UNIVERSIDAD POLITECNICA DE VALENCIA

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Departamento de Producción Vegetal

Study of nutrient solution management in soilless rose cultivation, through the analysis of physiological parameters and nutrient absorption

Estudio del manejo de la solución nutritiva en cultivo sin suelo de rosal, mediante el análisis de parámetros fisiológicos y de absorción de nutrientes

Tesis doctoral

Elisa Gorbe Sánchez

Valencia, 2009

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Memoria Presentada por

ELISA GORBE SÁNCHEZ

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DIRECTORES (IVIA):

Dra. Ángeles Calatayud Chover

Dr. Florentino Juste Pérez

TUTOR (UPV):

Dr. Alberto San Bautista Primo

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Apartado oficial 46113, Moncada (Valencia)

Dña. Ángeles Calatayud Chover, Doctora en Ciencias Biológicas y Colaborador Científico Adjunto del IVIA, y D. Florentino Juste Pérez, Doctor Ingeniero Agrónomo y Director del IVIA,

INFORMAN que:

Elisa Gorbe Sánchez, Ingeniera Agrónoma, ha realizado bajo nuestra dirección el trabajo que, con título "Estudio del manejo de la solución nutritiva en cultivo sin suelo de rosal, mediante el análisis de parámetros fisiológicos y de absorción de nutrientes", presenta para optar al grado de Doctora Ingeniera Agrónoma.

Para que así conste a los efectos oportunos, firman el presente documento en Moncada, a 25 de Noviembre de 2009.

Dña. Ángeles Calatayud Chover D. Florentino Juste Pérez

A mis padres y hermanos

A mi yaya

A Pablo

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CONTENTS

LIST OF PUBLICATIONS AND COMMUNICATIONS

List of publications derived from the work developed in this thesis:

Calatayud, A., Roca, D., **Gorbe, E**., Martínez, P.F., 2007. Light acclimation in rose (*Rosa hybrida* cv. Grand Gala) leaves after pruning: Effects on chlorophyll *a* fluorescence, nitrate reductase, ammonium and carbohydrates. Scientia Horticulturae 111: 152-159.

Calatayud, A., Roca, D., **Gorbe, E**., Martínez, P.F., 2008. Physiological effects of pruning in rose plants cv. Grand Gala. Scientia Horticulturae 116: 73-79.

Calatayud, A., **Gorbe, E**., Roca, D., Martínez, P.F., 2008. Effect of two nutrient solution temperatures on nitrate uptake, nitrate reductase activity, NH_4^+ concentration and chlorophyll *a* fluorescence in rose plants. Environmental and Experimental Botany 64: 65-74.

Gorbe, E., González-Mas, M.C., Calatayud, A. Nitrogen and carbohydrate dynamics under two different root temperatures in rose plants. Environmental and Experimental Botany (accepted).

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Gorbe, E., Zarzo, M. and Calatayud, A. Empirical models of nutrient and water uptake by rose plants for fertirrigation management. In progress.

Gorbe, E., Bermejo, A., Cano, A., Calatayud, A. Effect of preharvest nutrition on the quality of rose flowers and on postharvest evolution of anthocyanin, carbohydrates and essential oils in flower tissues. In progress.

Gorbe, E., Calatayud, A. Use of chlorophyll fluorescence imaging for detecting stress in agriculture. Review paper for Environmental and Experimental Botany. In progress.

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González-Mas M.C., **Gorbe E**., Roca D., Martínez P.F., Calatayud A. Efecto de dos temperaturas de la solución nutritiva de plantas de rosal sobre la absorción de nitrógeno. Poster. X Congreso Hispano-Luso de Fisiología Vegetal. XVII Reunión de la Sociedad Española de Fisiología Vegetal. Alcalá de Henares (Madrid, Spain). 18-21/09/2007.

Calatayud A., **Gorbe E**., Roca D., Martínez P.F. Efecto de dos temperaturas de la solución nutritiva sobre el contenido de azúcares solubles y almidón en rosa. Poster. X Congreso Hispano-Luso de Fisiología Vegetal. XVII Reunión de la Sociedad Española de Fisiología Vegetal. Alcalá de Henares (Madrid, Spain). 18-21/09/2007.

Gorbe E., Calatayud A. Influencia del uso de una solución nutritiva de menor concentración en el cultivo del rosal (variedad Grand Gala) en la posterior vida en vaso de los tallos florales. Oral communication. XI Congreso Hispano-Luso de Fisiología Vegetal. XVIII Reunión de la Sociedad Española de Fisiología Vegetal. Zaragoza, Spain. 8-11/09/2009.

Calatayud A., **Gorbe E**. Efecto de la concentración de la solución nutritiva y la estación climática en la calidad poscosecha de la rosa (variedad Grand Gala). Poster. XI Congreso Hispano-Luso de Fisiología Vegetal. XVIII Reunión de la Sociedad Española de Fisiología Vegetal. Zaragoza, Spain. 8-11/09/2009.

ABSTRACT

Optimization of crop nutrition is essential to avoid plant stress and to obtain high yields and qualities of horticultural products. In this respect, soilless systems are of great interest because they allow the management of the different factors affecting plant nutrition, such as nutrient solution composition and concentration or nutrient solution temperature. In this thesis, efforts have been done in this direction in order to optimize nutrition of rose plants cultivated for cut flower production. This general aim has been addressed in three chapters.

Chapter 3 has the aim of understanding the factors affecting daily water and nutrient uptake by rose plants and developing empirical models that could explain it. Five models for nutrient uptake (nitrate, phosphate, potassium, calcium and magnesium) and one for water uptake were developed with the interest of being practical for their application in real conditions. This is due to the fact that the models were developed with data of more than a year of cultivation and include the effects of some common practices in cut flower production of rose plants such as renewal of old bent shoots, the use of shade screen or the synchronization of flower shoot development for scheduling purposes. In addition, other independent variables were nutrient solution concentration, vapour pressure deficit, radiation integral inside the greenhouse, air temperature, nutrient solution temperature, flower shoot production or unknown internal factors. Nutrient uptake models also integrated the effect of water absorption.

Chapter 4 has the objective of testing the tolerance of rose plants to low nutrient solution temperatures by studying their effect on several physiological parameters. Rose plants were tolerant to a nutrient solution temperature of 10 °C during winter by means increasing the production of thin roots, nitrate uptake, nitrate reductase acivity, photochemical activity and carbohydrates content, and by enhancing the partitioning of N and carbohydrates towards the roots. Nevertheless, this response decreased in the beginning of spring, maybe because of the interaction between the effect of nutrient solution temperature and the improved air climatic conditions.

Chapter 5 aims at studying the effect of using a lower nutrient solution concentration compared to the standard on the subsequent vase life of cut roses. Although interesting from an environmental standpoint, a 40% dilution of the nutrient solution shortened vase life of rose flowers by one day. This resulted from a higher water loss to water uptake ratio during the first day after harvest, which was the main factor affecting vase life duration, and from a faster decrease of flower shoot fresh weight during postharvest life. The second objective was to apply chlorophyll fluorescence imaging to analyze the functioning of the photosynthetic apparatus

throughout postharvest life in order to understand the response mechanisms of the flower shoot to harvest. One day after harvest, an activation of the photoprotective mechanisms in the leaves was observed. These mechanisms began to be less operational with the progression of water loss throughout postharvest life and this eventually led to a decrease in the fraction of PSII centres that are capable of photochemistry. The best chlorophyll fluorescence parameter to describe the changes during rose vase life was ϕNPQ/ϕNO and the less informative was F_v/F_m.

RESUMEN

La optimización de la nutrición de los cultivos es vital para evitar estreses y obtener altos rendimientos y calidades de los productos hortícolas. Los sistemas de cultivo sin suelo son interesantes porque permiten el manejo de los diferentes factores que afectan a la nutrición vegetal, como la composición y concentración de la solución nutritiva o la temperatura de dicha solución. En esta tesis, se ha estudiado el manejo de algunos de estos factores con la finalidad de optimizar la nutrición de plantas de rosal cultivadas para la producción de flor cortada. Este objetivo general ha sido tratado en tres capítulos.

En el Capítulo 3 se expone el estudio de los factores que afectan a la absorción diaria de agua y nutrientes por las plantas de rosal. Cinco modelos de absorción mineral (nitratos, fosfatos, potasio, calcio y magnesio) y uno de absorción hídrica fueron desarrollados. El interés de estos modelos reside en la posibilidad de su aplicación en condiciones reales de producción debido a que fueron desarrollados con datos de más de un año de cultivo, y porque incluyen algunas de las prácticas más comunes en la producción de rosas para flor cortada como la renovación de tallos arqueados, el uso de malla de sombreo o la sincronización del desarrollo de los tallos florales para su cosecha en determinadas fechas. Además, otras variables independientes incluidas en los modelos fueron la concentración de la solución nutritiva, el déficit de presión de vapor, la integral de la radiación dentro del invernadero, la temperatura del aire y de la solución, la producción de tallos florales o factores internos desconocidos. Los modelos de absorción mineral también integraron el efecto de la absorción hídrica.

El Capítulo 4 tiene como objetivo evaluar la tolerancia o sensibilidad de las plantas de rosal a la baja temperatura de la solución nutritiva mediante el estudio de su efecto sobre parámetros fisiológicos. Las plantas de rosal fueron tolerantes a 10 °C de temperatura de la solución durante el invierno mediante el incremento en la producción de raíz fina, absorción de nitrato, actividad nitrato reductasa, actividad fotoquímica y contenido en carbohidratos, así como por el aumento de la movilización de N y carbohidratos hacia las raíces. Sin embargo, esta respuesta se vio disminuida a principios de primavera probablemente por la interacción del efecto de la temperatura de la solución con una mejoría en las condiciones climáticas ambientales.

El Capítulo 5 tiene como objetivo estudiar el efecto de la utilización de una menor concentración de la solución nutritiva en comparación con la estándar en la posterior vida en vaso de la flor cortada. A pesar del interés desde un punto de vista medioambiental, una dilución del 40% de la solución nutritiva acortó la vida en vaso de las rosas en un día. Esto fue el resultado de un mayor ratio agua transpirada/agua

absorbida durante el primer día después de la cosecha, que fue el factor principal de la duración de la vida en vaso, y de un descenso más rápido de peso de los tallos florales durante la etapa poscosecha. El segundo objetivo fue aplicar la técnica de la fluorescencia de imagen para analizar el funcionamiento del aparato fotosintético a lo largo de la vida en vaso, con el objeto de entender los mecanismos de respuesta del tallo floral a su corte. Un día después del corte se observó una activación de los mecanismos de fotoprotección de las hojas. Estos mecanismos empezaron a ser menos operativos a medida que la pérdida de agua de los tallos florales aumentaba durante la etapa poscosecha, lo que finalmente resultó en un descenso en la fracción de centros del PSII capaces de realizar fotoquímica. El mejor parámetro para describir la evolución durante la vida en vaso de los tallos florales fue ϕNPQ/ϕNO y el menos informativo fue F_v/F_m.

RESUM

L'optimització de la nutrició dels cultius és vital per a evitar l'estrés i obtenir alts rendiments i qualitats dels productes hortícoles. Referent a això, els sistemes de cultiu sense sòl són interessants perquè permeten el maneig dels diferents factors que afecten a la nutrició vegetal, com la composició i concentració de la solució nutritiva o la temperatura d'aquesta solució. En aquesta tesi, s'ha estudiat el maneig d'alguns d'aquests factors amb la finalitat d'optimitzar la nutrició de plantes de roser cultivades per a la producció de flor tallada. Aquest objectiu general ha estat tractat en tres capítols.

En el Capítol 3 s'exposa l'estudi dels factors que afecten a l'absorció diària d'aigua i nutrients per les plantes de roser. Cinc models d'absorció mineral (nitrats, fosfats, potassi, calci i magnesi) i un d'absorció hídrica van ser desenvolupats. L'interés d'aquests models resideix en la possibilitat de la seua aplicació en condicions reals de cultiu degut al fet que aquests models van ser desenvolupats amb dades de més d'un any de cultiu, i perque inclouen algunes de les pràctiques més comuns en la producció de roser per a flor tallada, com la renovació de tiges arquejades, l'ús de malla d'ombratge o la sincronització del desenvolupament de les tiges florals per a la seua collita en determinades dades. A més, altres variables independents incloses en els models van ser la concentració de la solució nutritiva, el dèficit de pressió de vapor, la integral de la radiació dintre de l'hivernacle, la temperatura de l'aire, la temperatura de la solució, la producció de tiges florals o factors interns desconeguts. Els models d'absorció mineral també van integrar l'efecte de l'absorció hídrica.

El Capítol 4 té com objectiu avaluar la tolerància o sensibilitat de les plantes de roser a la baixa temperatura de la solució nutritiva mitjançant l'estudi del seu efecte sobre paràmetres fisiològics. Les plantes de roser van ser tolerants a 10 °C de temperatura de la solució durant l'hivern, mitjançant l'increment en la producció d'arrel fina, absorció de nitrat, activitat nitrat reductasa, activitat fotoquímica i contingut en carbohidrats, així com per l'augment de la mobilització de N i carbohidrats cap a les arrels. No obstant això, aquesta resposta es va veure disminuïda a principis de primavera probablement per la interacció de l'efecte de la temperatura de la solució amb una millora de les condicions climàtiques ambientals.

El Capítol 5 té l'objectiu d'estudiar l'efecte de la utilització d'una menor concentració de la solució nutritiva en comparació de l'estàndard en la posterior vida en got de la flor tallada. Malgrat l'interés des d'un punt de vista mediambiental, una dilució del 40% de la solució nutritiva va acurtar la vida en got de les roses en un dia. Això va ser el resultat d'una major ràtio aigua transpirada/aigua absorbida durant el primer dia després de la collita, que va ser el factor principal de la durada de la vida en

got, i d'un descens més ràpid de pes de les tiges florals durant l'etapa postcollita. El segon objectiu va ser aplicar la tècnica de la fluorescència d'imatge per a analitzar el funcionament de l'aparell fotosintètic al llarg de la vida en got, per a entendre els mecanismes de resposta de la tija floral al seu tall. Un dia després del tall es va observar l'activació dels mecanismes de fotoprotecció de les fulles. Aquests mecanismes van començar a ser menys operatius a mesura que la pèrdua d'aigua de les tiges florals augmentava durant l'etapa postcollita, el que finalment va resultar en un descens en la fracció de centres del PSII capaços de realitzar fotoquímica. El millor paràmetre per a descriure l'evolució durant la vida en got de les tiges florals va ser $\phi NPQ/\phi NO$ i el menys informatiu va ser F_v/F_m.

ABBREVIATION LIST

(SOD) Superoxide dismutase

(POX) Peroxidase

(APX) Ascorbate peroxidase

(GR) Glutathione reductase

(MDAR) Monodehydroascorbate reductase

(CAT) Catalase

(WU) Water uptake

(NU) Nitrate uptake

(PU) Phosphate uptake

(KU) Potassium uptake

(CaU) Calcium uptake

(MgU) Magnesium uptake

(NUC) Nitrate uptake concentration

(PUC) Phosphate uptake concentration

(KUC) Potassium uptake concentration

(CaUC) Calcium uptake concentration

(MgUC) Magnesium uptake concentration

(NUE) Nitrate use efficiency

(PUE) Phosphate use efficiency

(KUE) Potassium use efficiency

(CaUE) Calcium use efficiency

(MgUE) Magnesium use efficiency

(C_p) Phosphate concentration in the nutrient solution

(C_{Ca}) Calcium concentration in the nutrient solution

(C_{Mg}) Magnesium concentration in the nutrient solution

(P_{Ca}) Indicator variable that equals 1 in the period of time between 20/04/05 - 05/08/05

(P_K) Indicator variable that equals 1 in the period of time between 19/05/05 - 08/07/05

(P_{Mg}) Indicator variable that equals 1 in the period of time between 15/04/05 - 02/06/05

(P_{P1}) Indicator variable that equals 1 in the period of time between 30/03/05 - 13/05/05

(P_{P2}) Indicator variable that equals 1 in the period of time between 28/03/06 - 12/04/06

(P_{PRUN}) Indicator variable accounting for the effect of the pruning practice of old bent shoots. Its value equals 1 in the period between 30/08/05 - 29/09/05

($P_{P\text{-}VB}$) Indicator variable accounting for effect of the physiological stage of the flower shoot between pruning and visible bud stage. Its value equals 1 in the periods 25/11/05 - 18/01/06 and 10/02/06 - 14/03/06

(P_{VB}) Indicator variable accounting for effect of the physiological stage of visible bud of the flower shoot. Its value equals 1 in the period between 29/12/05 - 26/01/06 and 28/02/06 - 24/03/06

(PROD) Flower shoot production calculated as the sum of flower shoots harvested on a specific day and the following 6 days

(R_i) Incident radiation integral inside the greenhouse

(R_o) Incident radiation integral outside the greenhouse

(T_a) Air temperature inside the greenhouse

(T_s) Nutrient solution temperature

(VPD) Vapour pressure deficit

(TR/SR) Ratio between thin-white roots and suberized-brown roots

(Ndf) Nitrogen derived from the fertilizer

(NR) Nitrate reductase

(ST) Starch

(SU) Soluble sugars

(FSW) Flower shoot fresh weight

(CF) Chlorophyll *a* fluorescence

(PSII) Photosystem II

(F_0) Minimum fluorescence yield in the dark

(F_0') Minimum fluorescence yield of the illuminated sample

(F_m) Maximal fluorescence yield after receiving the saturating pulse of light

(F_m') Maximum fluorescence yield during the actinic illumination

(F_s) Chlorophyll fluorescence yield during the actinic illumination before the saturating pulse

(F_v/F_m) Maximum quantum yield of PSII photochemistry

(ϕPSII) Actual quantum efficiency of PSII photochemistry

(q_L) Coefficient of photochemical quenching based on the lake model

(ϕNPQ) Quantum yield of regulated energy dissipation in PSII

(ϕNO) Quantum yield of non-regulated energy dissipation in PSII

1. INTRODUCTION

INTRODUCTION

General Problem Description

The achievement of high yields and product qualities is a priority in horticulture. However, when any factor affecting plant functions is at non-optimal conditions, plant heath may be affected and both yield and qualities may diminish. Optimizing the management of greenhouse climate and crop nutrition is, therefore, crucial for horticultural production to avoid possible stresses. Regarding crop nutrition, soilless systems are interesting because they allow the management of the factors affecting plant nutrition. For example, nutrient solution composition and concentration or root temperature may be controlled in soilless systems to optimize crop production. However, accurate guidelines about optimum and non-optimum levels of these factors are still poor and management is often based on grower's experience.

Roses are one of the most widely grown and valued of all ornamentals in the world. For that reason, it is important to learn how to optimize its nutrition so that high yields and qualities may be achieved by rose growers. In this thesis, efforts have been made in this direction. In particular, the obtention of the optimum nutrient solution composition for rose plants has been aimed through the development of empirical models that predict plant demand for nutrients. Moreover, the tolerance to low nutrient solution temperatures has been tested, and the effect of the concentration of the nutrient solution on vase life (a quality parameter) has been evaluated. The objectives of this work are explained below.

Objectives of the Thesis

The ultimate goal of this thesis is to optimize nutrition of a soilless rose crop and the focus is placed on the study of plant nutrient demand to formulate optimum nutrient solutions and on the management of concentration and temperature of the nutrient solution. This general aim is divided in the following specific objectives:

- To understand the factors affecting daily water and nutrient uptake by rose plants under commercial conditions
- To develop empirical models for water and nutrient uptake by rose plants with high applicability so that they could be implemented in decision support systems
- To test the tolerance of rose plants to low nutrient solution temperatures by studying their effect on several physiological parameters:

- linked to N metabolism (nitrate uptake, partitioning and re-translocation of N derived from the fertilizer in the plant, nitrate reductase activity and NH_4^+ concentration in leaves)
- associated with C metabolism (photochemical and non-photochemical activity, non-structural carbohydrates concentration and partitioning in plant tissues)

- To study the effect of the concentration of nutrient solution used during cultivation of a soilless rose crop, on the subsequent vase life of cut roses
- To find out whether the environment-friendly approach of diluting the nutrient solution affects yield and subsequent vase life of roses
- To apply chlorophyll *a* fluorescence imaging to analyze how the photosynthetic apparatus functions throughout vase life as a basis to better understand the response mechanisms of the flower shoot to the stress caused by harvest

Outline of the Thesis

The aforesaid objectives are addressed through the following chapters:

Chapter 2 presents a literature review about the different factors that need to be controlled to optimize crop nutrition in soilless culture. It analyzes the optimum levels that have been reported for each of these factors and it includes a section about formulating optimum nutrient solutions. Moreover, this review focuses in the physiological methods that can be used to diagnose plant stress when a given factor is at non-optimal conditions.

Chapter 3 shows the development, by multiple regression, of empirical models for nutrient (nitrate, phosphate, potassium, calcium and magnesium) and water uptake of greenhouse rose plants. Independent variables used for building the models vary from several climatic variables to the concentration of the nutrient solution, flower production, some common practices in rose cultivation or unknown internal factors. Data obtained along 14 months was used for modeling. A discussion about the strengths and weaknesses of the models is included.

Chapter 4 studies how a low nutrient solution temperature (10 °C) affects rose plants in comparison to a level considered as optimum (22 °C). To achieve this aim, several physiological functions of the plant are analyzed and, due to the number of techniques measured, this chapter has been divided in two. Section 4.1 includes the effect on biomass production, nitrate and water uptake, total nitrogen concentration

in roots and leaves, nitrate reductase activity, NH_4^+ concentration in leaves and chlorophyll *a* fluorescence. Section 4.2 contains the effect on the content, partitioning and re-translocation of N derived from the fertilizer in the plant, and on carbohydrates content, use and distribution within rose plants. This experiment was carried out along two flowering cycles, in winter and beginning of spring.

Chapter 5 focuses on the effect of the concentration of nutrient solution used for the cultivation of rose plants on the subsequent vase life of rose flowers. For this aim, two nutrient solution concentrations were used: one is the solution commonly used by local growers and the other is a 40% dilution of the former. To find out the mechanisms underlying the effect of nutrient solution concentration on flower shoot vase life, water balance and chlorophyll *a* fluorescence were measured during a period of 11 days after harvest. This experiment was carried out once in every season.

Finally, Chapter 6 highlights the conclusions derived from this work and presents some suggestions for future research.

2. OPTIMIZATION OF NUTRITION IN SOILLESS SYSTEMS: A REVIEW

OPTIMIZATION OF NUTRITION IN SOILLESS SYSTEMS: A REVIEW

Abstract

High yields and product qualities of crops grown in soilless systems are only possible if nutrition is optimized. This implies the accurate management of all factors involved in crop nutrition: nutrient solution composition, water supply, nutrient solution temperature, dissolved oxygen concentration, electrical conductivity and pH of the nutrient solution. If any of these factors is under non-optimal conditions, plants may suffer from stress leading to a decline of yields and product qualities. In order to specify the range of optimal conditions of a particular crop, a precise diagnosis of plant stress caused by an incorrect management of any of abovementioned factors is needed. This review analyzes, for every factor, the optimum ranges that have been reported and the physiological methods that can be used to diagnose plant stress at non-optimal conditions.

Introduction

Continuous cultivation of crops in soil throughout many decades has resulted in poor soil fertility, increase of salinity or infestations by pathogenic organisms. This situation has led to poor yields and qualities of crop products. Furthermore, some soils in the world are not suitable for plant growth for being poorly textured or shallow, degraded due to erosion or too close to metropolitan areas. Whenever soil conditions are unfavourable, soilless culture can be a solution.

Soilless culture is a method of growing plants in any medium different than soil. Many crops are grown in soilless systems: fruiting and leaf vegetables (e.g. tomato, sweet pepper, lettuce), cut flowers (e.g. rose, chrysanthemum), flowering bulbs (e.g. tulips, lily), flowering and foliage potted plants (e.g. cyclamen, ficus), among others (Van Os et al., 2008). Over the last decades, the progress of this technique has been rapid in many developed countries (e.g. the Netherlands, Japan, USA) linked to greenhouse building, automation and computerization development. However, the application of soilless culture for crop production is still limited in many countries such as the Mediterranean due to their lower technological development in agriculture (Olympios, 1999). A crucial factor in the progress of soilless culture is the level of technical knowledge of growers.

The classification of soilless systems proposed by the FAO Report (1990) is still widely accepted today. Plants can be grown in soilless culture with or without the use of artificial media to provide mechanical support. When no media is used, roots can develop in liquid culture (true hydroponics) or in a mist environment (aeroponics). These systems are generally closed, where drain is recycled in a closed circuit with continuous or intermittent irrigation. When artificial substrate is used, this can be inert (e.g. rockwool, vermiculite, perlite) or organic (e.g. peat, cocopeat, bark). Basic

requirements for the chosen substrate are: high water and air holding capacity, low salinity, high buffer capacity, free from pathogens and undesirable elements, among others (Abad et al., 2004). Cultivation in substrate can be done in open and closed systems. In open systems, drains are discarded, which may pollute the environment and should be avoided. Efforts have been done in several countries to promote the use of closed systems. However, closed systems have limited expansion in many countries (Olympios, 1999) due to their higher risk for propagation of pathogens, the cost of disinfection systems for the nutrient solution, and the difficulty of controlling nutrient solution composition because of the different absorption rates of different nutrients by plants.

When comparing soilless versus soil cultures, several benefits appear. Besides the advantage mentioned above on the fact that soilless systems can be used in places where soil cultivation is unfeasible, there is another benefit of the use of soilless systems: the possibility of an exhaustive control of nutrient solution (water and nutrient supply, pH, root temperature...) which allows the optimization of crop nutrition and the improvement of water and nutrient use efficiency. However, these advantages can turn into problems if a good management of the system is not carried out. This is due to the lower buffering capacity of soilless systems compared to soil systems, which involves that quick decisions should be taken when sudden deviations from optimum conditions appear. Therefore, personal in charge of soilless systems should be trained in the control of the technique and have some knowledge of plant physiology, elementary chemistry and plant nutrition. In addition to that, these systems imply a higher initial capital investment to the grower. Hence, when cultivating in soilless systems, it is very important to learn how to optimize crop nutrition so that advantages greatly exceed disadvantages. A description of the advantages and disadvantages in the use of soilless systems is shown by Olympios (1999).

In this review, a study of the most important factors that need to be controlled in soilless systems for an optimum management of nutrition is performed. These factors are nutrient solution composition and concentration, water supply, nutrient solution temperature, dissolved oxygen concentration, electrical conductivity (EC) and pH of the nutrient solution. An incorrect management of any of these factors can lead to stress in plants. Therefore, a precise detection of stress is essential in research to identify inadequate management strategies and to develop recommendations to growers about the abovementioned factors with the aim of obtaining the maximum yields and qualities of horticultural products. Hence, in this review, a list of the most important methods that can be used to diagnose plant stress due to an incorrect management of each studied factor is shown, and emphasis is given at why each method may be used to detect stress symptoms. Understanding the physiological

processes that underlie stress injury is of immense importance for agriculture (Taiz and Zeiger, 2002).

Nutrient solution composition

Optimization of nutrient solution composition

It is essential to have a good knowledge of plant mineral requirements in order to formulate optimum nutrient solutions. The ideal solution would provide the plant with the precise elements for producing the highest yield and/or quality and reduce the susceptibility to biotic and abiotic stresses. The way to formulate optimum nutrient solutions is discussed below. However, fertilization is often empirically based. Commercial greenhouse growers generally use high nutrient concentrations in an attempt to maximize crop yield (Rouphael and Colla, 2009), but this relationship is not necessarily straightforward. In general, crop yield responds positively to increasing concentrations until a level after which further increases often lead to no further increases in yield (luxury consumption). When concentrations are too high, yields may be even decreased (toxicity) (Salisbury and Ross, 1991).

Several studies have documented the advantage of using lower concentrations than the standard. Locascio et al. (1992) showed that the quality of chipping potatoes decreased with excessive potassium. Zheng et al. (2005) and Rouphael et al. (2008) proved that nutrient solution concentration used by growers can be reduced by 50% without any adverse effect on biomass and quality parameters in geranium and gerbera, respectively. Dufour and Guérin (2005) demonstrated that more than 60% of the nutrients supplied in the cultivation of *Anthurium andreanun* were lost in the leachate. This results in contamination of groundwater and is no longer permissible. Efforts should be made, from an environmental standpoint, in order to find out and use the less concentrated but optimum nutrient solution possible.

High concentrations, though, may be advisable for some crops to achieve high quality of the produce. For example, a high proportion of K^+ in the nutrient solution (14.2 meq L^{-1} vs 3.4 meq L^{-1}) increased fruit dry matter, total soluble solids content and lycopene concentration of tomato (Fanasca et al., 2006). In this thesis (Chapter 5) a dilution of the nutrient solution concentration of 40% with respect to the standard shortened vase life of rose flowers.

In addition to optimizing ion concentration, it is crucial to formulate nutrient solutions with a balanced relationship among the different ions (Cañamero et al., 2008). Some ions in excess can cause nutrient deficiencies in plants by interfering with the uptake of other ions, which is called ion antagonism. Studies of antagonisms that

may occur in soilless culture of horticultural crops have been reviewed by Mengel and Kirkby (2001), Pendias (2001) and Hall (2008). The importance of nutrient balance highlights the limitation of the current way of nutrient management by monitoring EC level, which is unable of distinguishing among different nutrients.

In order to formulate the optimum nutrient solution for a particular crop it is necessary to understand the factors that regulate nutrient absorption by the plant, and the first step is measuring plant absorption under different conditions.

Measurement of plant nutrient uptake

Plant nutrient uptake can be determined by measuring nutrient depletion in the root environment (1) and by quantifying nutrient content in plant tissues (2).

1) The first method consists of determining the difference in the amount of a certain ion in the root environment throughout a given period of time. This difference is associated with plant nutrient uptake, which is calculated using equation 1 (Cabrera et al., 1995):

$$Nutrient\ \ uptake\ \ rate = (V1 \cdot C1) - (V2 \cdot C2) \qquad (1)$$

In this equation V1 and V2 are the volume (L) of the nutrient solution on time 1 and 2, and C1 and C2 are the nutrient concentrations (mmol L^{-1}) on time 1 and 2.

This method allows a good accuracy of nutrient uptake over time (Kläring, 2001) and the results are comparable to those obtained by destructive long-term ^{15}N measurements (Barak et al., 1996). However, a correct methodology should be applied to avoid errors in the measurements. Obtaining samples from the root environment is difficult, and samples of the drainage might not represent the composition of the nutrient solution surrounding the roots. In that case, a soilless system with a low inertia should be used (e.g. NFT system, aeroponic system). Moreover, the system should avoid evaporation so that all volume losses can be attributed to water and nutrient uptake. Finally, this method is less accurate when the nutrient solution concentration is elevated (Le Bot et al., 1998a; Chapter 3 of this thesis) so diluted solutions are, thus, recommended.

2) The second method is based on measuring nutrient content in plant tissues. Not only can this method provide information about plant uptake, but it can also differentiate about the allocation of this uptake to different parts of the plant. This technique is very useful in crops with a growing period of several months, in which nutrient content in their tissues can be easily related to its uptake during a known period of time. However, in other crops such as woody plants in which their cultivation

lasts several years, the time when the nutrient content measured in plant tissues was absorbed is more difficult to be estimated. Redistribution processes among the different parts of the plant are common in woody plants. For example, in rose plants, endogenous N is redistributed within the plant during each flowering cycle (Cabrera et al., 1995). Therefore, in these cases, the measurement of nutrient content in plant tissues can be carried out by using isotopically labeled fertilizers and tracing the fate and recovery of these nutrients by the crop (Strong, 1995). Nitrogen is the element that has been most widely used as labeled ^{15}N for being quantitatively the most abundant in plant tissues. It represents about 2% of total plant dry matter and its availability is often an important limitation factor for plant growth and yield (Miller and Cramer, 2004). $^{15}NO_3^-$ and/or $^{15}NH_4^+$ fertilizers have been used in several crops (Dong et al., 2001; Quiñones et al., 2003; Chapter 4.2 of this thesis). The disadvantage of measuring the nutrient content in plant tissues is that it is a destructive technique and, in the case of using labelled fertilizers, it is expensive and requires qualified personal.

Factors that regulate nutrient uptake by the plant.

There are two theories that explain how plants absorb nutrients. One theory (1) states that plant nutrient uptake is proportional to nutrient supply. In that case, the optimum solution should equal the amount of nutrients that are found in the tissues of plants with the desired productions. The second theory (2) affirms that the plant regulates its uptake according to its needs. Hence, the optimum nutrient solution should exactly match the demand of the plant. Further details of both theories are explained below.

1) The first theory assumes that the only factor driving nutrient uptake is nutrient supply. This was supported by Bugbee (2003), who recommended adding nutrients to the solution depended on what one wanted the plant to absorb. This theory is based in the proved fact that nutrients transporters are induced by the concentration of its own substrate outside the root (Crawford and Glass, 1998; Glass et al., 2002). Actually, the high degree of specificity for individual ions is equivalent to the way enzymes do for a specific substrate (Bassirirad, 2000). Because of this analogy, Epstein and Hagen suggested in 1952 that carrier-mediated ion transport across the root can be described by the Michaelis-Menten kinetics:

$$v = \frac{V_{\max} \cdot c}{K_m + c} \qquad (2)$$

where c is the concentration of an individual ion whose uptake rate, v, is controlled by uptake capacity when all available carriers are occupied (V_{max}), and by the apparent affinity of the transporters (K_m). Although this hypothesis has been mainly proved for low external ion concentrations (<1 mM), the correlation between ions uptake and

external ions concentration also occurs for the high range (>1 mM) (Devienne-Barret et al., 2000; Kim et al., 2008). Many authors have adapted Epstein and Hagen's work (1952), to different crops like rose plants (Silberbush and Lieth, 2004; Mattson and Lieth, 2007; Kim et al., 2008; Massa et al., 2009) maize (Caassen and Barber, 1976), cotton (Brouder and Cassman, 1994) or tomato (Cardenas-Navarro et al., 1999) through the development of mathematical models.

According to this theory, the way to design the optimum solution would be by choosing plants having the best productions in terms of quantity and/or quality and/or having the highest resistance to stresses, and measuring the nutrient content in their tissues throughout the cultivation period. This would result in nutrient absorption curves based on which, optimum nutrient solutions may be formulated. Moreover, based on this theory, routine analysis of nutrient content in the leaf during the cultivation period may be used for corrections of the nutrient solution by comparison with the desired concentrations (Thomas, 1937). Several approaches have been suggested to diagnose plant nutritional status according to foliar analysis but Diagnosis and Recommendation Integrated System (DRIS), which was proposed by Beaufils in 1973, has been considered the most accurate of all (Rodriguez and Rodriguez, 2000; Cañamero et al., 2008).

The error of this theory is that it assumes that the plant would absorb the same amount of nutrients when keeping the same solution composition although other factors could change. However, it is well known that plant nutrient uptake changes with the season or its developmental stage among others. For example, in rose plants, a different pattern of N uptake was observed depending on the developmental stage of the flower shoot (Cabrera et al., 1995; Kim et al., 2008; Chapter 3 of this thesis). On the other hand, in summer, plant water uptake increases more than mineral requirement, and therefore, more diluted solutions are required during this season (Le Bot et al., 1998a; Chapter 3 of this thesis). Therefore, this approach alone cannot provide optimum nutrient solutions.

2) The second theory suggests an active role of the plant in nutrient uptake and establishes that plants regulate their uptake according to their demand. If that statement is true, one would have to predict plants demand to design the optimum nutrient solution. So next question would be: what does plants demand depend on? In order to answer this question, many authors have developed mechanistic or empirical models that try to predict nutrients uptake by different crops from several factors, either including or not nutrient solution concentration (Papadopoulos and Liburdi, 1989; Brun and Chazelle, 1996; Mankin and Finn, 1996; Kläring et al., 1997; Kläring and Cierpinski, 1998; Le Bot et al., 1998b; Zerche, 2000; Pardossi et al., 2005). Plant nutrient uptake depends on the transport rate of ions across root membrane, and this

is determined by the number of transporters in the membrane and the activity of those transporters (Smith, 2002). Besides, nutrient uptake needs energy to be carried out (Marschner, 1995). Then, any factor that affects directly or indirectly any of those parameters will affect nutrients uptake and will be a candidate for the model. The most interesting from the perspective of optimizing plant nutrition would be developing models that include simple measurable parameters so that they can be implemented in decision support systems for the management of nutrient solution in soilless culture (Marcelis et al., 1998; Carmassi et al., 2005; Massa et al., 2008). Examples of simple measurable parameters that have been related to nutrient uptake are water uptake (Del Amor and Marcelis, 2004; Pardossi et al., 2005) and climatic factors such as radiation (Brun and Chazelle, 1996; Mankin and Finn, 1996; Cedergreen and Madsen, 2003; Pardossi et al., 2005), vapour pressure deficit (VPD) (Kläring et al., 1997), air temperature (Adams, 1992; Kläring et al., 1997; Pardossi et al., 2005) and nutrient solution temperature (Adams, 1992; Brun and Chazelle, 1996; Bassirirad, 2000; Bougul et al., 2000; Dong et al., 2001).

Problems may arise when deviations between the output of these models and the real demand of the plant appear because of being applied in different conditions, i.e. when using different cultivars or when any kind of stress affects the plant (Kläring et al., 1997; Grattan and Grieve, 1998). This is due to the fact that there are other internal factors that control nutrients uptake (Imsande and Touraine, 1994). For that reason, in order to try to reduce over- or underestimation of nutrient uptake models, empirical models for rose plants were developed in this thesis (Chapter 3), which besides some of the abovementioned variables, they also include the effect of flower shoot production and of some common practices that significantly affect nutrients uptake. However, there are two additional limitations of this theory. On the one hand, as the concentration of the nutrient solution is an important factor that drives plant nutrient uptake, different recommendations about the optimum nutrient solution may result depending on the concentration used in the experiment. On the other hand, sometimes it might not be advisable to use a nutrient solution that exactly matches plant demand because the use of high concentrations may lead to a product of higher quality (Fanasca et al., 2006).

Therefore, it may be suggested that the best solution between these theories would include a combination of both approaches.

Diagnosis of plant stress caused by nutrient solution composition

An inadequate management of nutrient solution composition may be a consequence of the use of too high or too low concentration of the nutrient solution, or of imbalanced ions composition. The first situation involves a high EC of the nutrient

solution and, thus, a salt stress so this will be discussed below. The other two situations lead to a similar problem in plants: nutrient deficiency. One is due to insufficient supply and the other to ion antagonism, but both cases have similar consequences: a decrease of plant growth. A reduction of plant biomass has been reported under N, P, K, Ca, Mg, S, Cu, Zn or Mn deficiencies (Yu and Rengel, 1999; Fujita et al., 2004; Tewari et al., 2004; Zhao et al., 2005; Matcha, 2007; Ding et al., 2008).

It is well known that characteristic visual symptoms of specific nutrient deficiencies may appear in plant tissues. However, most of the classic deficiency symptoms described in textbooks are characteristic of acute deficiencies, i.e. when a nutrient is suddenly no longer available to a rapidly growing plant. In commercial cultivation in soilless systems, it is more common to find chronic deficiencies, which occur when there is a limited supply of a nutrient, at a rate that is insufficient to meet the growth demands of the plant (Berry, 2006). For chronic deficiencies, visual symptoms are not that clear so other methods have to be used to diagnose nutrient deficiencies. These methods are based on the key roles that nutrients play in plant metabolism, because limiting levels of a nutrient affect the metabolic role in which it is involved.

