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**Modelling Mineral Nutrition in Greenhouse Plants Grown in
Soilless Culture under Saline Conditions**

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PhD thesis

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Preface

The work reported in this manuscript is the result of three years of experimentation that has been conducted in collaboration with the Department of Plant Science (University of California, Davis USA) and Dipartimento di Biologia delle Piante Agrarie. (University of Pisa, Italy). A large part of the data collected in both laboratories has been included in this thesis, which has been written with the idea to assemble independent experiments on specific issues, also because different crop species (*Solanum lycopersicum* and *Rosa hybrida*) were considered. However, all experiments refer to the nutrition of plants grown in closed-loop soilless culture under saline conditions.

This PhD thesis mainly focuses on plant nutrition modelling. A large number of models have been developed and their application has been statistically evaluated. Such models are able to predict, with satisfactory accuracy: i) the variation of the concentration of both nutrients and non-essential elements (such as Na^+ and Cl^-) in the recirculating water of closed growing systems; ii) the uptake of nutrient and non-essential elements depending on the external salt concentrations.

Although the structure of the thesis involves several concepts of mathematics, statistics and computer programming, nevertheless most of the work is based on physiological and biological assumptions derived from experimental observations.

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List of publications

- Massa, D., Incrocci, L., Carmassi, G., Maggini, R., Pardossi, A., (in litteris). Water and nutrient use efficiency of tomato plants grown in closed-loop substrate culture with saline water: an analysis by simulation and greenhouse experiments.
- Massa, D., Mattson, N., S., Lieth., H., (submitted). Effects of saline root environment (NaCl) on nitrate and potassium uptake kinetics in roses grown in closed-loop soilless: a Michaelis-Menten modelling approach.
- Massa, D., Mattson, N., S., Lieth., H., (accepted). An empirical model to simulate sodium absorption in roses growing in a hydroponic system. *Sientia Horticulturae*.
- Pardossi, A., Incrocci, L., Massa, D., Carmassi, G., Maggini, R., (accepted). The influence of fertigation strategies on water and nutrient efficiency of tomato grown in closed soilless culture with saline water. *Acta Horticulturae*.
- Incrocci, L., Massa, D., Carmassi, G., Maggini, R., Bibbiani, C., Pardossi, A., (in press) SIMULHYDRO, a simple tool for predicting water use and water use efficiency in tomato soilless closed-loop cultivations. *Acta Horticulturae*.
- Incrocci, L., Massa, D., Carmassi, G., Maggini, R., Bibbiani, C., Pardossi, A., 2008. SIMULYDRO: uno strumento di simulazione delle relazioni idriche e minerali di colture fuori suolo a ciclo chiuso. *Incontri Fitoiatrici*, Torino.
- Pardossi, A., Malorgio, F., Incrocci, L., Carmassi, G., Maggini, R., Massa, D., Tognoni, F., 2006. Simplified models for the water relations of soilless cultures: what they do or suggest for sustainable water use in intensive horticulture. *Acta Horticulturae* 718, 425-434.
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ABBREVIATIONS

Abbreviation	Description	Unit
C^{CAT}	Cation concentration	eq m ⁻³
C_{D}	Drainage ion concentration	mol m ⁻³
C_{IW}	Raw water ion concentration	mol m ⁻³
C_{NS}	Nutrient solution ion concentration	mol m ⁻³
$C_{\text{NS}}^{\text{MIN}}$	Minimum value of nutrient solution ion concentration achieved after washing and flushing	mol m ⁻³
$C_{\text{NS}}^{\text{REF}}$	Nutrient solution ion concentration at full-strength	mol m ⁻³
C_{SS}	Stock solution ion concentration	mol m ⁻³
C_{U}	Ion uptake concentration	mol m ⁻³
Ca- R_{U}	Calcium uptake rate	mmol plant ⁻¹ day ⁻¹
DSS	Decision Support System	
g or kg DW	Grams or Kilograms of Dry Weight	g or kg
EC	Electrical conductivity	dS m ⁻¹
EC _D	Drainage water EC	dS m ⁻¹
EC _{NS}	Nutrient solution EC	dS m ⁻¹
EC _{NS} ^{MAX}	Maximum value of nutrient solution EC tolerated by the culture	dS m ⁻¹
EC _{NS} ^{REF}	Nutrient solution EC at full-strength	dS m ⁻¹
EC _{NS} ^{SP}	Nutrient solution EC set-point	dS m ⁻¹
EC _U	EC-represented nutrient uptake concentration	dS m ⁻¹
GDD	Growth Degree Day	°C
GR	Growth Rate	g day ⁻¹
I	Instantaneous ion influx	μmol gDW h ⁻¹
I _{max}	Maximum ion influx	μmol gDW h ⁻¹
I _{max2}	Minimum value of I _{max}	μmol gDW h ⁻¹
[Ir]	Inhibitor concentration	mol m ⁻³
K _m	M-M affinity constant for nutrients	mol m ⁻³
K _i	M-M affinity constant for inhibitor ions	mol m ⁻³
K- R_{U}	Potassium uptake rate	mmol plant ⁻¹ day ⁻¹
LAI	Leaf Area Index	dimensionless
LF	Leaching Fraction	dimensionless

Abbreviation	Description	Unit
LR	Leaching Requirement	dimensionless
Mg-R _U	Magnesium uptake rate	mmol plant ⁻¹ day ⁻¹
mgFW	Milligrams of Fresh Weight	mg
N-C _U	Nitrogen uptake concentration	mol m ⁻³
N-R _U	Nitrogen uptake rate	mmol plant ⁻¹ day ⁻¹
Na-R _U	Sodium uptake rate	mmol plant ⁻¹ day ⁻¹
P-R _U	Phosphorus uptake rate	mmol plant ⁻¹ day ⁻¹
PPFD	Photosynthetic Photon Flux Density	μmol m ⁻² s ⁻¹
RAD	Indoor global Radiation	MJ m ⁻²
RT	Ratio between V _T and V	dimensionless
SE	Stem elongation rate	cm day ⁻¹
T	Air temperature	°C
V _D	Drainage volume	mm (L m ⁻²)
V _F	Flushing volume	mm (L m ⁻²)
V _S	Substrate volume	mm (L m ⁻²)
V _T	Mixing tank volume	mm (L m ⁻²)
V _U	Volume of water uptake	mm (L m ⁻²)
V _{WS}	Raw water volume used for washing the substrate	mm (L m ⁻²)
W	Total used water	mm (L m ⁻²)
W _R	Total water runoff	mm (L m ⁻²)
W-R _U	Water uptake rate	L plant ⁻¹ day ⁻¹

1 INTRODUCTION

1.1 Generalities and work objects

Horticultural crops are widely diffused in the world since the demand of fruits, vegetables and ornamentals as well, is fundamental for the life of human beings and their health. Great benefits are associated to eat fruits and vegetables due to their nutritional and nutraceutical properties (Arab and Steck 2000). In the 2004 Italy was the sixth country among the main producers of fruits and vegetables after China, India, USA, Brazil and Turkey (<http://faostat.fao.org/>). As fruits and vegetables are diffused for their important use in human dietary, flower production represents one of the most profitable sectors in horticulture production because of its hedonistic value. An approximated number of about 2 millions of hectares could be given for worldwide protected horticulture; this is including tunnels, greenhouses and direct covers. In the last decade, since nineties, an increase of 30-40% has been detected in this sector. Main factors linked to the expansion of protected horticulture take place in the increase of demand of high-quality products, the improved technology of transportation and post-harvest storage, the development, coupled with cost decrease, of high technologies such us microcomputers and high-tech machineries for managing cultivation, harvest, post-harvest and commercialization of horticultural products (Carmassi, 2005).

This study is characterized by plant cultivation in soilless systems. Nowadays soilless is diffused and normally applied to many industrial cultures (tomato, cucumber, rose and others), especially in floriculture production. Moreover this technique offers experimental conditions that can be manipulated by researchers reducing the influence of variables which could interfere with experiment assumptions. Hydroponic technologies, which was originally developed as a tool for studying plant mineral nutrition (Savvas, 2002), is now considered one of the main components of sustainable production of vegetables and ornamental under greenhouse and in open air as well, if it is considered the growing sector of outdoor production of containerized nursery stocks (Pardossi et al., 2006). The application of closed-loop hydroponics may provide an excellent tool to reduce the consumption of water and fertilizers and the related environmental pollution that is generally associated to over-irrigation. In closed systems, the drainage water is captured and reused, normally after proper adjustment of the chemical characteristics and after disinfection in order to minimize the risks of root-pathogen diseases. Unfortunately, the application of closed-loop hydroponic technology is scarce on a commercial scale and, with the exception of The Netherlands where closed systems are compulsory, open (free-drain) substrate culture is

commonly used for intensive production of vegetables and ornamentals, on accounts of a much simpler management of fertigation in comparison to closed systems. In open systems, the plants are normally cultivated with abundant irrigation, just to avoid any stress conditions that may result from nutrient unbalance or the salinity build-up in the root zone. In effect, when the use of poor quality water is imposed, the nutrient solution recirculated in closed-loop soilless systems would results in accumulation of ballast ions. Such ions represent non-nutrients that are not or scarcely absorbed by the culture. The presence of ballast ions in the recirculating nutrient solution may bring on high EC (electrical conductivity) level, which induces osmotic stress (Munns, 2002; Parida and Das, 2005), and causes toxicity and nutrient imbalance in plant nutrition (Grattan and Grieve, 1999; Munns, 2002; Parida and Das, 2005). Along with root-pathogen diseases, salinity represents the main difficulty in horticulture in many world areas and especially in semi-arid and costal regions such as Mediterranean area where the effects of sea water coupled with soil erosion increase salinity level in ground water (Martinez-Beltran and Licona-Mazur, 2005; Rengasamy, 2006).

Proper management of water and nutrients is necessary to produce high quality greenhouse and nursery crops. Supplying fertilizer or water in either suboptimal or excessive levels can lead to diminished growth, low quality, or even damage. To maximize quality and productivity, adequate amounts of water and nutrients must be provided (Oki and Lieth, 2004). Under saline conditions plant nutrition changes (Grattan and Grieve 1999); hence excessive or erroneous fertilization may lead to plant stress as well as environmental pollution.

In the last years several DSSs (decision support systems) or simulation models have been developed for supporting growers. Most of them regards the adsorption of nutrient and applies for nutrient solution management (Carmassi et al., 2005; Bacci et al., 2005; Mattson et al., 2006; Savvas et al., 2007). Mathematical models enable to simulate plant behaviour giving suitable tools for predicting data; moreover they can apply for on-line and off-line controls that are helpful in crop management. Therefore, among others, simulation remains one of the most valuable approach for supporting plant cultivation in high-technology greenhouses (Pardossi et al., 2006).

This thesis focuses on plant nutrition modelling with particular emphasis towards soilless cultivation under saline conditions. The work was articulated on different modelling approaches that involved a large variety of environmental and plant variables. Such models attempt to simulate plant nutrition with an especial regard to saline environment. The use of such models, or at least an example of their practical application, is also reported trough a simulation study that aims to show in which way DSS-integrated models (e.g. SIMULYDRO, Incrocci et al., in

press) can improve the management of greenhouse-grown crops. Most of this work is addressed to rose (*Rosa* spp., L.) and tomato (*Solanum lycopersicon*, L.) plant that are species representative of large-scale greenhouse cultivated plants. However, the work is open to each field of horticulture, remarking issues, such as nutrient solution managements and fertilization, that are common to all species among hydroponic as well as soil cultivations.

1.2 General aspects of modelling

Human beings have a natural inclination to predict events. This behavior arises in part from the exigency to take under control the environment and manage it. The knowledge of how a certain event will perform, since ancient ages, has given people the possibility to choose among different alternatives and make decisions. The saying “red sky at night, shepherd’s delight” is a simple and empirical method for forecasting weather, but it well represents the logical process hidden behind the art of modelling that is based on the observation of a fact and its future implications. (Thornley and Johnson, 1990).

Basically, every system can be represented as a model or at least as logical algorithm or event sequence, since “for each action there is always an opposite and equal reaction”. Then we can assume that if we are able to observe, calculate and simulate a certain process we can predict, with a certain degree of error, the effects of this process. Clearly the art of modelling mainly applies for solving real problems; nevertheless researchers and scientists occasionally develop models which have only theoretical applications or philosophical justification.

It’s very hard to distinguish the limit that divides what is a real application and what is not; in fact many factors contribute to the realization and the utilization of a model. In this sense the complexity of system representation plays an important role. Generally complex systems need elaborated models which are based on theoretical applications; nevertheless, an apparently simple system or process could need more complicated procedures to be represented. On the contrary, sometimes empiricism and system simplification are considerable valid alternatives in modelling.

On other hand modelling is a natural mental process; when we wake up at morning and we observe the sky, we are elaborating a provisional model that will suggest us for dressing. Nevertheless, the accuracy of our predictions depends on many factors which can be better determined, analyzed and understood by means scientific tools. In recent years, the efforts of scientists and researchers are addressed to develop models in many scientific fields, proposing models as decision making useful tools.

A model as reported by De Wit (1982) can be defined as a simplified representation of a system, which is a limited part of reality that contains interrelated elements. Referring to “simulation model”, the term simulation represents the ability to build mathematical model which summaries the study and the properties of what ever system or process. Thus the modeller tries to obtain one adequate and complete quantitative description of the system, which contains the relationship between the system itself and the surrounding environment and, if possible, explains the underlying mechanisms.

Lieth (1999) describes a model as a tool providing information and knowledge for growers. In this sense, models should be packaged as management tools easy to use and increase profitability. Such tools can differ for complexity depending on the nature of the model; however they should represent an accessible decision-making method for users.

The capacity to communicate data provided by models relies on the ability to describe a certain event. Prior the introduction of microcomputers models were described by mathematical formula. Nowadays the “infinite” potential, offered by calculators, permits the simultaneous computation of several sub-routines involved in algorithm arranged models. Because computers programs are able to predict data they are often considered models. Nevertheless, it is useful remark that a model is a logical abstraction which deals with representing something real (e.g. plant growth, fruit development, crop systems).

The modelling process is based on the integration of information regarding the system observed. Quantitative collected data are used to calibrate the model which firstly attempts to establish a mathematical relationship between variables. Experimental work provides both to data used to run the model and to test different thesis or hypotheses. Modelling work involves the calibration of equations by estimating parameters; afterward model data verification and calibration follow. Basing on this framework, it is primary to figure out the object and aim of the modelling and the organization of the system.

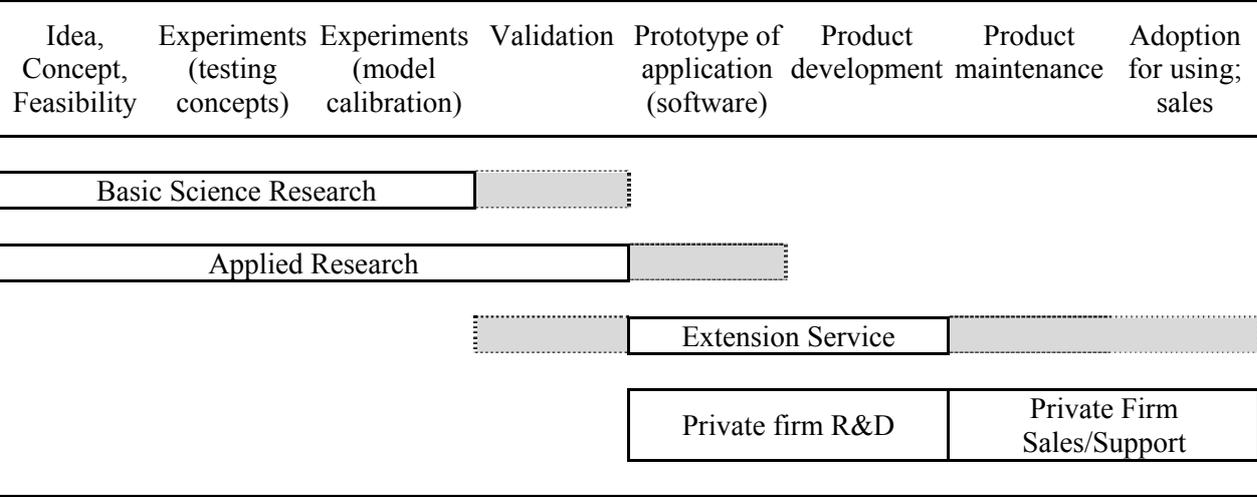
Most models are assembled to solve real issues such us production, nutrient uptake or product quality estimation. Rarely people responsible of model assembling are also the users. Figure 1.1 shows a graphical representation of people who deal with modelling and their rule in the modelling process (Lieth 1999).

Even if we restrict the work area to specific fields such as agronomy or horticulture, the number of modelling objectives can be theoretically unlimited. However it is possible to address different kind of models to different class of application.

Many models are developed as improving crop knowledge (Whisler et al. 1986; Reynolds et al. 1985); such models are often used by researcher or scientists and rarely are used by growers.

Models can deal with production optimization; in this sense models can be involved within input management procedures or specific production management tools. The former regards a large number of production variables such as climate parameters (climate greenhouse control) (Jolliet et al. 1991), nutrients, use of the water and others. The latter regards a large variety of models that can be very simple such as graphs showing patterns, ruler predicting growth or more complex such as decision support system (DSS) and computer software (Lieth 1999).

Figure 1.1. The graph shows relations among model assemblers, users and their respective work areas (Lieth 1999).



Legend: Probable Possible

In many cases models attempt to surrogate other tools that take place within production processes. In fact the use of sophisticated tools or procedures sometimes results difficult or too expensive; in these situations models can replace electrical instruments, analysis protocols and even human work.

The purpose of modelling systems implies the study of the structural system organization. It is existence of different system levels which give rise to great diversity. Thus, systems can be more or less complex (Thornley and Johnson, 1990). In this thesis we will mainly consider plant and crop level. Referring to the above description we can define the main properties of hierarchical systems (Thornley and Johnson 1990).

1. Different levels have different languages. Terminology used for a certain level might induce confusion for discussing about other levels. For instance terms like canopy, leaf area (LA) and plant density are adequate for crop levels whereas do not work at deeper level such as cellular levels.
2. Levels are related step by step and higher levels are the integration of lower levels.

3. Models developed for lower levels can work and be assembled to give an interpretation of higher levels. The *vice versa* is not possible.
4. Higher levels provide the constraints, boundary values and driving function to lower levels.
5. Temporal and spatial scales change according to higher or lower levels.

Models are generally classified as empirical or mechanistic depending on their nature. Empirical models are essentially direct descriptions of phenomenon which has been detected and measured. Therefore they are based on mathematical interpretation of experimental data. Such models, for their nature, attempt to describe and simulate plant behaviour without involve bio-physiological explanations but none the less they can be extremely useful (Thornley and Johnson 1990). In fact in many cases the empiricism results in a powerful tool to reproduce what has been observed experimentally. Carmassi et al. 2005, Savvas et al. 2007 proposed some empirical models to simulate both ion uptake/accumulation and water uptake in soilless-cultivated crops. Fisher et al. (1996) packaged a simple models to predict Easter lily flower harvest. Furthermore empirical models focus on fertilization strategies (Le Bot et al. 1998; Fink and Feller 1998), crop development and dry matter partitioning (Kage and Stutzel 1999) and estimating nutrient leaching (Simmelsgaard and Djurhuus 1998). The basic approach for these models, as explained by Thornley and Johnson (1990), is primarily one of examining data, deciding on an equation or set of equations, and fitting these to the data. All above mentioned implies that mathematics and statistics become the main supports and sometimes the only rule for empirical model evaluation. On the contrary mechanistic models are usually based on physiological laws or assumptions such as that of energy conservation or the laws of thermodynamics, on biological information and on the structure of the system. Hence, while empirical models attempt to simulate systems preceding directly to whole-plant variables, mechanistic model effort aims to reduce the system into components that relates with the lower hierarchical levels. Finally the integration of sub-systems gives explanation of whole-plant behaviour. It is very difficult to understand the limit that defines empiric versus mechanistic. Very often models consist of mechanistic ideas represented by empirical equations or *vice versa*. Sometimes DSSs involve both mechanistic and empirical models to accomplish their targets.

A plethora of different works shows that sometimes the use of empirical models is more suitable in those situations that require a low number of variables or assumptions, while a limit of mechanistic models would lie in their complexity. Nevertheless, empirical models are often based on specifics that deal with a particular system limiting the application of those models. Overall empirical models are declared fast and simple models with easy-to-estimate parameters.

Furthermore, predictive value of empirical models can be high, but there are important limitations; in fact when these types of models are used outside the range of input data, predictions can become unreliable and the use of these models to other species or locations is often unrealizable. On the other hand, mechanistic models usually involve a higher number of variables and or assumptions that need a large experimentation, but their applications can be addressed to several species and cultivation systems. Further model classifications deal with time variable; in this sense we talk about dynamic models which describe systems changing as a function of time or static models, which give prediction about not time-depending data. Then deterministic models assign a single value to considered variables while stochastic models include errors that give an idea of predicted data variability. In recent reviews, Lieth (1999), Gary et al. (1998) report other possible models classifications. Nevertheless here we will limit our excursus to what above said, with the aim of focusing reader's attention on specific aspects related to this work.

The identification and the knowledge of chosen approaches become a fundamental aspect for the successive work that consists in determining all the variables that define the system. Such variables can be classified in several ways. Most of them can be reassumed as independent variables, which basically represent model inputs and depending variable, which represent model outputs. More specifically we have driving variables, which determine and drive a phenomenon (temperature, global radiation, ion concentration, water transpiration) or state variable (carbohydrate accumulation, dry matter accumulation, nitrogen organication).

In a very simple explanation the best results of modelling lie in the ability of modeller to connect input variables with output variables which should be as more as suitable for giving an exhaustive impression of what happen in the reality.

To accomplish the onerous task of building a model, modellers usually 1) handle data by representing them with logical diagram (e.g. flow charts) and by fitting them with various statistic procedures. 2) By handling data, modellers obtain a model calibration that means parameter estimation.

Till now a modeller has not idea of model goodness, therefore a serious model valuation must be accomplished as follows:

1. Model performance:
 - a. comparing models versus data (verification);
 - b. understanding how variables and parameters can affect the model's behaviour (sensitivity analysis);
 - c. interpretation of parameters in relation to what is modelled.

2. Validation:

- a. collecting new data;
- b. testing model on new data set.

Such steps are often accomplished by the same team that proposed the model. Nevertheless, the solidity of models can be well detected only by their enlarged employment, especially whether their purpose is to simulate behaviours of different species and/or cultivation systems.

1.2.1 Modelling horticultural crops

Crop modelling commenced, as a branch of agronomy that involves specific engineering characteristics (computer programs, mathematics and others) in the early seventies with the significant contribution of microcomputer development. Afterward modelling studies spread around the globe as it appeared to be useful tool for research areas as well as on-field applications. Models usually provide information for making decisions on several aspects of cultivation such as fertilization and irrigation or for predicting events such as harvesting time.

There is a specific need for research models in horticulture. As for other areas of crop production, the development of models often starts as a natural continuation of the experimental approach to a problem. “As a branch of science progresses from the qualitative to the quantitative, one day it may be expected to reach the point where the connections between theory and experiment are most efficiently made using the language of mathematics” (Thornley and Johnson 1990; Gary et al. 1998). Grower’s commercial activity consists in making decisions that may strongly influence the economy of farms. Therefore, in agriculture as in other fields, a good decision is based on a clear picture of reality (Gary et al. 1998). In this sense models provide tools that help to simplify the complexity of agricultural systems giving a valid support to growers.

Models are powerful tools to increase efficiency of experiments, to test hypotheses, to synthesize knowledge, to describe and understand complex systems. Moreover, models may be used in decision support systems (DSS), for instance to compare different scenarios, to control greenhouse climate or to plan crop production. In the context of horticulture, which represents a field where yield prediction, policy evaluation or process optimization are crucial, the models may solve several problems (Carmassi, 2005).

Horticultural crop modelling has been reviewed by several authors in the late nineties. Le Bot et al. (1998) gave their contribution on nutrient absorption and plant fertilization, while water relation modelling was summarized by Jones and Tardieu (1998). Plant development and dry mass accumulation were objects of the works of Prusinkiewicz (1998) and Marcelis et al. (1998),

respectively. More general aspects of modelling horticultural crops, such as model classification and model application, have been reviewed by Gary et al. (1998) and Lentz (1998).

1.2.2 *Modelling plant nutrition*

In the literature, most of publications on modelling in horticulture reports works on the processes of plant growth and development, and evapotranspiration. The processes of nutrient uptake and quality formation, and the interactions between crop and pests have received much less attention (Gary et al., 1998).

Nutrient uptake is considered the results of consecutive steps in mechanistic models, as explained by Le Bot et al. (1998). Most of nutrition models attempt to simulate nutrient absorption involving processes that occur at root level as well as within plant tissues and their interaction (Siddiqi and Glass, 1986; Silberbush and Lieth, 2004; Mattson et al., 2006). In reviewing literature, three general mechanistic approaches to nutrient modelling uptake emerge: Michaelis-Menten kinetics, *relative addition rate of nutrients*, and plant nutrient demand. However, within these approaches, empirical relationships are used (Mathieu et al., 1999).

In Michaelis-Menten's approach nutrient absorption is defined as an ion influx into the plant; such influx depends on external nutrient concentration and follows a pattern that is mathematically described as a rectangular hyperbola (chapter 6). Parameters that characterize this curve may vary as a function of many plant and environmental variables. Siddiqi and Glass (1986) found an exponential correlation between maximum influx and tissue nutrient concentration. Wheeler et al. (1998) related this parameter to growth environmental ratio. Peuke and Jeschke (1999) and Bar-Yosef et al. (2004) proposed a modified Michaelis-Menten equation for simulating the action of inhibitor ions on nutrient uptake (e.g. Cl vs. N-NO₃)

Hellgren and Ingestad (1996) described the mathematical relationship used in the relative addition rate. This approach aims to maintaining constant plant nutrient concentration under nutrient limiting conditions, such that the plant has a stable relative growth rate (Mathieu et al., 1999).

As showed by Mankin et al. (1996) nutrient uptake can be related to plant nutrient demand, which in turn may depend on plant growth.

With regard to empirical approaches, there is a plethora of different equations that have been successfully proposed for modelling the uptake of nutrients and non-nutrients. Willits et al. (1992) proposed a linear regression in which nutrient uptake was function of relative growth rate. Pardossi et al. (2004) simulated nutrient uptake in melon grown in hydroponics by using multiple regression-based models (chapter 5) and an empirical model was proposed also by Fink and

Feller (1998) for simulating nitrogen absorption in white cabbage. Several empirical equations have been reviewed by Le Bot (1998).

In the last years, a particular interest has been shown versus models that simulate nutrient uptake basing on nutrient uptake concentration (Sonneveld 2000), that is the ratio between the ions and the water absorbed by the plant during the same time period (chapters 2 and 4).

Carmassi et al. (2005; 2007) developed a model which simulated nutrient and non-nutrient uptake (Na) in soilless tomato grown under saline conditions depending on uptake concentration. The same approach was satisfactorily adopted by Savvas et al. (2007) for simulating chloride and sodium accumulation in the recycling water of closed-loop substrate culture of cucumber. Moreover, some DSSs have been developed on the basis of what has been discussed previously (Bacci et al., 2005; Incrocci et al., in press). Although to couple water uptake and nutrient uptake has been object of several debates in the past (Le Bot et al., 1998), nevertheless several procedures to replenish nutrient solution have been proposed in recent years and most of them depend on water uptake (van Kooten et al., 2004) and the measurement of EC (Savvas and Manos, 1999). As a matter of fact, in soilless culture the frequency of nutrient solution supply to the crop is typically determined by plant water uptake, which may be measured directly or estimated on the basis of climatic parameters such temperature, relative humidity and incoming solar radiation (Le Bot et al., 1998; Carmassi et al., 2007).

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2 WATER AND NUTRIENT USE EFFICIENCY OF TOMATO PLANTS GROWN IN CLOSED-LOOP SUBSTRATE CULTURE WITH SALINE WATER: SIMULATION AND GREENHOUSE EXPERIMENT

2.1 Introduction

Along with the risk consequent to the possible diffusion of root pathogens, the salinity of irrigation water represents the main difficulty for the management of closed systems. When saline water is available, there is a more or less rapid accumulation of ballast ions, like sodium (Na) and chloride (Cl), which are dissolved at concentration higher than the uptake concentration (e.g. the ion/water root uptake ratio).

This phenomenon may result in a concomitant increase in the electrical conductivity (EC) of nutrient solution, if the management strategy aims to maintain constant nutrient concentration, or in a parallel depletion of nutrients, if the fertigation is based on a feed-back control of EC, as it is the case in many commercial greenhouses and nurseries. Under these conditions, the nutrient solution is normally recirculated till EC and/or the concentration of some potentially toxic ion reaches a maximum acceptable threshold value (according to plant exigencies), after that it is replaced, at least partially; the term ‘semi-closed’ is used for this system. In Holland, growers are allowed to leach their systems whenever a crop-specific ceiling of Na concentration is reached: for example, 10 mol m⁻³ for tomato, and 4 mol m⁻³ for cut roses (Baas and Van den Berg, 1999). According to the conclusions of a simulation study carried out by Stanghellini et al. (2005), there is no way that closed systems are financially viable under strict environmental rules (e.g. when irrigation water has a poor quality); under these conditions, the most valuable strategy is likely the improvement of water quality, by means of desalinization or the use of rainwater.

Nevertheless, on species with moderate salt tolerance (like tomato or melon), the application of some particular procedures, for the control of nutrient supply to the culture, may give positive results in terms of both crop sustainability and productivity. As a matter of fact, the growers should prolong the recirculation of the same nutrient solution and/or minimize the content of polluting agents, like nitrogen (N) and phosphorus (P), in the effluents, when the water is finally discharged.

Both review (Voogt and Sonneveld, 1997; Klaring, 2001; Savvas, 2002; Bugbee, 2004) and experimental papers (Baas and Ven den Berg, 1999; Savvas and Manos, 1999; Brun et al., 2001; Pardossi et al., 2002) were published on the procedures to control fertigation in closed hydroponic systems. To our knowledge, however, only a few works were conducted on the

possible strategies for managing the recycling of nutrient solution in the presence of saline water (Raviv et al., 1998; Bar–Yosef et al., 2001; Pardossi et al., 2002; Kempkes and Stanghellini, 2003). Among these, only the papers published by Raviv et al. (1998) and by Pardossi et al. (2002) reported a detailed study on the effect of fertigation strategies on crop yield, the use of water and fertilizers and the environmental impact provoked by nutrient leakage associated to periodical flushing. In particular, the strategies tested by Raviv et al. (1998) differed for the ratio among drainage, rain and tap water used to prepare the nutrient solution as well as for the EC at which the recycling solution was partially discharged.

