Thus, it was assumed an average value of 0.13 $mgCO_2m^{-2}s^{-1}$ for cuttings' net photosynthesis during propagation which is comparable to previous findings for

different cultivars of *Rosa hybrida* (Table 4.2.2). A big variation was fo[illegible] leaf dark respiration which had the highest values shortly (0.5h) after se[illegible] h a

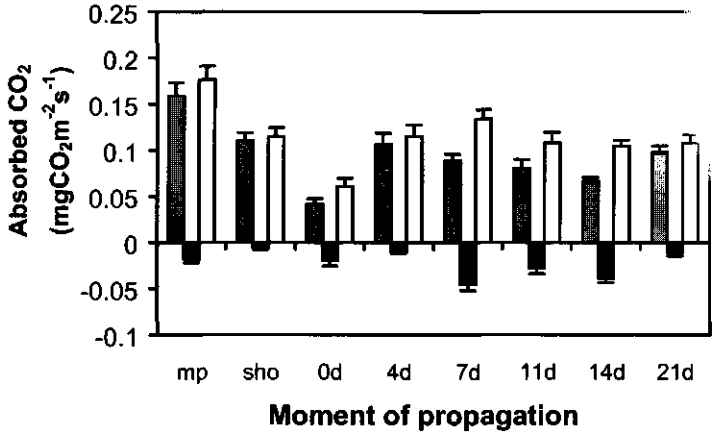

Figure 4.2.6 Net photosynthesis (■), leaf dark respiration (■) and gross photosynthesis (□) for the mother plant leaves (mp), leaves on detached flower shoot (sho) and cuttings of *Rosa hybrida* Madelon® during the first 21 days of propagation, measured at 85 μmol $m^{-2}s^{-1}$ (PPF) and T= 23.3- 26.1°C. Error bars indicate the SE of the mean (n=12).

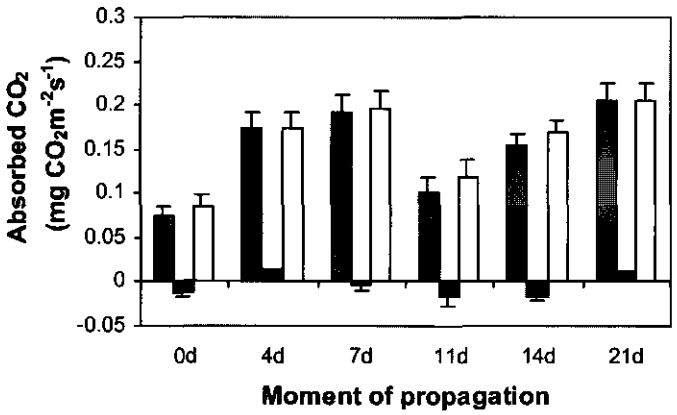

Figure 4.2.7 Net photosynthesis (■), leaf dark respiration (■) and gross photosynthesis (□) for single node leafy stem cuttings of *Rosa hybrida* Madelon® measured at 85 μmol $m^{-2}s^{-1}$ (PPF) and T= 23.1-25.2°C during the first 21 days of propagation. Error bars indicate the SE of the mean (n=12).

maximum value of 0.056 $mgCO_2m^{-2}s^{-1}$ (Fig. 4.2.5), although during propagation it remained low and in some cases close to 0 (Figs. 4.2.6 and 4.2.7).

Total respiration of cuttings increased during propagation (Table 4.2.1). No particular variation in response to root formation was found. However, the specific respiration (respiration per unit of fresh or dry weight) decreased since severance till day 21 of propagation (Table 4.2.1).

Table 4.2.1 Total respiration and specific respiration on fresh weight and dry weight basis of single node leafy stem cuttings of *Rosa hybrida* Madelon® measured at different moments of propagation. Values are means ± SE .

	Time after severance	Respiration		
		$mgCO_2cutting^{-1}h^{-1}$	$mgCO_2g^{-1}FWh^{-1}$	$mgCO_2g^{-1}DWh^{-1}$
Experiment 4 T=25°C n=8				
	0.5h	0.636±0.064	0.336	1.295[y]
	4h	0.850±0.064	0.384	1.488[y]
Experiment 5 T=27°C n=6				
	0.5 h	0.968± 0.088	0.486	1.779(1.281)[z]
	7 days	0.464± 0.038	0.269	0.894 (0.643)[z]
	11 days	0.717± 0.093	0.346	1.063 (0.765)[z]
Experiment 6 T=25°C n=8				
	0.5 h	0.763± 0.077	0.373	1.384
	11 days	0.627± 0.055	0.262	0.783
	21 days	1.017± 0.105	0.277	0.879

[y] considering a dry weight content of 27%

[z] corrected for T=25°C by a factor of 0.281 considering a Q_{10} (20-30°C) of 1.4 (Pasian and Lieth, 1989)

Table 4.2.2 Net carbon assimilation (Pn) for single leaves of different cultivars of *Rosa hybrida* measured or estimated from the light response curves.

Cultivar	Temperature °C	Light intensity $\mu molm^{-2}\ s^{-1}$	CO_2 ppm	Pn $mgCO_2m^{-2}s^{-1}$	Reference
Madelon®	20	100	450	0.1	Spaargaren, 1996
Madelon®	18	85-90	450	0.13[z]	Bakker et al., 1995
Samantha®		50-100	312	0.12-016[z]	Bozarth et al., 1982
Sonia®		100	375	0.11	Baille et al., 1996
Sonia®	28-32	50-85	110	0.088-0.13[z]	Gonzalez and Baille, 2000
Carl Red®	20-30	400		0.19-0.2	Yamaguchi and Hirata, 1998
Cara Mia®	20-30	50-100		0.08-0.25[z]	Pasian and Lieth, 1989; Lieth and Pasian, 1990
Samantha®		50		0.044	Jiao et al., 1991
Samantha®		85-100	350	0.087-0.11[z]	Jiao and Grodzinski, 1998

[z] estimated from the light response curve

Estimation of the daily carbon balance during the first days of propagation

In line with dry weight accumulation, the carbon balance of cuttings under our experimental conditions remained positive during propagation indicating that although the light intensity was rather low, leaves from cuttings were always above the light compensation point (Table 4.2.3). With the exception of the moments shortly after severance when carbon losses by respiration were about 80% of the gross CO_2 fixation, losses due to respiration varied between 30 and 55% (Table 4.2.3).

Table 4.2.3 Carbon balance during the first 21 days of propagation calculated for single node leafy stem cuttings of *Rosa hybrida* Madelon® based on data from gross photosynthesis (Pg) from Experiment 2 measured at 85 $\mu mol\ m^{-2}\ s^{-1}$ (PPF) and 16h photoperiod, T=25°C, and average leaf area of 70 cm^2. Total respiration at T=25°C.

Time	Pg[x] $mgCO_2cutting^{-1}day^{-1}$	Total respiration[y] $mgCO_2cutting^{-1}day^{-1}$	Daily carbon Balance $mgCO_2cutting^{-1}$
Severance (0.5h)	24.6	20.1	+ 4.5
Day 4	46.5	-[z]	
Day 7	54.1	15.8	+ 38.3
Day 11	43.6	17.2	+26.4
Day 14	42.1	-[z]	
Day 21	43.7	24.4	+19.3

[x] Gross photosynthesis from Experiment 2, Figure 4.2.6
[y] Considering the average of the specific respiration rates of Experiments 5 and 6 (Table 4.2.1) multiplied by the total DW of cuttings
[z] Not determined

Discussion

Our results show that rose cuttings are photosynthetically active since the early moments following severance and detachment of cuttings from the flower shoot appears only to have a short term negative effect on gross photosynthesis. It is possible that severance induces fast stomatal closure by disrupting the normal water relations as previously reported for leafy stem cuttings (Loach, 1988; Smalley et al., 1991; Fordham et al., 2001). The fast recovery of leaf photosynthesis (gross and net) after severance suggests that leaves were not suffering from substantial leaf water deficits. Moreover, cuttings had a positive variation of their total fresh weight since severance (Fig. 4.2.4) which could be interpreted as a sign of the good water status of the cuttings. The photosynthesis levels also did not increase in response to an increase in water uptake due to root formation which is a good indication that water stress was not a major limiting factor for growth. Still, leaves on cuttings did not reach the rates measured on the flower shoots on mother plants (Figs. 4.2.5 and 4.2.6) suggesting that a continuous limitation subsisted during propagation and was not related with cuttings' water relations. Thus, the apparent limitation of photosynthesis might have a non-stomatal origin and reduced sink activity may thus be a reason (Humphries and Thorne, 1964; Smalley et al., 1991).

The high water content of root and shoot tissues may justify that together they accounted for 70% of the increase in total fresh weight after 21 days whereas the remaining 30% increase was due to dry weight accumulation in the leaf and stem (Fig. 4.2.1b). However, if we consider that only 12% of the total dry weight of cuttings are allocated to roots and the primary shoot after 21 days (Fig. 4.2.3) we may conclude that the meristematic activity by the formed organs play a minor role on the sink activity of the cutting. In line with this view is the fact that fresh/dry weight ratio decreased mostly due to the reduced growth of roots and the primary shoot (only 3% of the total dry weight by day 14) during the first 11 days of propagation (Figs. 4.2.2 and 4.2.3). Reduced sink activity would cause carbohydrates accumulation in cuttings as previously found in leafy cuttings of other woody species (Hansen et al., 1978; Haissig, 1984; Leakey and Coutts 1989; Smalley et al., 1991). This change in the carbon metabolism of cuttings might negatively affect photosynthesis by feedback inhibition (Mauney et al., 1979; Myers et al., 1999) and avoid the increase in photosynthesis in response to root formation as reported for leafy stem cuttings of

different woody species (Okoro and Grace, 1976; Cameron and Rook, 1974; Davis and Potter, 1981; Yue and Margolis, 1993) or in response to root growth as found for detached leaves of *Phaseolus vulgaris* (Humphries and Thorne, 1964). Moreover, the present data on photosynthesis rates are in agreement with the linear relation between leaf area duration and total dry weight accumulation after 21 days of propagation (Chapter 3.1) which was supposed to imply rather constant levels of photosynthesis during propagation. The highest values of specific respiration found in the first 4 hours after severance were probably related to wounding. Leaf dark respiration seemed to be rather low during propagation although the variation observed between the experiments avoids any strong conclusion. However, this result is supported by previous literature indicating a strong decrease in light compensation point of cuttings during the first 3 days of propagation due to lower leaf dark respiration (Bertram and Veierskov 1989). Carbon losses due to respiration represented between 30-40% of the daily carbon fixation (Table 4.2.3) what is in accordance with previous literature (Amthor, 1989). The carbon accumulation after 21 days was estimated in 585 mg (CO_2 equivalents) which corresponded to approximately 400 mg of dry weight (CH_2O) after multiplication by the factor (30/44). This value is in the same order of magnitude of the real dry weight accumulation (514 mg).

In conclusion, under our propagation conditions severance seems to have a moderated negative effect on leaf photosynthesis because leaves recovered fast although partly. The photosynthetic activity of cuttings was apparently more affected by the drastic reduction of sink activity rather than by water unbalances. Further research should investigate the carbon dynamics in cuttings (leaves and stem) during propagation and study other photosynthetic parameters of cuttings besides CO_2 fixation (e.g. PSII efficiency) to better understand the relation between photosynthesis, carbohydrates and rooting of rose cuttings.

Acknowledgements

We thank Dr. Vadim Volkov and Dr. Ep Heuvelink for major comments during the elaboration of this chapter and to Oege Borsboom for his assistance in the photosynthesis measurements.

References

Amthor JS (1989) Respiration and crop productivity. Springer-Verlag, New York, 215 pp

Agbaria H, Heuer B, Zieslin N (1995) Effects of grafting on transpiration, CO_2 fixation and growth of rose plants (*Rosa x hybrida* cvs Ilseta and Mercedes). J Hort Sci 70(4): 651-656

Baille, M., Romero-Aranda, R., Baille, A (1996) Gas-exchange responses of rose plants to CO_2 enrichment and light. J Hort Sci 71: 945-956

Bakker, JA, Blacquière T, De Hoog J (1985) Effect van belichtingsregime en verrood licht op stekken van roos 'Frisco' en 'Madelon' en op Eustoma 'Fuji donker blauw', Rapport 197, Proefstation voor de Bloemisterij, Nederland, 30 pp

Bertram L, Veierskov B (1989) A relationship between irradiation, carbohydrates and rooting in cuttings of *Pisum sativum.* Physiol Plant 76: 81-85

Bozarth CS, Kennedy RA, Schekel KA (1982) The effects of leaf age on photosynthesis in rose. J Am Soc Hort Sci 107: 707-712

Cameron RJ, Rook DA (1974) Rooting stem cuttings of Radiata pine: environmental and physiological aspects. New Zeal J For Sci 4: 291-298

Davis TD, Potter JR (1981) Current photosynthate as a limiting factor in adventitious root formation on leafy pea cuttings. J Am Soc Hort Sci 106: 278-282

Dick J McP, Blackburn DG, McBeath C (1994) Stem respiration in leafy cuttings of *Prosopis juliflora* during the rooting process. New Forest 8: 179-184

Dutton RG, Jiao J, Tsujita MJ, Grodzinski B (1988) Whole plant CO_2 exchange measurements for non-destructive estimation of growth. Plant Physiol 86: 355-358

Eliasson L, Brunes L (1980) Light effects on root formation in aspen and willow cuttings. Physiol Plant 48: 261-265

Fordham MC, Harrison-Murray RS, Knight L, Clay CM (2001) Decline in stomatal response to leaf water deficit in *Corylus maxima* cuttings. Tree Physiol 21: 489-496

Gonzalez-Real MM, Baille A (2000) Changes in leaf photosynthetic parameters with leaf position and nitrogen content within a rose plant canopy (*Rosa hybrida*). Plant Cell Environ 23: 351-363

Hansen J, Strömquist L, Ericsson A (1978) Influence of the irradiance on the carbon content and rooting of cuttings of pine seedlings (*Pinus sylvestris* L.) Plant Physiol 61: 975-979

Hartmann HT, Kester DE, Davies FT, Geneve RL (1997) *Plant Propagation, Principles and Practices.* 7th ed. Prentice Hall Inc., Simon & Schuster, Upper Sadle River, New Jersey. 770 pp

Haissig BE (1984) Carbohydrate accumulation and partitioning in *Pinus banksiana* seedlings and seedling cuttings. Physiol Plant 61: 13-19

Hoad SP, Leakey RRB (1996) Effects of pre-severance light quality on the vegetative propagation of *Eucalyptus grandis* W. Hill ex Maiden. Cutting morphology, gas exchange and carbohydrate status during rooting. Trees 10: 317-324

Humphries EC, Thorne GN (1964) The effect of root formation on photosynthesis of detached leaves. Ann Bot 111: 391-400

Jiao J, Tsujita MJ, Grodzinsky B (1988) Predicting growth of Samantha roses at different light, temperature and CO_2 levels based on net carbon exchange. Acta Hort 230: 435-442

Jiao J, Tsujita MJ, Grodzinsky B (1991) Influence of temperature on net CO_2 exchange in roses. Can J Plant Sci 71: 235-243

Jiao J, Grodzinsky B (1998) Environmental influences on photosynthesis and carbon export in greenhouse roses during development of the flowering shoot. J Am Soc Hort Sci 123: 1081-1088

Knee M (2000) Selection of biocides for use in floral preservatives. Postharvest Biol Tec 18: 227-234

Leakey RRB, Coutts MP (1989) The dynamics of *rooting Triplochiton scleroxylon* cuttings: their relation with leaf area, node position, dry weight accumulation, leaf water potential and carbohydrate composition. Tree Physiol 5: 135-146

Loach K (1988) Water relations and adventitious rooting. In: TD Davis, BE Haissig, N Sankla (eds), Adventitious root formation in cuttings. Discorides Press, Portland Oregon, pp 102-116

Mauney JR, Guinn G, Fry KE, Hesketh JD (1979) Correlation of photosynthetic carbon dioxide uptake and carbohydrate accumulation in cotton, soybean, sunflower and sorghum. Photosynthetica, 13: 260-266

Myers DA, Thomas RB, DeLucia EH (1999) Photosynthetic responses of loblolly pine (*Pinus taeda*) needles to experimental reduction in sink demad. Tree Physiol 19: 235-242

Okoro OO, Grace J (1976) The physiology of rooting of *Populus* cuttings. I. Carbohydrates and photosynthesis. Physiol Plant 36: 133-138

Pasian CC, Lieth JH (1989) Analysis of the response of net photosynthesis of rose leaves of varying ages to photosynthetically active radiation and temperature. J Am Soc Hort Sci 114: 581-586

Pellicer V, Guehl, JM, Daudet, FA, Cazet M, Riviere LM, Maillard P (2000) Carbon and nitrogen mobilization in *Larix x eurolepis* leafy stem cuttings assessed by dual ^{13}C and ^{15}N labeling: relationships with rooting. Tree Physiol 20: 807-814

Smalley TJ, Dirr AM, Armitage AM, Wood BW, Teskey RO, Severson RF (1991) Photosynthesis and leaf water, carbohydrate and hormonal status during rooting of stem cuttings of *Acer rubrum*. J Am Soc Hort Sci 116: 1052-1057

Spaargaren, JW (1996) Fotosynthese bij de roos; de invloed van cultivar, sheutpositie en sinklimitering, Vakgroep Tuinbouplantenteelt WU

Svenson SE, Davies FT Jr, Sharon AD (1995) Gas exchange, water relations and dry weight partitioning during root initiation and development of poinsettia cuttings. J Am Soc Hort Sci 120: 454-459

Ueda Y, Nishirara S, Tomita H, Oda Y (2000) Photosynthetic response of Japanese rose species *Rosa bracteata* and *Rosa rugosa* to temperature and light. Sci Hort 84 : 365-371

Wiesman Z, Lavee S (1995) Relationship of carbohydrate source and Indole-3-butyric-acid in olive cuttings. Aust J Plant Physiol 22: 811-816

Yamaguchi H, Hirata Y (1998) Influence of high temperature on flower stem length and photosynthesis of roses. Acta Hort 454: 391-393

Yue D, Margolis HA (1993) Photosynthesis and dark respiration of black spruce cuttings during rooting in response to light and temperature. Can J Forest Res 23: 1150-1155

Zieslin N, Tsujita MJ (1990) Response of miniature roses to supplementary illumination. I. Effect of light intensity. Sci Hort 42:113-121

4.3 DYNAMICS OF STARCH AND SUGARS DURING ROOTING OF LEAFY STEM CUTTINGS OF ROSE

Abstract

Qualitative and quantitative methods to determine carbohydrates were combined to quantify the effects of severance and rooting on the dynamics of starch and sugars (glucose, fructose and sucrose) in single node leafy stem cuttings of *Rosa hybrida* Madelon® during the first 21 days of propagation. The iodium (IKI) test was applied to transversal and longitudinal sections of the stem and the intact leaf of cuttings. Quantitative analysis of carbohydrates concentrations in the original leaf, stem rooting zone (basal 15mm), upper stem, roots and primary shoot were determined by liquid chromatography on days 0, 4, 7, 14 and 21 of propagation. About 55% of the dry weight accumulated during the 21 days consisted of carbohydrates, in particular starch. Starch accumulated mainly in the first 7-14 days in the pith and medullar rays of the stem and less in the regions of the pith close to the basal callus . Glucose and fructose also accumulated in the stem and leaves whereas sucrose remained constant. Root tissues had the highest glucose and fructose concentrations. A decreasing basipetal gradient of total carbohydrates (sugars+starch) (leaf > upper stem > stem rooting zone) was found, but no radial concentration gradients were detected. It is concluded that severance reduces sink activity more than source activity in the first days of propagation of rose cuttings as demonstrated by pronounced starch accumulation. The lower concentration of starch at the rooting zone is possibly a consequence of callus formation and eventually root growth.

Key words: Severance, *Rosa hybrida*, starch accumulation, reduced sink activity, carbon gradients, callus, rooting

Introduction

Severance of a cutting from a mother plant disrupts the water and hormonal relations (Fordham et al., 2001) and modifies sink-source relations of cuttings (Hansen et al., 1978; Veierskov et al., 1982; Smalley et al., 1991).

Changes in the source-sink relations in cuttings following severance can be either attributed to reduced source activity (reduced photosynthetic activity) (Okoro and Grace, 1976; Davis and Potter, 1981; Yue and Margolis, 1993; Svenson et al., 1995; Fordham et al., 2001) or to limited sink activity caused by the removal of sinks and/or reduced growth (Hansen et al., 1978; Veierskov et al., 1982; Smalley et al., 1991). As a consequence, severance induces changes in the concentration of soluble and stored carbohydrates which can affect rooting (Haissig 1984; Haissig, 1989ab; Leakey and Coutts, 1989; Hoad and Leakey, 1996). Therefore, understanding the dynamics of carbon allocation in cuttings during propagation may help to understand the role of carbohydrates in the rooting process (Friend et al., 1994).

It has been shown that the total fresh /dry weight ratio of cuttings decreases during propagation which was interpreted as a sign of reduced sink activity. This possibility is investigated in this paper by quantifying the dynamics of carbohydrates in rose cuttings during the first 21 days of propagation. Moreover, root formation and growth in rose cuttings may be influenced by specific changes in carbohydrate concentrations or specific carbon gradients in the stem. We assumed that carbohydrates by the time of severance are homogeneously distributed in the stem tissues of cuttings and severance will induce changes in the carbon concentrations which may be directly related to callus and root formation.

Materials and methods

Qualitative analysis of starch gradients and amylase activity in stem tissues of cuttings during propagation

Mother plants and cuttings were grown as described in chapter 4.2. Three experiments were conducted observing starch patterns during rooting of rose cuttings. Observations were done on days 0, 4, 7, 11, 14 and 21 of propagation from different

parts of the stem and from the original leaf. One to two cuttings per day of observation were used for the qualitative determination of starch and amylase activity. Root distribution at the basal part of the rooting zone was also determined by counting number of roots formed on the side of the stem where the original leaf is standing and on the opposite side.

Starch determination: The iodine potassium iodide (IKI) test was applied to transversal and longitudinal sections of the stem to mark starch patterns. The IKI solution combined 3 grams of iodine (I_2) crystals, and 10 grams of potassium iodide (KI) crystals dissolved in 1L of destilated water. Tissues containing starch will be coloured dark blue by this staining solution. After harvesting the cuttings from propagators on days 0, 7, 11 and 14, roots when formed were removed, and the stem segment was sectioned with a razor blade into transversal sections from different zones of the stem: the basipetal end at callus level, the intermediate part and the region immediately above or beneath leaf insertion. Sections were further immersed in the IKI solution for about 0.5 to 1 h. Longitudinal sections from the most basal 15 mm of the stem (the rooting zone) or from the entire segment were made and stained. The starch patterns in tissues of intact leaves were also observed on days 4 and 21 of propagation. Before staining the leaves they were immersed in an ethanol solution of 96% to remove the chlorophyll pigments. Observations from the transversal sections of the stem segment were made with a stereomicroscope LEICA MZ8 Ocular 10x/21B with different magnifications. Photos were made using a video system framegrabber Panasonic n.3 Digital CCD or with a normal camera.

Amylase activity: The activity of alfa-amylase in the stem segment of cuttings was localized on days 14 and 21 of propagation. Starch agar at 3% was prepared by dissolving corn-starch SIGMA (S-4126) and agar (bacto-agar) DIFCO (014-01) in boiling destilled water at a pH of about 6.0. The starch agar was applied to petri dishes and let to solidify. After harvest, the cuttings were sectioned longitudinally in two halves and the stem sections were incubated for 24 hours in the petri dishes containing the white starch-agar already solidified. After incubation, the petri dishes were flooded with the IKI solution which colored the starch-agar dark blue. The regions of the stem with higher activity of amylase were localized one week later through the appearance of white spots in the starch agar. We used as control stem segments treated with ethanol at 96% before incubation in the starch-agar.

Quantitative analysis of carbohydrates in stem and leaf tissues during propagation

In another experiment (Experiment 4) glucose, fructose, sucrose and starch were analyzed from the basal 15mm of the stem segment (the rooting zone), the remaining upper part of the stem and from the original leaf on day 0, 4, 7, 14 and 21 of propagation. Ten cuttings were harvested (about 9 h after beginning of the photoperiod) at each sampling date. The stem was further longitudinally sectioned in two halves, one from the side of leaf insertion and the other, the opposite side. Roots and the axillary primary shoot were analyzed when formed. For carbohydrate analysis the samples were prepared and analysed as described in the previous Chapter 3.2. The total carbohydrate (TC) was calculated by summing sugars and starch. Total dry weight of cuttings and dry weight or the primary shoot and roots were also determined.

Experimental design and statistical analysis

The experimental design used in all the experiments was a completely randomised block design with a maximum number of blocks of 12. In Experiment 4, 10 blocks with 5 plots of a cutting per block were used. The effect of time on the carbohydrates concentration at different parts of the cutting were submitted to analysis of variance ($P<0.05$) by using the statistical package GENSTAT 5 (IACR, Rothamsted, UK). Data on root and shoot morphology and total dry weight accumulation are presented through the overall means from the 10 blocks for each day of propagation with the respective standard errors.

Results

Staining stem tissues with IKI solution showed that leaf and stem tissues of rose cuttings accumulate starch during propagation (Fig. 4.3.1). Starch accumulates within the first 11 days of propagation, preferentially in the medullar rays, cortex region and pith (Figs. 4.3.2a and 4.3.2b). No starch was visible in the pith cells close to the callus formed at the basipetal end of the stem segment (Figs. 4.3.2c and 4.3.2d). The absence of starch at the rooting zone of the stem, close to callus, was paralleled by higher amylase activity (Fig. 4.3.3). Dry weight accumulation during the first 21 days of

propagation (Figure 4.3.4a) occurred simultaneously with a significant accumulation of the total carbohydrates (Fig. 4.3.5a), and in particular of starch (Fig. 4.3.5b). About 55% of the increase in leaves or the upper stem dry weight after 21 days of propagation were due to TC accumulation (Table 4.3.1). At the rooting zone of the stem, the TC represented only 30% of the dry weight increase suggesting higher metabolic activity at this zone and/or high concentration of structural carbohydrates possibly related to callus formation.

Table 4.3.1 Contribution of the total carbohydrates (sugars+starch) (TC) for the dry weight on day 0 ($DW_{day\ 0}$) and on day 21 ($DW_{day\ 21}$) from the original leaf, the stem rooting zone and the upper part of single node stem leafy cuttings of *Rosa hybrida* Madelon®.

Part of the cutting	ΔDW ($DW_{day\ 21}$- $DW_{day\ 0}$) (mg)	$DW_{day\ 0}$ due to TC (mg)	$DW_{day\ 21}$ due to TC (mg)	ΔDW due to TC (mg)	ΔDW due to TC %
Leaf	251	38.3	157	118	47
Upper stem	136	11.2	95.7	84.5	62
Basal stem	88	4.2	30.7	26.5	30

The pattern of change in TC concentration in stem and leaf tissues were similar to those of starch because the sugars concentration remain rather stable (Figs. 4.3.5c, 4.3.5d and 4.3.5e).

Starch concentration significantly increased ($P<0.05$) within the first 7 to 14 days of propagation (more than doubled compared to severance) whereas between day 14 and 21 the concentration did not vary significantly (Fig. 4.3.5b). This coincided with the growth of buds and roots that occurred mainly after day 14 (Fig. 4.3.1).

Sucrose concentration in stem and leaf tissues did not change significantly ($P<0.05$) during propagation (Fig. 4.3.5c) suggesting that sucrose synthesis and consumption rates were in equilibrium, in particular in leaves where the variation was almost none (Fig. 4.3.5c). In contrast, glucose and fructose concentration increased significantly in leaves (almost doubled after 21 days) whereas no significant variation was found in the stem tissues (Figs. 4.3.5d and 4.3.5e).

The only significant concentration gradien ipetal. Leaves, showed the highest concentration in TC whereas it was lowe e rooting zone of the stem (Fig. 4.3.5a). This concentration gradient (leaf>u em> rooting zone) existed already at severance (day 0), although the differe in the TC concentration

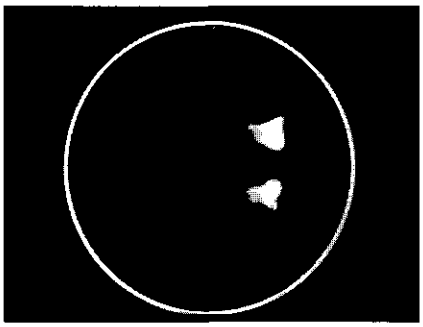

Figure 4.3.3 Starch-agar stained with IKI solution after incubation of two longitudinal sections of the stem of *Rosa hybrida* Madelon® cuttings, 14 days after severance. Note the white spots indicating higher activity of alfa-amylase at the rooting zone (basal end close to callus) of the cutting.

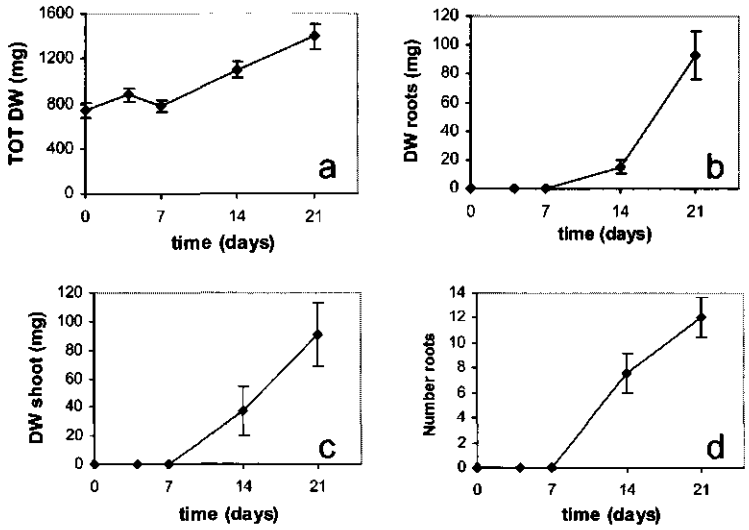

Figure 4.3.4. Total dry weight **(a)**, dry weight of roots **(b)** and the axillary shoot **(c)** and number of roots **(d)** for cuttings of *Rosa hybrida* Madelon® during the first 21 days of propagation. Vertical bars represent the standard error of the mean (n=10).

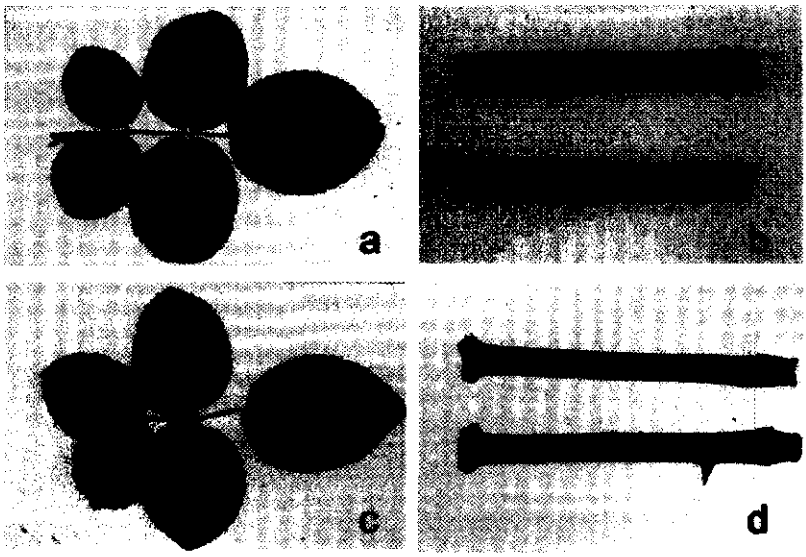

Figure 4.3.1 Leaves and longitudinal sections from the stem segment of cuttings of *Rosa hybrida* Madelon® stained with iodine potassium iodide (IKI) solution on day 4 **(a, b)** and day 21 **(c, d)** of propagation.

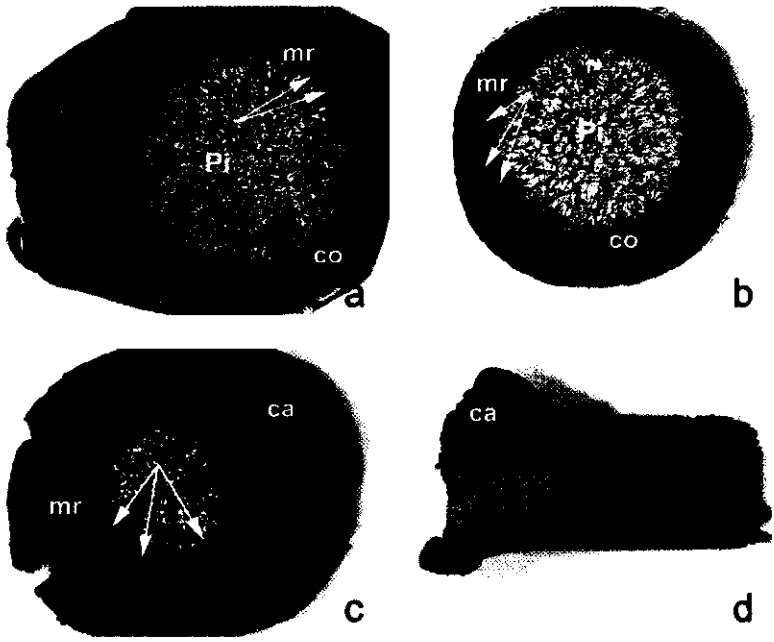

Figure 4.3.2 Starch accumulation in single node leafy stem cuttings of *Rosa hybrida* Madelon® stained with iodine potassium iodide (IKI) after 14 days of propagation in transversal sections from the stem at the level of leaf insertion **(a)** (16x), middle part of the stem segment **(b)** (16x) and basal end at callus (ca) level **(c)** (x12.5) and in from the longitudinal section of the rooting zone (basal 15 mm) of the stem after 11 days **(d)** (x6). Note in **(a)** and **(b)** starch accumulation in the medullary rays (mr), pith cells (Pi) and cortex (co) and the empty medullar rays (mr), cortex and pith, at the basal end of the stem close to the callus **(c and d)**.

between the upper part of the stem and the leaf became smaller in time possibly due to the accumulation of carbohydrates at the upper part of the stem. In the rooting zone, however, TC concentrations were always the lowest due to the lower starch concentration. More roots were found at the side of the stem where the leaf was inserted (Table 4.3.3), but no specific radial carbohydrates gradients were found (Fig 4.3.5).