For example, deficiencies of any of the essential mineral elements may affect photosynthesis (Dietz and Harris, 1997). A decrease in the rate of photosynthesis has been observed under N deficiency (Ciompi et al., 1996; Lima et al., 2000; Cruz et al., 2003; Fujita et al., 2004; Huang et al., 2004; Zhao et al., 2005; Matcha, 2007), under P deficiency (Lima et al., 2000) or under Mg deficiency (Ding et al., 2008). This has been attributed to the lower plant leaf area (Zhao et al., 2005), but also to a decrease of chlorophyll content (Cruz et al., 2003; Zhao et al., 2005), a reduced stomatal and/or mesophyll conductance (Natr, 1975; Cruz et al., 2003; Zhao et al., 2005) and to direct effects on light and dark reactions.

There are several cases of specific nutrients deficiency affecting photosynthetic light reactions. Mineral nutrients influence photosynthetic electron flow either for being constituents of the light harvesting complex, or for facilitating electron flow. For review about the specific roles of different nutrients in photosynthetic light reactions see Dietz and Harris (1997) and Cakmak and Engels (1999). This effect can be assessed by the chlorophyll fluorescence (CF) technique. A number of studies have shown that CF parameters are good indicators of nutrient deficiency. For example, Jacob (1995) stated that in P deficient plants, the ability of photosystem II (PSII) pigments to absorb and transfer light energy to the reaction centers is decreased, a phenomenon that is accompanied by an increase in non-photochemical quenching and linked to a higher dissipation of thermal energy, which is also associated with enhanced formation of the xanthophyll pigment zeaxanthin. This is considered a protective response against

overexcitation of PSII and destruction of photosynthetic apparatus (Demming-Adams and Adams, 1992). Likewise, N deficiency has been associated with a higher dissipation of the absorbed light energy and the formation of zeaxanthin, paralleled by a decrease in the quantum yield of electron transport that suggests a down-regulation of PSII photochemistry (Cakmak and Engels, 1999; Lu and Zhang, 2000; Cruz et al., 2003). This may occur in order to match the decreased demand in the Calvin cycle (Lu and Zhang, 2000) due to low CO_2 influx (i.e. closed stomata) or reduced carboxylation efficiency (Ciompi et al., 1996; Huang et al., 2004). Photoinhibition has been reported in some studies under different nutrient deficiencies (Lima et al., 2000; Huang et al., 2004) while it has not observed in others (Sun et al., 1989; Lima et al., 2000; Lu and Zhang, 2000; Cruz et al., 2003). One of the most useful indicators of N stress in plants is the ratio of UV excited blue fluorescence to chlorophyll fluorescence (BF/CF) (Cavender-Bares and Bazzaz, 2004). An increase in this ratio in stressed plants is due to an accumulation of phenolic or flavonoid compounds in leaf epidermis. Also, a dual fluorescence emission ratio of red fluorescence to far-red fluorescence excited at 355 and 532 nm was found to be strongly positively correlated with chlorophyll content, which decreases with mineral deficiencies (Cavender-Bares and Bazzaz, 2004).

Moreover, certain nutrient deficiencies can directly affect the dark reactions through non-stomatal factors. For instance, the rate of CO_2 fixation shows a strong positive correlation with leaf N content because the main portion of the leaf N is in RuBisCO, with the reminder is primarily localized within thylakoid proteins and the Calvin cycle enzymes (Dietz and Harris, 1997). Additionally, Mg is directly involved in the activation of RuBisCO (Dietz and Harris, 1997) and a decrease in the content of RuBisCO has been observed observed in S-deprived plants (Lunde et al., 2009).

Nutrient deficiencies may also affect the fate of the Calvin cycle products. The biosynthesis and degradation of starch and sucrose are affected by nutrient deficiencies (Cakmak and Engels, 1999; Lunde et al., 2009). Most nutrient limitations may result in starch accumulating in plant tissues (Loescher et al., 1990), although an increase in the sucrose/starch ratio has been observed in N-stressed sunflower plants (Ciompi et al., 1996). Moreover, nutrient deficiencies also affect the synthesis and accumulation of amino acid in plant tissues. Under N-limiting conditions, the levels of proline, asparagine and glutamine may decrease (Lemaître et al., 2008). In contrast, deficiencies of other nutrients different from N may increase amino acids content (Black, 1993).

Nutrient deficiencies also affect photoassimilates partitioning between plant organs. It is important to point out that although total dry matter production is similarly affected by different nutrient deficiencies, the effects on its partitioning are specific of the nutrient involved (Cakmak and Engels, 1999). For example, K and Mg

deficiencies influence phloem export of photosynthates (Cakmak and Engels, 1999), which results in higher accumulation of sucrose in leaves (Ding et al., 2008) and in lower accumulation of photosynthates in the sinks such as cereal grains or roots (Cakmak and Engels, 1999). On the other hand, P and N deficiencies stimulate the phloem export of photosynthates. This often results in a reduction of leaf area that decreases the sink strength of the shoots, leading to a preferential allocation of photoshynthates to the roots and to reduced shoot/root ratios (Ciompi et al., 1996; Cakmak and Engels, 1999; Fujita et al., 2004; Zhao et al., 2005; Matcha, 2007; Cakmak, 2008).

As a consequence of the decreased rate of photosynthesis and reduced ability of the plant to deliver photosynthates to the sinks, the number and metabolic activity of sink organs is negatively affected by nutrient deficiencies. Moreover, deficiencies of mineral nutrients severely limit flower initiation and development, development and viability of pollen grains and development of vegetative sink organs such as tubers, which eventually affects yield (Cakmak and Engels, 1999).

Nutrient deficiencies may also cause photooxidative damage, i.e., light-dependent generation of reactive oxygen species (ROS) in chloroplasts, which is a key process involved in cell damage of plants exposed to environmental stress factors (Cakmak, 2008). Any factor that reduces the capacity of photosynthetic electron transport, CO_2 fixation and protective mechanisms, combined with high light intensity may induce severe photooxidative damage to chloroplasts, and consequently cause further decrease in plant yield. For example, it has been proved that deficiencies of N, Mg, K and Zn increase the sensitivity of plants to photooxidative damage (Cakmak and Engels, 1999; Lu and Zhang, 2000; Cakmak, 2008). This higher susceptibility has been associated with the increased accumulation of inactivated PSII reaction centers, the decreased capacity of non-photochemical quenching, and the increased fraction of the reduction state of the primary quinone acceptor (Q_A) (Lu and Zhang, 2000).

ROS react with lipids on the cell membrane to form lipid peroxides such as ethane or malondialdehyde (MDA) (Kiyoshi et al., 1999). Enhanced lipid peroxidation, accumulation of MDA and hydrogen peroxide (H_2O_2), and premature senescence of older parts implying oxidative stress in plants has been observed in several crops under different nutrient deficiencies (Kiyoshi et al., 1999; Lima et al., 2000; Tewari et al., 2004; Ding et al., 2008). Plants have defense mechanisms to be protected against ROS, which include low molecular antioxidants and antioxidant enzymes (Kiyoshi et al., 1999). Particularly, an increase in the levels of ascorbic acid was observed under N deprivation (Kandlbinder et al., 2004), of ascorbate and glutathione under P starvation (Kandlbinder et al., 2004), of ascorbate under Mg deficiency (Anza and Riga, 2001) and of flavonoids and anthocyanins under S deprivation (Lunde et al., 2009). In addition,

stimulation of the activities of antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and other peroxidases (POX), glutathione reductase (GR), monodehydroascorbate reductase (MDAR), or catalase (CAT) has been observed under limiting supply of N (Polesskaya et al. 2004; Tewari et al., 2004), P (Kandlbinder et al., 2004; Tewari et al., 2004), K (Tewari et al., 2004), Mg (Anza and Riga, 2001; Ding et al., 2008), Ca (Tewari et al., 2004) and S (Kandlbinder et al., 2004; Tewari et al., 2004; Lunde et al., 2009). However, under N deficiency, a decrease in the activity of SOD (Polesskaya et al. 2004), APX (Kandlbinder et al., 2004) and CAT (Kandlbinder et al., 2004) have been reported, which may be related to a severe deficiency. Micronutrient deficiency (Cu, Zn or Mn) has been also observed to alter the activities of SOD depending on the kind and severity of the deficiency stress (Yu and Rengel, 1999).

Besides the effect of nutrient deficiency on the activity of antioxidant enzymes, there are other enzymes, either with an antioxidant role or not, which may be affected if that nutrient is part of the specific enzyme molecule. Therefore, the measurement of the activities of these enzymes may be used as indicators of nutrient deficiencies in plants (Lavon and Goldschmidt, 1999). For example, POX activity, for which Fe is a constituent, has been measured to distinguish iron deficiency from Mn deficiency in citrus (Bar Akiva, 1961). Carbonic anhydrase has been employed to identify Zn deficiency (Barker and Pilbeam, 2007). Ascorbic acid oxidase or cytochrome oxidase activities have been used to identify Cu deficiency (Bar Akiva et al., 1969; Walker and Loneragan, 1981). Mo and Fe deficiencies have been associated with low levels of nitrate reductase (NR) activity (Shaked and Bar Akiva, 1967; Alcaraz et al., 1986). NR activity has been also used for the assessment of N deficiency (Oosterhuis and Batea, 1983; Tanaka et al., 1987; Hall et al., 1990; Barker and Pilbeam, 2007; Lemaître et al., 2008), glutamate-oxaloacetate aminotransferase for P deficit and pyruvic kinase for K shortage (Lavon and Goldschmidt, 1999).

In conclusion, diagnosis of nutrient deficiencies can be carried out successfully by measuring the activity or resulting products of certain metabolic functions in which the limiting element is actively involved. This includes: measurements of biomass production and yield, photosynthetic activity, stomatal conductance, chlorophyll content, RuBisCO content or activity, CF, the formation of zeaxanthin, carbohydrates and amino acid content, sucrose/starch ratio, carbohydrates and dry matter partitioning in the plant, shoot/root ratio, lipid peroxidation and ROS species, the amount of antioxidant compounds and the activity of several enzymes.

Water supply

Optimization of water supply

In soilless culture, an accurate and dynamic control of the water supply is needed to meet plant water requirements due to the low water-holding capacity of the system (De Boodt and Verdonck, 1972). Optimum water supply should fulfill plant demand and also prevent salt accumulation in the substrate area surrounding the root. However, under conditions of high transpiration (e.g. at midday in summertime), supply of water may be often insufficient leading to temporal water stress in the plant. In order to avoid it, sometimes too much supply is given. This results in excessive ion lixiviation within the root environment and in loss of unabsorbed water, which should be avoided from an environmental standpoint because water is a scarce resource. For review about the environmental impact of irrigation see Stockle (2001).

In order to carry out an effective management of irrigation, precise information of water status of the group substrate-plant-environment is needed. Different methods try to approach this objective through measurements in the plant, in the substrate or by means of climatic sensors. An in detail review of these methods is included in Medrano (1999). Currently, most soilless systems rely on the measurement of a single sensor, normally a radiometer to determine solar radiation or a tensiometer to determine substrate water potential. When the level of water potential or cumulated radiation reaches a threshold, an irrigation event is activated. A higher level of precision, though, may be obtained through the integration of a more complex model in the irrigation control system, which estimates water demand according to several parameters. Many models have been developed with different levels of complexity (Medrano, 1999) but currently, most of them are based on Penman-Monteith equation, which include radiation, VPD and leaf area, among other parameters (Monteith and Unsworth, 2007). In this thesis (Chapter 3) a model for water uptake in a rose crop (cv. Grand Gala) cultivated for cut flower production has also been developed.

Due to water scarcity, new irrigation scheduling approaches designed to ensure the optimal use of water have appeared. Deficit irrigation and partial root-zone drying are two ways of maximizing water use efficiency for higher yields per unit of irrigation water applied. The expectation is that any yield reduction will be insignificant compared with water saved (Kirda, 2002). Although certain water stress might be suffered by plants irrigated through those strategies, sometimes a mild water stress may be advisable for obtaining a high quality of the product. For example, water stress conditions significantly affected xylem anatomy and functioning of two *Zinnia elegans* cultivars, which resulted in a longer vase life (Twumasi et al., 2004). Concerning fruit

quality, solute accumulation is a recognized physiological response to water stress. Moderate water stress late in the season improved kiwi fruit quality in terms of higher soluble solids and earlier accumulation of sucrose (Miller et al., 1998). Similarly, water stress also improved the quality of 'Merlot' grapes and wine (Peterlunger et al., 2005) and of wheat kernel (Ozturk and Aydin, 2004). Withholding irrigation water during certain periods of time may be a useful management tool to manipulate some quality attributes of the produce (Miller et al., 1998), but it is important to study when to apply water stress to avoid a significant yield reduction.

Diagnosis of plant stress caused by water supply

If water supply is higher that plant demand, salt may lixiviate from the root environment possibly leading to nutrient deficiencies if irrigation is excessive. In contrast, if water supply is lower than plant demand, plant water status may decrease. When water deficit is very limiting, plants wilt and visual symptoms are clear. However, water supply can be at suboptimum levels while showing no visual symptoms. In that case, several techniques based on the effects of water deficit on plant functions can help to evaluate the degree of the stress, which may vary depending on the cultivar and on the extent and duration of water deprivation.

The first process that might be affected by a decrease in plant water content is cell expansion (Munns and Tester, 2008). This results in a reduction of leaf expansion and root elongation, being leaf expansion a more sensitive process (Ball et al., 1994) which leads to a decrease in shoot/root ratio (Fageria et al., 2006). Besides leaf expansion, the number and growth rate of branches are reduced and old leaves abscission is stimulated. Therefore, whole plant leaf area decreases (Taiz and Zeiger, 2002).

The decrease in plant water status may be quantified as a decrease (i.e. more negative) of water potential (Verslues et al., 2006). Leaf water potential has been measured as an indicator of crop water status (Clark and Hiler, 1973; Chelab et al., 2009). In contrast, stem water potential has appeared as a better indicator of vine water status (Choné et al., 2001). For review about the measurement of water potential see Taiz and Zeiger (2006). Relative water content has been also used as a measure of plant water status. Plants can actively modify its water potential through osmotic adjustment, by which a reduction of osmotic potential may be achieved by increasing the cell concentration of a variety of common solutes (Taiz and Zeiger, 2002). Through osmotic adjustment, leaves may maintain turgor during certain time under water stress. This increases the lifetime of active tissues and extends the period of tissue preparation for drought (drought hardening) (Pugnaire et al., 1999). Under water stress conditions, high amounts of sugars like sorbitol, manitol, glucose or sucrose (Fredeen et al., 1991; Kameli and Losel, 1993; Wang et al., 1995; Arji and

Arzani, 2008; Chelab et al., 2009) and high levels of proline (Prasad et al., 1982; Kameli and Losel, 1993; Ramachandra Reddy et al., 2004; Arji and Arzani, 2008) have been measured in several crops a result of osmotic adjustment. The majority of drought-tolerant species have the ability to build up a high content of sugars in dry habitats, whereas drought-sensitive species accumulate far less. Several genes coding for enzymes associated with osmotic adjustment are up-regulated or down-regulated by water stress (Taiz and Zeiger, 2002). In addition, the expression of genes that encode proteins associated with membrane transport including H^+-ATPases and aquaporins are sensitive to water stress (Taiz and Zeiger, 2002; Galmés et al., 2007).

In order to prevent water loss, stomata can actively close when leaves and roots are dehydrating. This is triggered by ABA, which accumulates in stressed tissues (Jiang and Zhang, 2002; Taiz and Zeiger, 2002; Ramachandra Reddy et al., 2004). Stomatal closure reduces CO_2 intake and, thereby decreases net photosynthesis (Dejong and Phillips, 1982; Huber et al., 1984; Dubey, 1997; Tezara et al., 2008). In any case, the photosynthetic rate per unit leaf area is affected by water deficit in a lesser extent than leaf area (Taiz and Zeiger, 2002). In order to be adjusted to the reduced CO_2 assimilation, electron transport rate and photochemical quenching have to be down-regulated (Chaves et al., 2002; Tezara et al., 2008). As a result, a great proportion of incoming light energy is dissipated as heat and non-photochemical quenching increases (Cavender-Bares and Bazzaz, 2004; Calatayud et al., 2006; Tezara et al., 2008). Moderate stress does not induce a decrease in the PSII primary photochemistry as judged by the unchanged F_v/F_m in several crops (Fracheboud and Leipner, 2003; Calatayud et al., 2006; Tezara et al., 2008). In contrast, the steady-state parameter Fs appears useful in detecting water stress in plants. In well-watered plants, Fs increases with light intensity, but as water stress progresses it decreases with increasing light intensity (Flexas et al., 2000). Fs/F_0 is also an indicator of declining stomatal conductance, CO_2 assimilation, and generation of non-photochemical quenching during water stress (Flexas et al., 2002).

Under severe water deficit, photosynthetic activity may be affected by non-stomatal factors due to a strong dehydration of mesophyll cells (Fracheboud and Leipner, 2003). Decreased activity of many enzymes of the Calvin Cycle has been reported (Pugnaire et al., 1999), e.g. a strong decrease in RuBisCO activity in sunflower (Pankovic et al., 1999). This effect may be reversible if water stress is not too severe (Pugnaire et al., 1999). In addition, water stress may lead to ultrastructural changes in chloroplasts (Ackerson and Herbert, 1981; Dubey, 1997), which ultimately impair photosynthesis (Dubey, 1997). Concerning the light reactions, although leaf PSII photochemistry has been proved to be very resistant to water-stress conditions (Flexas et al., 2009), it may be completely lost if the stress is severe (Cavender-Bares and Bazzaz, 2004). In rose plants under severe water deficit, energy dissipation by non-

photochemical quenching, electron transport rate and the fraction of the oxidized state of Q_A decreased, while non-regulated energy dissipation increased (Calatayud et al., 2006) allowing, hence, a higher ROS production. It has been suggested that the weak tolerance of PSII photochemical capacity to severe water stress in desiccation-sensitive plants is related oxidative stress (Cavender-Bares and Bazzaz, 2004; Flexas et al., 2006). Down-regulation of PSII photochemistry is, hence, needed to prevent the generation of ROS within the chloroplast (Navari-Izzo and Rascio, 1999).

Accumulation of ROS or lipid peroxidation has been measured in several crops subjected to water stress (Sairam et al., 1998, Jiang and Zhang, 2002; Esfandiari et al., 2007). Crop species that are tolerant to water stress show reduced membrane damage due to increased synthesis of free radical scavengers (Dubey, 1997). An enhanced activity of GR, CAT, APX, SOD or MDAR (Sairam et al., 1998; Jiang and Zhang, 2002; Ramachandra Reddy et al., 2004; Esfandiari et al., 2007) and an increase in the content of antioxidant compounds such as ascorbic acid (Sairam et al., 1998, Ramachandra Reddy et al., 2004) have been measured in different crops under water stress. For review about oxidative stress under water deficit see Navari-Izzo and Rascio (1999).

Translocation of photosyntates may be unaffected until water deficit becomes severe. This relative insensitivity of translocation to mild water stress allows plants to mobilize and use reserves where they are needed (Taiz and Zeiger, 2002). Export of assimilates is less affected by water stress than carbon exchange rates (Huber et al., 1984). The decrease in the export of assimilates, which leads to the accumulation of carbohydrates in the leaves (Pugnaire et al., 1999), may be due to the dependence of phloem transport on turgor pressure (Taiz and Zeiger, 2002) and might depend on plant acclimation to water stress. For example, drought-adapted cotton plants exported sucrose whereas non-adapted plants accumulated sucrose at the same leaf water potential (Ackerson, 1981).

In addition to what has been said, water stress induces other responses in plants. The decreased transpiration rate under water deficit causes an increase in leaf temperature, which may lead to heat damage under hot conditions. A decrease of respiration has been measured in beans and peppers (González-Meler et al., 1997) and a decrease in ATP production was measured in sunflower (Tezara et al., 2008) and soybean (Ribas-Carbo et al., 2005). Water deficit has an important indirect effect on nutrient uptake, which may be as important as its effect on growth (Pugnaire et al., 1999). Maybe because of that, N content in the plant is reduced under water deficit (Dejong and Phillips, 1982; Mahieu et al., 2009) and the activity of NR is also depressed (Pugnaire et al., 1999; Correia et al., 2005; Fresneau et al., 2007). Finally, besides the reduction of yield due to reduced cell expansion and decreased export of assimilates

towards grains or fruits, water stress may also affect yield production by delaying flowering, leading to male sterility and reducing grain set (Acquaah, 2007).

In conclusion, diagnosis of water stress in plants can be assessed by different techniques that measure plant processes affected by the loss of turgor. Measurements of plant water potential or relative water content can be performed as indicators of plant water status. Measurements of biomass production and yield, leaf area, water uptake, photosynthetic activity, stomatal conductance, ABA accumulation, CF, RuBisCO activity, osmotic adjustment, carbohydrates content and partitioning in the plant, accumulations of several compounds in the leaves for osmotic adjustment (sugars, amino acids…), lipid peroxidation and ROS species, the amount of antioxidant compounds, the activity of antioxidant enzymes, the activity of NR, leaf temperature, nutrient uptake, N content, respiration or the expression of genes coding for H^+-ATPases and aquaporins may give clues to determine the severity of water stress in the plant.

Electrical conductivity and pH of the nutrient solution

Control of electrical conductivity and pH in the nutrient solution

EC is an index of salt concentration that informs about the total amount of salts in a solution. Hence, EC of the nutrient solution is a good indicator of the amount of fertilizer available to the plants in the root zone (Nemali and Iersel, 2004). When plants absorb nutrients and water from the solution, the total salt concentration, i.e. the EC of the solution changes, and measurements of EC level are easy, fast and economic so they can be carried out daily by growers. For that reason, fertirrigation management is currently based on the control of EC and pH in order to correct a pre-set nutrient solution prepared according to previous experience. This is a practical method but it is important to notice that EC does not inform about the concentration of specific ions in the solution so this way of managing nutrient solution may lead to nutrient imbalances.

The ideal EC range for soilless crops is between 1.5 and 2.5 dS/m. However, the effect of salinity on crops is specific on the specie and cultivar (Greenway and Munns, 1980). In general, EC>2.5 dS/m may lead to salinity problems while EC<1.5 dS/m may lead to nutrient deficiencies. In greenhouse culture, the high input of fertilizers is the main cause of the salinity problems (Li, 2000). In addition, a high EC may also be caused by the presence of specific ions such as Na^+ and Cl^- in the solution. In order to avoid salinity problems, growers add fresh water to reduce EC. However, in some regions there is the added problem of having irrigation water of bad quality, i.e. with high content of Na^+ and/or Cl^-. In that case, the addition of fresh water to the nutrient

solution would not alleviate the problem of salinity and the use of cultivars with salinity tolerance may be the solution.

In some cases, though, it may be advisable to use a high EC to improve the quality of the produce. For example, the quality of flavoring and health-promoting compounds in hydroponically grown tomatoes improves with increasing electrical conductivity in the nutrient solution (De Pascale et al., 2003; Krauss et al., 2007).

On the other hand, pH is a measure of the acidity or basicity of a solution and determines the availability of essential elements for plants. The pH is an essential parameter to control in soil and soilless system, but in the second one its correction should be done daily due to the lower buffering capacity of soilless systems (Urrestarazu, 2004). In fertirrigation, pH should be such that does not damage plant roots and allows that all essential nutrients are solved in the nutrient solution to prevent the formation of precipitates that block the irrigation systems and decrease nutrients availability by plants. The optimum nutrient solution pH depends on the plant but, in general, it ranges between 5.5 and 6.5, in which the maximum number of elements is at their highest availability for plants (Taiz and Zeiger, 2002). Chemical buffers can adjust the pH of a nutrient solution if it strays outside the ideal. The pH can be lowered by adding dilute concentrations of phosphoric or nitric acids and raised by adding a dilute concentration of potassium hydroxide. The incorporation of ammonium in the nutrient solution as another source of N (5-10%) may be also used to regulate pH. For review about of the management of pH in soilless systems see Urrestarazu (2004).

Diagnosis of plant stress caused by electrical conductivity and pH in the nutrient solution

The use of solutions with too low EC and the incorrect management of pH may lead to nutrient deficiencies, which have been reviewed above. In this section, we will discuss about how to detect salinity stress in plants. Depending on whether high EC is due to the use of highly concentrated solutions or to the use of water with high levels of Na^+ and Cl^-, the responses of plants are two-fold. First, the presence of high levels of salts in the solution reduces the ability of the plant to take up water, which is referred to as the osmotic or water-deficit effect of salinity. Second, if excessive amounts of injurious ions (e.g. Na^+ or Cl^-) enter the plant in the transpiration stream there may be injury to cells in the transpiring leaves, which is called the salt-specific or ion-excess effect of salinity (Greenway and Munns, 1980).

The osmotic effect of salinity induces metabolic changes in the plant identical to those caused by water stress (Munns, 2002). Specifically, the following effects have

been observed in different crops under salinity stress: a decrease of biomass production and growth (Soussi et al., 1998; Shani and Ben-Gal, 2005; Zhao et al., 2007; Giuffrida et al., 2008; Tavakkoli et al., 2008; Zribi et al., 2009); a decrease of leaf area (Terry et al., 1983; Taiz and Zeiger, 2002; Netondo et al., 2004; Zhao et al., 2007; Giuffrida et al., 2008); an increase of leaf abscission (Taiz and Zeiger, 2002); a decrease of root growth (Rodríguez et al., 1997) but to a lesser extent than the reduction in leaf growth (Munns, 2002); a lower shoot/root ratio (Meloni et al., 2004; Houlimi et al., 2008); a reduction in stomatal conductance (Terry et al., 1983; Sultana et al., 1999; Netondo et al., 2004; Zribi et al., 2009); an accumulation of ABA (He and Cramer, 1996); a decrease in CO_2 assimilation (Netondo et al., 2004; Maricle et al., 2007), being the effect in photosynthetic rate less important than the effect in leaf enlargement (Terry et al., 1983); a decrease of water uptake (Giuffrida et al., 2008); a decrease in water potential (De Pascale et al., 2003; Zribi et al., 2009); a decrease in relative water content (Meloni et al., 2004); an increase in osmotic adjustment (Taiz and Zeiger, 2002; De Pascale et al., 2003) due to accumulation of glycine betaine (Agastian et al., 2000; Meloni et al., 2004), proline (Mattioni et al., 1997; Soussi et al., 1998; Agastian et al., 2000) or sugars (Soussi et al., 1998, Agastian et al., 2000) among other compounds; down-regulation of photosynthetic electron transport (Netondo et al., 2004); a relative resistance of PSII primary photochemistry (Maricle et al., 2007; Zribi et al., 2009); an increased production of ROS (Cakmak, 2008); a stimulation of antioxidant enzymes such as SOD, APX, MDAR, CAT or GR (Tanaka et al., 1999; Hernández et al., 2000; Esfandari et al., 2007); a higher synthesis of antioxidant compounds like glutathione, carotenoids and lycopene (Ruiz and Blumwald, 2002; De Pascale et al., 2003); a decrease in RuBisCO activity (Miteva et al., 1992); a change in the ultrastructure of chloroplasts similar to that caused by water stress (Dubey, 1997); a lower translocation of photosynthates leading to an accumulation of carbohydrates in the photosynthesizing leaves (Dubey, 1997); an increase of leaf temperature (Kluitenberg and Biggar, 1992); a decrease of nutrient uptake (Dubey, 1997) and N content (Meloni et al., 2004); a decreased ATP synthesis (Dubey, 1997); a decrease of NR activity (Meloni et al., 2004); a reduced viability of reproductive organs (Munns, 2002); and, finally, a change in gene expression, similar to that caused by water stress (Taiz and Zeiger, 2002). Therefore, the same methods can be used for diagnosis of any osmotic effect, either caused by water or by salinity stress.

On the other hand, salt-specific effects may result in toxicity, deficiency, or changes in mineral balance. Firstly, plant deficiency of several nutrients and nutritional imbalance (i.e., extreme ratios of Na^+/Ca^{2+}, Na^+/K^+, Ca^{2+}/Mg^{2+}, and Cl^-/NO_3^- in plant tissues) may be caused by the higher concentration of Na^+ and Cl^- in the nutrient solution derived from ion antagonism (Grattan and Grieve, 1998). For example, Ca^{2+} and K^+ deficiency have been observed under salt stress, which affects membrane

integrity (Cramer et al., 1985) and root growth (Munns, 2002). Secondly, toxicity in plant cells may appear as a consequence of Na^+ and/or Cl^- accumulating in transpiring leaves. Plants are capable of compartmentalizing these ions in the vacuole up to a certain extent, but if the limit is exceeded, ions build up in the cytoplasm and inhibit enzyme activity, or they build up in the cell walls and dehydrate the cell, eventually causing cell death (Munns, 2002). The salt-specific effects of salinity depend on the concentration of salts, the duration of salinity exposure as well as on the plant species. Salt tolerant plants differ from salt-sensitive ones in having a low rate of Na^+ and Cl^- transport to leaves, and in the ability to compartmentalize these ions in vacuoles to prevent their build-up in cytoplasm or cell walls and, thus, avoid salt toxicity (Munns, 2002). Therefore, the resistance of salt-tolerant plants to salts is not consequence of salt-resistant metabolism but of strategies that avoid salt injury (Taiz and Zeiger, 2002).

The toxicity effects of salts have metabolic consequences. Photosynthesis may be inhibited when high concentrations of Na^+ and/or Cl^- accumulate in chloroplasts (Plaut et al., 1989; Taiz and Zeiger, 2002). For example, alterations in the photochemical activity have been observed under salinity in salt sensitive crop species (Dubey, 1997; Muranaka et al., 2002). Accumulation of injurious ions in the cytoplasm inactivates enzymes, inhibit protein synthesis and damage chloroplasts and other cell organelles (Taiz and Zeiger, 2002). These effects are more important in older leaves as they have been transpiring the longest so they accumulate more ions (Munns, 2002). This results in a progressive loss of the older leaves with time and reduces the photosynthetic leaf area of the plant to a level that cannot sustain growth. The rate at which leaves die becomes the crucial issue determining the survival of the plant (Munns, 2002). For example, vine mortality has been correlated with the increase in Na^+ and Cl^- content of leaves (Shani and Ben-Gal, 2005).

Plant growth might be, hence, reduced by both the osmotic and the salt-specific effect of salinity, being sometimes difficult to determine which of the effects is the responsible for growth reduction. For that reason, Munns et al. (1995) proposed a two-phase model of salt injury, where growth is initially reduced by osmotic stress and then by salt toxicity. According to these authors, the effect of salinity takes some time to develop and may become obvious over weeks, especially in the more sensitive species (Munns, 2002). This model has been proved in broccoli under salinity stress (López-Berenguer et al., 2006). However, it is difficult to assess with confidence the relative importance of the two mechanisms on yield reduction because they overlap (Tavakkoli et al., 2008). In brief, diagnosis of salinity stress in plants can be evaluated by the same techniques used for water stress in addition to the measurement of the concentration of Na^+ and Cl^- content in leaves. Special attention should be placed in the old leaves as they are the target of salt injury.

Dissolved oxygen concentration in the nutrient solution

Optimization of oxygen concentration in the nutrient solution

Oxygen is essential for roots functioning so its deficiency is an important concern. Problems with oxygen supply may periodically appear in soil conditions after rain showers. Also, in soilless systems, water and nutrients are supplied continuously and these wet conditions limit the diffusion of oxygen to the root system (Veen, 1988). An inadequate management of irrigation may lead to temporal hypoxia conditions (insufficient supply of oxygen) caused by inadequate aeration in some parts of the root system (Morard and Silvestre, 1996). In contrast, anoxia (complete lack of oxygen) is rare in soilless culture (Morard and Silvestre, 1996; Kläring and Zude, 2009). Oxygen deprivation stress in plants is distinguished by three physiologically different states: transient hypoxia, possible anoxia and reoxigenation (Blokhina et al., 2002).

In order to avoid oxygen deficiency in the root environment, it is essential to provide the nutrient solution with enough O_2. Possibilities for accurate control of root oxygen supply are more easily achieved in soilless cultures than in soils systems (Olympios, 1999). The best system regarding oxygenation of the root environment is the aeroponic system, which allows the roots to grow in air with a plentiful supply of oxygen so no extra mechanism is needed. In liquid systems, aeration might be needed by means of pumps if the solution culture is static. However, in continuous flow solution culture like the nutrient film technique, there is an abundant supply of oxygen to the roots of the plants if the system is well designed. In substrate systems, it is essential to choose a substrate that has a correct distribution of particle size, a low bulk density, a high porosity and a stable structure so that the supply of air to the roots is sufficient (Abad et al., 2004). If more aeration was needed, Urrestarazu and Mazuela (2005) have observed that the addition of potassium peroxide as chemical oxygenation improves water uptake and yield of different vegetables as sweet pepper, melon and cucumber. Also, the application of exogenous nitrate to plants under oxygen deprivation has been observed to improve their survival through the mechanism of 'nitrate respiration' (see below) (Morard et al. 2004).

In addition to the capacity of the system to provide the roots with enough aeration, the availability of oxygen in the root environment also depends on O_2 consumption by roots and microorganisms (Naasz et al., 2008). O_2 consumption increases with increasing nutrient solution temperature, root weight and photosynthates concentration in the roots, which leads to an increase in the relative CO_2 concentration in the root environment if root aeration is not adequate. The increased CO_2 concentration leads to an increase of anaerobic respiration which

continues releasing CO_2. Therefore, oxygen depletion is linked to the increase in the relative CO_2 concentration in the root environment (Morard and Silvestre, 1996).

Diagnosis of plant stress caused by dissolved oxygen concentration.

An insufficient supply of oxygen to the root has a negative effect in a number of metabolic processes, and its symptoms become visible, i.e. plants become wilted and defoliated (Morard and Silvestre, 1996), when plants are irreversibly damaged (Kläring and Zude, 2009). Growth may be decreased and sometimes impaired under oxygen deficiency (Wagner and Dreyer, 1997; Incrocci et al., 2000; Taiz and Zeiger, 2002; Kogawara et al., 2006; Parelle et al., 2006). Leaf growth is restricted (Pezeshki et al., 1996; Incrocci et al., 2000) and older leaves senesce prematurely because of reallocation of phloem mobile nutrients to younger leaves (Taiz and Zeiger, 2002), so an important reduction in plant leaf area occurs in the plant. Root growth is limited (Pezeshki et al., 1996; Mielke et al., 2003; Smethurst et al., 2005) even more than shoot growth (Smethurst and Shabala, 2003), which increases the shoot/root ratio (Kläring and Zude, 2009). Therefore, it is very important to detect the stress caused by hypoxia in time to prevent further yield reductions or even plant death (Kläring and Zude, 2009). The effect of oxygen deficiency and subsequent recovery in plant tissues depends on the duration and severity of oxygen deprivation, on the tolerance of the species or cultivars to oxygen deficiency, on the age and developmental stage of the plant, on the type of tissue and also on the light level and ambient temperature (Morard et al., 2000; Bragina et al., 2001; Blokhina et al., 2002; Fukao and Bailey-Serres, 2004; Smethurst et al., 2005; Kläring and Zude, 2009). Therefore, varied and sometimes contradictory plant responses have been recorded in literature.

The most immediate effect of the decline of oxygen concentration in the root environment is that root aerobic respiration is seriously restricted (Taiz and Zeiger, 2002; Islam and Macdonald, 2004). Pyruvate, the product of glycolysis, is then transformed to lactate, malic acid or mainly ethanol, which represent the main fermentation pathways in plants (Saenger, 2002; Sousa and Sodek, 2002). Fermentation involves a severe reduction of ATP synthesis that affects plant cell metabolism (Morard and Silvestre, 1996; Bertrand et al., 2003). It also leads to the accumulation of toxic compounds like ethanol or acetaldehyde (Morard and Silvestre, 1996; Schmull and Thomas, 2004; Kläring and Zude, 2009), but normally to levels that do not injure plant tissues (Lambers et al., 2008). Fermentation causes cytoplasm acidosis which decreases the activity of many enzymes, a possible cause of cell death (Vartapetian and Jackson, 1997). Despite its negative consequences, fermentation seems to ensure root survival under anaerobic conditions and it is very important for stress tolerance (Blokhina et al., 2002; Taiz and Zeiger, 2002; Fukao and Bailey-Serres, 2004). The early induction of the ethanolic fermentation pathway and sugar utilization

under hypoxia allows the maintenance of the energy status and, hence, improves anoxia tolerance (Blokhina et al., 2002). Acclimation to anaerobic conditions enhances the expression of genes that encode many of the anaerobic stress proteins, which are mainly related to enzymes of the glycolytic and fermentation pathways (Blokhina et al., 2002; Taiz and Zeiger, 2002; Lambers et al., 2008). A high activity the fermentative enzyme alcohol dehydrogenase (ADH) has been measured in many plants, whether tolerant to hypoxia or not (Pezeshki et al., 1996; Weng and Chang, 2004; Kogawara et al., 2006) and it is considered an indicator of hypoxia in plants (Kogawara et al., 2006). The activity of enzyme sucrose synthase is also promoted under hypoxia with the aim of sustaining the glycolytic flux (Parelle et al., 2006; Kläring and Zude, 2009). However, an inhibition of the sucrolytic, glycolytic and fermentative enzymes may occur under anoxia (Mustroph and Albrecht, 2003).

Fermentation accelerates the use of carbon reserves, so a prolonged period of oxygen deficiency may lead to the exhaustion of substrates (Bertrand et al., 2003). In order to protect root functions, plants tolerant to oxygen deficiency appear capable of sustaining photoassimilate transport to hypoxic roots (Kogawara et al., 2006). However, a reduction in distribution of photosynthates towards the roots has been reported in sensitive plants, which leads to an increased concentration of carbohydrates in the shoots (Islam and Macdonald, 2004; Kogawara et al., 2006) and may lead to feedback inhibition of photosynthesis (Smethurst et al., 2005). Once in the roots, photoassimilates may be partitioned among metabolic, structural and storage processes (Kogawara et al., 2006), being the partitioning into metabolically available forms the most advisable to maintain a high energy status, as occurs in highly tolerant species (Kogawara et al., 2006). However, in sensitive species, root hypoxia might increase photoassimilate partitioning into the storage fraction and decrease partitioning to metabolic processes and structural components in roots (Kogawara et al., 2006).

As a result of the reduced root biomass (Smethurst et al., 2005) and of the decrease of ATP in the roots due to both the inhibition of aerobic respiration (Morard and Silvestre, 1996; Morard et al. 2004) and the lower import of photosynthates in the roots, the absorption of nutrients may decrease under oxygen deprivation (Vartapetian and Jackson, 1997; Taiz and Zeiger, 2002; Smethurst et al., 2005). The depressive effects of oxygen deficiency on uptake have been classified by Morard and Silvestre (1996) in the following order: K>N>P>H_2O>Mg-Ca. Potassium uptake is the most sensitive and even efflux has been observed soon after the exposition to oxygen deficiency (Morard et al., 2000). It has been attributed to depolarization of root cell membranes, a direct consequence of H^+-ATPase inhibition (Morard and Silvestre, 1996). In addition, a low concentration of oxygen in the root environment decreases the selectivity of K^+/Na^+ uptake in favour of Na^+ and retards the transport of K^+ to the

shoots (Armstrong and Drew, 2002). Smethurst et al. (2005) observed nutrient deficiencies after 20 days of oxygen deficiency in *Medicago sativa* L. However, irreversible nutritional stress has not been detected in plants under these conditions (Morard and Silvestre, 1996).