With respect to the two papers cited previously, the originality of the present study consisted in the general approach and in the specific objectives. Indeed, the work aimed at evaluating, by simulation and by greenhouse experiment, three different strategies (A-C) for the replenishment of the nutrient solution in semi-closed substrate culture of greenhouse tomato (*Solanum lycopersicum* L., cv. Jama) conducted using only saline water ($9.0 \text{ mol m}^{-3} \text{ NaCl}$), as compared to open system. The Strategies A and B, described in details in the next paragraph, corresponded to two out of five different techniques for nutrient recycling reviewed recently by Savvas (2002), with the difference that for Strategy A EC (Electrical Conductivity) was not constant but increased with time. Instead, Strategy C was based on the simple expedient of discontinuing the nutrient replenishment for a few days before the expected time for the replacement of the nutrient solution, in order to drain off a nutrient solution with quite low concentrations of nitrogen, thus minimizing the environmental impact.

The simulation study was accomplished by means of different models for water and mineral relations of plants grown in soilless cultures, which were developed originally or in previous works (Carmassi et al., 2005; Carmassi, 2005; Carmassi et al., 2007). In this sense, the work also intended to build and validate a simulation tool that may be implemented in DSS (Decisional Support System) for optimal water management in soilless culture.

The relevant parameter that defined the strategies used in closed systems was the EC of the nutrient solution supplied to the plants, which was fairly constant (around 3.0 dS m^{-1}) in one case (Strategy A) and oscillated from 3.0 to 4.5 dS m^{-1} in the other ones. These values were selected in order to avoid any reduction in crop growth and fruit yield that, on the basis of previous works (D'Amico et al., 2003; Carmassi et al., 2005; Carmassi, 2005), were expected for Jama tomato plants in the experimental growing conditions considered by the study. The strategies also differed for the nutrient supply to the plants. Therefore, in the greenhouse experiment it was verified the possible effect of salinity oscillation and/or reduced nutrient supply on crop growth and the production and quality of fruits. Actually, the free-drain culture was included to provide

an idea of the crop performance under non-stressful conditions, and not to assess the well-known environmental impact of open system.

2.2 Materials and methods

2.2.1 Fertigation strategies and nutrient solution supply

Typically, in commercial closed-loop substrate culture with drip irrigation, the fertigation water is automatically prepared by mixing drainage nutrient solution, raw water, nutrient stock solutions and generally diluted acids as well, in order to achieve pre-set values of EC and pH (Savvas et al. 2002).

The simulation considered a growing system, used for greenhouse experiment as well, which resembles a commercial closed culture. In this experimental system, a mixing tank with a volume (V_T) of 6 mm ($L m^{-2}$) collected the nutrient solution drained from the substrate after each irrigation. The volume of water retained by the substrate and the irrigation lines (V_S) approximated 10 mm, thus the total volume of recycling water (V_{NS}) was 16 mm (Table 2.1).

Table 2.1. The relevant parameters for each fertigation strategy under investigation (see text and abbreviations for details and).

	EC_{NS}^{MAX}	EC_D	EC_{NS}^{MIN}	$N-NO_3^- C_{NS}^{FI}$ ($mmol L^{-1}$)	V_S (mm)	V_T (mm)	V_F (mm)
Strategy A	4.50	-	3.00	-	10	6	18
Strategy B	4.50	-	3.00	<1.0	10	6	18
Strategy C	4.50	-	3.00	<1.0	10	6	18
Strategy D	-	3.00	-	-	10	-	-

Whenever the water level in the tank decreased by 17% (1 mm) approximately, owing to crop water uptake (V_U), the tank was automatically replenished with raw water or a nutrient solution, depending on the fertigation strategy. The nutrient solution had been prepared manually simulating the operation of a typical fertigation system, in which two nutrient stocks are diluted with raw water or drainage depending on the strategy.

Every day, the recycling water was monitored for EC (EC_{NS}) and N concentration (C_{NS}) and flushed out, if the measured parameters were beyond the preset values. Throughout the paper, the

term ‘flushing’ refers to the discharge of the exhausted recycling nutrient solution, which is the responsible of water and nutrient losses. The different fertigation strategies are specified below.

Strategy A: V_U was systematically compensated with nutrient solution at full strength ($EC = 2.5 \text{ dS m}^{-1}$) in order to maintain a (relatively) constant nutrient concentration. This strategy resulted in a progressive increase of EC_{NS} till it reached a ceiling value ($EC_{NS}^{MAX} = 4.5 \text{ dS m}^{-1}$) tolerated by the crop, afterwards the recirculating nutrient solution was flushed out.

Strategy B: an EC set-point of approx. 3.0 dS m^{-1} was maintained; this produced a progressive depletion of nutrient content in the recycling water, which was flushed out whenever the N-NO_3^- concentration decreased below 1.0 mol m^{-3} , which was considered acceptable from the environmental viewpoint (approximately, it is the maximum acceptable nitrogen concentration established by the current Italian legislation for the disposal of wastewater to the soil).

Strategy C: V_U was initially compensated with fresh nutrient solution at full strength, as in Strategy A; when EC_{NS} reached 4.5 dS m^{-1} , the mixing tank was refilled with fresh water only till the N-NO_3^- concentration decreased below 1.0 mol m^{-3} .

A graphical representation of the above explained strategies is given as logical flow-charts in the Figure 2.1-3 and Figure 2.4.

The work included also an open system (**Strategy D**), in order to verify the possible influence of salinity build-up and/or nutrient depletion on crop growth and yield.

2.2.2 Model framework

The simulation was based on several assumptions, which were verified in previous works and/or derived from specific measures adopted in the experimental cultures: i) the substrate remained invariably at full container water capacity and the differences between the ion concentration in the substrate and in the mixing tank in closed systems, or drained out from open systems, were negligible in reason of frequent irrigations (up to 10 times per day) with an high drain fraction (LF, the ratio between drainage and irrigation water); ii) V_U was mostly determined by leaf transpiration; iii) no effect on leaf area, V_U and fruit yield was expected for the maximum salinity levels (4.5 dS/m) set up for model simulation and actually reached during the experiment (Carmassi et al., 2005); iv) the only source of ballast ions was the irrigation water, since high-purity soluble fertilisers were used for fertigation; v) the C_U for macronutrients was equal or very closed to their concentration ($C_{NS}^{REF}, EC_{NS}^{REF}$) in the full-strength (reference) nutrient solution; vi) the cationic-anionic balance maintains the electro-neutrality of the solution.

Basically, the simulation tool aggregated three major models that run on a daily basis to determine: i) V_U ; ii) the ion concentration (C_{NS}) and the electrical conductivity of recycling

nutrient solution (EC_{NS}) on the basis of the nutrient addition by fertigation device and the expected crop mineral uptake (namely, C_U); iii) the ion composition of drainage water as well of the recycling water after periodical flushing in semi-closed system. Different model parameters were used for vegetative and reproductive crop stage.

Crop water uptake. Daily V_U (mm) was simulated using the model developed by Carmassi et al. (2007):

$$V_U = 0.946 \cdot (1 - \exp^{-k \cdot LAI}) \cdot \frac{RAD}{\lambda} + 0.188 \quad \text{mm (2.1)}$$

where k is the canopy light extinction coefficient (0.69), LAI is leaf area index, RAD is the indoor cumulated global radiation and λ is the latent heat of vaporization (2.45 MJ Kg^{-1}).

LAI was modeled as a function of crop thermal time, as expressed as growing degree days (GDD):

$$LAI = -0.335 + \frac{(4.803 + 0.335)}{1 + \exp\left[\left(\frac{755.3 - GDD}{134.7}\right)\right]} \quad \text{dimensionless (2.2)}$$

GDD was computed since sowing from air temperature (T) with 8°C as basal temperature.

Recirculating water composition and EC. A linear equation was used to predict the C_{NS} of N-NO_3^- and other macronutrients at day n on the basis of the cumulated V_U , the ion concentration at the beginning of the period ($C_{NS, n-1}$) or in the refill water (C_{RW} , the water used to compensate V_U), the apparent plant uptake concentration (C_U) and the total volume of the recirculating nutrient solution (V), which included both the water in the mixing tank (V_T) and in the substrate (V_S):

$$C_{NS, n} = C_{NS, n-1} + \frac{(C_{RW} - C_U) \cdot V_U}{V} \quad \text{mmol L}^{-1} \text{ (2.3)}$$

with the condition that C_{NS} and ($C_{NS, n-1}$) were ≥ 0 .

The term C_{RW} has two components: the ion concentration of raw water (C_{IW}) and the one (C_{SS}) produced by the addition of nutrient stocks by fertigation device, which depends on the control Strategy. While in Strategies A and C the water-stock dilution ratio (then C_{SS}) was constant, in

Strategy B it was modulated on the basis of the actual EC_{NS} and the set-point value (EC_{NS}^{SP}). Therefore, equation (2.3) can be rewritten as:

$$C_{NS, n} = C_{NS, n-1} + (f \cdot C_{SS} + C_{IW} - C_U) \left(\frac{V_U}{V} \right) \quad \text{mol m}^{-3} \quad (2.4)$$

The coefficient f was 0 during the nutrient depletion in Strategy C (when fertigation device was used only for pH adjustment), while it was 1 in Strategy A, where V_U was compensated by nutrient solution at full strength EC_{NS}^{REF} . Diversely, in Strategy B f was calculated for every daily refill of the mixing tank as:

$$f = \frac{(EC_{NS}^{SP} - EC_{NS})}{(EC_{NS}^{REF} - EC_{IW})} \quad \text{dimensionless} \quad (2.5)$$

According to Carmassi et al. (2007), a first order differential equation was used to predict the C_{NS} of ballast ions (e.g. Na^+) at day n :

$$C_{NS, n} = \left(C_{NS, n-1} - \frac{C_{IW}}{p} \right) \cdot \exp\left(-p \cdot \frac{V_U}{V}\right) + \frac{C_{IW}}{p} \quad \text{mol m}^{-3} \quad (2.6)$$

where p describes the proportionality between C_{NS} and C_U for the considered ion

Finally, EC_{NS} was calculated from the total equivalent concentration of cations (C^{CAT} , eq m^{-3}) according to the following formula proposed by Sonneveld (2000):

$$EC = 0.19 + 0.095 \cdot C^{CAT} \quad \text{dS m}^{-1} \quad (2.7)$$

Drainage water composition and EC. When EC_{NS}^{MAX} (Strategy A) or a $N-NO_3^-$ concentration lower than 1.0 mol m^{-3} (Strategies B and C) was reached, the nutrient solution in the mixing tank was discharged and the salts accumulated in the substrate were washed out by means of free-drain irrigation with a pre-established volume of raw water (V_{WS}); therefore, the water loss in occasion of each flushing event (V_F) was the sum of V_T and V_{WS} . At the time of flushing, the concentration of each ion in the recirculating nutrient solution (C_{NS}^{FI}) and in the substrate (C_S^{FI}) was considered equal for the model assumptions reported previously. The concentration of any ion in the recirculating nutrient solution after flushing (C_{NS}^{MIN}) was determined as:

$$C_{NS}^{MIN} = C_{IW} \cdot \frac{V_T}{(V_T + V_S)} + C_S^{MIN} \cdot \frac{V_S}{(V_T + V_S)} \quad \text{mol m}^{-3} \quad (2.8)$$

where C_S^{MIN} is the ion concentration in the water retained by the substrate.

The latter was calculated with a model that describes the continuous depletion of salt concentration in the substrate slabs as function of increasing V_{WS} . The model was based on a mass balance equation that can be written in a differential form after rearrangement and for small increments of V_{WS} :

$$\frac{dC}{dV_{WS}} + \frac{C_S^{Fl}}{V_S} = \frac{C_{IW}}{V_S} \quad \text{mol m}^{-3} \quad (2.9)$$

The integration of equation (2.9), with the initial condition of $C = C_S^{Fl}$ for $V_{WS} = 0$, leads to the following equation:

$$C_S^{MIN} = C_{IW} + (C_S^{Fl} - C_{IW}) \cdot \exp\left[\frac{-V_{WS}}{V_S}\right] \quad \text{mol m}^{-3} \quad (2.10)$$

Finally, the model computed the LF in open system on the basis of a predetermined EC of drainage water (EC_D) using the equation proposed by Sonneveld (2000):

$$LF = \frac{(EC_{NS}^{REF} - EC_U)}{(EC_D - EC_U)} \quad \text{dimensionless} \quad (2.11)$$

where EC_U is the uptake concentration computed by converting the C_U for cations to EC by means of equation (2.7).

Model outputs. The most relevant outputs from the model were: i) the total water consumption (W), which is the sum of V_U and water runoff (W_R); ii) the leaching requirement (LR, the ratio between W_R and V_U); iv) the leakage of nutrients and ballast ions. In semi-closed systems W_R was computed as the product between V_F and the number of flushings, while in open system it was the sum of the daily drainage volume (V_D) deriving from the estimated V_U and the operational LF, as follows:

$$V_D = V_U \cdot \frac{LF}{(1-LF)} \quad \text{mm (2.12)}$$

The total leakage of mineral elements was calculated as follows: i) for Strategies A-C, by cumulating the loss at each flushing event, which was computed by a mass balance equation considering C_{NS}^{MIN} , C_{NS}^{FI} , V_{NS} and V_{FI} ; ii) in Strategy D, by cumulating the daily leakage determined as the product of V_D times C_D , the latter being calculated by means equation (2.11) for the established LF, obviously after rearrangement and by substituting ion concentration to EC

2.2.3 Greenhouse experiment

The experiment was conducted in a glasshouse (240 m²) in 2005 at the University of Pisa (Pisa, Italy, latitude 43°43'N, longitude 10°23'E). The experiments started on 2 May 2005 with 30-days old seedlings of tomato and lasted 84 days. The minimum (heating) and ventilation air temperature inside the glasshouse were 16 and 27 °C, respectively; maximum temperature reached up to 33–35 °C during sunny hours in the last weeks of cultivation. Maximum photosynthetic photon flux density (PPFD) ranged from 500 to 700 μmol m⁻² s⁻¹; global radiation (RAD) and daily air temperature averaged 12.5 MJ m⁻² and 25.2°C, respectively.

The plants were grown in standard rockwool slabs at density of approximately 3 plants m⁻². Three plants and five drippers were placed in each slab to ensure a uniform water application. The plants were grown vertically with single stem and stopped by cutting at the second leaf above the fifth truss. Bumblebees were kept in the glasshouse to improve flower pollination. In consideration of the short growing period, basal leaves were not removed.

Each fertigation strategy was applied to three separate growing systems, each containing 30 plants. The most relevant parameters for each strategy under investigation are shown in Table 2.1 and 2.2. The values for macronutrient C_U and p , in equations (2.6) and, were determined in previous works (Carmassi et al., 2005, 2007). In open system, a large LF was adopted in order to prevent any possible stress resulting from salt accumulation and/or nutrient depletion in the root zone; for that, irrigation frequency and dosage were adjusted quite frequently during the cultivation. Equation (2.11) was used to calculate the LF needed to produce an EC_D not higher than 3.00 or 2.70 dS m⁻¹, with EC_{NS}^{REF} of 2.64 or 2.31 dS m⁻¹ and EC_U of 1.78 or 1.42 dS m⁻¹, in crop stage I and II, respectively. Therefore, the operational LF was 0.70 and 0.65 in I and II stage, respectively, and these values were used for simulation. Similar values (0.60-0.75) were adopted for single water applications in semi-closed systems (Table 2.2).

Table 2.2. Ion concentration (mol m^{-3}) and EC (dS m^{-1}) of raw irrigation water and complete (reference) nutrient solution used during the vegetative (stage I) and reproductive (stage II) phase of tomato culture. The ion uptake concentration (C_U) used for simulation is also shown. For Na^+ and Cl^- , C_U was considered proportional to the concentration in the raw water (see text for details). The uptake concentration expressed as EC was applied to estimate ion concentration of the drainage water in open system.

	N- NO_3^-	P- H_2PO_4^-	Cl^-	K^+	Ca^{2+}	Mg^{2+}	Na^+	EC
Irrigation water (stage I and II)	0.00	0.00	9.50	0.00	1.50	0.80	9.50	1.53
Nutrient solution (stage I)	10.00	1.00	9.50	6.70	4.00	0.80	9.50	2.64
Nutrient solution (stage II)	7.00	0.70	9.50	4.70	3.25	0.80	9.50	2.31
C_U (stage I)	10.00	1.00	1.71	6.70	3.55	0.60	1.71	1.78
C_U (stage II)	7.00	0.70	1.71	4.70	2.80	0.45	1.71	1.42

Daily V_U was measured by recording with a flow-meter the amount of water or nutrient solution used to refill automatically the mixing tank. The accuracy of flow-meter was checked weekly. EC and nitrogen concentration of the recycling water in semi-closed system or in the water drained out of open system were frequently monitored by means, respectively, of an EC-meter and a reflectometer (Merck Reflectoquant® Reflectometer RQ Flex); the accuracy of the latter instrument was assessed preliminary using a colorimetric assay in the laboratories.

At least once or twice per week and in occasion of each flushing event in closed systems, samples of raw water, nutrient stocks and recycling or drainage nutrient solutions were collected for the laboratory determination of K, Ca, Mg, Na, N- NO_3^- concentration, which were performed by means of liquid chromatography (120 DX, Dionex, USA). These values were used to build the water and nitrogen balance sheet for each culture.

Moreover, three plants per each treatment were sampled every month for the destructive measurement of leaf area and dry biomass. Crop yield was determined by measuring the number and the fresh weight of harvested fruits. Fruit quality was assessed by measuring fresh weight, pH, total soluble solid (TSS) and K concentration, as well as by recording the incidence of non-marketable berries.

2.3 Results and discussion

As shown in Table 2.3, there was a good agreement between simulations and measurements for all considered quantities, including those not reported in this paper for the sake of brevity (e.g. the concentration of macronutrients and Na^+ in the recirculating or drainage water). The most important simulation errors are indicated in Table 2.3 by an asterisk, which designates a predicted value outside the confidence interval (95%) for the mean of measurements.

Table 2.3. The influence of fertigation strategies on water relations and N-NO_3^- leaching (see text for details and abbreviations). The mean value of the EC of the recirculating (Strategies A-C) or fertigation water (Strategy D) is also shown. In each row for measurements, different letters indicate a LSD significant difference for $P < 0.05$ (following ANOVA). The asterisk designates a simulation value outside the confidence interval (95%) for the mean of measurements.

		Strategy A	Strategy B	Strategy C	Strategy D
V_U (mm)	Simulated	341.5	341.5	341.5 *	341.5 *
	Measured	351.7 b	342.8 b	358.4 ab	364.3 a
Flushings (n°)	Simulated	10	14	6 *	-
	Measured	10 b	14 a	7 c	-
W_R (mm)	Simulated	180.0	252.0	108.0 *	732.1 *
	Measured	180.7 b	251.9 c	124.1 d	703.8 a
W (mm)	Simulated	521.5	593.5	449.5 *	1073.6
	Measured	532.4 c	594.7 b	482.5 d	1068.1 a
LR	Simulated	0.53	0.74	0.32	2.14 *
	Measured	0.51 c	0.73 b	0.35 d	1.93 a
N-NO_3^- leaching (g m^{-2})	Simulated	18.93 *	0.92	0.20	91.42 *
	Measured	13.02 b	0.58 c	0.67 c	70.75 a
Mean EC_{NS} (dS m^{-1})	Simulated	3.67	2.95	3.78 *	2.72
	Measured	3.63 a	2.95 b	3.55 a	2.66 c

The underestimation of V_U by the model, which was acceptable *per se* (5-6%), was not associated to a similar error in the simulation of LAI (data not shown) and accounted for the discrepancy between predictions and observations for the number of flushings and, then, for W_R , W and LR in Strategy C (Table 2.3). In Strategy D (open system), notwithstanding a predicted V_U lower than the actual one, the simulation slightly overestimated W_R and W, because the actual LF (then, LR) was lower (0.66, on average) than the operational one (0.68, on average)

due to the inevitably inaccuracy of manual irrigation management adopted in the experiment (Table 2.3).

The measured leakage of N-NO_3^- was also lower than the predictions, at least in Strategy A and D (Table 2.3). This was a consequence of the higher C_U for N-NO_3^- with respect to the expected values (see Table 2.2), in particular during the first crop stage (data not shown). This phenomenon reduced the actual C_{NS}^{Fl} or C_D of N-NO_3^- in Strategy A or D, respectively, and increased the frequency of water disposal in Strategy C, compared to the simulation (7 vs. 6 flushings; Table 2.3), since the condition for flushing (C_{NS} lower than 1.0 mmol L^{-1}) was reached more rapidly.

Strategies A and C reduced W compared to the others (Table 2.3), as indeed was expected from the nature of fertigation strategies under investigation and the model parameterisation. In all semi-closed systems, Na^+ accumulated at a rate of $2.0\text{-}2.2 \text{ mol m}^{-3}$ per day and the differences in the frequency of flushing (roughly, every 7, 5 and 10 days in Strategies A, B and C, respectively) were associated to a different Na_{NS}^{Fl} (roughly, 25, 22 and 31 mmol L^{-1} , in Strategies A, B and C, respectively) (Table 2.3).

Table 2.4. The influence of fertigation strategies on total soluble solid (TSS), titratable acidity, pH, EC, Na and K concentration measured on harvested fruit (2° and 4° truss). No significant difference was found (ANOVA, $P < 0.05$) for the mean of measurements ($n=3$).

	Strategy A	Strategy B	Strategy C	Strategy D
Total fruit yield (kg m^{-2})	10.2	9.7	10.4	10.3
TSS ($^\circ\text{Brix}$)	4.5	4.6	4.8	4.6
Titratable acidity (% citric acid)	0.5	0.5	0.6	0.5
pH	4.2	4.0	4.1	4.2
EC (dS m^{-1})	5.4	5.8	5.9	5.6
Na (mol m^{-3})	1.9	1.7	2.1	1.9
K (mol m^{-3})	59.4	63.2	65.5	61.7

As reported in Table 2.3, no important effect of the adopted strategies was observed on V_U and fruit yield (on average, 10.2 kg m^{-2}) and quality (Table 2.4), although V_U was significantly higher in open system than in the Strategies A and B. Indeed, this was expected, since: i) the salinity level in all semi-closed systems was maintained below the salinity threshold for Jama

tomato plants, as established in previous work (Carmassi et al., 2007); ii) the time during which the plants were grown at reduced or almost negligible N-NO_3^- concentration was much shorter (less than 3-4 days) than the 2-4 weeks that appeared necessary to reduce plant growth in a work with hydroponically-grown tomato, as observed by Le Bot et al. (2001). The absence of any effect of fertigation strategies on fruit yield was confirmed in a second experiment carried out in 2006 by growing plants for a much longer period (between late-March and mid-September; 146 days in total); in this experiment, the fruit yield averaged 19.2 kg m^{-2} .

2.4 Conclusions

Strategy C was even more efficient in term of water and nutrient use than Strategy A. The results also confirmed that a semi-closed system conducted following the strategy of full nutrient solution replenishment (Strategy A) may produce a massive environmental pollution due to nutrient leaching, although to a much lesser extent than open growing system. Experimental data confirmed the hypothesis that no important variation will be detected in terms of fruit yield and quality by using different strategies of nutrient solution replenishment. The explanation of this plant behaviour is strongly related to the choice of variables such us EC limit and NaCl concentration which should be ranged between values that are not prohibitive for plant development.

In conclusion, the proposed model appeared a valuable tool to predict the water use and the environmental impact of soilless cultures using a limited number of variables and parameters, the most important of which is the C_U for both macronutrients and ballast ions. Moreover, the results of both simulation and greenhouse experiment suggest the possibility to improve considerably the water and nutrient use efficiency of soilless culture carried out under saline conditions by adopting appropriate fertigation strategy.

Figure 2.1. In the picture a flow-chart describes the Strategy A as a logical sequence of events.

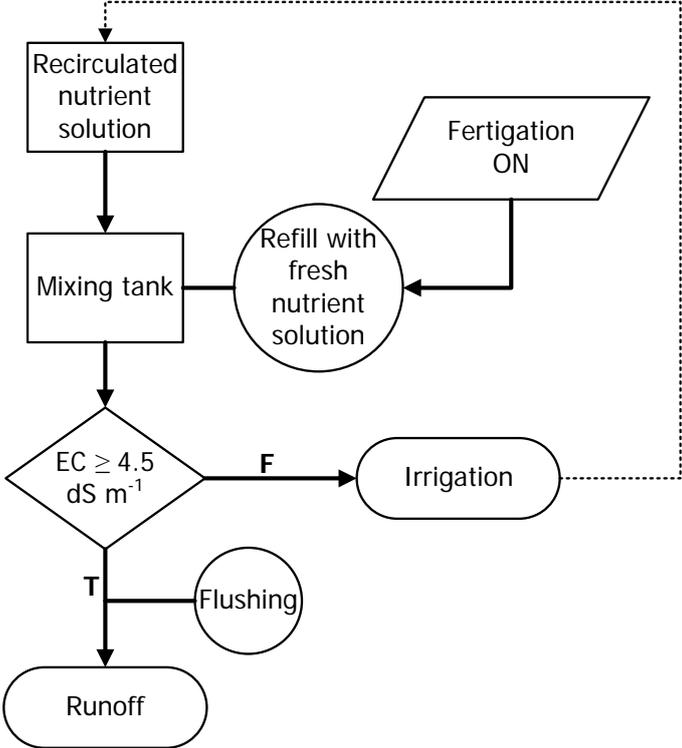


Figure 2.2. In the picture a flow-chart describes the Strategy B as a logical sequence of events.

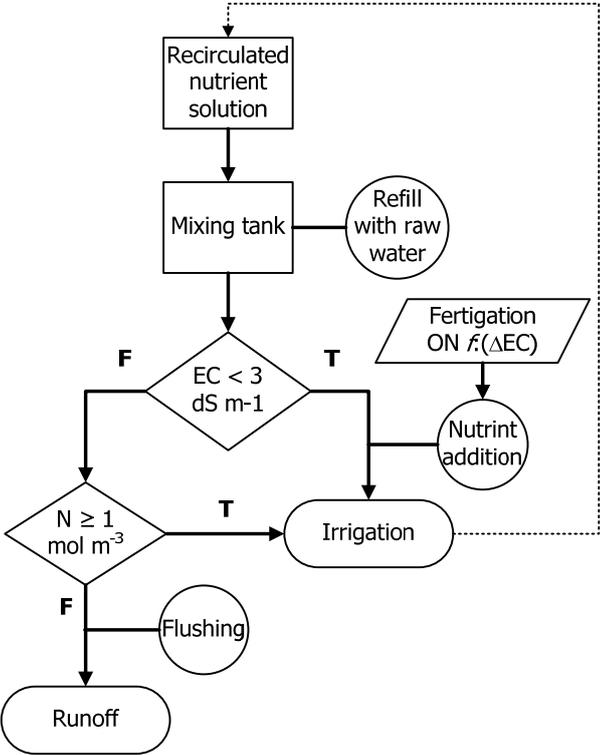


Figure 2.3. In the picture a flow-chart describes the Strategy C as a logical sequence of events.

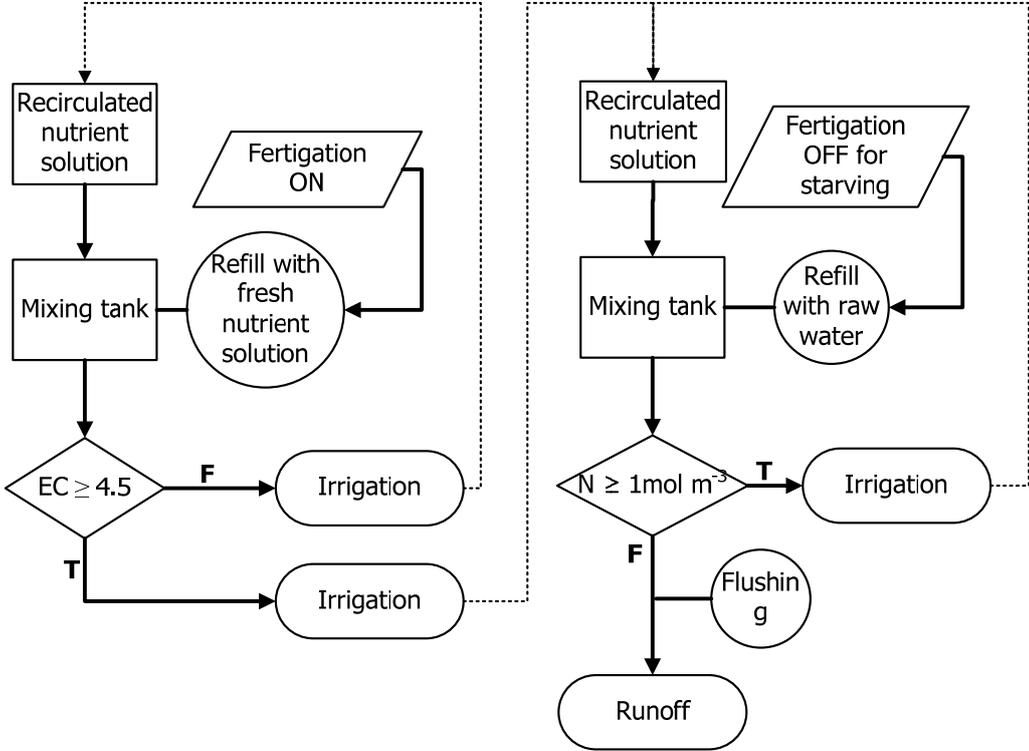
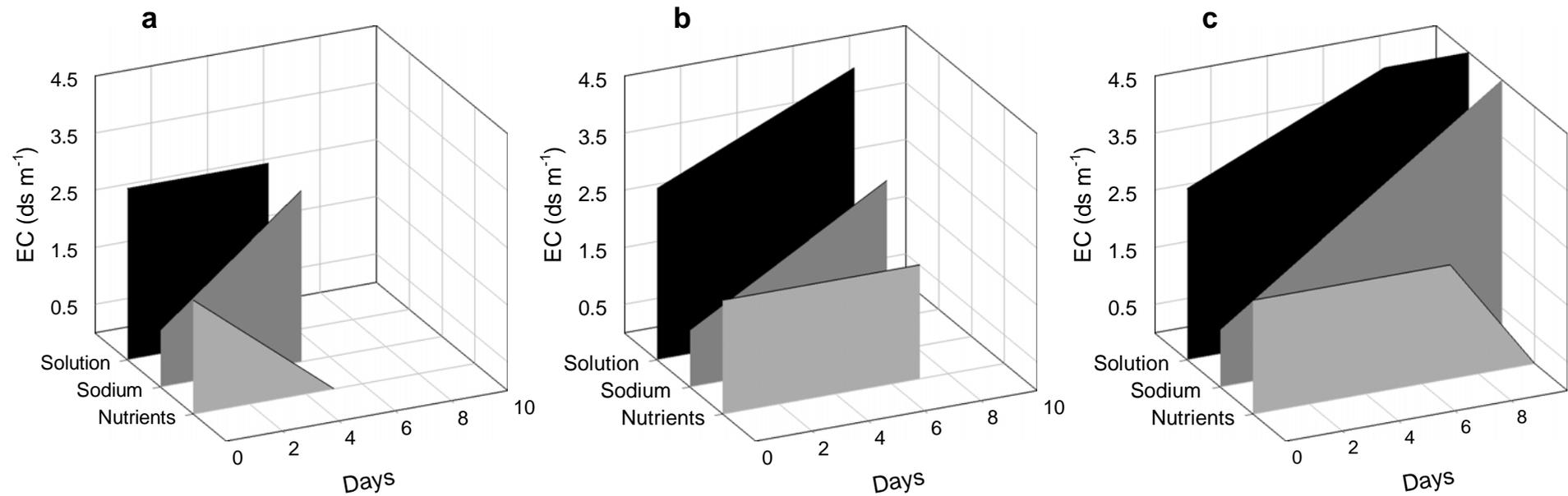


Figure 2.4. Influence of the different strategies (A, B, C respectively Graph 5b, 5a, 5c) on EC pattern and duration of the period between two flushing events. The contribution of nutrients (light grey) and sodium (grey) to the EC of nutrient solution (black) (in the picture solution=nutrients+sodium) was estimated by using equation 2.7. Reported values correspond to experimental mean values for both duration of periods between two different flushing events (5, 7, and 10 days, for the Strategy B, A and C respectively) and minimum and maximum EC value thresholds: 3-3 (Strategy B), 3-4.5 (Strategy A and C) dS m^{-1} .