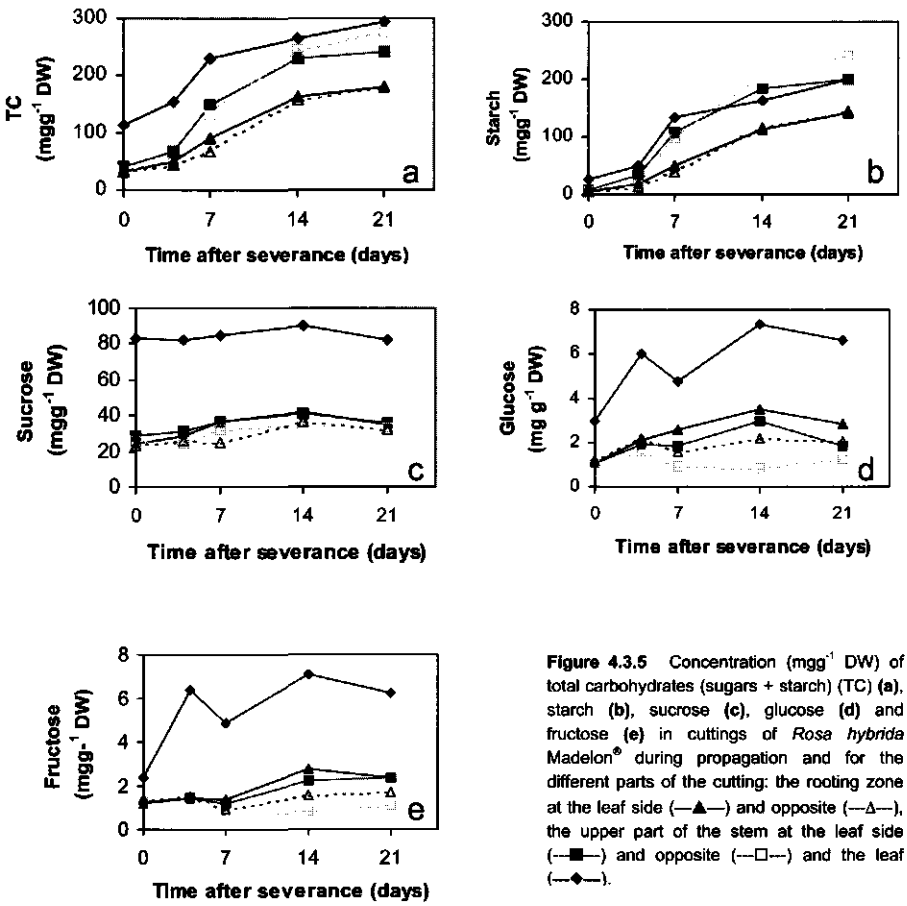

Figure 4.3.5 Concentration (mgg^{-1} DW) of total carbohydrates (sugars + starch) (TC) **(a)**, starch **(b)**, sucrose **(c)**, glucose **(d)** and fructose **(e)** in cuttings of *Rosa hybrida* Madelon® during propagation and for the different parts of the cutting: the rooting zone at the leaf side (—▲—) and opposite (---△---), the upper part of the stem at the leaf side (---■---) and opposite (---□---) and the leaf (---◆---).

Discussion

One of the first consequences of severance on carbon metabolism of rose cuttings was the pronounced increase in starch concentration in all the parts of the cutting

indicating that cuttings are photosynthetically active and that sink activity due to new formed organs (roots and the axillary shoot) is very low which is in agreement with previous findings for leafy cuttings of other species (Hansen et al., 1978; Loach and Gay, 1979; Veierskov et al., 1982; Smalley et al., 1991). In rose, the intermediate leaves on flower shoots have been shown to export photosynthates to support shoot growth and in particular development of the flower bud (Mor and Halevy, 1979; Jiao and Grodzinsky, 1998). The removal of these active sinks by detaching the cutting from the shoot coupled with a moderate decrease in CO_2 fixation by leaves on cutting (Chapter 4.2) enhanced storage of photosynthates as starch in both the leaf and the stem tissues. The fact that the starch concentrations increased 2 to 10 times more than sucrose concentrations is a typical indication of the low sink demand in plant tissues (Myers et al., 1999). However, as soon as the sink activity of new organs increased due to growth of the new formed organs the starch levels stabilised (Fig. 4.3.1). The lower accumulation of carbohydrates at the rooting zone of cuttings is a strong evidence that carbohydrates and carbon metabolism (starch hydrolysis) are directly involved in the preliminary events of rooting of rose cuttings, to support energy costs related to callus formation and eventually initial root growth. Starch hydrolysis is needed to sustain meristematic activity related to callus formation (Doud and Carlson, 1977), and it is possible that starch hydrolysis is required to provide a constant level of sugars at the rooting zone, which should be further allocated to the new roots and thus to support growth respiration of roots. This is supported by the fact that roots had very high concentration of glucose and fructose which are known to stimulate root growth by enhancing cell expansion (Pritchard and Rogers, 2000) or by promoting lateral root initiation like it happens with glucose (Bingham et al., 1997). Photosynthates may also be directly allocated to the new roots instead of being first converted into starch and afterwards metabolized in sugars as reported for conifer seedlings (Van der Driessche, 1987) or *Larix* cuttings (Pellicer et al., 2000). Although roots formed preferentially at the side of the stem where the leaf is inserted and where the leaf traces are located, we could not visualize any specific radial concentration gradient of carbohydrates in relation to the rooting patterns. This suggests the possibility that above a certain critical level of carbohydrate other factors (e.g. auxins), become preponderant in triggering root initiation (Davis and Potter, 1989).

Table 4.3.2 Carbohydrate concentration in the adventitious roots and in the axillary primary shoot of single node leafy stem cuttings of *Rosa hybrida* Madelon® after 14 and 21 days of propagation in Experiment 4. Values are means followed by the standard deviation in parenthesis.

Day of treatment	Carbohydrates (mg g^{-1} DW)				
	Glucose	Fructose	Sucrose	Starch	Total (sugars +starch)
			Roots		
14 (n=4)	115.9 (20.9)	43.5(4.1)	20.8(2.7)	9.2(2.8)	189.3(25)
21 (n= 9)	66.3(25.6)	12.7(5.3)	14.7(5.6)	5.8(3.8)	99.6(34.0)
			Axillary shoot		
14 (n= 5)	45.8(21.7)	27 (17.5)	45(7.1)	23(11.1)	140(43.6)
21 (n= 8)	45.3(25.6)	27.8(14.0)	40.8(5.4)	29.1(11.6)	142.9(30.0)

Table 4.3.3 Root distribution at the basal part of the stem in single node leafy stem cuttings of *Rosa hybrida* Madelon®, considering only the rooted cuttings. Values are means followed by the SE.

Day of observation		Number of roots at the leaf side of the stem	Number of roots at the side of stem opposite to the leaf
0		0	0
7		0	0
11	n=6	2.3(1.0)	0.8(0.3)
14	n=11	6.5(2.6)	3.1(1.3)
21	n=11	6.5(3.0)	3.6(1.5)

We may conclude that under the present propagation conditions, severance reduces more the sink rather than source activity of rose cuttings. Further research on the dynamics of carbohydrates in relation to rooting should be done under conditions of reduced source activity and limited photosynthate supply to clarify the role of carbohydrates on rooting of rose. The consequences of carbon accumulation in leaves for the photosynthetic apparatus should also be studied as it may influence the cuttings' photosynthetic efficiency (Harbinson, 1994).

References

Bingham IJ, Blackwood JM, Stevenson EA (1997) Site, scale and time-course for adjustments in lateral root initiation in wheat following changes in C and N supply. Ann Bot 80: 97-106

Doud S, Carlson R (1977) Effects of etiolation, stem anatomy and starch reserves on root initiation of layered *Malus* Clones. J Am Soc Hort Sci 102: 487-491

Davis D, Potter JR (1989) Relations between carbohydrate, water status and adventitious root formation in leafy pea cuttings rooted under various levels of atmospheric CO_2 and relative humidity. Physiol Plant 77: 185-189

Davis, TD, Potter JR (1981) Current photosynthate as limiting factor in adventitious root formation on leafy pea cuttings. J Am Soc Hort Sci 106: 278-282

Fordham MC, Harrison-Murray RS, Knight L, Clay CM (2001) Decline in stomatal response to leaf water deficit in *Corylus maxima* cuttings. Tree Physiol 21: 489-496

Friend AL, Coleman MD, Isebrands JC (1994) Carbon allocation to root and shoot systems of woody plants. In: TD Davis, B Haissig (eds) Biology of adventitious root formation. Basic Life Sciences. Vol. 62, pp 245-273

Haissig BE (1984) Carbohydrate accumulation and partitioning in *Pinus banksiana* seedlings and seedlings cuttings. Physiol Plant 61: 13-19

Haissig BE (1989a) Carbohydrate relations during propagation of cuttings from sexually mature *Pinus banksiana*. Tree Physiol 5: 319-328

Haissig BE (1989b) Removal of the stem terminal and application of auxin change carbohydrates in *Pinus banksiana* cuttings during propagation. Physiol Plant 77: 179-184

Hansen J, Strömquist L, Ericsson A (1978) Influence of the irradiance on the carbon content and rooting of cuttings of pine seedlings (*Pinus sylvestris* L.). Plant Physiol 61: 975-979

Harbinson J (1994) The responses of thylakoid electron transport and light utilisation efficiency to sink limitation of photosynthesis. In: Baker NR, Boyer JR (eds) Photoinhibition of photosynthesis, from molecular mechanisms to the field. Environmental Plant Biology Series. BIOS Scientific Publishers, Oxford, pp 273-295

Hoad SP, Leakey RRB (1996) Effects of pre-severance light quality on the vegetative propagation of *Eucalyptus grandis* W. Hill ex. Maiden. Cutting morphology, gas exchange and carbohydrate status during rooting. Trees 10: 317-324

Jiao J, Grodzinsky B (1998) Environmental influences on photosynthesis and carbon export in greenhouse roses during development of the flowering shoot. J Am Soc Hort Sci 123: 1081-1088

Leakey RRB, Coutts MP (1989) The dynamics of rooting *Triplochiton scleroxylon* cuttings: their relation with leaf area, node position, dry weight accumulation, leaf water potential and carbohydrate composition. Tree Physiol 5: 135-146

Loach K, Gay AP (1979) The light requirements for propagating hardy ornamental species from leafy cuttings. Sci Hort 10: 217-230

Myers DA, Thomas RB, De Lucia E (1999) Photosynthetic responses of lobolly pine (*Pinus taeda*) needles to experimental reduction in sink demand. Tree Physiol 19: 235-242

Mor Y, Halevy AH (1979) Translocation of ^{14}C-assimilates in roses. I. The effect of the age of the shoot and the location of the source leaf. Physiol Plant 45: 177-182

Okoro OO, Grace J (1976) The physiology of rooting *Populus* cuttings. Carbohydrates and photosynthesis. Physiol Plant 36: 133-138

Pellicer V, Guehl JM, Daudet FA, Cazet M, Riviere LM, Maillard P (2000) Carbon and nitrogen mobilization in *Larix x eurolepis* leafy stem cuttings assessed by dual ^{13}C and ^{15}N labelling: relationships with rooting. Tree Physiol 20: 807-814

Pritchard SG, Rogers HH (2000) Spatial and temporal deployment of crop roots in CO_2-enriched environments. New Phytol 147: 55-71

Svenson SE, Davies FT, Duray SA (1995) Gas exchange, water relations and dry weight partitioning during root initiation and development of Poinsettia cuttings. J Am Soc Hort Sci 120: 454-459

Smalley, TJ, Dirr AM, Armitage AM, Wood BW, Teskey RO, Severson RF (1991) Photosynthesis and leaf water, carbohydrate and hormonal status during rooting of stem cuttings of *Acer rubrum*. J Am Soc Hort Sci 116: 1052-1057

Van den Driessche R (1987) Importance of the current photosynthate to new root growth in planted conifer seedlings. Can J Forest Res 17: 776-782

Veierskov B, Andersen AS, Eriksen EN (1982) Dynamics of extractable carbohydrates in *Pisum sativum*,. I. Carbohydrate and nitrogen content in pea plants and cuttings grown at two different irradiances. Physiol Plant 55: 167-173

Yue D, Margolis HA (1993) Photosynthesis and dark respiration of black spruce cuttings during rooting in response to light and temperature. Can J Forest Res 23: 1150-1155

4.4 CHANGES OF PHOTOSYSTHEM II EFFICIENCY DURING ROOTING OF ROSE CUTTINGS

Abstract

Severance of a cutting from a mother plant for propagation imposes a stress, particularly to the leaves. There are short term effects on water status, and progressive, long term effects on the carbon, water and hormonal relations within the cutting which may affect leaf photosynthesis. The effect of severance and rooting on the functioning of the photosynthetic apparatus of leaves of single node cuttings of *Rosa hybrida* Madelon® was investigated by using images of the photosystem II efficiency (Φ_{PSII}) calculated from chlorophyll fluorescence images. Changes of Φ_{PSII} induced by severance and rooting were recorded during the first hours (0, 2, 4, 6 and 24 hours) or the first days (0, 3, 7, 11, 14 and 21) after severance. In another experiment starch accumulation in leaves and stems of cuttings was also determined on 0, 4, 7, 14 and 21 days of propagation. In the short term, severance decreased Φ_{PSII} by about 20% and increased heterogeneity (patchiness), but leaves re-achieved previous Φ_{PSII} values within 2 hours. In most cases a pronounced decrease of Φ_{PSII} and increased heterogeneity were found after one to two weeks of propagation after severance. Veins had higher values of Φ_{PSII} than the neighbouring mesophyll cells. Severance caused strong starch accumulation in leaves, beginning 4 days after severance. No increase of Φ_{PSII} was observed following rooting. The long term effect of severance or pruning on Φ_{PSII} may be related to drastic reduction in sink activity and starch accumulation in leaves.

Key words: Leaf, photosynthesis, severance, pruning, reduced sink activity, *Rosa hybrida*, propagation

Submitted as Costa JM, Marcakova J and Harbinson J

Introduction

Severance of cuttings from mother plants for propagation purposes results in a separation of a stem segment with attached leaf(ves) from the mother plant's vascular system. A cutting is therefore deprived of the normal supply of water and nutrients (Fordham et al., 2001), and of the root-derived hormones, such as cytokinins (Van Standen and Davey, 1979). These disturbances, acting either alone or in combination, change (reduce) stomatal conductance (Loach, 1988; Smalley et al., 1991; Fordham et al., 2001). Severance will also modify the normal source-sink relations of the foliar photosynthetic tissues by reducing sink activity and causing carbohydrates accumulation (Hansen et al., 1978; Veierskov et al., 1982; Smalley et al., 1991; Costa et al., 2001). As a result of these various mechanisms, the carbon dioxide uptake of leafy cuttings typically decreases following severance and remains depressed until roots are formed (Cameron and Rook, 1974; Okoro and Grace, 1976; Eliasson and Brunnes, 1980; Davis, 1988; Smalley et al., 1991; Svenson et al., 1995).

In recent years, chlorophyll fluorescence has been widely used to study the functioning of the photosynthetic apparatus in many plant species in response to stress (e.g. Snel et al., 1991; Meyer and Genty, 1999; Maxwell and Johnson, 2000). With regards to severed stems, chlorophyll fluorescence measurements have been used to estimate the condition of *Dendranthema* cuttings (Van Kooten and Peppelenbos, 1993), with the quantum yield of photosystem II electron transport (Φ_{PSII}) proving to be a good predictor of the rooting ability of the cuttings. Chlorophyll fluorescence has not been widely used to monitor physiological changes occurring in cuttings during propagation (Mésen et al., 1997; Bruce et al., 2001). Nontheless, the activity and distribution of CO_2 fixation during the period before and during the rooting of cuttings is of interest for several reasons. Light interception is known to be important for the rooting of roses, and of leafy stem cuttings in general (Moe, 1973; Davis, 1988; Costa et al., 2001), but the actual photosynthetic competence of cuttings is difficult to ascertain by conventional gas-analysis techniques because of the sensitive character of plant material deprived of roots. Knowing the photosynthetic competence of the cuttings is important for optimally controlling the environment of the cuttings to improve their rooting and further growth. The detached cutting is also a model with which it is possible to investigate the spatial responses of photosynthesis in stressed

systems, especially those in which the stress arises from the end-product (sink) limitation of CO_2 fixation. Measurements of the photochemical quantum yield of PSII (Φ_{PSII}) can give an accurate information on the photosynthetic activity of cuttings because Φ_{PSII} and gross CO_2 fixation are closely related (Genty et al., 1989; Harbinson et al., 1990; Hymus et al., 1999). Leaves whose stomata are functioning to maintain a constant intercellular CO_2 mole fraction (C_i) will commonly have a linear relationship between Φ_{PSII} and the quantum yield of gross CO_2 fixation though the precise relationship will depend on the intercellular CO_2 concentration (Genty et al., 1989; Fracheboud et al., 1999). Even in leaves with changing C_i or in leaves in an atmosphere with changing CO_2 or O_2 partial pressures, photosynthetic electron transport predicted from Φ_{PSII} agrees with estimates of electron transport derived from the total NADPH demand for both photorespiration and carboxylation calculated from biochemical models of photosynthetic metabolism (Cornic and Briantais, 1991; Peterson, 1991). Thus, measurements of Φ_{PSII}, may provide a sensitive indication of the functioning of the photosynthetic apparatus of cuttings during propagation. They would also complement the information given by CO_2 fixation measurements on the effects of severance and rooting on a cutting's photosynthesis during the first days of propagation.

Our first aim is to use chlorophyll fluorescence imaging to analyse the dynamics of spatial changes in Φ_{PSII} of the original leaf of rose cuttings in response to severance and root formation during the first 21 days of propagation. For that purpose we applied a method which allows visualisation of the distribution of Φ_{PSII} using leaf chlorophyll fluorescence images (Meyer and Genty, 1998).

A second goal of this research was to investigate the consequences of severance (pruning) on the homogeneity of photosynthesis in leaves of *Rosa* spp. It has been shown that water stress produces an uneven distribution of photosynthesis in rose leaves (Meyer and Genty, 1999). The development of heterogeneous photosynthesis has implications for our understanding of both the regulation and co-ordination of photosynthesis in leaves, and for the use of measurement procedures that rely upon a homogeneous distribution of photosynthesis across the area being measured.

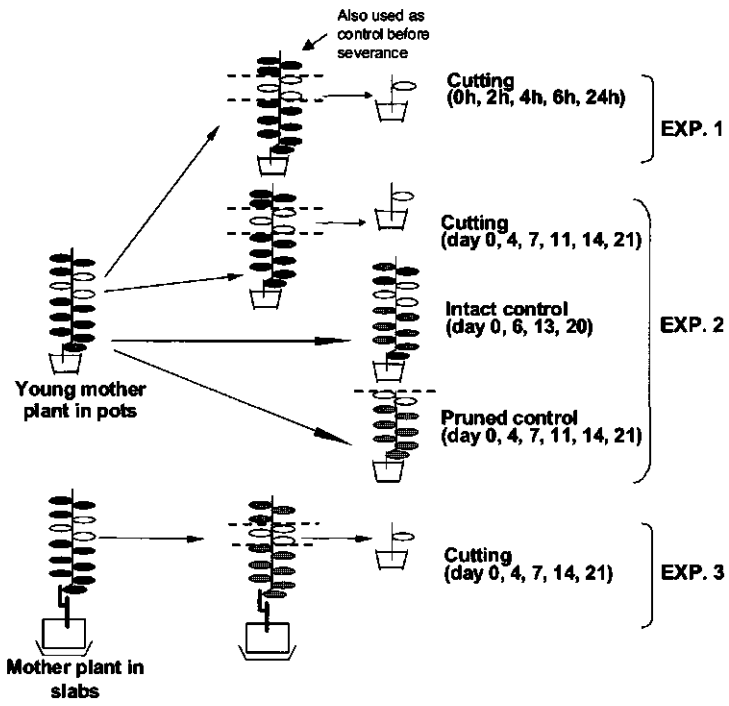

Figure 4.4.2 Schematic representation of the plant material and treatments applied in the 3 experiments to test the effect of severance and pruning on the Φ_{PSII} of single node leafy stem cuttings and plants of *Rosa hybrida* Madelon®.

To analyse the effect of severance on changes in the Φ_{PSII}, it was measured during the first 24 hours after severance. The terminal leaflets of the 4th to 6th attached leaf (counted from the top), still attached to the primary shoot of the young mother plants were used as controls (Fig 4.4.2). The "treatment" were similar terminal leaflets measured immediately after severance and 2, 4, 6, and 24 hours later (Fig. 4.4.2). After the leaves of each control had been measured, the shoots were used as source of cuttings (one cutting per shoot). These cuttings were inserted into pots with substrate and placed inside a propagator box with a plastic cover, at room temperature (20-22°C). Five replicates (mother plants and cuttings) were used. Measurements were made under a light intensity of 180-195 $\mu molm^{-2}s^{-1}$ (PAR).

Mapping of the PS II efficiency using chlorophyll fluorescence imaging system in leaves of cuttings

The chlorophyll fluorescence imaging system and associated equipment used in this procedure have been described previously (Vanacker et al., 1998). The design was based on that of Genty et al. (1989). To measure changes in quantum yield of electron transport *in vivo,* the imaging system calculated Φ_{PSII} images using a pixel by pixel calculation of the ΔF /Fm parameter (Genty et al., 1989). ΔF represents the difference between the maximum relative fluorescence yield (Fm) (Fig. 4.4.3a), reached during the saturating light pulse (7000 $\mu molm^{-2}s^{-1}$), and relative fluorescence yield under steady state irradiance (Fss) (Fig. 4.4.3b). The image of the Φ_{PSII} (Fig. 4.4.3c) is therefore obtained by combining two digitised fluorescence images: one of the steady state relative fluorescence yield (Fss) and the other of the maximum relative fluorescence yield (Fm) (Fig. 4.4.3). In both cases, the intensity of fluorescence from a fluorescent target was used to estimate the integrated irradiance during the exposures, which was then used to convert the raw fluorescence images into images of relative fluorescence yield. For visualisation the Φ_{PSII} are coloured according to a colour index (Fig. 4.3.3d).

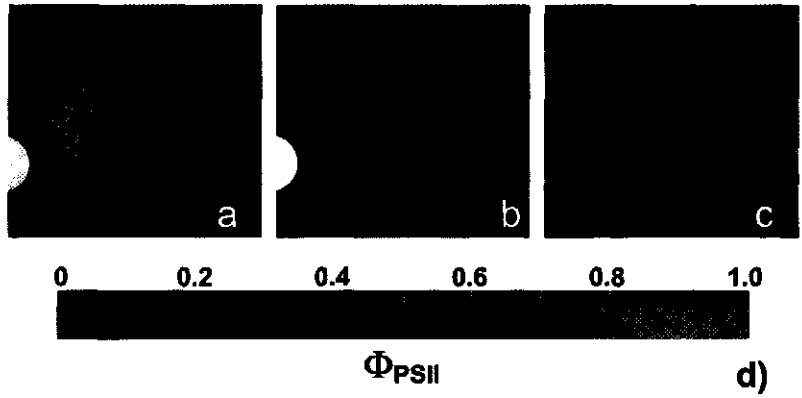

Figure 4.4.3 Images of chlorophyll fluorescence from the terminal leaflet of leafy stem cuttings of *Rosa hybrida* Madelon® during a saturating light pulse (7000 µmol m^{-2} s^{-1}) (Fm) **(a)** and under steady state irradiance (85- 125 µmol m^{-2} s^{-1} (Fss) **(b)**; these two images are used to calculate, pixel by pixel, (Fm-Fss)/Fm) also termed Φ_{PSII} **(c)**. A fluorescence reference (the white semicircle in both images **(a and b)** was used to estimate the integrated irradiance during the exposures and to allow the conversion of the raw fluorescence images to images of relative fluorescence yield. The scale **(d)** indicates the colour indexing coding used to colorise the Φ_{PSII} images.

Long term variation for the quantum yield for photosystem II electron transport in response to severance and rooting (Experiment 2)

To measure the long term effect of severance and root formation on Φ_{PSII} we measured the quantum yield for photosystem II electron transport during the first 21 days of propagation. Forty single node cuttings with a single leaf with five leaflets were harvested from the middle part of the primary shoots (4^{th}-6^{th} leaf from the top) of the young mother plants and were propagated as previously described (Fig. 4.4.2). These cuttings were allowed to root over a period of 21 days during which a series of measurements were made.

Rooting morphology

The number of roots, the fresh and dry weight of roots, the stem rooting zone (basal 1.5 cm of the stem), the entire stem segment, the original leaf, and the primary shoot were determined on day 0, 3, 7, 11, 14, and 21 of propagation from a sample of five cuttings randomly selected from each propagator box (one cutting from each propagator box).

Mapping of the PS II efficiency using chlorophyll fluorescence imaging system in leaves of young mother plants (controls) and cuttings

Images of Φ_{PSII} were made from the terminal leaflet of the original leaf of each of seven cuttings on day 0 (3.5h after severance), 3, 7, 11, 14 and 21 of propagation. To allow the effect of severance and rooting to be distinguished from the effects of leaf ageing or modified source-sink relations, two controls were used (Fig. 4.4.2): 1) pruned controls: the shoot was cut just above the 4^{th}-5^{th} fully developed leaf from the shoot tip and the terminal leaflet of the now terminal leaf, was used for measurements; and 2) unpruned controls: the terminal leaflet of the 4^{th}-5^{th} fully-developed leaf from the shoot-tip of an intact shoot was measured. Leaves from

pruned controls were measured on the same days as the cuttings, but due to a time limitation the leaflets of the unpruned controls were measured one day before the severance of cuttings and on days 6, 13 and 20 after the cuttings had been taken (Fig. 4.4.2).

To avoid stressing the freshly prepared cuttings, the steady-state irradiance used on day 0 for both cuttings and controls was 85-90 $\mu molm^{-2}s^{-1}$ PAR. Further measurements were made at light intensity of 180-195 $\mu molm^{-2}s^{-1}$ PAR.

Data analyses from leaf chlorophyll fluorescence imaging

Due to a large number of photosystem II efficiency images collected, representative samples were selected for data analysis. Profiles of the Φ_{PSII} across the leaf were plotted and analysed using the image analysis software ImageJ (http://rsb.info.nih.gov/ij/). This software allowed us to draw a sample line across an image and the values of the pixels along this line were then automatically plotted, thus providing a profile of pixel values (in our case Φ_{PSII}) across the image (Figs. 4.4.4-4.4.7). The profile selected for each image ran diagonally across the leaf, parallel to a vein on one side of the leaf, across the mid-rib, and then across veins on the other side of the leaf. Frequency-distribution histograms of PSII efficiency of the entire leaf surface were also calculated using ImageJ.

Long term variation for the starch content in leaves and stems in response to severance and rooting (Experiment 3)

Cuttings were harvested from a different batch of mother plants and propagated as previously described. The samples for carbohydrate analysis were taken on day 0, 4, 7, 11, 14 and 21 of propagation and analysed as described in Chapter 3.2.

Statistical analysis

The experimental design used in Experiments 2 and 3 was a randomised complete block design with 5 blocks in Experiment 2 and 10 blocks in Experiment 3, both with 6 plots of a single cutting per block. Data was subjected to an analysis of variance ($P<0.05$) using the statistical package GENSTAT 5 (IACR, Rothamsted, UK). Least significant differences by the *t*-test are presented whenever there was a significant effect. When no analysis of variance was performed, as occurred for data on the number of leaves and length of the primary shoot of young mother plants in Experiment 2, the standard error of the mean was calculated.

Results

Short term variation for the quantum yield for photosystem II electron transport in response to severance and rooting (Experiment 1)

Before severance, Φ_{PSII} is distributed homogeneously over the entire leaflet area, with a unimodal and narrow distribution between 0.73 and 0.76 and with a mean value of 0.74 (Fig. 4.4.4, mother plant). Severance caused a rapid, but transient 20% decrease in the mean values of Φ_{PSII} (from 0.74 to 0.69) as well a slight increase in the heterogeneity of Φ_{PSII} visible by the increased patchiness in the images and by the larger standard deviation and more skewed frequency distribution (Fig. 4.4.4, 0h image). Two hours later Φ_{PSII} was again at values between 0.72 and 0.76, as it had been on the leaflet before severance. Subsequently, the values of Φ_{PSII} remained stable until the first part of the experiment was concluded (Fig. 4.4.4, 2h-24h images).

Figure 4.4.4 Images, plots of pixel profile across the leaflet, and frequency distributions of PSII efficiency (Φ_{PSII}) of the terminal leaflet of the leaf of cuttings of *Rosa hybrida* Madelon® taken just before severance (0 h) and then 2, 4, 6 and 24h after severance in Experiment 1. The black line on the leaf images represents the line of pixels selected to obtain the pixel profile. Mean Φ_{PSII} ± standard deviation are presented.

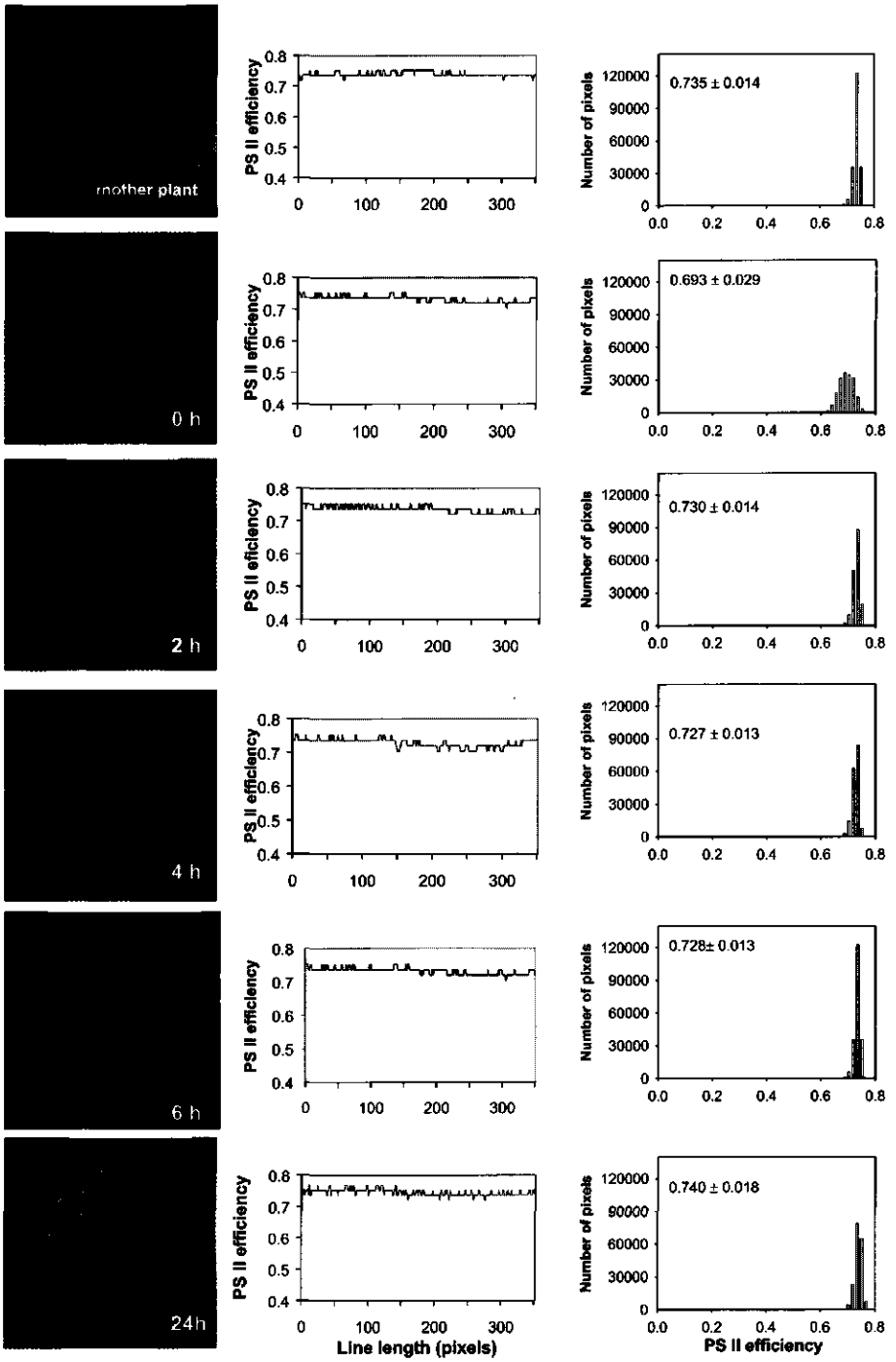
mother plant
0 h
2 h
4 h
6 h
24h
PS II efficiency
Number of pixels
0.735 ± 0.014
0.693 ± 0.029
0.730 ± 0.014
0.727 ± 0.013
0.728± 0.013
0.740 ± 0.018
Line length (pixels)
PS II efficiency

Figure 4.4.4

Long term variation for the quantum yield for photosystem II electron transport and starch in response to severance and rooting (Experiments 2 and 3)

A callus was visible at the basal end of the stem segment of cuttings from between day 7 and 14 (data not presented), with roots emerging between day 11 and day 21 (Table 4.4.1). The fresh and dry-weight of the stem and the leaf increased during propagation, particularly in the rooting zone, which is consistent with tissue proliferation related to callus growth (Table 4.4.1).