Stomatal closure has been observed under root oxygen deficiency in many species (Jackson and Hall, 1987; Bradford and Hsiao, 1982; Weng and Chang, 2004; Incrocci et al., 2000; Yordanova and Popova, 2001; Yordanova et al., 2003; Pezeshki et al., 1996; Mielke et al., 2003; Islam and Macdonald, 2004; Schmull and Thomas, 2004; Kogawara et al., 2006) often associated with a high concentration of ABA in their tissues (Jackson and Hall, 1987; Sojka, 1992; Incrocci et al., 2000). This has been mostly attributed to the production of ABA by the older lower leaves that wilt and export their ABA to the younger leaves, where stomata close (Zhang and Zhang, 1994). In addition, roots may stimulate ABA production or reduce cytokinin synthesis (Morard and Silvestre, 1996) under oxygen deficit. The decrease in stomatal conductance leads to a reduction of transpiration, water uptake and root hydraulic conductance (Jackson and Hall, 1987; Morard and Silvestre, 1996; Yoshida et al., 1996; Vartapetian and Jackson, 1997; Morard et al., 2000; Yordanova and Popova, 2001; Smethurst and Shabala, 2003; Yordanova et al., 2003; Islam and Macdonald, 2004; Schmull and Thomas, 2004; Weng and Chang, 2004; Nicolás et al., 2005). Unexpectedly, this has no negative consequences to leaf hydration since leaf water potential is unchanged (Bradford and Hsiao, 1982; Incrocci et al., 2000; Taiz and Zeiger, 2002; Weng and Chang, 2004) or even increased (Jackson and Hall, 1987).

In addition to the effect of stomatal closure on transpiration, it also reduces CO_2 intake and, thus, CO_2 assimilation (Pezeshki et al., 1996; Wagner and Dreyer, 1997; Mielke et al., 2003; Mustroph and Albrecht, 2003; Islam and Macdonald, 2004; Kogawara et al., 2006). Nevertheless, some species tolerant to oxygen deficiency can sustain photosynthesis under root hypoxic conditions (Kogawara et al., 2006). In addition to stomatal closure, other non-stomatal factors may affect photosynthesis. For example, a reduction of RuBisCO content or activity (Yordanova and Popova, 2001; Yordanova et al., 2003; Panda et al., 2008) and a decrease in leaf chlorophyll content (Wagner and Dreyer, 1997; Schlüter and Crawford, 2001; Yordanova and Popova, 2001; Smethurst and Shabala, 2003) have been measured under oxygen deficiency. Also, changes in the profile of carotenoids may occur and, accordingly, Kläring and Zude (2009) suggested that the measurement of leaf diffuse reflectance in the carotenoids absorption bands (at 550 and 455 nm) may provide a sensitive tool of stress diagnosis under these conditions.

Photochemistry might be also affected by oxygen deprivation as a consequence of the lower CO_2 assimilation rate (Mielke et al., 2003). Down-regulation of PSII has been

measured by CF as an increase of non-photochemical quenching (Schlüter and Crawford, 2001; Mielke et al., 2003) usually coupled with a decrease in photochemical quenching (Schlüter and Crawford, 2001). In the long-term, though, photochemistry may be affected by direct damage to components and membranes of the photosynthetic apparatus (Yordanova et al., 2003) or even by the nutrient deficiency caused by the impaired nutrient uptake (Smethurst et al., 2005). Then, the capacity for non-photochemical quenching may diminish, which leads to a permanent over-excitation of the thylakoids and enhanced danger of photoinhibitory damage (Schlüter and Crawford, 2001). As a result, a decrease of F_v/F_m has been measured in some species under oxygen deficit (Wagner and Dreyer, 1997; Schlüter and Crawford, 2001; Smethurst and Shabala, 2003; Smethurst et al., 2005, Panda et al., 2008). F_v/F_m and non-photochemical quenching have been considered as reliable indicators of tolerance to oxygen deficiency (Smethurst and Shabala, 2003; Smethurst et al., 2005).

In addition to the already explained consequences of oxygen deficiency, it also contributes to oxidative stress in plants. An in-depth review about oxidative stress in plants under oxygen deficiency has been performed by Blokhina et al. (2002). Generation of ROS can take place in hypoxic tissues under hypoxia and especially under reoxygenation. Hence, anoxic stress is always accompanied to some extent by oxidative stress (Blokhina et al., 2002). Hydrogen peroxide accumulation has been reported under hypoxic conditions (Yordanova et al., 2003). In order to protect membranes integrity, the antioxidant system is stimulated by oxygen deficiency (Blokhina et al., 2002). For example, an increase in the activities of several antioxidant enzymes like CAT, APX or SOD (Biemelt et al., 1998; Yordanova et al., 2003) or a higher level of antioxidant compounds like ascorbate and glutathione (Biemelt et al., 1998) have been measured under oxygen deprivation.

After hypoxia and/or anoxia conditions, physiological functions can eventually be recovered (Morard and Silvestre, 1996; Schlüter and Crawford, 2001; Smethurst et al., 2005; Panda et al., 2008), although, sometimes, growth may remain reduced (Smethurst et al., 2005). For example, although the photosynthetic apparatus could result damaged by oxygen deprivation, a complete recovery may be achieved once stress conditions disappear (Smethurst et al., 2005). This recovery may take different time depending on the duration of the stress or the tolerance of the species (Schlüter and Crawford, 2001) and might depend on the preservation of membrane integrity under anoxia (Blokhina et al., 2002). Under reoxigenation, plants suffer not only from weakening by anoxia stress, but they also have to endure the formation of ROS (Schlüter and Crawford, 2001).

Plants may adapt to the lack of oxygen in the root environment by a mechanism called ‘nitrate respiration’, where NO_3^- is reduced in root cells to NO_2^- by NR and acts

as an alternative electron acceptor to O_2 (Morard and Silvestre, 1996). This phenomenon has been observed in tomato when, after 12 hours of anoxia, nitrites were detected in the nutrient solution (Morard et al., 2000). An increase of NR activity has been also observed by Allègre et al. (2004) and by Morard et al. (2004) under oxygen deficiency. It has been suggested that nitrate reduction actually serves as an intermediate step of a respiratory pathway alternative to glycolytic fermentation: the haemoglobin (Hb) / nitric oxide (NO) cycle. In this cycle, NO produced from nitrate is oxidized back to nitrate in a reaction involving non-symbiotic Hb. The drop in ATP levels seems to stimulate the gene expression of Hb (Parelle et al., 2006), and enhance the activation of NR (for review see Igamberdiev and Hill (2004) and Igamberdiev et al. (2005)).

To sum up, in order to carry out a reliable diagnosis of oxygen deficiency in plants, the following techniques can be used: measurements of biomass production and yield, shoot/root ratio, leaf area, root respiration, accumulation of ethanol and acetaldehyde, measurements of lipid peroxidation and ROS species, the amount of antioxidant compounds, photosynthetic activity, chlorophyll content, stomatal conductance, transpiration, water uptake, root hydraulic conductance, ABA accumulation, CF, content, type and partitioning of carbohydrates, leaf diffuse reflectance, nutrient uptake, and measurements of the level, gene expression and/or activity of ADH, sucrose synthase, Hb, NR, RuBisCO or antioxidant enzymes.

Nutrient solution temperature

Optimization of nutrient solution temperature

Nutrient solution temperatures may reach injuriously high levels during summer, or damaging low levels in winter, which strongly influence growth and survival of whole plants. This parameter depends on solar radiation and aerial temperature, but also on the characteristics of the system. In general, soilless systems are exposed to larger daily variations in root temperature than soil systems (Kafkafi, 2001) but possibilities for accurate control of root temperature are more easily carried out in soilless cultures than in soils systems (Olympios, 1999), through cooling or heating systems. However, sometimes an excessive energy input is spent to protect the crop due to incorrectly established temperature ranges. In order to optimize the use of energy in greenhouse production it is necessary to know the range of nutrient solution temperatures, specific for each crop cultivar (Kafkafi, 2001), which permits plant growth and promotes high yields. In general terms, root zone temperatures below 18 °C and above 28 °C may seriously impair uptake and root growth so temperatures outside this range should be avoided (Bar-Yosef, 2008). In some cases, though, a higher product quality may be obtained when exposing roots to infra- or supra- optimum temperatures

during a short period of time. For example, a treatment of one week of low temperature stress in spinach plants increased the leaf concentrations of quality compounds like sugars, ascorbic acid and Fe^{2+}, while reduced the leaf concentrations of others considered harmful for human health like NO_3^- and oxalic acid (Hidaka et al., 2008).

Diagnosis of plant stress caused by nutrient solution temperature

If the root temperature, significantly affected by the management of nutrient solution temperature, strays from the optimum range several metabolic processes may be affected. This depends on the actual temperature, the duration of the stress, the physiological stage of the crop, the species and even cultivar (Sanders and Markhart, 2000; Rachmilevitch et al., 2006b; Kafkafi, 2008). In spite of the importance of root temperature to whole-plant responses, relatively little is known in comparison to the effect of air temperature, which has been studied extensively (Rachmilevitch et al., 2006a; Zhang et al., 2007). However, Xu and Huang (2000) suggested that root temperature appears to be more critical than air temperature in controlling plant growth.

One of the most widely observed symptoms of root temperature stress is that root growth is inhibited and number of roots and root dry weight may decrease. This has been observed in many plants with their roots subjected to supra-optimal (Sattelmacher et al., 1990; Rachmilevitch et al., 2006a; Lyons et al., 2007; Kafkafi, 2008) or infra-optimal temperatures (Bowen, 1970; Ali et al., 1996; Sanders and Markhart, 2000; Franklin et al., 2005; Apostol et al., 2007). Root viability decreases (Rachmilevitch et al., 2006b) and plants may die if the stress is very severe. The cause of the reduced root growth may be due to a reduced import of photosynthates from the shoots (see below), but in the case of supra-optimal root temperatures, the cause seems to be mainly related to the enhanced consumption by root respiration rather than to the reduced translocation.

Root respiration increases with root temperature (Xu and Huang, 2000; Rachmilevitch et al., 2006a; Lyons et al., 2007). Oxygen is consumed at a high rate and, accordingly, high root temperature is generally associated to hypoxia stress in soilless systems (Incrocci et al., 2000). Respiration is a major avenue of carbohydrates consumption and may lead to shortage of assimilates when temperatures are too high. Actually, this fact has been proposed to be a primary factor responsible for root growth inhibition and dysfunction at high root temperature (Rachmilevitch et al., 2006a; Kafkafi, 2008). The down-regulation of plant respiratory rates and the increase of respiratory efficiency by lowering maintenance and ion uptake costs are key factors

for plant acclimation to high root temperatures (Rachmilevitch et al., 2006a, b; Lyons et al., 2007).

In addition to the effect of root temperature on root growth, it also affects root morphology. Under low root temperature, roots might be more succulent (Kanda et al., 1994; Dieleman et al., 1998; Chapter 4.1 of this thesis), whiter (Dieleman et al., 1998; Chapter 4.1 of this thesis), with lower development of lateral roots (Bowen, 1970; Dieleman et al., 1998; Sanders and Markhart, 2000) and with higher content of unsaturated fatty acids in phospholipids (Kanda et al., 1994). The latter has been associated with tolerance to low root temperature (Lee et al., 2005b). In contrast, under high root temperature, roots may be shorter and highly branched (Stout et al., 1997). These differences in root morphology may lead to changes in hydraulic properties and in roots capacity for ion and water uptake.

The majority of the studies about the effect of root temperature on water uptake have been carried out under low temperatures, although water uptake may be affected by heat stress as well (Geater et al., 1997; McMichael and Burke, 1999). Many studies have reported a decrease in water uptake as root temperatures drop (Cornillon, 1988; Economakis, 1997; Pavel and Fereres, 1998; Sanders and Markhart, 2000; Abdel-Mawgoud et al., 2005; Murai-Hatano et al., 2008; Chapter 4.1 of this thesis). The decrease in water uptake seems to be immediate (Sanders and Markhart, 2000) and has been attributed to higher water viscosity (Abdel-Mawgoud et al., 2005; Affan et al., 2005) and to higher root hydraulic resistance (Pavel and Fereres, 1998). A decrease in the permeability of the root cell membranes (Yoshida and Eguchi, 1990) caused by a reduction in the activity of the plasma membrane H^+-ATPases and linked to changes in the activity (open/closed) of aquaporins (Radin, 1990; Yoshida and Eguchi, 1990; Sanders and Markhart, 2000; Lee et al., 2005a; Kafkafi, 2008; Murai-Hatano et al., 2008), have been suggested as the causes for the increase in root hydraulic resistance.

In addition to water uptake, nutrient uptake is very sensitive to nutrient solution temperature (Xu and Huang, 2006). A restriction of nutrient uptake has been observed under supra-optimal (Rachmilevitch et al., 2006a) or infra-optimal temperatures (Macduff et al., 1987; Ali et al., 1996; Dong et al., 2001). Actually, crops may suffer from nutrient deficiencies during long cold periods (Sanders and Markhart, 2000). However, in some studies no significant effect has been measured (Osmond et al. 1982) or even an increase of nutrient uptake has been determined under low temperatures (Chapter 4.1 of this thesis). This might be dependent on the tolerance of the species and the specific temperature used in the study. Nutrient uptake may be limited by uptake per unit root surface or by reduced root growth. The latter may become more significant over the long term (Sanders and Markhart, 2000). Regarding

supra-optimal temperatures, the reduction of nutrient uptake per unit root surface may be due to the shortage of root assimilates consumed by the enhanced respiration. With regard to the decrease of nutrient uptake per unit root surface under low root temperatures, it has been associated with the change in the structure of membrane lipids in roots and with the decrease in the activities of enzymes responsible for nutrient uptake such as H^+-ATPase (Dong et al., 2001). The uptake of different nutrients may have different sensitivities to root temperature. For example, NO_3^- absorption appears more sensitive than NH_4^+ absorption to low root temperatures (Clarkson and Warner, 1979; Macduff et al., 1987; Kafkafi, 2008) maybe due to the lower energy demand for NH_4^+ assimilation (Kafkafi, 2008).

The reduced nutrient uptake under non-optimal root temperatures may lead to a decrease in the leaf concentration of several nutrients (Kafkafi, 2008; Malcolm et al., 2008). Besides nutrient uptake, nutrient partitioning and assimilation are also altered by root temperature (Sanders and Markhart, 2000). For example, an increase of NR activity has been measured under low root temperature in leaves (Chapter 4.1 of this thesis) and roots (Sanders and Markhart, 2000), while nitrate assimilation rate seems to decrease under high root temperature (Rachmilevitch et al., 2006b). Besides, both an increase of ammonium content in leaves (Chapter 4.1 of this thesis) while a decrease of amino acid content (Kubota et al., 1987) have been measured under low root temperature. These divergences may depend on the specie and the specific temperature of the study.

Another root function that is influenced by root temperature is the synthesis and translocation of hormones like cytokinins, gibberellins and ABA (Ali et al., 1996; McMichael and Burke, 1999; Rachmilevitch et al., 2006a; Singh et al., 2007). A high level of cytokinins in the roots (Kanda et al., 1994) has been associated with tolerance to infra-optimal temperatures. Moreover, there is evidence that ABA is involved in cold-temperature signaling (Franklin et al., 2005), and that it may be a means of long-distance root-to-shoot signaling in plants with cooled root systems (Franklin et al., 2005).

The reduced water uptake at low root temperatures might decrease leaf water potential and leaf turgor (Radin, 1990; Sanders and Markhart, 2000). Nevertheless, plants can respond to their decreased water status by increasing ABA concentrations in the shoot (Udomprasert et al., 1995; Zhang et al., 2008), which triggers stomatal closure (Apostol et al., 2007; Zhang et al., 2008). The decrease in transpiration caused by stomatal closure has been indirectly determined by measuring leaf temperature (Ahn et al., 1999; Malcolm et al., 2008), which has been suggested as a very sensitive parameter in identifying stress caused by low root temperature (Ahn et al., 1999). In

sensitive species, stomata may be slow to respond and water stress may occur, which can result in transient or permanent wilting (Sanders and Markhart, 2000).

The closure of stomata results in a decrease of CO_2 assimilation rate (Zhang et al., 2008). A decline in photosynthetic rate has been measured under high (Xu and Huang, 2000; Rachmilevitch et al., 2006a, b; Lyons et al., 2007) and low root temperatures (Apostol et al., 2007; Malcolm et al., 2008), and a decrease in the maximum and effective quantum yield of photochemical efficiency of PSII and in the fraction of open PSII reaction centres has been observed at non-optimal temperatures (Repo et al., 2004; Rachmilevitch et al., 2006b; Zhang et al., 2007; Zhang et al., 2008). In contrast, the effective quantum yield and the fraction of open PSII reaction centres increased in rose plants with their root exposed at 10 °C (Chapter 4.1 of this thesis). In addition to the closure of stomata, changes in the ultrastructure of cortical cells that may affect the photosynthetic apparatus have been observed under low root temperature (Lee et al., 2002).

The decline in photosynthetic activity results in the reduction of shoot growth, shoot dry weight and/or leaf area under both supra-optimal (Kafkafi, 2008) and infra-optimal root temperatures (Ali et al., 1996; Sanders and Markhart, 2000; Franklin et al., 2005; Solfjeld and Johnsen, 2006; Apostol et al., 2007; Malcolm et al., 2008; Field et al., 2009). A high root temperature may also accelerate the senescence of aerial parts and may reduce the shoot dormancy period and the subsequent level of floral initiation (Guedira and Paulsen, 2002; O'Hare, 2004). In contrast, a low root temperature may prolong the shoot dormancy period and may cause developmental delay (Mowat, 1995; Lieten, 1997; Sanders and Markhart, 2000). As a result of any of these affected processes, a decrease of yield might be observed in plants exposed to non-optimal root temperature (Sanders and Markhart, 2000).

The assimilate use in plants is altered by root temperature but differently depending whether temperatures are above or below the optimum range. Under low temperatures, the leaf content of total nonstructural carbohydrates increases (Ali et al., 1996; Repo et al., 2004; Solfjeld and Johnsen, 2006). This been attributed to a lower partitioning of assimilates into structural carbohydrates (Solfjeld and Johnsen, 2006), a delayed loss of starch (Repo et al., 2004), a reduction of translocation (phloem loading/unloading) or a decrease of root sink demand (Sanders and Markhart, 2000). In contrast, some authors (Ali et al., 1996; Chapter 4.2 of this thesis) have measured an increase of carbohydrates in the roots, which has been associated with tolerance to low root temperatures (Kanda et al., 1994). On the other hand, at high root temperature total nonstructural carbohydrates decrease in shoots and roots (Kubota et al., 1987; Xu and Huang, 2000; Guedira and Paulsen, 2002) due to the imbalance between photosynthesis and respiration in which carbon consumption exceeds

production (Xu and Huang, 2000). Also, high root temperature leads to changes in allocation pattern favoring root growth at the expense of shoot growth (Rachmilevitch et al., 2006b).

The exposure of plant roots to non-optimal temperatures may lead to oxidative stress. Actually, membranes injury has been pointed as the cause of the inhibition of root functions (Sanders and Markhart, 2000). H_2O_2 (Rhee et al., 2007) and MDA (Zhang et al., 2007) have been detected in plant tissues under non-optimal root temperatures. In order to prevent the accumulation of ROS in root cells, plants may respond to unfavorable root temperatures by increasing their synthesis of ascorbate and glutathione, or the activity of SOD, CAT or APX (Zhang et al., 2007). Plants tolerant to non-optimal root temperatures should be capable of dealing with ROS (Rhee et al., 2007) and preventing membranes injury (Rachmilevitch et al., 2006a).

In conclusion, diagnosis of stress caused by non-optimal root temperatures in plants may be assessed by different techniques: measurements of biomass production and yield, leaf area, shoot/root ratio, root morphology, root respiration, water and nutrient uptake, nutrient content in plant tissues, photosynthetic activity, CF, stomatal conductance, transpiration, root hydraulic resistance, hormone accumulation in roots and shoots, carbohydrates content and partitioning in the plant, amino acid and ammonium content in plant tissues, lipid peroxidation and ROS species, the amount of antioxidant compounds, leaf temperature and the activity of several enzymes.

Conclusions

Optimization of nutrition in soilless systems can be achieved by means of an accurate management of all factors involved (i.e. nutrient solution composition and concentration, water supply, nutrient solution temperature, dissolved oxygen concentration, EC and pH of the nutrient solution). If any factor affecting plant nutrition is under non-optimal conditions, plants may suffer from stress, and yields (quantity and/or quality) may diminish. A precise diagnosis of plant stress caused by these factors is, hence, of great importance so that non-optimal levels of each factor could be determined and strategies for maximum benefits for growers can be planned. Regarding methods for diagnosis of plant stress, many physiological techniques are available. They are based on the fact that the above-mentioned factors affect the functioning of several plant physiological processes, and changes in these processes may be a sign of stress. It is important to point out, though, that the effect on these processes may depend on the tolerance of the specie or cultivar and on the duration and severity of the stress. In the short-term, plants may activate their defense mechanisms against stress. However, in the long-term, plants may acclimate to a mild stress or may be seriously damaged if the stress was severe. In addition, similar

symptoms might be the result of different stresses. Therefore, it is important to keep in mind the conditions of the measurement in order to give a correct diagnosis.

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3. EMPIRICAL MODELS OF NUTRIENT AND WATER UPTAKE BY ROSE PLANTS FOR FERTIRRIGATION MANAGEMENT

EMPIRICAL MODELS OF NUTRIENT AND WATER UPTAKE BY ROSE PLANTS FOR FERTIRRIGATION MANAGEMENT

Abstract

Empirical models for nutrient and water uptake of greenhouse rose plants were developed by multiple regression with data obtained along 14 months. The interest of this work lies in the high applicability of the models for their use in real conditions. Generally, independent variables included in the nutrient uptake models (nitrate, phosphate, potassium, calcium and magnesium) were water absorption, nutrient solution concentration, vapour pressure deficit, radiation integral inside the greenhouse, and several indicator variables related to periods of time were nutrient uptake was enhanced or diminished due to internal factors. The models also integrated the effect of flower shoot production and of some common practices such as renewal of old bent shoots, the use of shade screen or the synchronization of flower shoot development for scheduling purposes. The coefficient of determination ranged between 0.336 and 0.785 for magnesium and nitrate uptake models, respectively. However, a higher value (0.902) was obtained for the water uptake model, which included the variables vapour pressure deficit, air temperature, nutrient solution temperature, radiation integral inside the greenhouse, as well as renewal of old bent shoots and flower shoot production. The strong and weak points, usefulness and ways to improve the models are discussed.

Introduction

Current management practices in greenhouse rose cultivation include the use of luxuriant amounts of fertilizers in relation to that actually consumed by the crop. This is done to avoid any nutrient deficiency in the plant, but leaching of unused nutrients can lead to water contamination (Mankin and Finn, 1996). Besides, supplying fertilizers in high amounts can cause toxicity and nutrient imbalances in the crop. Therefore, in order to reduce the environmental impact and achieve optimum nutrient use efficiency, nutrient demand and supply should be synchronized (Kläring et al., 1997). Understanding the factors involved in nutrient uptake from a deterministic or empirical approach can help in this synchronization.

Regulation of nutrients uptake occurs through different mechanisms. Firstly, transport is driven by the H^+ gradient across the plasma membrane established by an H^+-ATPase pump, so ions uptake is thermodynamically uphill and thus dependent on metabolism (Marschner, 1995). Ion uptake is one of the major sinks for energy in roots (Van der Werf et al., 1988). For instance, nitrate uptake rate has been closely related to diurnal root respiration (Hansen, 1980). Secondly, uptake velocity has been associated with external nutrient concentration (Le Bot et al., 1998) because nutrients transporters are known to be induced by the concentration of its own substrate outside the root (Crawford and Glass, 1998; Glass et al., 2002). In contrast, ion transporters are down-regulated by the concentration of their specific ion within the root cells (Siddiqi and Glass, 1982; 1987; Glass et al., 2002). Finally, uptake rates of

most ions are seemingly controlled by specific demand-driven regulatory mechanisms. According to this idea, plant demand would result in the transport of feedback substances to the root transporters that would improve/reduce ions uptake (Imsande and Touraine, 1994; Glass et al., 2002; Smith, 2002).

Climatic factors affecting the regulation of nutrient transporters will affect nutrients uptake. The positive effect of radiation on nitrate uptake of different crops has been shown by many studies (Brun and Chazelle, 1996; Mankin and Finn, 1996; Cedergreen and Madsen, 2003; Pardossi et al., 2005). Air temperature (Adams, 1992; Kläring et al., 1997; Pardossi et al., 2005) and nutrient solution temperature (Adams, 1992; Brun and Chazelle, 1996; Bassirirad, 2000; Bougul et al., 2000; Dong et al., 2001; Chapter 4.1 of this thesis) also seem to affect nutrient uptake while air humidity, measured as relative humidity or vapour pressure deficit (VPD), has appeared in less studies (Kläring et al., 1997).

In addition, correlation between water and nutrient uptake of different crops has been proved on the large time scale (Le Bot et al., 1998; Pardossi et al., 2005) although at an hourly time scale, the correlation is not so clear (Le Bot and Kirkby, 1992; Cárdenas-Navarro et al., 1998). As far as water uptake is concerned, it has been mainly related to radiation, to VPD and to leaf area (Baille et al., 1994; Medrano, 1999; Suay et al., 2003), but also to air temperature (Medrano, 1999) and to nutrient solution temperature (Chapter 4.1 of this thesis).

A good knowledge of the factors affecting nutrient uptake allows the development of models that can be implemented in decision support systems for the management of nutrient solution in soilless culture (Marcelis et al., 1998; Carmassi et al., 2005; Massa et al., 2008). Roses are valuable for studying dynamics of nutrient uptake, storage and remobilization in woody crops because they exhibit many flushes of flower shoot growth every year (Cabrera et al., 1995; Mattson and Lieth, 2007a). These growth flushes may cause periods of high and low plant demand for mineral nutrients (Cabrera et al., 1995). In general, flowers at different stages of development can be found in the greenhouse at the same time but, when dealing with special dates, harvests are scheduled and flower development is homogenous in the greenhouse (Mattson and Lieth, 2007b). Most reported works on nutrient uptake of cut roses have focused largely on nitrogen, and information on uptake of other essential nutrients is quite limited (Brun and Chazelle, 1996; Bougoul et al., 2000; Silberbush and Lieth, 2004; Mattson and Lieth, 2007a; Kim et al., 2008; Massa et al., 2008; Massa et al., 2009). Most of these models were obtained from short-time experiments (Brun and Chazelle, 1996; Silberbush and Lieth, 2004; Kim et al., 2008; Massa et al., 2008; Massa et al., 2009), which would limit their validity in normal growing conditions. Moreover, many of them only include few factors, basically the concentration of the nutrient in

the root environment, and explain nutrient uptake according to the Michaelis-Menten kinetics (Silberbush and Lieth, 2004; Mattson and Lieth, 2007a; Kim et al., 2008; Massa et al., 2009).

The objective of this work was to develop empirical models for the uptake of five different nutrients (nitrate, phosphate, potassium, calcium and magnesium) and one model for water uptake by rose plants. Plants were grown in usual greenhouse conditions and managed using common practices, for an experimental trial of 14 months. Emphasis was given in building models with high applicability under real conditions that might be implemented in decision support systems.

Materials and methods

Plant growing conditions

A rose crop (*Rosa hybrida* L. cv. Grand Gala) in its third year after planting was grown in a polycarbonate greenhouse, equipped with convective heating (minimum 16°C), high pressure fogging and roof ventilation. Plants were grown following the bending technique as it is commonly done by local growers (Calatayud et al., 2007) and produced flower shoots all year-round. The experiment began on the 15th February 2005 and finished on the 12th April 2006. From the 1st of June until the 24th of October, coinciding with the period of highest incoming solar radiation, an external aluminized screen was placed over the greenhouse to reduce incoming solar radiation and temperature inside. Renewal of old bent shoots was done through pruning on the 29th of August.

The composition of the nutrient solution was slightly modified according to the season as it is commonly done by experience. We used a nutrient solution more diluted than the one commonly used by local rose growers in order to gain accuracy in the measurements, as explained below. Nevertheless, we observed that plants did not show any nutrient deficiency as it is also discussed below. The water for the nutrient solution was previously treated with reverse osmosis and ion columns in order to avoid variation of nutrient concentration in the solution. The actual concentration of NO_3^-, $H_2PO_4^-$, K^+, Ca^{2+} and Mg^{2+} in the nutrient solution along the whole experiment is shown in Fig. 1.

A set of 30 plants was grown in a closed-looped aeroponic system with a single tank that received the drain and from which solution was pumped again to feed all plants. Nutrient solution was recycled and was renewed once a week to ensure an optimum nutrient balance.

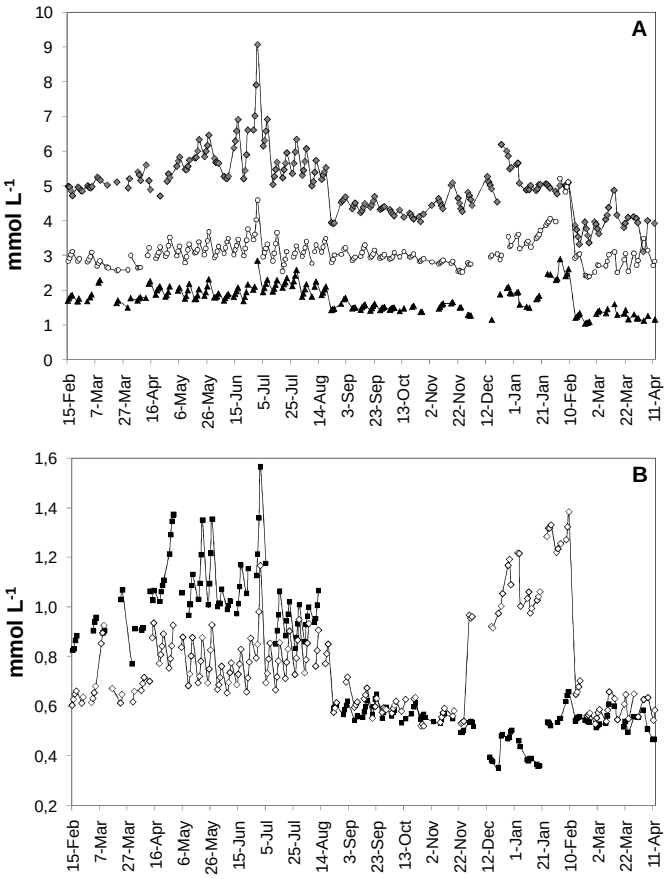

Fig. 1. Concentration of the nutrient solution along the experiment (mmol L^{-1}) (15/02/2005-12/04/2006). A: nitrate concentration (◆), potassium concentration (○) and calcium concentration (▲). B: phosphate concentration (■) and magnesium concentration (◇).

During the first 9 months of the experiment, flowers at different stages of development were found in the greenhouse at the same time. However, in order to include in the model two different situations in the greenhouse with respect to flower development, flower shoot development was synchronized during the last 5 months. For that purpose, the flowering shoots of all plants were pruned down to two nodes from their base on the 25th of November 2005 and on the 9th of February 2006 so that 2 flower cycles could be studied. For each cycle, 2 periods were differentiated according to its relative growth rate (Steininger et al., 2002): from flower soot pruning till the appearance of the visible bud of the immature flower shoot and from the latter until the open flower is ready to harvest. The fresh weight of all harvested flower shoots was quantified.

Solar radiation outside and inside the greenhouse, temperature of the air and solution, and relative humidity, were recorded every 15 s by means of electronic sensors placed over the canopy and connected to a data acquisition system. The evolution of the climatic parameters used for building the models is shown in Fig. 2. Lack of values appears in some dates when failure in the data acquisition occurred. All parameters followed the expected evolution for Mediterranean conditions. However, the external aluminized screen used around summertime had a profound effect on the incident radiation integral inside the greenhouse, lowering it down to values common for autumn and winter. VPD was also affected although in a lesser extent.

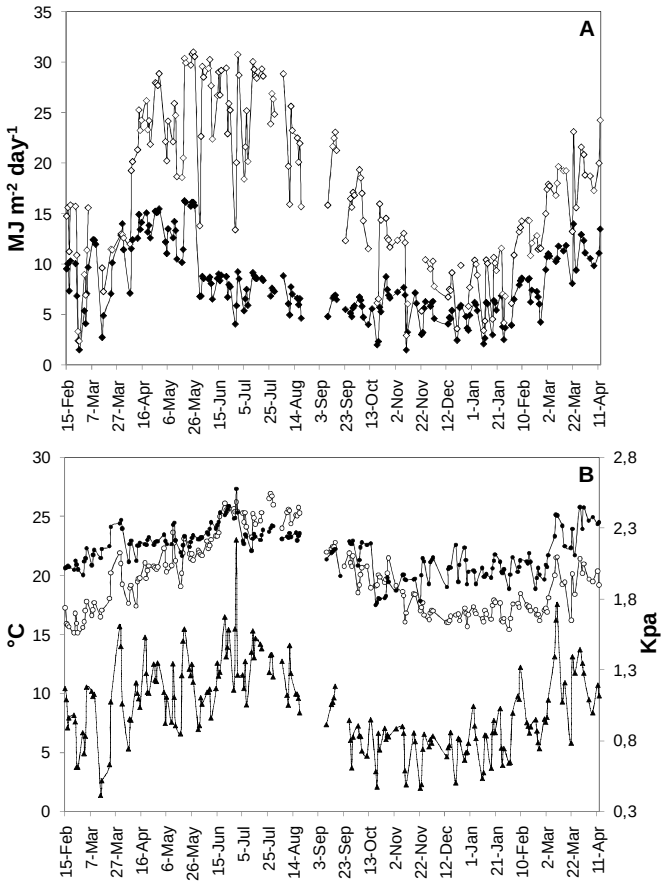

Fig. 2. Evolution of climate parameters along the experiment (15/02/2005 - 12/04/2006). A: incident radiation integral outside (◇) and inside (◆) the greenhouse (MJ m^{-2} day^{-1}). B: mean air (○) and nutrient solution temperature (●) inside the greenhouse (°C), and mean vapour pressure deficit inside the greenhouse (▲) (KPa).

Water and nutrient absorption by plants

Water and nutrient uptake rates were calculated by measuring depletion of nutrients in the nutrient solution tank, which represented the root zone solution since the inertia of the aeroponic system was assumed to be nonexistent.

The tank of the aeroponic system was associated with a precision weighing balance (±0.1 g resolution) connected to a data logging system, so that the weight of the tank was recorded every 15 s. A linear regression between weight an volume of the tank of R^2 equal to 0.99, was calculated before the beginning of the experiment assuming solution density as constant. Daily water uptake, expressed as L plant^{-1} day^{-1}, was calculated according to the volume difference in the system between two consecutive days. The system was watertight, so all volume losses were attributed to water and nutrient uptake. Every working day of the experiment at noon, 40 mL of nutrient solution were collected from the tank. The concentration of NO_3^- and $H_2PO_4^-$ was measured using Flow Injection Analyzer (FIASTAR 5000, Foss Analytical, Höganäs, Sweden), while the concentration of K^+, Ca^{2+} and Mg^{2+} was measured by an ion absorption spectrophotometer (Perkin-Elmer Analyst 200, USA). Daily nutrient uptake was expressed as mmol plant^{-1} day^{-1}, and was calculated using equation 1 of Chapter 2. Daily nutrient and water uptake were calculated between 12 noon of one day and 12 noon of the following day.

Nutrient uptake ratios

Several daily ratios of nutrient uptake were calculated along the whole experiment. The nutrient uptake to water uptake ratio (mmol nutrient absorbed/L water absorbed) was calculated daily for every ion. We studied as well the daily ratio between each nutrient uptake and its concentration in the nutrient solution [($mmol_{nutrient\ absorbed}$ plant^{-1} day^{-1})/(mmol L^{-1})]. Also, daily nutrient use efficiencies [(mmol nutrient absorbed)·(L water absorbed)$^{-1}$)/(mmol L^{-1})] were calculated according to equation 1 (Kläring, 2001):

$$Nutrient\ \ use\ \ efficiency = \frac{(1 - LF)\cdot C_u}{C_S} \qquad (1)$$

where C_u is the daily nutrient uptake to water uptake ratio, C_s is the concentration in the nutrient solution and LF is the leaching fraction, which can be considered zero when dealing with an aeroponic closed-loop system.

Statistical data analysis

In order to develop a statistical model for each nutrient, all experimental data were arranged in a matrix containing 210 observations (days) by 6 dependent variables (i.e.,

the daily absorption data of nutrients and water) and the following variables containing climatic values: incident radiation integral outside (Ro) and inside (Ri) the greenhouse, mean air temperature inside the greenhouse (Ta), mean nutrient solution temperature (Ts) and mean VPD inside the greenhouse. These parameters were calculated between 12 noon of one day and 12 noon of the following day. On the other hand, the concentration of every ion, the pH and electrical conductivity of the nutrient solution as well as the flower shoot production, through the variable PROD (see below), were also included in the matrix as additional variables. Moreover, several indicator variables were included to study the effect of factors such as pruning for renewal of old bent shoots or the stage of development of the flower shoots, and also to study the effect of factors not quantified in the experiment. These indicator variables take the values 0 or 1 to indicate the absence or presence of some effect that may be expected to shift the outcome. They were created according to the evolution versus time of the model residuals, which allowed the identification of periods of time when the average of the residuals was significantly different to zero.

For modeling purposes, data were subjected to stepwise multiple regression. The normality of the distribution of residuals was verified and a few outliers were removed. The possible differences between seasons in several nutrient ratios were analyzed by one-way ANOVA and means were compared by Fisher's least significant differences (LSD). The length of the different seasons considered in this study was only the standard for wintertime (22/12/05 - 21/03/06), but for the remaining seasons it depended on the use of the screen. Spring (21/03/05 - 31/05/05) and autumn (25/10/05 - 21/12/05) were shorter due to the fact that the period with screen (01/06/05 - 24/10/05) was longer than the standard summer. A significance level of α=0.05 was used in all cases. Statgraphics Plus 4.1 was used for statistical analysis.

Results

Flower production

Flower production was not constant along the 14 months but followed a cyclical pattern of flushes of flower shoot growth. This pattern can be seen in Fig. 3 that shows the production along the year. A new parameter called PROD was calculated as the sum of the fresh weight (g) of flower shoots harvested per plant on a specific day and on the following 6 days. This was done to obtain an estimation of the flower shoots present in a plant, either ready to harvest or almost, and therefore estimate sink strength and leaf area due to flower shoots. Actually, the value of PROD in one day was assumed to be approximately proportional to the leaf area of the plant in that day (data not shown).