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3 THE USE OF DSS IN SOILLESS-GROWN HORTICULTURAL CROPS: A SIMULATION STUDY

3.1 Introduction

In the last years many decision support systems (DSS) have been developed for horticultural crops (e.g. GLASSIM, <http://www.hpc.wur.nl/UK>). Most of them can simulate large-scale-cultivated crops such as tomato (TOMGRO, Jones et al., 1991; TOMSIM, Heuvelink, 1999). The main object of DSS may vary in relation to the investigated species; for instance DSSs that deal with floriculture production usually provide for information about harvesting time (Lieth and Carpenter, 1990; Lieth and Pasian, 1993). DSS approach attempts to provide the management with computer-based tools to analyze and use information in decision making. Therefore, a database management system and a model management system are usually combined. The main idea is that models might be used by decision makers if a single system provides all available data of a firm and a whole bunch of different types of models developed before in the field of operations research or econometrics (Lentz, 1998). To our knowledge only few DSSs are able to simulate crops grown under saline conditions and a very little number of them provides for data on nutrient solution management under such conditions (Bacci et al.2005).

As discussed previously in this manuscript there are different approaches for modelling plant nutrient absorption and each of them can be involved into a DSS. In effect a DSS is often composed of sub-models which might be replaced or implemented into DSSs depending on specific need. As an example of DSS-integrated models, in this manuscript we have already reported a DSS which involves three main sub-models (chapter 2). Such sub-models run for simulating crop water uptake, crop nutrient and non-nutrient uptake and water/nutrient runoff. The study reported in the chapter2 was carried out with the aim to support and validate a DSS originally developed by Incrocci et al. (2007) (SIMULHYDRO).

The current chapter aims to give an overview on the possible practical applications of DSSs such as SIMULHYDRO. An unlimited number of data can arise from simulation studies; however the main point of this section of the manuscript is to provide for practical examples and applications in soilless greenhouse production. To accomplish this task a series of simulation studies was run through SIMULHYDRO; such simulations mainly deal with EC (Electrical Conductivity) level of system-incoming water, cultivation system characteristics or rather the ratio between tank water volume (V_T) and total system volume (V) and different strategies for the nutrient solution

replenishment. The above mentioned model inputs would consist in the main independent variables of SIMULHYDRO.

Among the others, electrical conductivity is one of the most important variables influencing the management of closed-loop system (Pardossi et al., 2006). The main difficulty is related to the influence that EC exerts on osmotic potential which, for high absolute values, may affect negatively plant transpiration and development through osmotic stress (Munns, 2002). Nonetheless EC increases are often coupled with ballast ion accumulation (e.g. sodium, chloride or sulfate) in the recirculating nutrient solution. Such ions are responsible of salt toxicity and may negatively influence nutrient absorption (Grattan and Grieve, 1999; Munns, 2002; Parida and Das, 2005). Hence the presence of ballast ions must be kept under control in closed-loop systems. In the system the concentration of these ions ranges depending on raw water quality and plant salinity tolerance. When the level of ballast ion is deemed too high for the crop, a partial washing of the substrate is necessary. This procedure implies to open the system for washing ballast ions out (semi-closed loop). The optimization of this procedure, which has been accurately described in the previous chapter of this manuscript, is one of the main points of the current manuscript section. In fact loss of water (water runoff, W_R) and sometimes of nutrients (nutrient runoff) depends on flushing frequency and methodology.

SIMULHYDRO is able to simulate closed-loop systems taking into consideration different strategies for nutrient solution management. Such strategies are reported in chapter 2. However, simulations run in this study have been conducted by using only one of the possible strategies (Strategy A). In fact the current chapter aims to show in which way a DSS can be used for making decisions, while advantages and disadvantages of different strategies have been exhaustively discussed previously in the manuscript. Therefore the fundamental characteristics of the only Strategy A have been reported below.

Strategy A: V_U is systematically compensated with nutrient solution at full strength in order to maintain a (relatively) constant nutrient concentration. This strategy results in a progressive increase of EC_{NS} (see abbreviations for details) until it reaches a ceiling value (EC_{NS}^{MAX}) tolerated by the crop, afterwards the recirculating nutrient solution is flushed out.

3.2 Materials and methods

Object of the first simulation was to investigate EC and LR (leaching requirement) changes as a function of different NaCl concentration, different V_U/V ratios (RT) and different flushing methods (WS or NWS). Hence SIMULHYDRO was set in order to simulate data with three

different raw water NaCl concentrations, four different RT and two different flushing strategies which differed for washing method. Such combinations are all reported in Table 3.1.

Table 3.1. Input parameters used for running the simulation through SIMULHYDRO. Twenty-four combination of different raw water salinity levels, V_u/V ratios (RT) and flushing method (WS or NWS) were tested for calculating LR and average EC in the recirculated nutrient solution.

$[\text{NaCl}]$ (mol m^{-3})	RT	V_{ws} (mm)	$\text{EC}_{\text{NS}}^{\text{REF}}$ (dS m^{-1})	$\text{EC}_{\text{NS}}^{\text{MAX}}$ (dS m^{-1})
5	0.25	20.5	2.0	3.5
5	0.50	16.0	2.0	3.5
5	0.75	11.5	2.0	3.5
5	0.96	11.0	2.0	3.5
10	0.25	20.5	2.5	4.0
10	0.50	16.0	2.5	4.0
10	0.75	11.5	2.5	4.0
10	0.96	11.0	2.5	4.0
15	0.25	20.5	3.0	4.5
15	0.50	16.0	3.0	4.5
15	0.75	11.5	3.0	4.5
15	0.96	11.0	3.0	4.5
5	0.25	3.0	2.0	3.5
5	0.50	6.0	2.0	3.5
5	0.75	9.0	2.0	3.5
5	0.96	11.5	2.0	3.5
10	0.25	3.0	2.5	4.0
10	0.50	6.0	2.5	4.0
10	0.75	9.0	2.5	4.0
10	0.96	11.5	2.5	4.0
15	0.25	3.0	3.0	4.5
15	0.50	6.0	3.0	4.5
15	0.75	9.0	3.0	4.5
15	0.96	11.5	3.0	4.5

Raw water NaCl concentration ranged between 5 and 15 mol m^{-3} . Therefore, as a consequence of different cation concentration, EC range ($\text{EC}_{\text{NS}}^{\text{REF}}$ and $\text{EC}_{\text{NS}}^{\text{MAX}}$) was varying depending on raw water salinity level (Table 3.1). RT ranged between 0 and 1 for simulating different hydroponic environments (Table 3.1). With regard to the flushing time two different strategies were adopted. In one case at the flushing time only the volume of the tank was flushed out, whereas in the other substrate was washed with a volume of water (V_{ws}) variable as a function of the different RT

(Table 3.1). A total of twenty-four simulations were run for calculating LR and average EC in the nutrient solution. Simulation results were plotted against RT and analyzed graphically. For the second simulation, average EC and W (total water used) were determined as a function of different ΔEC (Table 3.2) or rather different EC_{NS}^{MAX} thresholds and different RT. In this simulation only one NaCl (10 mol m^{-3}) concentration was tested.

Table 3.2. Input parameters used for running the simulation through SIMULHYDRO. Twelve combination of different raw, ratios (RT) and flushing method (WS or NWS) were tested for calculating LR, W and average EC in the recirculated nutrient solution.

[NaCl] (mol m^{-3})	RT	V_{WS} (mm)	EC_{NS}^{REF} (dS m^{-1})	EC_{NS}^{MAX} (dS m^{-1})
10	0.25	3.0	2.5	4.0
10	0.50	6.0	2.5	4.0
10	0.75	9.0	2.5	4.0
10	0.96	11.5	2.5	4.0
10	0.25	3.0	2.5	5.0
10	0.50	6.0	2.5	5.0
10	0.75	9.0	2.5	5.0
10	0.96	11.5	2.5	5.0
10	0.25	3.0	2.5	6.0
10	0.50	6.0	2.5	6.0
10	0.75	9.0	2.5	6.0
10	0.96	11.5	2.5	6.0

This simulation study regards a soilless-grown tomato culture. Growing conditions such as plant density, cultivar and climate conditions were set as reported in the chapter 2. The only relevant assumption for all these simulations was that, within the investigated EC range, no significant difference was expected for plant evapotranspiration as discussed previously in this manuscript.

3.3 Results and discussion

In the first simulation we intended to investigate on how different raw water salinity levels, RT and flushing method (WS or NWS) influenced LR and average EC_{NS} in the root zone. The results of such analysis are reported in the Figure 3.1 where LR and average EC_{NS} are plotted against RT. Outputs chosen give us information about the efficiency of the system (LR) and the condition of the plant growth environment (EC_{NS}). With regard to the former, before discussing the results, it is useful to understand in which way leaching requirements can influence

hydroponically-managed greenhouse cultures. This parameter offers to the manager a valid tool to evaluate water use giving an idea of water loss as explained by the following equation:

$$LR = \frac{W_R}{V_U} = \frac{W - V_U}{V_U} \quad \text{dimensionless (3.1)}$$

where W_R represents total water runoff (mm) and V_U represents plant evapotranspiration (mm). Therefore the higher LR value results in lower water efficiency.

Salinity level induced an increase in average EC_{NS} and LR (Figure 3.1). This was because higher NaCl concentrations in the incoming water determined a faster ballast ion accumulation. As a result the Δt between two flushing events decreased, determining an higher number of flushing and then higher W_R and W . This apparently obvious conclusion in the practice becomes an essential tool for making decision. Let us suppose that government limitations impose a waste water threshold as it happens in The Netherlands. Assuming that such thresholds may vary as a function of different crops and water quality, by means of DSS managers could find the most valuable strategy for decreasing LR with the maximum profit. As an example we can consider an hypothetic limitation on total water use as follow: $W=540$ mm and $V_U=350$; hence by applying Equation 3.1:

$$LR = \frac{540 - 350}{350} = 0.54$$

This value can be used as a limit or alternatively as a target that must be achieved by testing different management strategies. Let us suppose that poor quality water use is imposed (15 mol m^{-3}). In the Figure 3.2 this value would exclude all technical solutions that require substrate washing and however those that require RT higher than 0.5. This implies that different RT may be adopted in order to maintain a certain LR value. As a practical consequence, the choice of a suitable type of soilless system should optimize the production in such conditions. To this purpose, a wide range of RT was tested during the simulation study. Such values ranged between 0 and 1; this range represents different hydroponic systems respectively substrate culture to liquid culture. Values that range between 0.2 and 0.5 are typically associated to substrate culture (e.g. perlite or rockwool) such as tomato or cucumber, while values close to 1 are typically associated to liquid culture (e.g. NFT or aeroponic) such as salads. In this sense SIMULYDRO and generally DSSs can be useful tools for planning production as well as dimensions and type of cultivation system. In effect, for the example above mentioned, if we consider lower NaCl

concentrations (e.g. 10 mol m^{-3} or below) several solutions are possible in terms of cultivation system dimensions as showed in the Figure 3.1. At the same NaCl concentration different RT affects LR in different ways depending on the strategy adopted for flushing. At the flushing time growers can choose between two option: the first consists in discharging the exhausted nutrient solution contained in the tank and replenishing it again (NWS); the second one consists in washing substrate-accumulated ballast ions out from the system with a water volume variable in relation to raw water quality (WS) (see chapter 2 for details). In terms of water efficiency there was evidence that WS strategy produced a higher loss of water compared to NWS strategy. This was due to the efficiency of washing procedure that decreased exponentially by increasing the volume of the substrate (V_s) as mathematically explained by Equation 2.10. In the practical use of water, LR limitation not only deals with government regulations or environmental impact. It is an interesting parameter that may be used for calculating variable costs in an economic balance. In this sense strategies that account for substrate washing (WS) should be deemed as profit-limiting strategies or rather cost-increasing strategies. However a serious economic balance must take into consideration production and costs as showed by Stanghellini et al. (2005). As a matter of fact a higher LR reduces average EC_{NS} in the root zone (Figure 3.1); this would affect positively yield as showed by Maas and Hoffman model (1977). In effect, as reported in the Figure 3.1, washing procedure kept average EC_{NS} quite constant in the root medium depending on salinity level, also induced lower average EC_{NS} compared to NWS strategy. The explanation of these patterns lies in the complexity of the simulation and strongly depends on RT. At the flushing time, WS strategy aims to decrease salt concentration in the system until a certain EC_{NS}^{MIN} that is $\approx EC_{NS}^{REF}$. Instead in NWS strategy, EC_{NS}^{MIN} results higher than EC_{NS}^{REF} depending on RT or rather V_T : the higher value of RT coupled with lower value of EC_{NS}^{REF} results in lower average EC_{NS} . For the above reasons in the Figure 3.1 EC decreases by increasing RT (NWS). With regard to EC system oscillation, a comparison between NWS and WS strategy is given in the Figure 3.2. In the picture the spikes of rapid decline in EC represent flushing events. The graph points out how washing procedure decrease EC_{NS} as an effect of a low EC_{NS}^{MIN} . Therefore, with regard to practical implications the choice of washing the substrate, which apparently decreases profits because of the larger water cost, may be a valid strategy for decreasing average EC_{NS} in the root zone that would result in yield increments (Maas and Hoffman, 1977). However in this last example we did not take into consideration possible water limitations due to government laws and/or scarcity of water availability that can reduce LR; in this case, EC increases inevitably occur if the use of poor quality water is imposed. Therefore

substrate washing would be possible only if water, although of poor quality, is not limited. In the common practice when water is a production-limiting factor two main procedures can be adopted: the improvement of water quality and the improvement of salinity crop tolerance. The former is realizable through inverse-osmotic water treatment or the collection of rain water. Then high quality water may be mixed with raw water that would result improved by the lower NaCl concentration or ballast ions concentration. Techniques which aim to improve water quality by mixing different types of water have been applied satisfactorily in horticulture (Raviv et al., 1998). On the other hand improving water quality implies higher production costs that have a negative effect on grower's profits (Stanghellini et al., 2005). To this purpose SIMULHYDRO and other DSSs may represent suitable tools to estimate EC target required to maximize profits.

With regard to crop salinity tolerance several studies have showed that salinity-tolerant crops (such as tomato) may be managed with saline water through different nutrient solution replenishment strategies (Pardossi et al., 2006) without significant loss of yield. In this scenario SIMULYDRO can work as a tool to evaluate the impact that different EC thresholds have on W and average EC_{NS} at root level. To this purpose a second series of simulations was run for predicting LR, W and average EC_{NS} in the root zone. Such simulations aimed to show how the efficiency of the system varies depending on EC_{NS}^{MAX} or rather on variations of ΔEC_{NS} that is $EC_{NS}^{MAX} - EC_{NS}^{REF}$. Graphs in the Figure 3.3 show the results of these simulations. As explained previously in this chapter, when no substrate washing is imposed at flushing time (NWS) RT increase influence negatively the efficiency of the system showing an higher LR and W (Figure 3.3). At the same time EC_{NS} decreases by increasing RT as an effect of higher volume of water discharged at flushing time (higher V_T). The expedient of setting higher EC_{NS}^{MAX} produced increase in ΔEC_{NS} that delayed flushing events. As a result curves representing LR and W were shifted to lower values, while curves representing EC were shifted to higher values. Although increases in EC decrease yield; nevertheless the use of salinity-tolerant crops can mitigate such loss of production. Hence, considering Figure 3.3, a small increase in EC, for example 3.7 to 4.2 $dS\ m^{-1}$ (e.g. in a substrate culture, RT=0.5), determines a significant reduction in LR (0.35 to 0.15) with important W saving (474 to 407 mm). As a practical consequence EC threshold modulation may be a valid strategy to maximize profits, especially for crops that tolerate small increments in EC in the root zone.

Figure 3.1. Effect of different RT, raw water salinity levels and flushing procedures (WS or NWS) on LR and EC. Twenty-four simulations were run through SIMULHYDRO. Such strategy implies that the nutrient solution absorbed by plants is recovered in the system with nutrient solution at full strength (EC_{NS}^{REF}). A partial or total replacement of the recirculating nutrient solution occurs whenever EC_{NS} is equal to EC_{NS}^{MAX} .

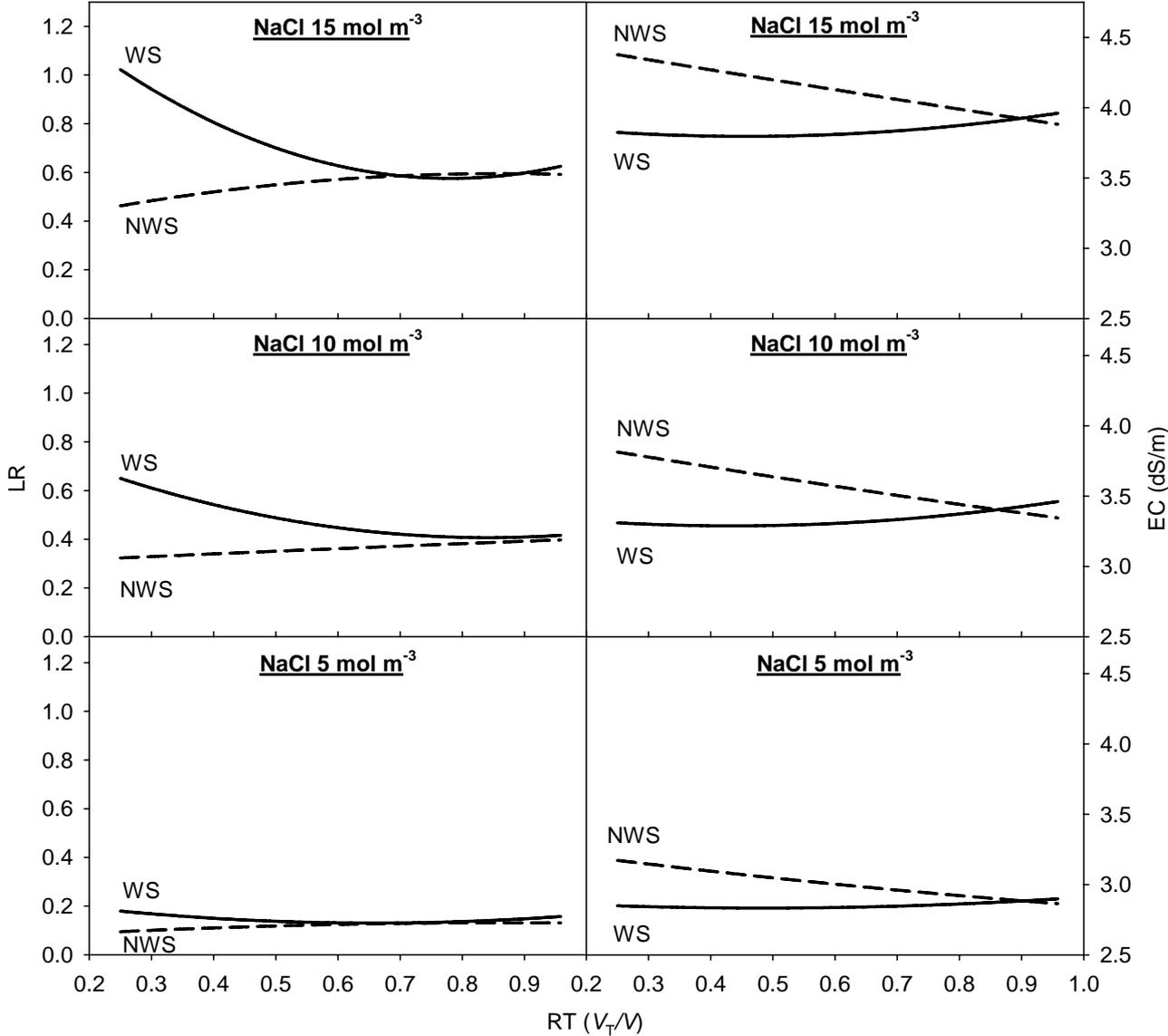


Figure 3.2. Effect of different flushing strategies on the nutrient solution EC. Flushing can be realized by discharging the only nutrient solution contained in the tank (NWS) or by washing the substrate (WS). The simulation reported in this figure was run with $RT=0.5$ and $NaCl=10 \text{ mol m}^{-3}$. In the figure the spikes of rapid decline in EC represent flushing events.

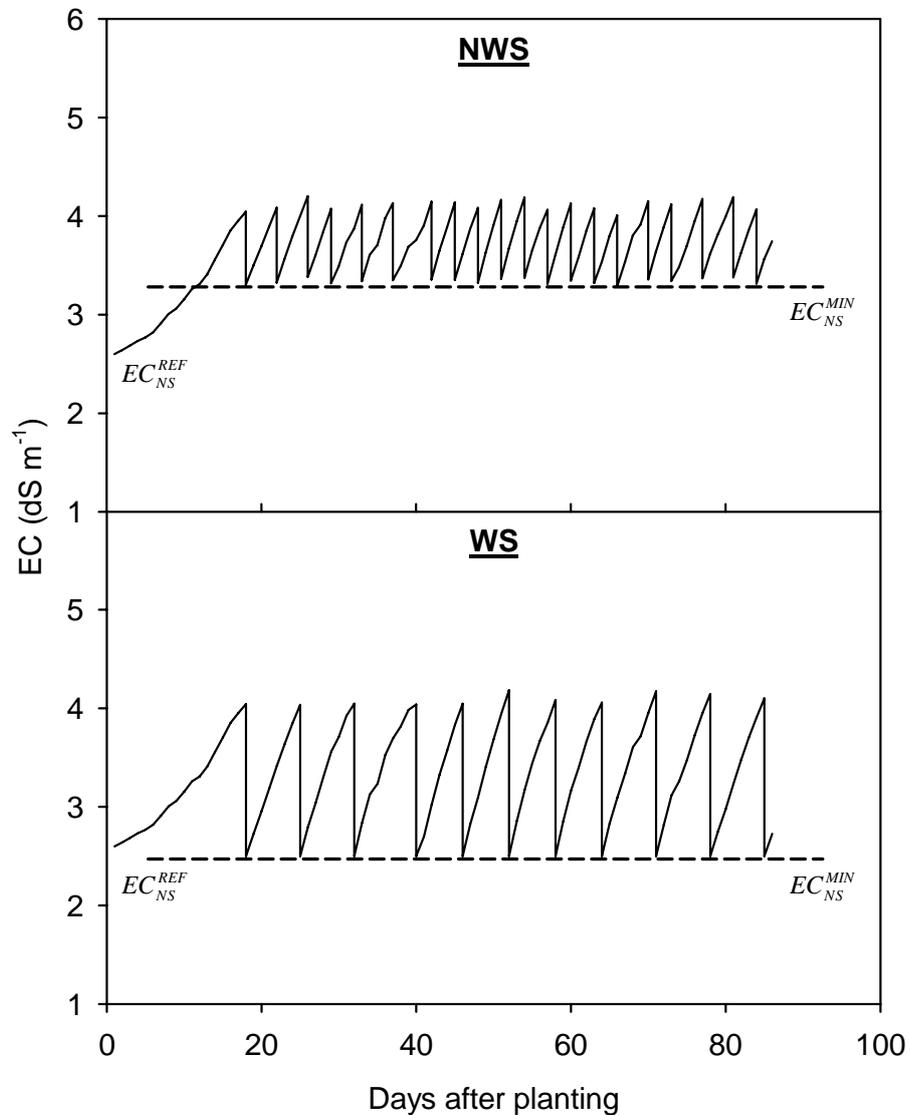
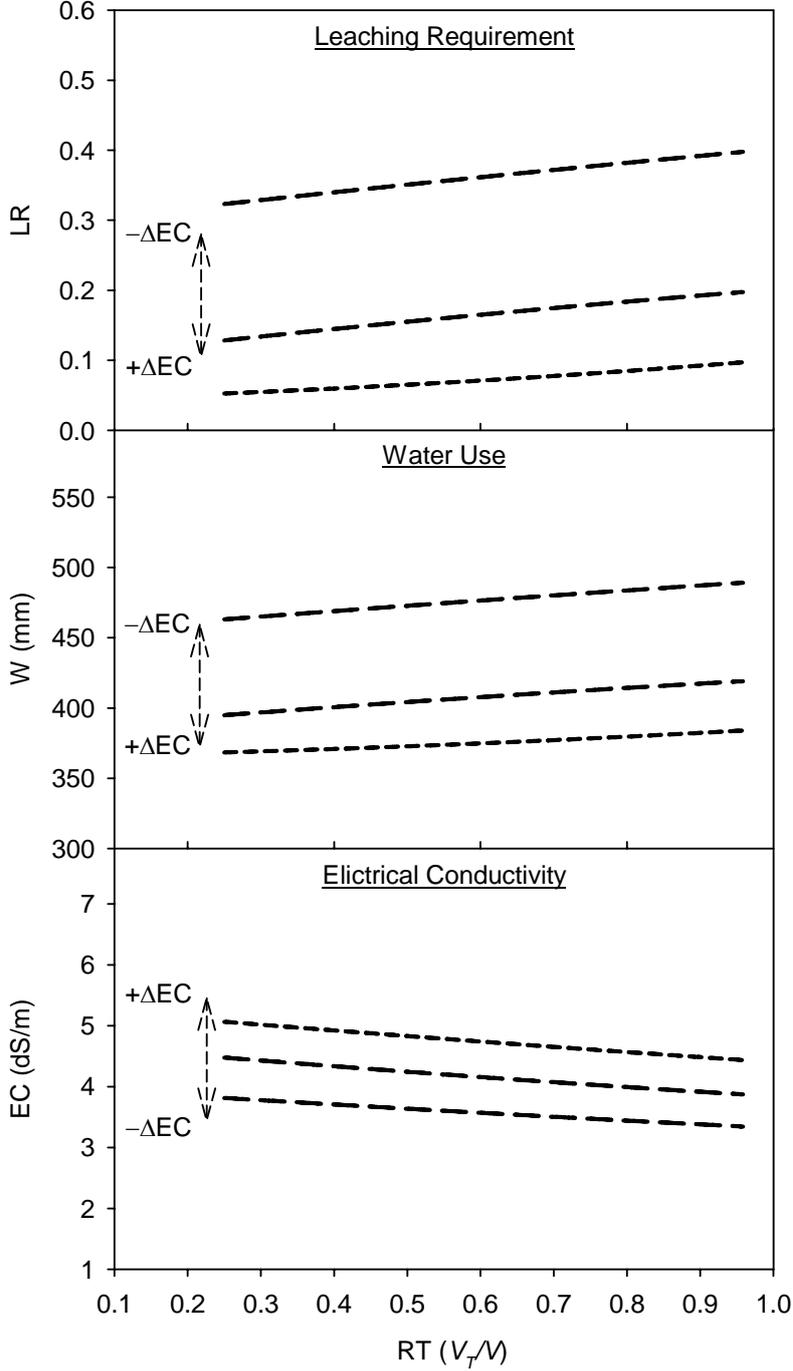


Figure 3.3. Influence of different ΔEC ($EC_{NS}^{MAX} - EC_{NS}^{REF}$) and different RT on LR, W and average EC in the root zone. Simulation was run with 10 mol m^{-3} of NaCl and variable ΔEC (Table 3.2).



3.4 Conclusions

In the last years several DSSs have been developed for simulating greenhouse-grown horticultural crops as reported by many authors (Jones et al., 1991; Lieth and Pasian, 1993; Lentz, 1998; Heuvelink, 1999; Pardossi et. al 2006). Most of them aim to predict data for crop management and cost optimization. However just few DSSs are able to provide data for crops growing under saline environments (Bacci et al., 2005).

SIMULYDRO (Incrocci et al., in press) is able to give information on crop growing in soilless culture under saline conditions. Such data may be handled by economists, growers and managers for making decisions. An idea of the potential use of DSSs arises from our simulation study that gives examples of DSS practical applications.