Table 4.4.1 Rooting and morphological aspects of single node leafy stem cuttings of *Rosa hybrida* Madelon® during the first 21 days of propagation, in Experiment 2. Total FW and DW include fresh and dry weights of the axillary bud (primary shoot).

Time (days after severance)	Num root	DW roots (mg)	FW Leaf (mg)	FW basal stem (mg)	FW stem (mg)	Total FW (mg)	DW basal stem (mg)	DW stem (mg)	DW leaf (mg)	Total DW (mg)	FW-DW ratio
0	0	0	707	129	419	1126	35	108	165	273	4.1
3	0	0	850	136	449	1298	36	125	227	352	3.7
7	0	0	893	160	497	1390	47	156	241	396	3.5
11	0.4	0	876	182	474	1358	58	164	253	419	3.2
14	4.2	-	857	196	582	1471	66	217	257	482	3.1
21	8.2	26	749	179	561	1573	78	225	273	548	2.9
LSD	1.5	8.6	184	59	68	207	9.2	25	58	61	0.3

The dry weight increase accounted for greater part of total (i.e. fresh) weight increase. As a result, the fresh-weight to dry-weight ratio after 21 days had diminished which was a sign that cuttings were storing carbohydrates rather then growing (Table 4.4.1). This was supported by the results from starch analysis showing that both stem and the original leaf accumulated starch during propagation, mostly between day 4 and day 14 of propagation (Fig. 4.4.8).

Figures (4.4.5-4.4.7) are images of Φ_{PSII}, and the corresponding profile plots and frequency distributions of the pixel values of Φ_{PSII}, for first, the terminal leaflet of leaves of the two controls; unpruned (Fig. 4.4.5) or pruned (Figs. 4.4.6a and 4.4.6b), and second for the terminal leaflet of the original leaf of the cuttings (Figs. 4.4.7a and 4.4.7b).

The intact controls (i.e. unpruned shoots) (Fig. 4.4.5) showed little change in Φ_{PSII} during the measurement period. Photosynthetic efficiency remained high and homogeneous through the measurement period. Only on day 13, was the mean photochemical yield over the all leaf slightly higher and more heterogeneously distributed. We have no explanation for this change.

The pruned controls differed somewhat from the unpruned controls in that they displayed with two types of response. If the initial measurement (day 0) of Φ_{PSII} was, on average low (e.g. mean value of 0.55, Fig. 4.4.6a), then during the subsequent 21 days of measurement the overall Φ_{PSII} mean was substantially unchanged, even though some variation in the distribution of Φ_{PSII} was apparent (Fig. 4.4.6a), and in some cases there was a short-term fluctuation in the overall Φ_{PSII} on a day to day basis.

Figure 4.4.5 Images, plots of pixel profile across the leaflet, and frequency distributions of PSII efficiency (Φ_{PSII}) of the terminal leaflet of the leaf (4th to 6th counted from the top) of unpruned young mother plant of *Rosa hybrida* Madelon® (intact control) taken on day 0, 6, 13 and 20 of Experiment 2. The black line on the leaf images represents the line of pixels selected to obtain the pixel profile. Mean $\Phi_{PSII} \pm$ standard deviation are presented.

Figure 4.4.6 Images, plots of pixel profile across the leaflet, and frequency distributions of PSII efficiency (Φ_{PSII}) of the terminal leaflet of the top leaf of prunned young mother plant of *Rosa hybrida* Madelon® (pruned control) taken since day 0 until day 21 of Experiment 2, for leaflets showing high heterogeneity and low values of Φ_{PSII} on day 0 **(a)** or high initial homogeneity and high Φ_{PSII} values **(b)**. The black line on the leaf images represents the line of pixels selected to obtain the pixel profile. Mean $\Phi_{PSII} \pm$ standard deviation are presented.

Figure 4.4.7 Images, plots of pixel profile across the leaflet, and frequency distributions of PSII efficiency (Φ_{PSII}) of the terminal leaflet of the leaf cuttings of *Rosa hybrida* Madelon® taken since day 0 until day 21 in Experiment 2, for cuttings showing the two main types of response observed : decreasing (Φ_{PSII}) **(a)** or relatively stable (Φ_{PSII}) **(b)**. The black line on the leaf images represents the line of pixels selected to obtain the pixel profile. Mean (Φ_{PSII}) $\pm$ standard deviation are presented.

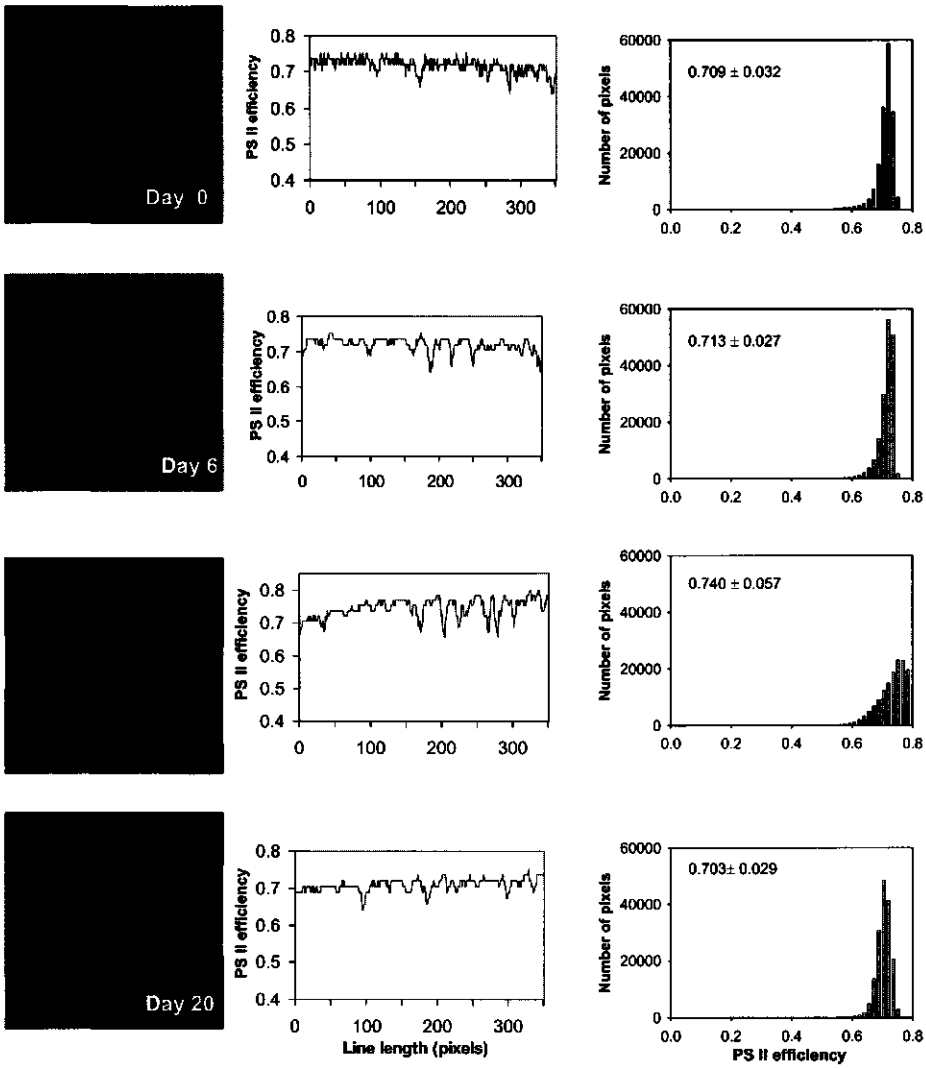
Day 0
Day 6
Day 20
PS II efficiency
Number of pixels
0.709 ± 0.032
0.713 ± 0.027
0.740 ± 0.057
0.703± 0.029
Line length (pixels)
PS II efficiency

Figure 4.4.5

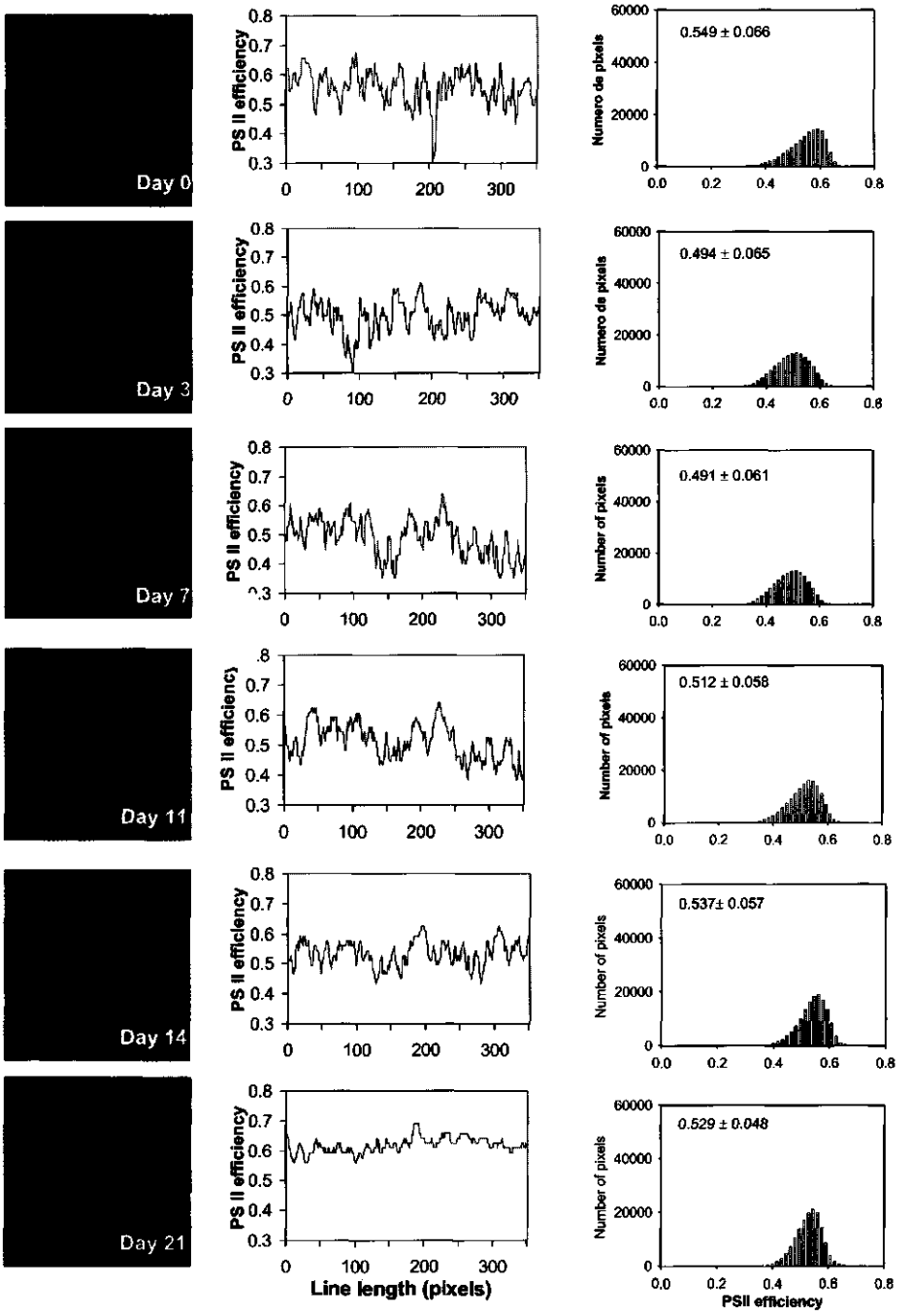
Day 0
Day 3
Day 7
Day 11
Day 14
Day 21
PS II efficiency
Line length (pixels)
Numero de pixels
Number of pixels
0.549 ± 0.066
0.494 ± 0.065
0.491 ± 0.061
0.512 ± 0.058
0.537± 0.057
0.529 ± 0.048
PSII efficiency

FIGURE 4.4.6a

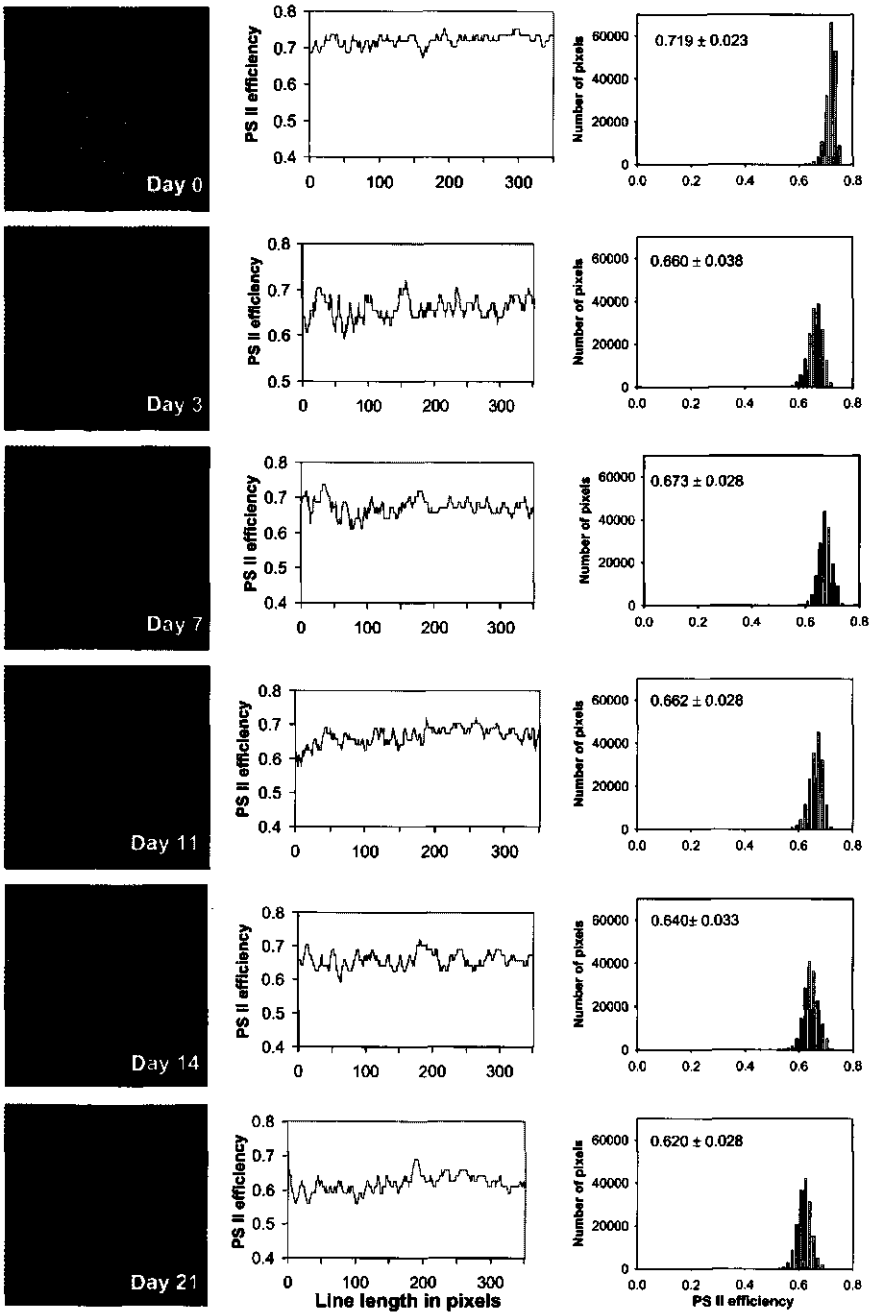
Day 0
Day 3
Day 7
Day 11
Day 14
Day 21
PS II efficiency
Number of pixels
0.719 ± 0.023
0.660 ± 0.038
0.673 ± 0.028
0.662 ± 0.028
0.640± 0.033
0.620 ± 0.028
Line length in pixels
PS II efficiency

Figure 4.4.6b

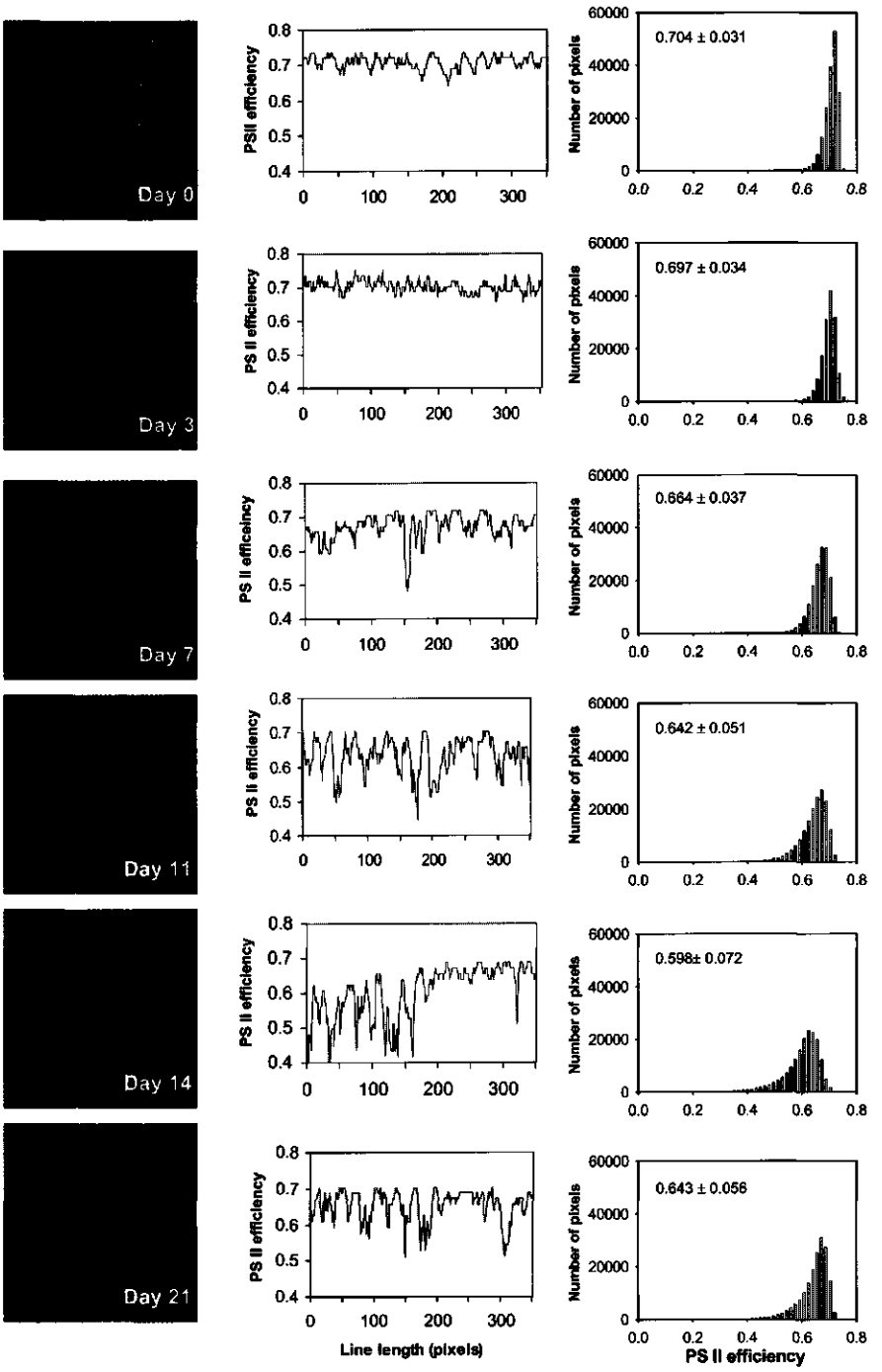
Day 0
Day 3
Day 7
Day 11
Day 14
Day 21
PSII efficiency
PS II efficiency
Number of pixels
0.704 ± 0.031
0.697 ± 0.034
0.664 ± 0.037
0.642 ± 0.051
0.598± 0.072
0.643 ± 0.056
Line length (pixels)
PS II efficiency

Figure 4.4.7a

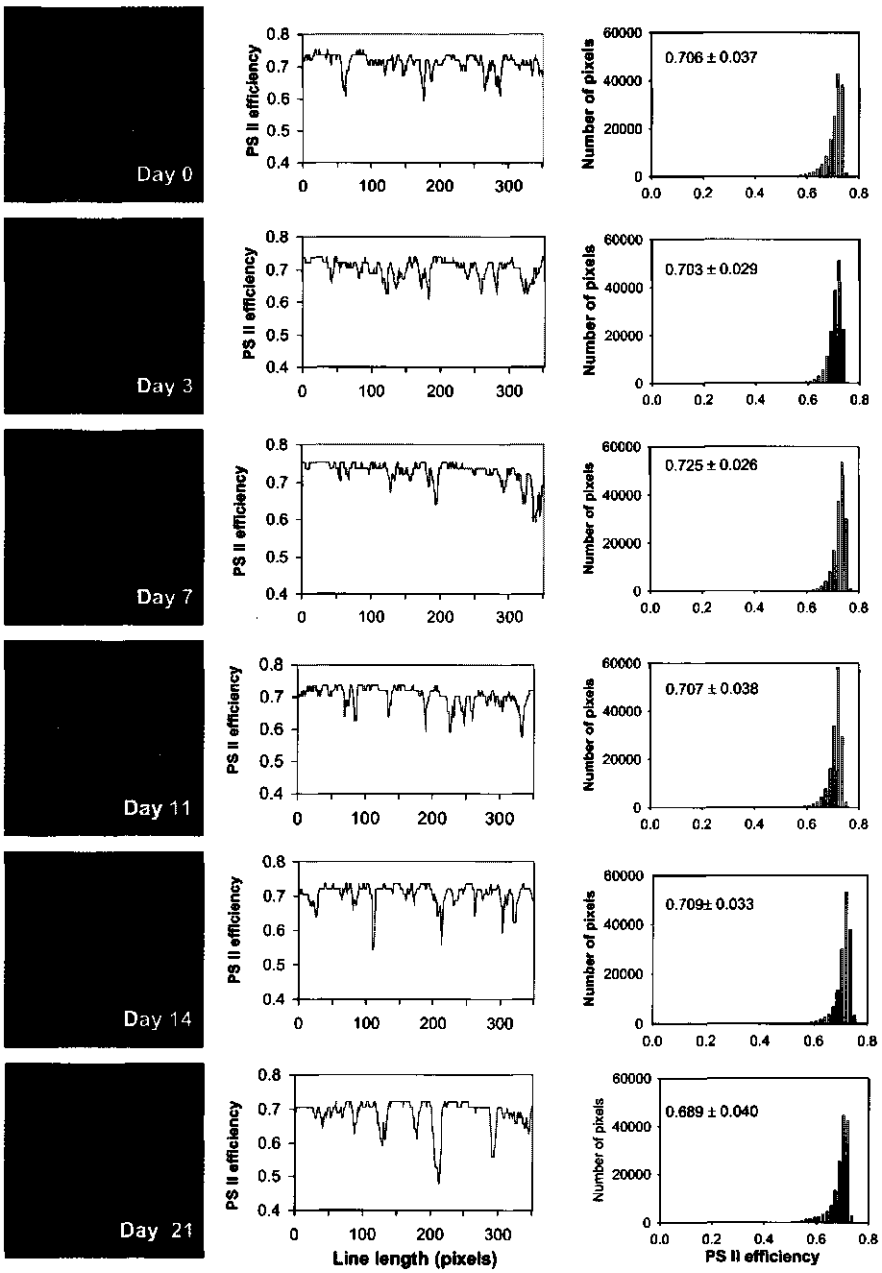
Day 0
Day 3
Day 7
Day 11
Day 14
Day 21
PS II efficiency
Line length (pixels)
Number of pixels
PS II efficiency
0.706 ± 0.037
0.703 ± 0.029
0.725 ± 0.026
0.707 ± 0.038
0.709± 0.033
0.689 ± 0.040

Figure 4.4.7b

On the other hand, if the initial overall mean value of Φ_{PSII} was high (e.g. 0.72, Fig. 4.4.6b), then during the subsequent 21 days of measurement the mean Φ_{PSII} over the all leaflet decreased so that by day 21 the difference between the two types of pruned control was decreased. Notably, in all those leaves of the pruned controls which showed a decreased overall Φ_{PSII} the values of Φ_{PSII} adjacent to the veins remained higher than that found in the lamina between the veins (Figs. 4.4.6a and 4.4.6b). In these leaflets there was no consistent trend of lower values of Φ_{PSII} towards the leaf tip.

The results obtained from the cuttings were substantially different to those of the controls. The distribution and overall values of Φ_{PSII} in the leaflets of cuttings displayed a range of responses during the measurement period, with most showing a decrease in the overall value of Φ_{PSII} and an increase in its heterogeneity. A smaller number showed a more stable overall mean value of Φ_{PSII} and with less development of heterogeneity. Examples of these two types of response in cuttings are shown in Fig. 4.4.7a (decreasing Φ_{PSII}) and Fig. 4.4.7b (relatively stable Φ_{PSII})

Leaves with decreasing Φ_{PSII} (Fig. 4.4.7a) had profiles of Φ_{PSII} across the leaf that showed little variation on days 0 and 3 (in a range between 0.65-0.75), demonstrating the high degree of photosynthetic homogeneity commonly observed in the unstressed leaves of the unpruned control (Fig. 4.4.5). Initially, the frequency distribution was slightly skewed to lower values due to the occurrence of a few patches with low Φ_{PSII} towards the leaflet tip (Fig. 4.4.7a). By day 7, however, Φ_{PSII} had decreased heterogeneously over the entire area of the leaflet of the cutting, producing a mosaic of patches with different efficiencies ranging between 0.47 and 0.72, resulting in the frequency distribution of Φ_{PSII} becoming more left-skewed (Fig. 4.4.7a). From day 7 until day 21 the profile plots and frequency distribution histograms for cuttings showed progressively greater heterogeneity, visible through increased patchiness of the Φ_{PSII} of the lamina between the veins (Fig. 4.4.7a).

In those cuttings with a relatively stable Φ_{PSII} during the rooting period (Fig. 4.4.7b) Φ_{PSII} values in profile plots varied initially between 0.6-0.75 (similar to results shown in Fig. 4.4.7a, days 0 and 3) and no overall change in the frequency distribution Φ_{PSII} occurred until day 14 when small patches with lower Φ_{PSII} values

developed. The frequency distributions of the pixel values were only slightly left-skewed during the first 14 days indicating that little heterogeneity was present overall, although by day 21 the degree of heterogeneity had increased and Φ_{PSII} decreased in some regions of the leaflet to about 0.5-0.55. So, by day 21, both classes of cuttings (represented in Figs. 4.4.7a and 4.4.7b) showed increasing heterogeneity with an increasing number of patches over the leaflets with low Φ_{PSII}. This implies that at least up to day 21 rooting had no positive effect on Φ_{PSII}, as by this time roots had formed and were functional. Characteristically the cuttings had higher values of Φ_{PSII} close the veins compared to the lamina between the veins (Figs. 4.4.6b and 4.4.7a and 4.4.7b)

Discussion

As in the experiments described in previous chapters, the weight changes of the cuttings, especially the dry weight changes, indicate assimilation continues after severance during the 21 day rooting period. Over this period, the total dry weight of the cuttings doubled, with all their components (leaf, stem, and the rooting zone of the stem) showing an increase in dry weight. The accumulation of dry matter in both the leaves and stems of cuttings suggests also that source activity was not limiting for the development of roots by the cuttings.

Comparison of cuttings and controls showed that pruning or severance is one of the important factors influencing efficiency of the photosynthetic electron transport (measured as Φ_{PSII} under conditions of constant irradiance) in leaves of rose cuttings (Figs. 4.4.7a and 4.4.7b) or young rose plants (Figs. 4.4.6a and 4.4.6b) during the subsequent three weeks. Whereas the unpruned control plants had a stable overall Φ_{PSII} during the three weeks after cuttings were taken or plants pruned, those leaves on the cuttings or the pruned plants often displayed a progressive decrease of Φ_{PSII} (Figs. 4.4.6-4.4.7). This decrease, when it occurred, was heterogeneous and affected the interveinal regions of the leaflet more than the veins and regions close to them.

We were not, in this experiment, able to estimate C_i over the leaf area, so we are unable to be certain whether a decrease in stomatal conductance as a result of

water stress began to limit CO_2 fixation, or if stomatal conductance decreased in parallel with photosynthesis as would be expected if CO_2 fixation were to be product (sink) limited. Some insight can, however, be gained by comparing the pruned controls (Figs 4.4.6a and 4.4.6b) with the cuttings (Figs. 4.4.7a and 4.4.7b). It is hard to see how the pruned control leaves could become water stressed, and thus might develop a degree of stomatal closure that would increase the stomatal limitation on CO_2 fixation. On the other hand, the apical leaves of the pruned controls during the period of the experiment developed only a small shoot from the axillary bud, so sink limitation of their photosynthesis in the absence of the shoot-tip would be still expected.

The quantum efficiency of photosystem II electron transport is closely related to the quantum efficiency of gross CO_2 fixation, but not to net CO_2 fixation around the light compensation point. This difference arises because gross CO_2 fixation is a measure of photosynthetic CO_2 fixation alone which is dependent on the regulation and operation of photosynthetic electron transport. Net CO_2 fixation depends on the regulation of both leaf respiration and photosynthesis. Consequently, Φ_{PSII} can predict gross, but not net CO_2 fixation, and it is impossible to predict what rate of net photosynthesis is achieved by the leaves based on Φ_{PSII} measurements alone especially at low light levels when the rates of respiration and gross CO_2 fixation will be similar. This might seem to make the Φ_{PSII} measurement of little use, but in these leaves we are interested in the regulatory state of the photosynthetic system - the extent of changes in the operation of gross photosynthesis as a result of the severance and the subsequent rooting process. The loss of Φ_{PSII} is evidence of down-regulation acting upon photosynthesis, thus reducing its capacity, even at the low light intensities used in this experiment.

The similarity between the pruned control leaves and the cuttings suggest that photosynthesis was becoming product, or sink, limited in both the cuttings and the pruned controls. The decrease in the fresh-weight to dry-weight ratio (Table 4.4.1) as well the starch accumulation in both the stem and the leaf of cuttings (Fig. 4.4.8) is consistent with the development of sink limitation. Identically, removal of a very important sink like the flower bud or the partial removal of the stem both important sinks for mature leaves in flower shoots of rose (Mor and Halevy, 1979; Jiao et al., 1989; Jiao et al, 2001) might have caused the development of sink limitation in the

top leaf of the pruned shoot. The effect of pruning on modified sink relations is mentioned in literature. In *Pinus silvestris* the removal of apical meristems from one and two-years old seedlings resulted in starch accumulation in the needles (Gezelius et al., 1981) and Myers et al. (1999) found for *Pinus taeda* that experimentally lowered sink strength by excision of the emerging terminal flush resulted in starch accumulation and fast feedback inhibition of leaf photosynthesis.

As described in previous chapters, single node leafy stem cuttings of rose are characterised by a reduced sink activity, at least during the first 21 days of propagation (Fig. 4.4.8).

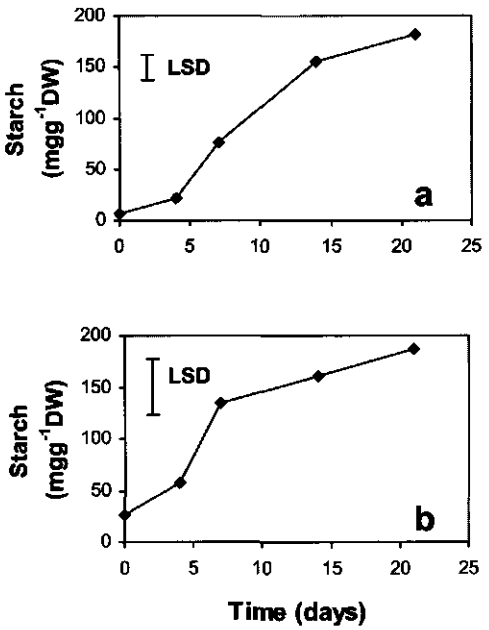

Figure 4.4.8 Starch concentration (mg g^{-1}DW) in the leaf **(a)** and the stem **(b)** of single node leafy stem cuttings of *Rosa hybrida* Madelon® measured during the first 21 days after severance in Experiment 3.

Our results are in line with previous findings that starch accumulation in leaves may decrease PSII efficiency (F_v/F_m) in dark adapted leaves via photoinhibition of some kind (Warner and Burke, 1993) and can down-regulate photosynthetic electron transport (Harbinson, 1994). Greer (1999) showed that

strongly sink-limited leaves of *Phaseolus vulgaris* were more sensitive to photoinhibition that slightly sink limited leaves. Therefore, it is possible that the negative effect of carbohydrates accumulation on PSII efficiency overcame any positive effect of root formation on Φ_{PSII}, explaining why root development was not related to increases in Φ_{PSII} nor to increases in net and gross photosynthesis (Chapter 4.2).

In comparison to the water stressed leaves of *Rosa rubiginosa* (Meyer and Genty, 1999) which had a patchy distribution of Φ_{PSII} that corresponded to the zones of the leaf lamina surrounded by veins (aureoles), the heterogeneity observed in our leaves was different in that it seemed to depend largely on the distance from the major leaf veins. There was no evident localisation of the heterogeneity on the aureoles. This dependency on distance from the veins may reflect gradients in sink-limitation of photosynthesis and the striking heterogeneity displayed by the leaves of both the unpruned controls and the cuttings might have many consequences for the evaluation of the photosynthetic properties of leaves.