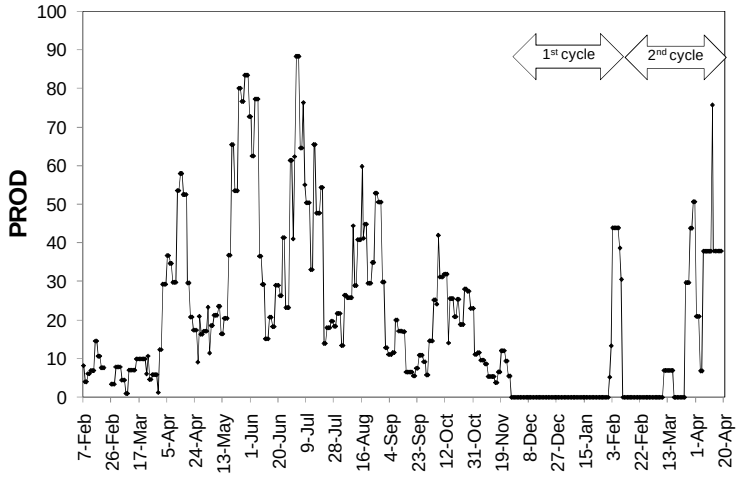

Fig. 3. Production of flower shoots along the experiment measured as the variable PROD, which is the sum of the fresh weight (g) of flower shoots harvested per plant on a specific day and on the following 6 days. Flower shoots were in continuous production till the end of November. From then, 2 flower cycles, indicated by arrows, were consecutively synchronized by pruning down all flower shoots at the beginning of each cycle.

In general terms, production was lower in winter and higher in June and July (Fig. 3). The length of the flower cycle and of the two phases of each flower cycle can be calculated from the two synchronized flower cycles at the end of the experiment (Fig. 3). The period from flower shoot pruning to visible bud lasted from 25/11/05 to 18/01/06 in the 1st cycle and from 10/02/06 to 14/03/06 in the 2nd cycle. The period from visible bud until harvest time lasted from 19/01/06 to 09/02/06 in the 1st flower cycle and from 15/03/06 to 12/04/06 in the 2nd cycle. Therefore, the average duration of the flower cycle was higher in winter (76 days) than in spring (62 days).

Daily water and nutrient uptake rates

Daily water and nutrient uptake rates along the experiment are shown in Fig. 4 and Fig. 5. They all followed a similar pattern. In general terms, daily rates increased in spring (highest values from April to July), decreased in summer, and remained relatively stable in autumn and winter (lowest values). Roughly speaking, daily water and nutrient uptake rates followed the evolution of climatic parameters (see Fig. 2).

Data dispersion was higher for the nutrients absorbed in a lower amount, that is, phosphate and magnesium (Fig. 5), and increased in the periods of higher concentration of phosphate and magnesium in the nutrient solution.

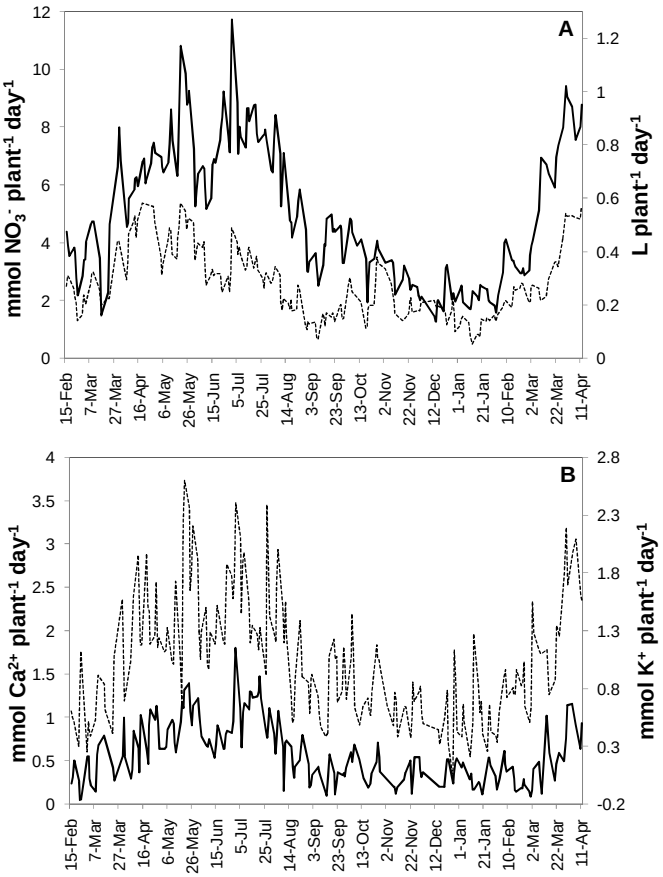

Fig. 4. Actual water uptake (L plant^{-1} day^{-1}) and nutrient uptake (mmol plant^{-1} day^{-1}) along the experiment. A: nitrate uptake (dashed line) and water uptake (solid line); B: potassium uptake (dashed line) and calcium uptake (solid line).

Nutrient uptake ratios

Nutrient concentration in the nutrient solution was not the only factor affecting nutrient uptake, given that the ratio of nutrient uptake to nutrient concentration was not constant along the seasons, and changed significantly. In spring, this ratio yielded the highest values for all nutrients (Fig. 6A).

The ratio of nutrient uptake to water uptake, also called nutrient uptake concentration (Schwarz et al., 2001), is shown in Fig. 6B. Nitrate uptake concentration (NUC) had the highest values and was the nutrient that changed the most, with statistically significant differences, along the year, in particular between the period when the screen was used (3.77) and autumn (7.18). However, no significant differences were found in NUC between spring and winter. Phosphate uptake concentration (PUC) was significantly higher in spring than in the rest of the year.

Potassium uptake concentration (KUC) showed significant differences between winter and the period with screen, while magnesium uptake concentration (MgUC) showed significant differences between winter and both spring and the period with screen. Conversely, in calcium uptake concentration (CaUC) no statistically significant differences were observed among the 4 periods.

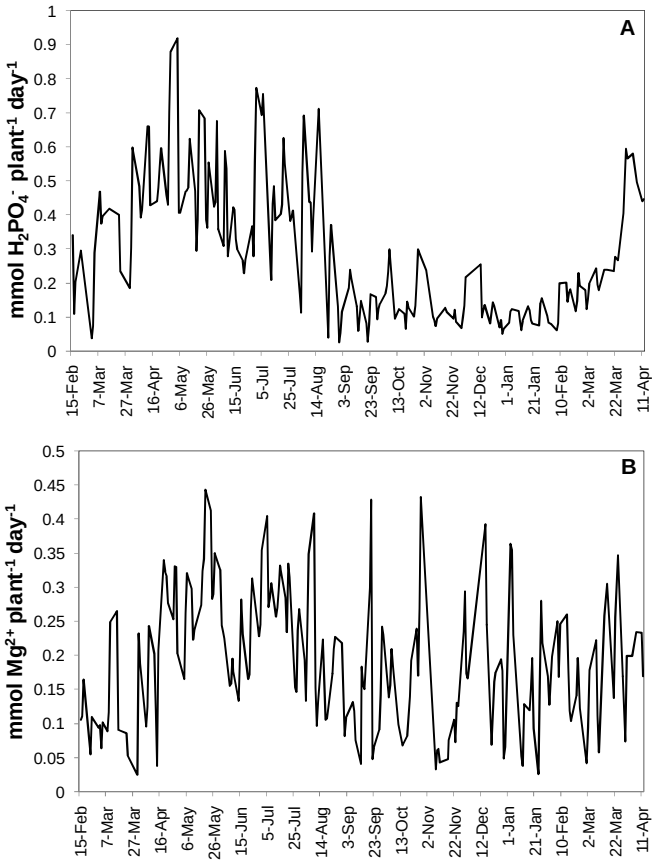

Fig. 5. Actual phosphate (A) and magnesium (B) uptake (mmol plant^{-1} day^{-1}) along the experiment.

In general, the highest nutrient use efficiency calculated by equation 1 was achieved in autumn, while the lowest was obtained in the period with screen, for all nutrients. Nitrate use efficiency (NUE) yielded the highest values while magnesium use efficiency (MgUE) was the lowest (Fig. 6C).

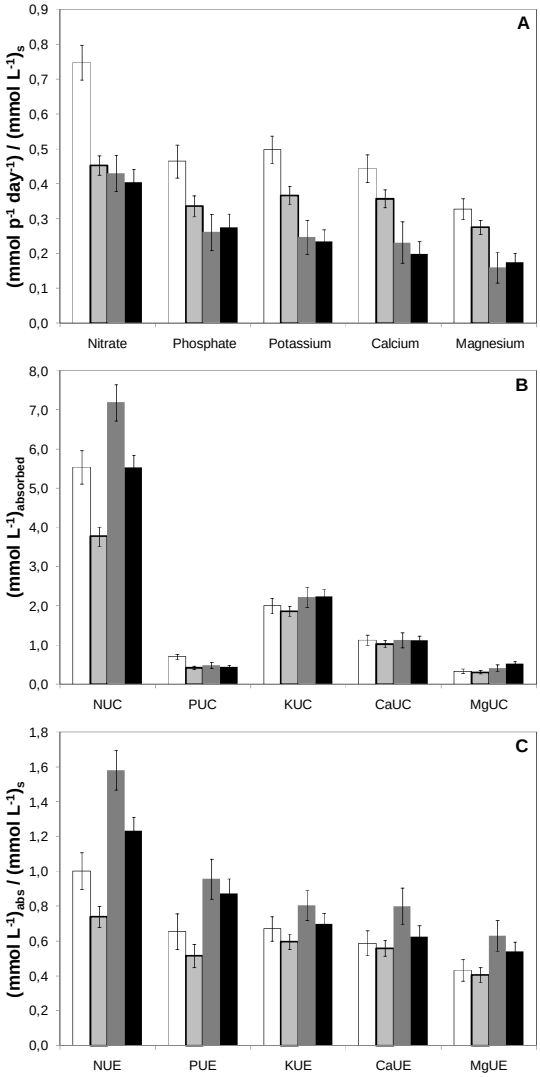

Fig. 6. Evolution of several nutrient ratios along the experiment. A: ratio of nutrient uptake to nutrient concentration, in [(mmol$_{\text{nutrient absorbed}}$ plant^{-1} day^{-1}) / (mmol L^{-1})$_{\text{solution}}$]. B: ratio of nutrient uptake to water uptake, i.e. nutrient uptake concentration, in (mmol nutrient absorbed/L water absorbed). NUC, PUC, KUC, CaUC and MgUC stand for nitrate, phosphate, potassium, calcium and magnesium uptake concentration, respectively. C: nutrient use efficiency, in [(mmol nutrient absorbed)·(L water absorbed)$^{-1}$) / (mmol L^{-1})$_{\text{solution}}$]. NUE, PUE, KUE, CaUE and MgUE stand for nitrate, phosphate, potassium, calcium and magnesium use efficiency, respectively. For each nutrient, one-way ANOVA was performed with factor season (spring (□): 21/03/05 - 31/05/05; the period with screen (▣): 01/06/05 - 24/10/05; autumn (▒): 25/10/05 - 21/12/05; winter (■): 22/12/05 - 21/03/06). Intervals indicate Fisher's least significant differences (LSD). Whenever LSD intervals overlap, not significant differences at 5% are found between those seasons.

Empirical models

The evolution of the actual nutrient and water uptake is shown in Fig. 4 and Fig. 5. These data were used for modelling nutrient and water uptake.

Nitrate uptake (NU)

The model of nitrate uptake developed in this work is the following:

$$NU = 1.422 + 3.038 \cdot WU + 0.174 \cdot Ri - 1.789 \cdot VPD + \\ + 0.0056 \cdot PROD - 0.619 \cdot P_{PRUN} - 0.46 \cdot P_{VB} \quad (2)$$

The effect of water uptake (WU) and the climatic parameters VPD and Ri were statistically significant (p<0.0001). A model built with these 3 parameters overestimated nitrate uptake during a period between the 30th of August and the 29th of September. This period takes place just after the renewal of old bent shoots, so it was attributed to this practice. Thus, pruning had a negative effect on nitrate uptake during one month (30/08/05 - 29/09/05) until the plant could recover from this practice. The effect of the indicator variable P_{PRUN}, that accounts for this period, was statistically significant (p=0.0035). Besides the pruning practice, the physiological stage of development of the flower shoot had also an effect on nitrate uptake. The indicator variable P_{VB}, which represented a period of time 15-20 days before and 7-9 days after the appearance of the visible bud (29/12/05 - 26/01/06 in the 1st cycle and 28/02/06 - 24/03/06 in the 2nd cycle), was statistically significant (p=0.0007). Finally, flower shoot production (PROD), calculated as explained above, had a positive significant effect on nitrate uptake (p=0.0437).

The coefficient of determination was quite high (R^2=0.79), which indicates that 79% of variability of the nitrate uptake could be explained by the statistical model. The standard deviation of the residuals was 0.564 and the standard deviation of the measurement error of nitrate uptake was estimated as 0.324. By replacing the residual variance by the variance of the measurement error in the R^2 formula, one would get the maximum R^2 that could be achieved with a given measurement method if all factors affecting nitrate uptake were known. In this case, maximum R^2 would be about 0.928, a value relatively close to that obtained in this work. The only way to enhance R^2 over 0.928 would be improving the accuracy of the measurement method.

Phosphate uptake (PU)

The model of phosphate uptake developed in this work is the following:

$$PU = -0.119 + 0.239 \cdot WU + 0.34 \cdot C_P - 0.068 \cdot P_{PRUN} + \\ + 0.114 \cdot P_{P1} + 0.226 \cdot P_{P2} \quad (3)$$

Phosphate uptake was predicted with an R^2 of 0.596. The effect of water uptake (WU), phosphate concentration (C_p) and of 2 indicator variables associated with periods of time taking place around April (P_{P1}: 30/03/05 - 13/05/05; P_{P2}: 28/03/06 - 12/04/06) were statistically significant (p<0.0032). Although the pruning-affected period (P_{PRUN}) was not clearly significant (p=0.072), it was not eliminated from the model since it also appeared in the model of low concentration, as explained below, with a lower dispersion of the data.

Residual variance of this model was very different depending on the concentration of phosphate in the nutrient solution which was reduced to almost the half by the end of August (Fig. 1B). Actually, residual variance of the model was 0.039 for the first group of data (high concentration; from the beginning of the experiment till the 23rd of August) while it was 0.0053 for the second group of data (low concentration; from the 24th of August till the end of the experiment). The reduction of phosphate concentration caused a decrease in the measurement error. The standard deviation of the measurement error was estimated as 0.195 for the first group and 0.071 for the second group. This fact justifies modelling the data variability separately for each data set.

When phosphate concentration was low (Eq. 4), the effect of water uptake was statistically significant (p<0.0001) and the pruning practice affected negatively (p=0.014). During one month just after pruning, plants absorbed less phosphate. However, this model underestimated a period of time in April 2006, at the end of the experiment, when the actual uptake was higher than the one predicted. This effect is explained by the indicator variable P_{P2}, which entered in the model with significance (p<0.0001).

$$PU = 0.0508 + 0.249 \cdot WU - 0.0432 \cdot P_{PRUN} + 0.225 \cdot P_{P2} \quad (4)$$

When phosphate concentration was high (Eq. 5), the effect of phosphate concentration was statistically significant (p=0.011). Although water uptake was not clearly significant (p=0.074), its coefficient was similar to that in Eq. 4 (+0.24), and we decided to keep it in the model. The effect of the indicator variable P_{P1} was also statistically significant (p=0.037).

$$PU = -0.2808 + 0.247 \cdot WU + 0.486 \cdot C_P + 0.114 \cdot P_{P1} \quad (5)$$

The coefficient of determination was quite different depending on the data set because as residual variance of the model increased, R^2 declined strongly (low concentration=0.718; high concentration=0.268).

Potassium uptake (KU)

The model of potassium uptake developed in this work is the following (R^2=0.626):

$$KU = 0.4253 + 2.236 \cdot WU - 0.655 \cdot VPD + 0.165 \cdot P_{P-VB} + 0.177 \cdot P_K \quad (6)$$

The effect of water absorption was statistically significant (p<0.0001) as well as for VPD (p=0.0006). However, a model with these two variables underestimated several periods of time, when the actual uptake was higher than the one predicted. The indicator variable P_K accounts for one of the periods that took place between the middle of May and the beginning of July (19/05/05 - 08/07/05) (p=0.034). Two additional periods were those from flower shoot pruning to visible bud of the 2 flower cycles taking place at the end of the experiment. The effect of the indicator variable P_{P-VB}, which accounts for these periods, was statistically significant (p=0.036).

Calcium uptake (CaU)

The model of calcium uptake developed in this work is the following (R^2=0.633):

$$CaU = -0.0876 + 1.1087 \cdot WU - 0.3623 \cdot VPD + 0.213 \cdot C_{Ca} + 0.1715 \cdot P_{Ca} \quad (7)$$

The effects of water absorption, calcium concentration in the nutrient solution (C_{Ca}) and VPD were statistically significant (p<0.0066). However, a model with these variables underestimated a period from the middle of April till the beginning of August (20/04/05 - 05/08/05). The indicator variable P_{Ca} associated to this period entered in the model with significance (p=0.0037).

Magnesium uptake (MgU)

The model of magnesium uptake developed in this work is the following:

$$MgU = -0.041 + 0.1877 \cdot WU + 0.154 \cdot C_{Mg} + 0.067 \cdot P_{Mg} \quad (8)$$

The effects of water absorption and magnesium concentration (C_{Mg}) in the nutrient solution were statistically significant (p<0.0001), but this model underestimated a period from the middle of April till the beginning of June (15/04/05 - 02/06/05). The indicator variable P_{Mg}, associated to this period, entered in the model with significance (p=0.0023). Residual variance was quite high as in the case of phosphate and, thus, R^2 was low (0.336).

Water uptake (WU)

The model of water uptake developed in this work is the following:

$$WU = -0.987 + 0.2637 \cdot VPD + 0.027 \cdot Ts + 0.0219 \cdot Ta + \\ + 0.0226 \cdot Ri + 0.002 \cdot PROD - 0.0838 \cdot P_{PRUN} \quad (9)$$

Water uptake followed a similar evolution as VPD (Fig. 2B and 4A). In this case, the following climatic parameters resulted statistically significant (p<0.0001): VPD, Ri, Ts and Ta. However, despite its high goodness-of-fit (0.86), the model did not forecast properly several cyclical periods with accuracy. We observed a cyclical pattern in the evolution of residuals versus time that resembled the evolution of flower production (Fig. 3). This suggested the inclusion in the model of additional variables. After including 2 variables in the model that referred to periods of different plant leaf area, the R^2 increased up to 0.902. One of the variables was the indicator variable P_{PRUN}, related to the pruning-affected period, which had a negative coefficient and a significance of 0.0116. The other variable was PROD, which had a positive coefficient and p<0.0001.

Discussion

In this work, empirical models were developed for nitrate, phosphate, potassium, calcium, magnesium and water uptake. The interest of these models lies in the fact that they were built in normal conditions of rose cultivation during 14 months, so that all different climatic situations and several common management practices could be reflected by the models. This makes them very practical to be used in real conditions.

Both nutrient and water uptake rates underwent seasonal variations, as observed in Fig. 4 and Fig. 5. As their seasonal pattern was similar to that of climatic parameters (Fig. 2), it suggested that nutrient and water uptake rates depended on climatic conditions so they might be modelled according to them. The highest values of nutrient and water uptake rates were obtained from April to July, and the lowest along autumn and winter. This agrees with Cabrera et al. (1995), who found that nutrient uptake of rose plants in summer was twice than in winter. The same was found by Ferrante et al. (2000) in gerbera.

Goodness-of-fit of nutrient uptake models

The highest goodness-of-fit of all nutrient uptake models developed in this work was that of nitrate uptake (R^2=0.79), which suggests that most factors affecting nitrate uptake were already included in the model. Thus, probably, the best way to improve it would be by enhancing the accuracy of the measurement method. The R^2 obtained

here for the nutrient uptake models were similar to those obtained in other studies with melon (Pardossi et al., 2005), which was 0.65, 0.56, 0.66, 0.72 and 0.57 for the models of nitrate, phosphate, potassium, calcium and magnesium weekly uptake, respectively. In a reported work with roses (Brun and Chazelle, 1996), R^2 of 0.71 was obtained for an hourly nitrate uptake model.

It is important to understand the effect of nutrient solution concentration on the accuracy of measuring depletion of nutrients in the root environment, since it influenced the goodness-of-fit of the models obtained. The higher the nutrient solution concentration, the lower the difference in nutrient solution concentration between two consecutive days, and the more difficult (i.e. less accurate) to measure nutrient uptake through Eq. 1 (Chapter 2). Some authors have also found difficulties that limit the accuracy of uptake rate measurements when the nutrient solution concentration is elevated (Le Bot et al., 1998). This problem is greater with nutrients that are absorbed at a low rate. Therefore, it is often necessary to use a nutrient solution more diluted than the standard (Fig. 1) in order to obtain accurate measurements. However, the dilution used in this work was not enough for nutrients that were absorbed at a low rate, i.e. phosphate and magnesium, where the residual variance of the models increased considerably, and the coefficients of determination decreased to a great extent. In the case of phosphate, it was clearly seen that when using a more diluted solution, residual variance of the models decreased and R^2 increased. For future work, a more diluted concentration of these nutrients should be used. The problem is that an adequate concentration to gain accuracy in the measurements might result in nutrient deficiencies that could affect nutrient uptake. Therefore, it could be appropriate to carry out physiological measurements in the plant to ensure that no stress is observed. In this work, plants did not show apparently any nutrient deficiency and the physiological measurements carried out in the plants during the last 4 months of the experiment proved that plants were in good conditions (Chapter 4 of this thesis).

Factors affecting nutrient uptake rate

Generally, independent variables included in the nutrient uptake models were water absorption, nutrient solution concentration, the climatic parameters vapour pressure deficit and radiation integral inside the greenhouse, and several indicator variables related to periods of time when nutrient uptake was enhanced or diminished due to internal factors. Next, the influence of each of the variables on the different nutrients uptake will be discussed.

One of the parameters most studied in relation to nutrient uptake is nutrient solution concentration. In fact, most of the nutrient uptake models for rose plants are based on the Michaelis-Menten kinetics (Silberbush and Lieth, 2004; Mattson and Lieth, 2007a;

Kim et al., 2008; Massa et al., 2009) and include basically the concentration of the nutrient in the root environment to explain nutrient uptake. In our work, concentration of phosphate, calcium and magnesium appeared in their respective models with significance. Although this parameter did not have apparently an effect on nitrate and potassium uptake, it was probably implicit in another variable. Actually, the simple correlation between nitrate uptake and nitrate concentration, and between potassium uptake and potassium concentration was statistically significant (p<0.016). The concentration of a nutrient in the root environment has been verified to induce a higher capacity for uptake on its transporter (Crawford and Glass, 1998; Glass et al., 2002). However, this work clearly proves that nutrient solution concentration is important but it is not the only factor affecting nutrient uptake as seen by the statistically significant variation of the ratio nutrient uptake to nutrient concentration among seasons (Fig. 6A).

Water uptake appears in every nutrient uptake model with high significance, so it might affect all nutrients uptake through a common mechanism. Several studies have found a significant correlation between water and nutrient uptake of different crops on a large time scale (Le Bot et al., 1998; Pardossi et al., 2005). In contrast, the ratio of nutrient uptake to water uptake was not constant along the year, as seen in Fig. 6B. We suggest that water uptake may affect nitrate and other nutrients uptake via 2 ways. Once absorbed, ions are transported through the xylem with the transpiration flux. Transpiration from leaves and the rate of water uptake in the root, determines flow within the xylem and the concentration of its solutes (Marschner, 1995). As nutrient uptake may be down-regulated by its accumulation in the root cells (Siddiqi and Glass, 1982; Siddiqi and Glass, 1987; Glass et al., 2002), a higher water uptake would prevent ion accumulation in root cells by transporting ions far from the roots. Alternatively, nutrients in the solution tend to concentrate around the root surface due to water uptake (Wallach, 2008). As nutrients transporters are induced by the concentration of its own substrate outside the root (Crawford and Glass, 1998; Glass et al., 2002), a higher water uptake that would lead to a higher nutrient concentration in the root surface, would in turn increase root nutrient uptake capacity.

Incident radiation integral inside the greenhouse had a positive effect on nitrate uptake but did not have a significant effect on the rest of nutrients studied. However, other authors have shown that total uptake of P, K, Ca or Mg increased with increasing irradiances (Chu and Toop, 1975; Magalhaes and Wilcox, 1983). In our results, in general terms, the uptake of these nutrients also increased with increasing irradiances but the effect was not significant. The positive effect of radiation on nitrate uptake of different crops has been reported by many studies (Brun and Chazelle, 1996; Mankin and Finn, 1996; Cedergreen and Madsen, 2003; Pardossi et al., 2005). As Mankin and Finn (1996) showed, radiation affects nitrate uptake via its effect on photosynthesis.

According to Cedergreen and Madsen (2003), plants grown at high irradiance have higher growth rates and higher nitrate uptake rates and reduction capacity, and vice versa. It can be suggested that radiation may affect nitrate uptake through different mechanisms. Firstly, a high radiation, up to a threshold level, increases CO_2 fixation and, thus, production of assimilates, which may be used in growth and also, in root respiration. Ion uptake is one of the major sinks for ATP in roots (Van der Werf et al., 1988), which is produced by root respiration (Johnson, 1990). Actually, root respiration has been closely related to nitrate uptake rate (Hansen, 1980). Secondly, the higher photosynthetic rate induced by high radiation may result in a higher growth rate (Poorter, 1999), and nitrate uptake has been suggested to be driven by growth rate (Willits et al., 1992) and regulated in response to plant demand through feedback control (Imsande and Touraine, 1994). According to this idea, plant demand would result in the transport of feedback substances to the root nitrate transporters that would improve/reduce nitrate uptake. Finally, root nitrate reductase, which reduces nitrate to nitrite and, thus, decreases nitrate concentration in the root cytoplasm, is induced by light (Cerezo, 1998; Tischner, 2000; Cedergreen and Madsen, 2003). There is evidence that accumulated nitrate in the root cells down-regulates transporter function (Glass et al., 2002). Therefore a high nitrate reductase activity in the root would prevent the inhibition of nitrate transporters by nitrate accumulation. In brief, the positive effect of radiation on nitrate uptake may be due to its effect on root respiration, plant demand and nitrate reductase activity, which would in turn affect nitrate transporters uptake capacity.

Vapour pressure deficit had a negative coefficient in the models of nitrate, potassium and calcium uptake. However, the simple correlation between VPD and each of the five nutrients studied was positive ($r>0.36$) and significant ($p<0.0001$), so the negative sign makes no physiological sense. Actually, VPD may affect positively nutrient uptake via its effect on water uptake (see Eq. 9). When a model is built with correlated variables, as it happens in this case, the interpretation of the coefficients may sometimes result confusing and lead to wrong conclusions. This should be, thus, interpreted as the best lineal combination of variables that maximizes the R^2.

Flower shoot production represented by the variable PROD affected nitrate uptake positively. Plant demand was higher during periods of high production, which would enhance nitrate uptake through feedback control (Imsande and Touraine, 1994). Besides, the higher leaf area of the plant during the periods of high production would increase net assimilation rate, which would increase availability of assimilates in the roots and, thus, nitrate uptake (Hansen, 1980). None of the other nutrients uptake was significantly affected by this parameter, but this does not mean that plant demand did not affect the uptake of these nutrients as it is explained below.

Besides these quantitative variables, some indicator variables appeared in the models with significance. They were related to periods of time in which some known (pruning practice and developmental stage of the flower shoot) or unknown factors had an effect on nutrient uptake.

The practice of pruning old bent shoots had an effect on nitrate and phosphate uptake. The pruning-affected period lasted one month and its negative effect was maybe due to the reduction of leaf area that reduced photosynthetic rate in the plant (Macduff and Jackson, 1992). Also, pruning may have caused death of some roots or may have reduced relative growth rate of roots, thus reducing uptake surface (Fuchs, 1986). A negative effect of shoot pruning on N and P uptake was also observed by Cadisch et al. (2004) in *Peltophorum dasyrrhachis* and *Gliricidia sepium*, and by Caradus and Snaydon (1986) in white clover, respectively. Nurseries suggest carrying out the pruning of old bent shoots in summer but before August, when metabolic activity of the plant is the highest (Real, 1997). Maybe, pruning in another date according to Nurseries' suggestion would have affected nitrate and phosphate uptake in a lesser extent, but this was not tested in this work.

The developmental stage of the flower shoot had an effect on nitrate and potassium uptake but in a different way. On the one hand, the indicator variable P_{VB} indicates that nitrate uptake was significantly lower approximately 2 weeks before and a week after the appearance of the flower bud (i.e. visible bud stage), compared with the rest of the flower cycle. This phase approximately coincides with the period with highest elongation rate of the flower shoot (Cabrera et al., 1995). Cabrera et al. (1995) and Kim et al. (2008) found that the pattern of N uptake depended on the developmental stage of the flower shoot. According to these authors, N uptake was minimum when the flower buds became visible and elongation rate was maximum. Cabrera et al. (1995) attributed this pattern to competition within the plant for photoassimilates because of the dependence of N uptake on the availability of assimilates in the roots. They also found a similar pattern for potassium, calcium, magnesium and phosphate, but this was not observed in our work. In contrast, according to the positive effect of the indicator variable P_{BB_VB} in Eq. 6, an opposite pattern was observed for potassium uptake suggesting that, during the stage between flower shoot pruning and visible bud, an extra amount of potassium was required. Potassium plays central roles in plant growth and development, including maintenance of turgor pressure and cell elongation (Fox and Guerinot, 1998). Silberbush and Lieth (2004) stated that the flower contained the highest amount of K of all branch parts, which would lead to an enhanced requirement for K at visible bud stage. Maybe, that is why an extra amount of potassium was required in the period of higher elongation rate of the flower shoots.

Finally, some indicator variables related to periods of time when nutrient uptake was enhanced by unknown factors had a significant effect on the models of phosphate, potassium, calcium and magnesium. In all cases, the period of extra uptake took place in spring and summer, which is the time of plant reactivation and highest production, that is, a period of high demand for photoassimilates and mineral nutrients. Kim et al. (2008) stated that due to the cyclical nature of rose flower shoot production in flushes, it is difficult to optimize the supply of nutrients. Shoot demand has been considered as the driving force in nutrient uptake (Silberbush and Lieth, 2004). Therefore, these indicator variables included in the models may be related to an internal plant factor related to the demand of the plant for a specific nutrient, which induces a feedback response in the roots in order to improve the absorption of those ions. This feedback response may be similar to that described for nitrate uptake, where an enhancement of transporter activity and/or the synthesis of new transporters are stimulated to fulfill plant demand (Imsande and Touraine, 1994).

Factors affecting water uptake rate

Water uptake was modelled according to the climatic parameters VPD, R_i, T_s and T_a. Besides, pruning of old bent shoots and periods of high flower shoot production had an effect on water uptake. Although both factors had opposite effects, the physiological basis is the same since they are related to the leaf area of the plant. Leaf area constitutes the surface of heat and vapour transfer of the plant (Allen et al., 1998) so a lower leaf area would result in a lower transpirating surface and lower water uptake rate, as was observed in tomato by Schwarz and Kuchenbuch (1998). Periods of high production, in which there is a high number of flower shoots per plant that results in a high leaf area in the plant, affected water uptake positively. According to Allen et al. (1998), leaf area of a crop reaches its maximum before or at flowering. By contrast, pruning reduced leaf area of the plant considerably, which affected water uptake negatively as was also observed by Cadisch et al. (2004).

In different studies (Baille et al., 1994; Medrano, 1999; Suay et al., 2003), water uptake models have been based in Penman-Monteith equation (Monteith and Unsworth, 2007) and have been related to radiation, to VPD and to leaf area. The positive effect of T_s on water uptake is shown in Chapter 4.1 (this thesis), although solution temperatures higher than 30°C can reduce transpiration (Medrano, 1999). This author stated that, between a range of temperatures, the higher the T_a, the higher the VPD and stomatal conductance, so the higher the water absorbed by the plant. R^2 was much higher in the water uptake model than in any of the nutrient uptake models. The reason for that may lie in the fact that water uptake by plants is often described as a purely physical process, where water moves passively through the roots in response

to a water potential gradient set up by transpiration (Kirkham, 2006), while nutrient uptake is under feedback control by plant demand (Imsande and Touraine, 1994).

Evaluation of the fertilization strategy

Nutrient use efficiency was calculated in order to evaluate the fertilization strategy used in this experiment. The ratio should be as close to 1 as possible to match nutrient uptake concentration with nutrient solution concentration and, thus, synchronize demand to supply (Kläring et al., 1999; Kläring, 2001). Fertilizing in this way would reduce groundwater contamination. A value lower than 1 shows that nutrient solution is too concentrated, and both an increase in electrical conductivity of the nutrient solution and contamination through leaching of unused nutrients may occur. A value higher than 1 indicates that nutrient solution is too diluted and a problem of nutrient deficiency may appear if not well controlled. In our results, even though the nutrient solution was more diluted than the one used by local growers, in most cases the nutrient solution concentration was higher that nutrient uptake concentration, so nutrient use efficiency was lower than 1 (Fig. 6C). Nitrate was the nutrient with highest nutrient use efficiency and even in autumn and winter it was higher than 1, so a higher nitrate concentration could have been used.

The ratio nutrient uptake to water uptake rate (Fig. 6B) has been used to give recommendations of the optimum nutrient solution concentration (Bougoul et al., 2000; Kläring, 2001; Mattson and Lieth, 2007a) with the premise that nutrient concentration should equal nutrient uptake concentration (Kläring et al., 1999). Based on this idea, in this work, the recommendation for each season of the optimum nutrient solution concentration for a greenhouse rose crop grown in aeroponic system would be equal to the values shown in Fig. 6B.

Main advantages and disadvantages of the models

In this study, empirical models for nitrate, phosphate, potassium, calcium, magnesium and water uptake of a greenhouse rose crop were developed in normal growing conditions with data acquired during 14 months. This makes them suitable for being integrated in decision support systems for fertirrigation according to plant demand in every season, although a validation of the models in place would be needed beforehand. In addition, they are based on simple measurable parameters since sensors for climatic variables are available at a reasonable price. The models integrate the effect of nutrient solution concentration and flower shoot production as well as of some common practices in rose cultivation such as renewal of old bent shoots, the use of shade screen in summer or the synchronization of flower shoot development for scheduling purposes. The resulting equations might provide useful clues for better understanding nutrient uptake mechanisms in plants from a global point of view

because most of the factors affecting nutrients uptake are taken into account. In contrast, many of the models that have been reported in literature only included some of these factors.

On the other hand, the models have some weak points that should be discussed. Firstly, except for the model of nitrate and water uptake, the R^2 of the others is medium-low and quite low in the case of magnesium uptake. This can lead to over- or underestimations when making predictions. In any case, the R^2 obtained here are similar to those reported in other studies as stated above. In a study from Mattson and Lieth (2007a), magnesium uptake results were also the most erratic. In their work, authors pointed out that another factor that can have an influence on the predictive power of the nutrient models is the capacity of the rose plant to store nutrients and redistribute them to new growth when needed, which would affect nutrients uptake. In any case, the crops have tolerance thresholds for optimum nutrient concentration in plant tissues, also called sufficiency ranges (Plank, 1989), which means that the short-time deviations between fertirrigation and exact plant demand may not have any negative consequence on plant nutrient status. Thus, these models, when tested as tools to steer fertirrigation, should be combined with physiological measurements of plant nutrient status to check if they are good enough or should be improved.

Secondly, because dealing with empirical models, they might not be suitable out of the range of conditions where the model was fitted. For instance, if plants are suffering from stress such as salinity stress, nutrient uptake may change (Kläring et al., 1997; Grattan and Grieve, 1998). In any case, as the parameters that appear in the model have a physiological background, it is likely that most of the parameters would also appear although with different coefficients. Even though they have been developed in normal growing conditions, this is not exactly true for the concentration of the nutrient solution given that a more diluted solution was used to improve the accuracy of the measurements. An aeroponic system was used also for that purpose, which is different to the perlite system commonly used by local growers. Maybe, other results would have been obtained in commercial conditions. However, a test that was carried out before in our group, using the common nutrient solution concentration and a system with perlite as substrate, showed that measurement errors were excessive. The strong influence of using a nutrient solution highly concentrated on the measurement error was apparent in the phosphate uptake model. In line with the latter, the models of phosphate and magnesium uptake could be improved if a lower nutrient solution concentration is used.

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4. EFFECTS OF LOW NUTRIENT SOLUTION TEMPERATURE IN SOILLESS ROSE CULTIVATION

4.1. EFFECT OF TWO NUTRIENT SOLUTION TEMPERATURES ON NITRATE UPTAKE, NITRATE REDUCTASE ACTIVITY, NH_4^+ CONCENTRATION AND CHLOROPHYLL *a* FLUORESCENCE IN ROSE PLANTS.

Abstract

The effect of two nutrient solution temperatures, cold (10 °C) and warm (22 °C), during two flowering events of rose plants (*Rosa hybrida* cv. Grand Gala grafted on *Rosa Manetti*) were examined by measuring chlorophyll *a* fluorescence, ammonium content and NR activity in four different leaf types, that is, external and internal leaves of bent shoots and lower and upper leaves of flowering stems. Besides, nitrate uptake and water absorption, total nitrogen concentration in the plant, dry biomass, and the ratios shoot/root and thin-white roots/suberized-brown roots were determined. Generally, cold solution decreased water uptake but increased NO_3^- uptake and thin-white roots production, so plants grown at cold solution had to improve their NO_3^- uptake mechanisms. The higher NO_3^- uptake can be associated with the increase in NR activity, NH_4^+ content and total N concentration at cold solution. Nutrient solution temperature also had an effect on the photosynthetic apparatus. In general terms, the effective quantum yield (ϕPSII) and the fraction of open PSII reaction centres (q_L) were higher in rose plants grown at cold solution. These effects can be associated with the higher NO_3^- uptake and total N concentration in the plant and were modulated by irradiance throughout all the experiment. Plants could adapt to cold solution by enhancing their metabolism without a decrease in total dry biomass. Nevertheless, the effect of nutrient solution temperature is not simple and also affected by climatic factors.

Introduction

Soilless culture techniques are used for commercial production of several high value ornamental plants in the Mediterranean area. In this region, in winter, air temperature can get down below the rose plant biological minimum of 14 °C to 16 °C (Tesi, 1969) in unheated greenhouses at night. The temperature of the nutrient solution can frequently get to 7-9 °C considered critical for root functions (Mortesen and Gislerød, 1996). In soilless systems, root temperature can be controlled by warming or cooling the nutrient solution (Moss and Dalgleish, 1984) providing the energy requirements for optimum plant development. Sometimes an excessive energy input is spent to protect the crop from climatic constraining conditions due to poorly established guidelines (Willits and Peet, 2001). In order to reduce energy costs in greenhouse production it is necessary to know the range of temperatures that permits plant growth and the production of high yields. However, results from temperature studies are sometimes difficult to understand because temperature may affect in a different way depending on the physiological process and on the plant organ that is studied (Theodorides and Pearson, 1982).