The applications showed in this section of the manuscript represent a limited example of the capacity of SIMULYDRO. Actually a huge number of simulations and combinations are possible by changing inputs parameters. Moreover our simulation was limited to LR, W and EC_{NS} as dependent variables that are model outputs; nevertheless the adopted DSS can provide for data about nutrient and non-nutrient leaching that is important for environmental impact evaluations.

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4 AN EMPIRICAL MODEL TO SIMULATE SODIUM ABSORPTION AND ACCUMULATION IN ROSES GROWING IN A HYDROPONIC SYSTEM¹

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4.1 Abstract

Nutrient modeling currently contributes to the management of hi-tech cultivation system in greenhouse horticultural production. Nevertheless, previous studies to understand the kinetics of nutrient absorption, rarely take into consideration the accumulation of salt ions in the nutrient solution. In this project we develop and validate an empirical model for sodium uptake concentration and sodium accumulation in hydroponic rose (*Rosa* spp. cv. Kardinal) production. Model development and validation was conducted using a series of experiments in both greenhouse and growth chamber climate conditions. The model framework takes into account plant developmental stage and external sodium concentration. While model calibration data were collected at levels of sodium up to 40 mol m³ NaCl as root environment salt concentration, model validation was carried out at lower ranges.

The proposed model not only shows a high predictive capability, but also provides useful output parameters such as EC (electrical conductivity), which is the main parameter currently monitored for managing nutrient solution in greenhouse cultivation. Incorporated as part of a larger DSS, our model can be used to improve nutrient solution management in production regions that do not have economically valid alternatives other than the use of poor quality irrigation water (salt water).

Key words

Rosa spp., sodium absorption, salinity, hydroponic, closed systems.

¹ Scientia Horticulturae, manuscript number: HORTI2190R1

4.2 Introduction

In hydroponic horticultural production, beside the risk of root pathogen infections, nutrient solution management remains the primary difficulty, especially when poor quality water is used. The dilemma between open or closed systems, which was the subject of several studies in previous years (Klaring 2001, Savvas 2002), is no longer an issue with respect to environmental consideration. In order to limit environmental pollution caused by greenhouse productions, upcoming and current restrictions and regulations imposed by numerous governments around the world limit or prohibit the presence of nutrients in discharged irrigation water (Voogt and Sonneveld, 1997). Therefore, in this scenario, the only possibility, both to optimize fertilization and keep environmental contamination under control, is to adopt cultivation systems which collect and reuse the extra irrigation water (closed-loop systems). Hence, closed-loop systems have been declared “environmentally friendly” because they drastically improve the use of water and fertilizer use efficiency as compared with systems allowing drainage water runoff. Nevertheless, an accumulation of ballast ions (Na, Cl, sulphates and others), which occurs when poor quality water is used, may require a partial discharge of the recirculated nutrient solution (semi-closed loop system) (Carmassi et al., 2005). Recently Stanghellini et al. (2004, 2005) have pointed out that the improvement of the water quality (desalinization and rain water collecting) is the most valuable strategy against high salinity; nevertheless other authors have shown that it is possible to improve the water and nutrient use efficiency by using different strategies to manage the recirculated nutrient solution (Raviv et al. 1998). Optimization of nutrient solution management requires knowledge of how nutrient and non-nutrient concentrations change in the system during the cultivation period. In this sense, the simulation of nutrients and non-nutrients absorbed by plants remains a powerful tool to know how the composition of the nutrient solution changes over the time. For this capacity, several models developed for nutrient uptake simulation are integrated into a DSS (Decisional Support System) which can be adopted to manage the nutrient solution in high-technology greenhouses. Most of the nutrient solution controlling systems are currently based on EC (electrical conductivity) control (Savvas and Manos, 1999; Sonneveld, 2000). Because the EC value is a relationship of the total macrocation concentration (Sonneveld, 2000) (K, Ca, Mg and Na), an accurate simulation of the accumulation rate of these elements is required to estimate their concentration in the nutrient solution and eventually to determine the degree to which measured EC is influenced by ballast ions and/or nutrient presence. For this reason, sodium is reported to play an important role for the estimation of the EC in environments in which a high salinity level (NaCl) occurs in the irrigation water source. Currently, most fertigation devices used for nutrient solution preparation and delivery work on

the basis of an imposed EC value threshold, chosen in relation to the response of the crop to salinity in terms of yield and product quality. When the nutrient solution drained from the system is reused, the fertigation system first tests the EC value. The system then provides for a nutrient reintegration if $EC_{Dr} < EC_{Tr}$, where EC_{Dr} is the EC value in the drained nutrient solution and EC_{Tr} is the EC threshold value, or non-reintegration (release of solution) if $EC_{Dr} \geq EC_{Tr}$. With regard to the former, the integration value is directly proportional to $\Delta EC = EC_{Tr} - EC_{Dr}$ where ΔEC is assumed to correspond to the quantity of nutrient absorbed by the crop (Savvas and Manos, 1999). When ballast ions build-up in the recirculated nutrient solution, ΔEC approaches 0 more or less rapidly depending on the uptake rate of non-nutrient ions by the plants and of their concentration in the irrigation water. This can result in a large reduction of nutrients present in the medium growth and even nutrient starvation periods. Although the salinization of large areas in the world is increasing (Rengasamy, 2006; Martinez-Beltran et al., 2005), models developed for nutrient solution management rarely point out how the sodium uptake changes in closed or semi-closed system (Savvas et al, 2005; Carmassi et al., 2005) as a function of the external sodium concentration and/or the cultivation stage.

In this work we present a series of experiments carried out during the summer 2006 to spring 2007 on rose plants (*Rosa* spp. cv. Kardinal) grown in a liquid cultivation system. Data collected were analyzed in order to obtain a calibration and validation of an empirical model. The objective of this work is to provide a simple tool to simulate the absorption and accumulation of sodium in a closed or semi-closed cultivation system in order to obtain an estimation of the contribution of sodium to nutrient solution EC. Knowledge of the composition of the nutrient solution in the root environment, in relation to the electrical conductivity, is essential to optimize the fertilization in the greenhouse environment, to avoid nutrient imbalance in the growth medium and to limit the environmental pollution caused by the waste water. Because roses are generally considered sensitive to salinity (Raviv and Blom, 2001), an investigation on how sodium accumulates in the nutrient solution could be a useful tool for making decisions on nutrient solution management and irrigation water use.

4.3 Materials and Methods

4.3.1 Growing conditions

A series of experiments was carried out on rose plants (*Rosa* spp. cv. Kardinal grafted on “Natal Briar” rootstock) during the summer-autumn 2006 and spring 2007 at the Department of Plant Science, University of California Davis (USA). One year-old plants were grown in a glasshouse where temperature was kept under control by using an automatic cooling and ventilation system.

During the summer the roof was covered with shading compound (shading percentage ca. 50%) to avoid greenhouse warming due to high light intensity. Minimum, maximum and mean daily greenhouse temperature were 21.9, 24.8 and 22.8 °C respectively; minimum and maximum daily greenhouse RAD (global radiation) were respectively 1.7 and 2.9 MJ m⁻² while the mean RAD over the whole summer-autumn period was 2.4 MJ m⁻².

Data used for model development and calibration were collected during a first experiment carried out over the summer-autumn period: plants were moved into a hydroponic liquid medium system 2 months before commencement of the experiment and were left growing, under optimal environment growth conditions (no salinity treatments), during the first cultivation cycle (pruning to harvest of new flower shoots). After the first cycle, salinity treatments commenced and pH and EC data, in the nutrient solution, were collected to monitor the cultivation medium environment. The initial two crop cycles were deemed necessary for plant acclimation to solution culture prior to experiment initiation. Plants were cultivated in 8 L pots (liquid medium) where the oxygenation of the nutrient solution was accomplished by air pumps continuously bubbling air into solution. Nutrient solution concentration of the first treatment was N-NO₃⁻ 10.00; N-NH₄⁺ 2.53; P-PO₄⁻ 1.50; S-SO₄²⁻ 1.95; Cl 2.38; K 4.45; Ca 2.95; Mg 0.83; Na 4.00 mol m⁻³ (EC = 1.9 dS m⁻¹) and micronutrients were added according to Hoagland and Arnon (1950); pH value was 6.0±0.5. NaCl was added in order to obtain the other salinity levels: 16, 28 and 40 mol m⁻³ in which EC values were 3.0, 4.3 and 5.5 dS m⁻¹. After the second crop cycle, plants were divided into four treatments with five replicates for each treatment. Salinity level and nutrient concentrations were kept close to constant by frequent replacement of the entire nutrient solution in containers. In order to avoid high salt concentration, the nutrient solution was replaced whenever the remaining volume was 90% of initial volume (ca. every 6 days). At replacement time, evapotranspiration was measured in terms of lost-water weight and a sample of nutrient solution was collected for each replicate. EC and pH values were measured on the samples collected and an atomic absorption spectrophotometer (Varian SpectrAA Model 55 Atomic Absorption Spectrometer; Varian Inc., Palo Alto, CA, USA) was used to analyze Na⁺ through flame emission. Environmental data were collected during the experiment by datalogger (Cambell 21X datalogger; Campbell Scientific Inc., Logan, UT, USA) which recorded air temperature (°C), relative humidity (%) and solar radiation (PPFD).

Two different experiments were carried out to complete data validation. The first experiment was conducted in growth chambers to have climate conditions different from the greenhouse environment. The scope of this validation was to test the model under condition of relatively high GR. In the growth chambers, temperature was maintained at 24 °C during the light hours

and 20 °C during the dark hours (mean daily temperature was equal to 22.5 °C) while daily GR was 6.46 MJ m⁻². The second experiment was conducted in the greenhouse under climate conditions very close to the first greenhouse calibration experiment (climate data were not significantly different between the two greenhouse periods).

4.3.2 Model development

Absorption of sodium has been estimated by multiplying the sodium uptake concentration rate for the plant water uptake according to Carmassi et al. (2005) and Sonneveld et al. (1999):

$$V_U \cdot C_U = \frac{\text{Ion}}{V_{UR}} \cdot V_U = \text{number of moles absorbed} \quad (4.1)$$

where C_U is the ion uptake concentration rate (mol m⁻³), V_U is the water uptake volume (L), Ion is any ion absorbed as number of moles absorbed over a certain time (t_i) and V_{UR} is the water volume absorbed as liters in the same time (t_i). Then, the concentration of sodium at the end of any cultivation period is calculated by the following formula:

$$[\text{Na}]_n = \frac{[\text{Na}]_{n-1} \cdot V_{n-1} - (C_U \cdot V_U)}{V_n} \quad (4.2)$$

where $[\text{Na}]_{n-1}$ and $[\text{Na}]_n$ are respectively the initial and final sodium concentration, V_{n-1} and V_n are respectively the initial and final nutrient solution volume in the system; then, if a constant volume is imposed in the system, the previous equation becomes:

$$[\text{Na}]_n = [\text{Na}]_{n-1} - \frac{(C_U \cdot V_U)}{V_{NS}} \quad (4.3)$$

where V_{NS} is the volume of the recirculated nutrient solution. The following formula, proposed by Sonneveld (2000), was used to calculate the contribution of the Na⁺ to the EC value in the nutrient solution included in the growth medium:

$$\text{EC} = \sum C^+ \cdot 0.095 + 0.19 \quad (4.4)$$

where C^+ is the macrocations concentration (mol m^{-3}) and EC is the electrical conductivity of the nutrient solution expressed as dS m^{-1} . In order to simulate the C_U of sodium, over the crop cycle, two independent variables were taken into account: GDD (growing degree days) expressed as $^{\circ}\text{C}$ and the sodium concentration in the root zone (mol m^{-3}). Because the ratio between ion uptake and water uptake may be different over the crop cycle, (especially for perennial crops such as rose) the uptake concentration could vary within different plant stages. For this reason an independent variable, which follows the crop cycle such as GDD, is required in order to place the C_U values within the context of the whole cultivation cycle.

GDD were calculated from temperature data collected during the crop cycle and the method used involves the computation of thermal units (GDD), from daily average of temperature values (T_j):

$$\text{GDD} = \sum_{j=1}^r \max[(T_j - T_b), 0] \cdot \Delta t_j \quad (4.5)$$

where Δt_j is the length of the period j (days) and the T_b is the base temperature below which plant development does not occur (Thornley and Johnson, 1990). Base temperature was assumed to be 5.2°C according to Pasian and Lieth (1994). While three different equations were assumed to simulate the C_U over the crop cycle as a function of the GDD, a quadratic equation was assumed to calculate the C_U of sodium in relation to the external sodium concentration (according to a best-fit procedure); then a non-linear regression procedure was run using a statistical software (SAS, 1987) and coefficients were calculated. The equations assumed are specified below:

$$C_U = \begin{cases} a_1 S^2 + a_2 S \dots \text{if} \dots G \leq G_1 \\ b_1 S^2 + b_2 S \dots \text{if} \dots G_1 < G \leq G_2 \\ \lambda_1 S^2 + \lambda_2 S \dots \text{if} \dots G > G_2 \end{cases} \quad (4.6)$$

where S is the sodium concentration in the root zone (mol m^{-3}), G is the cumulative temperature (GDD) at any time expressed as $^{\circ}\text{C}$, G_1 and G_2 are constant values and λ_1 , λ_2 are parameters; the following equations were used to estimate a_1 , a_2 , b_1 and b_2 :

$$a_1 = \alpha_1 G + \alpha_2 \quad (4.7)$$

$$a_2 = \alpha_3 G + \alpha_4 \quad (4.8)$$

$$b_1 = \beta_1 G^2 + \beta_2 G + \beta_3 \quad (4.9)$$

$$b_2 = \beta_4 G^2 + \beta_5 G + \beta_6 \quad (4.10)$$

After replacing equations 4.7, 8, 9, 10, equation 4.6 becomes:

$$C_U = \begin{cases} (\alpha_1 G + \alpha_2) S^2 + (\alpha_3 G + \alpha_4) S \dots \text{if} \dots G \leq G_1 \\ (\beta_1 G^2 + \beta_2 G + \beta_3) S^2 + (\beta_4 G^2 + \beta_5 G + \beta_6) S \dots \text{if} \dots G_1 < G \leq G_2 \\ \lambda_1 S^2 + \lambda_2 S \dots \text{if} \dots G > G_2 \end{cases} \quad (4.11)$$

According to other authors (Yin et al. 2003), since truncated curves, involved in empirical modelling, can show “non-elegant” patterns when they are forced out the calibration range, it was imposed that:

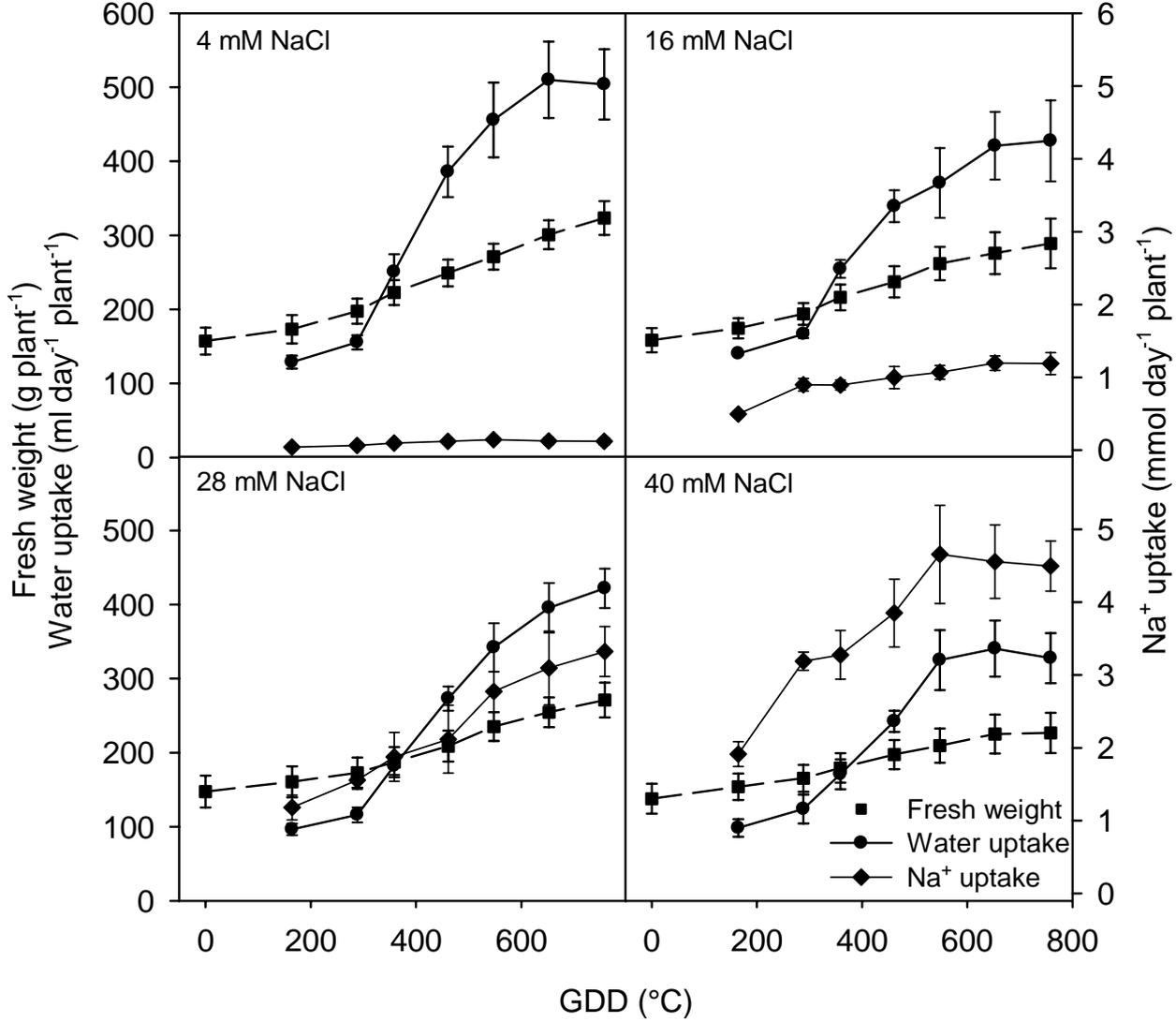
if $G_1 < G \leq G_2$ and $(\beta_1 G^2 + \beta_2 G + \beta_3) S^2 + (\beta_4 G^2 + \beta_5 G + \beta_6) S < \lambda_1 S^2 + \lambda_2 S$ then $C_U = \lambda_1 S^2 + \lambda_2 S$

4.4 Results and Discussion

4.4.1 Model development

In crop simulation models, thermal units are often taken into account as independent “time” variables because they are strongly correlated to plant development. In fact, all biological systems need a certain number of GDD (growth degree days) to complete their own life cycle; therefore, temperature is one of the most important driving variables to estimate plant growth (Thornely and Johnson, 1990). The influence of temperature on the tomato plant has been reported by Van Der Ploeg and Heuvelink (2005) and the computation of thermal units is already involved in several models and DSS such as TOMGRO (Jones et al., 1991), TOMDAT and others. Lieth and Pasion (1991) found a strong correlation between plant development stages and GDD and developed a model to predict the harvest time, for several rose cultivars, as a function of greenhouse mean daily temperature. During our study, the duration of the plant cycle required 758 GDD according to Mattson et al. (2006); this represents harvest about a week later than commercial harvest. The relationship between plant development and GDD is reported in (Figure 4.1). GDD is not directly related to C_U (it is a parameter calculated dynamically over the crop cycle) but it is related to plant development as discussed above. Because C_U is the ratio between the amount of ion absorbed (mmol) in a certain time (Δt_i) and the amount of water absorbed (L) in the same Δt_i for a certain plant stage (Δt_i), then GDD computation is necessary to place a certain C_U within the context of the cultivation cycle.

Figure 4.1. Plant growth, water and sodium uptake versus GDD in the different treatments. Data are from the first greenhouse experiment.



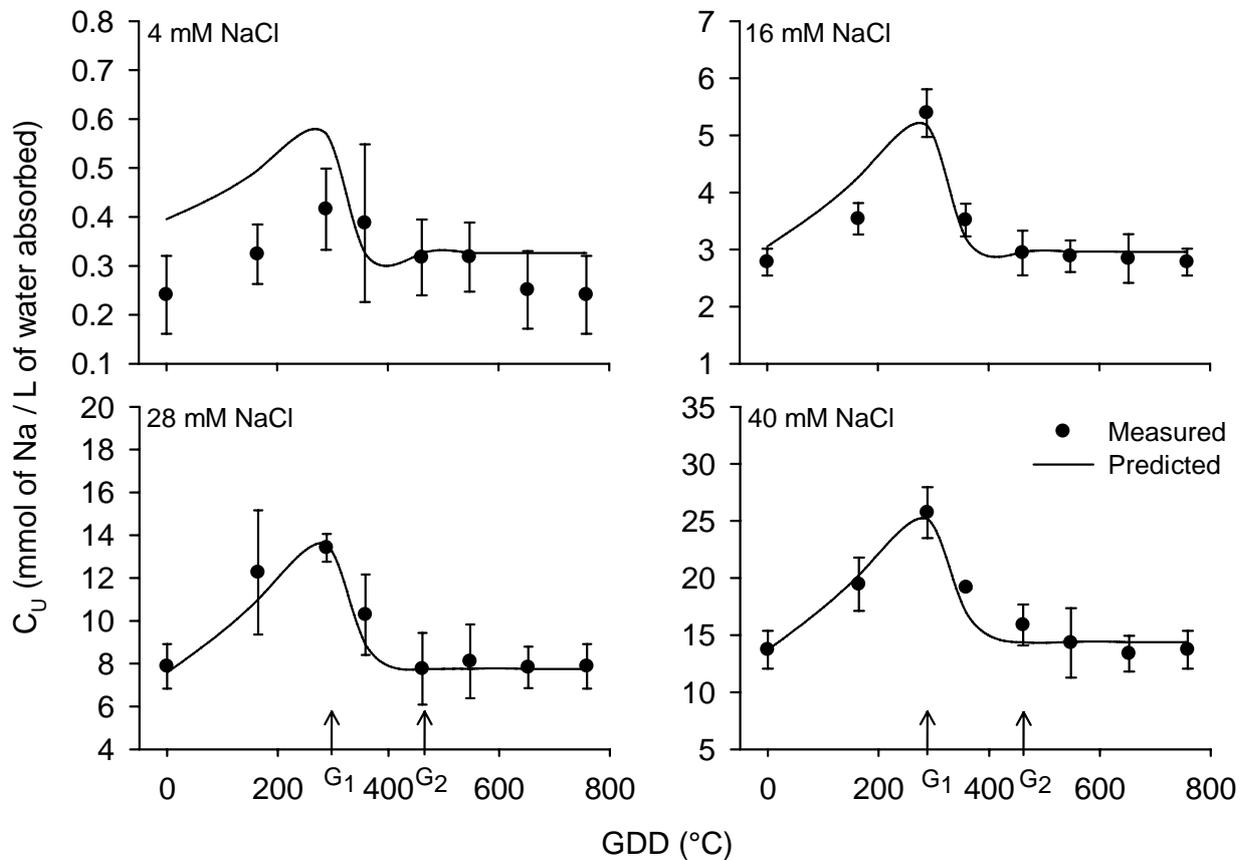
The uptake concentration is a simple parameter previously used for modelling ion uptake, with regard to nutrients and non nutrients, by other authors (Carmassi et al., 2007; Savvas et al. 2005, 2007; Sonneveld 1999). For many species, the uptake concentration becomes stable because the rate of water uptake follows the variations of plant size similarly to the rate of the ion uptake. Therefore, in situations of optimal nutrient concentration, the fluctuation in ion/water uptake is negligible (Savvas et al. 2005). While this behavior is largely accepted for annual plants, different patterns have been found for perennial plants such as rose in which the base of the plant influences variably the absorption of ions during the crop cycle. Hence, the prediction of this parameter is evaluated as uncertain by some authors who found uptake concentration fluctuations during the cultivation cycle (Silberbush and Ben-Asher, 2001). Such fluctuations should be related to plant size variability and environmental conditions.

The sodium uptake concentration calculated for the model (C_U) shows the same pattern in the four different salt treatments, over the whole cultivation cycle (Figure 4.2). In order to draw an equation, which represents the C_U value calculated at a certain developmental phase of the cultivation cycle, two different constant values were found (G_1 , G_2) as showed in Table 4.1 and Figure 4.2; G_1 and G_2 are very close to the initial and final points of the growth curve linear phase. For the first part of the cycle a linear function was assumed; before flower shoot harvest sodium C_U shows a relative low value because of high transpiration due to the large leaf surface of flower shoots. After flower shoot harvest, a rapid rise in sodium C_U value was observed. The pattern is due to a relatively low plant transpiration rate, that occurs soon after pruning/harvest which removes much leaf area. As a result of an enhanced sodium uptake rate the C_U value rises ($G_0 < G < G_1$) until the rate of leaf area development increases rapidly ($G > G_1$) (Figure 4.1, 4.2). During the next phase, a rapid decrease of the ratio between sodium and water absorption occurs; therefore a quadratic equation (Figure 4.2) was assumed over the period $G_1 < G \leq G_2$. Since in the last part of the cycle both the plant water absorption and the plant sodium absorption are stable, a constant relationship between C_U and GDD was assumed for $G > G_2$. The mathematical representation of the C_U pattern is the result of a best fit statistical analysis and it is based on the assumption that the C_U of ions can change during different plant stage (as a result of different transpiration rates coupled with a different ion uptake rate over the crop cycle) as discussed below. While the relationship between plant transpiration rate and environmental parameters is well-known (Carmassi et al., 2005, 2007; Allen et al., 1998; Stanghellini, 1987), it is difficult to understand the physiological implication involved in sodium uptake and the relationships between Na^+ and other ions. Physiologically the influence of K^+ on Na^+ absorption is not well understood. Even though different transporters have been identified for K^+ (HAK) and Na^+ transport (HKT), Horie and Schroeder (2004) and Rodríguez-Navarro and Rubio (2006) report that the function of HKT transporters, as high-affinity Na^+ uptake systems, conflicts with the wheat HKT1 transporter which shows high-affinity K^+ uptake. As explained by Rodríguez-Navarro and Rubio (2006) the versatility of HKT transporters probably lies in their capacity for transporting two cations simultaneously. These descriptions apply when Na^+ and K^+ are present at micromolar concentrations; when the cations are present at millimolar concentrations some of these transporters may uniport Na^+ or K^+ . Tester and Davenport (2003) remark the influence of Ca^{2+} availability and concentration on Na^+ uptake and accumulation. Our results show a progressive increase of sodium uptake, along the crop cycle, likely related to the plant development (Figure 4.1). Summing up, the uptake of sodium can varies during the crop cycle as an effect of the flux/availability of other ions (such us K^+ and Ca^{2+}); nevertheless, in this

experiment we assume that the sodium uptake rate is mainly related to the plant development because nutrient solution concentration was maintained within a constant range over the crop cycle.

With regard to the external sodium concentration, a quadratic function was assumed to calculate the C_U value as a function of the sodium concentration average in the root zone (Figure 4.3); the graph reports the average of sodium C_U during the whole cultivation cycle against the average of the external sodium concentration. Once again, the mathematical representation of the C_U pattern is the result of a best fitting statistical analysis and it is based on the assumption that the C_U of ions can increase by increasing external ion concentration. A similar pattern ($C_U = bx^c + d$) was also observed by Savvas (2005) on cucumber cultivated in a closed-system using polyurethane slabs as a growing system; while Carmassi et al. (2005, 2007) found a linear correlation ($C_U = mx$), between sodium C_U and sodium external concentration, for tomato cultivated in rockwool. This relationship is the result of a higher uptake of Na^+ and lower transpiration at higher sodium external concentrations (Figure 4.3). When the medium contains Na^+ at relatively high concentrations, a passive influx of these ions into the root cells will inevitably occur (Munns, 1985). More recent studies show that HKT transporters enable a passive influx of Na^+ . Amtmann et al. (2001) reports that one of the candidate for Na^+ influx is a non-selective cation (NSC) transporter represented by the wheat protein LCT1 which is known to be permeable a wide range of cation. At root level NSC permits Na^+ influx (Tyerman and Skerrett, 1999; Davenport and Tester, 2000). More specific studies show how sodium influx, mediated by NSC, is positively related to external sodium concentration (Essah et al., 2003). Lorenzo et al. (2000) found that, in rose leaf tissues, the K^+/Na^+ ratio decreased by increasing external sodium concentration as consequence of a higher sodium accumulation. As shown, a larger amount of sodium can be absorbed by increasing the external concentration. Furthermore secondary mechanisms for sodium cell storage and translocation may occur when plants are under a salinity stress condition and significant part of the Na^+ taken up is likely accumulated into the vacuole (Carden et al. 2003). Some authors report a negligible sodium uptake for rose (Sonneveld 2000) cultivated on substrate. Nevertheless, referring to the cultivation system, Shi et al. (2002) found a larger amount of sodium accumulated in hydroponic cultivation compared to turface soil. Furthermore climate conditions and especially different genotypes can greatly increase the accumulation of sodium in the plant; in a recent work focusing on rose rootstocks, Cabrera (2002) reports that Natal Briar rootstock (the same used in this experiment) accumulates more sodium (40%) in comparison with other rose rootstocks.

Figure 4.2. Simulated and measured Na uptake concentration (C_U) versus GDD in the different treatments. Data are from the first greenhouse experiment used for the calibration of the model.