Water stress is known to induce heterogeneous photosynthetic properties across the leaf lamina of rose (Meyer and Genty, 1999). Here we present evidence that heterogeneous photosynthetic properties can be induced by a process as simple as pruning a shoot or by severance of a cutting from the mother plant shoot. Many of the techniques used to evaluate the photosynthetic properties of leaves are based on the homogeneous operation of photosynthesis across the leaf area being measured. One of the best known of these measurements is the estimation of C_i based on measurements of leaf transpiration to calculate stomatal conductance. Once heterogeneity develops these techniques can be no longer reliably applied. Correlations between gas-exchange and biophysical measurements of leaf physiological processes (such as Φ_{PSII}) are likewise vulnerable to distortion if there is heterogeneity of leaf photosynthesis and if the regions of the leaf used for gas-exchange and for chlorophyll fluorescence measurements are not identical.

Thus, we conclude that severance has a negative effect on PSII efficiency and induces heterogeneity in leaves of rose cuttings during propagation. This besides being a sign of reduced sink activity, may also conditionate the accuracy of the techniques commonly used to evaluate the photosynthetic properties of leaves which

require a homogeneous operation of photosynthesis across the leaf area being measured.

Acknowledgements

The authors thank Dr. Hugo Challa, Dr. Eva Rosenqvist, Dr. U. Van Meeteren for major comments during the elaboration of this paper and Arjen v.d. Peppel and Sonja Moorhoff for their technical assistance in the carbohydrate assays.

References

Bruce SE, Rowe DB, Flore JA (2001) Chlorophyll fluorescence and vegetative propagation of *Taxus*. HortScience 36: 971-975

Cameron RJ, Rook DA (1974) Rooting stem cuttings of Radiata pine: environmental and physiological aspects. New Zeal J For Sci 4: 291-298

Cornic G, Briantais, JMB (1991) Partitioning of photosynthetic electron flow between CO_2 and O_2 reduction in C_3 leaf (*Phaseolus vulgaris* L.) at different CO_2 concentrations and during drought stress. Planta 183: 178-184

Costa JM, Challa H, Van Meeteren U, Van de Pol PA (2001) Photosynthates: mainly stored and yet limiting in propagation of rose cuttings. Acta Hort 547: 167-174

Davis TD (1988) Photosynthesis during adventitious rooting, In: Davis TD, Haissig BE and Shankla, N (eds) Adventitious root formation in cuttings. Discorides Press, Portland, Oregon, pp 79-87

Eliasson L, Brunnes L (1980) Light effects on root formation in aspen and willow cuttings. Physiol Plant 48: 261-265

Fracheboud Y, Haldimann P, Leipner J, Stamp P (1999) Chlorophyll fluorescence as a selection tool for cold tolerance of photosynthesis in maize (*Zea mays L.*). J Exp Bot 50: 1533-1540

Fordham MC, Harrison-Murray RS, Knight L, Clay CM (2001) Decline in stomatal response to leaf water deficit in *Corylus maxima* cuttings. Tree Physiol 21: 489-496

Genty B, Briantais JM, Baker, NR (1989) The relationship between quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990: 87-92

Gezelius K, Ericsson A, Hallgren, JE, Brunes L (1981) Effects of bud removal in Scots pine (*Pinus silvestris*) seedlings. Physiol Plant 51: 181-188

Greer DH (1998) Photoinhibition of photosynthesis in dwarf bean (*Phaseolus vulgaris* L.) leaves: effect of sink limitations induced by changes in daily photon receipt. Planta 205: 189-196

Hansen J, Strömquist L, Ericsson, A (1978) Influence of the irradiance on carbohydrate content and rooting of cuttings of pine seedlings (*Pinus sylvestris* L.). Plant Physiol 61: 975-979

Harbinson J, Genty B, Baker, NR (1990) The relationship between CO_2 assimilation and electron transport in leaves. Photosynth Res 25: 312-324

Harbinson J (1994) The responses of thylakoid electron transport and light utilisation efficiency to sink limitation of photosynthesis. In: Baker NR, Boyer JR (eds) Photoinhibition of photosynthesis, from molecular mechanisms to the field. Environmental Plant Biology Series. BIOS Scientific Publishers, Oxford, pp 273-295

Humphries, EC, Thorne, GN (1964) The effect of root formation on photosynthesis of detached leaves. Ann Bot 28: 391-400

Hymus GJ, Ellsworth DS, Baker NR, Long, SP (1999) Does free-air carbon dioxide enrichment affect photochemical energy use by evergreen trees in different seasons? A chlorophyll fluorescence study of mature loblolly pine. Plant Physiol 120: 1183-1191

Jiao J, Gilmour M, Tsujita MJ, Grodzinski B (1989) Photosynthesis and carbon partitioning in Samantha roses. Can J Plant Sci 69:577-584

Jiao J, Grodzinsky B (1998) Environmental influences on photosynthesis and carbon export in greenhouse roses during development of the flowering shoot. J Am Soc Hort Sci 123: 1081-1088

Loach K (1988) Water relations and adventitious rooting. In: Davis TD, Haissig BE, Shankla N (eds) Adventitious root formation in cuttings. Discorides Press, Portland, Oregon, pp 102-116

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence - a practical guide. Review Article, J Exp Bot 51: 659-668

Mésen F, Newton, AC, Leakey RRB (1997) The effects of propagation environment and foliar area on the rooting physiology of *Cordia alliodora* (Ruiz&Pavon) Oken cuttings. Tree 11: 404-411

Meyer S, Genty B (1998) Mapping intercellular CO_2 mole fractions (C_i) in *Rosa rubiginosa* leaves fed with abcissic acid by chlorophyll fluorescence imaging. Plant Physiol 116: 947-957

Meyer S, Genty B (1999) Heterogeneous inhibition of photosynthesis over the leaf surface of *Rosa rubiginosa* L. during water stress and abscisic acid treatment: induction of a metabolic component by limitation of CO_2 diffusion. Planta 210: 126-131

Mor Y, Halevy AH (1979) Translocation of ^{14}C-assimilates in roses. I. The effect of the age of the shoot and the location of the source leaf. Physiol Plant 45: 177-182

Myers DA, Thomas RB, DeLucia EH (1999) Photosynthetic responses of loblolly pine (*Pinus taeda*) needles to experimental reduction in sink demand. Tree Physiol 19: 235-242

Moe R (1973) Propagation, growth and flowering of potted roses. Acta Hort 31: 35-51

Okoro O, Grace J (1976) The physiology of rooting *Populus* cuttings. I. Carbohydrates and photosynthesis. Physiol Plant 36: 133-138

Peterson RB (1991) Effects of O_2 and CO_2 concentrations on quantum yields of photosystems I and II in tobaco leaf tissue. Plant Physiol 97: 1388-1394

Sawada S, Usuda H, Tsukui, T (1992) Participation of inorganic orthophosphate in regulation of the ribulose-1,5-bisphosphate carboxylase activity in response to change in the photosynthetic source-sink balance. Plant Cell Physiol 33: 943-949

Sawada S, Arakawa O, Muraki I, Echigo H, Miyashita M, Iwafune M, Kasai M, Usuda H (1999) Photosynthesis with single-rooted *Amaranthus* leaves. I. Changes in the activities of the RuBP-1,5-biphosphate Carboxylase and Phosphoenolpyruvate carboxylase and the amounts of intermediates in photosynthetic metabolism in response to changes in the source-sink balance. Plant Cell Physiol 40: 1143-1151

Smalley TJ, Dirr AM, Armitage AM, Wood BW, Teskey RO, Severson, RF (1991) Photosynthesis and leaf water, carbohydrate andhormonal status during rooting of stem cuttings of *Acer rubrum*. J Am Soc Hort Sci 116: 1052-1057

Snel J, van Kooten O, Van Hove L (1991) Assessment of stress in plants by analysis of photosynthetic performance. Trends Anal Chem 10: 26-30

Svenson SS, Davies FT Jr, Duray SA (1995) Gas exchange, water relations, and dry weight partitioning during root initiation and development of Poinsettia cuttings. J Am Soc Hort Sci 120: 454-459

Sonneveld C, Voogt W (1994) Het berenken van voedingsopplosingen, voor plantenteelt zonder arde. PBG Serie: Voedingsopplosingen glastuinbouw, 10, 21 pp

Van Kooten O, Peppelenbos H (1993) Controlled atmosphere storage of chrysanthemum cuttings. Proc Sixth Int Controlled Atmosphere Res Conference, 2: 610-619

Vanacker H, Harbinson J, Ruijsch J, Carver TLW, Foyer CH (1998) Antioxidant defences of the apoplast. Protoplasma 205: 129-140

Van Staden J, Davey, JE (1979) The synthesis, transport and metabolism of endogenous cytokinins. Plant Cell Environ 2: 93-106

Veierskov, B, Andersen, AS, Eriksen, EN (1982) Dynamics of extractable carbohydrates in *Pisum sativum*. I. Carbohydrate and nitrogen content in pea plants and cuttings grown at two different irradiances. Physiol Plant 55: 167-173

Warner DA, Burke JJ (1993) Cool night temperatures alter leaf starch and photosystem II chlorophyll fluorescence in cotton. Agron J 85: 836-840

CHAPTER 5

DYNAMICS OF ROOTING AND GROWTH OF ROSE CUTTINGS UNDER CONDITIONS OF MANIPULATED PHOTOSYNTHESIS

5.1 EFFECT OF LIGHT INTEGRAL AND CO_2 ON THE DYNAMICS OF ROOTING, DRY WEIGHT AND CARBOHYDRATES OF LEAFY STEM CUTTINGS OF ROSE

Abstract

To investigate the role of leaf photosynthesis in rooting of leafy stem cuttings of rose the effects of light integral and CO_2 concentration on rooting and growth of single node stem cuttings of *Rosa hybrida* Madelon® were quantified and compared. Cuttings were subjected to light integrals varying between 0 and 20MJ m^{-2} and CO_2 concentrations varying between 80-100ppm to 600 ppm and leaf area varying between 0 and 90cm^2. Rooting, dry matter accumulation and partitioning, and concentration of starch and glucose, fructose, sucrose were measured during the first 21 days of propagation. The histology of the rooting process under low and standard CO_2 concentrations was followed during the first 14 days of propagation. Without light rooting was not possible, whereas at low light integrals (2.0 MJ m^{-2}) or low CO_2 concentrations (80-100 ppm) callus and root formation were delayed and reduced. Larger light integrals and higher CO_2 concentrations fastened rooting and increased number and dry weight of roots. The lowest starch concentrations were found at the most basal 7mm of the stem segment likely due to callus and root growth. Cambial activity and proliferation of the parenchyma were strongly diminished under low CO_2, probably due to the very low carbohydrate concentrations at the rooting zone compared to the standard CO_2 treated cuttings. The number, and in particular, the dry weight of roots were significantly related with the overall dry weight accumulation. It is concluded that leaf photosynthesis has a strong regulatory effect on rooting of leafy stem cuttings of roses. Although root initiation (number of roots) was considered less sensitive to photosynthesis than root growth both processes were linearly related with the overall net CO_2 fixation during propagation.

Key words: leaves, total dry weight accumulation, carbohydrates, rooting

Introduction

The influence of leaves on rooting of rose softwood cuttings has been attributed to different factors. However, leaf photosynthesis and the supply of carbohydrates to the rooting zone of cuttings during propagation may be considered one of the most important factors. This hypothesis is based on previous findings relating the cutting leaf area (Howard, 1965; Leakey and Coutts, 1989; Newton et al., 1992; Ofori et al. 1996; Chapter 3.1 of the thesis), light intensity (Howard and Sykes, 1966; Eliasson, 1968; 1978; Hansen et al., 1978; Elliasson and Brunnes, 1980; Veierskov et al., 1982; Harrison-Murray and Howard, 1998), air CO_2 concentration (Davis and Potter, 1983; Grant et al., 1992) or dry weight accumulation (Howard and Harrison-Murray, 1995) with rooting of leafy stem cuttings.

Indeed, carbohydrate metabolism in cuttings (Tschaplinski and Blake, 1989; Dick and Dewar, 1992; Hoad and Leakey, 1996; Pellicer et al., 2000; Druege et al., 2000), and in particular, starch metabolism has been related with rooting (Nanda and Ananda, 1970; Breen and Muraoka, 1974; Haissig, 1974; Doud and Carlson, 1977; Wiesman and Lavee, 1995; Li and Leung, 2000). Moreover, external application of sugars like glucose, fructose and sucrose also improved rooting of cuttings (Howard and Sykes, 1966; Nanda et al., 1971; Eliasson, 1978; Farina and Veruggio, 1979; Haissig, 1986).

Adventitious root formation is a multi-step process, which can be divided, in two main phases: root initiation and root growth (Lovell and White, 1986). These two phases are considered to have distinct requirements either in carbohydrates (Haissig, 1986) or hormones (De Klerk et al., 1999). Root initiation is supposed to be primarily regulated by hormones (e.g. auxin) (Jarvis, 1986; Blaskeley, 1994; Mohen, 1994; Hackett et al., 1998), whereas further growth of roots should depend on carbohydrates as observed in rooted cuttings (Eliasson, 1968; Middleton et al., 1980; Fournioux, 1997), seedlings (Van den Driessche, 1987) and plants in general (Tinus et al., 2000; Muller, 1998; Pritchard and Rogers, 2000),

Rooting of leafy stem cuttings of rose is positively related to the leaf area duration which is approximately proportional to the integral of leaf photosynthesis during propagation (Chapter 3.1). External application of auxin only promoted rooting of rose cuttings when leaves were present and exposed to light suggesting that auxin was not the prime limiting factor (Costa et al., 2001). Moreover, larger carbohydrate

concentration at the rooting zone of rose cuttings was associated with larger number and dry weight of roots after 11 days of propagation (Chapter 3.2).

The aim of the present chapter is to investigate the hypothesis that the effect of leaf size on rooting (initiation and growth) and further growth of rose leafy stem cuttings is essentially through the role of leaves as suppliers of photosynthates. Following partly the approach of Davis and Potter (1981) to study the effect of photosynthates on rooting of leafy pea cuttings, we subjected rose cuttings to different light integrals, air CO_2 concentrations and different leaf area (LA) in order to observe to what extent the effects of variations of photosynthesis brought about by these three parameters (light, CO_2, LA) on rooting and growth of cuttings are comparable during the first 21 days of propagation.

Material and methods

Plant material grown in greenhouse and in climate chamber

Growth of mother plants of *Rosa hybrida* Madelon® and the procedure of taking the cuttings and the rooting conditions were the same as described in chapter 3.1. No auxin was applied to the cuttings.

CO_2 supplying system

Pure CO_2 was mixed with outside air in a mixer. The mixer was calibrated to mix outside air and pure CO_2 till a concentration of 600-650 ppm (Experiment 4) or 300-350 ppm (Experiment 5) is achieved. The low CO_2 air concentration in Experiment 5 was obtained by using a CO_2 scrubber with soda lime pellets with an indicator (Merck, Germany). The air was supplied to the propagators by 2 flexible plastic tubes (Rauclair), with 7-mm diameter and 1.5 mm thick. Propagators were sealed with aluminium tape to minimise mix with the outside air. The system worked continuously.

Monitoring CO_2, photosynthetic active radiation and leaf area

CO_2 concentration inside propagators was monitored on days 2, 6, 14 and 21 for Experiment 4 and daily in Experiment 5. Samples from the air inside the propagators were collected using a 3-mL plastic syringe through two septa on the top of the propagators. At least two samples were collected per propagator and were analyzed by injecting 1 mL of air into a gas chromatograph Micro GC CP 2002 (Chrompack, the Netherlands) equipped with a column module Haye Sep A. Radiation was measured with a energy-response PAR-sensor (TFDL, Wageningen, the Netherlands). Leaf area was determined with a LI-3100 Area meter (Li-Cor Inc., Lincoln, NE, USA).

Experiments

Effect of the light integral (LI) and leaf area (LA) on rooting and growth of rose cuttings (Experiments 1, 2, 3)

Cuttings of Experiment 1 were harvested on 9 September 1997 and propagated in a greenhouse. Total LI (natural plus the supplementary light) was 27.2 MJ m^{-2} for the 14 days of propagation. The natural light at cuttings level was calculated, considering PAR $\approx$ 45% of global radiation, a greenhouse light transmission of 60% and a light transmission of the plastic cover of the rooting benches of 50%. The supplementary light was provided by high-pressure sodium lamps (Phillips SON/T plus 70 W) extending the photoperiod to 18h and providing a minimum radiation level of about 3.5 Wm^{-2} (PAR) for at least 5 hours. Cuttings were propagated under 4 LIs: (1) 27.2 MJm^{-2} (control, 100% LI) , (2) 10.2 MJm^{-2} (35-40% LI), (3) 4.1 MJm^{-2} (15% LI) and (4) 0 MJm^{-2} (0%, covered leaf). Variation in LIs was created using different shadowing nets or covering the leaf with aluminium foil (treatment 4).

Cuttings from Experiment 2 were harvested on 26 February 1998 and were propagated in a greenhouse. LI (including supplementary light) was 20.2 MJm^{-2} over 21 days. High-pressure sodium lamps (SON-T 150W) supplemented natural light with about 7 Wm^{-2} (PAR) at cuttings level for at least 8 hours. There were seven treatments: 1) cuttings with a LA of 64 cm^2 and LI of 20.2 MJm^{-2} (control); (2) 27 cm^2 (40% LA) and 20.2 MJm^{-2}; (3) 15cm^2 (20% LA) and 20.2 MJm^{-2}; (4) leafless

cutting (0% LA) and 20.2 MJm^{-2}; (5) 73 cm^2 and 6.05 MJm^{-2} (30% LI); (6) 67cm^2 and 2.02 MJm^{-2} (10% LI); (7) 62 cm^2 and 0 MJm^{-2} (covered leaf, 0% LI).

In Experiment 3, cuttings were rooted in climate chambers and subjected to two LIs: 1) 20.1 MJ m^2 obtained under a photosynthetic active radiation of 16.6 Wm^{-2} and 2) 3.4 MJ m^2 obtained under 2.8 Wm^{-2} (PAR). The photoperiod was 16h.

Data on the effects of leaf area on rooting and growth of cuttings described in the Chapter 3.1 is used to complement that described in the Experiments 1, 2 and 3 and will be used in further regression analysis.

Effect of air CO_2 concentration on rooting and growth of rose cuttings (Experiments 4 and 5)

Cuttings were propagated under a standard air CO_2 concentration of 300-350ppm and compared to cuttings propagated under high (600-700ppm) (Experiment 4) or low (80-100 ppm) (Experiment 5) CO_2 concentration. Observations were made on day 0, 3, 7, 11, 14 and 21 of propagation. The total light integral was 20.6 MJ m^{-2}.

Measurements: The percentage of cuttings with visible callus and roots were determined. Number and dry weight of roots, dry weight of total stem, of the stem rooting zone (most basal 15 mm), of the original leaf, and of the axillary bud were determined. Callus growth at the base of the stem segment was scored in Experiment 5 according to the following scale regarding the amount and the distribution of the callus tissue: 0- no callus; 1-small amount of irregular callus; 2–medium amount of callus; 3- large amount of regular callus).

Carbohydrate analysis: Glucose, fructose, sucrose and starch concentrations from the rooting zone of the stem, the remaining upper stem part, the leaf, roots and the axillary bud were determined during propagation (on day 21 in Experiment 2 and on days 0, 3, 7, 11, 14 and 21 in Experiment 5). Cuttings were harvested about 6 (Experiment 2) to 9 hours (Experiment 5) after the beginning of the photoperiod and the carbohydrates were extracted and analyzed according to the method described in Chapter 3.2. Four cuttings were used in Experiment 2, whereas Experiment 5 used 6 cuttings per treatment and sampling date. In Experiment 2 the stem was longitudinally divided in two halves, one from the side where the leaf was inserted and the other from the opposite side. In Experiment 5, the stem was not sectioned longitudinally but the rooting zone was divided in two shorter sections of about 7 mm each.

Histological analysis: The histology of the rooting process in cuttings subjected to different CO_2 concentrations (standard and low CO_2) was studied by using fresh sections from the most basal 7 mm of the stem segment. Sections were collected on days 3, 7, 11 and 14 of propagation and placed in a fixative, formalin-acetic acid-ethanol (FAA)(5:5:90). Cross sections from the most basal 2.5-3mm were cut at 40-50μm using a slide microtome. Sections were mounted on object glasses and covered with glycerine-gelatine and a coverslip. Observations and photographs were made as described in Chapter 4.1.

Experimental design and statistical analysis

In the majority of the experiments a randomized complete block design was used with 2 blocks in Experiments 3 and 5, 4 blocks in Experiments 1 and 2, 6 blocks in Experiment 4. Each block had 2 plots (Experiments 3 and 4), 4 plots (Experiment 1), 6 plots (Experiment 5) and 7 plots (Experiment 2). One cutting per plot was used in Experiments 4 and 5, whereas Experiments 1 and 2 used 4 cuttings and Experiment 3 used 5 cuttings. The percentage of cuttings with visible callus or rooted were analysed by binomial regression ($P<0.05$) using a logit link function. For means separation, confidence intervals were calculated by multiplying the standard errors of the predicted means by the *t* value at 5% level and considering the degrees of freedom of the residuals. Means and the standard error of the number and the dry weight of roots and buds were determined. The other figures (dry weight and carbohydrates concentration of the different parts of cuttings) were analysed by one-way ANOVA ($P<0.05$) calculated for each day of observation, except for day 0. When no significant block effect was observed data were reanalysed as a complete randomised block design in order to gain one degrees of freedom for the residuals. Least significant differences (LSD) were calculated by the *t*-test. The Wilcoxon non-parametric test (single tailed) ($P<0.001$) was used to analyse the differences between callus development at different CO_2 concentration. The relation between the total dry weight accumulation as percentage of the control and the number and dry weight of roots as percentage of the control was analysed by linear regression. The statistical analysis was performed by using the statistical packages GENSTAT 5 (IACR, Rothamsted, UK) and SPSS (SPSS Inc., Chicago, USA).

Results

Cuttings without the original leaf or deprived of light (0% LI or 0%LA) did not form roots (Table 5.1.1). Although not always significant, the number and dry weight of roots showed the tendency to be lower at lower LI and low LA (Table 5.1.1). Cuttings responded responded to increased LI and LA by forming heavier root system (larger dry weight) (Table 5.1.1).

Table 5.1.1 Effect of light integral and leaf area on callus and root formation and dry weight of the different parts of leafy stem cuttings of *Rosa hybrida* Madelon®.

Treatment (light integral)	Callus (%)	Rooted cuttings (%)	Root num.	Root DW (mg)	Bud DW (mg)	Total DW (mg)
Experiment 1 (14 days duration)						(471)[w]
27.2 MJ m^{-2}(100%)	100a	88a	5.7	6.3	-[z]	629
10.2 MJ m^{-2}(35-40%)	100a	94a	4.2	6.0	-	512
4.08 MJ m^{-2} (10-15%)	100a	12b	0.5	0.3	-	473
0 MJ m^{-2} (0%, covered leaf)	25b	0b	0	0	-	445
LSD			1.8	2.9	-	82
Experiment 2 (21 days duration)						(557)[w](402)[x](338)[y]
64cm^2 /20.2MJ m^{-2} (Control)	94a	75ab	3.8	30	2	1063
73cm^2/ 6.05MJ m^{-2}	100a	88a	3.7	36	8	786
67cm^2/ 2.02MJ m^{-2}	100a	63b	2.0	8	0	553
62cm^2/ 0MJ m^{-2} (leaf covered)	50b	0c	0	0	0	471
27 cm^2 / 20.2MJ m^{-2}	94a	75ab	3.2	20	1	653
15cm^2/ 20.2 MJ m^{-2}	100a	75ab	2.8	16	3	555
0cm^2/ 20.2 MJ m^{-2} (leafless)	6c	0c	0	0	18	199
LSD			2.1	19	11	170
Experiment 3 (21 days duration)						(594)[w]
20.1MJ m^{-2}		100	8.3	38	19	980
0.34 MJ m^{-2}		100	4.3	13	0	707
LSD			0.9	24	6.8	268
Data Chapter 3.1 (21 days duration)						(581)[w]
93 cm^2		100	10.3(1.0)[z]	42(6.0)[z]	32 (8.7)[z]	1119 (38)[z]
61 cm^2		100	8.5(1.2)[z]	29(5.1)[z]	27 (1.4)[z]	1034 (30)[z]
22 cm^2		90	4.6(0.7)[z]	7 (1.2)[z]	30 (9.9)[z]	648 (39)[z]
0 cm^2		0	-	-	-	560 (44)[z]

[w] *mean total dry weight on day 0 considering the original leaf intact*
[x] mean total dry weight on day 0 considering 65% leaf area reduction
[y] mean total dry weight on day 0 considering 85-90% leaf area reduction
[z] standard error of the mean (n=11)

Very low CO_2 (80-100 ppm) also resulted in less number and dry weight of roots compared to cuttings propagated at 350 ppm (Fig. 5.1.1).

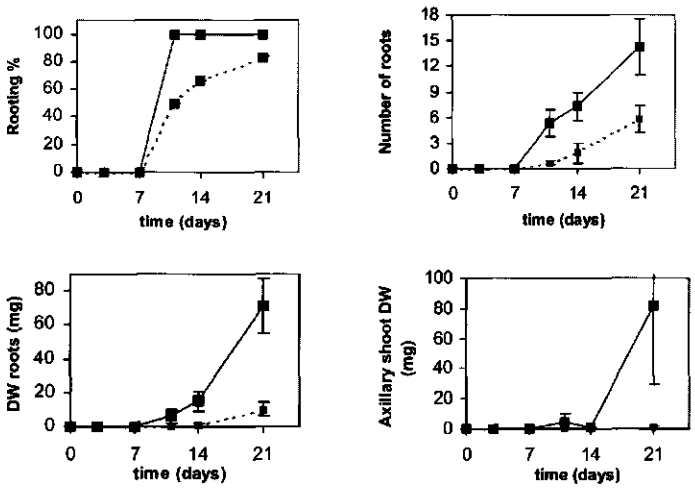

Figure 5.1.1 Rooting percentage, number and dry weight of roots and dry weight of the axillary bud from single node leafy stem cuttings of *Rosa hybrida* Madelon® measured on days 0, 3,7,11,14 and 21 of propagation and propagated at a CO_2 concentration of 300-350 ppm (—■—) or 80-100ppm (----■----) in Experiment 5. Vertical bars indicate the SE (n=6).

Callus formation and dry weight accumulation were also improved at higher CO_2 concentration (Tables 5.1.2 and 5.1.3). However, at CO_2 concentration of 600-700 ppm only the dry weight of roots was significantly higher than at 300-350ppm (Table 5.1.4). Plotting the number and dry weight of roots after 21 days as percentage of the control against the total dry weight accumulation as percentage of the control showed that the dry weight (Fig. 5.1.2a) and the number (Fig. 5.1.2b) of roots are positively and significantly correlated with the total dry weight accumulation. No significant relation between dry weight accumulation and axillary bud growth was found (data not presented).

Table 5.1.2 Effect of CO_2 concentration on callus development, for leafy stem cuttings of *Rosa hybrida* Madelon® during 21 days of propagation in Experiment 5. Data was recorded according to a qualitative scale: 0-no callus; 1-small amount of irregular callus; 2–medium amount of regular callus; 3- large amount of regular callus. Letters within the same column indicate significant differences calculated by the Wilcoxcon non-parametric test (single tailed) (n=6)

CO_2 air concentration						
	Day 0	Day 3	Day 7	Day 11	Day 14	Day 21
			Callus development			
300-350ppm	-	0	1.3a	3.0a	3.0a	3.0a
80-100 ppm	-	0	0.7b	2.0b	2.0b	1.9b

Table 5.1.3 Effect of CO_2 concentration on total dry weight and on dry weight of the stem and the leaf of leafy stem cuttings of *Rosa hybrida* Madelon® during the first 21 days of propagation in Experiment 5.

CO_2 air concentration	Day 0	Day 3	Day 7	Day 11	Day 14	Day 21
				Total DW (mg)		
300-350ppm	(579)[y]	563a	647a	680a	802a	1068a
80-100 ppm		523a	573a	498b	581b	460b
LSD		168	170	142	136	267
				Basal stem DW (mg)		
300-350ppm	(70)[y]	60a	84a	89a	104a	138a
80-100 ppm		58a	72a	64b	70b	56b
LSD		19	26	16	14	34
				Stem DW (mg)		
300-350 ppm	(201)[y]	224a	317a	334a	391a	444a
80-100 ppm		243a	279a	242b	288b	212b
LSD		95	89	69	58	122
				Leaf DW (mg)		
300-350ppm	(309)[y]	320a	330a	335a	395a	470a
80-100ppm		299a	294a	256a	293b	238a
LSD		83	102	87	102	182

[y] means are the average of a sample of 8 cuttings

Table 5.1.4 Effect of CO_2 enrichment on dry weight of roots of leafy stem cuttings of *Rosa hybrida* Madelon® during the first 21 days of propagation in Experiment 4.

CO_2 air concentration	Day 0	Day 3	Day 7	Day 10	Day 14	Day 21
				Dry weight of roots (mg)		
300-350ppm	-	0	0	0.2a	14.9a	62.2a
600-700 ppm	-	0	0	1.3a	24.1a	90.0b
LSD		0	0	ns	ns	7.3

Concentration of carbohydrates in cuttings, in particular that of starch, decreased in response to lower LI, LA or low CO_2. This decrease was always more pronounced in leaves (Table 5.1.5 and Fig. 5.1.3). The concentrations of glucose, fructose or sucrose were less affected than starch by changes in the LI and air CO_2 concentration and only decreased significantly in the stem tissues when there was no source activity (Table 5.1.6 and Fig. 5.1.3).

Table 5.1.5 Concentration (mg/gDW) of the total carbohydrates (glucose, fructose, sucrose and starch) in the different parts of leafy stem cuttings of *Rosa hybrida* Madelon® measured 21 days after severance for different treatments in Experiment 2.

Treatments	Total carbohydrates				
	Leaf	Upper stem leaf side	Low 1.5cm leaf side	Upper stem opposite leaf	Low 1.5cm opposite leaf
64cm^2 /20.2 MJ m^{-2} (Control)	394	256	205	249	173
73cm^2/ 6.05 MJ m^{-2}	118	167	132	156	117
67cm^2/ 2.02 MJ m^{-2}	79	54	50	56	50
62cm^2/ 0 MJm^{-2} (leaf covered)	19	32	69	24	13
27 cm^2 / 20.2MJ m^{-2}	198	147	137	153	134
15cm^2/ 20.2 MJm^{-2}	194	126	110	95	117
0cm^2/ 20.2 MJ m^{-2} (leafless)	-	36	38	13	13
LSD	56	46	41	50	51
	Starch				
	Leaf	Upper stem leaf side	Low 1.5cm leaf side	Upper stem opposite leaf	Low 1.5cm opposite leaf
64cm^2 /20.2 MJ m^{-2} (Control)	285	214	158	210	122
73cm^2/ 6.05 MJ m^{-2}	24	137	102	125	81
67cm^2/ 2.02 MJ m^{-2}	5	32	24	33	23
62cm^2/ 0 MJm^{-2} (leaf covered)	14	17	54	10	4
27 cm^2 / 20.2 MJ m^{-2}	98	103	95	116	99
15cm^2/ 20.2 MJm^{-2}	82	95	77	69	80
0cm^2/ 20.2 MJm^{-2} (leafless)	-	10	29	5	10
LSD	44	38	36	45	54

Considering the concentrations measured on day 0 of propagation in a previous experiment (Chapter 4.3) for the stem (about 6mg g^{-1}DW) and the original leaf (26 mg g^{-1}DW), cuttings accumulated starch under conditions of reduced LI or LA (Table 5.1.5). Contrary to the stem, leaves did not accumulate starch under reduced LI (Table 5.1.5). The roots had high concentrations of soluble sugars compared to the other parts of the cutting (data not presented).

Table 5.1.6 Concentration (mg/gDW) of the soluble sugars (glucose, fructose and sucrose) in the different parts of leafy stem cuttings of *Rosa hybrida* Madelon® measured 21 days after severance in Experiment 2.