Root temperature has been shown to have pronounced effects on shoot growth of a number of plant species (Bowen, 1991) but optimal values vary among them (Barr

and Pellet, 1972). Research about root temperature in rose plants is sometimes contradictory and depends on the cultivar, climatic conditions, the combination root-air temperatures, among other factors. Some studies indicate that 18 °C root-zone temperature is the optimal for shoot growth of 'Better Time' and 'Sonia' roses grafted on the rootstock *Rosa indica* (Shanks and Laurie, 1949; Zeroni and Gale, 1982). However, no effects of root temperature increases from 18 °C to 25 °C on stem length and flower production have been reported (Kohl et al., 1949; Zeroni and Gale, 1987). In contrast, other studies show that soil heating is beneficial for roses (Brown and Ormrod, 1980; Zeroni and Gale, 1982) because when root temperature is lowered from 18 °C to 10-12 °C, shoot growth is reduced (Moss and Dalgleish, 1984; Mortensen and Gislerørod, 1996).

Main root functions are water and nutrient uptake and synthesis of plant hormones (Dieleman et al., 1998). Soil temperature affects water and nutrient uptake, root and shoot growth and metabolic processes (Dong et al., 2001). Among them, nutrient uptake is one of the most sensitive processes to temperature (Xu and Huang, 2006). Dong et al. (2001) have shown that low soil temperatures (8 °C) reduced absorption of ^{15}N by roots of apple trees. In contrast, Osmond et al. (1982) reported that the whole root system of soybean plants absorbed NO_3^- similarly at both cool (14 °C) and warm temperatures (22 °C). On the other hand, root temperature also influences water uptake. This may be due to the fact that both the viscosity of water and root hydraulic resistance increase at low root temperature, causing a decrease of water flow to the root (Pavel and Fereres, 1998).

Nitrogen absorption is directly related to the reduction rate of nitrate nitrogen ($N\text{-}NO_3^-$) to nitrite. This reduction is the first step of N assimilation and involves enzyme NR (Toseli et al., 1999), which is sensitive to high temperature (Lauri and Stewart, 1993). Younis et al. (1965) found that an increase in temperature from 30 to 35 °C caused a 60 to 70 % decrease in NR activity in young corn plants. Increase of NR activity after low temperature treatment has been reported in wheat (Yaneva et al., 2002) and in oil-seed rape (Macduff and Trim, 1986).

Low root temperature can also affect photosynthesis. Chlorophyll *a* fluorescence, an indicator of the fate of excitation energy in the photosynthetic apparatus, has been used as early indication of many types of plant stress (Calatayud et al., 2004). We propose the use of CF imaging technique as a tool to detect the possible stress in rose plants under low root temperature. It has been shown that sensitivity to low temperatures may be verified by measuring CF in potato (Greaves and Wilson, 1987), tomato (Willits and Peet, 2001), lettuce (He et al., 2001), cucumber (Ahn et al., 1999), maize (Fracheboud et al., 1999) or roses (Hakan et al., 2000).

The objective of this work was to test root chilling tolerance of rose plants (*Rosa x hybrida* cv. Grand Gala grafted on *Rosa Manetti*) by studying the physiological response of the plant, with the final aim of optimising nutrient solution temperature and reducing energy cost in winter. To reach this objective, CF, NR activity and NH_4^+ concentration were measured in different types of leaves, as well as NO_3^- and water absorption by the roots, total biomass produced and total nitrogen concentration in roots and leaves under two root-zone temperature conditions, i.e. a level supposedly limiting root activity (10 °C) and a non-limiting level for root processes (22 °C).

Materials and methods

Plant management and greenhouse conditions

A three-year-old rose crop (*Rosa x hybrida*), cv. Grand Gala, grafted on the rootstock *Rosa Manetti,* was grown in a polycarbonate greenhouse, equipped with convective heating (minimum 16 °C), high pressure fogging and roof ventilation. Two units of closed aeroponic growing system were used.

Thirty plants were grown in each aeroponic unit at two different nutrient solution temperatures while their aerial parts were subjected to the same climate conditions, i. e. radiation, air temperature and relative humidity. In the cold solution treatment, a heat exchanger placed in the solution tank and connected to a cooling equipment, cooled the solution down to 9 °C, automated by means of a thermostat. The average values and standard deviation of nutrient solution temperatures along the whole experiment were 10.5 $\pm$ 1.02 °C in the cold solution treatment and 21.72 $\pm$ 2.22 °C in the warm one, which was the control treatment. Climate variables inside the greenhouse such as temperatures of the air and nutrient solution, air humidity and VPD were recorded. Solar radiation integral per period (MJ m^{-2} $period^{-1}$) (see period length in Table 1) and the average radiation (W m^{-2}) when the physiological measurements were done (11:00 to 13:00), defined here as growth radiation, are shown in Table 1. They increased as the experiment progressed and were higher in the 2nd flowering event.

Plants were grown following the bending technique as it is commonly done by local growers (Calatayud et al., 2007). The experiment began at the end of November and was finished at the beginning of April, after two complete flowering cycles. The 1st flowering cycle started at the end of November (25/11/05) and finished at the beginning of February (09/02/06), when the 2nd one began, which finished at the beginning of April (12/04/06). Just before the beginning of both flower cycles, all flowering shoots, either mature or immature, were pruned down to two nodes from

their base. The physiological measurements (see below) were carried out at the same physiological stages independently of the date:

T0: Flower stems with a small visible flower bud (in the middle of January; first floral cycle).

T1: Flower stems in commercial harvesting stage (at the beginning of February; first floral cycle).

T2: Flower stems with a small visible flower bud (at the beginning of March; second floral cycle).

T3: Flower stems in commercial harvesting stage (at the beginning of April; second floral cycle).

All measurements done in the aerial part of the plant were carried out in fully developed leaves. Samples were taken from 4 different locations within the plant: external (sunny) and internal (dark) position of the bent shoots, basal (second leaf from the base) and upper (leaf below the flower or bud) position of flower shoots (Calatayud et al., 2007).

At the end of each flowering cycle (at times T1 and T3), 5 plants from each treatment were taken for destructive measurements. Fresh and dry weight (FW and DW respectively), and total N concentration of roots and leaves, which was done by using a C/N analyser (NC 2500, Eager 300 software®, CE instruments, ThermoQuest Italia, Rodano, Italy), were measured.

Following previously described methodology (Chapter 3 of this thesis), daily water and nitrate uptake were measured throughout the experiment.

Measurements of chlorophyll a fluorescence imaging

CF imaging of rose leaves was performed using an imaging-PAM fluorometer (Walz, Effeltrich, Germany). Leaflets were darkened for 15 min prior to measurement. Then, a saturating pulse of light (blue light, 800 ms, 2400 µmol m^{-2} s^{-1}) was applied and the maximum quantum yield of PSII photochemistry, F_v/F_m, which is equivalent to (F_m-F_0)/F_m (Schreiber et al., 1989), was determined being F_0 the minimum fluorescence yield in the dark and F_m the maximal fluorescence yield after receiving the saturating pulse of light. Both F_m and F_0 yields were measured by the equipment. Next, in order to assess light-adapted parameters, actinic illumination (blue light, 200 µmol m^{-2} s^{-1}) was switched on and saturating pulses were applied at 20 s intervals for 5 min in order to determine the maximum chlorophyll fluorescence yield (F_m'), and the chlorophyll fluorescence yield during the actinic illumination before the saturation pulse (F_s). The

minimum fluorescence yield of the illuminated sample (F_0') was calculated according to Oxborough and Baker (1997). After 5 minutes, it was assumed that the steady state had been reached. The actual quantum efficiency of PSII photochemistry, ϕPSII, was calculated according to Genty et al. (1989) by the formula: (F_m' − F_s)/F_m'. The coefficient of photochemical quenching based on the lake model, q_L, was defined by Kramer et al. (2004) as:

$$qL = \frac{\left(F_m^{'} - F_s\right)}{\left(F_m^{'} - F_0^{'}\right)} \cdot \frac{F_0^{'}}{F_s} \qquad (1)$$

The quantum yield of regulated energy dissipation in PSII, ϕNPQ, was calculated according to Kramer et al. (2004) by the equation:

$$\phi NPQ = 1 - \phi PSII - \phi NO \qquad (2)$$

The quantum yield of non-regulated energy dissipation in PSII, ϕNO, was calculated according to Kramer et al. (2004) by the equation:

$$\phi NO = \frac{1}{\left[NPQ + 1 + qL \cdot \left(\frac{F_m}{F_0} - 1\right)\right]} \qquad (3)$$

where NPQ is defined as (F_m-F_m')/F_m'. For more details about this technique see Calatayud et al. (2006).

CF was measured in the central part away from the midrib of the terminal leaflet of n ≥ 6 leaves of each of the four types, and between 11:00 and 13:00 h to minimize diurnal fluctuations. CF kinetics was determined at times from T0 to T3, in plants at each nutrient solution temperature treatment.

Nitrate reductase activity

Nitrate reductase activity (EC 1.6.6.1) was determined *in vivo* with methods described by Hageman and Hucklesby (1971) and Jaworki (1971). Discs of 1 cm diameter were punched out from fully expanded mature leaf tissue. 200 mg of discs per sample were introduced in a glass vial containing 10 mL of 100 mM potassium phosphate buffer (pH=7.5), 1% (v/v) n-propanol and 100 mM KNO_3. The glass vial was subjected to vacuum infiltration in order to induce anaerobic conditions in the incubation medium. Plant samples were incubated in a water bath at 30 °C for 60 min in the dark and then placed in a boiling water bath for 5 min to stop the enzymatic reaction. Nitrite released

from plant material was determined colorimetrically at 540 nm (spectrophotometer Uvikon XS, Bio-Tek, USA) by adding 0.02% (w/v) N-(1-naphthyl)-ethylenediaminehydrochloride and 1% sulphanilamide. In order to calculate the amount of NO_2^- contained in the samples a standard curve with KNO_2 was prepared. NR activity was measured in $n \geq 4$ leaves of each type at times from T0 to T3 in both nutrient solution temperature treatments.

Ammonium determination

Ammonium was analysed in a FIA system (FIASTAR 5000, Foss Analytical, Höganäs, Sweden). Extraction of NH_4^+ from leaves was performed by Husted et al. (2000) with slight modifications for adaptation to rose leaves. One gram of leaves was frozen in liquid N_2 and homogenized with 10 mL of 10 mM cold formic acid. The homogenate was centrifuged twice at 25000 g (2 °C) for 10 min. The clarified supernatant was used for analysis in FIA system. Ammonium standard curve (0.010-1mg/L) was prepared with the extraction media as solvent. NH_4^+ concentration was measured in $n \geq 4$ leaves of each type at times from T0 to T3 in plants at both nutrient solution temperature treatments.

Statistical analysis

The effect of the nutrient solution temperature was evaluated using nine response variables, i.e. NH_4^+ content, activity of the enzyme NR, NO_3^- uptake, water absorption, total N concentration in the plant and four fluorescence parameters: F_v/F_m, ϕPSII, ϕNPQ y q_L. All measurements were carried out in leaves except for NO_3^- and water uptake that were done from nutrient solution samples and total N determination that was measured in both leaves and root parts.

Nutrient solution temperature had 2 levels: cold and warm solution. Three more factors were included in the study. One factor was the position in the plant of the leaf sample analysed, with 4 levels: external and internal leaves of bent shoots, and highest and lowest leaves of the flowering stems. The two other factors were related to time, and were the flowering event (1^{st} and 2^{nd}) and the stage of development of the flowering shoot (stage 1: visible flower bud; stage 2: harvest time). The first reflected the variability in the parameters due to the change of the climatic conditions, and the second one, was related to the internal behaviour of the plant in two different development stages. When the main effect of stage of development of the flowering shoot was statistically not significant, both time factors were reduced to one, time event with 4 levels: T0, T1, T2 and T3 which was only related to the change in climatic characteristics.

The normality of the distribution was verified by Q-Q plot for most of the response variables, but F_v/F_m and ϕPSII showed non-normal behaviour. Because of that, F_v/F_m and ϕPSII populations were analysed by the Wilcoxon test for median comparison.

For the rest of parameters, one-, two-, three- and four-way ANOVA were performed to compare the means of these parameters among the levels of the factors involved. For all inferences, 5% significance was specified. Calculations were made with the aid of a statistical software (Statgraphics Plus for Windows 4.1).

Results

Effect of nutrient solution temperature on nitrate and water uptake and total nitrogen in the plant

For the analysis of NO_3^- and water uptake, one-way ANOVA, with nutrient solution temperature as factor, was performed for each period of time (Table 1). The stage of development of the flowering shoot was not a significant factor. NO_3^- uptake was statistically higher in the plants grown at cold solution, in all the periods of the experiment, except at the end of the second flowering event (T2-T3). Water absorption (Table 1) was statistically higher in plants grown at warm solution during all the experiment. NO_3^- and water uptake were affected by the change in climate characteristics and increased with radiation.

One-way ANOVA, with nutrient solution temperature as factor, was carried out to analyse the effect of temperature on total N concentration in both roots and leaves at the end of each flowering cycle. Plants grown at cold solution showed statistically higher levels of N concentration in the roots at the end of both flower cycles, but only at the end of the 1st one in the leaves (Table 1).

Effect of nutrient solution temperature on biomass parameters

The effect of nutrient solution temperature on total biomass per plant, on the thin-white roots/suberized-brown roots ratio (TR/SR) and on the shoot/root ratio was studied (Table 1). At the end of the first flowering event, the total DW per plant was higher in the plants growing at cold solution (Table 1) with significant differences, while at the end of the 2nd one, no significant differences between temperature treatments were found.

Plants growing at cold solution showed more succulent and white roots than plants grown at warm solution, where roots were mainly browner and more suberized.

Accordingly, the ratio between TR/SR (Table 1) was higher in plants grown at cold solution at the end of both flowering events.

Table 1. Effect of cold (10 °C) and warm (22 °C) nutrient solution temperature (T_S) on daily mean values of nitrate uptake (mmol NO_3^- plant day^{-1}) and water absorption (L plant day^{-1}) in each period. Also, effect on total nitrogen in roots (N_{total} roots) and leaves (N_{total} leaves) (g N g DW^{-1}), on dry weight per plant (g DW $plant^{-1}$), on the relation between thin-white roots and suberized-brown roots (TR/SR) and on shoot/root ratio (both ratios in dry weight basis) at the end of each flowering event for *n*=5 plants. Besides, solar radiation integral per period inside the greenhouse (Rad $period^{-1}$, MJ m^{-2} $period^{-1}$) and mean radiation during physiological measurements (Growth Rad) between 11:00 to 13:00 h (W m^{-2}). The periods are: from flower soot pruning till the appearance of the visible bud of the immature flower shoot ((P-T0) in the 1st flower event and (T1-T2) in the 2nd flower event) and from the latter until the open flower is ready to harvest ((T0-T1) in the 1st flower event and (T2-T3) in the 2nd flower event). For each period or flower event, one-way ANOVA was performed with solution temperature as factor. Values (means±SE) followed by the same letter within each period indicate not significant differences at 5%.

Periods	T_S	NO_3^- uptake	H_2O absorption	Rad $period^{-1}$	Growth Rad
P-T0	10	1.724±0.09a	0.154±0.01b	139.39	298.95
	22	1.377±0.10b	0.229±0.01a		
T0-T1	10	2.083±0.13a	0.199±0.02b	62.70	370.79
	22	1.499±0.11b	0.271±0.02a		
T1-T2	10	2.588±0.10a	0.261±0.04b	121.63	461.30
	22	2.279±0.07b	0.434±0.03a		
T2-T3	10	4.044±0.38a	0.653±0.04b	222.68	444.32
	22	4.410±0.41a	0.842±0.05a		

Flower event	T_S	N_{total} roots	N_{total} leaves	g DW $plant^{-1}$	TR/SR	Shoot/Root
1st	10	3.011±0.07a	2.837±0.07a	258.11±10.6a	1.06±0.06a	6.41±0.35a
	22	2.484±0.07b	2.532±0.08b	198.38±10.6b	0.78±0.06b	5.66±0.39a
2nd	10	3.224±0.09a	3.030±0.13a	291.06±16.1a	1.50±0.12a	6.67±0.53a
	22	2.792±0.09b	2.711±0.13a	333.83±16.1a	0.45±0.12b	7.39±0.59a

Shoot/root ratio showed no statistical differences between temperature treatments in any of the two samplings.

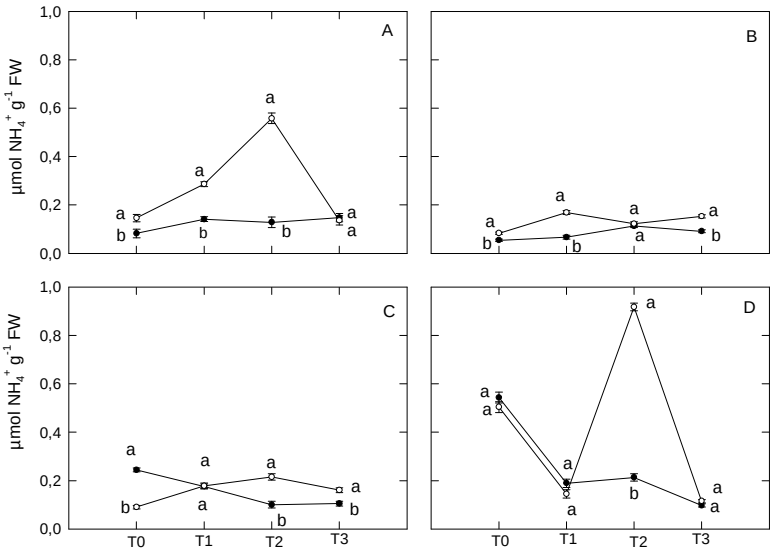

Fig. 1. Effect of two different nutrient solution temperatures: cold (○) and warm (●), on NH_4^+ concentration (µmol NH_4^+ g^{-1} FW) in internal and external leaves of bent shoots (A, B) and lower and upper leaves of the flowering stems (C, D) at four time events (T0: Shoots with visible flowering bud in the middle of January, T1: Flower stems in commercial harvesting stage at the beginning of February, T2: Shoots with visible flowering bud at the beginning of March and T3: Flower stems in commercial harvesting stage at the beginning of April). Data are means±SE of *n*=4. For each time event one-way ANOVA was performed with temperature of nutrient solution as factor. Values followed by the same letter within each time event indicate not significant differences at 5%.

Effect of nutrient solution temperature on ammonium concentration

The variation with time in the amount of NH_4^+ in the leaves, at four leaf positions in plants grown at both nutrient solution treatments is shown in Fig. 1. A four-way ANOVA with solution temperature, leaf position, flowering event and stage of development of the flowering shoot as factors, showed that the stage of development of the flowering shoot was highly significant (p<0.0001). On average over levels of the rest of the factors, there was a higher amount of NH_4^+ in the leaves at the visible bud stage (T0 and T2). Besides, the statistically significant 2-way interaction between solution temperature and flowering event (p=0.0004) suggested a different effect of solution temperature depending on the flowering event. On average, there was a significantly higher amount of NH_4^+ in all leaf types of plants at cold solution during the second flower cycle, but only in the leaves of the bent shoots during the first one.

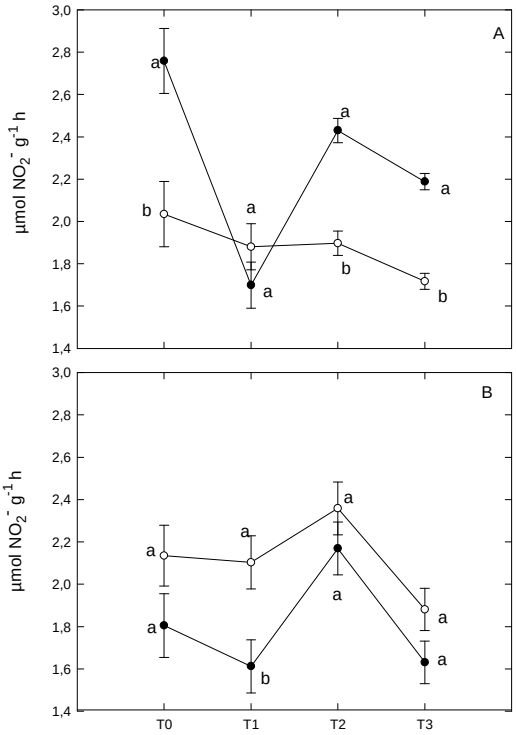

Fig. 2. Effect of nutrient solution temperatures: cold (○) and warm (●), on NR activity ((µmol NO_2^- g^{-1} FW h^{-1}) in external leaves of bent shoots (A) and in the rest of the leaves sampled (internal leaves of bent shoots, lower and upper leaves of the flowering stems) (B) along the experiment between the time event T0 and T3. Data are means±SE of *n*=4. For each time event one-way ANOVA was performed with temperature of nutrient solution as factor. Values followed by the same letter within each time event indicate not significant differences at 5%.

Effect of nutrient solution temperature on NR activity

The variation of NR activity in the leaves with time in plants grown at both nutrient solution treatments is shown in Fig. 2.

A four-way ANOVA showed a significant interaction between solution temperature and leaf position (*p*=0.0019), which led to a separate analysis for the data of two parts of the plant where the effect of solution temperature on NR activity was opposite: external leaves of bent shoots (Fig. 2A), on the one hand, and the rest of the leaves (Fig. 2B), on the other hand. In plants at cold solution, the NR activity of the plants was higher (*p*=0.002) in the internal leaves of bent shoots and flowering stems, but lower (*p*=0.0023) in the external leaves of bent shoots. Additionally, stage of development of the flowering shoot had a significant effect (*p*=0.0001): NR was higher at the visible bud stage (T0 and T2) than at harvest time (T1 and T3).

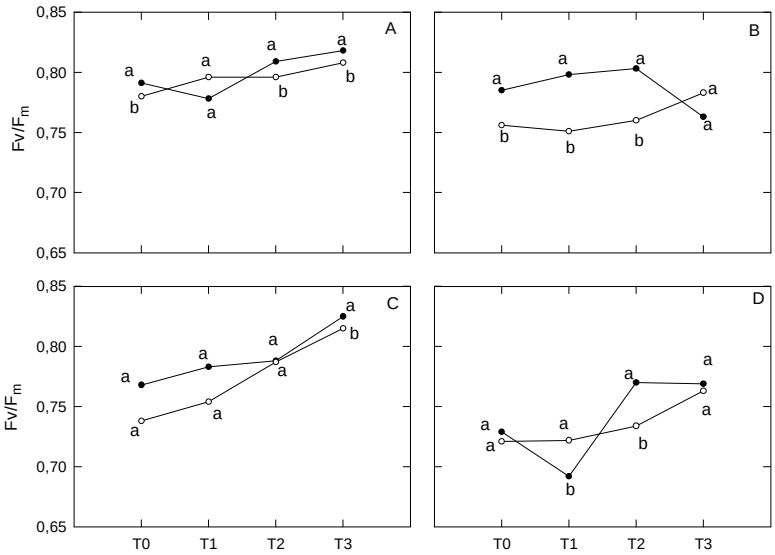

Fig. 3. Changes in dark-adapted chlorophyll fluorescence parameter F_v/F_m at four time events (T0, T1, T2 and T3) with respect to nutrient solution temperatures (cold (○) and warm (●)) in internal and external leaves of bent shoots (A, B) and lower and upper leaves of the flowering stems (C, D). Data are medians of *n*= 6. For each time event the Wilcoxon test was performed with temperature of nutrient solution as factor. Values followed by the same letter within each time event indicate not significant differences at 5%.

Effect of nutrient solution temperature on F_v/F_m

F_v/F_m and ϕPSII were not analysed by ANOVA because of their non-normal distribution. Thus, the medians for each solution temperature treatment were compared by Wilcoxon test.

F_v/F_m (Fig. 3) was higher in the leaves of plants grown at warm solution, and this difference was mainly due to the bent shoots (p=0.0016 (internal leaves) and p<0.0001 (external leaves)). Differences between temperature treatments in the leaves of flowering shoots, were significant only at T3 in lower leaves and T1 and T2 in upper leaves), but not on average over all (p=0.2339 (lower leaves) and p=0.2914 (upper leaves)).

Effect of nutrient solution temperature on ϕPSII

Averaged over leaf position levels and times, ϕPSII was higher (p=0.0004) in the leaves of plants grown at cold solution. Significant differences occurred on average over leaf positions in the first three measurement times (p=0.0205, 0.0035 and 0.014, respectively), but not in the last one (p=0.804). Comparing results for each leaf position, solution temperature had a significant effect only in the external leaves of bent shoots (p=0.001) and the upper leaves of the flowering stem (p=0.0036) (Fig. 4).

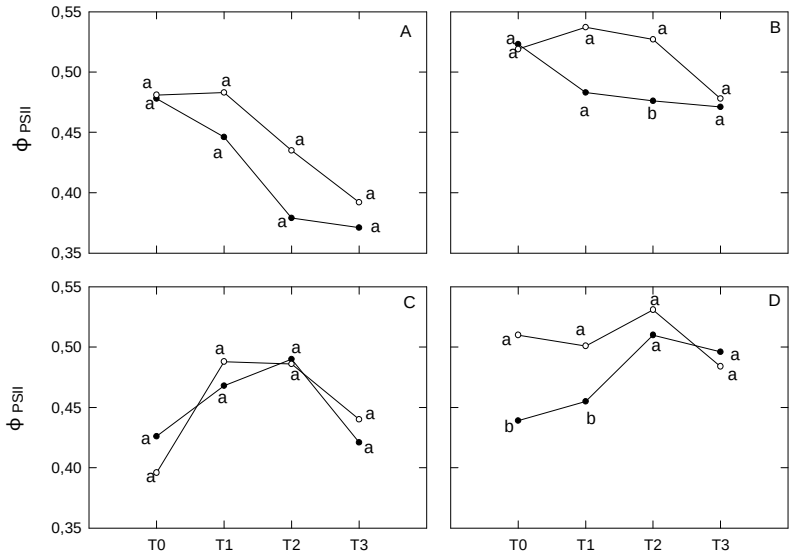

Fig. 4. Changes in chlorophyll fluorescence parameter ϕPSII in internal and external leaves of bent shoots (A, B) and lower and upper leaves of the flowering stems (C, D) showing steady-state values after fluorescence induction kinetics, at two nutrient solution temperatures (cold (○) and warm (●)) and at four time events (T0 to T3). Data are medians of *n*= 6. For each time event the Wilcoxon test was performed with temperature of nutrient solution as factor. Values followed by the same letter within each time event indicate not significant differences at 5%.

Effect of nutrient solution temperature on q_L

q_L and ϕNPQ followed the normal distribution, so the ANOVA test was applied.

Averaged over the other factors, q_L (Fig. 5) was higher in the leaves of plants grown at cold solution (p<0.0001). The effect was statistically significant at all time events (p<0.0028) and for all leaf types, except for the lower leaves of the flowering stem (p=0.0836). On the other hand, the effect of the stage of development of the flowering shoot was not significant.

Effect of nutrient solution temperature on ϕNPQ

Averaged over all measurement times, the effect of solution temperature on ϕNPQ was significant only in internal and external leaves of the bent shoots (p<0.009), where ϕNPQ was higher in the leaves of the plants grown at warm solution (Fig. 6).

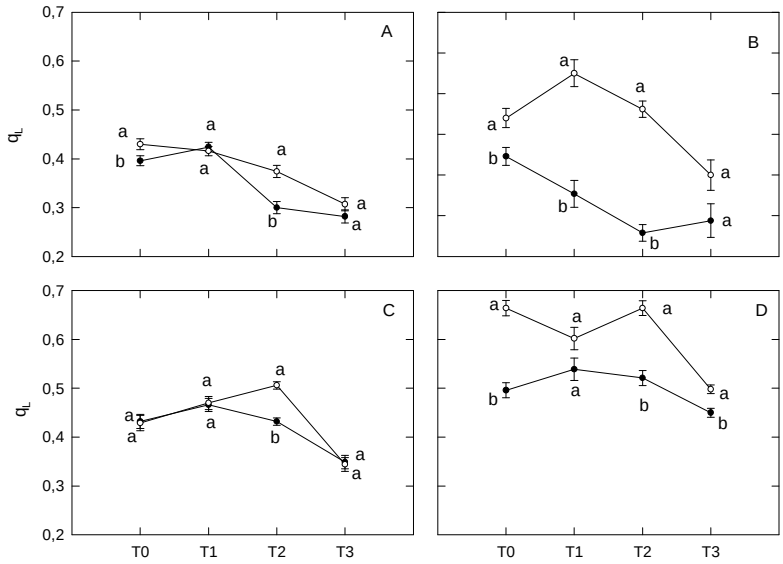

Fig. 5. Changes in chlorophyll fluorescence parameter q_L showing steady-state values after fluorescence induction kinetics, at two different nutrient solution temperatures ((cold (○) and warm (●)) and at four time events (T0 to T3). Data are means±SE of *n*=6 internal and external leaves of bent shoots (A, B) and lower and upper leaves of the flowering stems (C, D). For each time event one-way ANOVA was performed with temperature of nutrient solution as factor. Values followed by the same letter within each time event indicate not significant differences at 5%.

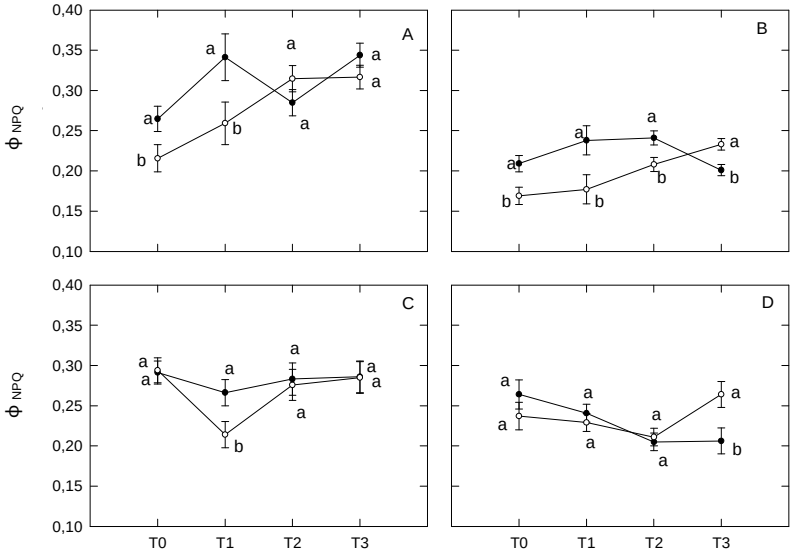

Fig. 6. Quantum yield of regulated energy dissipation in PSII (ϕNPQ) showing steady-state values after fluorescence kinetics induction in internal and external leaves of bent shoots (A, B) and lower and upper leaves of the flowering stems (C, D) from T0 to T3 under two different nutrient solution temperatures (cold (○) and warm (●)). Data are means±SE of *n*=6. For each time event

one-way ANOVA was performed with temperature of nutrient solution as factor. Values followed by the same letter within each time event indicate not significant differences at 5%.

Discussion

Plants sense transient temperature fluctuations and may respond to these changes by actively adjusting their biology to fit the subsequent temperature regime. In our results, root morphology was considerably changed by nutrient solution temperature. A great proportion of roots at cold solution were white and succulent, while at warm solution roots were mainly brown and more suberized. Accordingly, the ratio obtained in our experiment between thin-white roots and suberized-brown roots was higher in plants grown at cold solution in both flowering events (Table 1). These morphological characteristics were also found by Dieleman et al. (1998) in rose plants and they were, maybe, the result of an adaptation process against the limitation of plant water absorption at low temperature, possibly caused by an increase of water viscosity and root hydraulic resistance (Pavel and Fereres, 1998; Toselli et al., 1999). Shanks and Laurie (1949) observed that rose plants grown at high temperatures produced fewer root hairs and the endodermis cells were filled with tannins. Early studies suggest that suberized roots are somewhat less permeable to water (Chapman and Parker, 1942; Kramer and Bullock, 1966) but, in our experiment, water uptake was higher in the warm solution treatment, where roots were more suberized.

With respect to the influence of cold solution on NO_3^- uptake, the results in the bibliography are contradictory. A decrease in N uptake has been measured at low temperatures (Dong et al., 2001). In contrast, after long-term exposure to low temperature, roots of rye, barley and maize increased their capacity for ion uptake (Siddiqi et al., 1984; Clarkson, 1986; White et al., 1987; Engels et al., 1992). This has been attributed to an increased number of ion transporters in the root plasma membrane (Siddiqi et al., 1984) maybe the consequence of higher shoot demand per unit root fresh weight (Clarkson et al., 1986; White et al., 1987; Engels et al., 1992). Also, increased plasma membrane H^+-ATPase gene transcription and translation was shown in roots of cucumber at low temperature (Ahn et al., 1999). In our results (Table 1), we have observed an increase in NO_3^- uptake at cold solution with respect to warm solution, associated with an increase of total N in the plant. The higher capacity for NO_3^- uptake while lower for water uptake indicates that plants grown at cold solution had to stimulate their NO_3^- uptake mechanisms. This may reflect the first step of an adaptation process to suboptimal conditions. However, in the last period of the experiment (T2-T3), NO_3^- uptake became similar in both temperature treatments suggesting an interaction between the increase of light intensity and photoperiod (spring) and the response to nutrient solution temperature (Browse and Xin, 2001). NO_3^- and water uptake increased with radiation in this study.

The higher NO_3^- uptake by roots at cold solution resulted in an increase of total N concentration in the leaves at the end of the first flowering event. In the same way, total N concentration of shoots and roots (Clarkson et al., 1986) and NO_3^- concentration in root xylem exudates (Bigot and Boucaud, 1996) have been referred to be positively affected by cold root treatment. Toselli et al., (1999) stated that the higher NO_3^- uptake by roots at cold solution may increase the xylem flow of NO_3^- to the leaves where it is reduced. The enzyme NR, which is involved in the reduction of NO_3^- to nitrite, the first step of N assimilation, is induced by its own substrate (NO_3^-) (Crawford, 1995). It hence follows that the higher the NO_3^- concentration, the higher the activity of the enzyme. As expected in our case, a higher activity of NR was shown, on average, in leaves of rose plants grown at cold solution. The external leaves of bent shoots are the exception, where a higher activity was obtained at warm solution (Fig. 2) and for which behaviour we do not have an explanation. NR activity was higher in the visible bud stage (T0 and T2) than in the commercial harvesting stage, for all leaf types. This effect can be associated with the support of bud growth demand (Mor and Halevy, 1979; Jiao and Grodzinski, 1998). The effect of this factor was more important than the effect of radiation, where the relation with NR activity is not so clear since it seems to be hidden by the former.

The product of NR, nitrite, is converted into ammonium by nitrite reductase in the chloroplast, which is later transformed into amino acids (Husted et al., 2000). In our results with rose plants a higher NH_4^+ concentration was obtained at cold solution (Fig. 1). This was probably the consequence of a higher NR activity but also, it may be a result of the adaptation process. NH_4^+ may be kept as a store of soluble N (Miller and Cramer, 2004). This would explain the behaviour of the external leaves of bent shoots, where a lower NR activity was linked to a higher NH_4^+ concentration at cold solution compared to the warm treatment. On average, NH_4^+ content was higher in the visible bud stage than in the commercial harvesting stage, which correlated with a higher NR activity in this stage too. As for NR activity, the effect of radiation seemed to be disguised by the effect of the stage of development of the flowering shoot, being the latter a more important factor affecting NH_4^+ content. The upper leaves of flower stems had the highest NH_4^+ concentration at the visible bud stage and this effect could indicate a major demand of NH_4^+ for flower development (Jiao and Grodzinski, 1998; Gonzalez-Real and Baille, 2000). In contrast, external leaves of bent shoots had the lowest concentration of NH_4^+, which suggests that part of the NH_4^+ produced there was probably sent to the growing flower stems.

Measurements of CF have been used to detect effects of stress on the functioning of the photosynthetic apparatus (Calatayud et al., 2004, 2006, 2007). In our results, the maximum quantum efficiency of PSII photochemistry (F_v/F_m) was, generally, higher in plants at warm solution (Fig. 3). The lower values of F_v/F_m in rose plants grown at cold

solution, were associated with a decrease in F_0 paralleled to a decrease in F_m (data not shown). The decrease in F_v/F_m ratio could result from a reduction in the fraction of PSII centres that are capable of photochemistry and/or down-regulation (an increase in non-photochemical quenching, ϕNPQ) (Baker and Oxborough, 2004). However, except for the measurements at the end of the experiment, ϕNPQ was lower in plants grown at cold solution in some leaf types. Despite the possible decrease in the percentage functional PSII centres, the obtained ϕPSII values were high enough in plants grown at cold solution. Actually, the effective PSII quantum yield (Fig. 4) and the fraction of open PSII reaction centres (Fig. 5) were higher in plants grown at cold solution, which meant that the majority of photons absorbed by PSII were used in photochemistry and that PSII centres were maintained in an oxidised state. ϕPSII gives a measure of the rate of non-cyclic electron transport so it is an indication of overall photosynthesis. A positive correlation between the N content of leaves and photosynthetic activity has been reported in many plants (He et al., 2001; Chen et al., 2003). Therefore, the increase in photochemical activity experimented by plants at cold solution treatment, may be associated with the higher N content in leaves (Table 1), which is the result of a higher NO_3^- uptake, a higher NR activity and NH_4^+ content in those leaves.

At the end of the experiment (T3) a change in the CF parameters with respect to solution temperature was observed. The parameters ϕPSII and q_L became similar in both treatments and, for some types of leaves, ϕNPQ was significantly higher and F_v/F_m was significantly lower in plants at cold solution. These results suggest that at T3, there may have been a down-regulation of the capacity of PSII electron transport in plants at cold solution with respect to their capacity in the previous measurements, which involved increases in the thermal dissipation of excess excitation energy and a decrease in the percentage functional PSII centres. This was related to a similar NO_3^- uptake and total N concentration in the leaves at both temperature treatments during this period. In the beginning of spring, light intensity and photoperiod improved, which may be involved in this response (Browse and Xin, 2001).

CF parameters were not affected by the stage of development of the flower stem but all were modulated by radiation. This can be clearly observed in arched stem leaves. ϕPSII (Fig. 4) and q_L (Fig. 5) decreased throughout the experiment with similar shape following an increase of sunlight radiation, which was associated with photoprotective thermal energy dissipation, that is, to an increase of ϕNPQ. This has been also reported by other authors (He et al., 1996; Calatayud et al., 2007). Maximum quantum efficiency of PSII photochemistry (F_v/F_m) increased with radiation as well (Fig. 3). The pattern of the leaves in the flowering stems throughout the experiment is not that clear, maybe due to their higher sink strength. It seems that radiation is not the

only factor driving photosynthesis, but another internal factor may also have a strong impact on it.

The highest values of ϕPSII and q_L were obtained in leaves receiving high radiation, that is, upper leaves of flower stems and external leaves of bent shoots. This agrees with Jiao and Grodzinski (1998), who attributed the highest photosynthetic capacity to the upper leaves below flower bud measured at high PAR. In contrast, in the same type of leaves, the minimum values of F_v/F_m and ϕNPQ were obtained. These results were similar to those obtained by Calatayud et al. (2007).

In conclusion, rose plants were well adapted to cold solution in winter in our experimental conditions without any decrease in dry biomass or photochemical activity. However, other climatic factors may modify this response.

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4.2. NITROGEN AND CARBOHYDRATE DYNAMICS UNDER TWO DIFFERENT ROOT TEMPERATURES IN ROSE PLANTS.