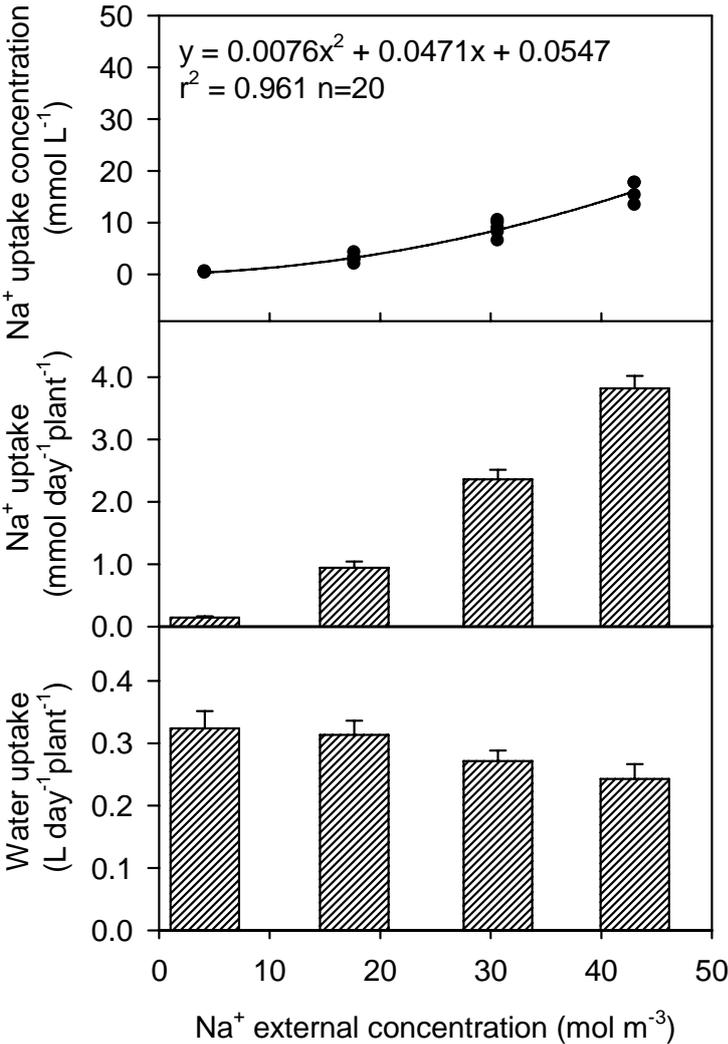


Along with the increase of sodium uptake a lower water uptake determines higher sodium C_U . As well known, salt stresses induce high stomatal resistance which limits transpiration (Figure 4.3) hence the water uptake in rose plants (Raviv and Blom, 2001). In summary higher C_U , in saline environments, is given by a larger sodium uptake coupled with a less water uptake per plant (Figure 4.3).

In modelling horticultural crops several approaches have been proposed for nutrient and non-nutrient absorption Mathieu et al. (1999). Most of them are based on the concept of ion flux (Michaelis-Menten kinetics) (Silberbush and Lieth, 2004), nutrient tissue concentration (nutrient demand strategy) (Mankin and Fynn, 1996) and relative growth rate (relative addition rate) (Hellgren et al., 1996). Such model approaches require the knowledge of plant biomass production which would require destructive plant analyses or models for calculating plant growth (Marcelis et al., 1998). In contrast, the use of the uptake concentration enables the estimation of ion uptake simply by measuring crop water uptake which can be easily measured with a water meter or can be estimated with mathematical models. Although, the concept of

relating ion uptake only to water uptake (C_U) is a simplification that does not base on a mechanistic approach, several researchers have shown its importance and relevance in developing easy-to-use empirical models for greenhouse nutrient solution management (Carmassi et al. 2005, 2007; Savvas et al. 2005, 2007).

Figure 4.3. The influence of the external sodium concentration on sodium uptake, water uptake and sodium uptake concentration (C_U). Data are from the calibration experiment.



The current model is not able to simulate the effect of salinity on plant growth or yield. However data collected showed a pattern of decreasing stem elongation, dry matter production and leaf area by increasing NaCl concentration although the differences were not significant (ANOVA $p=0.05$) between treatments (data not shown). Cabrera (2001) and Cabrera and Perdomo (2003) found no significant differences in terms of dry matter production until 30 mol m⁻³ of NaCl present in the nutrient solution, whereas, Oki and Lieth (2004) found a reduction of the stem elongation, but non significant dry matter variation when short salinity stress periods were imposed on rose plants (cv. Kardinal).

4.4.2 Calibration

The model was calibrated on a total of 152 C_U values measured during the whole cultivation cycle in the four different salinity treatments. Measured data were fit to equation 4.11 using non-linear regression in SAS 9.0 (Statistical Analysis System, SAS Institute Inc., Cary, NC, USA, Version 9.1). In Table 4.1 parameters and constant values used for running the model are reported as well as the main statistical analysis results. The three sub-equations, used for the C_U computation, show a significant capability to fit the experimental data ($P<0.0001$). Analyses, for the three different sub-equations, are reported in Figure 4.4; a sensitivity analysis was calculated by comparing SSQ (sum of squares) as parameters values were changed by steps of -50, -25, 0, +25 and +50 %. In agreement with other authors (Yin et al. 2003), the coefficients of polynomial equations rarely involve parameters which represent biological behaviors or have biological meanings. Furthermore the developed model shows an empirical nature; such statements suggest a mathematical interpretation of the data presented in the Figure 4.4. Thus, parameters found are parts of a mathematical representation of a biological system and all of them are necessary for the correct use of the model. Specifically the model is fairly sensitive to those parameters involved in square terms ($\alpha_1, \alpha_2, \beta_4, \lambda_1$), representing intercept value ($\alpha_2, \alpha_4, \beta_6$) or that take into account a negative x sign (β_2, β_5).

The correlation between predicted data versus actual data is shown in the Figure 4.5. Both predicted Na uptake concentration and predicted EC, versus the actual data, are significantly correlated. Overall the model tends to underestimate EC at the highest values, whereas it tends to overestimate when measured EC is low.

Figure 4.4. Sensitivity analysis for the three different equations used to simulate the sodium uptake concentration during the whole cultivation cycle respectively for the stage GDD=0 to G1, G1 to G2 and G2 to end cycle. Parameters were changed for 0 to 50% of their own value.

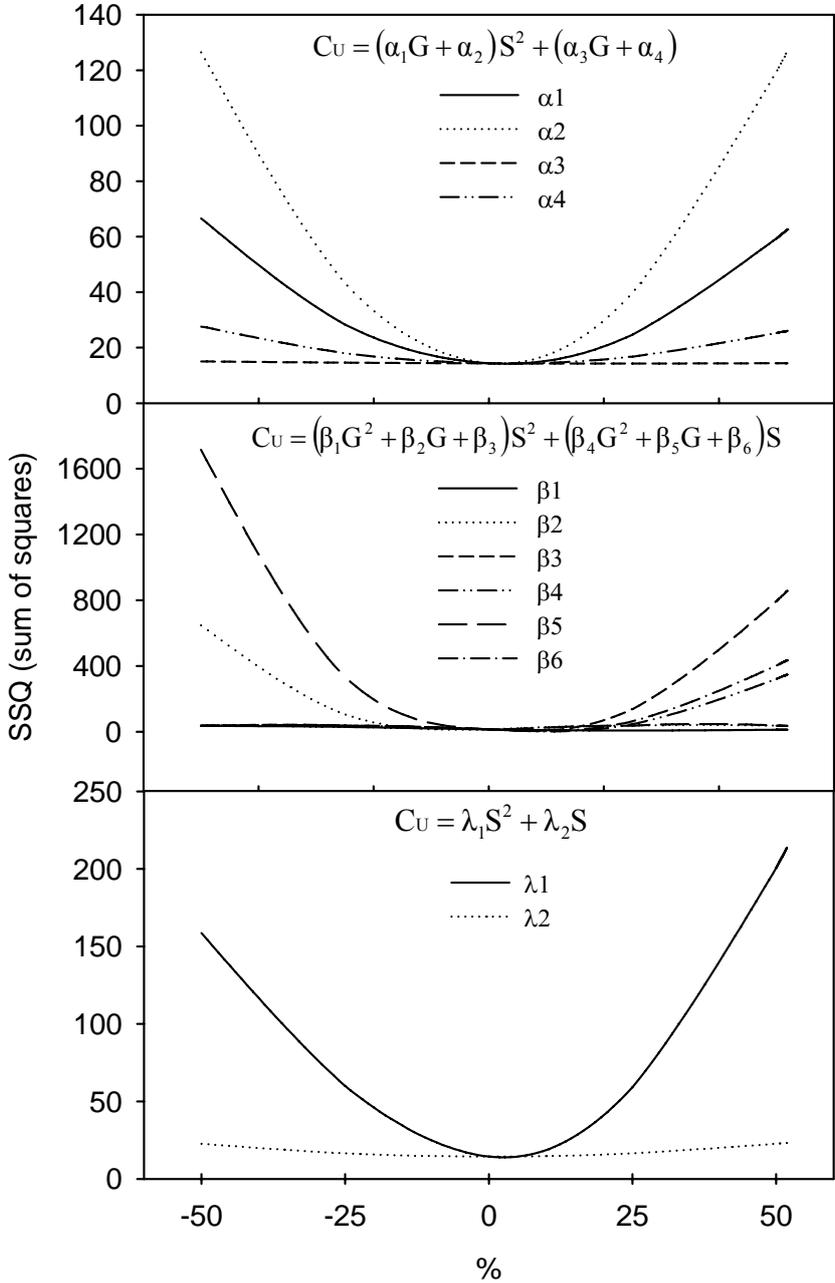
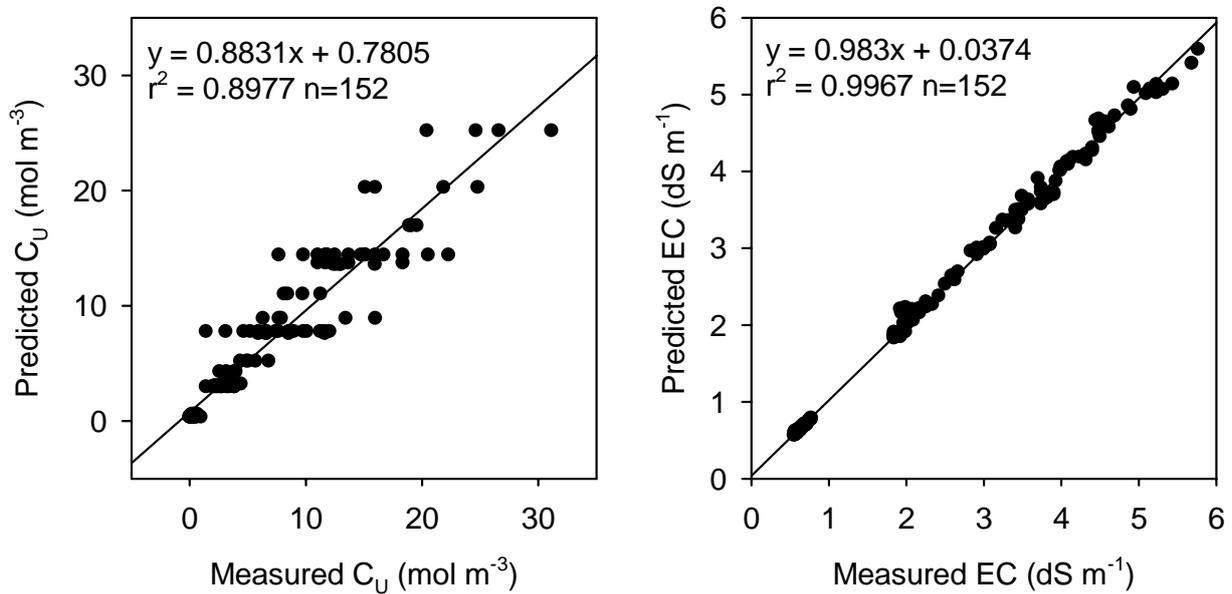


Figure 4.5. The predicted uptake concentration (C_U) and predicted EC value are plotted against the respective measured values. Data are from the first greenhouse experiment used for the calibration of the model.

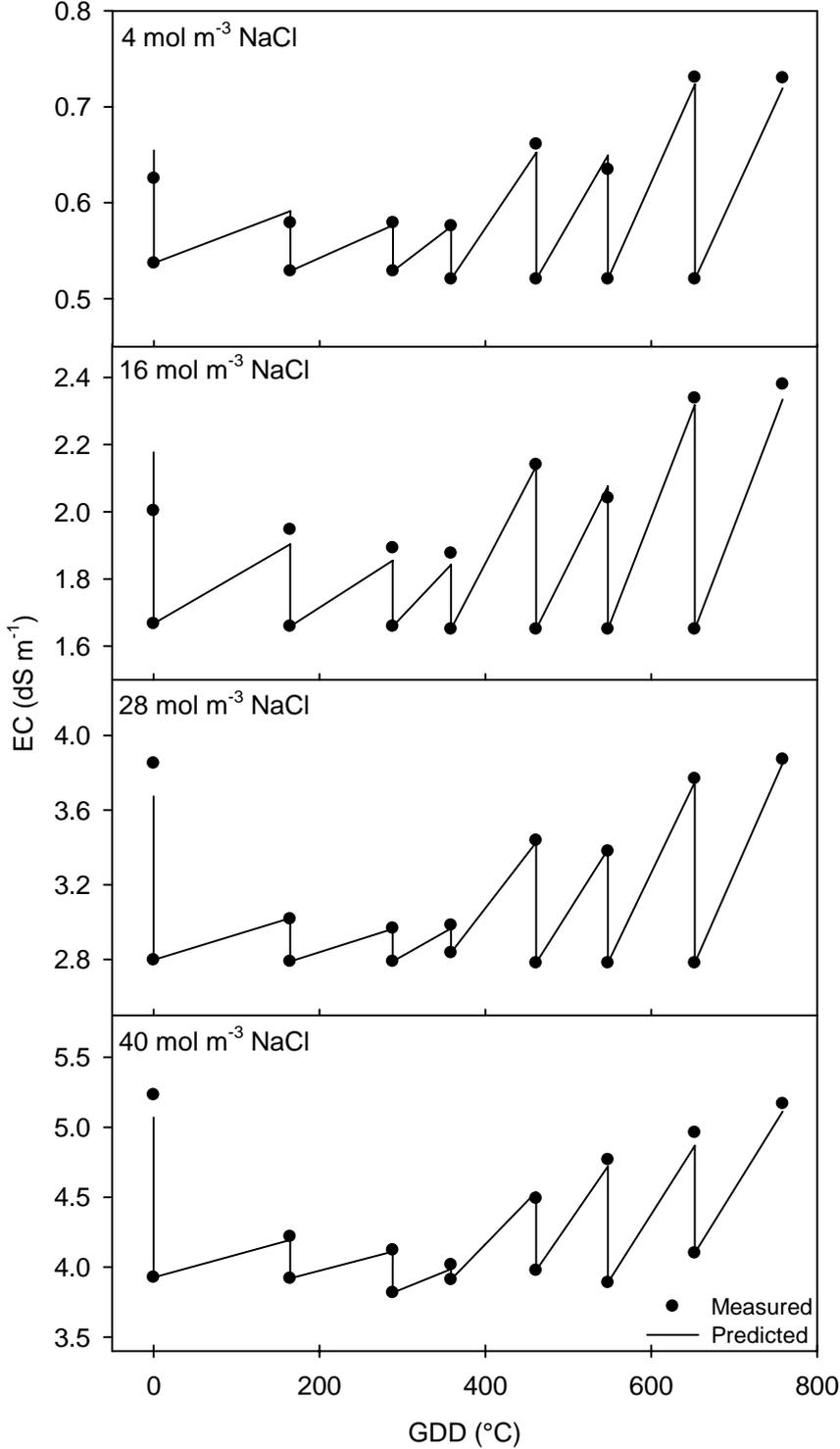


4.5 Simulation and Validation

In order to show the patterns of both predicted and measured values a spreadsheet was built to simulate the C_U (Figure 4.2) and its effect on solution EC (Figure 4.6) against the GDD in the four different treatments. The model was able to predict accurately the C_U value during the cycle, showing the best results for sodium concentration averaging above 4 mol m^{-3} . With regards to the lowest sodium concentration treatment, there was a slight overestimation over the whole cultivation period. This is likely due to the fact that there are not many calibration points for sodium concentrations below 4 mol m^{-3} ; for this reason, the model was forced to zero (no intercept for salinity equation) as showed in equation 4.11. Our assumption is that, when the concentration of the sodium is close to zero, the absorption of sodium is negligible. Moreover at very low concentrations, the contribution of the sodium to the nutrient solution EC value also becomes negligible. In Figure 4.6, the pattern of the sodium concentration in the cultivation system, expressed as the corresponding EC (dS m^{-1}) value, is plotted against the GDD for the different treatments. The graph shows an uneven oscillation due to the frequent solution replacement. This procedure was imposed in order to avoid nutrient depletions and to keep the sodium concentration close to constant. The flush time is easily identifiable in the graph because it occurs whenever the line and the experimental data suddenly drop at the same GDD value.

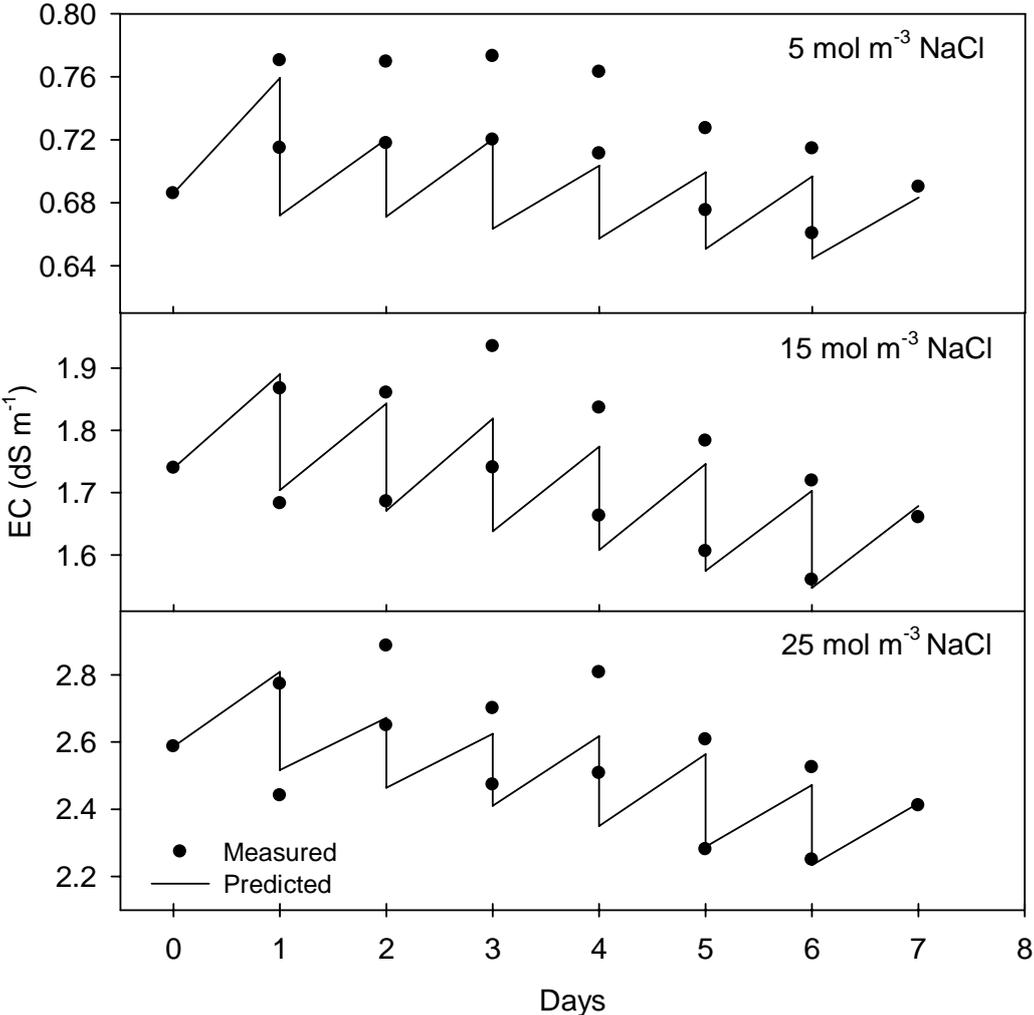
Overall, the simulation well interpolates the experimental data giving an accurate estimation of the EC value for all treatments as already anticipated in the calibration description.

Figure 4.6. Predicted and measured Na⁺ concentration expressed as EC (dS m⁻¹) against GDD in the different treatments. Data are from the first greenhouse experiment used for the calibration of the model. Error bars are not reported to avoid plot overlaps. The spikes of rapid decline in EC represent solution replacement.



Because evapotranspiration is mainly influenced by GR, as showed by Carmassi et al. (2007), it was of interest to evaluate the model under environmental conditions different than the greenhouse climate. To this extent, a growth chamber experiment was used for the model validation. Figure 4.7 shows, for these experiments, a model simulation of EC for one week.

Figure 4.7. Predicted and measured EC against in three different treatments from the growth chamber experiment which had been used for the model validation. Error bars are not reported to avoid plot overlaps. The spikes of rapid decline in EC represent solution replacement.



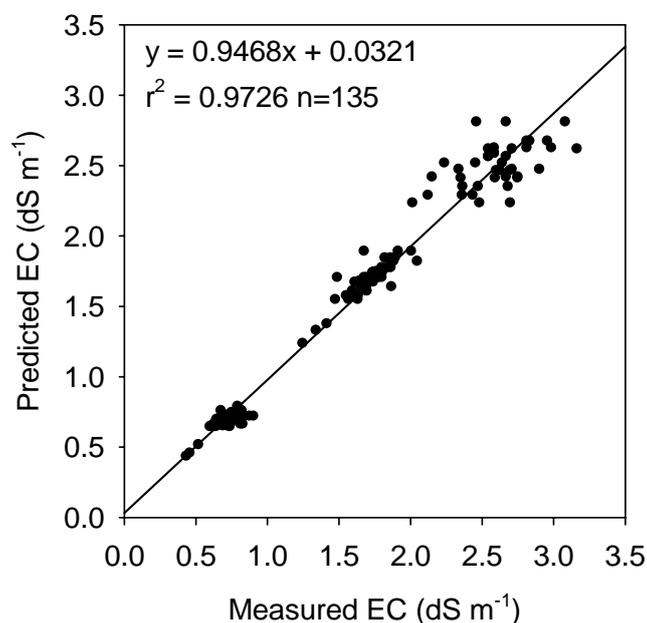
Simulated EC slightly underestimates the measured data. This is due to the high light intensity: when transpiration increases, the model predicts higher sodium consumption and hence a lower sodium accumulation which results in a lower EC value in the remaining nutrient solution. However, the data simulated show a similar pattern with the measured EC and the interpolation results are reasonably acceptable. The measured and predicted Na and EC levels in the nutrient solution are shown in Table 4.2 for the validation experiment conducted in the greenhouse in spring 2007). Simulated data show a high capability to estimate sodium uptake and thus to

predict the EC value at the end of the experimental period. Even though sodium concentration, in the nutrient solution, is below the lowest concentration level used for the calibration of the model, data collected show good fitting between predicted and measured data. This confirms that, the mathematical strategy of forcing to zero the quadratic equation (proposed to represent the relationship between C_U and external sodium concentration) was helpful. Finally, Figure 4.8 reports all predicted data against measured data originated from the validation experiments in terms of EC contributed by Na^+ . The regression still shows a slight underestimation of the data and a small positive intercept was found. Overall the model showed a satisfactory capability in the validation experiment to interpolate and predict the sodium uptake concentration and hence the contribution of sodium to the EC increases in the nutrient solution remaining in the root zone.

Table 4.2. Predicted and measured Na concentration and EC values for the second greenhouse experiment used for the model validation.

[Na] average in the system (mol m ⁻³)	[Na] at the time n-1 (mol m ⁻³)	Measured [Na] at the time n (mol m ⁻³)	Predicted [Na] at the time n (mol m ⁻³)	Measured EC at the time n (dS m ⁻¹)	Predicted EC at the time n (dS m ⁻¹)
2.5	2.0	3.0 ± 0.3	2.9	0.47 ± 0.03	0.47
5.0	4.0	6.0 ± 0.2	5.9	0.76 ± 0.02	0.75
10.0	8.0	12.1 ± 0.5	11.8	1.34 ± 0.05	1.31

Figure 4.8. Predicted EC values are plotted against the respective measured values. Data are from the second greenhouse experiment and the growth chamber experiment used for the validation of the model



4.6 Conclusion

To our knowledge there are no existing reports on the sodium uptake during the course of an entire rose crop cycle and therefore our results are pioneering in this crop.

Although a few authors report negligible sodium uptake for rose (Sonneveld 2000), nevertheless cultivation systems (Shi et al., 2002), climate conditions and especially different genotypes can greatly increase the accumulation of sodium in the plant (Cabrera 2002). As other studies show, generally, the increase of sodium concentration in the medium is positively related to plant sodium absorption (Savvas et al., 2005, 2007; Carmassi et al., 2005; Essah et al., 2003; Lorenzo et al., 2000). This behavior can affect EC estimation of the recycling nutrient solution and ultimately the management of the nutrient solution. Therefore, it becomes important to have an idea on how sodium concentration affects EC value in order to keep under control the nutrient concentration in the recirculated nutrient solution by optimizing the fertigation system.

In recent years EC level, in the nutrient solution, has become a parameter of interest to manage hydroponic systems for rose cultivation (Baas and van den Berg, 1999; Brun et al., 2001). Depending on nutrient solution replenishment strategies and procedures as well as water quality (Pardossi et al., 2006; Raviv, et al., 1998), the contribution of sodium to salinity and finally to EC value can represent a chief component of the total nutrient solution EC during the crop cycle; to this extent the model can be an useful tool for growers in making decision. Moreover the use of simplified empirical models does not require a background in high-technologies.

In summary, the empirical model we presented predicts well the concentration of sodium in the recirculated nutrient solution. The integration of this tool in a nutrient solution DSS may allow further optimization of nutrient and salinity management with regard to plant sodium accumulation.

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5 MODELLING NUTRIENT UPTAKE IN ROSES GROWN IN CLOSED-LOOP SOILLESS SYSTEM

5.1 Introduction

In the past roses have been usually labeled as salinity-sensitive plants (Hughes and Hanan, 1978). Nevertheless soilless conditions (Bass and van den Berg, 1999) and different strategies of nutrient solution management (Raviv et al., 1998) have showed improvements in salinity tolerance of roses. Further studies suggested that relatively high salinity can be tolerated in rose cultivation without significantly or minimally affecting flower yield and quality (Cabrera, 2001; Cabrera and Perdomo, 2003). Therefore there is a need of reassessing salinity tolerance in rose greenhouse cultivation (Cabrera and Perdomo, 2003).

In hydroponic horticultural production, beside the risk of root pathogen infections, nutrient solution management remains the primary difficulty, especially when poor quality water is used (Carmassi et al., 2007). The optimization of nutrient solution management requires knowledge of how nutrient and non-nutrient (“ballast ions”) concentrations change in the system during the cultivation period. In this sense, the simulation of nutrients and non-nutrients absorbed by plants remains a powerful tool to know how the composition of the nutrient solution changes over the time. For this capacity, several models developed for nutrient uptake simulation are integrated into a DSS (Decisional Support System) which can be adopted to manage the nutrient solution in high-technology greenhouses. As an example, most of the nutrient solution controlling systems are currently based on EC (electrical conductivity) control (Savvas and Manos, 1999; Sonneveld, 2000). Because the EC value is a relationship of the total macrocation concentration (Sonneveld, 2000) (K, Ca, Mg and Na), an accurate simulation of the absorption and eventually the accumulation or depletion rate of these elements is required to estimate their concentration in the nutrient solution. Although the salinization of large areas in the world is increasing (Rengasamy, 2006; Martinez-Beltran et al., 2005), models developed for nutrient solution management rarely point out how the uptake of nutrients and non-nutrients changes in closed or semi-closed soilless culture (Savvas et al, 2005; Carmassi et al., 2005) as a function of the external salt concentration (NaCl) during the cultivation cycle.

In this work we present an experiment carried out during the summer 2006 to spring 2007 on rose plants (*Rosa* spp., cv. Kardinal) grown in a liquid cultivation system. Collected data were analyzed and fitted in order to obtain a calibration of a series of empirical models. Hence, the

objective of this work is to provide a simple tool to simulate the absorption of nutrient and non-nutrient (Na^+) for rose plants.

5.2 Materials and methods

5.2.1 Growing conditions

The experiment was carried out on rose plants (*Rosa* spp. cv., Kardinal grafted on “Natal Briar” rootstock) during the summer-autumn 2006 and spring 2007 at the Department of Plant Science, University of California Davis (USA). One year-old plants were grown in a glasshouse where temperature was kept under control by using an automatic cooling and ventilation system. During the summer the roof was covered with shading compound (shading percentage ca. 50%) to avoid greenhouse warming due to high light intensity. Minimum, maximum and mean daily greenhouse temperature (T) were 21.9, 24.8 and 22.8 °C respectively; minimum and maximum daily greenhouse RAD (indoor global radiation) were respectively 1.7 and 2.9 MJ m⁻² while the mean RAD over the whole summer-autumn period was 2.4 MJ m⁻².

Plants were moved into a hydroponic liquid medium system 2 months before commencement of the experiment and were left growing, under optimal environment growth conditions (no salinity treatment), during the first cultivation cycle (pruning to harvest of new flower shoots). After the first cycle, salinity treatments commenced and pH and EC (Electrical Conductivity) in the nutrient solution were collected to monitor the cultivation medium environment. The initial two crop cycles were deemed necessary for plant acclimation to solution culture prior to experiment initiation. Plants were cultivated in 8 L pots (liquid medium) where the oxygenation of the nutrient solution was accomplished by air pumps continuously bubbling air into solution. Nutrient solution concentration of the first treatment was N- NO_3^- 10.00; N- NH_4^+ 2.53; P- PO_4^- 1.50; S- SO_4^{2-} 1.95; Cl 2.38; K 4.45; Ca 2.95; Mg 0.83; Na 4.00 mol m⁻³ (EC = 1.9 dS m⁻¹) and micronutrients were added according to Hoagland and Arnon (1950); pH value was 6.0±0.5. NaCl was added in order to obtain other salinity levels: 16, 28 and 40 mol m⁻³ in which EC values were 3.0, 4.3 and 5.5 dS m⁻¹. After the second crop cycle, plants were divided into four treatments with five replicates for each treatment. Salinity level and nutrient concentrations were kept close to constant by frequent replacement of the entire nutrient solution in the containers. In order to avoid high salt concentration, the nutrient solution was replaced whenever the remaining volume was 90% of initial volume (ca. every 6 days). At replacement time, evapotranspiration was measured in terms of lost-water weight and a sample of nutrient solution was collected for each replicate.

5.2.2 Analysis and modelling

EC and pH values were measured in the samples collected and K^+ , Ca^{2+} , Mg^{2+} and Na^+ were analyzed through flame emission by means of an atomic absorption spectrophotometer (Varian SpectrAA Model 55 Atomic Absorption Spectrometer; Varian Inc., Palo Alto, CA, USA). Nitrogen content was analyzed by diffusion conductivity method with a Timberline Model TL200 Ammonia/Nitrate Analyzer (Timberline Instruments L.L.C., Boulder, CO, USA) whereas chloride was analyzed by using an ion-specific sensor (Cole-Parmer chloride electrode, Cole-Parmer Instrument Company, IL, USA). Phosphorus analysis was accomplished by stannous chloride colorimetric method with a Brinkmann PL800 colorimeter (Brinkmann Instruments, West Bury, NY, USA).