Treatments	Leaf	Upper stem leaf side	Low 1.5cm leaf side	Upper stem opposite leaf	Low 1.5cm opposite leaf
			Glucose		
$64cm^2$ /20.2 MJm^{-2} (Control)	8.2	1.6	3.0	2.4	4.1
$73cm^2$/ 6.05 MJm^{-2}	8.1	0.9	1.6	0.8	8.2
$67cm^2$/ 2.02 MJm^{-2}	6.6	1.1	1.2	1.1	1.4
$62cm^2$/ 0 MJm^{-2} (leaf covered)	0.4	0.6	2.8	0.8	0.4
27 cm^2 / 20.2 MJm^{-2}	9.2	2.5	2.6	1.7	2.1
$15cm^2$/ 20.2 MJm^{-2}	10.8	1.7	4.5	0.8	4.2
$0cm^2$/ 20.2 MJm^{-2} (leafless)	-	5.3	0.6	0.8	0.3
LSD	4.9	3.0	2.6	1.2	7.7
			Fructose		
$64cm^2$ /20.2 MJ m^{-2} (Control)	7.6	1.9	2.1	2.0	2.8
$73cm^2$/ 6.05 MJ m^{-2}	10.0	1.2	1.7	0.8	2.4
$67cm^2$/ 2.02 MJ m^{-2}	8.1	1.7	1.8	0.5	2.3
$62cm^2$/ 0 MJm^{-2} (leaf covered)	0.2	0.5	1.2	1.1	0.7
27 cm^2 / 20.2 MJm^{-2}	11.7	3.4	3.3	2.0	2.5
$15cm^2$/ 20.2 MJm^{-2}	13.4	2.4	3.4	1.3	2.8
$0cm^2$/ 20.2 MJm^{-2} (leafless)	-	4.5	0.6	1.0	0.2
LSD	5.1	1.9	1.4	1.1	1.6
			Sucrose		
$64cm^2$ /20.2MJ m^{-2} (Control)	92.9	38.7	41.7	35.3	43.5
$73cm^2$/ 6.05MJ m^{-2}	75.5	27.3	27.1	29.2	25.2
$67cm^2$/ 2.02MJ m^{-2}	58.6	19.4	23.1	21.1	23.1
$62cm^2$/ 0MJ m^{-2} (leaf covered)	4.6	14.4	10.9	11.8	8.2
27 cm^2 / 20.2MJ m^{-2}	79.5	37.5	35.8	33.0	35.9
$15cm^2$/ 20.2MJm^{-2}	87.4	19.4	24.9	24.4	30.4
$0cm^2$/ 20.2MJ m^{-2} (leafless)	-	16.4	8.3	6.5	2.7
LSD	25.4	10.7	9.2	8.0	9.4

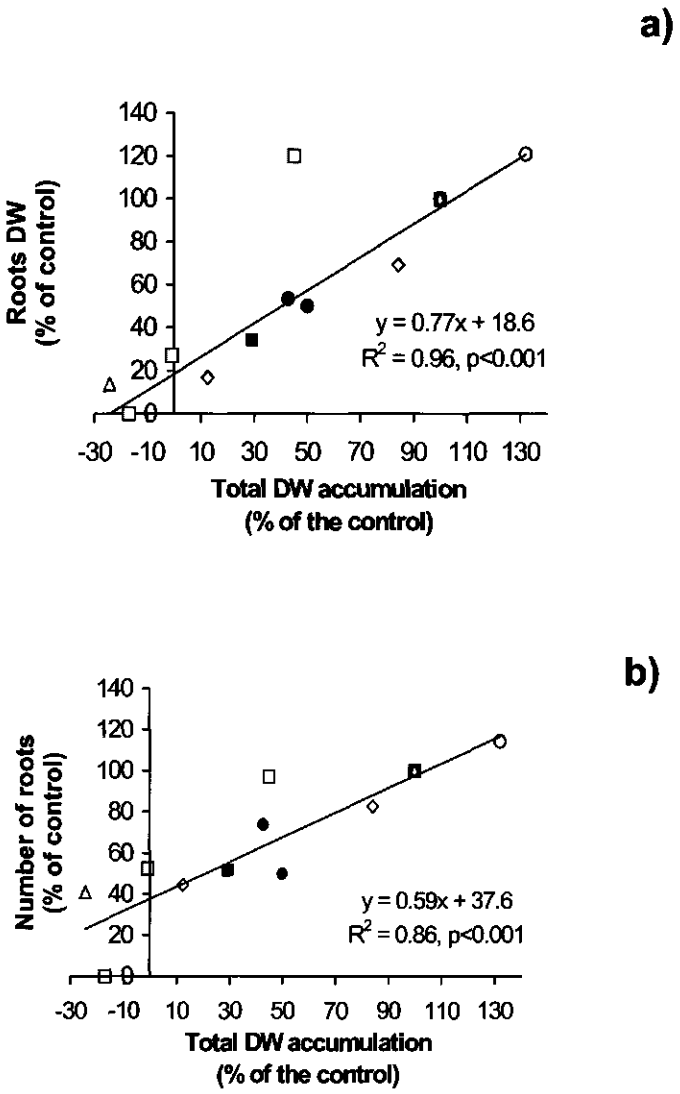

Figure 5.1.2 Relationship between the total dry weight accumulation (Final Total DW - Initial Total DW) expressed as percentage of the control for Experiment 2 (leaf area effect) (•), Experiment 2 (light integral effect) ( ), Experiment 3 (light integral effect) (■); Experiment 4 (high CO_2 effect) (O), Experiment 5 (low CO_2 effect) (Δ); data from Chapter 3.1 (leaf area effect) (◊), and the dry weight **(a)** and number **(b)** of roots also as percentage of the control measured for leafy stem cuttings of *Rosa hybrida* Madelon® after 21 days of propagation. The out-lier from Experiment 2, was not included in the regression analysis.

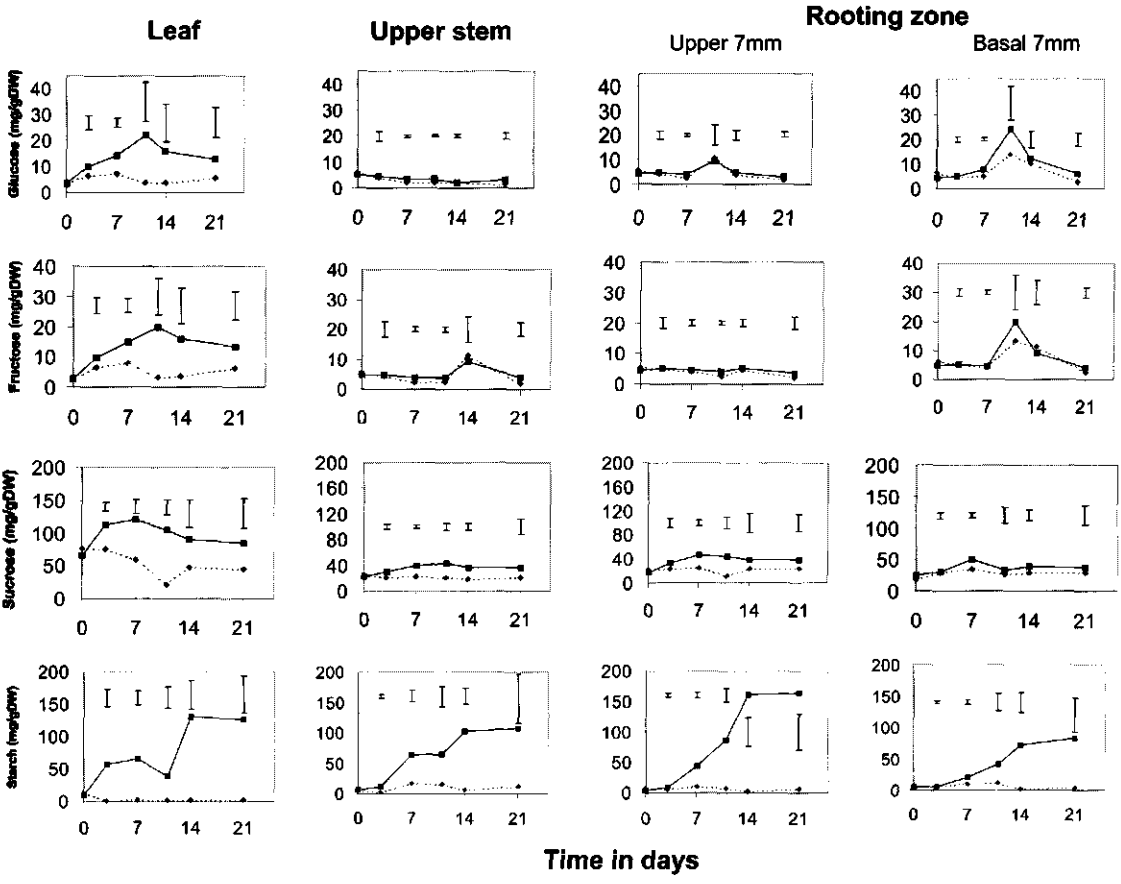

Figure 5.1.3 Concentrations (mg/g DW) of glucose, fructose, sucrose, and starch on day 0, 3, 7, 11, 14 and 21 of propagation from the leaf, the upper part of the stem (upper stem), the upper 7 mm of the rooting zone (upper 7 mm) and the basal 7mm of the rooting zone (basal 7mm) for cuttings of *Rosa hybrida* Madelon® propagated under an air CO_2 concentration of 300-350 ppm (control) (—) and at 80-100 ppm (----) in Experiment 5. Vertical bars indicate the LSD values at 5% level of confidence.

Time course of the histological events occurring in cuttings propagated under ambient and low CO_2 air concentrations

On day 3, cambial activity was already visible at the rooting zone of control cuttings through proliferation of parenchyma tissue between the cambial zone and the secondary phloem or the sclerenchyma (Fig. 5.1.4a) whereas cuttings under low CO_2 showed no cambial activity (Fig. 5.1.4b). Observations on day 7 showed more pronounced anatomical differences between control and low CO_2 treated cuttings due to proliferation of parenchyma tissue in control cuttings and increased thickness of the cortex (Fig. 5.1.4c). On day 7 it was also possible to distinguish groups of meristematic cells within the parenchyma tissue as well differentiated dome-shaped root primordia pushing through the sclerenchyma fiber ring or root primordia already elongating through the cortex, almost emerging (Fig. 5.1.4c). By day 7, cuttings propagated under low CO_2 kept a limited cambial activity, and consequently, reduced proliferation of parenchyma tissue (Fig. 5.1.4d). On day 11, the initial anatomical structure of the stem of control was distorted by proliferation of new phloem parenchyma and phloem tissue as well by the expansion of roots and adjacent tissues and external callus (Table 5.1.2 and Fig. 5.1.4e) in opposite to low CO_2 treated cuttings which kept their initial anatomical structure rather intact (Fig. 5.1.4f). By day 14, cortex of controls had enlarged, the new-formed vessels had larger diameters and the medullar rays were also differentiating new cells and elongating (Fig. 5.1.4g). Cuttings under low CO_2 presented a moderated proliferation of the parenchyma tissue and a less distorted structure, with root primordia (Fig. 5.1.4h). These results suggest that root initiation is not inhibited, but simply delayed by low CO_2. Changes in the anatomical structure of control cuttings were visible within the most basal 2-2.5mm of the stem whereas low CO_2 treated cuttings had them confined to the most basal 1.5mm of the stem segment.

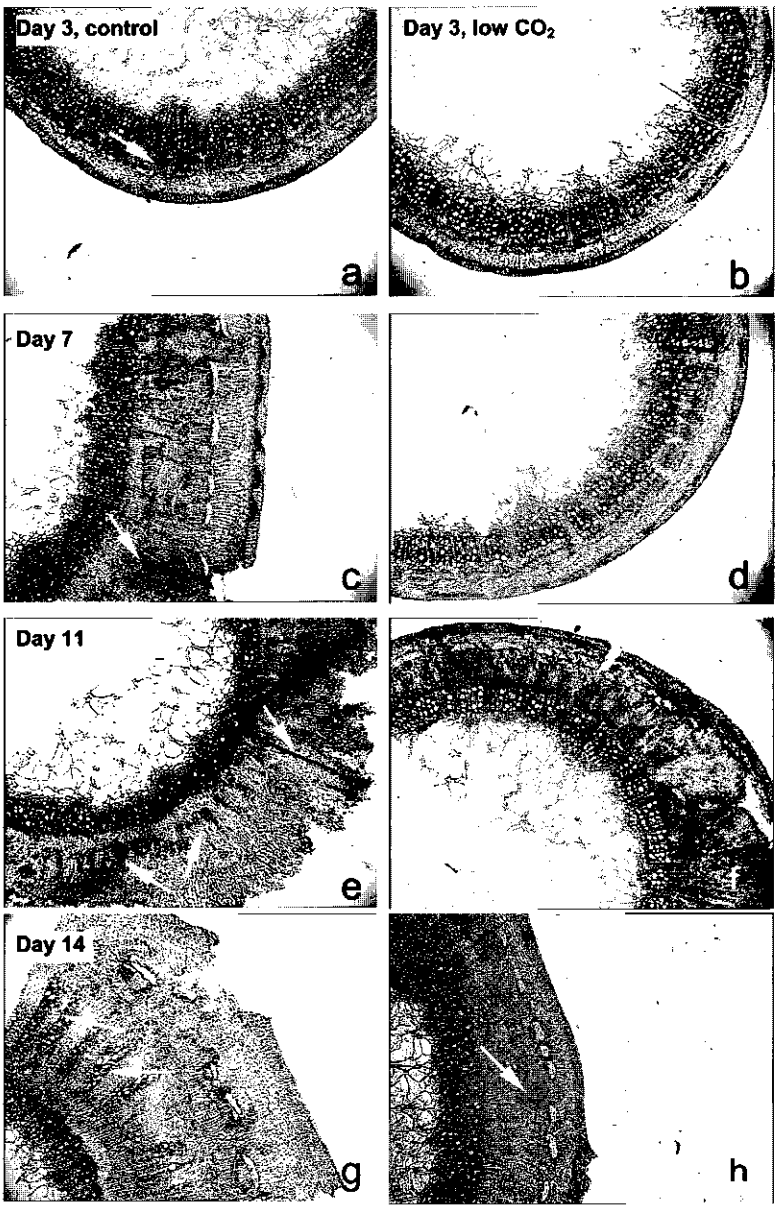

Figure 5.1.4 Cross sections (50µm) (30X) from the most basal 7mm of the stem segment of leafy stem cuttings of *Rosa hybrida* Madelon® on days 3, 7, 11, 14 of propagation for cuttings propagated under ambient (control) **(a, c, e, g)** or low CO_2 (80-100ppm) **(b, d, f, h)**. **(a)** Cross section from control from day 3 showing cambial activity and initial proliferation of phloem parenchyma (arrow); **(b)** Cross section from low CO_2 treated cuttings on day 3 with none or reduced cambial activity (arrow); **(c)** Meristematic centers within the new phloem/xylem parenchyma tissue (pa) and a root primordia almost emerging (arrow) on day 7; **(d)** Cross section from low CO_2 treated cuttings on day 7 showing reduced cambial activity; **(e)** Meristematic centers (vessels) on day 11 within the new parenchyma tissue; **(f)** Cross section from a low CO_2 treated cutting on day 11with a moderate proliferation of parenchyma tissue; **(g)** Enlarged xylem vessels (arrows) and medullar rays (mr) elongating on day 14. **(h)** Cross section from low CO_2 treated cuttings with a moderate proliferation of parenchyma tissue and a root primordia (arrow) on day 14.

Discussion

The present results are in line with previous ones showing that single node leafy stem cuttings of rose need the original leaf to be present and exposed to light in order to form roots (Chapters 4.1 and 4.2). Our results demonstrate that the overall leaf photosynthesis, independently of the cause of variation, CO_2 or light, is directly influencing rooting and root growth (Fig. 5.1.2). In fact, changes in the dry weight of roots (root growth) were almost proportional to changes on the overall net photosynthesis expressed as an increase in dry weigh of the total cutting. This agrees with previous findings showing that root growth depends on the supply of current photosynthates (Middelton et al., 1980; Eliasson, 1968; Eliasson and Brunnes, 1980; Van den Driessche, 1987; Muller, 1998; Tinus et al., 2000). The number of roots, which can be considered the macroscopical expression of root initiation, was also linearly related with the total dry weight accumulation although roots were even formed when there was no net increase in dry weight (Fig. 5.1.2b).

This is in accordance with previous findings showing that roots can be formed under low light levels (Howard, 1965) or even under situations of dry weight loss as reported for *Syringa* cuttings (Howard and Harrison-Murray, 1995).

Carbohydrates might have been limiting or strongly delaying initiation when leaf photosynthesis was totally suppressed either by leaf covering or removal or strongly reduced by subjecting cuttings to a 90% reduction of the LI or decreasing CO_2 concentrations to 80-100ppm.

Reduced photosynthesis and decreased carbohydrates concentrations at the rooting zone (Fig. 5.1.3) might have contributed for the reduced cambial activity and proliferation of phloem parenchyma during the first 11 days of propagation and consequently further root initiation (Fig. 5.1.4). In fact, the basic process of callus formation is cell division which depends on the available carbohydrate (Muller et al., 1998) and cambial activity (Kramer, 1964) and callus growth in cuttings or graftings (Shippy, 1929; Waard and Zambin, 1983) have been shown to require adequate carbon supply.

We conclude that rooting of rose leafy stem cuttings correlates with the integral of the photosynthetic capacity of cuttings during propagation rather than with dry weigth accumulation or carbohydrates concentration at the rooting zone. To clarify the roles of photosynthesis and carbohydrates on the rooting of rose leafy stem

cuttings further research should quantify their effects on each phase of the rooting process (root initiation and root growth).

References

Bertram L, Veierskov B (1989) A relationship between irradiation, carbohydrates and rooting in cuttings of *Pisum sativum*. Physiol Plant 76: 81-85

Bingham IJ, Blackwood JM, Stevenson EA (1997) Site, scale and time-course for adjustments in lateral root initiation in wheat following changes in C and N supply. Ann Bot 80: 97-106

Blakesley D (1994) Auxin metabolism and adventitious root initiation, In: Davis TD, Haissig BE (eds) Biology of adventitious root formation. Basic Life Sciences,Vol. 62, Plenum Press, New York, pp 143-154

Breen, PJ, Muraoka T (1974) Effect of leaves on carbohydrate content and movement of ^{14}C-assimilate in plum cuttings. J Am Soc Hort Sci 99(4): 326-332

Costa JM, Challa H, Van Meeteren U, Van de Pol PA (2001) Photosynthates: mainly stored and yet limiting in propagation of rose cuttings. Acta Hort 547: 167-174

Costa JM, Challa H (2002) The effect of the original leaf area on growth of softwood cuttings and planting material of rose. Sci Hort 95: 111-121.

Davis TD, Potter JR (1981) Current photosynthate as a limiting factor adventitious root formation on leafy pea cuttings. J Am Soc Hort Sci 106: 278-282

Davis TD, Potter JR (1983) High CO_2 applied to cuttings: effects on rooting and subsequent growth in ornamental species. HortScience 18: 194-196

De Klerk G, Van der Krieken W, De Jong J (1999) Review the formation of adventitious roots: new concepts and possibilities. In Vitro Cell Dev Pl 35: 189-1999

Dick, J McP, Dewar RC (1992) A mechanistic model of carbohydrates dynamics during adventitious root development in leafy cuttings. Ann Bot 70: 371-377

Doud S, Carlson R (1977) Effects of etiolation, stem anatomy and starch reserves on root initiation of layered *Mallus* Clones. J Am Soc Hort Sci 102: 487-491

Druege U, Zerche S, Kadner RD, Ernst M (2000) Relation between nitrogen status, carbohydrate distribution and subsequent rooting of Chrysanthemum cuttings as affected by pre-harvest nitrogen supply and cold-storage. Ann Bot 85: 687-701

Eliasson L (1968) Dependence of root growth on photosynthesis in *Populus tremula*. Physiol Plant 21: 806-810

Eliasson L (1978) Effects of nutrients and light on growth and root formation in *Pisum sativum* cuttings. Physiol Plant 43: 13-18

Eliasson L, Brunnes, L (1980) Light effects on root formation in aspen and willow cuttings. Physiol Plant 48: 261-265

Farina E, Veruggio (1979) Livello dei carboidrati e potenziale rizogeno di talee di rosa (*Rosa hybrida*). Effetto di trattamenti glucosidici. Annalle Instituto Sperimentale Floricoltuora 15: 47-66

Ford YY, Bonham EC, Cameron RWF, Blake PS, Judd HL, Harrison-Murray, RS (2001) Adventitious rooting: examining the role of auxin in an easy and a difficult-to-root plant. Plant Growth Reg 00: 1-11

Fournioux JC (1997) Adult leaves of grapevine cuttings stimulate rhizogenesis. Vitis 36: 49-50 Research note

Grant WJR, Fan HM, Downton WJS, Loveys BR (1992) Effects of CO_2 enrichment on the physiology and propagation of two australian ornamental plants, *Chamelaucium unicanatum* (Schauer) x *Chamelaucium floriferum* (MS) and *Correa schlechtendalli* (Beher). Sci Hort 52: 337-342

Gregory, FG, Samantarai B (1950) Factors concerned in the rooting responses of isolated leaves. J Exp Bot 1: 159-193

Hansen J, Strömquist L, Ericsson A (1978) Influence of the irradiance on carbohydrate and rooting of pine seedlings (*Pinus sylvestris* L.). Plant Physiol 61: 975-979

Harrison-Murray RS, Howard BH (1998) Environmental requirements as determined by rooting potential in leafy cuttings. In: KE Cockshull, D Gray, GB Seymour, B Thomas (eds). Genetical and environmental manipulation of horticultural crops. CAB International 1998, pp 75-94

Haissig BE (1974) Metabolism during adventitious root primordium initiation and development. New Zeal J For Sci 4: 324-337

Haissig BE (1986) Metabolic processes in the adventitious rooting of cuttings. In: MB Jackson (ed) New root formation in plants and cuttings. Long Ashton Research Station University of Bristol, UK, pp 141-190

Haissig BE (1989) Removal of the stem terminal and application of auxin change carbohydrates in Pinus banksiana cuttings during propagation. Physiol Plant 77: 179-184

Hoad SP Leakey RRB (1996) Effects of pre-severance light quality on the vegetative propagation of *Eucalyptus grandis* W. Hill ex. Maiden. Cutting morphology, gas exchange and carbohydrate status during rooting. Trees 10: 317-324

Howard BH (1965) Regeneration of the hop plant (*Humulus lupulus* L.) from softwood cuttings. I. The cutting and its rooting environment. J Hort Sci 40: 181-191

Howard BH, Sykes JT (1966) Regeneration of the hop plant (*Humulus lupulus* L.) from softwood cuttings. II. Modification of the carbohydrate resources within the cutting. J Hort Sci 41: 155-163

Howard BH, Harrison-Murray RS (1995) Responses of dark-pre conditioned and normal light grown cuttings of *Syringa vulgaris* 'Madame Lemoine' to light and wetness gradients in the propagation environment. J Hort Sci 70: 989-1001

Jarvis BC (1986) Endogenous control of adventitious rooting in non-woody cuttings. In MB Jackson (ed), New root formation in plants and cuttings. Long Ashton Research Station University of Bristol, UK, pp 191-222

Kramer P.J. (1964) The role of water in wood formation. In: Zimmermann MH (ed) The formation of wood in forest trees. Academic Press, NY

Leakey RRB, Coutts MP (1989) The dynamics of rooting *Triplochiton scleroxylon* cuttings: their relation with leaf area, node position, dry weight accumulation, leaf water potential and carbohydrate composition. Tree Physiol 5: 135-146

Li M, Leung DWM (2000) Starch accumulation is associated with adventitious root formation in hypocotyl cuttings of *Pinus radiata*. J Plant Growth Reg 19: 423-428

Lovell PH, Illsley A, Moore KG, Moore (1972) The effect of light intensity and sucrose on root formation, photosynthetic ability, and senescence in detached cotyledons of *Sinapsis alba* L. and *Raphanus sativa* L. Ann Bot 36: 123-134

Lovell PH, White J (1986) Anatomical changes during adventitious root formation. In MB Jackson (ed), New root formation in plants and cuttings. Long Ashton Research Station University of Bristol, UK, pp 141-190

Middleton W, Jarvis BC, Booth A (1980) The role of leaves in auxin and boron-dependent rooting of stem cuttings of *Phaseolus aureaus* Roxb. New Phytol 84: 251-259

Muller B, Stosser M, Tardieu F (1998) Spatial distributions of tissue expansion and cell division rates are related to irradiance and to sugar content in the growing zone of maize roots. Plant Cell Environ 21: 149-158

Nanda KK Anand VK (1970) Seasonal changes in auxin effects on rooting of stem cuttings of *Populus nigra* and its relationship with mobilization of starch. Physiol Plant 23: 99-107

Nanda KK, Jain MK, Malhotra S (1971) Effect of glucose and auxins in rooting etiolated stem segments of *Populus nigra*. Physiol Plant 24: 387-391

Newton, AC, Muthoka PN, Dick J McP (1992) The influence of leaf area on the rooting physiology of leafy stem cuttings of *Terminalia spinosa Eng.* Trees 6: 210-215

Ofori DA, Newton AC, Leakey RRB, Grace J (1996) Vegetative propagation of *Milicia excelsa* by leafy stem cuttings: effects of auxin concentration, leaf area and rooting medium. Forest Ecol Manag 84: 39-48

Pellicer V, Guehl, JM, Daudet, FA, Cazet M, Riviere LM, Maillard P (2000) Carbon and nitrogen mobilization in *Larix x eurolepis* leafy stem cuttings assessed by dual ^{13}C and ^{15}N labeling: relationships with rooting. Tree Physiol 20: 807-814

Pritchard SG, Rogers HH (2000) Spatial and temporal deployment of crop roots in CO_2-enriched environments. New Phytol 147: 55-71

Shippy WP (1929) Influence of environment on the callusing of apple cuttings and grafts. Am J Bot 17: 290-327

Tinus RW, Burr KE, Atzmon N, Riov J (2000) Relationship between carbohydrate concentration and root growth potential in coniferous seedlings from theree climates during cold hardening and dehardening. Tree Physiol 20: 1097-1101

Tschaplinski TJ, Blake TJ (1989) Correlation between early root production, carbohydrate metabolism, and subsequent biomass production in hybrid poplar. Can J Bot 67: 2168-2174

Van de Pol, PA (1988) Partial replacement of the rooting procedure of *Chrysanthemum morifolium* cuttings by pre-rooting storage in the dark. Acta Hort 226: 519-524

Van den Driessche R (1987) Importance of the current photossynthate to new root growth in planted conifer seedlings. Can J Forest Res 17: 776-782

Van Overbeek J, Gordon SA, Gregory LE (1946) An analysis of the influence of the leaf in the process of root formation in cuttings. Am J Bot 33: 100-107

Veierskov B, Andersen AS, Stummann, BM, Henningsen KW (1982) Dynamics of extractable carbohydrates in *Pisum sativum.* I. Carbohydrate content and photosynthesis of pea cuttings in relation to irradiance and stock plant temperatures and genotype. Physiol Plant 55: 174-178

Veierskov B (1988) Relations between carbohydrates and adventitious root formation. In: TD Davis, BE Haissig N, SanKhla N (eds). Adventitious root formation in cuttings. Discorides Press, Portland Oregon, pp 70-78

Waard PWF, Zaubin R (1983) Callus formation during grafting of woody plants. A concept for the case of black peper (*Piper nigrum* L.). Abst Trop Agri Vol 9: 9-19

Wiesman Z, Lavee S (1995) Relationship of carbohydrate source and Indole-3-butyric Acid in olive cuttings. Aust J Plant Physiol 22: 811- 816

5.2 THE EFFECT OF LIGHT AND CO_2 AT DIFFERENT PHASES OF THE ROOTING PROCESS IN SINGLE NODE LEAFY STEM CUTTINGS OF ROSE

Abstract

This chapter addresses the question whether and to what extent the different phases of the rooting process (initiation and growth) react differently to photosynthesis rates and carbohydrates concentration. Single node leafy stem cuttings of *Rosa hybrida* Madelon® were subjected, in two separated experiments, to low light intensity (7 μmol $m^{-2}s^{-1}$) (PPF) and to low CO_2 concentration (80-100ppm) in four different ways: 1) standard light (76μmol$m^{-2}s^{-1}$) or standard CO_2 (300-350ppm) during the 21 days (HH) (controls); 2) low light or low CO_2 during the 21 days (LL); 3) standard light or standard CO_2 during the first 11 days followed by low light or CO_2 till day 21(HL); 4) low light or low CO_2 during the first 11 days followed by standard light or CO_2 till day 21 (LH). Cuttings were observed on day 11 and 21 of propagation. LL treatments were the most detrimental for callus formation, rooting percentage, number and dry weight of roots. The results show that most of the growth process occurring in cuttings responded to the integral of photosynthesis when comparing LH and HL treatments. Root formation, and particularly growth of the axillary shoot, were sensitive to the moment cuttings were photosyntheticaly active during propagation. Root initiation, expressed by the number of roots, and growth of the axillary shoot were negatively influenced by low photosynthetic activity in the first 11 days of propagation, whereas root growth was mainly affected by the integral of cuttings photosynthesis. It is suggested that the low level of reserves at severance was the major cause of higher reduction in growth observed in the LH treatments (low photosynthesis during the first 11 days of propagation) compared to the HL treatments.

Key words: leaf, photosynthesis, propagation, root initiation, root growth

Introduction

The process of adventitious root formation can be divided in two main phases: initiation and growth (Eriksen, 1973; Lovell and White, 1986). These two phases are considered to have different requirements either in carbohydrates (Haissig, 1986) and hormones (De Klerk et al., 1999). Root initiation would be mainly regulated by hormones (e.g. auxin) (Jarvis, 1986; Blaskeley, 1994; Mohen, 1994; Hackett et al., 1998), whereas further root growth should rather depend on carbohydrates (Eliasson, 1968; Middleton et al., 1980; Van den Driessche, 1987; Muller, 1998; Pritchard and Rogers, 2000; Tinus et al., 2000).

Rooting of leafy cuttings is usually assumed to be positively influenced by photosynthesis (Davis, 1988), but the way photosynthesis influences each of the phases of the rooting process has not been clarified. Photosynthesis has been considered a pre-requisite for root initiation in detached cotyledons of *Synapsis alba* (Lovell et al., 1972) and the formation of root primordia in stem cuttings of Larix was shown to depend on the supply of current photosynthates (Pellicer et al., 2001). Moreover, carbohydrates influence the frequency of lateral root initiation in wheat (Bingham et al., 1997). In contrast, other authors, consider root initiation as a hormonal dependent process not limited by carbohydrates (Middleton et al., 1980; Veierskov et al., 1982). There is also the evidence that initiation may occur in darkness (Van de Pol, 1988). Fournioux (1997) observed in cuttings of *Vitis vinifera*, that root growth (expressed by the fresh weight of roots) depended more on light than did the number of formed roots.

Less controversial is the regulatory effect of carbohydrates on root growth (Humphries and Thorne, 1964; Eliasson, 1968; Middelton et al., 1980; Menoud et al., 1991; Muller et al., 1998). Root length was related to cumulative intercepted radiation (Vincent and Gregory, 1989).

In rose, the overall photosynthesis during propagation influenced rooting of cuttings (Chapters 3.1 and 5.1). However, its specific effect on initiation and growth has not been fully quantified. Therefore, our aim is to quantify to what extent does photosynthesis influence root initiation and growth of roots in rose cuttings. Based on previous own results (Chapter 4.1) we assumed that root initiation (phase including

induction till the formation of the root primordia) occurs mostly during the first 11 days of propagation whereas root growth (phase including growth of the root primordia and further emergence and elongation of roots) occurs mainly thereafter.

Materials and methods

Experiment 1: Influence of low light intensity at different phases of the rooting process on rooting and carbohydrate dynamics in rose cuttings

Experiment 1 started on 23 February 2000. The growth of mother plants of *Rosa hybrida* Madelon® and the procedure of taking the cuttings and the rooting conditions were as described in Chapter 3.1 for Experiment 2. Cuttings were subjected to four treatments: 1) standard light (76μmol $m^{-2}s^{-1}$ /PPF) during the 21 days of propagation (HH) (control); 2) standard light during the first 11 days followed by low light (7 μmol $m^{-2}s^{-1}$/PPF) till day 21(HL); 3) low light during the first 11 days followed by standard light till day 21 (LH) and 4) low light during the 21 days of propagation (LL).

Experiment 2: Influence of low CO_2 concentration on rooting and carbohydrate dynamics in rose cuttings.

Experiment 2 started on 11 November 1999. The growth of mother plants of *Rosa hybrida* Madelon® and the procedure of taking the cuttings and the rooting conditions were as described in Chapter 3.1 for Experiment 2. Cuttings were subjected to four treatments: 1) standard CO_2 air concentration (300-350ppm) during the 21 days of propagation (HH) (control); 2) standard CO_2 during the first 11 days followed by low CO_2 concentration (80-100 ppm) till day 21(HL); 3) low CO_2 during the first 11 days followed by standard CO_2 till day 21 of propagation (LH) and 4) low CO_2 concentration during the 21 days (LL). The CO_2 supplying system was as described in the previous chapter and CO_2 was daily monitorized as described in chapter 5.1.

Measurements: Cuttings were observed on day 0, 11 and 21. Data from day 0 were based on a sample of 9 (Experiment 1) or 16 cuttings (Experiment 2). Percentage of rooted cuttings and of cuttings with stem rot was determined. Number of roots, length of the longest root, length of the axillary shoot, fresh and dry weight of roots, of the primary shoot of the total stem segment and of the stem rooting zone (basal 15mm) and of leaves were determined.

Carbohydrate analysis: Glucose, fructose, sucrose and starch concentrations from the stem (the most basal 7 mm, the above 7mm and from the remaining upper part), original leaf, roots and primary shoot were determined according to the method described in Chapter 3.2. Three cuttings were analysed per treatment (one cutting per block) in both experiments.

Samples with dry weights lower than 15 mg were analysed for carbohydrates by following another procedure than the one described in Chapter 3.2. After weighing the samples, they were placed in a mortar containing 2 mL of ethanol with internal standard and were crushed with a pestle. The sample was then transferred to a centrifuge tube. Pestle and mortar were rinsed with 3 mL of ethanol with internal standard and the washing was added to the sample till a volume of 5 mL is achieved. The samples were stored in a freezer at -20°C and then analysed according the method described in Chapter 3.2.

Experimental design and statistical analysis

Both experiments used a randomized complete block design with 3 blocks, each with 4 plots (6 cuttings per plot). Percentage of the rooted cuttings, stem rot were analyzed by binomial regression ($P<0.05$) using a logit link function. For mean separation, confidence intervals were calculated by multiplying the standard errors of the predicted means by the t value at 5% level and considering the degrees of freedom of the residual.

Data on fresh and dry weight of the different parts of cuttings and carbohydrate concentrations from the different parts of the cuttings were analyzed by one-way ANOVA ($P<0.05$) calculated for each sampling date (day 11 and 21). Least

significant differences were calculated by the t-test. When no significant block effect was observed data were reanalysed as a complete randomised block design in order to gain two degrees of freedom for the residual. The statistical analysis was performed by using the statistical package GENSTAT 5 (IACR, Rothamsted, UK).

Results

Dry weight accumulation was proportional to the integral of leaf photosynthesis (Table 5.2.1 and Fig. 5.2.1). Fresh weight varied in a different way and the largest fresh weight increments occurred mainly between day 11 and day 21, (Table 5.2.2 and Fig. 5.2.1), a consequence of the growth of roots and the axillary primary shoot. This was also visible by the large increments of dry weight verified between day 11 and 21 (Fig. 5.2.2).

Most of the growth process occurring in cuttings responded to the integral of photosynthesis when comparing LH and HL treatments. However, it did affect significantly the number of roots formed (Table 5.2.3). The LH treatments showed significantly less roots by day 21 than the HL treatment (Table 5.2.3), although for dry weight of roots no significant differences were found between the HL and LH treatments (Table 5.2.3).