Abstract

The effect of two different nutrient solution temperatures, cold (10 °C) and warm (22 °C), on nitrogen absorption, partitioning and dynamics, and on carbohydrates concentration and distribution throughout two flowering cycles (winter-spring) was studied in rose plants (*Rosa hybrida* var. Grand Gala grafted on *Rosa Manetti*). The ^{15}N study showed that the level of N derived from the fertilizer (Ndf) in every plant compartment was higher in plants grown at cold nutrient solution during the 1st flower cycle. Also, during this cycle, a higher aerial biomass was observed in this treatment. The study of subsequent re-translocation of Ndf within the plant during the 2nd cycle showed that, in general terms, the roots, the structure and the stems of the bent shoots acted as sources while the rest of the aerial compartments acted as sinks. The solution temperature differenciated the Ndf dynamics of the thin root: at 10 °C they were sinks while at 22 °C they were sources. The concentration of soluble sugars and starch was higher at cold solution during the first cycle, mainly in the roots. However, the effect of low solution temperature depended on the season. At the end of the 2nd flower cycle, biomass and the concentration of soluble sugars and starch were similar at cold and warm solution. The differences in carbohydrates levels between the ends of the 1st and 2nd flowering cycle suggest that there might have been a substantial consumption of carbohydrates at cold solution during the 2nd cycle. Also, during this cycle, a higher aerial biomass production was observed at warm solution. Regarding yield, there were no differences between treatments at any cycle. These results suggest that rose plants have high capacity to adapt to cold solution in winter by increasing nitrogen absorption and carbohydrate levels in the roots.

Introduction

Roses are one of the most important cut flowers in greenhouse production of ornamentals in the world. Rose plants have multiple fast cycles of flower shoot growth over a year (Zieslin and Moe, 1985). Therefore, the year round production of cut roses demands a continuous supply of sugar and nitrogen to the developing flower shoots (Calatayud et al., 2007). Soilless cultivation techniques, often used for rose plants, enable the control of the root environment, including nutrition, irrigation, root temperature and aeration (Moss and Dalgleish, 1984). In spite of recent developments in these techniques, the nitrogenous fertilizers have been used in excess, with serious repercussions on both the environment and agricultural crops. The general strategy of soilless culture is based on supplying nutrient solution with an excess of 20-30% respect to the estimated needs of the crop (Cid et al., 2001). Therefore, rational guidelines that respect the environment and, at the same time, maintain production and flower quality, must be established for nitrogen fertilization. The first step is to understand how rose plants control the absorption and distribution of acquired nitrogen. Nitrogen fertilizer recovery by plants is influenced by many factors such as the nature of the plants and its roots system (Tamimi et al., 1999). In Chapter 4.1, it

has been shown that root temperature modified root morphology and nitrate uptake in rose plants. Limited information is available on the rate of uptake and distribution of nitrogen within rose plants exposed to low nutrient solution temperature, which is a common situation in greenhouses without heating in winter. Labeled ^{15}N fertilizers have been used in several fruit species (Dong et al., 2001; Quiñones et al., 2003) to study the uptake, distribution of N and sink/source organs within the plant. In greenhouse roses, there are few studies about nitrogen uptake and partitioning (Cabrera et al., 1995) but, as far as we know, there is no research about nitrogen translocation in plants.

Along with nitrogen, carbohydrates reserves play a crucial role in supporting growth (Zapata et al., 2004). The levels and translocation of carbohydrates are considered the main factors affecting the development of rose flowers (Kumar et al., 2007). Carbon assimilation rate and export are influenced by the environmental conditions (Jiao and Grodzinski, 1996), by the demand for photosynthates in the sinks (Wardlaw, 1990) and by nitrogen supply (Druege et al., 2004). Storing nitrogen in the form of either protein or free amino acids requires carbon inputs to provide a carbon skeleton and energy supply (Cheng et al., 2004).

The objective of this work was to study the effect of a plant external factor such as nutrient solution temperature on nitrogen uptake, partitioning and re-translocation, and on carbohydrate content, use and distribution within rose plants (*Rosa x hybrida* cv. Grand Gala) during two flower cycles under Mediterranean winter-spring greenhouse conditions. N uptake and distribution was studied in the 1st flower cycle, while re-translocation of N acquired during the 1st cycle was assessed in the 2nd flower cycle. Two different nutrient solution temperatures were chosen: 22 °C as the control temperature since it has been described as the optimum one (Zeroni and Gale, 1982), and 10 °C as the cold temperature, which can be achieved in winter in greenhouses without heating under Mediterranean conditions.

Materials and methods

Plant management and greenhouse conditions

The experimental conditions and treatments were the same as described in Chapter 4.1. The nutrient solution, which was renewed every week, had the following composition (mmol L^{-1}): 5 NO_3^- (isotopic enrichment of 4 % atom ^{15}N excess); 0.5 $H_2PO_4^-$; 0.5 SO_4^{2-}; 2.5 K^+; 1.5 Ca^{2+}; 0.5 Mg^{2+}. ^{15}N-enriched solution was only applied during the first floral cycle (winter). The water for the nutrient solution was previously treated with reverse osmosis and ion columns.

The yield in fresh weight (FW) of all flower shoots harvested during each cycle was measured. At the end of each flowering cycle (1st and 2nd), 5 plants from each treatment were taken for destructive measurements 3 hours after sunrise. All samples of plant material were washed, divided into different compartments, frozen in liquid nitrogen, lyophilized-dried and milled. They were, then, ready for the measurements about nitrogen and carbohydrates. Dry weight of aerial and root biomass and of all plant compartments were measured. All the plants were separated in nine compartments (for each compartment, a number between brackets was assigned): thin roots (1), suberized roots (2), structural part (3), leaves of bent shoots (4), stems of bent shoots (5), leaves of flower shoots (6), stems of flower shoots (7), peduncle (8) and flower bud (9).

Nitrogen measurements and calculations

Three different measurements were carried out concerning nitrogen: nitrate (NO_3^-) uptake, concentration of total nitrogen (N) and nitrogen derived from fertiliser (Ndf) in the plant.

The measurement protocol of daily NO_3^- uptake rate was described in Chapter 3. Nitrate uptake concentration (NUC) was calculated as the relation between the mmol of NO_3^- absorbed and the L of nutrient solution absorbed by the plant. It is expressed as mmol NO_3^- L^{-1} (Chapter 3 of this thesis).

The allocation of nitrogen derived from the mineral N absorbed during the 1st flower cycle was determined by measuring the abundance of ^{15}N in the different plant compartments of *n*=5 plants from each temperature treatment harvested at the end of this cycle (Dong et al., 2001; Meuriot et al., 2004). Total N (the sum of ^{14}N and ^{15}N) concentration (g N g DW^{-1} (%)) and ^{15}N concentration in each compartment (g ^{15}N g N^{-1} (%)) were measured in continuous flow using a C/N analyser linked to an isotope ratio mass spectrometer (IRMS) (NC 2500, Eager 300 software®, CE instruments, ThermoQuest Italia, Rodano, Italy, and DELTA^{plus}, Isodat NT 2.0 software®, ThermoQuest Finigan, Bremen, Germany, respectively). Natural ^{15}N abundance (0.3663) of atmospheric N_2 was used as reference for ^{15}N analysis. The amount of N derived from fertilizer (g Ndf) in each compartment was calculated as:

$$g\ \ Ndf\ = \frac{\left(atom\%^{15}N_{excess}\right)_{compartment}}{\left(atom\%^{15}N_{excess}\right)_{fertilizer}} \times g\ \ N_{compartment} \qquad (1)$$

The amount of Ndf was also calculated on dry weight basis as g Ndf g DW^{-1} (%).

To analyse N partitioning within the plant in the 1st cycle, the percentage of Ndf in a compartment over the total amount of Ndf absorbed by the plant and on dry weight basis (g Ndf_c g Ndf_t^{-1} g DW^{-1} (%), where c means compartment and t means total) was calculated. All these equations were calculated for all compartments of plants at both temperature treatments sampled at the end of the 1st flower cycle since it was the cycle where ^{15}N-enriched solution was applied.

In order to analyse N re-translocation within the plants at any temperature treatment during the 2nd flower cycle, the difference of the percentage of Ndf in every compartment over the total Ndf in the plant between both cycles was calculated as:

$$\Delta\ gNdf_c\ \ gNdf_t^{-1} = \left(\frac{gNdf_c \times 100}{gNdf_t}\right)_{2nd} - \left(\frac{gNdf_c \times 100}{gNdf_t}\right)_{1st} \quad (2)$$

where 1st and 2nd are the flower cycles.

Soluble sugars and starch

Soluble sugars (SU) and starch (ST) were analysed using a colorimetric method based on McCready et al. (1950). For each plant compartment, 100 mg of lyophilised and milled sample were used for the analysis. The samples were mixed with heated ethanol and centrifuged. The liquid part contained the SU and the precipitate contained the ST. Anthrone-acid solution was added to the precipitate, which was placed in a boiling water bath. The resulting mixtures of SU and ST were read at 630 nm (spectrophotometer Uvikon XS, Bio-Tek, USA). SU and ST were measured in all plant compartments of *n*=5 plants from each temperature treatment sampled at the end of both flower cycles.

The amount of SU and ST in each compartment was expressed as g SU and g ST, and also on dry weight basis as g SU g DW^{-1} (%) and g ST g DW^{-1} (%).

To analyse SU and ST partitioning within the plant in each cycle, the percentage of SU and ST in a compartment over the total amount of SU and ST in the plant and in dry weight basis (g SU_c g SU_t^{-1} g DW^{-1} (%) and g ST_c g ST_t^{-1} g DW^{-1}(%), where c means compartment and t means total) was calculated.

The relationship between carbon (C) and N levels in the plant was studied by calculating C/N ratio in every compartment. C content was measured by a C/N analyser (NC 2500, Eager 300 software®, CE instruments, ThermoQuest Italia, Rodano, Italy).

All these equations were calculated for all compartments of plants at both temperature treatments sampled at the end of both flower cycles.

Statistical analysis

The effect of the temperature of the nutrient solution on the concentration and distribution of soluble sugars, starch and Ndf was evaluated in rose plants by means of one-way ANOVA with nutrient solution temperature as factor. It had 2 levels: warm temperature, which was the control treatment, and cold temperature. In order to analyse the change in the content of carbohydrates in each nutrient solution treatment during the 2nd flowering event, the flowering event was considered as factor, with 2 levels: 1st and 2nd flowering event. Normality of distribution was verified by Q-Q plot. For all inferences, α=0.05. Calculations were made with the aid of a statistical software (Statgraphics Plus for Windows 4.1).

Table 1. Effect of cold (10 °C) and warm (22 °C) nutrient solution temperature (T_S) on daily mean values of nitrate uptake concentration (NUC, mmol NO_3^- L^{-1} nutrient solution) throughout each flowering cycle. Also, effect on values of aerial and root biomass on dry weight basis (g DW) and on yield of flower stems (Yield, g FW $plant^{-1}$) at the end of each flowering cycle for *n*=5 plants. For each flowering cycle, one-way ANOVA was performed with solution temperature as factor. Values (means±SD) followed by the same letter within each cycle indicate not significant differences at 5%.

Flowering cycle	T_S	NUC	Aerial biomass	Root biomass	Yield
1st	10	11.7±3.2a	221.16±20a	36.94±6.4a	50.50±14a
	22	5.98±2.3b	165.64±10b	32.74±8.6a	47.70±17a
2nd	10	7.58±3.3a	251.13±16a	39.94±7.7a	172.4±30a
	22	5.37±1.8b	289.96±41a	43.86±7.7a	203.2±18a

Results

Biomass parameters

At the end of the 1st flower cycle, plants grown at 10 °C had developed statistically higher aerial biomass than plants grown at 22 °C, while root biomass and yield of flower shoots were similar between treatments (Table 1). At the end of the 2nd flower cycle, there were not significant differences between treatments regarding yield of flower shoots and aerial and root biomass. Between the 1st and 2nd cycle both aerial and root biomasses increased in both treatments, with statistical differences in the case of aerial biomass (*p*=0.0005). The relation between produced biomasses of the

second and the first cycle was higher for plants at warm solution (175%) than at cold solution (113.5%) (Table 1).

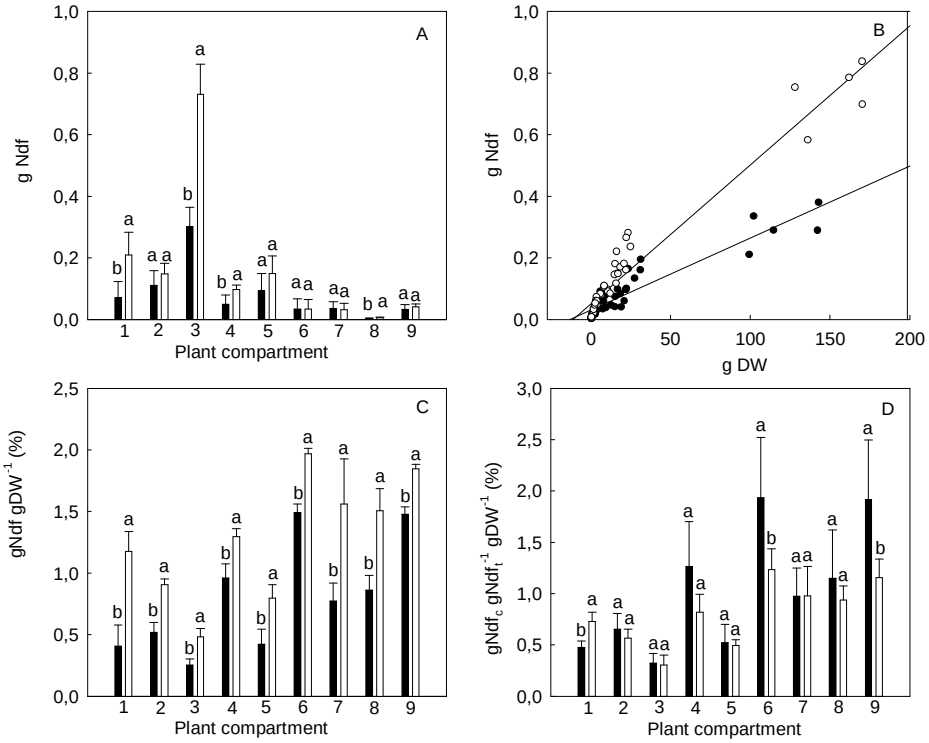

Fig. 1. Effect of nutrient solution temperature ((cold (□) and warm (■)) on the amount of nitrogen derived from fertiliser (g Ndf) in every compartment (A); the relationship between g Ndf and g DW in plants at cold solution (O) (g Ndf = 0.0045 g DW + 0.0507; R^2 = 0.946) and warm solution (●) (g Ndf = 0.0023 g DW + 0.0308; R^2 = 0.872) (B); the effect of nutrient solution temperature on the concentration of Ndf in every compartment per 100 g of dry weight of the compartment (g Ndf g DW^{-1}(%)) (C), and on the percentage of Ndf in a compartment over the total amount of Ndf absorbed by the plant and on dry weight basis (g Ndf_c g Ndf_t^{-1} g DW^{-1} (%), c means compartment and t means total) (D). Moreover, In A, C and D, the numbers on the X-axis are related to the different plant compartments: 1 (thin roots), 2 (suberized roots), 3 (structural part), 4 (leaves of bent shoots), 5 (stems of bent shoots), 6 (leaves of flower shoots), 7 (stems of the flower shoots), 8 (peduncle) and 9 (flower bud). For each compartment, one-way ANOVA was performed with solution temperature as factor. Values are means±SD of *n*=5. Columns with the same letter within each compartment indicate not significant differences at 5% between temperature treatments.

N absorption by the plant

The destination of N absorbed by the roots during the first flower cycle was tracked using ^{15}N in the fertiliser. It revealed that plants at cold solution had statistically more Ndf (1.628 ± 0.253 g Ndf) than plants at the warm one (0.87 ± 0.418 g Ndf). In Fig. 1A, it is possible to see how many Ndf had ended up in each compartment of the plant at

the end of the 1st flower cycle. There were statistically more g of Ndf in the thin roots (1), the structural part (3), the leaves of the arched shoots (4) and the peduncle (8) of plants at cold solution. However, this parameter can be misleading since the amount of Ndf in a compartment is strongly related to the amount of biomass of it, being this regression much steeper for the cold solution (see Fig. 1B). So, to get rid of the overlapping effect of biomass, the parameter g Ndf was calculated on dry weight basis (Fig. 1C).

The ratio g Ndf g DW^{-1} (%) was statistically higher in all compartments of plants at cold solution at the end of the 1st flower cycle. The greatest difference between treatments was found in the thin roots (1) and the lowest in the flowering bud (9), where g Ndf g DW^{-1} (%) was 65.4% and 20% lower in the warm treatment, respectively. The parameter g Ndf g DW^{-1} (%) was generally higher in the 4 compartments related to the flower shoots (6, 7, 8, 9), and lower in the structural part of the plant (3) (Fig. 1C). The whole plant grown at cold solution absorbed much more g Ndf per g (DW) than plants at warm solution (0.67 ± 0.032 vs 0.375 ± 0.078 g Ndf g DW^{-1} respectively). This is in agreement with the results about nitrate uptake per plant (Chapter 4.1 of this thesis), which was statistically higher in plants at cold solution during the 1st flower cycle. However, at the end of the 2nd flower cycle, no differences were found between treatments. In contrast, nitrate uptake concentration (NUC) was statistically higher, for both flower cycles, in plants at cold solution (Table 1).

N partitioning in the plant

To avoid the overlapping effect of biomass in Ndf partitioning, the percentage of Ndf on a compartment over the total amount of Ndf absorbed by the plant was calculated on dry weight basis for each of the treatments at the end of the first flower cycle, and the comparison between them can be seen in Fig. 1D. The low temperature of the nutrient solution affected N partitioning in the plant by increasing the percentage of Ndf distributed to 1 g of thin roots (1) and by decreasing the percentage of Ndf destined to 1 g of leaves of the flowering shoots (6) and to 1 g of flower bud (9). Even though the percentage destined to the leaves of the flowering shoots (6) and to the flower bud (9) was lower in the cold treatment, as the whole plant absorbed more Ndf, the final amount in each gram (DW) of the compartment was higher in plants at cold solution (Fig. 1C).

Nitrogen dynamics in time

Once N absorbed by the plant has arrived to its destiny, it can remain there or it can be re-translocated to another compartment. In order to know how the absorbed N during the 1st cycle moved in the 2nd cycle within the plant, no ^{15}N was used in the fertiliser during the 2nd cycle. All the ^{15}N measured in the plants at the end of the 2nd flower

cycle had been absorbed in the 1st one. Thus, except for the ^{15}N contained in few plant parts that may had died during the 2nd cycle, mainly some leaves of bent shoots and thin roots, the amount of Ndf in the whole plant at the end of both cycles was very similar (data not shown). What could have changed was the percentage of Ndf in each of the compartments over the total Ndf in the plant, which would be related to re-translocation of acquired Ndf within the plant. The difference between this percentage in the 2nd and 1st flower cycle is shown in Fig. 2.

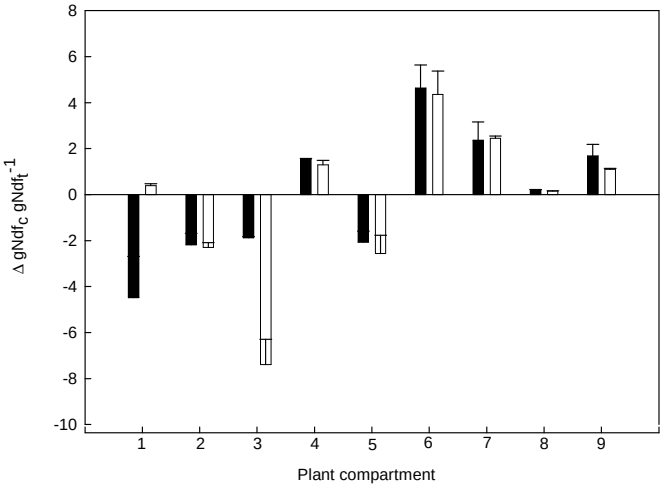

Fig. 2. Difference of the percentage of nitrogen derived from fertiliser (Ndf) in every compartment over the total Ndf in the plant between the 2nd and 1st flower cycle in the cold (□) and warm (■) treatment (Δ g Ndf_c g Ndf_t^{-1}, c means compartment and t means total). The numbers on the X-axis are related to the different plant compartments: 1 (thin roots), 2 (suberized roots), 3 (structural part), 4 (leaves of bent shoots), 5 (stems of bent shoots), 6 (leaves of flower shoots), 7 (stems of the flower shoots), 8 (peduncle) and 9 (flower bud). Values are means±SD of *n*=5.

As the aim is to represent what happens in the 2nd flower cycle, in the case of the calculation of N dynamics in the flower shoots, the starting point was Ndf=0, since at the end of the 1st flower cycle all flower shoots of the plants were cut. The positive values indicate that the percentage of Ndf in that compartment over total Ndf in the plant had increased during the 2nd cycle. This means that the compartment was a sink of N as it attracted N from the other plant parts. The negative values denote that the percentage of Ndf in that compartment had decreased during the 2nd cycle, which means that either some death had occurred in that compartment during the 2nd cycle, or that the compartment was a source of N towards other plant parts that acted as sink. According to that reasoning, the whole flower shoots (6, 7, 8, 9) and the leaves of the arched shoots (4) were sinks of N, while the suberized roots (2), the structural part (3) and the stems of the arched shoots (5) were sources of N. On the other hand, the temperature of the nutrient solution differentiated the dynamics of the thin roots (1).

At the cold solution, thin roots were sinks, while in the warm one they behaved as sources. Alternatively, N losses may have occurred due to root death in this treatment. Besides, the structure (3) was a stronger source at cold than at warm solution.

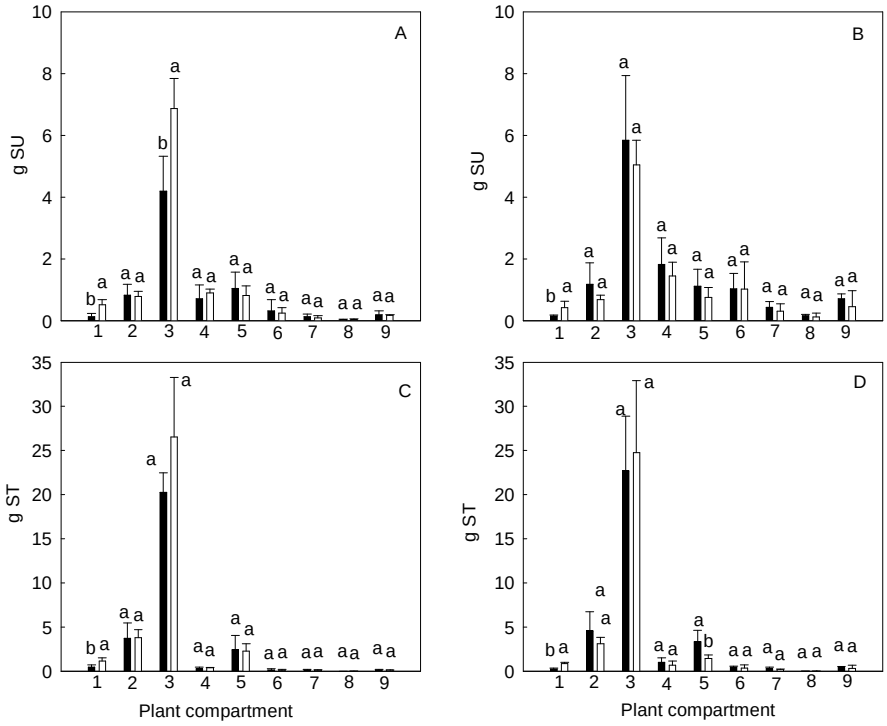

Fig. 3. Effect of nutrient solution temperature ((cold (□) and warm (■)) on the amount of soluble sugars (g SU) at the end of the 1st flower cycle (A) and 2nd flower cycle (B), and on the amount of starch (g ST) at the end of the 1st flower cycle (C) and 2nd flower cycle (D) in every plant compartment. The numbers on the X-axis are related to the different plant compartments: 1 (thin roots), 2 (suberized roots), 3 (structural part), 4 (leaves of bent shoots), 5 (stems of bent shoots), 6 (leaves of flower shoots), 7 (stems of the flower shoots), 8 (peduncle) and 9 (flower bud). For each compartment, one-way ANOVA was performed with solution temperature as factor. Values are means±SD of *n*=5. Columns with the same letter within each compartment indicate not significant differences at 5% between temperature treatments.

Soluble sugars and starch content in the plant

Concerning the whole plant, at the end of the 1st flower cycle, the amount of soluble sugars was statistically higher in plants at cold than at warm solution (11.156 ± 1.698 vs 7.757 ± 2.311 g SU, respectively) while the amount of starch was similar in both treatments (38.076 ± 9.656 (cold) vs 29.378 ± 12.308 (warm) g ST). At the end of the 2nd flower cycle, no differences were found between treatments related to the amount of soluble sugars (11.232 ± 2.992 (cold) vs 14.347 ± 3.736 (warm) g SU) and starch

(35.516 ± 10.817 (cold) vs 36.588 ± 8.113 (warm) g ST). However, regarding each compartment separately (Fig. 3), the amount of SU (Fig. 3A, 3B) and ST (Fig. 3C, D) were statistically higher at the end of both flower cycles in the thin roots (1) of plants at cold solution. In addition, at the end of the 1st flower cycle, there was a higher amount of SU in the structural part (3) of plants at cold solution (Fig. 3A, C). The content of ST was lower, at the end of the 2nd flower cycle, in the bent stems (5) of plants at the cold treatment (Fig. 3D).

As for Ndf, the problem of using the amount of SU and ST to compare treatments can be confusing since both are strongly linked to biomass. There is a linear relationship (data not shown) between g SU and g (DW) and also between g ST and g (DW) very similar to the one seen in Fig. 1B, with R^2 between 0.88 and 0.99, and with a higher slope for the cold treatment. That is why g SU and ST were calculated for each compartment on dry weight basis (Fig. 4).

At the end of the 1st flower cycle, g SU g DW^{-1} (%) was statistically higher in whole plants at cold solution (4.607 ± 0.415 vs 3.447 ± 0.534 g SU g DW^{-1} (%)) while g ST g DW^{-1} (%) did not show significant differences between treatments concerning whole plants (15.446 ± 1.667 (cold) vs 12.826 ± 3.283 (warm) g ST g DW^{-1} (%)). At the end of the 2nd flower cycle, no differences were found between treatments on the amount of soluble sugars (3.801 ± 0.853 (cold) vs 4.293 ± 0.237 (warm) g SU g DW^{-1} (%)) and starch on dry weight basis (11.963 ± 2.817 (cold) vs 11.058 ± 0.925 (warm) g ST g DW^{-1} (%)) of complete plants.

The g SU and ST g DW^{-1} (%) for each compartment are represented in Fig. 4. At the end of the 1st cycle (Fig. 4A), g SU g DW^{-1} (%) was statistically higher in thin and suberized roots (1, 2), the structural part (3) and the flower stems (7) of plants at cold solution, while it was lower in the leaves of the arched shoots (4). Also, g ST g DW^{-1} (%) was statistically higher in thin and suberized roots (1, 2) and the peduncle (8) of plants at cold solution (Fig. 4C). At the end of the 2nd flower cycle, g ST g DW^{-1} (%) was statistically higher in suberized roots (2) and structure (3) at the cold treatment (Fig. 4D). For the rest of the compartments, no differences were found between temperature treatments concerning SU and ST concentration (Fig. 4B, D).

Carbohydrates partitioning in the plant

So as to understand the distribution of carbohydrates within the plant, the percentage of SU or ST in a compartment over the total amount of SU or ST in the plant and on dry weight basis (to avoid the overlapping effect of biomass in carbohydrates partitioning), was calculated for each treatment at the end of both flower cycles, and the comparison between them can be seen in Fig. 5.

At the end of the 1st flower cycle, the percentage of SU and ST distributed to 1 g (DW) of thin roots (1) over the total amount of SU (Fig. 5A) and ST (Fig. 5C) in the plant, was statistically higher in the cold treatment, while the percentage of SU distributed to 1 g of bent shoots (4, 5) and to 1 g of flower shoot (6, 7, 9) except for the peduncle (8) (p=0.0675) was statistically lower in this treatment. However, at the end of the 2nd flower cycle, no differences were found between treatments (Fig. 5B, D).

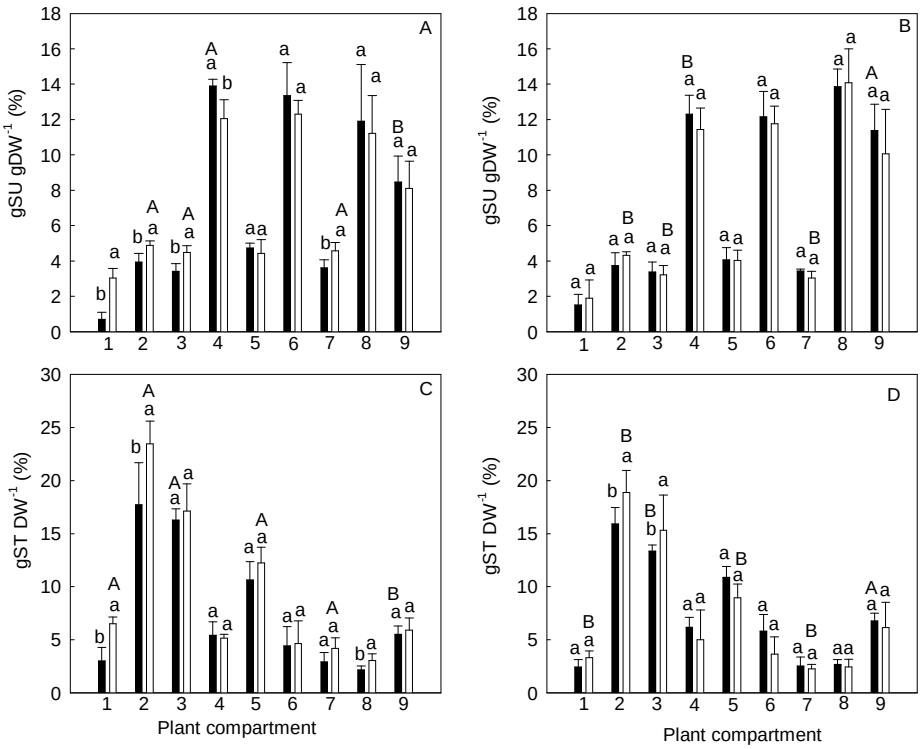

Fig. 4. Effect of nutrient solution temperature ((cold (□) and warm (■)) on the concentration of soluble sugars per 100 g of dry weight of the compartment (g SU g DW^{-1}(%)) at the end of the 1st flower cycle (A) and 2nd flower cycle (B), and on the concentration of starch per 100 g of dry weight of the compartment (g ST g DW^{-1} (%)) at the end of the 1st flower cycle (C) and 2nd flower cycle (D) in every plant compartment. The numbers on the X-axis are related to the different plant compartments: 1 (thin roots), 2 (suberized roots), 3 (structural part), 4 (leaves of bent shoots), 5 (stems of bent shoots), 6 (leaves of flower shoots), 7 (stems of the flower shoots), 8 (peduncle) and 9 (flower bud). For each compartment, one-way ANOVA was performed with solution temperature as factor. In the same graph, a pair of columns within a compartment with the same lower-case letter indicates not significant differences at 5% between temperature treatments. Also, for each compartment, one-way ANOVA was performed with the flower cycle as a factor with 2 levels (1st and 2nd). A pair of columns placed each one in a different graph (A and B for SU, or C and D for ST) within the same compartment, belonging to the same temperature treatment, and having a different capital letter indicates significant differences at 5% between the end of both flower cycles. The absence of capital letters means

that there were not significant differences at 5% between the end of both flower cycles. Values are means±SD of *n*=5.

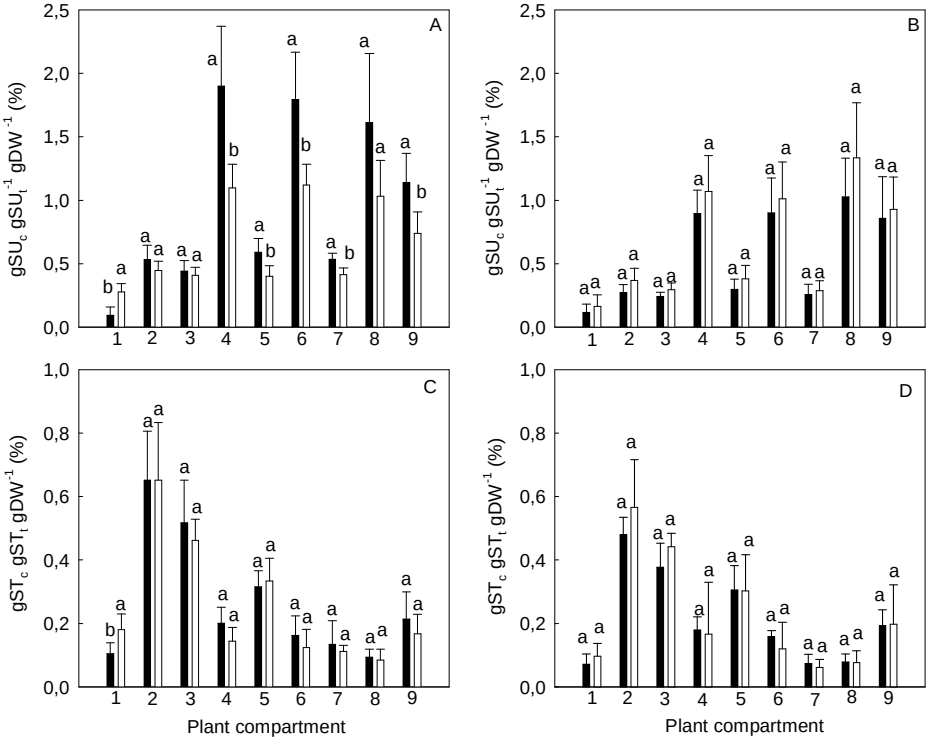

Fig. 5. Effect of nutrient solution temperature ((cold (□) and warm (■)) on the percentage of soluble sugars in a compartment over the total amount of soluble sugars in the plant and on dry weight basis (g SU_c g SU_t^{-1} g DW^{-1}(%), c means compartment and t means total) at the end of the 1st flower cycle (A) and 2nd flower cycle (B), on the percentage of starch in a compartment over the total amount of starch in the plant and on dry weight basis (g ST_c g ST_t^{-1} g DW^{-1}(%), c means compartment and t means total) at the end of the 1st flower cycle (C) and 2nd flower cycle (D) in every plant compartment. The numbers on the X-axis are related to the different plant compartments: 1 (thin roots), 2 (suberized roots), 3 (structural part), 4 (leaves of bent shoots), 5 (stems of bent shoots), 6 (leaves of flower shoots), 7 (stems of the flower shoots), 8 (peduncle) and 9 (flower bud). For each compartment, one-way ANOVA was performed with solution temperature as factor. Values are means±SD of *n*=5. Columns with the same letter within each compartment indicate not significant differences at 5% between temperature treatments.

Changes in carbohydrate content between the end of two successive cycles

When comparing the levels of sugars and starch at the end of each cycle, differences were found, which depended on the temperature treatment. One-way ANOVA was carried out for each compartment taking the flower cycle as factor and g SU or ST g DW^{-1} (%) as response variables (Fig. 4, see capital letters). As plant samples for these measurements were taken at the end of both flower cycles, whenever significant

differences were found, they illustrated that during the 2nd flower cycle some increase or decrease in the concentration of SU or ST had happen in a given compartment.

In the cold treatment, a significant decrease of g SU g DW^{-1} (%) (Fig. 4A, B) between cycles was observed in some compartments such as the suberized roots (2), the structural parts (3) and the flower stems (7), while a decrease of ST g DW^{-1} (%) (Fig. 4C, D) was seen in the roots (1, 2), the bent stems (5) and flower stems (7)

On the other hand, plants at warm solution did not experience such decrease except for the leaves of bent shoots (4) and structural part (3), where g SU and ST g DW^{-1} (%) decreased, respectively, during the 2nd cycle. What is more, the % g SU and ST g DW^{-1} increased during the 2nd cycle in the flower bud (9).

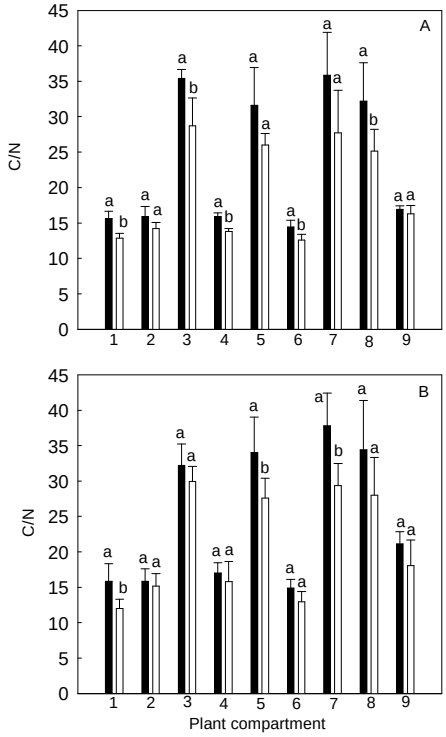

Fig. 6. Effect of nutrient solution temperature ((cold (□) and warm (■)) on the carbon (g) and nitrogen (g) ratio (C/N) at the end of the 1st flower cycle (A) and at the end of the 2nd flower cycle (B). The numbers on the X-axis are related to the different plant compartments: 1 (thin roots), 2 (suberized roots), 3 (structural part), 4 (leaves of bent shoots), 5 (stems of bent shoots), 6 (leaves of flower shoots), 7 (stems of the flower shoots), 8 (peduncle) and 9 (flower bud). For each compartment, one-way ANOVA was performed with solution temperature as factor. Values are means±SD of *n*=5. Columns with the same letter within each compartment indicate not significant differences at 5% between temperature treatments.

Relation between N and C in the plant

C/N ratio was calculated to know how the temperature of the nutrient solution had affected the relationship between C and N levels in the plant (Fig. 6).

In both treatments, C/N ratio (Fig. 6A, B) was higher for the structural part (3), stems (5, 7) and peduncle (8). Comparing temperature treatments, this ratio was statistically higher in plants at warm solution in different compartments: thin roots (1), structural part (3), leaves of bent and flower shoots (4, 6) and peduncle (8) at the end of the 1st flower cycle (Fig. 6A), and thin roots (1) and bent and flower stems (5, 7) at the end of the 2nd one (Fig. 6B).

Discussion

Nitrogen demand depends on the stage of development of the plant (Cabrera et al., 1995) and on the climatic conditions (Imsande and Touraine, 1994), among other factors. For instance, in Chapter 4.1 of this thesis we observed, in general terms, that roots of rose plants absorbed more nitrate when growing at cold nutrient solution (10 °C) than if grown at 22 °C. In the cold treatment, roots were white and succulent and absorbed less water, while leaves increased their nitrate reductase activity, NH_4^+ concentration and the activity of the photosynthetic light reaction with respect to rose plants grown at warm solution (22 °C). In this work we have shown that this improved metabolism leads to a higher concentration in the plant of carbohydrates and N derived from the fertiliser (Ndf), with a clear increase in the distribution of them, mainly, towards the thin roots. Nitrogen levels in the plant seemed to be more affected by the cold nutrient solution than carbon levels but, in any case, the effect of nutrient solution temperature is not simple but depends on air climatic variables linked to the change of the season.