Environmental data were collected during the experiment by datalogger (Cambell 21X datalogger; Campbell Scientific Inc., Logan, UT, USA) which recorded air temperature ($^{\circ}C$), relative humidity (%) and solar radiation (PPFD).

Moreover, at each nutrient solution sampling, plant weight, stem length as well as LA (leaf area) were measured.

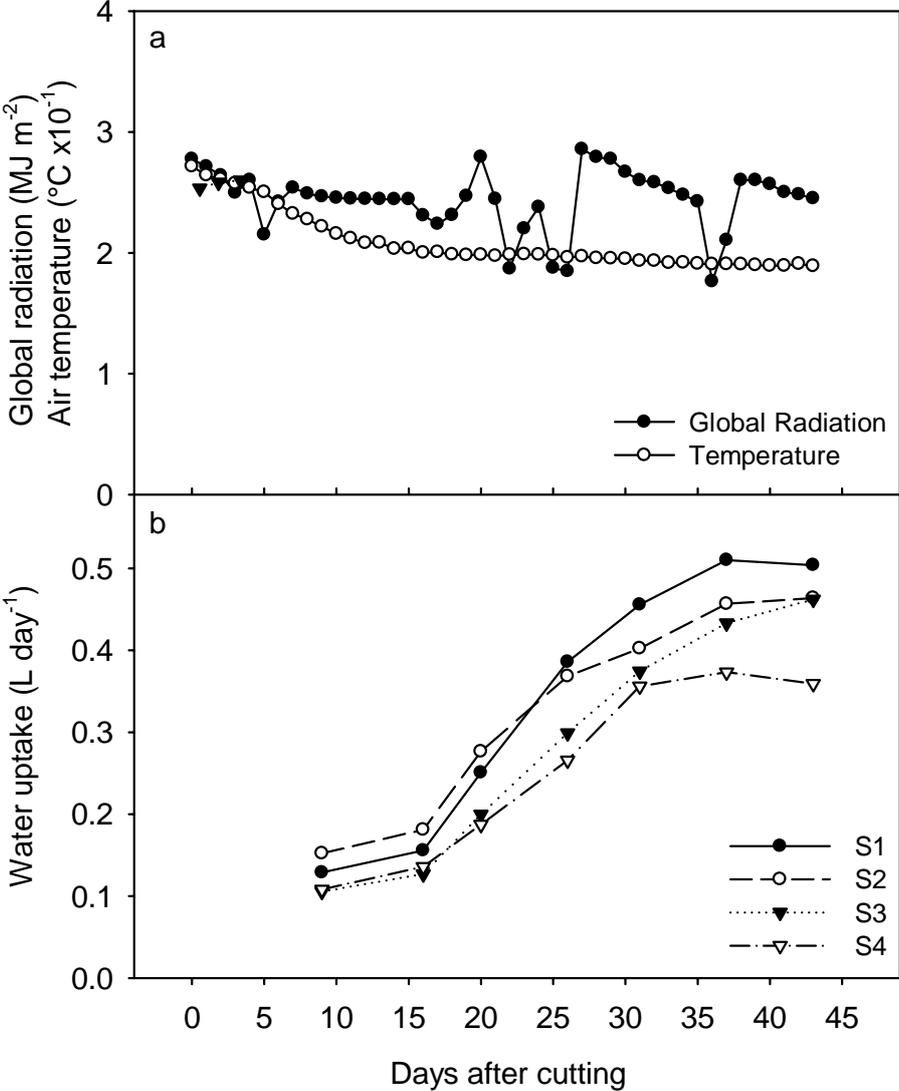
Experimental data were analyzed in order to establish possible correlations between environmental and biologic variables such as FW, GR, SE, W-R_U, [NaCl] (see abbreviations for details) and nutrient/non-nutrient absorption. Then a multiple regression analysis (forward stepwise method, F -value ± 4) was used for model calibration..

5.3 Results and discussion

Climate data are reported in Figure 5.1a where daily-averaged RAD and T show a light tendency to decrease approaching the colder season. Although sharp oscillations of RAD values were sporadically detected during cloudy days, overall the homogeneity of T and RAD let us suppose that climate conditions enabled a regular plant growth and development (Figure 5.2). As well known, while RAD influences productivity and product quality, T is the main factor influencing plant cycle duration (Lieth and Pasian, 1991). During our study, plant cycle required 758 GDD according to Mattson et al. (2006); this represents harvest about a week later than commercial harvest.

W-R_U increased along the time (Figure 5.1b) showing a typical sigmoidal pattern due to LA (Leaf Area) expansion (data not shown) and plant development (Figure 5.2a and 5.2b). However, although saline treatments did not influence significantly final LA (ANOVA, $P < 0.05$) (data not shown), water uptake resulted significantly reduced by NaCl treatments (Figure 5.1b) as an effect of the lower osmotic potential (more negative) (Munns, 2002; Parida and Das, 2005).

Figure 5.1. Climate data recorded during the cultivation cycle are showed in the graph 5.1a; while graph 5.1b shows plant water uptake rate plotted against time. The different saline treatments affected negatively water uptake determining a significant reduction in the highest salinity level (ANOVA, $P < 0.05$). Error bars are not reported to avoid line overlaps.



According to Cabrera et al. (1995), SE showed its maximum rate in the middle of the flowering cycle which corresponds to the linear phase of plant growth (Figure 5.2a and 5.2c). NaCl treatments did not affect neither stem elongation nor stem length but it caused a reduction of stem diameter (data not shown) likely due to the lower amount of water taken up by salt-stressed plants (Figure 5.1b).

GR followed the same pattern of SE with the difference that two main peaks occurred (Figure 5.2d). The first one corresponds to maximum SE (ca. 20 days after cutting) while the second one (ca. 33 days after cutting) is likely due to the large amount of water accumulated into the flower

tissues for blooming. NaCl treatments influenced significantly (ANOVA, $P < 0.05$) GR and fresh weight as it appears in Figure 5.2b and 5.2d. This behavior, apparently in contrast with SE pattern, is likely explained by the lesser amount of water taken up by salinity-stressed plants (Figure 5.1b); such hypothesis would be confirmed by the positive correlation between dry matter percentage and salinity level (data not shown). (Munns, 2002; Parida and Das, 2005).

Figure 5.2. Influence of different saline treatments on stem length (5.2a), cumulated fresh weight (5.2b), stem Elongation (5.2c) and growth rate (5.2d). Saline treatments negatively effected growth rate and fresh weight without influencing neither stem length nor stem elongation (ANOVA, $P < 0.05$). Error bars are not reported to avoid line overlaps.

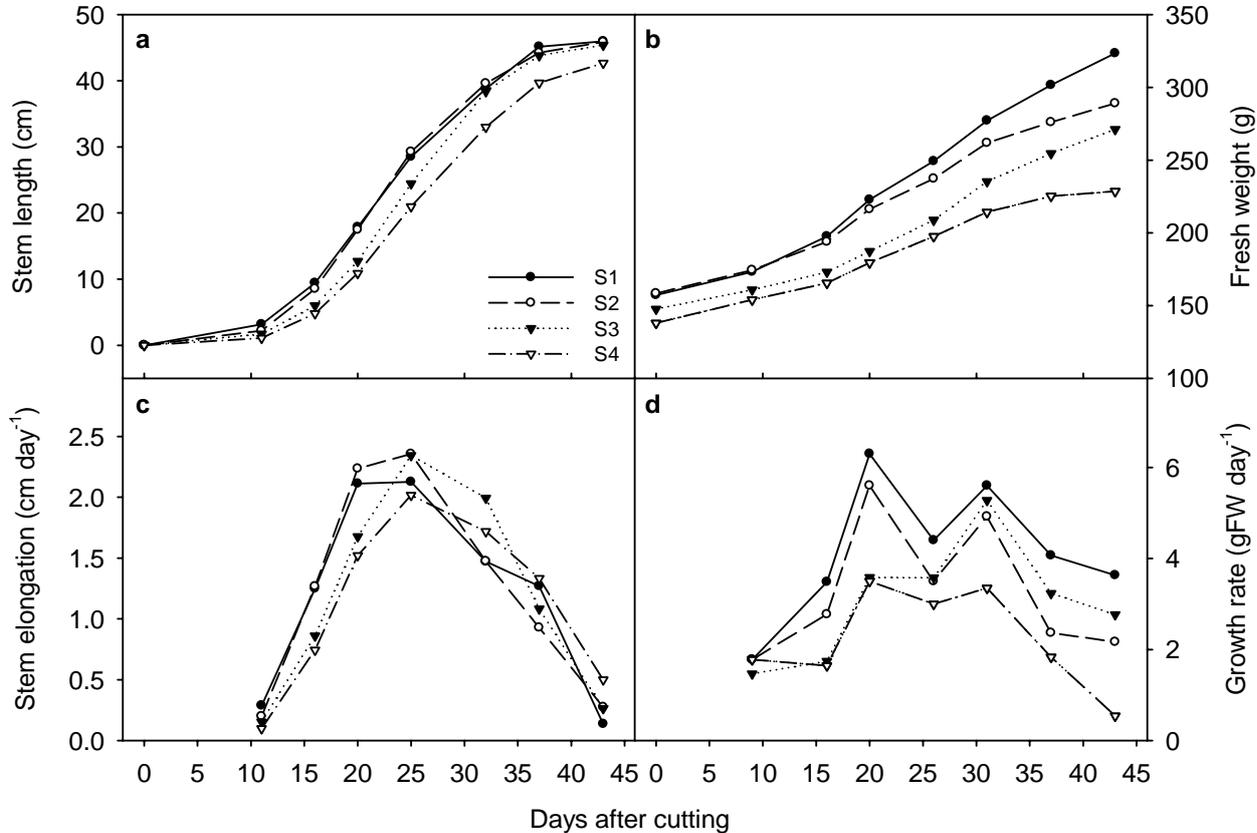
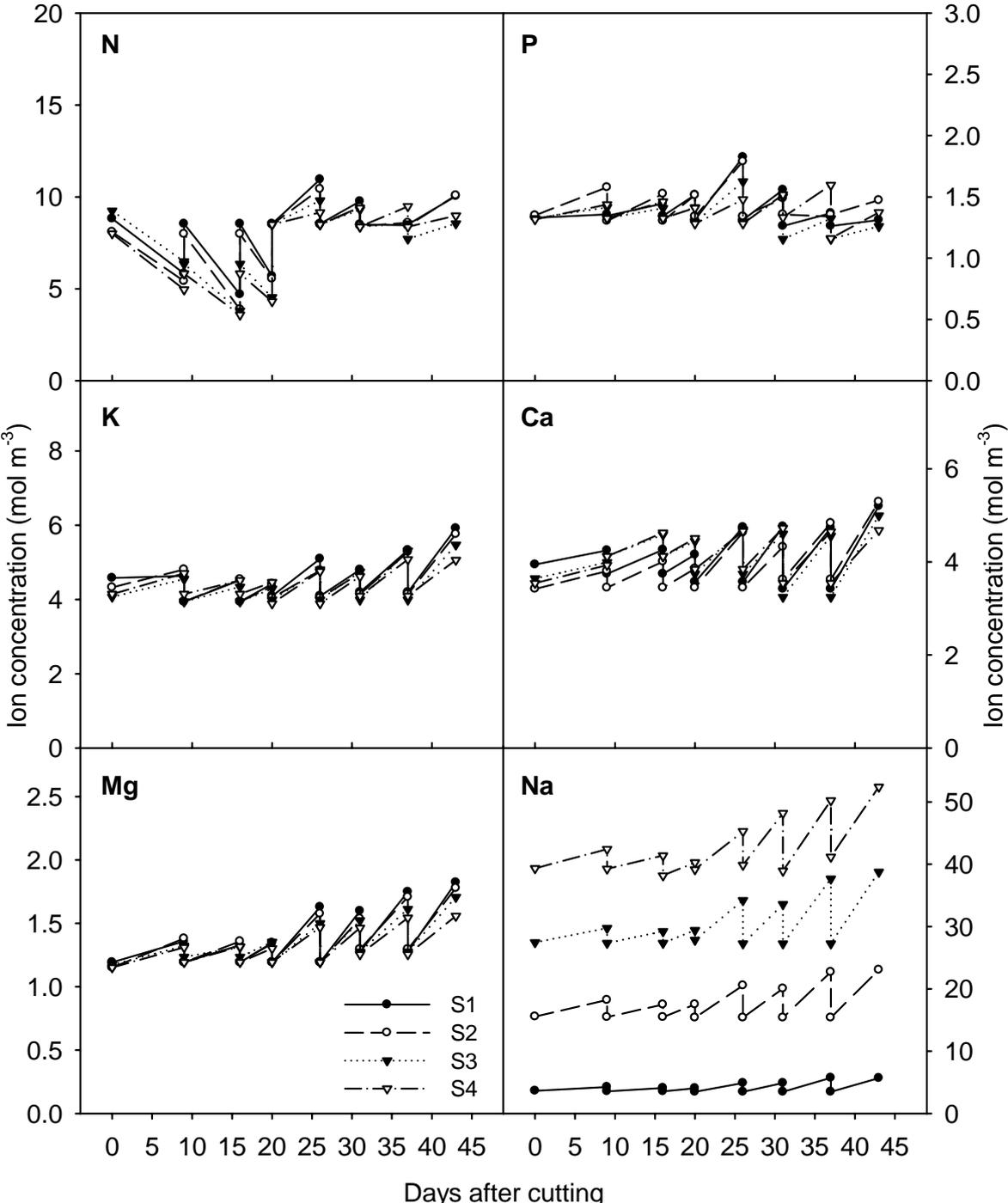


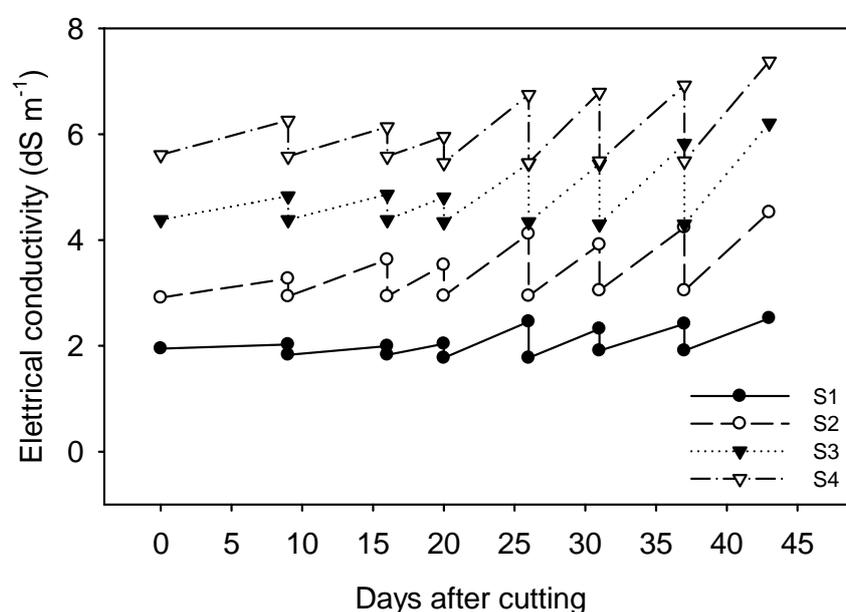
Figure 5.3. Nutrient and non-nutrient concentration in the system during the whole crop cycle. Sudden variations at the same time represent nutrient solution replenishments.



During the experiment frequent replenishments (ca. 6 days) of the nutrient solution were imposed to accomplish two essential experimental goals. The first one was obviously to collect as more as possible samples in order to determine accurately how the ion uptake rate changes over the entire flowering cycle. The second one deals with possible variations of ion concentrations that may occur in a system with variable volumes. In fact, ion concentration, in

such systems, can vary depending on both plant water uptake and ion absorption. Since nutrient and non-nutrient concentration may largely influence ion and water uptake kinetics (Le Bot, 1998; Parida and Das, 2005), therefore frequent nutrient replenishments accomplished the goal of keeping constant the ion concentration at root level. Overall ion concentration tended to increase in the system during the time (Figure 5.3). This pattern was likely due to an overestimation of the nutrient solution concentration that resulted higher than ion uptake concentration (the ratio between ion and water absorbed in the same period) (Sonneveld, 2000; Carmassi et al., 2007; Savvas et al., 2007). In effect, ion uptake concentration was lower than expected causing ion accumulation in the nutrient solution. This tendency was more marked for Ca^{2+} and Mg^{+} than K^{+} and P. On the contrary nitrogen concentration varied with a different pattern during the flowering cycle showing an initial decrease until the middle of the flowering cycle, then a constant value occurred. However no evidence of significant differences was detected among the different saline treatments. This is likely due to the fact that salinity influenced at the same levels both nutrient and water absorption. On the contrary Na^{+} concentration ranged more or less widely depending on the saline treatments. The evidence of ion accumulation in the nutrient solution was also given by EC value which showed an evident tendency to increase during the time (flowering cycle) (Figure 5.4). Since EC value is mainly related to cationic sum (Sonneveld, 2000), hence EC oscillation was mainly influenced by sodium concentration rather than nutrient concentration. The oscillation detected let us suppose that nutrient concentration could not affect ion absorption or plant growth and development.

Figure 5.4. Influence of different saline treatments on EC (Electrical Conductivity) patterns. Sudden variations at the same time represent nutrient solution replenishments.



Ion uptake for rose plants consists in a complex phenomenon that involves many internal and external variables. Cabrera et al. (1995) remarked the importance of carbohydrates availability for nutrient uptake finding a negative correlation between SE and nutrient influx. A strong correlation has been found between N-R_U and W-R_U by Brun and Chazelle (1996). Mattson et al. (2006) simulated nutrient uptake as a function of tissue and external nutrient concentration. Cyclic patterns of nutrient absorption have been related to growth variables as well as climate variables during the entire cultivation cycle (Silberbush and Lieth 2004) and nonetheless during a single day (Bougoul et al. 2000).

Nutrient absorption modelling was based on a series of variables that represent environment factors as well as plant development. Correlations between such independent variables and ion absorption are summarized as a correlation matrix in Table 5.1 where correlation coefficients (*R*) and *P*-values are reported for each combination of variables.

Table 5.1. Correlation matrix resulting from the analysis of variables. To each combination (n=133) of variables correspond its own correlation coefficient (*R*) and *P*-value

		FW	GR	SE	W-R _U	[NaCl]
GR	<i>R</i>	0.3282				
	<i>P</i> -value	0.0001				
SE	<i>R</i>	0.0003	0.4832			
	<i>P</i> -value	0.9975	0.0000			
W-R _U	<i>R</i>	0.7980	0.4075	0.1139		
	<i>P</i> -value	0.0000	0.0000	0.1918		
[NaCl]	<i>R</i>	-0.2343	-0.3042	-0.0628	-0.1311	
	<i>P</i> -value	0.0066	0.0004	0.4729	0.1325	
K-R _U	<i>R</i>	0.6323	0.3285	0.0531	0.8241	-0.2784
	<i>P</i> -value	0.0000	0.0001	0.5436	0.0000	0.0012
Na-R _U	<i>R</i>	-0.0122	-0.2148	0.0355	0.0711	0.8652
	<i>P</i> -value	0.8892	0.0130	0.6848	0.4160	0.0000
Ca-R _U	<i>R</i>	0.5097	0.3662	0.0762	0.6182	-0.2171
	<i>P</i> -value	0.0000	0.0000	0.3830	0.0000	0.0121
Mg-R _U	<i>R</i>	0.6762	0.1099	-0.1936	0.7960	-0.0447
	<i>P</i> -value	0.0000	0.2081	0.0256	0.0000	0.6097
N-R _U	<i>R</i>	0.0565	0.3373	-0.0013	-0.0969	-0.2973
	<i>P</i> -value	0.5182	0.0001	0.9882	0.2671	0.0005
N-C _U	<i>R</i>	-0.4563	-0.1220	-0.2244	-0.7284	-0.1155
	<i>P</i> -value	0.0000	0.1618	0.0094	0.0000	0.1857
P-R _U	<i>R</i>	0.5981	0.2185	-0.2609	0.6890	-0.1222
	<i>P</i> -value	0.0000	0.0115	0.0024	0.0000	0.0012

Nutrient uptake (rate and concentration), of all investigated nutrients and sodium, is reported in Figures 5.5-5.10 respectively for N, P, K, Ca, Mg Na. Next to nutrient uptake, in the figures we can see represented water uptake and growth rate as well; this is because these variables resulted in a high correlation with nutrient and non-nutrient absorption (Table 5.1). N uptake (Figure 5.5) shows a low correlation with both $W-R_U$ and GR with regard to the $N-R_U$ while $N-C_U$ results in a visible negative correlation with water uptake. According to Cabrera (2005) the adsorption of N is strongly influenced by carbohydrates availability; moreover requirements of this nutrient are influenced by the nutrient balance resulting between shoots and base part of the plant. According to this author we found a negative correlation between N absorption and GR. Unlike $P-R_U$ (Figure 5.6) shows a pattern that can be roughly represented by $W-R_U$ while $P-C_U$ is better correlated to GR. The same was observed for the other nutrients Figure 5.7-5.9 while Na (Figure 5.10) seems not to show any correlation between GR and $Na-R_U$ or $Na-C_U$.

Although what said suggests correlations between nutrient uptake rate and water uptake and between nutrient uptake concentration and growth rate, the analysis of correlation among the considered variable did not show any strong correlation if nutrient uptake concentration was considered as dependent variable. The only high correlation was found for $N-C_U$. Because of the low correlations found between independent variables and nutrient and non-nutrient uptake concentration, in Table 5.1 we report a matrix which considers just nutrient uptake rate as independent variable for most of the nutrients and Na, while N absorption is represented by the two investigated dependent variables. In the matrix the highest values, expressed as correlation coefficients, can be found between nutrient uptake and water uptake. Although to couple water uptake and nutrient uptake has been object of several debates in the past (Le Bot et al., 1998), nutrient uptake concentration has been addressed as a suitable concept for modelling the nutrient uptake of several species (Sonneveld, 2000, Carmassi et al., 2005 Savvas et al., 2007). In effect several procedures to replenish nutrient solution have been proposed in recent years, most of them are coupled with water uptake (van Kooten et al., 2004) and EC control (Savvas and Manos, 1999). On the other hand in the practice of soilless culture, the frequency of delivery of nutrient solution done to the crop is determined almost solely by plant water demand estimated from incoming solar radiation (Le Bot et al. 1998; Carmassi et al. 2007). As a matter of fact water uptake is a flux that can contribute to supply nutrients to plants (soil cultivation) and facilitates within-plant nutrient transport.

Significant correlations were found between nutrient absorption and GR for all investigated ion while FW appeared strongly correlated to K, Ca, Mg and P uptake rate. $N-R_U$ did not show any significant correlation with FW; on the contrary, $N-C_U$ resulted in a strong correlation with FW.

As water uptake can help ionic influx, GR and FW can represent the requirement of nutrients on the basis of the concept of nutrient demand (Le Bot et al., 1998; Mathieu et al., 1999). In other words, plants need nutrients for growing, hence nutrients are generally absorbed as a function of plant growth.

Nutrient absorption showed negative correlations with NaCl external concentration (Table 5.1). Nevertheless Mg absorption seemed to be not significantly correlated to NaCl. However increases in salinity are not always associated with decreases in Mg absorption (Grattan and Grieve, 1999). On the contrary, as expected, the absorption of Na showed a strong positive correlation with its external concentration. Under salinity stress and particularly in presence of NaCl plants tend to reduce nutrient uptake activity (Grattan and Grieve, 1999; Parida and Das, 2005). This is due to many physic and biological factors (Munns, 2002). NaCl limits nutrient absorption in different way: a) directly, by uptake inhibition and ionic antagonisms (Grattan and Grieve 1999) indirectly as a consequence of reduced growth and water uptake (Parida and Das, 2005). Lorenzo et al. (2000) found a significant reduction in nitrogen and potassium uptake and their tissue concentration in rose plants growing in saline root environment (NaCl).

Experimental data were fitted by using a multiple linear regression with stepwise forward method. The results of such analysis are reassumed in Table 5.2. Proposed models explain the variability of predicted data ranging between 0.22 and 0.78 as R^2 . Two different equations are proposed for N absorption; this was because the model that took into account N uptake concentration, as a dependent variable, showed an higher capability in predicting data compared to the model that took into account N uptake rate (Table 5.2). In effect, the former was able to greatly improve R^2 giving evidence of a good interpolation of experimental data as reported in Figure 5.11. Nevertheless, the spot produced by predicted data versus measured data let us suppose some model deficiencies, highlighting an improper simulation of lower values. Moreover the regression line shows an overestimation of low values (positive intercept) with a tendency to underestimate higher values (slope lower than 1). As N adsorption, the models proposed for P and Ca resulted in the lowest R^2 when the uptake was considered as uptake rate. Nevertheless, contrarily to N uptake, no benefit arose by considering uptake concentration as dependent variable (data not shown). Thus, although spots produced by plotting predicted data against measured data show a regular point distribution, model calibrations show for both P and Ca positive intercepts coupled with slope values much lower than 1 (Figure 5.11). The best model performances were detected by modelling K, Mg and Na uptake rate (Table 5.2). High R^2 values have correspondence with model calibration analysis showed in Figure 5.11. The regular

distribution of data, the low intercept values and the slope values closed to 1 make multiple regression an appropriate method to model such nutrient under our experimental conditions. Modelling plant absorption by multiple regression analysis can be a powerful tool; nevertheless it involves all advantages and disadvantages of empirical models. Firstly resulting models have not biological or physiological interpretation because the established correlations are the results of mathematical or statistic procedures and the final result is computed by them multiplication. As a result, not all nutrient absorption patterns can be explained by using this kind of approach. In effect, although several independent variables were taken into consideration, proposed models did not enable a suitable simulation of P and Ca absorption. Moreover an appropriate simulation of N was possible only if $N-C_U$ was considered as dependent variable. Secondarily a series of assumptions can limit the application of this model: a) plants must be on production phase because calibration has been accomplished on adult plants, b) plants must be in good health condition (no stresses) because no feed-back control is involved in the proposed models. On other hand the use of empirical models can show high capability in predicting data as reported for N (uptake concentration) K, Mg, and Na (uptake rate) absorption.

5.4 Conclusion

Cut rose production is mainly done with hydroponics systems (Mattson et al., 2006). Nevertheless, when poor quality water is used an accumulation of “ballast” ions can occur in closed-loop soilless systems causing both increase of toxic ion and EC value which is the main responsible of osmotic stress (Munns, 2002; Parida and Das, 2005). Therefore there is a need of researches for reassessing the salinity tolerance of greenhouse roses under soilless production conditions (Cabrera and Perdomo, 2003). To this extent the use of models that simulate nutrient absorption under saline condition may be a profitable support to optimize plant nutrition.

Saline treatments reduced water uptake, plant weight without affecting stem rate elongation. Moreover negative correlations were found between NaCl external concentration and nutrient absorption. On the contrary saline treatments greatly increased Na uptake.

Proposed models show a high capability in simulating N, K, Mg and Na absorption giving a powerful tool for estimating ion concentrations in the recirculated nutrient solution. Nevertheless P and Ca uptake were not simulated in an appropriate way. These results suggest that further investigations and efforts should be done before involving such models in DSS (Decisional Support System) or nutrient solution management programs.

Table 5.2. Models proposed for nutrient uptake in rose growing in closed-loop soilless. Equation parameters were estimated by multiple linear regressions by using a stepwise forward method (Statgraphics Centurion Version XV, Stat Point, Inc., Herndon, VA, USA). R^2 explains the variability of predicted data ranging between 0.22 and 0.78.

Ion	Unit	Model	R^2	P -value
N- R_U	mmol plant ⁻¹ day ⁻¹	3.73991 + 0.30754GR - 3.1241 W- R_U - 0.0249[NaCl]	0.2223	0.0000
N- C_U	mol m ⁻³	26.2267 + 0.0546FW + 0.91624GR - 79.2289 W- R_U - 0.1041[NaCl]	0.6250	0.0000
P- R_U	mmol plant ⁻¹ day ⁻¹	-0.0840 - 0.0108GR + 1.1839 W- R_U - 0.0009[NaCl]	0.4822	0.0000
K- R_U	mmol plant ⁻¹ day ⁻¹	0.0113 - 0.0127GR + 2.21412 W- R_U - 0.0053[NaCl]	0.7121	0.0000
Ca- R_U	mmol plant ⁻¹ day ⁻¹	0.0139 + 0.0122GR + 0.9204 W- R_U - 0.0019[NaCl]	0.4092	0.0000
Mg- R_U	mmol plant ⁻¹ day ⁻¹	-0.0290 - 0.0127GR + 0.6171 W- R_U	0.6888	0.0000
Na- R_U	mmol plant ⁻¹ day ⁻¹	-1.2116 - 0.0220GR + 2.2565 W- R_U + 0.1048[NaCl]	0.7837	0.0000

Figure 5.5. Influence of NaCl treatments on water uptake (a), growth rate (b), nitrogen uptake rate (c) and nitrogen uptake concentration (d). Error bars are omitted to avoid line overlaps, statistical significance is reported in the text.