Growth of the axially bud/shoot was probably the most affected parameter by the timing of the treatments. Low light and low CO_2 during the first 11 days had a strong negative effect on growth of the axially bud after 21 days compared to the HL treatment (Table 5.2.4). This was also visible by the small dry weight increments verified for LH treatments compared to HL (Fig. 5.2.2).

Table 5.2.1 Effect of low light (7μmol $m^{-2}s^{-1}$) and low CO_2 (80-100 ppm) when applied to cuttings in different periods of propagation (HH, HL, LH and LL) (see details in material and methods) on the total dry weight and on the dry weight of the different parts (the total stem, the leaf, the stem rooting zone) of single node leafy stem cuttings of *Rosa hybrida* Madelon®, during the first 21 days of propagation. Until day 11 it is considered only 2 treatments (H, control) or low (L). LSD represents the least significant difference at 5% level by the student *t*-test.

Treatments	Light treatment			CO_2 treatment		
	Day 0	Day 11	Day 21	Day 0	Day 11	Day 21
			Total DW (mg)			
H → HH		617	844		664	898
H → HL	(433±19)[x]		638	(482±28)[y]		589
L → LH		442	663		449	658
L → LL			430			448
LSD		39	113		56	92
			DW stem (mg)			
HH		303	346		343	385
HL	(205±12)[x]		311	(251±19)[y]		281
LH		205	315		217	273
LL			207			216
LSD		27	66		47	50
			DW leaf (mg)			
HH		303	315		313	309
HL	(228±11)[x]		265	(260±13)[y]		233
LH		237	316		231	329
LL			216			227
LSD		41	66		37	65
			DW basal stem(mg)			
HH		88	113		88	103
HL	(52±4)[x]		93	(56±4)[y]		79
LH		57	91		53	74
LL			56			50
LSD		9	19		11	13

[x] means are the average of a sample of 13 cuttings (±SE)
[y] means are the average of a sample of 16 cuttings (±SE)

Table 5.2.2 Effect of low light (7µmol $m^{-2}s^{-1}$) and low CO_2 (80-100 ppm) when applied to cuttings in different periods (HH, HL, LH and LL) (see details in material and methods) on the total fresh weight or on the fresh weight of the different parts (the total stem, the leaf, the stem rooting zone) of single node leafy stem cuttings of *Rosa hybrida* Madelon®, during the first 21 days of propagation. LSD represents the least significant difference at 5% level by the student *t*-test.

Treatments	Light treatment			CO_2 treatment		
	Day 0	Day 11	Day 21	Day 0	Day 11	Day 21
			Total FW (mg)			
H HH		1837	3328		1959	3649
H HL	(1630±77)[x]		2700	(1833±92)[y]		2578
L LH		1662	2219		1620	2201
L LL			1638			1474
LSD		195	620		334	461
			FW stem (mg)			
HH		843	964		934	1143
HL	(753±46)[x]		998	(797±46)[y]		945
LH		718	932		741	778
LL			735			844
LSD		111	199		133	200
			FW leaf (mg)			
HH		913	1029		941	1016
HL	(877±41)[x]		1070	(1036±51)[y]		867
LH		943	1009		765	942
LL			832			576
LSD		150	391		173	310
			FW basal stem (mg)			
HH		275	368		271	339
HL	(172±10)[x]		330	(182±10)[y]		298
LH		208	292		195	244
LL			210			231
LSD		28	65		35	52

[x] means are the average of a sample of 13 cuttings (±SE)
[y] means are the average of a sample of 16 cuttings (±SE)

Table 5.2.3 Effect of low light (7μmol $m^{-2}s^{-1}$) and low CO_2 (80-100 ppm) when applied to cuttings in different periods (HH, HL, LH and LL) (see details in material and methods) on the rooting percentage, on the number and fresh and dry weight of roots for cuttings of *Rosa hybrida* Madelon®, during the first 21 days of propagation Different letters within the same column represent significantly different treatments. LSD represents the least significant difference at 5% level by the student *t*-test.

Treatments		Light treatment			CO_2 treatment		
		Day 0	Day 11	Day 21	Day 0	Day 11	Day 21
				Rooted cuttings (%)			
H	HH		100a	100a		94a	100a
	HL			100a			100a
L	LH		5b	89a		16b	100a
	LL			77a			22b
				Number of roots			
HH			4.9	16.4		5.7	15.2
HL				12.4			11.6
LH			0.06	6.2		0.1	8.1
LL				2.2			1.2
	LSD		1	4.1	2.0		3.1
				Max. root Length (mm)			
HH			17.8	68.4		18.9	65.1
HL				65			59.7
LH			0.3	51.6		0.6	55.3
LL				32.2			10.6
	LSD		3.1	23.7	11.6		26.1
				FW roots (mg)			
HH			31.2	790		43	817
HL				396			479
LH			0	225		0.7	360
LL				71			50
	LSD		12.4	164	28		217
				DW roots (mg)			
HH			2.6	77		4.3	72
HL				31			33
LH			0	24		0	35
LL				0.6			4
	LSD		0.7	16	2.3		13.5

Table 5.2.4 Effect of low light (7μmol $m^{-2}s^{-1}$) and low CO_2 (80-100 ppm) when applied to cuttings in different periods (HH, HL, LH and LL) (see details in material and methods) on the length, the fresh weight and the dry weight of the axillary primary shoot for cuttings of *Rosa hybrida* Madelon®, during the first 21 days of propagation. LSD represents the least significant difference at 5% level by the student *t*-test.

Treatments	Light treatment			CO_2 treatment		
	Day 0	Day 11	Day 21	Day 0	Day 11	Day 21
			Primary shoot length (mm)			
HH			64.3			71.2
H		4.1			3.7	
HL			34.4			38.5
LH			9.4			17.4
L		0			0	
LL			0			1.3
LSD		3.2	25.7		4	20.3
			FW primary shoot (mg)			
HH			641			673
		49.5			20	
HL			236			287
LH			53			121
		0			0	
LL			0			4
LSD		39	26.8		24	185
			D.W. primary shoot (mg)			
HH			105			100
		7.5			3.5	
HL			30.4			42
LH			8			20
		0			0	
LL			0			0.6
LSD		4.3	43.2		5.3	26.4

Dynamics of carbohydrates in cuttings propagated under standard and low light intensity or under ambient and low CO_2 air concentrations

Low light and low CO_2 treatments reduced carbohydrate concentration in all the parts of the cutting although the decrease was faster and more pronounced in leaves (Figs. 5.2.3 and 5.2.4). Characteristic of the controls was the peak in soluble sugars (glucose and sucrose), and in some cases starch by day 11 whereas cuttings growing under low light and low CO_2 had a slight decrease or kept the levels measured at severance (Figs. 5.2.3 and 5.2.4). This concentration peak observed for controls on day 11 was also observed for LH treatments on day 21. In contrast, the HL treatments showed a fast decrease in starch since day 11 until day 21.

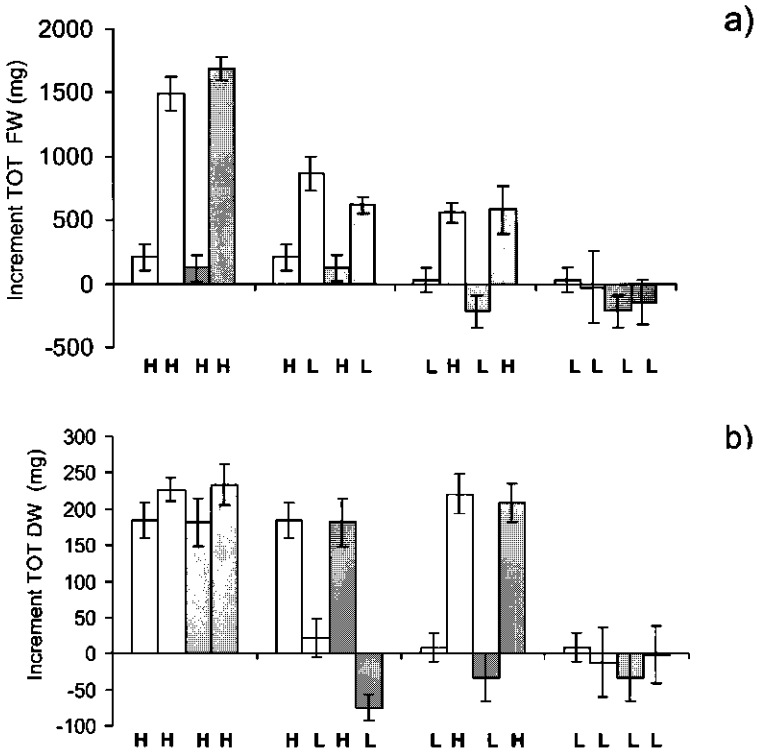

Figure 5.2.1 Increments in the total fresh **(a)** and total dry weight **(b)** of leafy stem cuttings of *Rosa hybrida* Madelon® subjected to different light (white bars) and CO_2 (grey bars) treatments when applied to cuttings in different periods (HH, HL, LH or LL) (see details in material and methods) during the first 21 days of propagation. H indicates standard light intensity (76 μmol $m^{-2}s^{-1}$) or standard CO_2 concentration (300-350ppm) and L represents low light intensity (7μmol $m^{-2}s^{-1}$) or low CO_2 concentration (80-100 ppm).

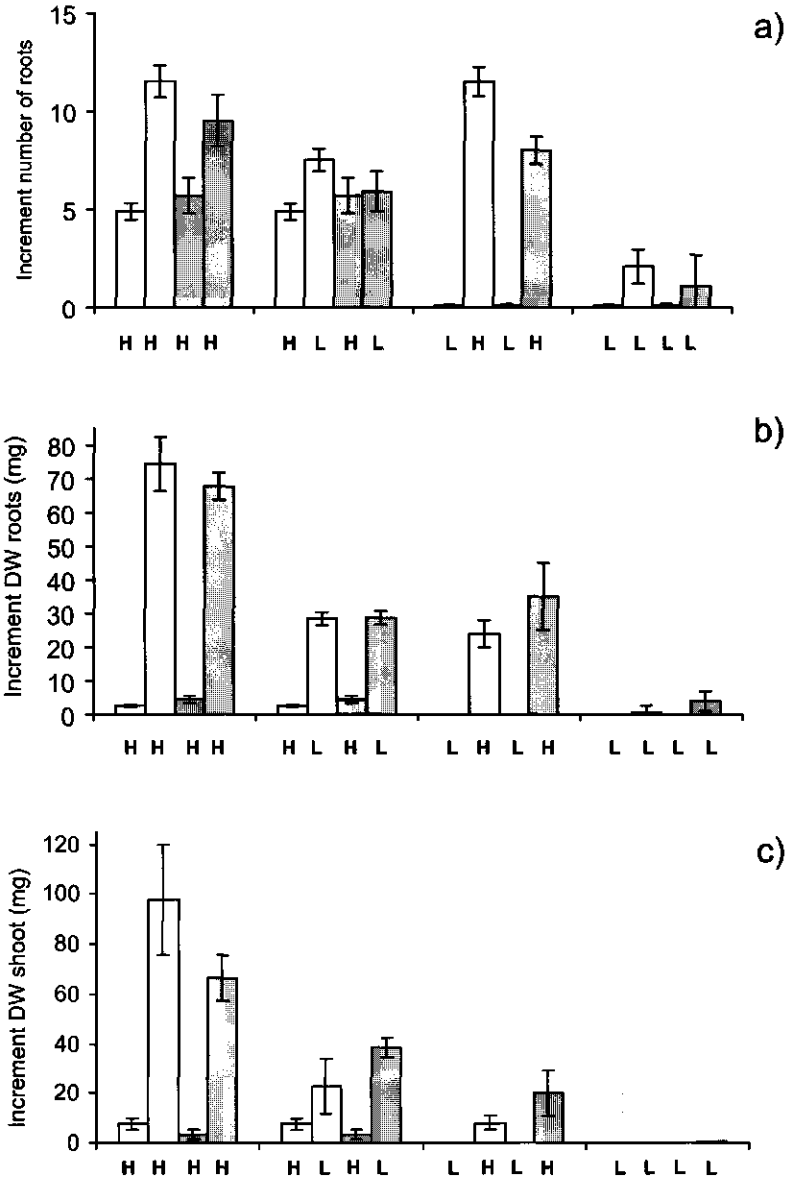

Figure 5.2.2 Increments in the number of roots **(a)**, the dry weight of roots **(b)** and the dry weight of the axillary shoot **(c)** of leafy stem cuttings of *Rosa hybrida* Madelon® subjected to different light (white bars) or CO_2 (gray bars) treatments when applied in different periods (HH, HL, LH or LL) (see details in material and methods) during the first 21 days of propagation. H indicates standard light intensity (76 µmol $m^{-2}s^{-1}$) or standard CO_2 concentration (300-350ppm) and L represents low light intensity (7µmol $m^{-2}s^{-1}$) or low CO_2 concentration (80-100 ppm).

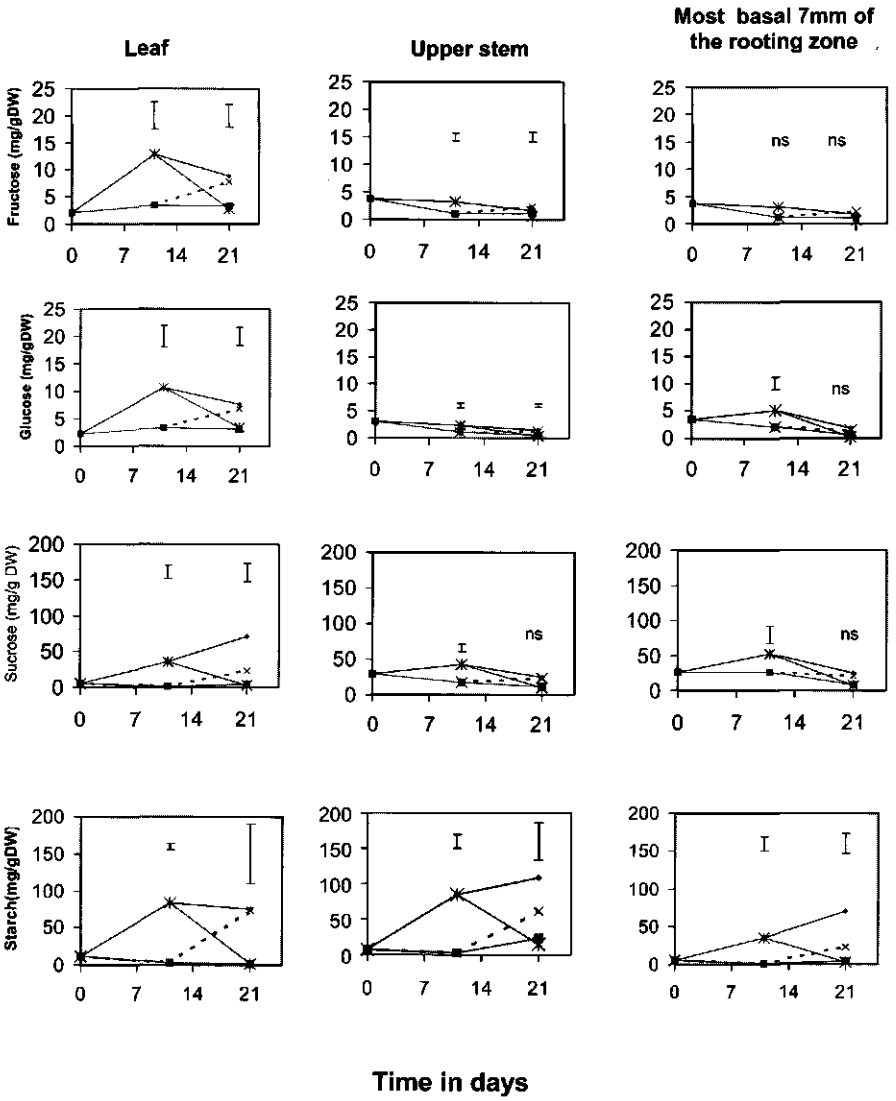

Figure 5.2.3 Concentrations (mg/gDW) of fructose, glucose, sucrose, and starch on day 0, 11 and 21 of propagation for the leaf, the stem (upper stem) and the rooting zone of the stem (most basal 7mm) of leafy stem cuttings of *Rosa hybrida* Madelon® subjected to different CO_2 treatments: control (HH) (—◆—), LH (----x----), HL (—✳—) and LL (—■—) (see details in material and methods). H indicates standard CO_2 concentration (300-350ppm) and L represents low CO_2 concentration (80-100 ppm). Vertical bars indicate the LSD values at 5% level of confidence.

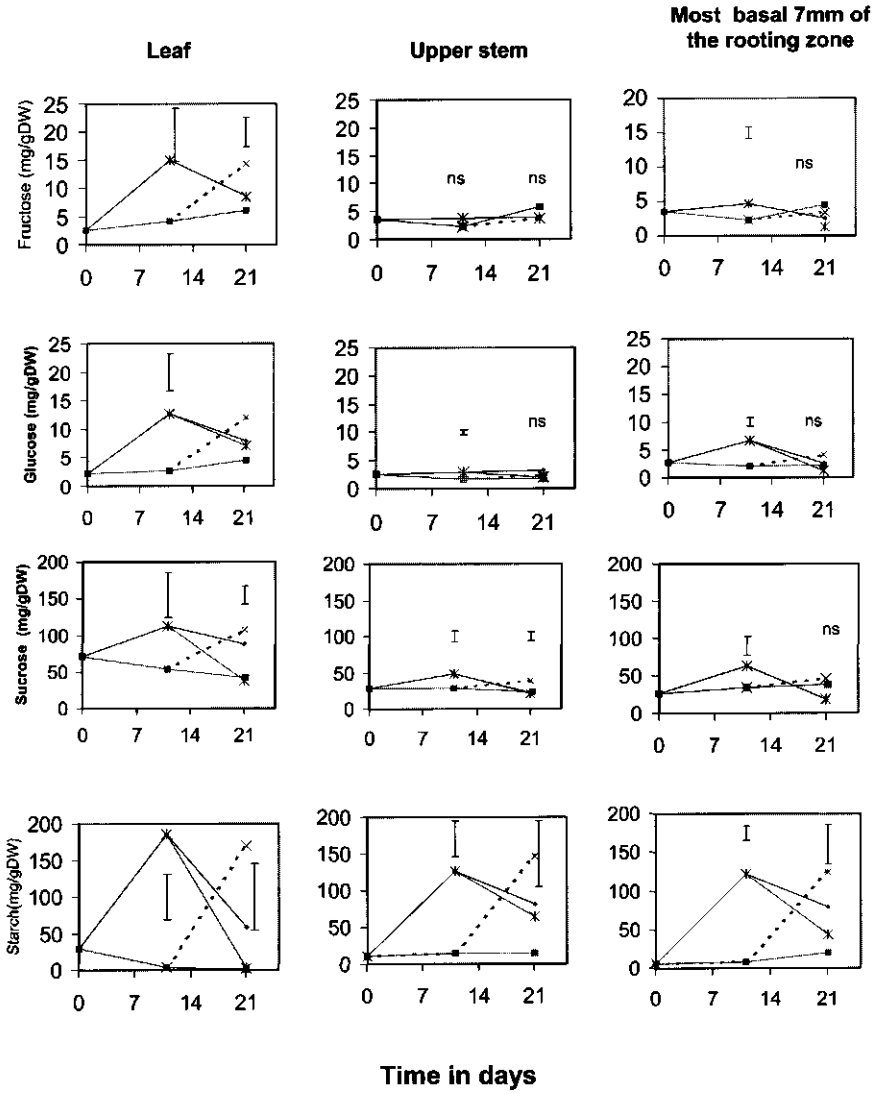

Figure 5.2.4 Concentrations (mg/gDW) of fructose, glucose, sucrose, and starch on day 0, 11 and 21 of propagation for the leaf, the stem (upper stem) and the rooting zone of the stem (most basal 7mm) of leafy stem cuttings of *Rosa hybrida* Madelon® subjected to different light intensity treatments: control (HH) (—◆—), LH (---x---), HL (—✳—) and LL (—■—) (see details in material and methods). H indicates standard light intensity (76 μmol $m^{-2}s^{-1}$) and L represents low light intensity (7μmol $m^{-2}s^{-1}$). Vertical bars indicate the LSD values at 5% level of confidence.

Discussion

Rooting and growth of the primary shoot responded differently to the period cuttings were photosynthetically active during propagation. Rooting was less sensitive to the timing of the treatments than growth of the axillary primary shoot.

Root initiation (number of roots) was strongly diminished by low photosynthesis during the first 11 days of propagation although, root growth (dry weight of roots) responded mostly to the integral of the leaf photosynthesis in line with previous own findings from Chapter 5.1 and previous literature (Eliasson, 1968; Middleton et al., 1980; Van den Driessche, 1987; Vincent and Gregory, 1989; Pritchard and Rogers, 2000).

Contrary to root growth, the axillary shoot responded more negatively to the LH than the HL treatments. This may be attributed to the fact that the response time of the axillary shoot differs from that of the roots. Roots have probably shorter lag phase compared to the axillary shoot which made possible to roots to use the photoassimilates available since day 11. Because of longer lag phase, the axillary bud did not have the opportunity to use fully the photosynthates available since day 11 resulting in less growth in the LH situation.

The strong negative effect of low photosynthesis during the first 11 days of propagation on rooting may be the combined result of low initial level of reserves and reduced photosynthate supply that would strongly limit growth. Reduced photosynthesis decreased carbohydrate concentrations at the rooting zone (Figs. 5.2.3 and 5.2.4) which could reduce cambial activity and proliferation of phloem parenchyma during the first 11 days of propagation and consequently further root initiation as found in Chapter 5.1. Moreover, limited carbohydrate supply is also supposed to reduce axillary bud growth. In rose plants, growth of buds was found to depend on carbon supply (Marcellis Van-Acker, 1994; Van Labeke, 2001). This is in line with the results for the HL treatment which presented a concentration peak of carbohydrates by day 11 followed by a decrease either in leaves as well in the stem (Figs. 5.2.3 and 5.2.4), most probably to support growth of roots and of the axillary shoot which occur mainly after day 11.

Our results show evidence that rooting, and particularly growth of the primary shoot of rose cuttings, are sensitive to the moment cuttings are photosyntheticaly active during propagation. Root initiation (expressed by the number of roots) as well growth of the axillary bud are strongly negatively influenced by low photosynthetic activity during the first 11 days of propagation whereas root growth (fresh and dry weight of roots) is mainly affected by the integral of cuttings photosynthesis.

Other main conclusion, was that the role of the initial reserves in cuttings for quality of the planting material in terms of growth of roots and the axillary shoot seems to be irrelevant if compared to the photoassimilates synthesized in leaves.

Nevertheless, reserves accumulated during propagation were also efficiently used in growth of roots and in particular of the primary shoot when photosynthesis was suppressed by leaf removal, low light levels or low CO_2.

References

Bingham IJ, Blackwood JM, Stevenson EA (1997) Site, scale and time-course for adjustments in lateral root initiation in wheat following changes in C and N supply. Ann Bot 80: 97-106

Costa JM, Challa H, Van Meeteren U, Van de Pol, PA (2001) Photosynthates: mainly stored and yet limiting in propagation of rose cuttings. Acta Hort 547: 167-174

Cowling SA, Sage RF (1998) Interactive effects of low atmospheric CO_2 and elevated temperature on growth, photosynthesis and respiration in *Phaseolus vulgaris*. Plant Cell Environ 21: 427-435

Davis TD (1988) Photosynthesis during adventitious rooting. In: TD Davis, BE Haissig and N Sankhla (eds) Adventitious root formation in cuttings. Discorides Press, Portland Oregon, pp 79-87

De Klerk G, Van der Krieken W, De Jong J (1999) Review the formation of adventitious roots: new concepts and possibilities. In Vitro Cell Dev Biol -Pl 35: 189-1999

Dubois LAM, De Vries DP (1991) Variation in adventitious root formation of softwood cuttings of *Rosa chinensis* minima (Sims) Voss cultivars. Sci Hort 47: 345-349

Eliasson L (1968) Dependence of root growth on photosynthesis in *Populus tremula*. Physiol Plant 21: 806-810

Eriksen EN (1973) Root formation in pea cuttings. I. Effects of decapitation and disbudding at different developmental stages. Physiol Plant 28: 503-506

Fournioux JC (1997) Adult leaves of grapevine cuttings stimulate rhizogenesis. Vitis 36: 49-50 Research note

Hartmann HT, Kester DE, Davies FT, Geneve RL (1997) Plant Propagation, Principles and Practices. 6th ed. Prentice Hall Inc., Englewood Clifs, New Jersey, 770 pp

Humphries EC, Thorne GN (1964) The effect of root formation on photosynthesis of detached leaves. Ann Bot 28: 391-400

Lovell PH, Illsley A, Moore KG, Moore (1972) The effect of light intensity and sucrose on root formation, photosynthetic ability, and senescence in detached cotyledons of *Sinapsis alba* L. and *Raphanus sativa* L. Ann Bot 36: 123-134

Lovell PH, White J (1986) Anatomical changes during adventitious root formation. In: MB Jackson (ed), New root formation in plants and cuttings. Long Ashton Research Station University of Bristol, UK, pp 141-190

Marcelis-van Acker CAM (1994) Effect of assimilate supply on development and growth potential of axillary buds in roses. Ann Bot 73: 415-420

Menoud, MA, Mauget JC, Frossard., JS (1991) Effect of clod storage duration on budbreak, root regeneration and on carbohydrate content of "Mme A. Meilland" rose plants. Acta Hort 298: 237-245

Middleton W, Jarvis BC, Booth A (1980) The role of leaves in auxin and boron-dependent rooting of stem cuttings of *Phaseolus aureaus* Roxb. New Phytol 84: 251-259

Muller B, Stosser M, Tardieu F (1998) Spatial distributions of tissue expansion and cell division rates are related to irradiance and to sugar content in the growing zone of maize roots. Plant, Cell and Environ 21: 149-158

Okoro OO, Grace J (1976) The physiology of rooting *Populus* cuttings. Carbohydrates and

photosynthesis. Physiol Plant 36: 133-138

Pritchard SG, Rogers HH (2000) Spatial and temporal deployment of crop roots in CO_2-enriched environments. New Phytol 147: 55-71

Van de Pol, PA (1988) Partial replacement of the rooting procedure of *Chrysanthemum morifolium* cuttings by pre-rooting storage in the dark. Acta Hort 226: 519-524

Van den Driessche R (1987) Importance of the current photossynthate to new root growth in planted conifer seedlings. Can J Forest Res 17: 776-782

Van Labeke MC, Dambre P, Bodson M, Pien H (2001) Developmental changes in carbohydrate content in young rose shoots (*Rosa hybrida* 'Frisco'). Acta Hort 547: 193-201

Vincent CD, Gregory PJ (1989) Effects of temperature on the development and growth of winter wheat roots. II. Field studies of temperature, nitrogen and irradiance. Plant Soil 19: 99-110

Wilkins LC, Graves WR, Townsend AM (1995) Development of plants from single node cuttings differs among cultivars of Red Maple and Freeman Maple. HortScience 30: 360-362

Wilson PJ (1994) Contributions of the leaves and axillary shoots to rooting in *Eucalyptus grandis* Hill ex.Maid. stem cuttings. J Hort Sci 69: 999-1007

CHAPTER 6

GENERAL DISCUSSION

Introduction

Leaves are essential for propagation of leafy stem cuttings and this has often been related to the role of leaves as source of carbohydrates via photosynthesis (Reuveni and Raviv, 1981; Wilson, 1994a; Wiesman and Lavee, 1995) although in many cases, including rose (Moe, 1973; Dubois and De Vries, 1985; 1991), that relation has not been fully quantified nor tested.

Thus, one of the aims of this thesis was to study the role of the original leaf (area) of cuttings in rooting and further growth of cuttings and to investigate the importance of leaf photosynthesis and the supply of carbohydrates. Simultaneously, a major practical aim was to obtain insight in the role of the original leaf in quality of planting material as related to morphological parameters of growth such as rooting, total plant size or growth of the axillary bud into the primary shoot (Chapter 1).

Quantitative effect of leaves and carbohydrates on survival and growth of single-node leafy stem cuttings of rose

One of the very preliminary results of this thesis was that leaves have to be present for a certain period to guarantee survival and rooting of cuttings (Chapters 3.1, 3.2 and 5.1) and these leaves have also to be illuminated to promote rooting (Chapters 3.1, 3.2 and 5.1). One of the initial assumptions was that the leaf influences the performance

of rose cuttings during propagation via the supply of carbohydrates. During propagation, carbohydrates (reserves and/or current photosynthates) should primarily support respiration costs and guarantee survival, an aspect that can be particularly decisive for success in propagation during the first week of propagation. Based on the respiration measurements for the whole cutting (Chapter 4.2) and carbohydrate concentrations for stem and leaves (Chapter 4.3) the total amount of carbohydrates present at severance (about 60 mg CH_2O) was enough to support respiration costs of cuttings for about 8 days (1 day respiration would represent the consumption of about 7 mgCH_2O considering an average dry weight of 450 mg and an average maintenance respiration for leaves and stem of 0.015 gCH_2Og^{-1}DW d^{-1} (Spitters et al., 1989). This agrees with the observations that carbohydrates were depleted in leafless cuttings (Chapter 3.2) or cuttings subjected to total light exclusion (covered leaf), low irradiation or low CO_2 after 7 or 11 days (Chapter 5), where finally stem rot occurred.

Once survival is guaranteed, photosynthesis was found to promote cambial activity at the rooting zone of cuttings and further proliferation of parenchyma tissue where roots are expected to initiate (Chapter 4.1.). This has been considered a pre-requisite for improved rooting, in line with previous findings for other woody species (Lovell and White, 1986; Hamann, 1998; Schwarz et al., 1999). Moreover, the relevance of cambium and cambial activity has been observed in cuttings of *Rosa rugosa*, where a thicker cambial zone was related with better rooting ability (Schmidt and Fouda, 2000). Callus formation was related to the photosynthetic activity (dry matter accumulation) of the cuttings during propagation (Chapters 3.1. and 5.1.) which is probably is related with the need of carbohydrates (current photosynthates).

Root formation and initial growth during the first 21 days of propagation represent a small sink. However, the consistent positive linear relation between the number of roots and the integrated net photosynthesis of the cutting (expressed by the total dry weight accumulation) achieved under different conditions of light, CO_2, leaf area, (Chapter 5.1) supports the conclusion that carbohydrates influence root initiation. Root growth (expressed by dry weight of roots) stopped when the dry weight increase of the cutting approach 0, but some roots were still formed. This indicates that root growth was apparently more negatively affected by limited supply of current photosynthate than root initiation (number of roots) in accordance with previous findings showing that root growth depends on the availability of carbohydrates (Van den Driessche, 1987; Pritchard and Rogers, 2000). The positive

effect of photosynthesis and availability of carbohydrates may also be extended to improvement on lateral root initiation (Bingham and Stevenson, 1993; Bingham et al., 1997) and increase the number of secondary roots. Although growth of roots and primary shoot growth were positively affected by current photosynthesis, in particular primary shoot growth (Fig. 5.2.2), they were both positively influenced by the previous build up of reserves when the conditions for current photosynthesis were limiting.

The amount of reserves at severance was normally much lower than the needs for propagation and to achieve optimal rates of rooting and growth. In fact, the reserves in the leaf and stem of cuttings should be rather high to fully compensate the lack of current photosynthesis. As an example, even when the original leaf was removed after 7 to 11 days of propagation (Chapter 3.1) or subjected to low CO_2 and low light intensity after 7 or 11 days of propagation (Chapter 5.2) cuttings would survive and also form roots showing that the reserves can also be used for growth of roots and the axillary primary shoot as soon as photosynthetic activity ceased (Chapters 5.1 and 5.2). This proves that cuttings are able to use the pool of starch as well as the one of the photosynthates for growth.

The discrepancy between our results and previous literature reporting that root formation may occur in absence of leaves or light (Van Overbeek et al., 1946; Davis, 1988; Van de Pol, 1988) can be explained by the type of cuttings and the type of carbon pools supporting the rooting process. Hardwood cuttings of rose (e.g. *Rosa multiflora*) root easily without leaves (Davies et al., 1987; Hambrick et al., 1991) and that is to a great extent explained by the fact that their main source of carbohydrates is the pool of stored carbohydrates. The big amount of starch existing in 25 cm long canes compared to the amounts in 4-6 cm long stems of softwood material can largely justify the ability of leafless hardwood cuttings of *Rosa* or other species (Okoro and Grace, 1976; Hartmann et al., 1997) to root. However, we may consider the possibility that hardwood cuttings would root better with leaves due to a possible synergetic effect of larger availability of reserves and larger supply of current photosynthate as suggested by Marcelis-van Acker and Leutscher (1993) to justify that double node cuttings of *Rosa hybrida* Motrea® with two leaves rooted better and gave rise to heavier plants than single node and single leaf cuttings.

In fact, in species characterised by long rooting periods the synergetic effect of reserves and current photosynthates is visible. Cuttings with substantial carbohydrate

reserves such as *Picea abies* L or *Shorea leprosula* required additional photosynthates to compensate for the depletion of carbohydrate reserves during the rooting process (Strömquist and Eliasson, 1979; Aminah et al., 1997).

Severance, leaf photosynthesis and rooting

Severance has been assumed to reduce photosynthetic activity of leafy stem cuttings mainly by decreasing leaf stomatal conductance (Loach, 1988; Svenson et al., 1995; Fordham et al., 2001). It is shown in Chapter 4, however, that photosynthesis, although experiencing a fast decrease in response to severance, recovered fast and up to 70% of the rates of net photosynthesis values measured on the mother plants. This contradicts previous reports indicating that net photosynthesis decreases after severance and remains low until roots emerge (Okoro and Grace, 1976; Davis and Potter, 1983; Cameron and Rook, 1974; Davis, 1988; Smalley et al., 1991). It is not likely that under the present propagation conditions water stress has affected growth of cuttings significantly. It is possible that the environmental conditions inside propagator boxes, high R.H. and low light intensities minimised transpiration compared to other propagation procedures described in literature where cuttings are rooted in greenhouse.