In this study, at the end of the first flowering cycle that took place in winter, plants at cold solution had statistically higher concentration of Ndf in all their compartments (Fig. 1), in relation to a higher NUC (Table 1) and a higher NO_3^- uptake during that period (Chapter 4.1 of this thesis). In other studies, low root temperature increased leaf N concentration in tomatoes (Gosselin and Trudel, 1983) but reduced N content in peppers (Gosselin and Trudel, 1986). The Ndf in dry matter is frequently used as an indication of how adequate N supply is for crop growth (Lawlor et al., 2001). Most of the leaf N is present within the chloroplast, with a large proportion being a component of RuBisCO (Evans, 1989; Jensen, 2000). Thus, the higher concentration of Ndf measured might have contributed to a higher photochemical activity as observed in the leaves of plants at cold solution (Chapter 4.1, this thesis). The improved NO_3^- uptake in the cold treatment may be due to an enhancement of the active NO_3^- uptake

mechanisms of the roots. At cold solution the assimilate supply to the roots was not inhibited and even it was improved as indicated by the higher non-structural carbohydrates concentration (Fig. 4). Similar results were obtained in maize and wheat by Engels (1994). The addition of carbohydrates to the nutrient solution is known to increase NO_3^- uptake (Delhon et al., 1996).

Once absorbed, Ndf was distributed within the plant depending on the biomass of the compartment, that is, the bigger the compartment, the more Ndf destined to it, and on the sink strength of the compartment (Fig. 1). The flower shoots and the leaves of the bent shoots, organs with a high growth rate, appeared to be the compartments with the highest sink strength since they received a higher amount of Ndf per gram (DW) than the other compartments, both directly by root absorption or indirectly through re-translocation from other parts of the plant. Besides biomass and sink strength, the temperature of the nutrient solution modulated the distribution of Ndf by increasing the percentage of Ndf destined to each gram of thin roots and reducing the percentage destined to each gram of leaves of the flower shoots and the flower bud in plants with their roots at 10 °C (Fig. 1). In this treatment, thin roots strongly increased their sink strength with respect to the warm treatment. The greatest difference between treatments concerning the amount of Ndf per gram (DW) was observed in this compartment (Fig. 1) and, in the cold solution, thin roots seemed to receive Ndf through re-translocation from other compartments while, at the warm treatment, they did not (Fig. 2). Some studies have demonstrated that low temperature induces changes in source-sink relationships for N leading to a preferential allocation of N to the roots associated with an increase in the total soluble protein pool (Noquet et al., 2001).

In rose plants, endogenous N is redistributed within the plant during each flowering cycle (Cabrera et al., 1995). In our results (Fig. 2), suberized roots, structure and stems of arched shoots represented the major source organs in rose plants at cold and warm solution, and N was redistributed from these organs to support, mainly, the flower shoot demand. Cabrera et al. (1995) showed that the N absorbed by the roots could supply 16% of the total N content of the flower shoot. But, not only were the flower shoots the sinks, but also the leaves of the bent shoots, which received N possibly from the bent stems. The bent shoot has been described as a promoter of the initiation and growth of vigorous flower stems (Calatayud et al., 2007). As stated before, the N remobilisation within the plant was affected by solution temperature, and this effect was observed mainly in two compartments: thin roots and structure. At cold solution, thin roots acted as sinks while at warm solution they were sources of N. This can be explained by the fact that, at cold solution, the plants had a higher proportion of thin root versus suberized root, being the first one more effective in mineral uptake (Murisier, 1996; Dong et al., 2003). As new tissues act as sinks for N

(Faust, 1989; Dong et al., 2001), the mobilization of N from source organs was necessary to support the growth and enhanced metabolism of thin roots.

On the other hand, the structure was a more important N source at cold than at warm solution. The structure, as all perennial organs of woody plants, may have a storage function (Kool et al., 1997). In the cold treatment during the first cycle, the structure accumulated more amount of Ndf (Fig. 1), and the higher capacity of accumulation of N may have provided subsequently higher source (export) capacity in the 2nd cycle (Fig. 2). Nutrient translocation rate in the xylem may depend on several processes: 1) net uptake of nutrients by the roots from the external solution, 2) the fraction of 1) which is retained in the roots for storage or root growth, 3) the amount of nutrient recycled in the phloem from the shoot to the root and 4) the fraction of 3) which is stored in the roots or utilized for root growth (Engels et al., 1992). Any of these processes may be affected by cold solution temperature in order to get Ndf through re-translocation in the thin roots.

Along with nitrogen, carbohydrates reserves play a crucial role in supporting plant metabolism and growth (Zapata et al., 2004). At the end of the first flower cycle, whole plants had higher concentration of soluble sugars at cold than at warm nutrient solution. This means that there may be a higher production of carbohydrates during the first cycle in the cold treatment, which agrees with the fact that the photosynthetic light reaction was stimulated (Chapter 4.1, this thesis). Low temperature has been described to increase the carbohydrates content in roses (Kool et al., 1997). Looking into the soluble sugars and starch concentrations of the different plant compartments (Fig. 4), it could be suggested that some of the extra soluble sugars accumulated at cold solution in the less photosynthesizing compartments such as roots, structure or stems of flower shoots, were probably produced in the leaves of bent shoots and remobilized. Furthermore, the higher accumulation of carbohydrates in the form of starch in the roots and peduncle (Fig. 4) could indicate either that the concentration of soluble sugars was too high that a conversion to starch was promoted or that the possible mild stress caused by the cold solution stimulated a conversion of soluble sugars to starch to better keep carbohydrate reserves. The latter was observed in Norway spruce by Repo et al. (2004). Alternatively, if respiration increases exponentially with temperature, at cold solution, a lower respiration would consume fewer amounts of reserves.

The distribution of soluble sugars and starch within the plant depended on the biomass of the compartment and source strength, i.e. net carbohydrate production of the photosynthetically active tissues. In addition, the temperature of the nutrient solution had an effect on the partitioning of carbohydrates. For both treatments, the higher soluble sugar concentration was in the photosynthetic compartments, in the

peduncle and in the flower bud (Fig. 4), which can be due to mobilization reserves from root to leaves with starch hydrolysis and/or synthesis of sugars *in situ.* Mor and Halevy (1979) reported that rose shoots are strong sinks for photoassimilates. For both treatments, the starch concentrations were higher in suberized roots and structural parts. These results confirm those previously reported for roses (Roca et al., 2005) and for other woody species like grape (Zapata et al., 2004), apple (Loescher et al., 1990) or birch (Soljeld and Johnsen, 2006). Carbohydrates reserves in roots are essential for flower production. With regard to the temperature of the nutrient solution, the percentage of soluble sugars and starch given to the thin roots increased in plants at cold solution during the 1st cycle, while this priority was done by distributing a lower percentage of soluble sugars to almost all the aerial compartments compared to plants at 22 °C (Fig. 5).

C and N metabolism are linked by shared intermediates and products and also by a complex network of cross-talking signal pathways (Miller and Cramer, 2004). An evaluation of C/N balance (Fig. 6) indicates that it was lower in plants at cold solution which means that, the low solution temperature stimulated N uptake in a greater extent than C production. Similar results were obtained in rose plants by Hambrick et al. (1991). Compartments with lower C/N ratio have been described (Haneklaus and Schung, 2004) as having rapid growth and more plasticity in allocation, and this corresponds in our studies with thin roots, suberized roots, leaves of bent and flowering stem and flower bud, which are compartments with high growth potential. A higher C/N value may reflect a more active carbon import by the structure, stem of bent shoot, flowering stem and peduncle. C/N ratio of the different compartments, showed the same shape in 1st and 2nd cycle. Between treatments, there were more significant differences during the 1st cycle, in association with a higher NO_3^- uptake and higher biomass in the aerial part of plants at cold solution.

At the end of the 2nd flower cycle that took place at the end of winter and beginning of spring, the situation observed in the plant was quite different since the differences between temperature treatments diminished. For example, NO_3^- uptake, which increased with respect to the 1st cycle in both treatments, was similar in plants at 10 °C and 22 °C (Chapter 4.1, this thesis). The concentration of soluble sugars and starch were also similar between treatments, and the only significant difference was found in the concentration of starch of suberized roots and structure. There was no priority in the destiny of carbohydrates towards the thin roots and the partitioning percentages were similar in plants at cold and warm solution. Aerial and root biomass were also similar between treatments. Moreover, the photochemical activity became similar between treatments (Chapter 4.1, this thesis). However, some differences were observed between treatments when comparing the 1st and 2nd cycle. On the one hand, in the cold treatment, a significant decrease of soluble sugars and starch between the

1st and 2nd cycle took place in many plant compartments, while in plants at warm solution there was some decrease but not so strong (Fig. 4). On the other hand, between the 1st and 2nd cycle, there was an increase in the production of aerial biomass in both treatments, but it was much higher in the warm treatment. The assimilates production is generally higher in spring than in winter due to higher light and air temperature, and a use of reserves is normal in woody plants in this period. An increase in the content of carbohydrates has been described in many species under winter conditions: maize and wheat (Engels, 1994; Guedira and Paulsen, 2002), birch (Solfjeld and Johnsen, 2006) and roses (Schrock and Hanan, 1981; Kool et al., 1997). Storage and mobilization of carbohydrates reserves are essential for winter survival and regrowth of perennial plants in spring (Loescher et al., 1990). Therefore, it could be suggested that the decrease in the levels of carbohydrates in spring could be related to the use of reserves, being this higher for plants at cold solution. Plants of this treatment decreased their carbohydrate levels much more than plants at warm solution in order to obtain the same yield at the end of the cycle. Besides, even though the decrease in carbohydrates was higher at cold solution, the increase in aerial biomass during the 2nd cycle was much lower in this treatment.

The differences in the effect of nutrient solution temperature depending on the flower cycle may be due to a) the result of the different current environmental conditions (e.g. light, air temperature), which entails different growth rate and assimilate production by the plant and b) the result of the pre-conditioning to the different temperatures, which already occurred in cycle 1.

In general terms, nutrient solution temperature influences the mechanisms controlling plant development and distribution of carbon and nitrogen into the different plant compartments. Plants grown at cold solution in winter increased the level of nitrogen derived from the fertiliser in every compartment, the carbohydrate concentration in some compartments and also increased their aerial biomass, respect to rose plants grown at warm solution. Therefore, rose plants showed plasticity and could adapt to grow without root heating in winter up to 10 °C under Mediterranean conditions. On the other hand, the change in the response of plants to nutrient solution temperature in the 2nd flower cycle might depend on other parameters such as air climatic variables and internal plant factors.

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5. EFFECTS OF PREHARVEST NUTRITION ON VASE LIFE OF CUT ROSES AND ON POSTHARVEST CHANGES OF CHLOROPHYLL *a* FLUORESCENCE

EFFECTS OF PREHARVEST NUTRITION ON VASE LIFE OF CUT ROSES AND ON POSTHARVEST CHANGES OF CHLOROPHYLL *a* FLUORESCENCE

Abstract

Rose plants (cv. Grand Gala) were grown using a perlite based soilless culture system under Mediterranean greenhouse conditions over a period of one year, and two different nutrient solution concentrations were used (control treatment: solution commonly used by local growers; low concentration treatment: 40% dilution with respect to the control). Flower shoot production and days of flower shoot vase life were determined in both treatments throughout the four seasons. Water balance of the flower shoots, chlorophyll *a* fluorescence and relative water content of the fifth uppermost leaf were measured over an 11-day period following harvest in each season. Although no relevant differences in flower production were observed between concentration treatments, the vase life of flower shoots at the low concentration treatment was, on average, shorter by one day. This was due to an inadequate regulation of the flower shoot water balance, resulting in an earlier loss of turgor that led to bent neck and petal wilting. Water balance during the first day after harvest was decisive for the subsequent duration of vase life. Vase life was found to be shorter in winter and in summer, in connection with a lower incoming radiation and higher daily minimum relative humidity. This work is pioneer in the in-depth analysis of the photochemical and non-photochemical processes occurring during vase life. Both treatments and all seasons showed that the stress caused by the excision activated a response of the photoprotective mechanisms in the leaves as soon as one day after harvest. However, when flower shoots began to wilt because of the progression of water loss, photoprotection mechanisms could not function properly, entailing a loss of regulation in the energy absorbed. After the end of vase life, there was an even higher loss of regulation that led to a decrease in the fraction of operational centres of photosystem II and ended up with the death of the flower shoot. In conclusion, the better the regulation of water balance and the longer the functioning of photoprotective mechanisms, the higher the duration of rose vase life.

Introduction

Roses (*Rosa hybrida* L.) make up about 33% of the total cut-flower production in the world (Kras, 1999). Several postharvest quality parameters -such as flower vase life duration- are very important for its commercial value. Therefore, an optimum management of the factors affecting this parameter is required in order to extend rose vase life. In particular, preharvest factors such as seasonal climatic conditions (light, relative humidity and temperature), as well as mineral nutrition, may be studied for this purpose.

In general, with respect to the influence of seasonal climatic changes on vase life, increasing photosynthetic photon flux during rose cultivation may reduce cut rose vase life due to the increased transpiration rate caused by incomplete stomatal closing at night (Slootweg and Van Meeteren, 1991), or it may improve vase life by increasing the level of accumulated carbohydrates (Fjeld et al., 1994). An optimal management of relative humidity (RH) is critical for the subsequent vase life of flower shoots. In this

respect, a rise in the air RH from 75% to 91% reduced the vase life of 14 rose cultivars (Mortesen and Gilslerod, 1999). According to Torre and Fjeld (2001), plants grown under high air humidity experience no water stress and may lose the function of stomatal closure in postharvest conditions when the RH is lower. On the other hand, although preharvest temperature per se may not have an effect on vase life, the combination of effects of high temperature, high irradiance, low RH and high vapour pressure deficit (VPD) (i.e., dry conditions) leads to the production of cut flowers with a long vase life (In et al., 2007).

Regarding the effect of preharvest nutrition on vase life, nutrition may affect osmoregulation, water content in plants, leaf water potential, chlorophyll content and hormone levels; it may also lead to sugar and organic acid accumulation, while excessive fertilization may cause secondary salt stress in plants, thus affecting postharvest life (Drüge, 2000; Bernstein et al., 2005). In particular, the effect of preharvest nutrition on abscisic acid (ABA) levels at harvest has been studied, yet the findings pertaining to the effect on vase life vary. High ABA levels at harvest have been related to a short vase life (Menard et al., 1995), although they have also been considered to possibly favour postharvest behaviour through a reduction in water loss and increased stress tolerance (Drüge, 2000). Concerning the effect of preharvest nutrition on osmoregulation, high mineral concentrations in the leaf seem to better maintain leaf water status due to the osmotic adjustment mechanism (Mahouachi, 2009). Nitrogen is an important element for plant nutrition but it is not the only factor capable of affecting rose flower vase life. For instance, an optimum addition of micronutrients has been observed to have a positive effect on vase life (Khoshgoftarmanesh et al., 2008). An optimum management of the nutritional status of plants may be a means capable of delaying turgor loss of cut flowers, while prolonging their vase life.

The effect of preharvest fertilization on flower vase life has been studied in several types of cut flowers. Bernstein et al. (2005) investigated the effect of nitrogen on growth, flower production and flower quality of *Ranunculus asiaticus* L. The longest vase life was obtained with the lowest N concentration (50 ppm vs. 100 ppm N), regardless of the level of NH_4^+ applied. In contrast, the longevity of cut *Pteris* leaf was increased when increasing the nitrogen supply (Drüge, 2000), while contradictory results were obtained for *Dendranthema grandiflorum* in relation to nitrogen supply and vase life (Röber and Reuther, 1982; Drüge et al., 1998). In connection with cut roses, as far as we know, there is only one study about the effect of nutrient concentration on their vase life (Menard et al., 1995). This work showed that a preharvest nitrogen concentration of 21.4 mmol L^{-1} decreased the subsequent vase life in “Royalty” roses as compared to using 10.7 mmol L^{-1}. However, as the concentration used in this study was very high (21.4 mmol L^{-1}), the differences between treatments

were attributed to salinity rather than to a specific nitrogen effect. Moreover, the effect of nutrients other than nitrogen was not studied. To the best of our knowledge, no studies have ever approached the issue of how certain nutrient concentrations contained in the solution may affect the subsequent vase life of roses, under conditions in which the possible influences cannot be confused with salinity-induced effects and within the range of commercial concentrations under Mediterranean conditions. Accordingly, this became the objective of our work.

Efforts attempting to determine the optimum concentration of nutrient solution are essential in order to improve the yield and quality of flower shoots, but are also necessary from an environmental standpoint. The nutrient solution is generally supplied in an amount exceeding the estimated needs of the crop in 20-30% (Cid et al., 2001). Drainage containing high amounts of not absorbed fertilizers is then discarded, this resulting in the pollution of superficial water and groundwater. Hence, rational environment-friendly guidelines must be established in connection with the concentration of nutrient solution. Accordingly, based on the usual levels of nutrient solution concentration used by local growers in Mediterranean conditions, the present study considers whether the environment-friendly approach of diluting the solution affects yield and subsequent vase life of roses.

On the other hand, the harvest practice leads to progressive stress throughout the vase life of harvested flower shoots due to both the injury caused by the excision and the decreased water content of the tissues, which may affect leaf photosynthesis. However, as far as we know, currently there are no studies on the development of photosynthesis and chlorophyll *a* fluorescence during the vase life of cut roses. Understanding photochemical and non-photochemical processes taking place after harvest may be useful to develop future strategies to prolong rose vase life. In this respect, CF imaging is a sensitive and non-destructive technique that allows a rapid quantification of any alteration in the light photosynthetic reaction. It permits the visualization of the spatial and temporal changes in photochemistry and non-photochemistry on leaves under different types of stress (Calatayud et al., 2006, 2007). Hence, another objective of this work was to apply CF imaging to analyze how the photosynthetic apparatus functions throughout vase life as a basis to better understand the response mechanisms of the flower shoot to the stress caused by harvest.

Materials and methods

Plant growing and harvest conditions

A rose crop (*Rosa hybrida* var. Grand Gala) in its second year after planting was grown in a polycarbonate greenhouse, equipped with convective heating (minimum 16°C), high pressure fogging and roof ventilation. Plants were grown following the bending technique commonly used by local growers (Calatayud et al., 2007). The experiment lasted one year and was divided in 4 seasons; in each one the same measurements were carried out. Plants produced flower shoots all year-round. Throughout the experiment, the number and fresh weight of all harvested flower shoots as well as the percentage of those that suffered from crooked neck were quantified. Crooked neck is a quality defect whereby the peduncle of the flower shoots grows in the plant with a downward bend.

Two sets of 150 plants were grown in a closed perlite system, each one having a different nutrient solution tank. Therefore, two treatments were differentiated according to the concentration of the nutrient solution: the control treatment, where the nutrient solution concentration was that generally used by local growers, and the low concentration treatment, which represented a 40% dilution with respect to the control. Following common practice, a different composition was used for every season, but the differences of concentration between the control and low concentration treatment were kept. The water for the nutrient solution was previously treated with reverse osmosis and ion columns in order to avoid variation of nutrient concentration in the solution. The nutrient solution was recycled but renewed once a week to ensure an optimum nutrient balance. The composition of the nutrient solution in each treatment during the experiment is shown in Table 1.

Table 1. Nutrient solution concentration (mmol L^{-1}) in every season for the two treatments (C: control treatment; L: low concentration treatment (dilution of 40% with respect to the control)) and composition of the tap water (mmol L^{-1}) used in vase life measurements. The water for the nutrient solution was previously treated with reverse osmosis and ion columns.

	Winter		Spring		Summer		Autumn		Tap
	C	L	C	L	C	L	C	L	Water
NO_3^-	12	7.2	10	6	8.6	5.16	10	6	0.12
$H_2PO_4^-$	1	0.6	1.3	0.78	1.3	0.78	1.3	0.78	1.00
SO_4^{2-}	0.5	0.3	1.2	0.72	0.5	0.3	0.6	0.36	2.18
NH_4^+	0.7	0.42	0.6	0.36	0.6	0.36	0.6	0.36	-
K^+	5	3	4.8	2.88	3.7	2.22	4.7	2.82	0.09
Ca^{2+}	3.5	2.1	6	3.6	3	1.8	3	1.8	3.69
Mg^{2+}	1.1	0.66	1.6	0.96	0.5	0.3	0.7	0.42	2.48

Solar radiation, air temperature, and relative humidity of air inside the greenhouse were recorded every 15 s by means of electronic sensors placed over the canopy and connected to a data acquisition system. From the 26th of June until the 1st of October, coinciding with the period of highest incoming solar radiation, an external aluminized screen (40% shading factor) was placed over the greenhouse to avoid stress due to high radiation and inside temperature.

Once each season (3rd of March, 26th of May, 28th of July and 17th of November), 38 flower shoots from each treatment were harvested for vase life assessment. Shoots were immediately placed in tap water after harvest and taken to the laboratory within 15 minutes. A summary of the climatic conditions taking place during the 15-day period prior to harvest in each season is shown in Table 2. The aforementioned timeframe was chosen as it has been considered to have an important influence on vase life (In et al., 2007).

Table 2. Summary of the climatic conditions inside the greenhouse taking place 15 days before flower shoot harvest in every season: 15-day mean of the maximal daily solar radiation (Max. Rad.), 15-day mean of the solar radiation during the daytime (Mean Rad.), 15-day mean of the day and night temperature (Tª day and Tª night, respectively), 15-day mean of the minimal relative humidity (Min. RH), and 15-day mean of vapour pressure deficit, VPD (Mean VPD).

	Max. Rad. (W m^{-2})	Mean Rad. (W m^{-2})	Tª day (°C)	Tª night (°C)	Min. RH (%)	Mean VPD (KPa)
Winter	463.0	216.2	24.39	15.69	50.77	0.518
Spring	729.5	264.7	26.24	16.89	46.33	0.625
Summer	356.7	162.5	29.34	22.06	56.68	0.647
Autumn	425.0	243.3	26.66	15.74	35.41	0.776

Vase life assessment

Flower stem ends were re-cut to a length of 75 cm after harvest. Flower shoots of each treatment were distributed in 11 groups of 3 and one group of 5 shoots in every season. Each one of the 11 groups was placed in a plastic flask containing 1 L tap water (Table 1). Tap water was used instead of distilled water to simulate real postharvest conditions. In the group of 5 shoots, each shoot was weighed (balance of ±0.01 g resolution) and all 5 were placed together in a plastic flask containing 1 L tap water, which was weighed before placing the shoots. Also, two flasks with no shoots, filled with 1 L tap water, were weighed and placed next to the flasks with the flower shoots in order to estimate water loss through evaporation. In every season, all flasks were placed in a test-room at 23 °C, 50% RH and a 12-h photoperiod with 25 µmol m^{-2} s^{-1} irradiance from cool-white fluorescescent lamps.

From harvest (day 0) and over 11 days (day 1-11), vase life of the group of 5 flower shoots from each treatment and season was assessed visually according to the association of Dutch flower auctions and research station for floriculture and glasshouse vegetables (VBN, 2005). Our work showed that the first symptoms that determined the end of vase life appeared in the flower bud, these being bent neck and visible petal wilting. The number of days until each rose flower shoot reached the end of its vase life was recorded. On day 11 and after all measurements had finished, the leaf area and stem diameter of the 5 flower shoots from each treatment and season were measured. A leaf area meter was used for leaf area measurements (model LI-COR 3100). The transpiration surface was calculated in cm^2 as the sum of leaf area and stem area, which was assumed to be cylindrical.

Every day, from day 0 until day 11, at 9:00 a.m., every flower shoot from both groups of 5 and their flasks were weighed. The control flasks containing no shoots were also weighed. Flower shoot fresh weight (FSW) was calculated in percentage values with respect to FSW at day 0 (FSW_{D0}) through the following equation,

$$Flower\ \ shoot\ \ fresh\ \ weight_{D_i}(\%) = \frac{FSW_{D_i} \cdot 100}{FSW_{D_0}} \qquad (1)$$

where D_i is a day of vase life from 0 to 11.

Daily water uptake per unit surface area of flower shoot (mL cm^{-2}) was calculated for each treatment as:

$$Daily\ \ water\ \ uptake = \frac{\left[\left(WF_{D_i} - WF_{D_{i+1}}\right) - \left(WC_{D_i} - WC_{D_{i+1}}\right)\right]}{Total\ \ transpiration\ \ surface} \qquad (2)$$

In this equation, D_{i+1} stands for the day after D_i; WF means weight of the flask containing flower shoots, which was weighed without them in order to calculate any variation of the water content in the flask; WC is the average of the weight of the 2 control flasks with no flowers. It was assumed that the density of water is 1 g/mL so, in the measurements, g is equivalent to mL. Total transpiration surface refers to the sum of the transpiration surfaces of the 5 flower shoots contained in the flask.

Daily water loss per unit surface area of flower shoot (mL cm^{-2}), which was assumed to be due to transpiration, was calculated for each treatment as follows:

$$Daily\ \ water\ \ loss = Daily\ \ water\ \ uptake + \\ + \frac{\left(Total\ \ FSW_{D_i} - Total\ \ FSW_{D_{i+1}} \right)}{Total\ \ transpiration\ \ surface} \qquad (3)$$

Total FSW refers to the sum of the fresh weights of the 5 flower shoots in the flask.

In the same 5 flower shoots per treatment and season, just after carrying out the daily measurements of water balance, CF was measured in the lamina of the external leaflet of the 5th uppermost leaf using an imaging-PAM fluorometer (Walz, Effeltrich, Germany). The method to measure CF is described in Chapter 4.1 (this thesis). The following parameters were determined: F_v/F_m, ϕPSII, q_L, ϕNPQ and ϕNO.

On the other hand, at 9:00 a.m. each day, from day 0 until day 11, the relative water content (RWC) of the external leaflet of the 5th uppermost leaf was calculated through the following equation:

$$RWC = \frac{FW - DW}{TW - DW} \qquad (4)$$

In this equation FW, DW and TW are fresh, dry and turgid weight (g) respectively. Turgid weight was obtained after 4 h floating in distilled water (Smart and Bingham, 1974). After that, the leaflet was oven-dried at 75°C for 24 hours to obtain its dry weight. For RWC measurements, each day a different flask of the 11 groups of 3 flower shoots of each treatment was used. Thus, from day 0 to day 10, RWC measurements had 3 repetitions. On day 11, however, RWC was measured in the same 5 leaflets per treatment where CF had been measured during 12 consecutive days (days 0-11).

Statistical analysis

In order to compare nutrient concentration treatments and seasons, 2-way ANOVA, paired-sample comparison or multiple regression were carried out depending on the variable.

A paired-sample comparison was performed on those variables related to flower production (weekly number of flower shoots harvested per plant, weekly fresh weight of harvested flower shoots per plant and weekly percentage of non-commercial flower shoot due to crooked neck over the total weekly amount of harvested flower shoots) due to the strong influence of the time factor on the evolution of these variables (Fig. 1). Thus, this analysis was carried out for each variable and for every season with *n*=13.

Two-way ANOVA (*n*=5) was performed on variables such as days of flower shoot vase life and percentage of fresh weight of flower shoots, using the nutrient solution concentration (control and low) and the season (winter, spring, summer and autumn) as factors. In actual fact, two separate analyses were required to assess the evolution of the second variable: one referred to the percentage of fresh weight of flower shoots on the first day after harvest, and another regarding the falling rate in the percentage of fresh weight of flower shoots from day 2 until day 11 after harvest. In addition to that, simple linear regression (*n*=8) was performed on vase life (Table 3) and climate parameters shown in Table 2.

In relation to other variables such as water uptake, water loss and the ratio between them, a multiple regression (*n*=88) was performed with the following independent variables: days of postharvest life (linear and quadratic component), the concentration of nutrient solution and the season. An indicator variable was created for the solution concentration, having the values 1 for the control treatment and 0 for the low concentration treatment. The seasons were also represented through three indicator variables (winter, spring and summer).

Moreover, a multiple regression (*n*=42) was also performed between RWC of the leaf and the steady state values of several CF parameters (ϕPSII, ϕNPQ, ϕNO, q_L and F_v/F_m) for each nutrient concentration treatment. Also, an indicator variable having the values 1 for the control treatment and 0 for the low concentration treatment was created and included in the regression analysis. The mean value of RWC at the end of vase life, averaged for all seasons and both concentration treatments, was 89.6 (with a standard deviation of 2.7), whereby only data of RWC>89.6 was used for the analysis to focus exclusively on the days of vase life.

Finally, a multiple regression (*n*=8) was performed to determine the most important factors affecting vase life. The independent variables were the water loss to water uptake ratio, the concentration of nutrient solution and the season. As explained above, to include the effect of the last two variables, four indicator variables were used.

Results

Production of flower shoots

According to our data, the concentration of the nutrient solution significantly affected the number and fresh weight of harvested flower shoots per plant and week in some seasons (Fig. 1A, B). However, no significant differences, at α=0.05, were observed

between concentration treatments regarding non-commercial production of flower shoots due to crooked neck in any season (Fig. 1C).

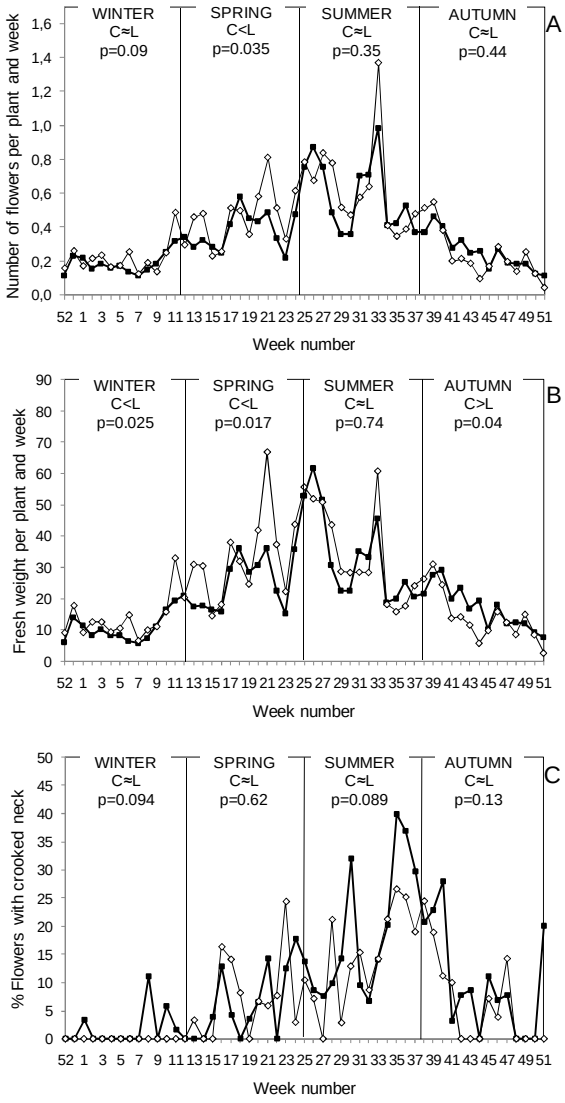

Fig. 1. Annual variation in the weekly number of flower shoots harvested per plant (A), in the weekly fresh weight (g) of harvested flower shoots per plant (B), and in the weekly percentage of non-commercial flower shoot due to crooked neck over the total weekly amount of harvested flower shoots (C). Different points refer to each concentration treatment (control (C, ■); low concentration (L, ◇)). For each variable, a paired-sample comparison between concentration treatments was performed for each season (n=13), and the results are shown in each graph (α=0.05).

In particular, both the weekly number (Fig. 1A) and fresh weight (Fig. 1B) of harvested flower shoots per plant were significantly higher in the low concentration treatment during spring. This was also observed for the fresh weight of harvested flower shoots in winter, but the opposite was observed in autumn. It is important to note that, although the overall data for the whole season showed significant differences, these resulted from specific periods of time in which differences were more remarkable (Fig. 1). In fact, when considering the overall data for the whole year, no significant differences were observed between treatments regarding any variable.

Table 3. Effect of the nutrient solution concentration and the season on the days of rose vase life, on the percentage of fresh weight of flower shoots in the first day after harvest, with respect to fresh weight at harvest (FSW_{D1}), and on the slope of the decrease of the percentage of fresh weight of flower shoots from day 2 till day 11 after harvest (means±sd, *n*=5). For each variable, 2-way ANOVA (α=0.05) was performed and the result is shown. Values followed by a different letter in the same column indicate statistically significant differences among seasons. Means were compared by the Tukey-Kramer's multiple range test.

		Vase life (days)		FSW_{D1} (%)		Slope (% day^{-1})	
Winter	C	4.8±0.4	b	109.0±2.5	ab	-3.60±0.87	b
	L	3.6±0.5		106.7±0.6		-4.43±0.69	
Spring	C	7.2±0.6	a	107.7±1.9	bc	-3.91±0.48	ab
	L	6.4±0.5		105.5±1.5		-4.36±0.44	
Summer	C	5.3±1.0	b	105.6±1.1	c	-4.68±0.66	a
	L	3.9±0.4		104.3±0.6		-5.03±0.37	
Autumn	C	6.4±2.0	a	109.6±1.2	a	-3.69±0.60	b
	L	5.8±1.8		108.4±2.5		-4.14±0.54	
P-value	Concentration	0.0067		0.0019		0.0106	
	Season	<0.0001		<0.0001		0.0059	
	Concentration x Season	0.839		0.814		0.821	

Vase life of flower shoots

The duration of vase life was significantly lower (approximately 1 day shorter) in the low concentration treatment (Table 3). On the other hand, the factor season affected vase life significantly, which was longer in spring and autumn than in summer and winter (Table 3). Additionally, vase life was positively correlated with the 15-day mean of solar radiation during the daytime (p=0.047; r=0.71).

Water balance during vase life

Water balance of flower shoots during vase life changed according to the season and the concentration treatment (Fig. 2).

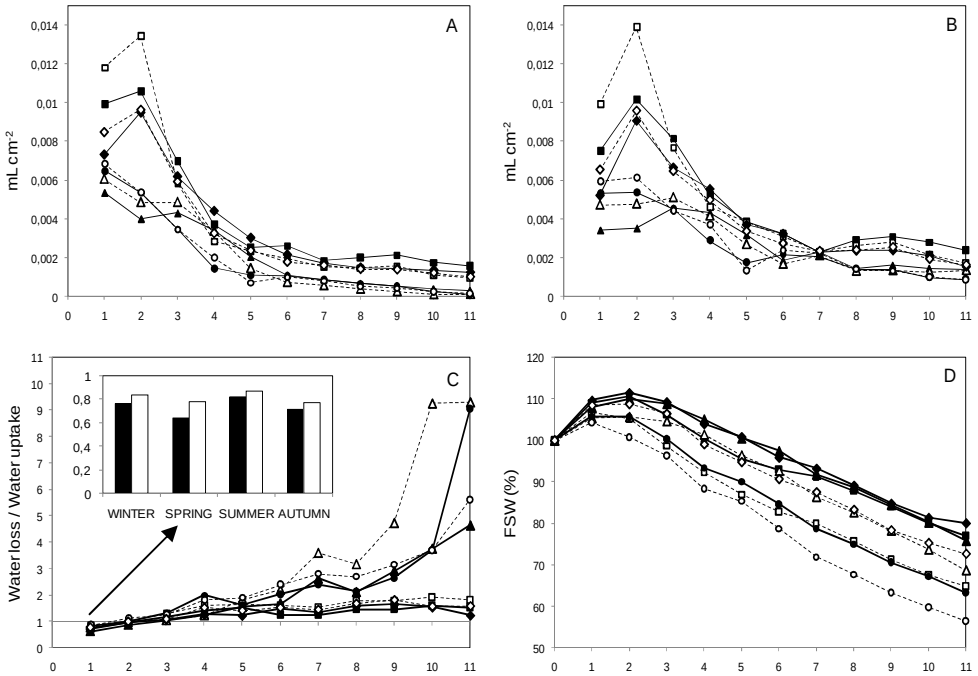

Fig. 2. Water balance of the flower shoots during 11 days after harvest according to the different seasons (winter (□); spring (Δ); summer (O); autumn (◇)) and nutrient solution concentration treatments (control: filled points, solid lines; low concentration: white points, dashed lines). (A) Daily water uptake and (B) daily water loss in mL cm^{-2} (n=1); (C) water loss to water uptake ratio (n=1) (a separate bar graph is shown for the first day after harvest; control: filled bars, low concentration: white bars); (D) fresh weight of flower shoots (FSW) (n=5), in percentage values with respect to FSW at harvest day (day 0, FSW=100%).

In general, the first days after harvest, water uptake (Fig. 2A) and water loss (Fig. 2B) were high but began to decrease around day 2. From approximately day 5 after harvest, water uptake and loss became very low and stable (Fig. 2A, B). Although a similar pattern of water balance was observed in all seasons, the multiple regression performed revealed that water uptake and loss were, on average, significantly different in every season (p<0.0001 in all three indicator variables associated with the season factor, for both water uptake and loss) and decreased in the following order: winter, autumn, summer and spring. In addition, in the low concentration treatment, water uptake and loss were, on average, significantly higher than in the control treatment (p=0.0075 for water uptake; p=0.0048 for water loss) (Fig. 2A, B). The ratio between water loss and uptake was <1 on the first day after harvest but, after the 3rd day it was >1 in all seasons and concentration treatments (Fig. 2C). It increased considerably in spring and summer during the days following the end of vase life. According to the multiple regression performed with this variable, no differences were observed between concentration treatments. However, when considering days 1 or 2

only, this ratio was significantly higher for the low concentration treatment (p=0.0337 for day 1 and p=0.0287 for day 2).

This pattern of water balance resulted in a different evolution of the percentage of FSW over 11 days following harvest depending on the season and the concentration treatment (Fig. 2D). In general, as a result of the relationship between water uptake and water loss, the percentage of FSW of a certain day with respect to FSW at harvest (FSW=100%), increased during the first days after harvest but decreased, on average, after day 2. Both the increase of fresh weight one day after harvest (FSW_{D1}) and the slope of subsequent decrease were significantly affected by the concentration treatment (Table 3). Flower shoots from the control treatment gained more weight one day after harvest and the subsequent loss of weight was slower. The largest difference in FSW (%) between concentration treatments was observed in winter, when on day 11 after harvest the difference was of 12% as compared to the 7% difference recorded for the other seasons (Fig. 2D). Besides, both the increase of fresh weight one day after harvest and the following decrease rate varied significantly from one season to another. In particular, the highest FSW (%) on the first day after harvest was observed in autumn and the lowest, in summer; moreover, summer was the season with the heaviest decrease rate of FSW (%) observed during postharvest life (Table 3).