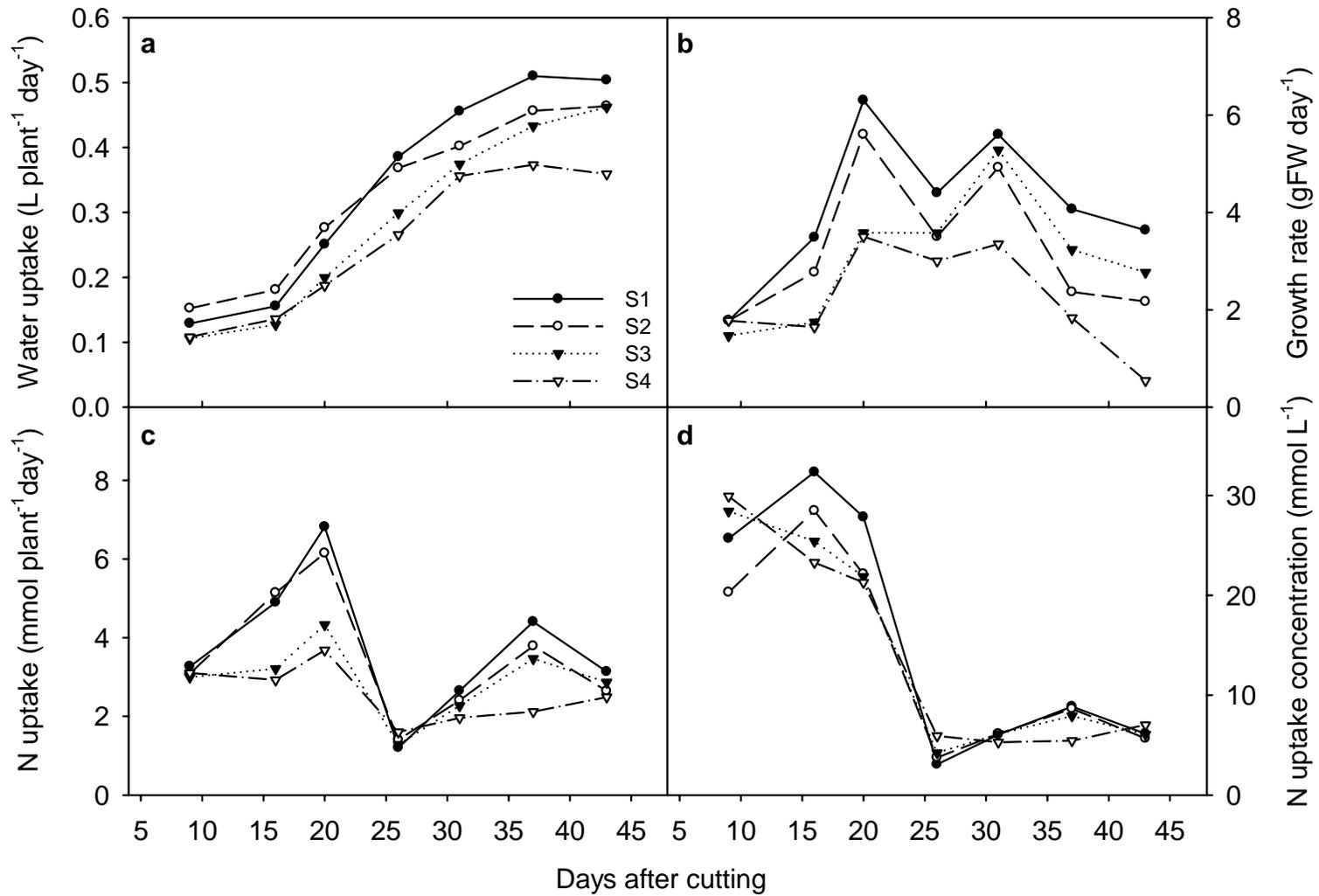


Figure 5.6. Influence of NaCl treatments on water uptake (a), growth rate (b), phosphorus uptake rate (c) and phosphorus uptake concentration (d). Error bars are omitted to avoid line overlaps, statistical significance is reported in the text.

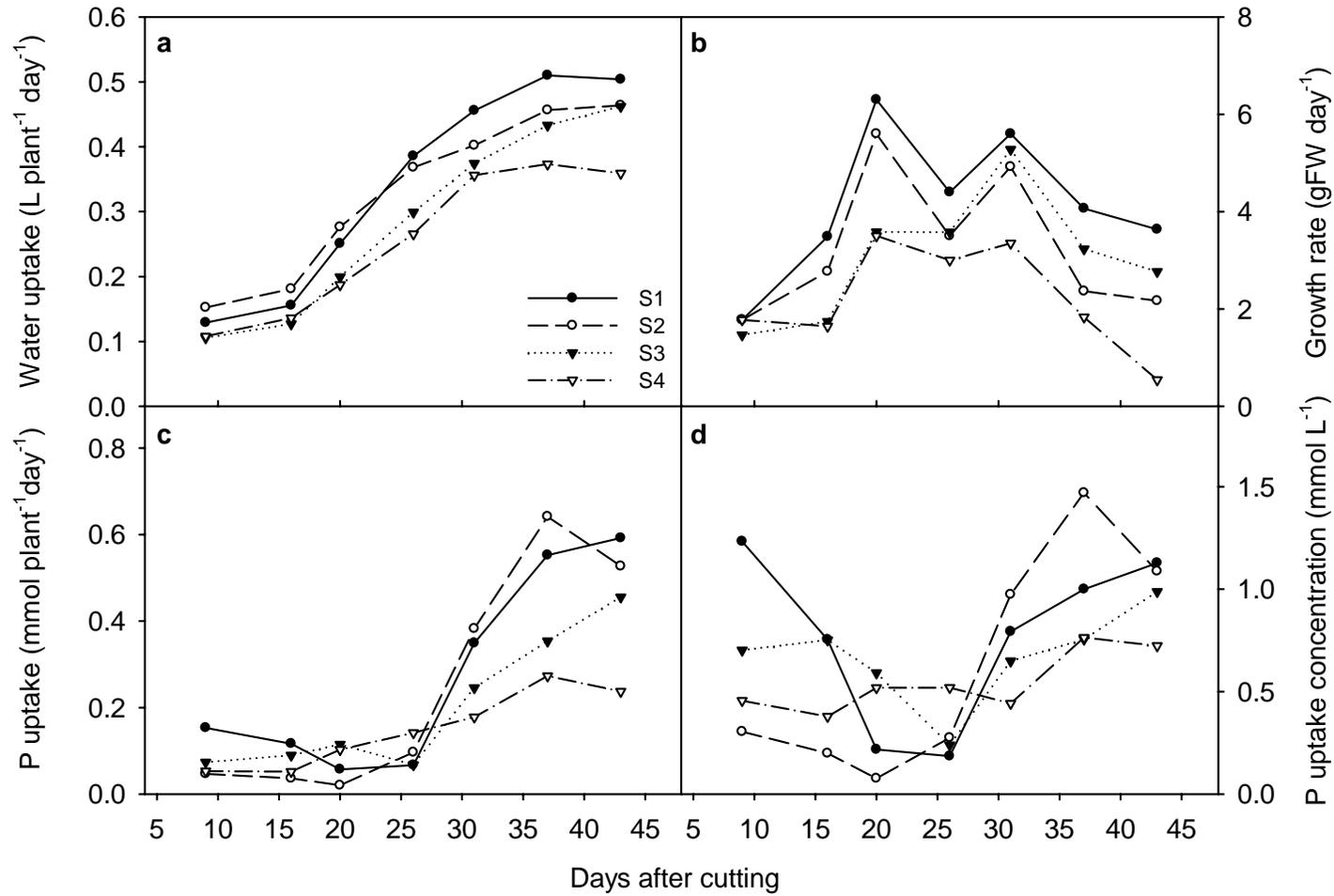


Figure 5.7. Influence of NaCl treatments on water uptake (a), growth rate (b), potassium uptake rate (c) and potassium uptake concentration (d). Error bars are omitted to avoid line overlaps, statistical significance is reported in the text.

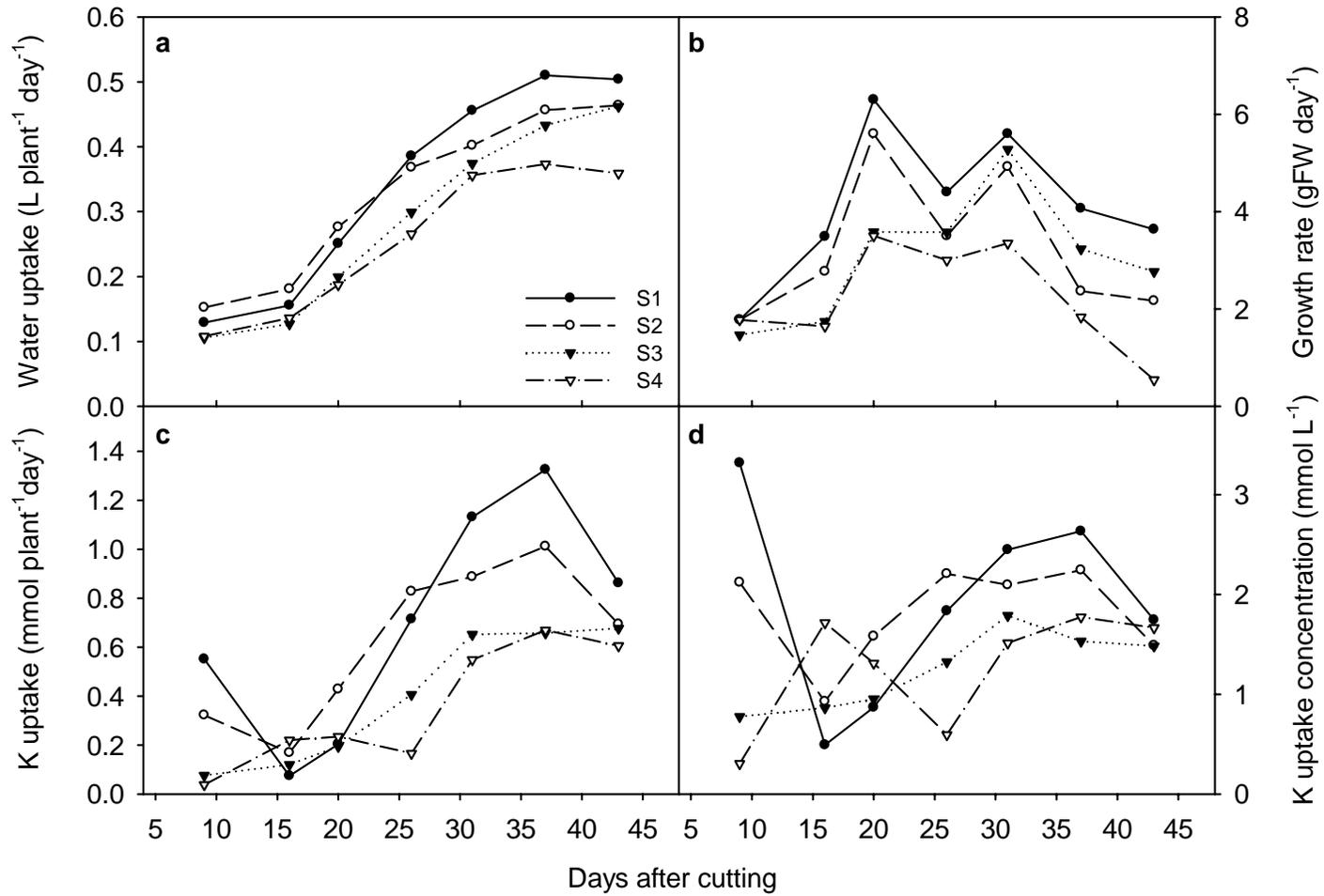


Figure 5.8. Influence of NaCl treatments on water uptake (a), growth rate (b), calcium uptake rate (c) and calcium uptake concentration (d). Error bars are omitted to avoid line overlaps, statistical significance is reported in the text.

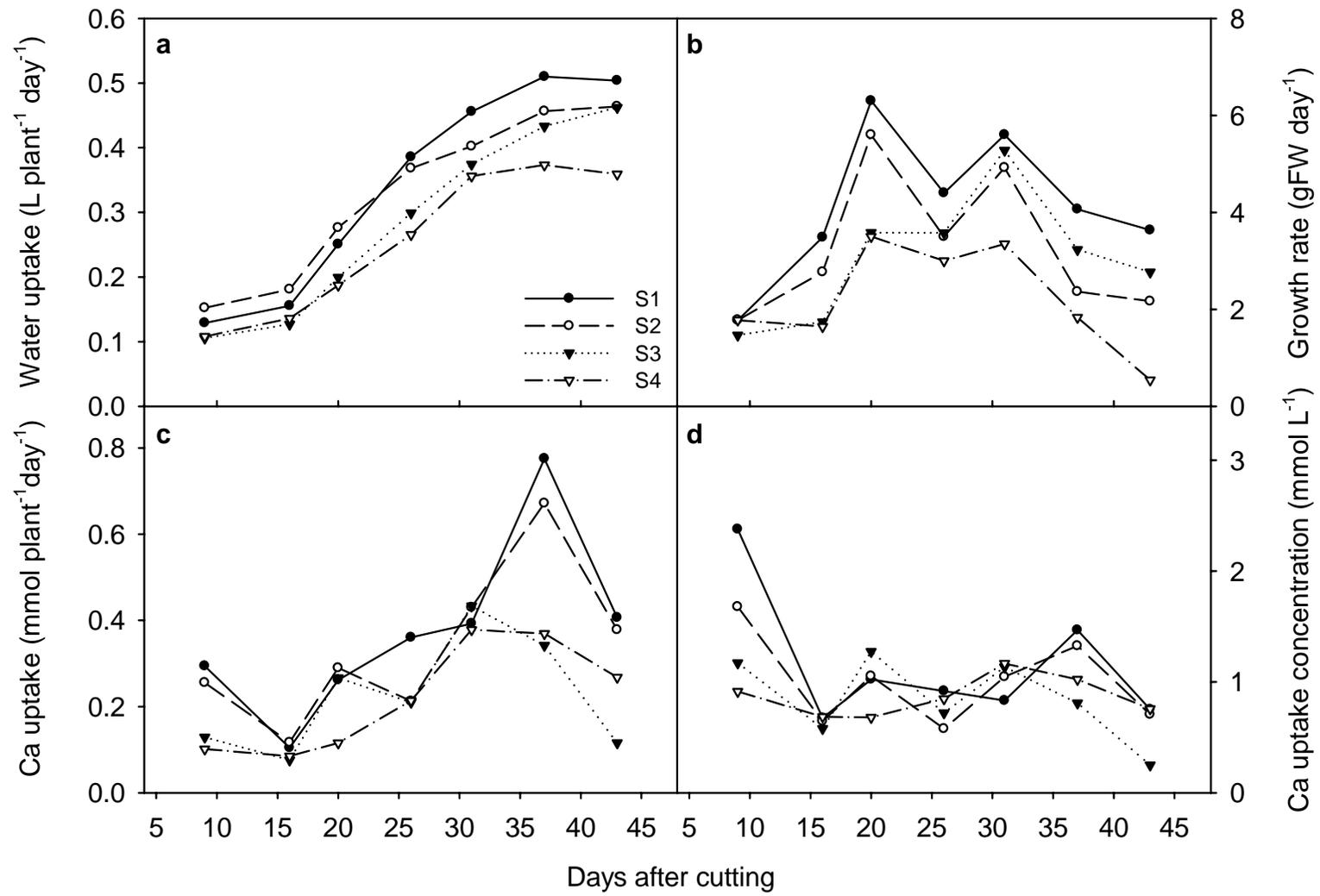


Figure 5.9. Influence of NaCl treatments on water uptake (a), growth rate (b), magnesium uptake rate (c) and magnesium uptake concentration (d). Error bars are omitted to avoid line overlaps, statistical significance is reported in the text.

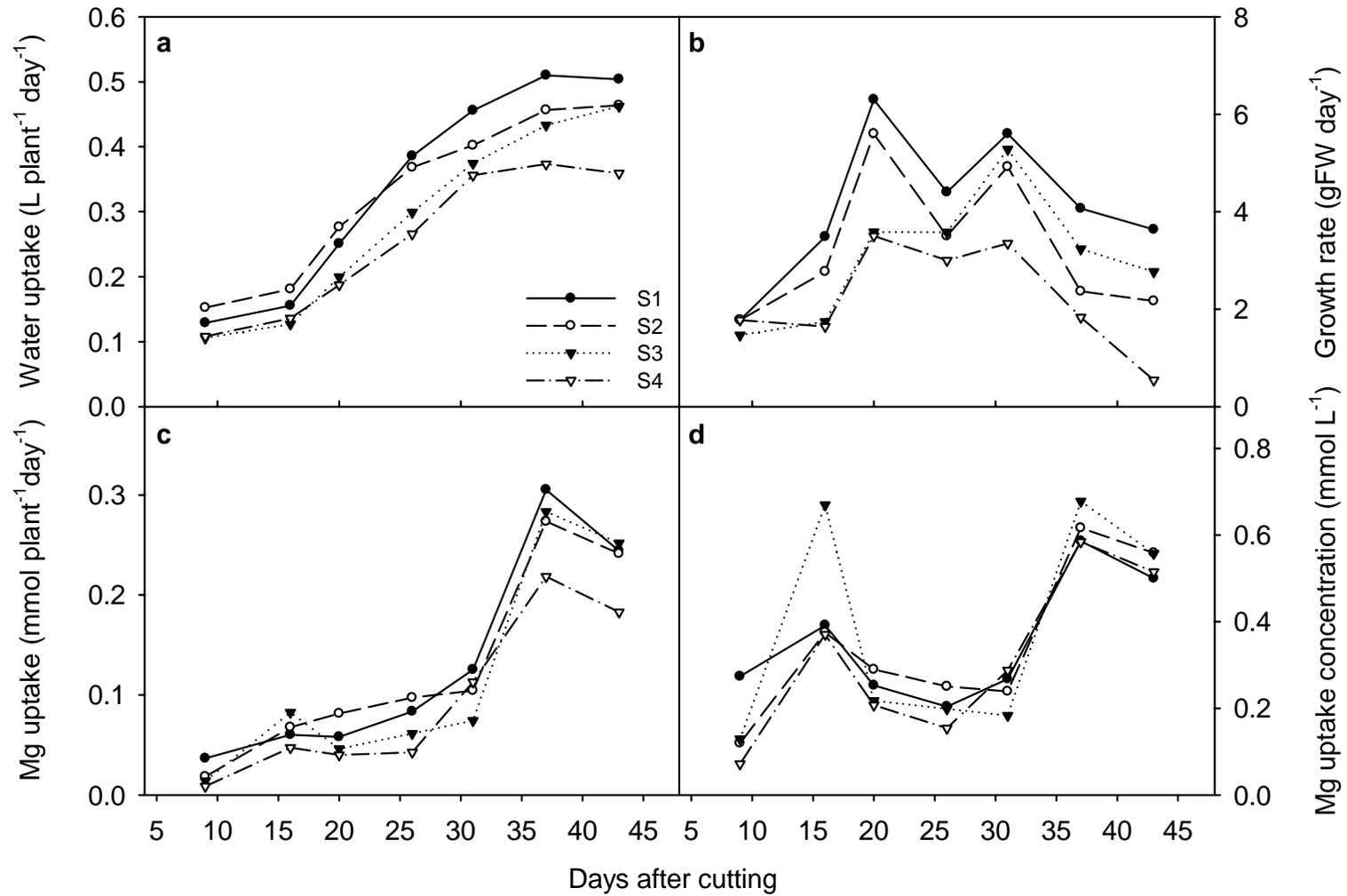


Figure 5.10. Influence of NaCl treatments on water uptake (a), growth rate (b), sodium uptake rate (c) and sodium uptake concentration (d). Error bars are omitted to avoid line overlaps, statistical significance is reported in the text.

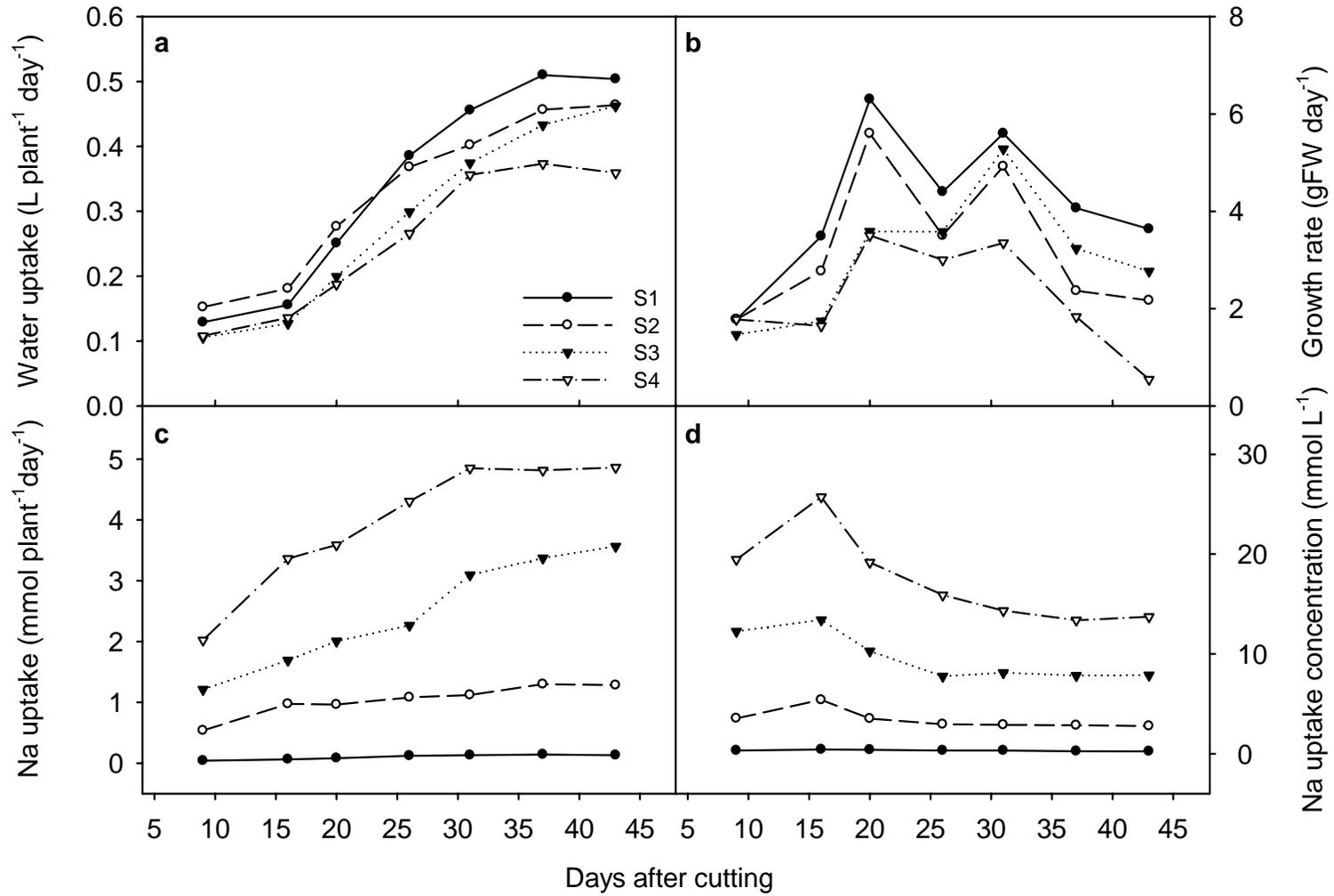
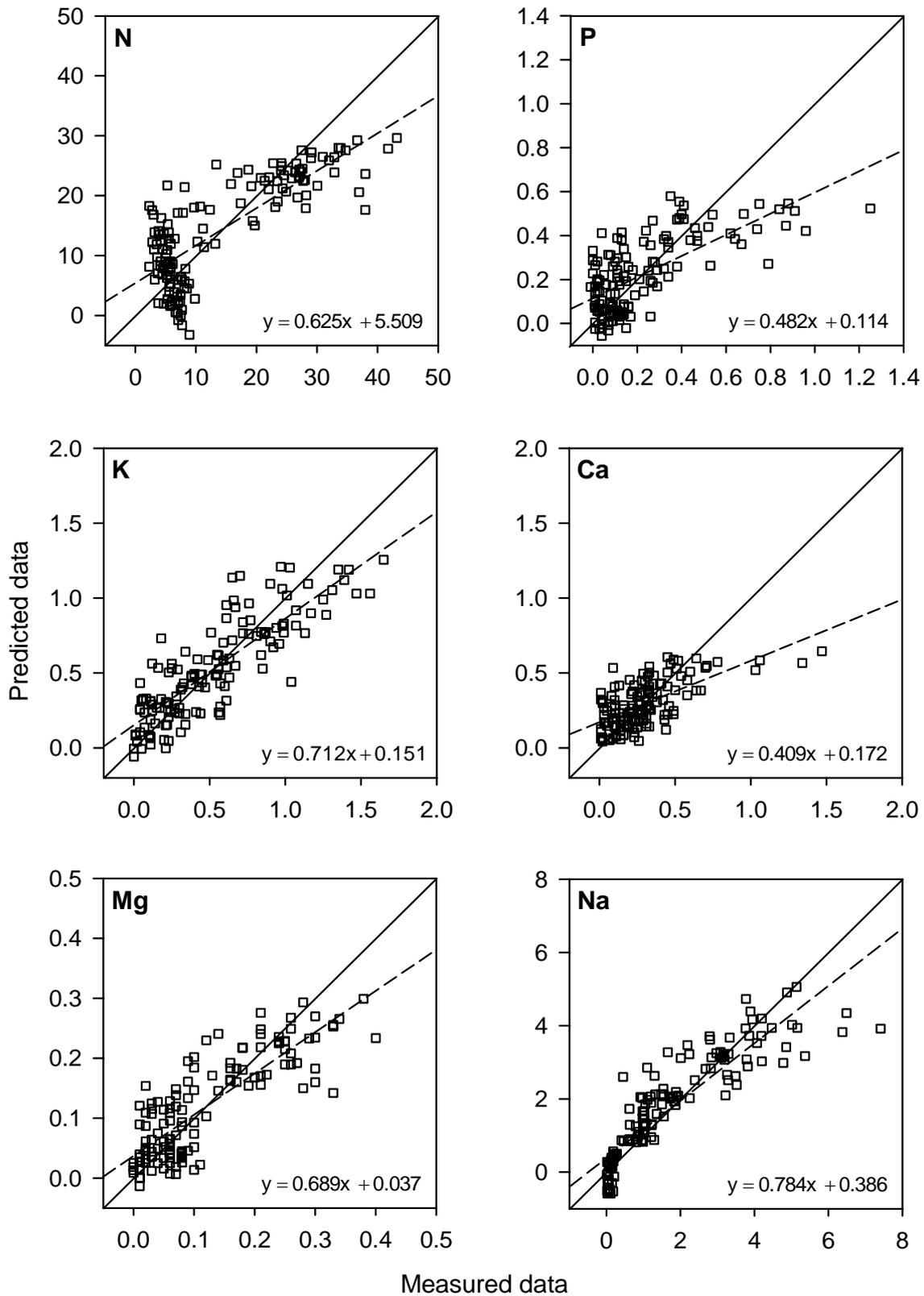


Figure 5.11. Predicted data against measured data are reported for the different nutrients and sodium uptake. Dashed line represents linear regressions ($n = 133$), while solid line represents $y = x$.



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6 EFFECTS OF SALINE ROOT ENVIRONMENT (NaCl) ON NITRATE AND POTASSIUM UPTAKE KINETICS IN ROSES GROWING IN CLOSED-LOOP SOILLESS: A MICHAELIS-MENTEN MODELLING APPROACH

6.1 Introduction

Soil and groundwater salinization is increasing continuously in many countries around the globe and mainly in semi-arid regions (Martinez-Beltran and Licona-Mazur, 2005; Rengasamy, 2006). This phenomenon is due to massive fertilization activity, soil erosion, and the incursion of sea water into ground water in coastal areas. Salinity is considered one of the major factors limiting crop production, because of its negative effects on plant development and nutrition (Parida and Das, 2005; Grattan and Grieve, 1999). Further, there is interest in using reclaimed urban water that is often high in dissolved salts for landscape, nursery, and greenhouse irrigation. As it is well known, a saline root-zone environment induces osmotic changes in plants, affects water and nutrient absorption, and can cause tissue toxicity (Halperin et al., 2003; Munns, 2002; Parida and Das, 2005). Several studies attempt to develop strategies that may improve, water and nutrient use efficiency, product quality and yield under salinity stress for rose plants (Raviv et al., 1998; Baas and Van den Berg, 1999; Brun et al., 2001), while others focus on the effects produced by salt ions on plant growth, product quality and nutrient absorption (Lorenzo et al., 2000; Cabrera, 2003).

With regard to fertilization strategies, several studies have demonstrated that higher fertilizer concentrations in the root medium can mitigate the competitive action of salt ions on nutrient ion absorption (Grattan and Grieve, 1999). Potassium concentration can influence sodium influx by reducing the affinity between sodium and non selective channels, while a positive relationship occurs between calcium absorption and its external concentration under saline conditions (Rodriguez-Navarro and Rubbio, 2006; Rengel, 1992). Grattan and Grieve (1999) report that the use of nitrogen salts with a high $\text{NO}_3^-/\text{NH}_4^+$ ratio limits the competition of chloride on nitrogen absorption compared to nitrogen salts which consist of high percentage of NH_4^+ . High Cl^- concentrations are known act as an antagonist ion to decrease NO_3^- influx, and can indirectly inhibit nitrate reductase activity which is positively correlated to influx of NO_3^- influx (Barber et al., 1989; Debouba et al., 2007). Nevertheless, increased fertilizer has a negative environmental impact and increases production costs. Greenhouse rose production already uses fertilizers intensively without even regard to ions that effect nutrient absorption. Cabrera et al. (1993)

reported potential nitrogen losses in greenhouse rose production of 2,000 kg of N per hectare per year.

An increased knowledge of nutrient influx kinetics of plants under saline stress is important for improving nutrient uptake efficiency and to optimizing fertilization procedures.

In the past rose plants have been reported to be salt sensitive (Hughes and Hanan, 1978). However, more recent research has found roses to be relatively tolerant of salt stress, but that this depends on cultivar, rootstock, the cultivation system, and the specific salt constituents (Cabrera, 2002; Cabrera and Perdomo, 2003). Several authors have demonstrated that soilless-grown plants may tolerate higher salinity levels without relevant loss of yield and quality (Baas and Van den Berg, 1999; Brun et al., 2001; Cabrera and Perdomo, 2003; Pardossi et al., 2006).

Current cut flower rose production typically use soilless media that is kept moist through irrigation with a nutrient solution (constant liquid feed) (Mattson et al. 2006). In recirculating nutrient solution systems the concentration of nutrients is kept constant by an equilibrium resulting between fertilizer additions and plant nutrient demand. When poor quality water is used, salt ions would build up in the recirculating nutrient solution, because of their scarce absorption by plants. As a consequence, the accumulation of “ballast” ions increases and this is reflected in increasing solution salinity and measured EC (electrical conductivity). Hence ballast ion accumulation negatively impacts nutrient and water absorption. The use of poor quality water is declared economically unacceptable in terms of reduced crop yield or the cost of filtering water (Stanghellini et al. 2005). However, the use of salt-stress tolerant cultivars, coupled with different water management strategies may become one of the most valuable technique for the cultivation of horticultural crops such as tomato or rose in closed hydroponic systems when the access to high quality water is not possible (Raviv et al., 1998; Brun et al., 2001; Pardossi et al., 2006). For the above mentioned reasons, the knowledge of nutrient absorption kinetics under salinity stress is becoming a crucial point to manage fertilization of rose crop cultivated in hydroponic as well as in the open field.