Cutting a source limited system but still storing carbohydrates

Unexpected, and apparently contradicting the findings showing a promotive role of photosynthesis on rooting (Chapter 5.1 and 5.2), was the large fraction of photosynthates driven to storage (Chapter 4.3). The concentrations measured in rose plants of *Rosa hybrida* Motrea® (about 50 $mgg^{-1}DW$) (Kool, 1996) were comparable to the concentrations measured at severance of cuttings of the rose cultivar Madelon®, but were only half of the concentration measured in cuttings after 21 days. This has been explained by a drastic reduction of sink activity following severance as suggested in literature for cuttings (Humphries and Thorne, 1964; Veierskov et al., 1982; Feldman et al., 1989, Wilson, 1994b). In fact, in the present case, the meristematic sinks (new formed roots and axillary primary shoot) represented

together less than 10% of the total dry weight after 21 days (Chapter 4.2) showing that the remaining weak sinks, stem growth and storage organelles, became major competing sinks, much stronger than the meristematic sinks (root initials or the axillary bud/primary shoot). This could explain why cuttings, nevertheless, responded positively (by increasing rooting) to increased current photosynthesis.

Variations in rooting induced by variations in the leaf area can be explained to a great extent by variations in the photosynthetic capacity (Chapter 5.1) and carbohydrate supply. Therefore, photosynthesis and carbohydrates are capable to explain to a great extent variations in rooting and growth caused by different leaf area. However, considering that the majority of the current photosynthate supply was stored (about 55 % of the dry weight accumulation in cuttings was due to carbohydrates accumulation after 21 days of propagation (Chapter 4.3.)) we can also not exclude the possibility that rooting is being influenced in other ways than only by the supply of current photosynthates. Current photosynthesis may sustain the continuous concentration gradient and overcome the sink strength of storage cells (of the cortex), from the stem segment.

The positive effect of photosynthesis and/or the supply of on root initiation, could be a direct effect of it could be mediated by other factors like auxin by a synergetic effect between carbohydrates and auxin (Jarvis, 1986). Photosynthesis may be needed to promote leaf export activity and thus transport of other rooting factors besides carbohydrates as suggested by different authors (Davis, 1988; Aminah et al., 1997; Druege et al. 2000). Therefore, the relation between rooting and carbohydrates could be a more complex relation than a simple substrate-rate relation (Pritchard and Rogers, 2000), although this does not affect the main conclusions of this thesis.

Some considerations on limitations of the present research and relevant points for practice

Rooting besides being affected by environment or by the interaction of the environment and the morphological characteristics of cuttings is also a genetically determined trait in roses (Van de Pol, 1988; De Vries, 1993;Van der Salm, 1996) as is growth of the axillary primary shoot (De Vries, 1993; Dieleman, 1997; De Hoog, 1998). The cultivar used in this study, *Rosa hybrida* Madelon® can be considered a

medium or easy-to-root cultivar, which develops first the root system and afterwards the axillary primary shoot. Therefore, it is possible that the present findings with *Rosa hybrida* Madelon® are not totally valid for all cut-rose cultivars, specially the difficult-to-root ones, where rooting is genetically limited.

There is a tendency of the breeding companies to have easy-to-root cultivars. Thus, like suggested for pot roses by Scagel (2001), the ability to form roots in cut-roses may not be the major limiting factor for quality of planting material of cut-roses. Instead, growth rate of roots and of the axillary bud into a primary shoot can be, as it will influence final size of the planting material and the time needed to achieve a saleable plant. Moreover, optimisation of growth rates during propagation may be a step to increase uniformity as it implies diminishing the probability of exposure to stress or variation factors. Thus, practice should focused on finding ways to optimise growth of cuttings, and therefore, photosynthesis by optimising light and/or, CO_2 conditions as function of the moment of propagation. The present results show that photosynthesis and reserves favoured rooting and growth of the axillary bud/shoot. Therefore, when water stress can be avoided growth of cuttings should be favoured by higher light levels since early moments of propagation which would increase photosynthesis as well as the reserves, without causing any clear negative effect on the condition of the leaf in terms of the PSII efficiency.

A future line of research, with a possible direct application to practice, includes the building up of a model in order to predict growth of single node leafy stem cuttings of rose during propagation, based on the photosynthetic capacity of the original leaf. Measurements on leaf photosynthesis are not necessarily representative for the photosynthetic behaviour of the entire canopy, plant growth and crop productivity (Dutton et al., 1988). Also the respiration of a single leaf does not represents the dark respiration of the entire plant. However, in single leaf cuttings this is not a problem, because the canopy is the single leaf and the plant is simply the cutting. This gives the possibility to integrate data on dry matter and carbohydrates accumulation, photosynthesis and respiration and further estimate and predict rooting and growth of cuttings based on the photosynthetic capacity of the original leaf of cuttings like it has been previously done for leafy stem cuttings of *Triplochyton scleroxylon* (Dick and Dewar, 1992). In Chapters 3 and 5 it is demonstrated that it is possible to relate photosynthetic activity and carbon dynamics with external quality of the planting

material (rooted cuttings) mostly in terms of the size of the rooting system and the total plant dry weight.

Considering quality assessment in cut-rose propagation, the original leaf may be considered the prime determinant of quality of cuttings and the planting material of cut-roses, considering that their photosynthetic capacity is the main determining process of rooting and further growth of roots and the axillary primary shoot during the first 21 days of propagation. Thus, good quality cuttings should have the original leaf intact and healthy to be able to resume and maintain photosynthesis since the early days of propagation. Considering the stem part of the unrooted cuttings, because the role of initial reserves on growth has been found limited compared to that of the photosynthates, except in situations where photosynthesis is suppressed (Chapter 5.2), thinner and less woody stems should give better rooting results rather than thicker and more mature material that contain larger amount of initial reserves but will be simultaneously more difficult to root due to the higher legnification as mentioned for other species (Hartmann et al., 1997).

The tentative conceptual model presented in Chapter 2 seems to be in line with the reality of the dynamics of rooting and growth as influenced by photosynthesis. We should moreover consider that the relevance of photosynthesis or of the pool of stored carbohydrates can vary in function of the source activity of the original leaf. Finally, it should be mentioned that other factors besides photosynthesis and carbohydrates could play a role in the rooting behaviour of cuttings although their role has not been considered in the aim and approach of this thesis.

References

Aminah H, Dick JMcP, Grace J (1997) Influence of irradiance on water relations and carbon flux during rooting of *Shorea leprosula* leafy stem cuttings. Tree Physiol 17: 445-452

Bingham IJ, Stevenson (1993) Control of root growth: effects of carbohydrates on the extension, branching and rate of respiration of different fractions of wheat roots. Physiol Plant 88: 149-158

Bingham IJ, Blackwood JM, Stevenson EA (1997) Site, scale and time-course for adjustments in lateral root initiation in wheat following changes in C and N supply. Ann Bot 80: 97-106

Cameron RJ, Rook, DA (1974) Rooting stem cuttings of Radiata pine: environmental and physiological aspects. New Zeal J For Sci 4: 291-298

Davis TD (1988) Photosynthesis during adventitious rooting. In: TD Davis, BE Haissig and N Sankhla (eds) Adventitious root formation in cuttings. Discorides Press, Portland Oregon, pp 79-87

Davies, FT, Hembrik, CE Pemberton, HB FaanY (1987) Grafting and adventitious root formation of

Texas field rose bushes. Acta Hort 189: 89-100

Davis TD, Potter JR (1983) High CO_2 applied to cuttings:effects on rooting and subsequent growth in ornamental species. HortScience 18: 194-196

De Hoog J (1998) Teelt van kasrozen. Proefstation voor Bloemisterij en Glasgroente. The Netherlands, 218 pp

De Vries DP (1993) The vigour of glasshouse roses: scion-rootstock relationships: effects of phenotypic and genotypic variation. PhD Thesis Wageningen University, 169 pp

Dick, J McP, Dewar RC (1992) A mechanistic model of carbohydrates dynamics during adventitious root development in leafy cuttings. Ann Bot 70: 371-377

Dieleman JA (1998) Cytokinins and bud break in rose combination plants, PhD Thesis Wageningen University, 109 pp

Druege U, Zerche S, Kadner RD, Ernst M (2000) Relation between nitrogen status, carbohydrate distribution and subsequent rooting of Chrysanthemum cuttings as affected by pre-harvest nitrogen supply and cold-storage. Ann Bot 85: 687-701

Dubois LAM, De Vries DP (1985) Onderzoek met stekken van 'Sonia'. Vakblad voor de Bloemisterij 45: 26-31

Dubois LAM, De Vries DP (1991) Variation in adventitious root formation of softwood cuttings of *Rosa chinensis* minima (Sims) Voss cultivars. Sci Hort 47: 345-349

Dutton RG Jiao J, Tsujita MJ, Grodzinski B (1988) Whole Plant CO_2 Exchange Measurements for Non-Destructive Estimation of Growth. Plant Physiol 86: 355-358

Feldman WR, Palzkill DA, Dobrenz AK, Hogan L (1989) Carbon dioxide exchange during mist propagation of jojoba cuttings. Agron J 81: 233-236

Fordham MC, Harrisson-Murray RS, Knight L, Clay CM (2001) Decline in stomatal response to leaf water deficit in *Corylus maxima* cuttings. Tree Physiol 21: 489-496

Hamann A (1998) Adventitious root formation in cuttings of loblolly pine (*Pinus taeda* L.) developmental sequence and effects of maturation. Trees 12: 175-180

Hambrick CE, Davies FT, Pemberton HB (1991) Seasonal changes in carbohydrates/nitrogen levels during field rooting of *Rosa multiflora* 'Brooks 56' hardwood cuttings. Sci Hort 46: 137-146

Hartmann HT, Kester DE, Davies FT, Geneve RL (1997) Plant Propagation, Principles and Practices. 6th ed. Prentice Hall Inc., Englewood Clifs, New Jersey, 770 pp

Humphries EC, Thorne GN (1964) The effect of root formation on photosynthesis of detached leaves. Ann Bot 28: 391-400

Jarvis BC (1986) Endogenous control of adventitious rooting in non-woody cuttings. MB Jackson (ed.), New root formation in plants and cuttings. Long Ashton Research Station University of Bristol, UK, pp 191-222

Kool MTN (1996) System development of glasshouse roses. PhD Thesis Wageningen University, 143 pp

Loach K (1988) Water relations and adventitious rooting. In: TD Davis, BE Haissig, N Sankla (eds), Adventitious root formation in cuttings. Discorides Press, Portland Oregon, pp 102-116

Lovell PH, White J (1986) Anatomical changes during adventitious root formation. In: MB Jackson (ed) New root formation in plants and cuttings. Long Ashton Research Station University of Bristol, UK, pp 141-190

Marcelis-van Acker CAM, Leutscher KJ (1993) Effect of the type of cutting on heterogeneity and growth of *Rosa hybrida* cv. 'Motrea' and *Schefflera arboricola* cv. 'Compacta'. Sci Hort 54: 59-67

Moe R (1973) Propagation, growth and flowering of potted roses. Acta Hort 31: 35-51

Okoro OO, Grace J (1976) The physiology of rooting *Populus* cuttings. Carbohydrates and photosynthesis. Physiol Plant 36: 133- 138

Pritchard SG, Rogers HH (2000) Spatial and temporal deployment of crop roots in CO_2-enriched environments. New Phytol 147: 55-71

Reuveni O, Raviv M (1981) Importance of leaf retention to rooting of avocado cuttings. J Am Soc Hort Sci 106: 127-130

Scagel CF (2001) Cultivar specific effects of mycorrhizal fungi on the rooting of miniature rose cuttings. J Environ Hort 19: 15-20

Schwarz JL, Glocke PL, Sedgley M (1999) Adventitious root formation in *Acacia baileyana* F. Muell. J Hort Sci Biotech 74: 561-565

Schmidt G, Ramadan, F (2000) Histological studies on the above and underground stem portions of two suckering *Rosa* species. Pub Hort Food and Landscape Sciences, Szent István University, Budapest, Vol 60, pp 65- 68

Smalley TJ, Dirr AM, Armitage AM, Wood BW, Teskey RO, Severson RF (1991) Photosynthesis and leaf water, carbohydrate and hormonal status during rooting of stem cuttings of *Acer rubrum*. J Am Soc Hort Sci 116: 1052-1057

Strömquist LH, Eliasson B (1979) Light inhibition of rooting in Norway spruce (*Picea abies*) cuttings. Can J Botany 57: 1314-1316

Svenson SE, Davies FT Jr, Sharon AD (1995) Gas exchange, water relations and dry weight partitioning during root initiation and development of poinsettia cuttings. J Am Soc Hort Sci 120: 454-459

Spitters CJT, Van Keulen H, Van Kraalingen DWG (1989) A simple and universal crop growth simulato: SUCROS 87. In: Rabingge R, Ward SA, Van Laar HH (eds) Simulation and systems management in crop protection. Wageningen Pudoc pp.147-181.

Van de Pol PA (1988) Partial replacement of the rooting procedure of *Chrysanthemum morifolium* cuttings by pre-rooting storage in the dark. Acta Hort 226: 519-524

Van den Driessche R (1987) Importance of the current photosynthate to new root growth in planted conifer seedlings. Can J Forest Res 17: 776-782

Van der Salm TPM (1996) Introduction of ROL genes in *Rosa hybrida L.* for improved rootstock performance. PhD Thesis, Wageningen University, 114 pp

Van Overbeek J, Gordon SA, Gregory LE (1946) An analysis of the influence of the leaf in he process of root formation in cuttings. Am J Bot 33: 100-107

Veierskov B, Andersen AS, Stummann, BM, Henningsen KW (1982) Dynamics of extractable carbohydrates in *Pisum sativum*. Carbohydrate content and photosynthesis of pea cuttings in relation to irradiance and stock plant temperatures and genotype. Physiol Plant 55: 174-178

Wiesman Z, Lavee S (1995) Relationship of carbohydrate source and Indole-3-butyric-acid in olive cuttings. Aust J Plant Physiol 22: 811-816

Wilson PJ (1994a) Contributions of the leaves and axillary shoots to rooting in *Eucalyptus grandis* Hill ex.Maid. stem cuttings. J Hort Sci 69: 999-1007

Wilson PJ (1994b) Contributions of the stem to rooting in *Eucalyptus grandis* Hill ex.Maid. stem cuttings. J Hort Sci 69: 1009-1017

SUMMARY

In the Netherlands about 20 million roses are propagated every year to provide the needs for planting material of the Dutch and foreign cut-rose sector. Propagation by single-node leafy stem cuttings is relatively easy, and cheap and, therefore, commonly used. Moreover, the use of own-rooted plants expanded rapidly in the Netherlands following the introduction of cultivation in substrate (cocos peat and rockwool, which represents about 80% of the total cultivation area of 921 ha).

Although high degrees of success in propagation have been achieved in the last years, losses and lack of uniformity in rooting and further growth of cuttings and planting material still occur. To increase uniformity and efficiency, knowledge of the main factors regulating growth of leafy stem cuttings is required.

Leaves are essential for propagation of cut-roses by leafy (semi-hardwood) stem cuttings and they are also the most vulnerable part of the cutting to external factors and stress during propagation. Therefore, Chapter 2 presents a literature review on the role of leaves as photosynthetic structure and as source of carbohydrates. A conceptual model relating the original leaf, its photosynthesis and the available carbohydrate pools to rooting and growth is proposed to describe the possible role of leaves and carbohydrate dynamics (synthesis, partitioning) in propagation of single node leafy stem cuttings of rose.

In chapter 3 the quantitative effect is described of the original leaf on rooting and growth of rose cuttings until a maximum of 10 weeks after severance. This analysis was extended using the concept of leaf area duration (LAD), defined as leaf area x the number of days the leaf remains attached to the cutting. In physiological terms it indicates the total potential of cuttings for photosynthesis during propagation. Ten weeks after severance, the total plant dry weight, the total plant leaf area and the length of the primary shoot were proportional to the area of the original leaf, but, were not affected significantly by a 30% reduction of the leaf area. However, root dry weight after 10 weeks was linearly related to the original leaf area, irrespective of the level of reduction. After 21 days of propagation total plant dry weight was linearly related to LAD indicating a direct relation with the integral of photosynthesis over this period. LAD was also linearly related with the number and dry weight of roots after 21 days, and explained at least 50% of the rooting of cuttings after 21 days. Leaf removal during the first seven days of propagation caused stem rot and suggesting that the initial content in reserves was not able to support the basic needs of the cutting. This was confirmed by experiments reported in Chapter 3.2, where it is showed that carbohydrate depletion at the rooting zone of cuttings is associated to increased sensitivity of cuttings to stem rot. Moreover, external application of sugars (e.g. glucose and sucrose) delayed the appearance of stem rot symptoms in leafless cuttings.

Adventitious root formation is a multi-step developmental process, each of which may have different requirements for carbohydrates or hormones. Therefore, the timing of the different rooting phases (initiation and growth) in a controlled environment (23-25°C,

irradiance of 85 μmol $m^{-2}s^{-1}$, 16 h day^{-1}, and without auxin was measured during the first 14 days of propagation. Cambial activity was detected at the basal part of cuttings stem on day 3 by proliferation of undifferentiated cells between the cambium and the sclerenchyma. It was concluded that root initiation occurred mainly between days 3 and 7 but continued until day 11, whereas differentiation into root primordia and further growth of the root primordia until emergence would mainly occur between days 7 and 11. Growth (elongation) of the functional roots and enlargement of the newly formed vessels were the main anatomical events occurring after day 14.

To quantify to what extent severance and rooting influence CO_2 fixation during propagation, net photosynthesis was measured during the first hours and days after severance. Net photosynthesis of leaves on shoots of the mother plant, of leaves of detached shoots and of leaves of cuttings during propagation was measured and gross photosynthesis was estimated. Total respiration of cuttings was measured in order to establish the daily carbon balance. After severance net photosynthesis of cuttings decreased by 75 to 95 %, but partial recovery (up to 70% relatively to leaves on the mother plants) occurred within a few hours. During propagation, net photosynthesis at about 85μmol $m^{-2}s^{-1}$ (PPF) varied between 0.09 and 0.17 mg CO_2 $m^{-2}s^{-1}$. Losses due to respiration varied between 30 to 55% of the daily gross CO_2 uptake, but the daily carbon balance remained positive following severance. Root and shoot tissues accounted for 70% of the increase in total fresh weight after 21 days whereas the remaining 30% increase was due to dry weight accumulation in leaf and stem. A first indication that cuttings were storing photosynthates rather than using them for growth of new organs was given by a decrease in the total fresh weight/total dryweight ratio until day 14 (Chapter 4.2). Cuttings accumulate starch, particularly in the first 7-14 days in the pith and medullar rays of the stem, but less in the regions of the pith close to the basal callus, probably due to higher metabolisation. Glucose and fructose also accumulated in the stem and leaves, whereas levels of sucrose remained constant over time. Root tissues had the highest glucose and fructose concentrations. No radial gradient of carbohydrates (leaf side compared to the opposite side) was detected, but there was a clear basipetal gradient (leaf > upper stem > stem rooting zone).

The photochemistry of the photosynthetic apparatus of the original leaf as influenced by severance and rooting was investigated in Chapter 4.4 using images of the photosystem II efficiency (Φ_{PSII}) calculated from chlorophyll fluorescence imaging. Changes of Φ_{PSII} induced by severance and rooting were recorded during the first hours (0, 2, 4, 6 and 24 hours) or the first days (0, 3, 7, 11, 14 and 21) after severance. In the short term (immediately after severance), Φ_{PSII} decreased by about 20% in response to severance and there was an increase in heterogeneity (patchiness), but leaves re-achieved previous Φ_{PSII} values within 2 hours. In most cases a pronounced decrease of Φ_{PSII} and increased heterogeneity were found after one to two weeks after severance. The leaf veins had higher values of Φ_{PSII} than the neighbouring mesophyll cells. No increase of Φ_{PSII} was observed following rooting. The long-term negative effect of severance or pruning on Φ_{PSII} may be related to the small sink activity of the new-formed organs and carbohydrate accumulation in leaves.

Leaf photosynthesis was investigated as possible major regulator of rooting of leafy stem cuttings of rose in Chapter 5.1. Cuttings were subjected to light integrals varying between 0 and 20MJ m^{-2} and CO_2 concentrations varying between 80-100ppm to 600 ppm and leaf area (LA) varying between 0 and 90 cm^2. Rooting, dry matter accumulation and carbohydrates (starch and glucose, fructose, sucrose) were measured during the first 21 days of propagation. Without light, rooting was not possible whereas at low light integrals (0.2 MJ m^{-2}) or low CO_2 concentrations (80-100 ppm) callus and root formation were delayed and reduced. Higher light integrals and higher CO_2 concentrations hastened rooting and increased the number, and particularly, the dry weight of roots. Reduced photosynthesis and carbohydrate concentrations were directly related to diminished cambial activity in the rooting zone and to diminished callus formation. The number and dry weight of roots were linearly related to the overall dry weight accumulation and thus with the integral of leaf photosynthesis. Although cuttings accumulated starch during propagation, they responded positively to photosynthesis (by forming more and heavier roots). Root initiation and growth were almost proportional to the overall CO_2 fixation during propagation, but even at zero net carbon gain some roots were still initiated (Chapter 5.1).

After observing that leaf photosynthesis during propagation positively influences rooting, we investigated whether the different phases of the rooting process (initiation and growth) would react differently to photosynthesis and to carbohydrates. Cuttings were subjected, in two separate experiments with a duration of 21 days, to low light intensity ($7\mu mol\ m^{-2}s^{-1}$)(PPF) or low CO_2 concentration (80-100ppm) during the first half (LH), the second half (HL) or the whole 21-day period (LL). Controls remained at standard light ($76\mu molm^{-2}s^{-1}$) or standard CO_2 (300-350ppm) during the 21 days of the experiment (HH). Cuttings were observed on day 11 and 21 of propagation. Treatments with low light or CO_2 during the whole period were the most detrimental for callus formation, rooting percentage, number of roots, and dry weight of roots. Generally, the results showed evidence that most of the growth processes occurring in cuttings increased as the integral of photosynthesis increased irrespective of when the integral was decreased. Exceptionally growth of the primary shoot was impaired only by low photosynthesis during the first 11 days of propagation. This has been justified by a delay in response or a longer lag phase compared to rooting. Likewise, root initiation as well as growth of the axillary bud are strong negatively influenced by low photosynthetic activity in the first 11 days of propagation. Root growth is mainly affected by the integral of cutting's photosynthesis. Reserves accumulated during propagation were used efficiently in growth when photosynthesis was strongly reduced.

A general discussion on the relevance of photosynthesis for propagation of leafy stem cuttings is presented in Chapter 6. It is concluded that variation in growth and rooting performance of single node leafy stem cuttings of rose due to variations in the leaf area is mainly explained by the changes in the photosynthetic activity of the original leaf. The apparent contradiction that cuttings accumulated starch, whereas still responding positively to photosynthesis (larger leaf area, higher CO_2 concentration, and higher light integral) is discussed. A possible explanation is that roots and the axillary primary shoot during the first

21 days of propagation are minor sinks relative to the sink activity of the storage cells of the stem or leaves. Another main conclusion, was that the role the initial reserves of the cuttings plays in the quality of the material in terms of growth of roots and the axillary shoot seems irrelevant when compared to the photoassimilates synthesised in leaves. Nevertheless, reserves accumulated during propagation were also efficiently used in growth of roots and in particular of the primary shoot, when photosynthesis was strongly decreased by low light levels or low CO_2.

The results of this research confirm the significance of photosynthesis for propagation of leafy stem cuttings of cut-rose and provides relevant information for optimisation of growth and improving the uniformity of planting material of cut-roses derived form cuttings.

SAMENVATTING

In Nederland worden jaarlijks ongeveer 20 miljoen snijrozenstruiken vermeerderd voor eigen behoefte aan plantmateriaal en export. Vermeerdering door stekken is relatief eenvoudig en goedkoop en wordt daarom algemeen toegepast. Als stek wordt een samengesteld blad met okselknop en onderliggend internodium gebruikt. Het gebruik van rozen op eigen wortel is in Nederland vooral snel toegenomen door de introductie van teelt op kunstmatig substraat (voornamelijk steenwol en cocos en dit beslaat ongeveer 80 % van het huidige teeltareaal van 921 ha).

De laatste jaren is er een goed niveau van vermeerdering bereikt. Toch zijn er nog steeds uitval en onvoldoende uniformiteit in beworteling en verdere groei van stekken. Om de uniformiteit en de efficiëntie te verbeteren is kennis van de belangrijkste ontwikkelingsfactoren van rozenstekken vereist.

Bladeren zijn essentieel voor de vermeerdering van snijrozen door middel van stekken. Zij zijn ook het deel van de stek dat het meest kwetsbaar is voor uitwendige omstandigheden en stress tijdens de vermeerdering. Daarom geeft Hoofdstuk 2 een literatuuroverzicht over de rol van het blad als fotosynthese structuur en als bron van koolhydraten. Er wordt een verklarend model voorgesteld, waarin verband gelegd wordt tussen het oorspronkelijke blad, diens fotosynthese en de beschikbare koolhydraatbronnen voor beworteling en groei. Hierbij gaat het dus vooral om de betekenis van het blad voor de koolhydraatdynamiek (synthese en verdeling) bij rozenstekken.

In Hoofdstuk 3 wordt het kwantitatieve effect beschreven van het oorspronkelijke stekblad op de beworteling en groei van rozenstekken tot 10 weken na knippen van de stek van de moederplant. Deze analyse werd uitgebreid met het concept: blad oppervlak duur (in het Engels: leaf area duration, afgekort tot LAD). Dit begrip LAD wordt gedefinieerd als: bladoppervlak x aantal dagen dat het blad aan de stek zit. In fysiologische termen geeft dit de totale potentiële fotosynthese van de stek tijdens de vermeerdering aan. Tien weken na het stekken was er een positieve correlatie tussen het totale drooggewicht van de plant, diens totale bladoppervlak en primaire scheutlengte met het oppervlak van het oorspronkelijke stekblad. Echter 30 % reductie van het stekblad gaf geen significant effect. Wel had het drooggewicht van de wortels na tien weken een lineaire relatie met het oppervlak van het stekblad, ongeacht het niveau van reductie. Drie weken na het stekken was er een lineair verband tussen het drooggewicht van de plant en de LAD. Dit wijst op een direct verband met de integraal van de fotosynthese gedurende deze periode. De LAD bleek ook een lineair verband met aantal en drooggewicht van de wortels na drie weken te geven en kon tenminste 50% van de beworteling van stekken tijdens deze periode verklaren. Bladverwijdering tijdens de eerste zeven dagen van de vermeerdering veroorzaakte stengelrot. Dit is een aanwijzing, dat het oorspronkelijke niveau van reserves niet in staat is om te voorzien in de basisbehoefte van de stek. Dit kon worden bevestigd in experimenten, die in Hoofdstuk 3.2 beschreven zijn. Hier wordt aangetoond dat de uitputting van koolhydraten in de bewortelingszone gepaard gaat met toegenomen gevoeligheid voor stengelrot van de rozenstek. Bovendien kon door

externe toediening van suikers (glucose en saccharose) het optreden van stengelrot van stekken zonder blad worden vertraagd.

Adventieve wortelvorming kent meerdere stappen van ontwikkelingsprocessen, die ieder verschillende behoefte aan koolhydraten of hormonen kunnen hebben. Daarom werd de tijdsduur van de verschillende bewortelingsfasen (initiatie en groei) waargenomen tijdens de eerste twee weken van de beworteling onder gecontroleerde omstandigheden (23-25°C, een lichtniveau van 85 μmol $m^{-2}s^{-1}$, 16 h dag^{-1}, en zonder auxine). Cambiumactiviteit in de basale stengelwond kon worden waargenomen op dag drie door deling van ongedifferentieerde cellen tussen cambium en sclerenchym. Geconcludeerd wordt, dat de wortelinitiatie vooral gebeurde tussen dag drie en zeven maar doorging tot dag 11, terwijl differentiatie in wortelprimordia en verdere groei van deze primordia tot aan naar buiten groeien voornamelijk gebeurt tussen dag 7 en 11. Groei (lengtegroei) van de wortels en vergroting van de nieuwgevormde vaten waren de belangrijkste anatomische gebeurtenissen vanaf dag 14.

De netto fotosynthese werd gemeten tijdens de eerste uren en dagen na knippen van de stekken om te kwantificeren in welke mate scheiding van de moederplant en beworteling de CO_2 fixatie beïnvloeden. De netto fotosynthese van bladeren van scheuten aan de moederplant, van bladeren van afgeknipte scheuten, en van bladeren van stekken tijdens de vermeerdering werd gemeten en de bruto fotosynthese werd geschat. Ook werd de totale ademhaling van stekken gemeten om de dagelijkse koolstofbalans vast te stellen. Na het knippen nam de netto fotosynthese van stekken af met 75-95%, maar een gedeeltelijk herstel (tot 70% van het niveau van bladeren aan de moederplant) trad op binnen enkele uren.

Tijdens de vermeerdering varieerde de netto fotosynthese tussen 0.09 en 0.17 mg CO_2 $m^{-2}s^{-1}$ bij een lichtniveau van ongeveer 85 μmol $m^{-2}s^{-1}$ (PPF). Ademhalingsverliezen varieerden tussen 30 tot 55 % van de dagelijkse bruto CO_2 opname, maar de dagelijkse koolstofbalans bleef positief vanaf het knippen van de moederplant. Wortel en scheutweefsels waren voor 70% verantwoordelijk voor de toename in versgewicht na 21 dagen, terwijl de overige 30 % veroorzaakt werd door toename van het drooggewicht van blad en stengel van de oorspronkelijke stek. Een eerste aanwijzing, dat stekken eerder assimilaten opslaan dan gebruiken voor de groei van nieuwe organen werd gegeven door een daling van de totale verse/drooggewicht verhouding tot aan dag 14 (Hoofdstuk 4.2). Stekken slaan zetmeel op, vooral gedurende de eerste 7-14 dagen, in het merg en de mergstralen van de stengel. Dit gebeurt in mindere mate in het merggedeelte dichtbij het basale callus, waarschijnlijk door een intensievere stofwisseling. Glucose en fructose hopen zich ook op in stengel en bladeren, terwijl de niveaux van saccharose in de tijd constant blijven. Wortelweefsels hadden de hoogste glucose en fructose concentraties. Er kon geen radiale gradiënt van koolhydraten (de zijde van het blad vergeleken met de overstaande zijde) worden aangetoond, maar wel was er een duidelijke basipetale gradiënt (blad > bovendeel van de stengel > bewortelingszone).

In Hoofdstuk 4.4 werd de fotochemie van het fotosynthese apparaat van het stekblad onderzocht. Hierbij werd gebruik gemaakt van beelden van de fotosysteem II efficiëntie Φ_{PSII} die berekend werd vanuit de chlorofyl fluorescentie beeldvorming. Veranderingen van de Φ_{PSII}, die geïnduceerd waren door de verwijdering van de moederplant en beworteling werden

geregistreerd gedurende de eerste uren (0, 2, 4, 6 en 24 uur) of de eerste dagen 0, 3, 7, 14, en 21) vanaf verwijdering. Op korte termijn (direct na verwijdering) daalde de Φ_{PSII} met ongeveer 20 % als reactie op de isolatie en er was een toename van de heterogeniteit ("patchiness"), maar de bladeren bereikten de oorspronkelijke waarden van de Φ_{PSII} weer binnen twee uren. In de meeste gevallen werd er een uigesproken daling van de Φ_{PSII} en toename van de heterogeniteit gevonden vanaf 1-2 weken na stekken. De vaatbundels van de bladeren hadden hogere waarden voor de Φ_{PSII} dan de aangrenzende mesofylcellen. Na de beworteling werd er geen toename van de Φ_{PSII} gevonden. De negatieve invloed op de lange termijn van isolatie of snoei op de Φ_{PSII} zou kunnen samenhangen met de geringe sink activiteit van de nieuw gevormde organen en de opslag van koolhydraten in de bladeren.

In Hoofdstuk 5.1 werd de bladfotosynthese als mogelijke hoofd regulator voor de beworteling van rozenstekken onderzocht. Stekken ontvingen lichthoeveelheden variërend van 0-20 MJm^{-2} en CO_2 concentraties variërend van 80-100 ppm en 600 ppm en bladoppervlaktes (LA) variërend van 0-90 cm^2. Beworteling en toename van drogestof en koolhydraten (zetmeel, glucose, fructose en saccharose) werden gemeten gedurende de eerste 21 dagen van het vermeerderingsproces. Zonder licht bleek beworteling niet mogelijk, terwijl bij lage lichtniveaux (0.2 MJ m^{-2}) of lage CO_2 concentraties (80-100 ppm) de callusvorming en de beworteling werden vertraagd en beperkt. Hogere lichtniveaux en hogere CO_2 concentraties versnelden de beworteling en bevorderden het aantal en vooral het drooggewicht van de wortels. Gereduceerde fotosynthese en koolhydraatconcentraties waren direct gerelateerd aan afgenomen cambiumactiviteit en callusvorming in de bewortelingszone. Het aantal en drooggewicht van de wortels bleek een lineair verband te vertonen met de totale drooggewicht toename en dus met de integraal van de bladfotosynthese. Hoewel stekken zetmeel opslaan tijdens het vermeerderingsproces, reageren ze positief op toegenomen fotosynthese (door de vorming van meer en zwaardere wortels). Wortelinitiatie en groei waren bijna evenredig met de totale CO_2 fixatie tijdens de vermeerdering, maar zelfs op een niveau van nul koolstoffixatie werden er toch enkele wortels geïnitieerd.