According to the multiple regression performed to determine the most important factors affecting flower shoot vase life, the ratio between water loss and water uptake in the 1st day after harvest (Fig. 2C) was critical for the subsequent vase life of the flower shoot. The best model (R^2= 0.91) was obtained with the quadratic component of the ratio and the indicator variable linked to wintertime (W):

$$Vase\ \ life = 11.035 - 1.191 \cdot W - 8.808 \cdot \left(\frac{Daily\ \ water\ \ loss}{Daily\ \ water\ \ uptake} \right)_{D1}^{2} \qquad (5)$$

Measurements of chlorophyll a fluorescence during postharvest life

In general, the evolution of the mean steady state values (n=5) of several CF parameters throughout postharvest life followed relatively similar patterns in every season (Fig. 3). ϕPSII and q_L (Fig. 3A) decreased progressively in the days following harvest. The pattern was usually steeper after the end of vase life, particularly in summer (both treatments), autumn and winter (low concentration treatment) (Table 3). ϕNPQ and ϕNO (Fig. 3B) showed opposite patterns one from the other. During the first days following harvest, ϕNPQ increased while ϕNO decreased until they respectively reached a maximum and minimum point after which they then evolved

oppositely. The gradient after the end of vase life was steeper. On the other hand, the evolution of the values of F_v/F_m (Fig. 3A) throughout the 11 days of the experiment was quite different when compared to the aforementioned parameters. F_v/F_m remained around 0.8 during vase life and, in some cases, during the 11 days of the experiment. However, in summer (both treatments), autumn and winter (low concentration treatment), values suddenly dropped to around 0.4-0.5 some days after the end of vase life.

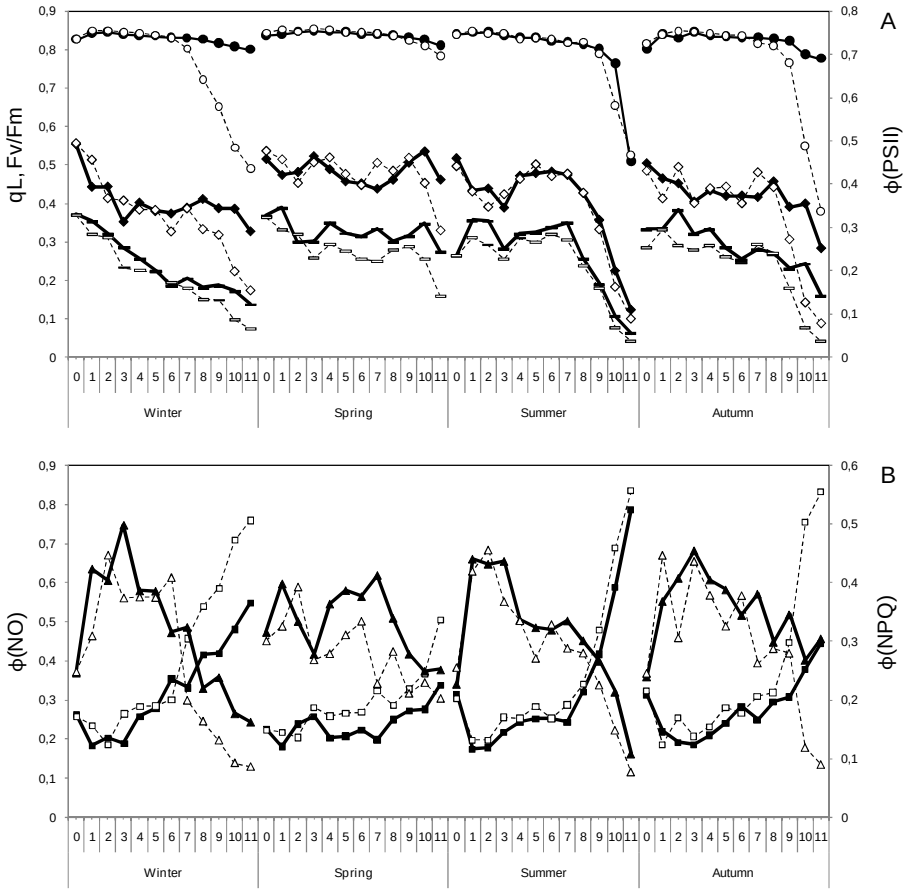

Fig. 3. Variation in the mean steady state values (*n*=5) of several chlorophyll fluorescence parameters (A: q_L (▭),F_v/F_m (○), ϕPSII (◇); B: ϕNO (□), ϕNPQ (Δ)) over a period of 11 days after harvest in every season and at 2 nutrient solution concentration treatments (control: solid lines, filled points; low concentration: dashed lines, white points). Harvest corresponds to day 0.

The same values of the parameters shown in Fig. 3 were plotted against leaf RWC, and a regression was established between RWC and each CF parameter (Fig. 4). The type of regression was polynomial ($y=a+bx+cx^2$). Fig. 4 represents the regressions based on data from all the days of the experiment in order to graphically assess the evolution during and after vase life; however, only data of RWC>89.6 was used for another analysis to determine if the concentration treatment had affected the relationship between RWC and CF parameters during vase life only. For each of the CF parameters, a multiple linear regression analysis was performed with RWC and the indicator variable linked to the concentration treatment as independent variables. The indicator variable had a statistically significant effect on ϕNPQ (p=0.025), ϕNO (p=0.009) and q_L (p=0.024). On average, for the same level of RWC and during vase life, ϕNPQ was 0.04 units higher, ϕNO was 0.029 units lower and q_L was 0.024 units higher in the control treatment as compared to the low concentration treatment.

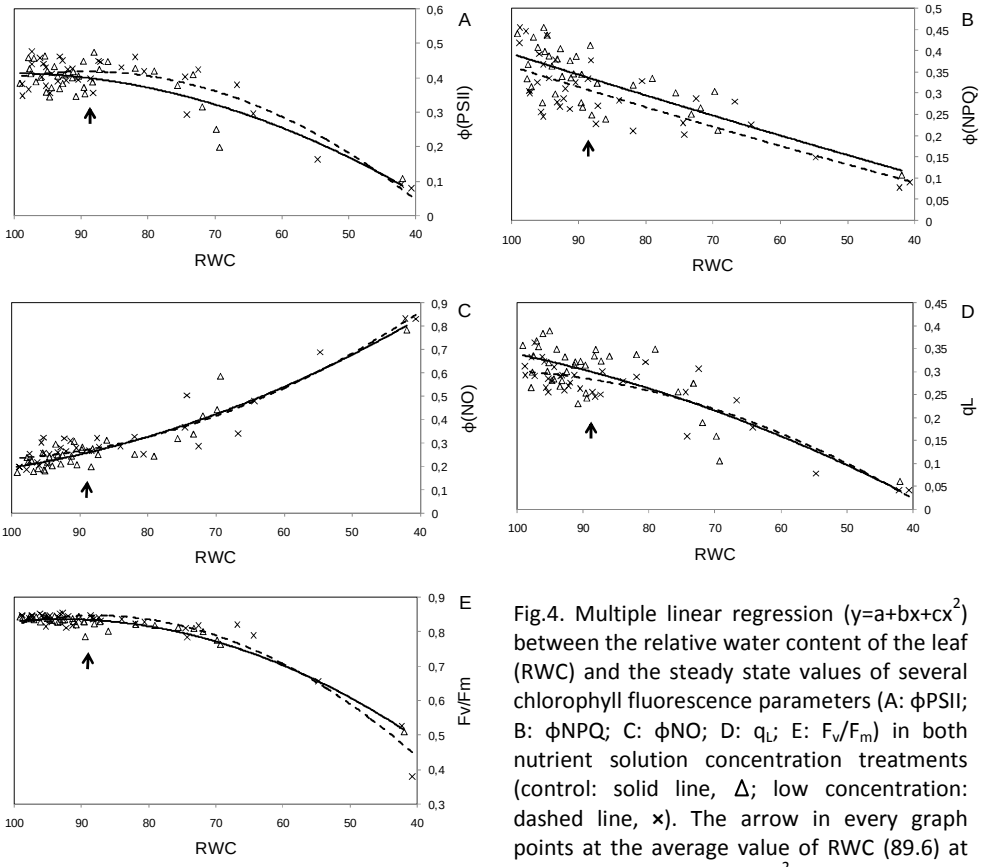

Fig.4. Multiple linear regression ($y=a+bx+cx^2$) between the relative water content of the leaf (RWC) and the steady state values of several chlorophyll fluorescence parameters (A: ϕPSII; B: ϕNPQ; C: ϕNO; D: q_L; E: F_v/F_m) in both nutrient solution concentration treatments (control: solid line, Δ; low concentration: dashed line, ×). The arrow in every graph points at the average value of RWC (89.6) at the end of vase life. The R^2 of the regression models were 0.629 (ϕPSII), 0.559 (ϕNPQ), 0.861 (ϕNO), 0.594 (q_L) and 0.947 (F_v/F_m) for the control treatment and 0.828 (ϕPSII), 0.619 (ϕNPQ), 0.904 (ϕNO), 0.804 (qL) and 0.94 (Fv/Fm) for the low concentration treatment.

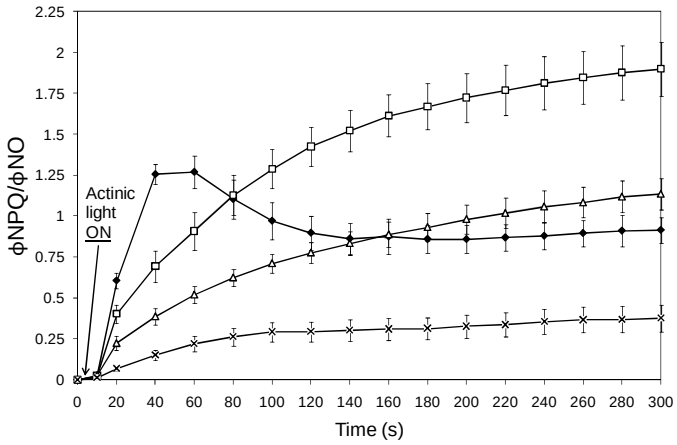

Fig. 5. General pattern of the dark-light induction curve for the parameter ϕNPQ/ϕNO. Four curves are distinguished corresponding to different phases of vase life. Phase 1 (◆) corresponds to the day of harvest (day 0). The dark-light induction curves corresponding to the remaining days from 1 to 11, were classified as phase 2 (□), phase 3 (△) and phase 4 (×) as indicated in Table 4. Phases were characterized according to the mean values of ϕNPQ/ϕNO at time=60 s and at time=300 s of the induction curve. In all cases, the end of vase life takes place in phase 3. Values are means±SE.

Besides analyzing the steady state conditions, it is interesting to verify how these conditions were achieved. Actually, the dark-light induction kinetics curve changed substantially throughout the days after harvest. The parameter ϕNPQ/ϕNO was chosen to describe these changes during and after the end of vase life as it is an indicator of the photoprotection capacity of the photosystem (Klughammer and Schreiber, 2008). After a careful visual inspection of all 96 curves (i.e. 12 days x 4 seasons x 2 concentration treatments), they were classified in 4 groups, corresponding to 4 phases of postharvest life (Fig. 4). All the curves belonging to a particular phase had, statistically, the same value of ϕNPQ/ϕNO at t=60 s, i.e. no significant differences were shown by one-way ANOVA (α=0.05), and the same value of ϕNPQ/ϕNO at t=300 s (one-way ANOVA, α=0.05). However, in certain transitional cases a different result was obtained at t=60 and at t=300, and the conclusion was then visually assessed according to the pattern of the whole induction curve. Day of harvest or day 0 always corresponded to phase 1 and, in all cases, the end of vase life took place in phase 3.

With regard to the 1st phase, ϕNPQ/ϕNO increased far more during the first minute than in any of the other 11 days of the experiment; after reaching a maximum at t=60, ϕNPQ/ϕNO decreased asymptotically until a steady state value (Fig. 5). In phases 2, 3 and 4, no relative maximum was observed and ϕNPQ/ϕNO progressively reached lower values during the first minute of the induction kinetics curve. With regard to the steady state value of ϕNPQ/ϕNO, while in the 2nd phase, it was much

higher than for phase 1, during the 3rd phase it became similar. Finally ϕNPQ/ϕNO decreased to the lowest values in the last phase (Fig. 5).

Table 4. Distribution of days of vase life in the different seasons and nutrient solution concentration treatments (C: control; L: low concentration) among phases of vase life according to Fig. 5. Phase 1 stands for the day of harvest (day 0). The end of vase life takes place in phase 3 in all cases.

		Phase 2	Phase 3	Phase 4
Winter	C	1-3	4-7	8-11
	L	1-2	3-6	7-11
Spring	C	1-6	7-11	-
	L	1-4	5-10	11
Summer	C	1-3	4-7	8-11
	L	1-2	3-8	9-11
Autumn	C	1-5	6-9	10-11
	L	1-3	4-9	10-11

The evolution of F_v/F_m and the steady state values of ϕPSII, ϕNPQ, ϕNO and q_L throughout the 4 phases of postharvest life can be seen in Fig. 6. A leaf from the low concentration treatment analyzed in winter was randomly taken as an example. In the images, different colours code for different values of the CF parameters ranging from 0 (black) to 1 (pink), as indicates the code bar showed in the top of the figure. Based on these images, it is possible to study spatial differences of CF parameters within the leaf, which stand for a different photosynthetic performance across the leaf surface. In this figure, it is important to observe whether the values of a given parameter are homogenous or heterogeneous across the leaf and if these spatial differences change or not during postharvest life. It is also important to observe which areas develop more severe damage as flower shoots wilt and the temporal evolution of local wounds throughout postharvest life. The evolution of each parameter along a line perpendicular to the midrib is represented in graphs underneath each image.

Discussion

Vase life duration of cut flowers is an important quality parameter of great commercial value (In et al., 2007). It is important to increase flower shoot production but also to improve the quality of the product. However, a given preharvest factor may affect production and quality parameters differently. Our study showed that a dilution of the nutrient solution concentration in 40% with respect to that regularly used by local growers did not affect, over the whole year, commercial and non-commercial production of flower shoots, however it shortened vase life in 1 day. The physiological changes throughout postharvest life using two different nutrient solution concentrations are discussed below.

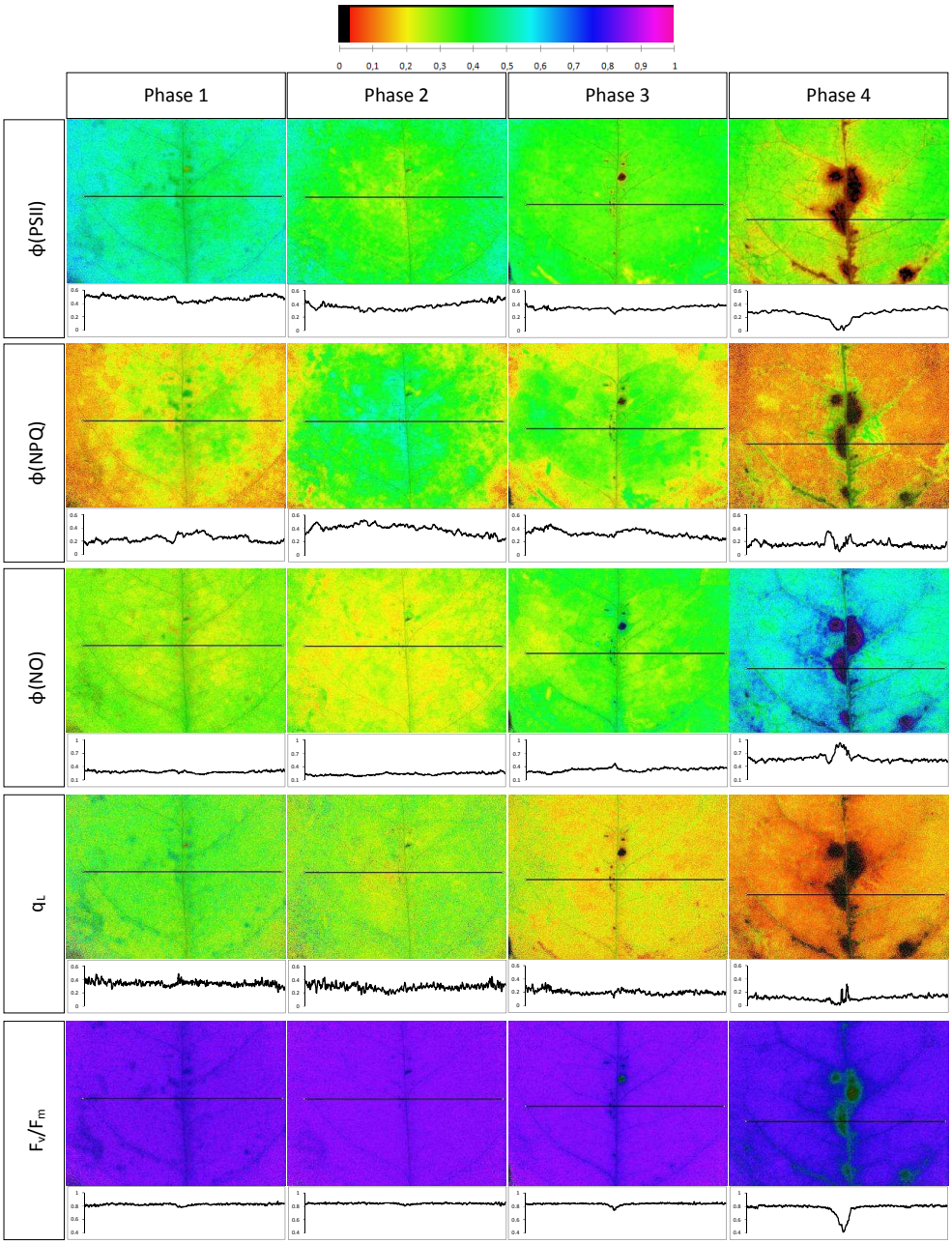

Fig. 6. Images of the same leaf throughout the 4 phases of vase life, which are described in Fig. 5, showing the values of the CF parameters ϕPSII, ϕNPQ, ϕNO and q_L at t=300 s of the induction curve, and F_v/F_m after dark adaptation. The images correspond to a leaf from the low concentration treatment analyzed in winter. The different colours stand for values from 0 (black) to 1 (pink) according to the code showed in the top of the figure. The evolution of each parameter along a line perpendicular to the midrib is represented in graphs underneath each image. This line corresponds to a length of 2 cm.

Water balance of flower shoots during postharvest life

Flower shoots from plants grown using a 40% dilution of nutrient solution, showed poorer water balance regulation compared to those from plants at control treatment. This resulted in an earlier loss of turgor that led to bent neck and petal wilting, characteristic symptoms of the end of cut rose vase life. Mortensen and Giserøld (1999) also associated a shorter vase life with excessive water loss from leaves at harvest in 14 cultivars of rose plants.

The ratio between water loss and water uptake during the first two days after harvest was higher in the low concentration treatment (Fig. 2). This means that a lower amount of the water absorbed during that period was retained in the tissues of shoots from this treatment. In flower shoots, water is lost mainly from the leaves (In et al., 2007). As any water stressed plant, flower shoots can prevent water loss through two mechanisms: stomatal closure to reduce transpiration, and osmotic adjustment to avoid water loss from cells and to maintain tissue turgor (McCree and Richardson, 1987). An inadequate water balance was noted in flower shoots from the low concentration treatment, possibly due to any of these mechanisms. Plant nutrition has been shown to play an important role in drought stress (Davis and Quick, 1998; Mahouachi, 2009) and, thus, in postharvest life of ornamentals (Drüge, 2000; Bernestein et al., 2005) through both mechanisms. Specifically, nutrient supply may indirectly affect stomata conductance via the increase or decrease of ABA or cytokinin levels in the leaves, or it may act directly through the K^+ balance in guard cells (Davis and Quick, 1998). In fact, potassium deficient plants have lower tolerance for water stress due to the role of K^+ in stomatal regulation (Davis and Quick, 1998), but also in osmotic adjustment (Mahouachi, 2009). Ca^{2+} (Chari et al., 1986) and NO_3^- (Kusaka et al., 2005) accumulation in leaf cells also seem to contribute to osmotic adjustment.

From approximately the 5^{th} until the 11^{th} day after harvest water uptake and water loss were strongly reduced (Fig. 2), probably due to the development of xylem blockage that hinders water uptake (Mayak et al., 1974; De Stitger, 1980). As the low concentration treatment was characterized by a heavier decrease of FSW during this period (Table 3), it is possible that shoots from this treatment might have suffered from xylem blockage more than shoots at control treatment. Torre and Fjeld (2001) observed that cut roses with low water loss were not so sensitive to vessel blockage as roses with high water loss. The water balance regulation in plants grown with a 40% dilution may be poorer than that of plants at control treatment due to a combination of a higher water loss to water uptake ratio during the first days after harvest and a faster decrease of FSW during postharvest life.

In addition to the concentration of nutrient solution, the season factor affected water balance and vase life significantly. This factor was linked to the change of climate conditions (Table 2). Slootweg et al. (2001) and Pompodakis et al. (2005) also found major differences between seasons, whereby the shortest vase life was recorded in winter. However, the longest was found in summer, which is apparently in disagreement with our results. This was due to the fact that those authors did not use a shade screen in summer, hence the incoming radiation reached maximum levels. In actual fact, our results offered a positive relationship between vase life and the 15-day mean of the solar radiation during the daytime. A similar relationship was also found by Pompodakis et al. (2005), who associated this result with higher carbohydrate levels as radiation increased. Such findings lead to a practical conclusion: although the shade screen used in our work during summertime was useful to reduce extreme conditions of temperature and radiation, its commercial shading factor (40%) may have been too high to produce flower shoots with a long vase life.

The mean of the minimum relative humidity during 15 days before harvest had a negative correlation (r=-0.58) with vase life, although it was not significant (p=0.133) probably due to the low number of observations (n=8). This preharvest climate parameter has been considered to have a high repercussion on subsequent vase life of flower shoots (Mortensen and Giserøld, 1999; Marissen and Benninga, 2001; In et al., 2007). For instance, the shorter vase life in winter has been put down to the high RH of this season (Slootweg et al., 2001). RH affects water loss of flower shoots through its effect on ABA levels, which influence stomatal anatomy and functionality (Franks and Farquhar, 2001; Torre and Fjeld, 2001; Torre et al., 2003; In et al., 2007). Rose plants grown under low RH conditions have smaller functional stomata and are able to regulate their water relations properly after harvest, resulting therefore in a longer vase life than rose plants grown under high RH conditions (Torre et al., 2003; In et al., 2007). Also, Torre and Fjeld (2001) observed that the osmotic concentration of roses grown at RH=90% was 10% lower than in roses grown at RH=70%, both in leaves and petals, suggesting a lower ability to prevent loss of turgor when water deficit develops in the tissue. However, the primary reason for the shorter vase life of flowers grown under high RH conditions was assigned to stomatal malfunctioning (Torre and Fjeld, 2001).

Although the season factor was associated with the change of climate conditions, it is important to note that, in this study, the seasonal influence may also include the change of nutrient solution concentration during the experiment (Table 1). In any case, although the climate influence may be confused with solution concentration up to a certain extent, the main effect is in fact climate as confirmed by previous literature in line with the above discussion.

Ultimately, even though plugging or cavitation of xylem vessels has been identified as the cause of the end of vase life in some varieties of cut roses (Mayak et al., 1974; De Stitger, 1980; Ichimura et al., 2002; Ichimura 2006), our study of Grand Gala showed that the end of vase life took place just before water uptake was seriously limited (Fig. 2). Hence, it was probably more influenced by factors taking place before xylem blockage was fully developed. This is in agreement with the findings of In et al. (2007). The water loss to water uptake ratio on the 1st day after harvest was negatively and highly correlated with rose commercial longevity (Eq. 5). This means that if flower shoot tissues were able to retain a high amount of the water absorbed during the first day after harvest, they would be able to prolong vase life. A similar conclusion was obtained by Torre and Fjeld (2001), who observed that the water loss during the first 30 minutes after subjecting the recently harvested flower shoot to the dry conditions of vase life were crucial in connection with subsequent neck bending, while the mechanical strength of the pedicel tissue was not a relevant factor.

Although the nutrient solution concentration and the season had a major influence on vase life (Table 3), they did not appear in the model that better predicts the duration of vase life (Eq. 5). This suggests that their effect was hidden by the effect of the water loss to water uptake ratio, which means that the differences between concentration treatments and seasons were due to variations in this ratio on the first day after harvest. In winter, besides the effect of the water loss to water uptake ratio, an additional factor contributed to reduce the length of rose vase life in 1.19 days: the lower radiation in winter and, as a consequence, lower levels of carbohydrate might explain this fact.

Variations of chlorophyll a fluorescence parameters throughout postharvest life

Both the water loss in the flower shoot and the wound caused by the excision, involve the generation of a progressive stress in the shoot that ends up with its death after several days. The better the shoot can deal with this stress, the longer its vase life. Therefore, understanding the processes that take place in the photosynthetic apparatus, which is very stress-sensitive, may be useful to understand how the flower shoot tries to overcome this critical situation. CF imaging is a technique that can give information about plant stress. This technique provides images of the leaf showing the distribution of its CF values. It is therefore possible to study spatial differences showing a different performance of the photosynthetic apparatus depending on the part of the leaf. This technique has been used to study the impact of numerous stress types such as cold (Savitch et al., 2001; Ehlert and Hincha, 2008), extreme brightness (Muller-Moule et al., 2004), wounding (Quilliam et al., 2006) and drought (Calatayud et al., 2006; Woo et al., 2008).

The CF parameter that was less affected by changes throughout vase life was F_v/F_m (Fig. 3). F_v/F_m remained close to 0.83 during vase life, the typical value for non-photoinhibited leaves in vascular plants (Björkman and Demmig, 1987). This means that the fraction of centres of the PSII that are capable of photochemistry was not affected by the progressive stress during vase life. Only after the end of vase life, in some seasons and when using certain concentration treatments, -when shoot water reserves declined to critical levels (RWC around 64%)-, did this parameter fall heavily, probably due to a decrease in the fraction of operational PSII centres caused by photodamage. The same pattern of F_v/F_m during increasing water deficit was also observed by Woo et al. (2008) in *Arabidopsis thaliana*, by Tezara et al. (1999) in sunflowers, by Giardi et al. (1996) in peas, and by Calatayud et al. (2006) in rose plants. This fact reveals that studies which only measure F_v/F_m as a stress indicator provide incomplete information that may lead to incorrect conclusions. Should we have measured this parameter only, we would have concluded that no stress was developed in PSII during vase life; yet, this is not true as explained hereunder.

Among several other CF parameters, ϕNPQ/ϕNO is an indicator of the photoprotection capacity of the photosystem. ϕNPQ accounts for the fraction of energy dissipated in the form of heat via the regulated photoprotective non-photochemical quenching mechanism, whereas ϕNO corresponds to the fraction of non-regulated energy that is passively dissipated in the form of heat and fluorescence, mainly due to closed PSII centres (Klughammer and Schreiber, 2008). Under a given set of environmental conditions, successful regulation is aimed at maximal values of ϕPSII and maximal ratio ϕNPQ/ϕNO (Klughammer and Schreiber, 2008). From the values of ϕNPQ/ϕNO at the first minute and at steady state of the dark-light induction kinetics, it was possible to assess the development of the photoprotection capacity of PSII throughout rose postharvest life, and four phases were accordingly suggested (Fig. 5). This pattern is valid for both concentration treatments and for all seasons. However, as the duration of vase life was different depending on the concentration treatment and the season, each phase covered different days according to every combination of factors (Table 4). The shorter the vase life, the faster the evolution through the different phases.

The first phase corresponded to harvest day, when no signs of stress were observed in the CF parameters. The light induction of ϕNPQ/ϕNO after dark adaptation was very fast and achieved very high values within the 1st minute, but decreased rapidly afterwards reaching medium values at steady state (Fig. 5). This kinetics suggests that the leaf was in very good conditions since the photoprotective mechanisms were operational within seconds avoiding any possible damage by incoming light. Besides, the fast quenching after the 1st minute was related to the activation of the Calvin cycle, which means that production of photoassimilates was

not affected. The fast activation of the Calvin cycle required to use the products of photochemistry (Calatayud et al., 2002). This agrees with the high steady state values of ϕPSII and q_L (Fig. 3 and Fig. 6), which means that most photons absorbed by PSII were used in photochemistry and that PSII centres were maintained in an oxidized state. With regard to the spatial distribution of the CF parameters (Fig. 6), steady state values of ϕPSII and ϕNO were lower in the area of the midrib, maybe motivated by an increase of photoprotective thermal energy dissipation as reflected by ϕNPQ. The reduction of ϕPSII was not due to a change in the reduction state of the first acceptor of PSII (quinone A) as reflected by a homogenous q_L across the leaf, so it may have been related to a diminished trapping efficiency of the photosystem (Lu and Zhang, 1999). Actually, the higher values of ϕNPQ confirm this idea. Similar results were shown by Calatayud et al. (2006) in rose plants subjected to water stress. Several authors (Bro et al., 1996; Meng et al., 2001) have observed a low ϕPSII along the main veins and neighbouring areas, which suggests that pigment composition and concentration, water potential and stomatal function differ in cells from different regions of the leaf, contributing to spatial differences in photochemical activity (Terashima, 1992; Chaerle et al., 2003; Rezaei Nejad et al., 2006).

The second phase comprised the first days after harvest (Table 4) and was characterized by the highest steady state values of ϕNPQ/ϕNO (Fig. 5). During this phase, flower shoots were turgid and in visible good conditions. The induction by light of ϕNPQ/ϕNO after dark adaptation was slower than in the previous phase. This result indicates that, within 1 day after harvest, the stress caused by this harvest had affected the speed of the photochemical reactions because photoprotective mechanisms needed more time to be operational. However, once activated, these mechanisms were strongly promoted, which proves the response of the flower shoot to stress conditions. It is important to highlight that this response could only be motivated by the stress caused by the excision because, in this phase, FSW was higher than that at harvest (Fig. 2), so the leaf could not be suffering from water deficit. Quilliam et al. (2006) reported similar results with leaves of *Arabidopsis thaliana* that had been wounded. Only 1 day after the wound, NPQ increased in the parts of the leaf furthest from the wound. The high steady state values of ϕNPQ/ϕNO in our results were achieved by an increase of ϕNPQ and a subsequent decrease of ϕNO (Fig. 3 and Fig. 6). As a consequence of the increase of ϕNPQ, the exciton trapping efficiency of PSII might have decreased (Lu and Zhang, 1999); this circumstance, together with the decrease in the fraction of open centres (q_L), resulted in a decrease of the quantum efficiency of PSII photochemistry ϕPSII (Fig. 3 and Fig. 6). Klughammer and Schreiber (2008) stated that a high ϕNPQ can compensate for the down-regulation of PSII and even cause a lowering of ϕNO. Antenna pigments may have turned from energy funnels into quenchers that dissipate the excitation energy as heat in order to protect

the PSII from stress (Horton et al., 1996). This does not mean that the Calvin cycle was inhibited, but it was considerably slowed down according to the lack of quenching in the values of ϕNPQ after the 1st minute of induction towards the steady state.

During the 3rd phase, flower shoots began to wilt and, soon after the beginning of this phase, vase life ended. FSW was lower than the levels recorded at harvest (Fig. 2) and water loss from shoot tissues began to cause stress. This phase was characterized by a slower induction by light after dark adaptation of ϕNPQ/ϕNO and a decrease of its steady state values as compared to the previous phase (Fig. 5), due to a decrease of ϕNPQ and an increase of ϕNO (Fig. 3 and Fig. 6). This fact, together with the reduction of ϕPSII (Fig. 3 and Fig. 6), suggests a reduction in the ability of the plant to protect itself against damage by stress (Klughammer and Schreiber, 2008). Woo et al. (2008) also observed that the rapid decline in CF parameters occurred concurrently with the appearance of physical symptoms of drought stress (e.g. loss of turgor). The fate of excitation energy through regulated pathways such as photochemistry or thermal energy dissipation was reduced, and consequently, plant's ability to cope with excess excitation energy diminished. This eventually results in photodamage of PSII reaction centres or associated chlorophylls due to the production of singlet $^1O_2$ (Öquist et al., 1992; Klughammer and Schreiber, 2008). However, this was not yet observed in this phase, as reflected by F_v/F_m (Fig. 3 and Fig. 6). Maybe the stress due to water loss up-regulated the activity of antioxidant systems such as ascorbate peroxidase (APX), in order to scavenge active oxygen species as observed by Jin et al. (2006) in the rose cultivar Samantha.

In the last phase, vase life was over but flower shoots were still alive, albeit considerably wilted and dry. This phase was characterized by a progression from the previous phase with respect to energy deregulation (Fig. 5). The decrease rate of ϕNPQ, ϕPSII and q_L, and the increase rate of ϕNO were steeper in this phase (Fig. 3). This led in summer (both treatments), winter and autumn (low concentration treatment) to photodamage of membranes that reduced the fraction of operational PSII centres, as reflected by F_v/F_m (Fig. 3). This could have been caused by the degradation of the PSII reaction centre protein D1 motivated by the decrease of photoprotection mechanisms (Pieters et al., 2003). During water deficit, severe reduction of cellular water content results in elevated levels of reactive oxygen intermediates and chlorophyll degradation (Flexas et al., 1998; Rivero et al., 2007). Spatial differences were evident in this phase (Fig. 6). Leaf damage was greater next to the midrib. Besides, there were also certain areas that at harvest presented local damage which was invisible to the naked eye; the evolution of this damage was slow during the first three phases, but very rapid in the last phase. A similar development pattern of CF parameters throughout water stress was observed by Calatayud et al. (2006) in rose plants as well as by Lu and Zhang (1999) in wheat.

The variations of CF parameters after harvest were related to the variations of RWC in the leaves as shown by the polynomial correlations obtained when relating each CF parameter with RWC (Fig. 4). Every model decreased progressively as RWC became lower (i.e. as the leaf dried), with the exception of ϕNO which evolved oppositely. This means that RWC of the leaf, as an indicator of its water status and related to the water status of the whole shoot, had an important effect on the performance of PSII. However, it was not the only influencing factor since the correlation between some CF parameters (ϕNPQ, ϕNO and q_L) and RWC was slightly but significantly different according to the concentration treatment during vase life. For the same level of RWC, ϕNPQ and q_L were higher in the control treatment, and the opposite applied to ϕNO, which means that shoots from this treatment were able to deal with stress better than shoots from the low concentration treatment for the same leaf water status. Gupta and Berkowitz (1988) stated that although RWC declines during the first part of water stress, if chloroplast stromal volume was kept constant through osmotic adjustment, photosynthetic capacity could be maintained. It is likely that a higher accumulation of solutes in the chloroplasts of flower shoots from the control treatment could have led to a better capacity for photochemical reactions by keeping chloroplast stromal volume constant despite RWC decreased.

Conclusion

The use of a nutrient solution concentration with a 40% dilution with respect to that commonly used by local growers shortened vase life in about 1 day, which was derived from an inadequate regulation of water balance. Water balance during the first day after harvest was decisive for subsequent vase life, so the water loss to water uptake ratio might be used as an early predictor of rose vase life duration. With regard to the effect of climatic conditions, vase life was shorter with lower incoming radiation and higher daily minimum relative humidity. Only one day after harvest, the stress caused by the wound activated a response of the photoprotective mechanisms in the leaf. However, as water loss progressed in the flower shoots, they began to wilt and these mechanisms could not function correctly leading to a loss of regulation in the energy absorbed by pigments, which eventually led to a decrease in the fraction of operational PSII centres. In conclusion, the better the regulation of water balance and the longer the functioning of photoprotective mechanisms, the longer the vase life. The best CF parameter to describe the evolution of rose vase life was ϕNPQ/ϕNO, while F_v/F_m did not show any change during vase life. Finally, a study of the balance between the environmental benefits of diluting nutrient solution concentration and the economical impact of the reduction of rose vase life should be carried out to determine whether the dilution applied in this work could be recommended or not to local growers.

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6. CONCLUSIONS OF THE THESIS

CONCLUSIONS OF THE THESIS

The optimization of rose cultivation may be achieved by means of an accurate management of nutrition factors such as concentration and temperature of the nutrient solution, and through the formulation of optimum nutrient solutions. These are the conclusions of this thesis:

1) Nutrient solution composition may be optimized by matching supply and plant demand. In this thesis (Chapter 3) empirical models that predict plant demand for nutrient (nitrate, phosphate, potassium, calcium and magnesium) and water were developed. These models are interesting for their high applicability in real conditions because they integrate the effect of a high number of factors affecting nitrate and water uptake during one-year period of commercial cultivation. This makes them practical and suitable for being integrated in decision support systems for fertirrigation management. R^2 ranged between 0.336 and 0.785 in the nutrient uptake models, and equalled 0.902 in the water uptake model.

2) The factors affecting nutrients uptake by rose plants under commercial conditions are represented by those variables included in the models (Chapter 3). These variables are water absorption, nutrient solution concentration, some climatic parameters (vapour pressure deficit and radiation integral inside the greenhouse), flower shoot production, some common practices in rose cultivation (renewal of old bent shoots, the use of shade screen and the synchronization of flower shoot development for scheduling purposes) and unknown internal factors.

3) Factors affecting water uptake and, hence, included in the water uptake model were vapour pressure deficit, air temperature, nutrient solution temperature, radiation integral inside the greenhouse, as well as renewal of old bent shoots and flower shoot production (Chapter 3).

4) In order to know the optimum range of nutrient solution temperatures for rose plants, it is necessary to study the limits of this range. In this thesis (Chapter 4), rose plants showed tolerance to 10 °C of root temperature during winter. This tolerance was achieved by increasing the production of thin roots, nitrate uptake, nitrate reductase activity, photochemical activity and carbohydrates production, and by enhancing the partitioning of N and carbohydrates towards the roots. However, this response to low solution temperatures was reduced in the beginning of spring maybe as the result of improved air climatic conditions.

5) The optimization of nutrient solution concentration has two viewpoints: the plant and the environment. A 40% dilution of the nutrient solution is advisable for reducing groundwater pollution but, in this thesis (Chapter 5), it shortened vase life of rose

flowers by one day. This was due to an inadequate regulation of the flower shoot water balance caused by a combination of a higher water loss to water uptake ratio during the first day after harvest (key factor) and a faster decrease of flower shoot weight during postharvest life.

6) Flower harvest results in a progressive stress in shoot tissues during portharvest life, which can be described with accuracy by chlorophyll fluorescence. This thesis shows (Chapter 5) that the stress caused by the excision activated a response of the photoprotective mechanisms in the leaves one day after harvest. The progression of water loss led to wilting of flower shoots and photoprotection mechanisms became less operational, eventually leading to a decrease in the fraction of PSII centres that are capable of photochemistry. The best CF parameter to describe the evolution of rose vase life was $\phi NPQ/\phi NO$ and the less informative was F_v/F_m.

Future work may be directed at: 1) validating the nutrient and water uptake models at commercial conditions, 2) improving the models of phosphate and magnesium uptake by using a lower nutrient solution concentration, 3) verifying whether the nutrient solution composition derived from the models results in the highest yields and qualities of rose flower shoots, 4) identifying the temperature from which root heating should be used in rose plants in wintertime, 5) finding out the highest optimum nutrient solution temperature in summer conditions, and the level from which root cooling should be used to avoid stress, 6) Analyzing the causes underlying the higher water loss to water uptake ratio in flower shoots from plants grown at lower nutrient solution concentration, and 8) integrating the study of vase life with complementary research fields such as economics and environmental impact, in order to analyze the balance between the environmental benefits of diluting nutrient solution concentration and the economical impact of the reduction of rose vase life.