Na de waarneming, dat de bladfotosynthese tijdens de vermeerdering de beworteling positief beïnvloedt, onderzochten we of de verschillende fasen van het bewortelingsproces (initiatie en groei) verschillend zouden reageren op fotosynthese en koolhydraten. In twee aparte experimenten werden stekken gedurende 21 dagen behandeld met laag lichtniveau (7 $\mu mol\ m^{-2}s^{-1}$) of lage CO_2 concentratie (80-100ppm) gedurende de eerste helft (LH), de tweede helft (HL) of de gehele periode van 21 dagen (LL). De controles stonden bij standaard licht (76 $\mu mol\ m^{-2}s^{-1}$) of standaard CO_2 (300-350ppm) tijdens de 21 dagen van het experiment (HH). De stekken werden waargenomen op dag 11 en dag 21 tijdens de vermeerdering.

De behandelingen met lage lichten CO_2 niveaux tijdens de gehele periode (LL) werden het meest benadeeld voor callusvorming, bewortelingspercentage, aantal wortels en drooggewicht van de wortels. Over het algemeen gaven de resultaten een aanwijzing, dat de meeste van de groeiprocessen, die in de stekken plaatsvinden bevorderd worden bij een toename van de integraal van de fotosynthese, onafhankelijk van het moment, waarop deze

integraal daalt. De groei van de primaire scheut werd vooral geremd bij lage fotosynthese tijdens de eerste 11 dagen. Dit kan worden verklaard door een uitgestelde reactie (bij een laag fotosynthese niveau in de tweede helft) of een langere werkingsduur, vergeleken met beworteling. Bovendien werden zowel wortelinitiatie als groei van de okselknop sterk negatief beïnvloed door lage fotosynthese activiteit tijdens de eerste 11 dagen van het vermeerderingsproces. Wortelgroei wordt voornamelijk beïnvloed door de integraal van de fotosynthese van de stek. Reserves, die tijdens de vermeerdering waren opgeslagen werden efficiënt gebruikt voor de groei, wanneer de fotosynthese onderdrukt werd.

Een algemene discussie over de betekenis van de fotosynthese voor vermeerdering van stekken met bladeren wordt gegeven in Hoofdstuk 6. Geconcludeerd wordt, dat variatie in beworteling en groei van rozenstekken met blad als gevolg van variatie in oppervlak van het stekblad hoofdzakelijk verklaard kan worden door veranderingen in de fotosynthese activiteit van dit oorspronkelijke blad. De ogenschijnlijke tegenstrijdigheid dat stekken zetmeel opslaan en toch positief reageren op toename van de fotosynthese (groter blad oppervlak, hoger lichtniveau en hogere CO_2 concentratie) wordt bediscussieerd. Een mogelijke verklaring is dat wortels en de primaire okselscheut tijdens de eerste 21 dagen van het vermeerderingsproces zwakke sinks vormen ten opzichte van de sink sterkte van de opslagcellen van de stengel en het blad. Een andere hoofd-conclusie is, dat de betekenis van de aanvankelijk aanwezige reserves van de stek, voor de kwaliteit van het materiaal in de zin van wortel- en scheutgroei, irrelevant is ten opzichte van de assimilaten, die gevormd worden in het blad tijdens het vermeerderingsproces. Toch werden reserves, die tijdens de vermeerdering waren opgeslagen ook efficient gebruikt voor de groei van wortels en vooral van de primaire scheut, wanneer de fotosynthese onderdrukt werd door bladverwijdering of verlaagde licht- of CO_2 niveaux.De resultaten van dit onderzoek bevestigen de betekenis van fotosynthese voor de vermeerdering van rozenstekken met blad en verstrekt relevante informatie voor optimalisatie van de groei en verbetering van de uniformiteit van plantmateriaal voor snijrozen afkomstig van stekken.

RESUMO

Na Holanda cerca de 20 milhões de roseiras são propagadas anualmente para satisfazer as necessidades de material de plantação do sector produtor de rosa cortada holandês e estrangeiro. A propagação de roseira por estacas caulinares com folhas é um método fácil e barato, e por isso, habitualmente utilizado. Paralelamente, o uso de plantas derivadas de estacas enraizadas (não enxertadas), cresceu rapidamente nos últimos anos na Holanda em consequência da expansão do cultivo em substrato (ex. lã de rocha, fibra de coco) o qual representa actualmente 80% dos 921 hectares destinados à produção de rosa para corte.

Embora as taxas de sucesso alcançadas na propagação de roseira por estaca tenham atingido níveis elevados, as perdas de material e a falta de uniformidade no enraizamento das estacas, e posterior crescimento das planta jovens verificam-se ainda na prática. Deste modo, para aumentar a uniformidade e a eficiência na propagação é necessário aprofundar o conhecimento sobre os principais mecanismos e factores reguladores do crescimento das estacas. As folhas são um orgão imprescindível na propagação de roseira por estacas caulinares, mas são também a parte mais sensível da estaca à acção de factores externos e ao stress imposto durante a propagação. No Capítulo 2 faz-se uma revisão bibliográfica sobre o papel das folhas na propagação abordando em pormenor o efeito da actividade fotossintética e dos hidratos de carbono no enraizamento. No mesmo capítulo, é também proposto um modelo conceptual para relacionar a folha original de estacas caulinares (semi-lenhosas e com um gomo axilar), a sua actividade fotossintética e as "pools" de hidratos de carbono disponíveis com o enraizamento e o crescimento das estacas. O modelo tenta também descrever o papel da dinâmica dos hidratos de carbono (síntese e partição) na propagação.

No Capítulo 3 o efeito da folha original no enraizamento e crescimento das estacas de roseira é analisado e quantificado até 10 semanas após a colheita das estacas da planta mãe. Neste estudo aplicou-se o conceito de duração da área foliar (DAF) que é definido pelo produto entre a área foliar e o número de dias durante os quais a folha subsiste na estaca Em termos fisiológicos, este conceito pode ser considerado como um indicador do potencial fotossintético da estaca durante a propagação. Dez semanas após colheita, o peso seco total, a área foliar total bem como o comprimento do lançamento axilar primário das plantas revelaram-se proporcionais à área da folha original da estaca, embora reduções da ordem dos 30% da área não diminuíssem significativamente o crescimento das plantas comparativamente com a testemunha. Contudo, o peso seco das raízes, manteve-se linearmente relacionado com a área da folha original independentemente do nível de redução.

Foi também encontrada uma relação linear significativa entre o peso seco total das plantas e a DAF vinte e um dias após a colheita das estacas,, o que indica uma forte relação entre o integral de fotossíntese durante a propagação e o crescimento das estacas nesse período. A variação observada no enraizamento (número e peso seco das raízes) foi explicada em cerca de 50% pela variação da DAF. A remoção da folha durante a primeira semana de propagação causou o apodrecimento basal das estacas sugerindo que o nível inicial de reservas não era suficiente para suportar a manutenção da estaca. Esta hipótese foi confirmada

no Capítulo 3.2, onde se demonstrou que a exaustão dos hidratos de carbono está estreitamente associada ao aumento da sensibilidade da estaca ao apodrecimento basal . Além disso, a aplicação externa de soluções açucaradas de glicose e sacarose, atrasou o apodrecimento de estacas desprovidas da folha original.

O enraizamento adventício é um processo composto por várias fases, cada uma das quais com necessidades específicas em hidratos de carbono e/ou hormonas. Por isso, a sequência dos diferentes eventos e alterações anatómicas relacionadas com a iniciação e crescimento radiculares, foi estudada durante os primeiros 14 dias de propagação em estacas propagadas à temperatura de 23-25°C, intensidade luminosa de 85 μmol m^{-2} s^{-1}, fotoperíodo de 16 h e sem qualquer tratamento com auxinas. A actividade cambial na zona basal da estaca foi detectada ao terceiro dia de propagação, através da proliferação de células indiferenciadas (callus) entre a zona cambial e o esclerênquima. Concluiu-se que a iniciação radicular ocorre preferencialmente entre os dias 3 e 7 de propagação, podendo prolongar-se até ao dia 11. A diferenciação dos primórdios radiculares, até à emergência, ocorre principalmente entre os dias 7 e 11 de propagação enquanto que o alongamento das raízes e o crescimento em diâmetro dos novos vasos xilémicos são os principais eventos anatómicos a ocorrer depois do dia 14.

De forma a quantificar os efeitos da colheita das estacas da planta mãe e da formação de raízes na actividade fotossintética das estacas, a taxa de fotossíntese aparente foi determinada durante as primeiras horas e dias de propagação. As medições efectuaram-se sequencialmente em folhas de lançamentos florais da planta mãe, em folhas de lançamentos florais destacados da planta mãe e na folha original das estacas. Nas estacas, as medições foram feitas durante os primeiros 21 dias de propagação. As taxas de fotossíntese real foram estimadas igualmente. A respiração total das estacas foi medida durante a propagação e o balanço de hidratos de carbono estimado. A taxa de fotossíntese aparente diminuiu cerca de 75 a 95% imediatamente após colheita, embora tal fosse seguido por uma recuperação parcial (até cerca 70% dos valores medidos em folhas da planta mãe) durante as primeiras horas de propagação. Durante a propagação e à intensidade luminosa de 85μmol m^{-2} s^{-1} (PPF), os valores médios da taxa de fotossíntese aparente oscilaram entre 0.09 e 0.17 $mgCO_2$ m^{-2} s^{-1}. As perdas de carbono por respiração variaram entre 30 e 55% da fotossíntese real embora o balanço de hidratos de carbono se mantivesse positivo ao longo dos 21 dias de propagação. Cerca de 70% do aumento do peso fresco total ao fim de 21 dias, foi devido ao aumento de peso das raízes e do lançamento axilar, cabendo os restantes 30% à acumulação de peso seco na folha e no caule da estaca. Uma primeira indicação de que as estacas estariam a acumular fotoassimilados em vez de os aplicarem no crescimento de novos orgãos, foi dada pela diminuição do rácio entre o peso fresco total e o peso seco total (Capítulo 4.2). Efectivamente, as estacas acumularam amido na zona medular assim como nos raios medulares do caule, principalmente durante os primeiros 14 dias de propagação. A acumulação de amido foi menos intensa nas regiões da medula central localizadas na vizinhança do *callus* formado na base da estacas. A concentração de sacarose nos tecidos foliares e do caule permaneceu constante durante a propagação e os tecidos radiculares

apresentaram as concentrações mais elevadas de glicose e frutose. Não foi observado qualquer gradiente radial de hidratos de carbono (entre a zona do caule do lado da folha e a zona oposta) embora fosse evidente a existência de um gradiente basipétala (folha >caule> zona de enraizamento do caule).

No Capítulo 4.4. investigou-se o efeito da colheita e do enraizamento na fotoquímica do aparelho fotossintético da folha original de estacas de roseira. Para isso, analisaram-se imagens da eficiência do fotossistema II (Φ_{PSII}) calculadas a partir de imagens da fluorescência clorofilina. As alterações dos níveis de eficiência do fotossistema II (PSII) induzidas pela colheita da estaca e posterior formação de raízes, foram medidas durante as primeiras horas (0, 2, 4, 6 e 24 horas) ou primeiros dias (0, 3, 7, 11, 14 e 21) de propagação. Imediatamente após a colheita da estaca (a curto prazo) registou-se uma diminuição em cerca de 20% dos valores médios do Φ_{PSII} com o aumento simultâneo da heterogeneidade na distribuição do Φ_{PSII}. Todavia, 2 horas após a colheita, os valores iniciais de Φ_{PSII} foram novamente atingidos. Uma ou duas semanas após colheita (a longo prazo), os valores de Φ_{PSII} encontrados foram na generalidade mais baixos e mais heterogéneos comparativamente com os valores médios e distribuição observados nos primeiros dias de propagação. As nervuras foliares apresentaram valores mais elevados de Φ_{PSII} que as células vizinhas do mesófilo foliar e não se observou qualquer aumento da eficiência do fotossistema II em resposta à formação de raízes. O efeito negativo a longo prazo da colheita da estaca, ou da poda dos lançamentos florais, no Φ_{PSII} pode estar relacionada com a redução da actividade de "sink" e consequente acumulação de hidratos de carbono nas folhas.

No Capítulo 5.1, a actividade fotossintética da folha original de estacas caulinares semi-lenhosas de roseira foi estudada para testar a hipótese da fotossíntese da folha original ser o principal mecanismo regulador do enraizamento. Para isso, as estacas foram submetidas a diferentes integrais de luz (entre 0 e 20 MJm^{-2}), diferentes concentrações de CO_2 (entre 80-100 ppm e 600 ppm), assim como a diferentes níveis de redução da área foliar (LA) (entre 0 e 90cm^2). O enraizamento e a acumulação de matéria seca e de hidratos de carbono (amido, glicose, frutose e sacarose), foram medidos durante os primeiros 21 dias de propagação. Sem luz as estacas não enraizaram, enquanto que integrais de luz reduzidos (0.2 MJm^{-2}) ou baixas concentrações de CO_2 (80-100 ppm), diminuiram ou atrasaram significativamente a formação de callus e raízes. Integrais de luz maiores e concentrações de CO_2 mais elevadas aceleraram e aumentaram o número, e principalmente, o peso seco das raízes. A redução da actividade fotossintética em consequência das baixas concentrações de CO_2 diminuiu a actividade cambial na zona de enraizamento da estaca e consequente a proliferação de callus. Quer o número quer o peso seco das raízes estiveram linearmente relacionados com a acumulação total de matéria seca nas estacas durante o período de propagação e, consequentemente, com o integral da actividade fotossintética da estaca durante os primeiros 21 dias após a sua colleita. Embora ocorresse acumulação de amido nas estacas durante o período de propagação, houve uma resposta positiva ao aumento da actividade fotossintética traduzida na formação de um maior número de raízes e de um sistema radicular mais pesado. A iniciação radicular e o crescimento das raízes foram quase proporcionais à fixação de CO_2 durante o

período de propagação (expressa pela acumulação total de peso seco nos 21 dias). Contudo, a formação de raízes ocorreu mesmo quando não se registou qualquer aumento de peso seco.

Demonstrado o efeito regulador da actividade fotossintética das folhas no enraizamento das estacas caulinares de roseira com folhas, investigou-se no Capitulo 5.2 a possibilidade das diferentes fases do enraizamento (iniciação e crescimento) reagirem diferentemente à actividade fotossintética da estaca e à disponibilidade de hidratos de carbono. Desta forma, dois ensaios, cada um com a duração de 21 dias, foram levadas a cabo, submetendo-se as estacas ou a baixas intensidades luminosas (7 $\mu mol^{-1}m^{-2}s^{-1}$) (Ensaio 1) ou a baixas concentrações de CO_2 (80-100 ppm) (Ensaio 2) durante a primeira metade do período de propagação (LH), durante a segunda metade (HL) ou durante todo o período de propagação (LL). As testemunhas (HH) permaneceram a níveis normais de luz (85$\mu mol\ m^{-2}\ s^{-1}$, PPF) ou de CO_2 (300-350ppm) durante os 21 dias. As estacas foram observadas nos dias 11 e 21 da propagação. Intensidade luminosas reduzidas ou baixas concentrações de CO_2 durante todo o período de propagação tiveram o efeito mais negativo na formação de callus, na percentagem de enraizamento, no número e peso seco de raizes. O crescimento das estacas foi em geral, regulado pelo integral da actividade fotossintetica, dada a acumulação de peso seco ser idêntica nos tratamentos HL e LH. A excepção coube ao crescimento do gomo axilar primário, o qual foi fortemente reduzido quando a actividade fotossintética foi limitada durante os primeiros 11 dias de propagação. Isto foi justificado por um atraso na resposta à síntese de fotoassimilados consequência de uma "lag phase" mais prolongada que a do enraizamento. A iniciação radicular tal como o crescimento do gomo axilar são negativamente afectados por uma reduzida actividade fotossintética durante os primeiros 11 dias de propagação enquanto que o crescimento das raizes, expresso em termos do seu peso seco, é afectado pelo integral da actividade fotossintética. Outro resultado igualmente relevante foi o facto das reservas acumuladas durante a propagação poderem ser usadas eficientemente no crescimento em situações em que a fotossíntese das estacas é drasticamente reduzida .

A importância da fotossíntese para a propagação de roseira por estacas caulinares semi lenhosas com folhas é discutida no Capítulo 6. Neste capítulo é concluído que a variação obtida no enraizamento e posterior crescimento das estacas, é largamente explicada pôr variações da actividade fotossintética da folha original. A aparente contradição entre o facto das estacas acumularem amido, e ainda assim, responderem positivamente a aumentos da capacidade fotossintética (maior área foliar, maiores integrais de luz, concentrações mais elevadas de CO_2), é discutida. Uma possível razão reside no facto das raizes e do lançamento primário actuarem como "sinks" menores nos primeiros 21 dias de propagação comparativamente à capacidade de "sink" das células armazenadoras de amido existentes nas folhas e caule. Outra importante conclusão é a de que as reservas iniciais, isto é, as reservas existentes à altura da colheita, terem um efeito muito limitado no crescimento das estacas e consequentemente na futura qualidade do material de plantação (estacas enraizadas) quando comparado com o efeito dos fotoassimilados sintetizados durante a propagação. Mesmo assim, as reservas acumuladas durante a propagação foram eficientemente usadas no

crescimento de raízes, e em particular, do lançamento primário, especialmente quando a actividade fotossintética é praticamente suprimida.

Os resultados desta investigação confirmam a importância do processo fotossíntetico para o sucesso na propagação de estacas caulinares semi-lenhosas de roseira e disponibiliza informação que poderá permitir a optimização do crescimento das estacas durante a propagação e assim aumentar a uniformidade do material de plantação de roseira para flor de corte derivado de estacas enraizadas.

RESUMEN

En los Países Bajos cada año se propagan aproximadamente 20 millones de rosales con el fin de cubrir las necesidades de material de plantación de rosa cortada del sector holandés y extranjero. La propagación de rosales mediante el uso de estacas de tallo con hojas es relativamente fácil y barata y por eso, comúnmente usada. Además, el uso de plantas derivadas de estacas enraizadas se ha extendido rápidamente en los Países Bajos con la introducción del cultivo en sustrato (fibra de coco y lana de roca), lo que se estima representa aproximadamente un 80% de las 921 ha dedicadas actualmente al cultivo de rosa para corte.

A pesar del elevado éxito alcanzado en la propagación que se ha llevado a cabo en los últimos años, todavía se producen pérdidas y falta de uniformidad en el enraizamiento y crecimiento de las estacas y material de plantación. Para incrementar la uniformidad y la eficacia de la propagacion de rosales, se requiere un conocimiento más profundo de los principales factores y mecanismos reguladores del crecimiento de las estacas.

Las hojas son esenciales en la propagación de rosales por estacas de tallo semi-leñoso y éstas son también la parte de la estaca más vulnerable a factores externos y stress durante la propagación. Por eso, en el Capítulo 2 se presenta una revisión de la literatura sobre la función de las hojas en el proceso de enraizamiento, enfocando el papel de la fotosíntesis y las "pools" de hidratos de carbono disponibles para el enraizamiento. En el mismo capítulo se propone un modelo conceptual para relacionar la hoja original de estacas de tallo semi-leñoso con una yema, su fotosíntesis, y las "pools" de hidratos de carbono disponibles, con el enraizamiento y el crecimiento. El modelo intenta también describir el papel de la dinámica de los hidratos de carbono (síntesis, división) en la propagación.

En el capítulo 3 se describe el efecto cuantitativo de la hoja original en el enraizamiento y crecimiento de la estaca hasta un máximo de 10 semanas después de la cosecha de la estaca de la planta madre. Este análisis se desarrolló usando el concepto de duración del área foliar (DAF), definido como el área de la hoja x el número de días que la hoja permanece en la estaca. En términos fisiológicos es una indicación del potencial total de la estaca para la fotosíntesis durante la propagación. Diez semanas después de la cosecha, el peso seco total de la planta, el área foliar total de la planta y la longitud del primer retoño fue proporcional al área de la hoja original, así como la reducción del área de la hoja en un 30% no afectó significativamente el crecimiento de la planta comparada con el testigo. Sin embargo, el peso seco de la raíz después de 10 semanas fue linearmente relacionado con el área de la hoja del original, independiente del nivel de reducción. Después de 21 días de propagación el peso seco total de la planta fue linearmente relacionado con DAF lo que indicó una relación directa con la integral de fotosíntesis en este período. El DAF también fue relacionado linearmente con el número de raíces y su peso seco después de 21 días, explicando al menos el 50% de la variación en el enraizamiento de las estacas después de 21 dias de propagación.

El retirar la hoja durante los primeros siete días de propagación causó pudrición de la base del tallo, lo que sugiere que el contenido inicial de reservas no fue suficiente para suplir las necesidades básicas de la estaca. Esto hecho fue confirmado con los experimentos

descritos en el Capítulo 3.2, donde se muestra la asociación entre el agotamiento de carbohidratos en la zona de enraizamiento de las estacas y un incremento en la susceptibilidad del tallo a la pudrición. Además, la aplicación externa de azúcares (por ejemplo glucosa y sacarosa) retardó la aparición de síntomas de pudrición en el tallo en alas estacas sin hoja.

El proceso de enraizamiento adventicio es un proceso compuesto por varias fases, cada una de las cuales presenta requerimientos específicos en carbohidratos y/u hormonas. Por esto, la secuencia temporal de las diferentes fases de enraizamiento (iniciación y crecimiento) fue observada en estacas propagadas en un ambiente controlado (23-25°C, irradiación de 85 μmol $m^{-2}s^{-1}$ durante 16 h $día^{-1}$ y sin tratamiento con auxina) durante los primeros 14 días de propagación. La actividad cambial fue detectada en la parte basal del tallo en el día 3 por proliferación de las células indeferenciadas entre el cambium y el esclerénquima. Se concluyó que el processo de iniciacion radicular ocurrió principalmente entre los días 3 y 7 pero continuó hasta el día 11, mientras que la diferenciación en el primordio radicular y crecimiento hasta la emergencia, ocurrió principalmente entre los días 7 y 11. El crecimiento (elongación) de las raíces y alargamiento de los nuevos vasos xilemáticos fueron el principal evento que ocurrió después del día 14.

Para cuantificar la magnitud de la influencia de la cosecha (corte) de la estaca de la planta madre en el enraizamiento y en la actividad fotosintética de las estacas durante la propagación, la tasa de fotosíntesis aparente (neta) fue medida durante las primeras horas y días de propagación. La fotosíntesis neta de las hojas en los tallos florales de la planta madre, de hojas de tallos desprendidas de la planta madre y de hojas de estacas fueron determinadas durante la propagación. La fotosíntesis real fue igualmente estimada. La respiración total de las estacas fue medida para establecer el balance diario del carbono.Después de la cosecha, la tasa de fotosíntesis neta de las estacas decreció cierca de 75% al 95% pero se recuperó parcialmente (sobre el 70% en las hojas de la planta madre) en unas pocas horas. Durante la propagación, la tasa de fotosíntesis neta varió entre 0.09 y 0.17 mg de CO_2 $m^{-2}s^{-1}$ con una irradiancia alrededor de 85 μmol $m^{-2}s^{-1}$ (PPF). Las pérdidas debido a la respiración variaron entre 30 a 55% de la fijación diaria real de CO_2, pero el balance diario del carbono permaneció positivo durante la propagación. Después de 21 días de propagación, los tejidos de las raíces y los tallos aportaron el 70% del incremento en el peso fresco total mientras que el 30% restante se debió a la acumulación de peso seco en la hoja y tallo de la estaca. Una primera indicación de que las estacas estuvieron almacenando fotoasimilados en lugar de usarlos para el crecimiento de nuevos órganos fue dada por la disminución en la relación entre el peso fresco total y el peso seco total hasta el día 14 (Capitulo 4.2). Las estacas acumularon almidón, particularmente en los primeros 7-14 días en la médula y radios medulares del tallo, pero menos en las regiones de la médula cercanas al callo basal, probablemente debido a una mayor metabolización. La glucosa y la fructosa también se acumularon en los tallos y hojas, mientras que los niveles de sucrosa permanecieron constantes a través del tiempo. Los tejidos radiculares presentaron las concentraciones de glucosa y fructosa más altas. No se detectó un gradiente radial de carbohidratos (un lado de la hoja comparado con el lado opuesto), pero hubo un claro gradiente basipetalo (hoja>tallo superior>zona de enraizamiento).

En el Capítulo 4.4. se estudió el efecto de la cosecha de las estacas de la planta madre y del enraizamiento en la fotoquímica del aparato fotosintético de la hoja original usando imágenes de la eficiencia del fotosistema II (Φ_{PSII}) calculado en función de las imágenes de la fluorescencia clorofílica. Cambios del Φ_{PSII} inducidos por el corte y el enraizamiento fueron registrados durante las primeras horas (0, 2, 4, 6 y 24 horas) o primeros días (0, 3, 7, 11, 14 y 21) después de la cosecha. Inmediatamente después de la cosecha (a corto plazo) la Φ_{PSII} decreció en un 20%, a la vez que hubo un incremento en la heterogeneidad de la distribución del Φ_{PSII}, pero los valores iniciales de la eficiencia del fotosistema II fueron obtenidos dentro de 2 horas. Una o dos semanas después de la cosecha (a largo plazo) los valores observados del Φ_{PSII} fueron generalmente más bajos y la heterogeneidad fue mayor que al inicio de la propagación. Las nervaduras de las hojas tuvieron valores más altos del Φ_{PSII} que las células mesófilas cercanas. No se observó un incremento del Φ_{PSII} en respuesta a la formación de raíces. El efecto negativo de largo plazo de la cosecha de la estaca en los valores del Φ_{PSII} puede estar relacionado con la reducida actividad del "sink" de los nuevos órganos formados y con la acumulación de carbohidratos en las hojas.

En el capitulo 5.1, la fotosíntesis de las hojas de las estacas fue investigada para probar la hipótesis de que la actividad fotosintética es el mecanismo más importante como regulador del enraizamiento de estacas de tallos semi-leñosos de rosales. Las estacas fueron sometidas a variaciones de luz entre 0 y 20MJ m^{-2} y a concentraciones de CO_2 que variaron entre 80, 100 y 600 ppm, mientras que el área de la hoja (LA) varió entre 0 y 90 cm^2. El porcentaje de estacas con pudrición, la acumulación de materia seca y la acumulación de carbohidratos (almidón, glucosa, fructosa y sucrosa) fueron determinados durante los primeros 21 días de propagación. Sin luz no fue posible el enraizamiento mientras que con bajas intensidades de luz (0.2 MJm^{-2}), o bajas concentraciones de CO_2 (80 a 100ppm), la formación de callos y de raíces fue retrasada y reducida. Mayores intensidades de luz y mayores concentraciones de CO_2 aceleraron el enraizamiento e incrementaron el número, y particularmente, el peso seco de las raíces. La reducida fotosíntesis y las bajas concentraciones de carbohidratos estuvieron relacionadas directamente con la disminución de la actividad cambial en la zona de enraizamiento así como de la formación de callos. El número y peso seco de raíces fue relacionado linearmente con la acumulación total de materia seca, y consecuentemente, con la integral de la actividad fotosintética durante la propagación. Aunque las estacas acumularon almidón durante la propagación, el proceso de iniciación radicular (expresado por el número de raíces) y el crecimiento radicular (expresado en peso de las raíces) fueron casi proporcionales a la fijación total de CO_2 durante la propagación, aunque algunas raíces se formaron con un nivel cero de ganancia de carbono(Capitulo 5.1).

Después de observar que la fotosíntesis de las hojas durante la propagación influye positivamente en el enraizamiento, se investigó si las diferentes fases del proceso de enraizamiento (iniciación y crecimiento) reaccionarían diferentemente a la fotosíntesis y los carbohidratos. Para eso, las estacas fueron estudiadas en dos experimentos separados, con una duración de 21 días cada uno, a baja irradiancia (7 $\mu mol\ m^{-2}s^{-1}$)(PPF) o a bajas

concentraciones de CO_2 (80-100ppm) durante la primera mitad (primeros 11 días) (LH), la segunda mitad (HL) o durante todo el periodo de 21 días (LL). El testigo permaneció a niveles normales de irradiancia (76 $\mu mol\ m^{-2}s^{-1}$) y CO_2 (300-350) ppm durante los 21 días del experimento (HH). Las estacas fueron observadas en el día 11 y 21 de propagación.

Los tratamientos con baja intensidad de luz o baja concentración de CO_2 durante todo el periodo de propagación fueron los más perjudiciales para la formación de callos y para el porcentaje de enraizamiento, el numero de raíces y el peso seco de las mismas. Los resultados mostraron en general evidencia de que el crecimiento de las estacas es regulado por la integral de la fotosíntesis sin considerar cuando esta decreció. La excepción se debe al crecimiento excepcional de la yema axilar (tallo primario) aunque fue particularmiente debilitado por una baja actividad fotosintética en los primeros 11 días de propagación. Esto ha sido justificado por un retraso en respuesta a la síntesis de fotoasimilados a causa de una fase "lag" más larga que la del enraizamiento. El proceso de iniciación radicular así como el desarrollo de las yemas axilares estuvieron fuertemente influidos de forma negativa por la baja actividad fotosintética observada en los primeros 11 días de propagación mientras que el crecimiento de las raíces fue afectado principalmente por la integral de la fotosíntesis durante la propagación. También se observó que las reservas acumuladas durante la propagación fueron usadas eficientemente en el crecimiento cuando la fotosíntesis fue suprimida.

La importancia de la fotosíntesis para la propagación de rosales por estacas de tallo semileñosos en una yema axilar es discutida en el Capitulo 6. Se concluye que la variación en la formación de raíces y posterior crecimiento de estacas es en gran parte explicada por variaciones de la capacidad fotosintética de la hoja original. Se discute la aparente contradicción de que las estacas acumularan almidón mientras todavía estaban respondiendo positivamente a aumentos de la capacidad fotosintética (mayor área foliar, mayor concentración de CO_2 y mayor intensidad de luz). Una posible explicacion es que las raíces y los tallos primarios axilares actúan como pequeños sumideros (durante los primeros 21 días de propagación comparados con la capacidad de sumidero ("sink") de las células de almacenamiento de los tallos u hojas. Otra conclusión importante fue que las reservas iniciales de las estacas juegan un papel irrelevante en la calidad del material de plantación en términos de crecimiento de las raíces y yemas (tallos) axilares cuando son comparados con el papel de los fotoasimilados sintetizados durante la propagación. Sin embargo, se verificó también que las reservas acumuladas durante la propagación pueden tambien ser eficientemente usadas para sustentar el crecimiento de las raíces, y particularmente, del tallo primario, cuando la fotosíntesis es limitada por los bajos niveles de luz o la baja concentración de CO_2.

Los resultados de esta investigación confirman la importancia de la fotosíntesis para la propagación de estacas de tallo semi-leñoso y con hojas de rosales para flor cortada y provee relevante información para la optimización del crecimiento y mejoramiento de la uniformidad del material de plantación del rosas de corte derivadas de estacas enraizadas.

Curriculum Vitae

Joaquim Miguel Rangel da Cunha Costa was born on 28 April 1971, in Oporto, Portugal. In 1989 he completed his high school in the Colégio Internato dos Carvalhos (CIC) and started the Agricultural Engineering course in the University of Trás-os-Montes e Alto Douro (UTAD) in Vila Real, the Northeast Portugal. The final stage was done in 1994, at the Department of Horticulture (Vakgroep Tuinbouwplantenteelt) of Wageningen Agricultural University, the Netherlands, with an Erasmus scholarship. In October 1994, he started the Master Course (Mestrado) in the Instituto Superior de Agronomia (Technical University of Lisbon), Lisbon which was founded by a scholarship from the Fundação para a Ciência e Tecnologia (FCT), Portugal. The second year of the Mestrado was carried out at the Department of Horticulture of the WAU. In May 1997, he started his Ph.D. program at the same department (today called Horticultural Production Chains Group), founded with scholarship from the FCT (Programa PRAXIS XXI). This book is the result of this work.

List of publications

Papers

Costa JM, Challa H, Van Meeteren U, Van de Pol PA (2001) Photosynthates: mainly stored and yet limiting in propagation of rose cuttings. Acta Hort 547: 167-174

Costa JM, Challa H (2002) The effect of the original leaf area on growth of softwood cuttings and planting material of rose. Sci Hort 95: 111-121.

Schouten R, Costa M, Kooten, O (2002) External quality measurements reveal internal processes. Acta Hort (in press)

Costa, JM, Marcakova J, Harbinson J (2002) Changes of the photosystem II efficiency during rooting of rose cuttings (submitted)

Chapter in Book

Costa JM, Van de Pol P (2002) Propagation of rose by cuttings (Submitted as Chapter in the Encyclopedia of Rose Science, Academic Press)

Book Edition

Greenhouse horticulture in Almeria (Spain). **JM Costa and E Heuvelink (eds)** - 109 pp. ISBN 90 6754 593 7, 109pp.

Professional journals

Costa, JM Guerner J, Marques ML 1999 Alguns Aspectos Práticos do Cultivo e Fitossanidade da Roseira. O Minho a Terra e o Homem 41: 22-28

Costa M 2002 Rooting of cut roses directly related to leaves. Flowertech 5 (3):16-17

Present addresses:

Marijkeweg 22
6709 PG Wageningen
The Netherlands

Rua Gonçalves de Castro, 537
4415- Carvalhos
Portugal

The work presented in this thesis was carried out at the Wageningen University, Horticultural Production Chains Group, Marijkeweg 22, 6708 PG Wageningen, The Netherlands, and was founded by:

FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA CIÊNCIA E DO ENSINO SUPERIOR Portugal

The printing costs of this thesis were financially supported by FCT and Wageningen University and the generous donations from Dutch and Portuguese companies:

Horto - Florícola de Santo Antão, Lda.

Anton Verbeek Roses Int.
MEMBER OF THE POUW GROUP

De Ruiter's
New Roses International B.V.
MEMBER OF THE POUW GROUP

TECNIFLORA
Plantas Limitada

Van Kleef Roses bv

MÁRIO MOTA SUC.s