6.2 Michaelis-Menten kinetics

Mechanistic models such as M-M (Michaelis-Menten) kinetics are typically used to express ion influx (I) mathematically. The large interest in short-term uptake studies has evolved from the early work of Epstein (1972) showing that the M-M equation fits the relationship between uptake rate and external ion concentration (Adamowicz and Le Bot, 1999). The main evidence for this hypothesis came from the similarity in the behaviour of ion uptake versus external ion concentration and that of enzyme activity versus substrate concentration. According to this

mechanism, transporters are membrane-bound proteins that exhibit a high degree of specificity for individual ions in much the same way enzymes do for a specific substrate (Bassirirad, 2000). Therefore, ion uptake rate follows a rectangular hyperbola pattern as a function of the external ion concentration. The general M-M equation is reported in Table 6.1 (Eq. M-M), where I is the flux of the ion into the plant, I_{\max} is the asymptotic maximum value at ion concentration approaching infinite, K_m is a constant value which represents the concentration of ion at which $I = \frac{1}{2} I_{\max}$ and $[N]$ is the concentration of the ion in the root zone.

The I_{\max} and the K_m terms represent the maximum uptake velocity and the affinity between carriers and ion, respectively. These constants may change as a function of internal factors (e.g. internal tissue ion concentration) (Siddiqi and Glass, 1986), climate conditions (e.g. temperature or light intensity) (Wheeler et al., 1998) and interaction with other ions (e.g. ion uptake inhibition) (Peuke and Jeschke, 1999). Hence interaction between ions can be represented empirically as altered M-M parameters. This is expressed as an increase in K_m or a decrease in I_{\max} , which can occur separately or in combination depending on the type of inhibition. An ion (inhibitor) may interact with another (nutrient) in three different ways. The first is a competitive inhibition which takes place between the carrier and the inhibitor. This interaction does not change the total amount of carriers but limits the amount of nutrient taken up, by competing with the nutrient for the binding site on the carrier. Competitive inhibition is represented in M-M kinetics as a linear increase in a nutrient's K_m parameter (a decreased nutrient affinity) as a function of the inhibitor concentration, without changes in I_{\max} . In noncompetitive inhibition an ion interacts with the carrier by changing its activity without performing any competition for the binding site. As a result I_{\max} decreases, following an hyperbolic pattern, as a function of the inhibitor concentration. In this case, the process does not affect K_m value. A third mechanism, mixed competitive inhibition, is the combination of the two. It is typically represented by changes in both I_{\max} and K_m . Typical M-M competitive and noncompetitive equations, proposed by Dixon and Webb (1979), are presented in Table 6.1.

Experimentally, data collection for fitting M-M curve necessitates exposing plant roots to several different ion concentrations. As is often reported in the literature, there are different mechanisms, for absorbing ions at different external ion concentrations. Therefore the molecular interpretation of influx kinetics over a wide range of concentration has led to considerable debate but the dual transport mechanism of Epstein (1972) is the most widely accepted. At relatively low concentration (mmol m^{-3}) mechanism I (or HATS, High Affinity Transport System) operates for the most important macronutrients which show very low I_{\max} and K_m values. Mechanism II (or

LATS, Low Affinity Transport System) operates at higher ion concentration range and its kinetics are not well characterized (Le Bot et al. 1998).

Several studies focus on Michaelis-Menten-simulated rose nutrition (Silberbush and Lieth 2004; Mattson et al. 2006) but most of them do not quantify or report the influence of antagonistic/ballast ions on primary nutrient absorption. The main objective of this paper is to mathematically represent rose plant NO_3^- and K^+ absorption under a saline (NaCl) root environment. The approach is to fit modified Michaelis-Menten equations on experimental data, of the under saline (NaCl) root environment, with particular emphasis on hydroponic closed systems.

Table 61. Michaelis-Menten kinetic fundamental equations used for modelling nutrient uptake rate.

Equation name	Formula	Inhibitor term
Michaelis-Menten (M-M)	$I = \frac{I_{\max} [N]}{K_m + [N]}$	
Competitive inhibition (CM-M)	$I = \left(\frac{[N]}{[N] + K_m \left(1 + \frac{[Ir]}{K_i} \right)} \right) I_{\max}$	$K_m \left(1 + \frac{[Ir]}{K_i} \right)$
Non-competitive inhibition (NCM-M)	$I = \left(\frac{[N]}{([N] + K_m) \left(1 + \frac{[Ir]}{K_i} \right)} \right) I_{\max}$	$\frac{I_{\max}}{\left(1 + \frac{[Ir]}{K_i} \right)}$
Mixed non-competitive inhibition (MM-M)	$I = \left(\frac{[N]}{K_m \left(1 + \frac{[Ir]}{K_i} \right) + [N] \left(1 + \frac{[Ir]}{K_{i2}} \right)} \right) I_{\max}$	

6.3 Materials and methods

6.3.1 Growing conditions

The experiment was conducted on rose plants (*Rosa* spp. cv. Kardinal grafted on “Natal Briar” rootstock) at the Department of Plant Science, University of California Davis (USA). One year old plants were moved from the nursery to solution culture systems and grown in a glasshouse where temperature was kept under control by using an automatic cooling system and ventilation.

During the summer the roof was covered with shading compound to avoid greenhouse warming due to high light intensity. Minimum, maximum and mean daily temperature recorded were 21.9, 24.8 and 22.8 °C respectively; minimum and maximum daily RAD (indoor global Radiation) average were respectively 1.7 and 2.9 MJ m⁻² while the mean R over the whole summer period was 2.4 MJ m⁻². After a period of acclimation to the hydroponic environment, plants, which were previously trimmed to achieve homogeneous weight and shape, were moved into growth chambers where the experiment took place. At all times while plants were in solution culture, oxygenation of the nutrient solution was accomplished by air pumps continuously bubbling air into solution. This air bubbling also served to maintain a homogenous nutrient solution.

Growth chamber climate conditions were kept under control by means of a computer which monitored and recorded temperature, PPF (Photosynthetic Photon Flux Density) and humidity. Temperature average during dark and light hours were respectively 20 and 24 °C, relative humidity value was kept close to 85% and PPF ranged between 350 and 550 μmol m⁻² s⁻¹. Before the experiment, plants were grown in optimal nutrient conditions (a modified Hoagland's solution) and no saline stress was inducted for 7 days. Then, 24 hours before experiment initiation, plants were moved in four litres pots filled up with deionized water.

The experiment was arranged in three replicates (complete randomized blocks) and 8 treatments which differed for initial NaCl concentration (0, 5, 15, 25, 35, 45, 55, 65 mol m⁻³). The base nutrient solution had two different initial nitrate and potassium concentrations, respectively 7.0 and 2.6 mol m⁻³ (HC nutrient solution) used for 5, 25, 45, 65 NaCl treatment; 3.5 and 1.3 mol m⁻³ (LC nutrient solution) used for 0, 15, 35, 55 NaCl treatment. The initial EC (Electrical Conductivity) values were respectively for each NaCl treatment 0.73, 1.54, 2.15, 3.44, 4.05, 5.34, 5.95, 7.24 dS m⁻¹ while initial pH value was 5.5. The concentration of macronutrients present in the HC solution was N-NO₃ 7.0, P-PO₄ 1.0, S-SO₄ 0.2, K 2.6, Ca 2.4, Mg 1.0 mol m⁻³; and in the LC solution N-NO₃ 3.5, P-PO₄ 1.0, S-SO₄ 0.2, K 1.3, Ca 1.2, Mg 1.0 mol m⁻³. The difference between the sum of anions and cation was less than 0.1 eq m⁻³, which was due to carbonates.

6.3.2 *Sample collection and laboratory analysis*

During the seven day experimental period, water consumption and plant fresh weight were measured and nutrient solution samples were collected daily. Following these daily measurements, deionized water was used to replace solution lost to evapotranspiration.

At experiment termination, plants were destructively harvested and root volume, stem length and shoot stem, base stem, flower and leaf dry matter were measured.

Nutrient solution samples were analyzed for sodium and potassium by using an atomic absorption spectrophotometer (SpectrAA Model 55; Varian Inc., Palo Alto, CA, USA), nitrogen by diffusion conductivity method with an ammonia/nitrate Analyzer (Model TL200, Timberline Instruments L.L.C., Boulder, CO, USA) and chloride by using an ion-specific sensor (Cole-Parmer Instrument Company, IL, USA).

6.3.3 Experimental conditions and data collection

In order to have a suitable data set we must make the assumption that during the experimental period climate factors and plant development had a negligible influence on the uptake rate of the investigated ions. With regard to the former, plants were moved into the controlled environment chambers a week before experiment initiation and with simulated day and night conditions so as to not interfere with the circadian cycles. This period was necessary for plant acclimatation and stabilization to growth chamber environment.

The quite high relative humidity (85%) was imposed to limit water stress by reducing stomatal activity. As water uptake can influence ion absorption rates (Brun and Chazelle, 1996) we attempted to maintain constant water uptake rates regardless of NaCl treatment. Because plants grown in long-term saline conditions express reduced leaf surface area (Parida and Das, 2005), and hence reduced water uptake rates, our experimental period occurred at the stage where shoots had nearly open flowers and had reached stable leaf surface area (Dayan et al., 2002).

Further benefits were gained by working with plants at early flowering stage. At this stage, very little dry matter accumulation occurred, hence the dry matter accumulation rate and the stem elongation rate both were close to zero (Cabrera et al., 1995; Gutierrez-Colomer et al., 2006). Since plant nutrition is greatly related to plant growth rate (Cabrera et al., 1995; Schenk, 1996; Mathieu et al., 1999), the nutrient uptake was assumed, in this experiment, uninfluenced by this plant variable. This last aspect was fundamental in our experiment because lower nutrient (NO_3^- and K^+) concentrations (in the nutrient solution) were obtained by nutrient depletion technique which constantly occurred during the time (six days) depending on plant uptake rate. In summary we executed the experiment so that nutrient uptake rate was primarily influenced only by external salt and nutrient ion concentration.

Nutrient solution samples were collected on daily basis at the same time of the early morning hours so that the uptake of nutrient and water was normalized with respect to daily patterns. Previous research has shown that the rate of nutrient uptake changes over an entire day following the same pattern as water uptake rate (Brun and Chazelle, 1996; Bougoul et al., 2000). Furthermore nutrient uptake is often coupled to the carbohydrate availability (Cabrera et al., 1995) which increases during light hours (Peuke and Jeschke, 1998).

Different ion concentrations were obtained continuously as a consequence of their removal from solution through plant uptake. This permitted the determination of nutrient and salt ion uptake under varying solution concentrations.

Before experiment initiation, plant roots were maintained 24 hours in deionized water. This accomplished the goal of washing out possible excesses of nutrients from the apoplast. As a result of this procedure we found that uptake rates during the first 24 hours were much higher than the rest of the experimental period (data not shown). This period was considered necessary to establish equilibrium between apoplastic absorption and the external nutrient solution concentration. Therefore the first set of data was not considered for fitting the proposed model equations.

Collecting data for model fitting M-M kinetics can easily lead to systematic errors such as to collect samples from non-homogeneous nutrient solution. It happens because during short-term experiment the nutrient solution can result not well stirred leading to dissimilar ion concentration between the closest root zone and the rest of the nutrient solution. To solve this problem, the nutrient solution within the pots was continuously moved by pumps bubbling air into the solution. Adopting this procedure improved both the regular oxygenation of the nutrient solution and its homogeneity degree.

6.3.4 Model development

The experimental data on ion and nutrient solution concentration and uptake rates were fit using non-linear regression (Statgraphics Centurion Version XV, Stat Point, Inc., Herndon, VA, USA). Nutrient uptake kinetics were first analyzed with a general M-M approach in which I_{\max} and K_m changes represent respectively non-competitive and competitive inhibition in ion interactions. Isotherm changes were discussed in relation to the different salinity treatment also considering water uptake rate, nutrient and salt ion uptake rate and growth rate. Then non-linear regression procedure was used to fit nitrogen and potassium uptake rate (dependent variables) with a function that took into account for both nutrient external concentration and NaCl external concentration (independent variables) or only nutrient external concentration depending on the nutrient investigated (N or K).

6.4 Results and discussion

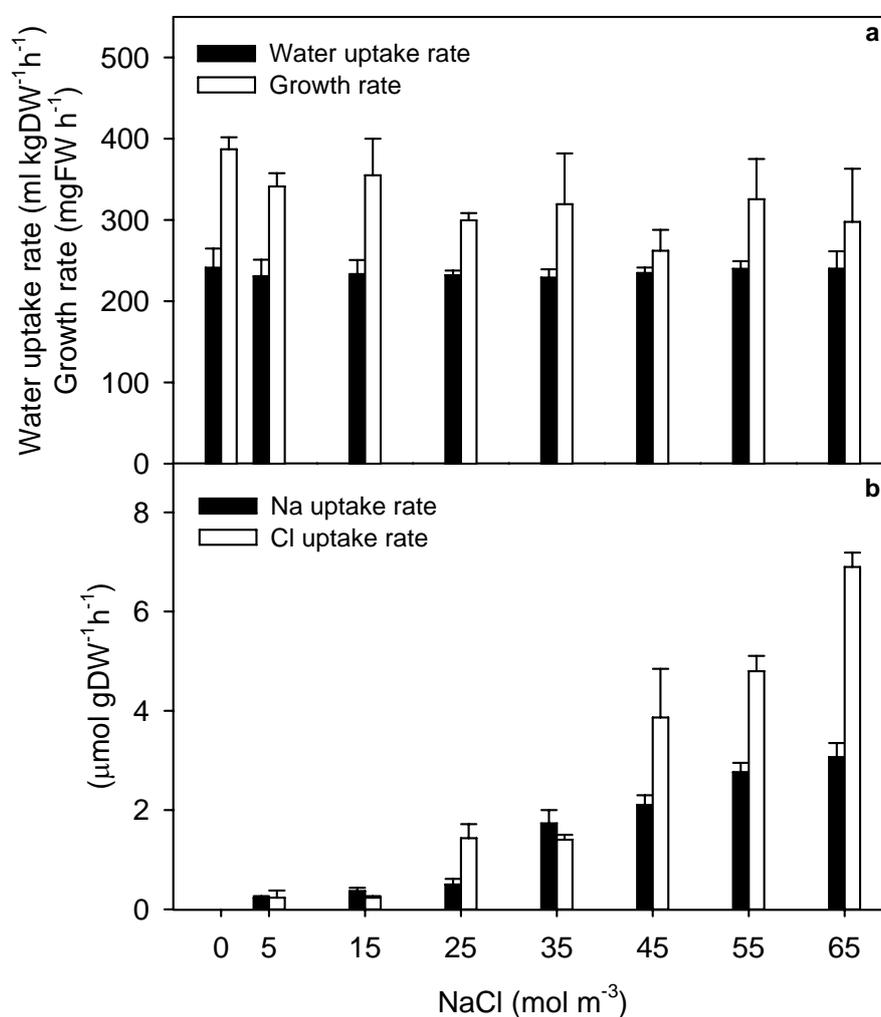
6.4.1 Growth, water uptake and ion uptake rate

Water uptake rate was not affected by the different treatments. As discussed above, salinity affects plant transpiration primarily through a reduced leaf area and secondarily by increasing

stomatal resistance (Parida and Das, 2005). Thus the choice of using plant at the end of the flowering cycle mitigated the influence of salinity stress on evapotranspiration because the total leaf area was already developed (Dayan et al., 2002). Assumptions stated previously in the paper were then confirmed by data analysis graphically reported in Figure 6.1a. The chart does not show any significant difference (ANOVA test, $P < 0.05$) neither for water uptake rate nor for growth rate respectively expressed as ml of water absorbed per kg of total plant dry weight per hour and mg of total plant fresh weight per hour. As reported by other authors (Gutierrez-Colomer et al., 2006), at incipient harvesting time plant dry matter accumulation is basically complete. Therefore, it is reasonable to assume that the slight increment observed as a difference of fresh weight between the end and the beginning of the experiment is due to water accumulated into the maturing flower. In conclusion, no significant difference could be found for the growth rate (expressed as fresh weight in the Δt) because water uptake occurred among the different saline treatments.

A positive correlation was found between the external concentration of NaCl and the uptake rate of sodium and chloride (ANOVA test, $P < 0.05$) (Figure 6.1). The apparent uptake of salt ions was measured during the whole experiment period by the difference between the initial ion amounts and the final one. Influx rate of Na^+ and Cl^- show an exponential relationship with the external NaCl concentration. A similar relationship between the external NaCl concentration and the uptake concentration (the ratio between ion absorbed and water absorbed in the same Δt) of sodium and chloride was reported by Savvas et al. (2007) for a hydroponically-grown bean crop; the relationship was exponential and linear for Na^+ and Cl^- , respectively. HKT transporters (Rodriguez-Navarro and Rubio, 2006), which characterize the high affinity transport system for sodium, and NSC (non-selective cation) transporters (Amtmann et al., 2001) represent the main vectors that enable sodium influx and transport within the plant. Essah et al. (2003) report that the NSC transporters would be the main channels responsible for sodium influx. Further studies show that sodium influx increases depending on the external sodium concentration (Carmassi et al., 2005; Cabrera and Perdomo, 2003) and the cultivation system (Shi et al. 2002). Generally saline environments cause homeostasis imbalance in higher plants (Parida and Das 2005). Therefore most of the excess sodium is rapidly stored into the vacuole (Carden et al. 2003). The results of our study show a clear difference between the uptake rate of sodium and chloride; the former shows a progressive increase that is much less stressed than chloride uptake rate.

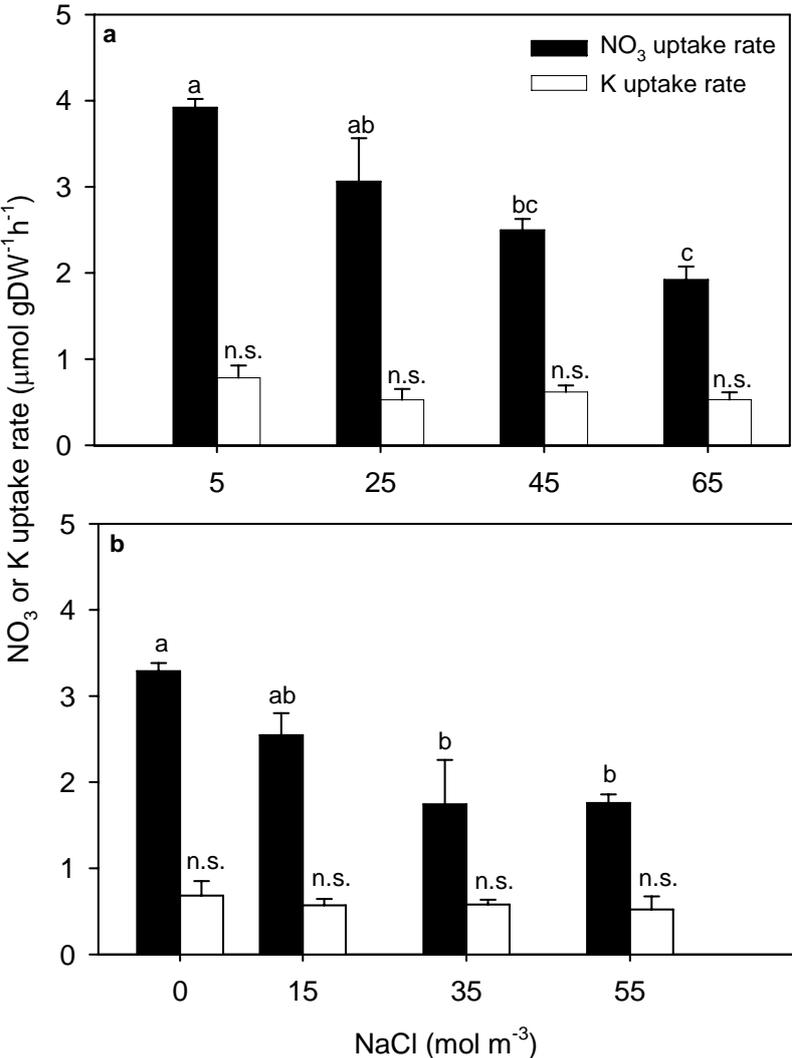
Figure 6.1. Effects of NaCl treatments on rose (cv. Kardinal grafted on Natal Briar rootstock) water uptake rate, growth rate, Na^+ and Cl^- uptake rate calculated over the whole experiment period. One year old plants were moved, at the incipient harvesting time, into growth chambers where water uptake and growth rate were measured on a daily basis by means of a scale (6.1a); kgDW and mgFW represent respectively kilograms of total plant dry weight and milligrams of total plant fresh weight. Figure 6.1b reports the uptake rate of Na^+ and Cl^- calculated over the entire experiment period by solution depletion; gDW represents grams of total plant dry weight. While Figure 6.1a show no significant difference according to ANOVA test ($P < 0.05$), Figure 6.1b show a significant increase of ion uptake related to NaCl treatments (P -value 0.0000). Data are means ($n=3$) \pm standard error.



Differing evidence has been found for rose plants with regard to chloride and sodium uptake and their accumulation within plant tissues. Sonneveld (2000) found a negligible sodium uptake against a larger chloride uptake depending on the external NaCl concentration. Cabrera et al. (2002) report a high variability among rose rootstocks that show a differing capacity to exclude

or to accumulate chloride and sodium depending on genotypes. In particular this work reports that Natal Briar rootstock (the same used in this work and one of the most important rootstock used in greenhouses-produced cut roses) allows a larger accumulation of sodium and chloride (respectively 40% and 100%) than other commercial rootstocks. The same author concludes saying that there is a need for studies which investigate the behavior of rose rootstock in saline environments (Cabrera and Perdomo 2003). To this purpose it would be useful to apply the methods described in this experiment to screen rose rootstocks for salinity tolerance.

Figure 6.2. Effects of NaCl treatments on the early (24 hours) NO_3^- and K^+ uptake rates of rose plants (cv. Kardinal grafted on Natal Briar rootstock). One year old plants were moved, at the incipient harvesting time, into growth chambers where uptake rate of NO_3^- and K^+ were calculated by nutrient depletion for the two initial nutrient concentrations (HC and LC, Fig. 6.2a and 6.2b respectively); gDW represents grams of total plant dry weight.. Data are means ($n=3$) \pm standard error. Means labelled with the same letter are not significantly different based on LSD test ($P<0.05$).



The initial 24-hr potassium and nitrate absorption rates are reported under treatment HC (Fig. 6.2a) and LC (Fig. 6.2b). Nitrate uptake rate was promptly inhibited by the presence of NaCl showing a negative correlation with the external NaCl concentration (ANOVA test $P < 0.05$). Under the greatest NaCl concentration, nitrate uptake was reduced to 50% of the initial value. The increase of NaCl concentration in the cultivation medium is typically coupled with decrease of NO_3^- uptake and tissue concentration (Grattan and Grieve, 1999). The presence of Cl^- in the external medium inhibits NO_3^- absorption. Decrease of NO_3^- uptake is generally accompanied by a high Cl^- uptake and accumulation within plant tissues (Parida et al., 2004). Therefore in the presence of NaCl, NO_3^- uptake is mainly inhibited by the antagonist action exerted by Cl^- . Although some authors focus on the role of cations as inhibitor of NO_3^- uptake (Lorenzo et al., 2000), nevertheless most of them remark that, under saline conditions, the main role as inhibitor is played by anion concentration which would effect the absorption of the nutrient more than cation composition; as well as affecting the water uptake rate (Aslam et al., 1984; Cerezo et al., 1999). With regard to the latter, our results exclude that the inhibition found was influenced by water uptake because no significant difference was detected among the treatments (Fig. 6.1a). Instead it was not possible to figure out whether other salts or compounds could influence NO_3^- uptake by osmotic effect as other researchers report (Peuke and Jeschke, 1999).

The initial 24-hr K^+ uptake rates show a slight decrease with increasing NaCl concentration (Figure 6.2). However it did not result significantly affected by the NaCl treatments (ANOVA test, $P < 0.05$). Under saline conditions, potassium uptake is typically limited by increasing NaCl concentration (Parida and Das, 2005). Nevertheless, several studies report improvement in potassium absorption depending on the saline ion composition and time of exposure (Bloom and Finazzo, 1986; Manchanda et al., 1991), presence of adequate Ca^{2+} concentration in the substrate (Cachorro et al., 1994), and nitrogen-salt composition. Grattan and Grieve (1999) state that nitrate salts, as a source of nitrogen, have positive effect on potassium uptake compared to ammonium salts. With regard to this issue, differing responses have been reported for rose plants. Lorenzo et al. (2000) found a progressive decrease in potassium uptake during the crop cycle and a higher Na^+/K^+ ratio (tissue concentration) related to sodium external concentration. On the contrary Cabrera and Perdomo (2003) did not find any significant change for potassium tissue concentration on roses grown under saline conditions. Recent works (Rodriguez-Navarro and Rubio, 2006) remark that potassium and sodium uptake are accomplished by different cation transporters respectively labeled as HAK and HKT transporters; which implies that sodium and potassium uptake could not influence each other especially in the short-term period. The same authors report that Na^+ influence K^+ uptake only if the latter is scarcely present in the root

environment. To our knowledge there is no other existing work that shows potassium uptake kinetic changes for a rose crop under short-term saline conditions. In conclusion, in this work different kinetic models perform for potassium and nitrate uptake under saline conditions for the experimental period tested. The discrepancy arisen between nitrogen and potassium absorption likely lies in the rose plant nutrient demand which is variable during the crop cycle depending on the absorbed nutrient, the carbohydrate availability and plant growth rate (Cabrera et al., 1995). According to Cabrera et al. (1995) and basing on our previous studies (data not yet published) at the very end of the flowering cycle rose plants show a tendency to decrease potassium uptake rate. In this sense the presence of NaCl could have influenced much less the uptake of potassium compared to the uptake of nitrate which, in the same period, is strongly required by the plant because its replenishment in the older plant tissues.

6.4.2 Nitrate and potassium uptake kinetics

Non-linear regression was used to fit data on solution concentration of ions versus uptake rates during the entire experimental period for each treatment. M-M general equation (Tab. 6.1) was used for a first interpretation of nutrient absorption kinetics. The results are summarized in the Table 6.2. Estimated parameters show a significant reduction (according standard error) of the estimated nitrate uptake I_{\max} , whereas no difference was detected for nitrate uptake K_m by increasing NaCl concentration. I_{\max} decreased following a hyperbolic pattern and showing a clear decline until a NaCl concentration of 35 mol m^{-3} , after which an asymptotic pattern occurred (Figure 6.3). Other authors noticed that I_{\max} is the primary factor influencing net influx of NO_3^- (Sharifi and Zebarth, 2006). To this extent several works focus on I_{\max} reduction, as a consequence of external stress conditions or internal plant feed-back controls, for simulating nutrient uptake by using M-M approach. Mattson et al (2006) relates the variation of I_{\max} to tissue nutrient storage; Sharifi and Zebarth (2006) found a negative relationship between I_{\max} and plant/root age for potato plants; Wheeler et al. (1998) proposed a model which related I_{\max} changes to GER (Growth Environment Ratio). Decrease in I_{\max} value combined with constant K_m value is typically associated with non-competitive inhibitions as discussed previously in this paper. Hawkins and Lewis (1993) found a non-competitive inhibition exerted by NaCl on NO_3^- and NH_4^+ for wheat. Such inhibition was characterized by a negative relationship between I_{\max} and NaCl concentration whereas no significant difference was found for estimated K_m . Peuke and Jeschke (1999) in a similar experiment, found the same kinetics for HATS nitrate uptake in barley seedlings, whereas a competitive relationship was established between LATS nitrate uptake and NaCl. We did not found any difference between LC and HC nutrient solution with

regard the variation of I_{\max} and K_m . Therefore we handled all data in the same way with the result of modelling the total NO_3^- uptake.

Table 6.2. Effects of NaCl (mol m^{-3}) treatments on the kinetics of nitrate (NO_3^-) and potassium (K^+) uptake in a rose culture (cv. Kardinal grafted on Natal Briar rootstock). One year old plants were moved at the end of the crop cycle into growth chambers where nitrogen and potassium depletion were monitored during six days. Two different initial nitrate and potassium concentration (mol m^{-3}) and eight different NaCl treatments were tested. I_{\max} ($\mu\text{mol gDW}^{-1} \text{h}^{-1}$ where gDW corresponds to grams of total dry weight) and K_m (mol m^{-3}) were estimated via non-linear regression with a statistical software package. Because of incomplete solution depletions (HC nutrient solution), potassium M-M kinetic was sometimes not estimable (n.e.). The corresponding standard errors are given as \pm for the estimated parameters.

Initial nutrient solution concentration		Nitrate uptake kinetic			Potassium uptake kinetic		
NaCl	NO_3^- or K^+	I_{\max}	K_m	R^2	I_{\max}	K_m	R^2
0	LC	5.1±0.4	0.8±0.1	0.99	1.1±0.4	0.4±0.1	0.74
5	HC	4.3±0.3	0.8±0.2	0.89	n.e.	n.e.	-
15	LC	3.8±0.5	0.7±0.2	0.96	1.1±0.3	0.6±0.2	0.73
25	HC	3.4±0.4	0.8±0.3	0.59	n.e.	n.e.	-
35	LC	2.5±0.6	0.7±0.3	0.71	0.9±0.4	0.4±0.1	0.83
45	HC	2.6±0.3	0.7±0.3	0.68	n.e.	n.e.	-
55	LC	2.7±0.2	0.6±0.1	0.99	0.9±0.6	0.6±0.2	0.64
65	HC	2.5±0.2	0.8±0.3	0.69	n.e.	n.e.	-

As mentioned above, few researchers have investigated M-M-simulated nutrient uptake under saline environments and the effect of inhibitor ions on nutrient uptake kinetics (Hawkins and Lewis, 1993; Peuke and Jeschke, 1999) and, to our knowledge, previous research has not focused on rose. Potassium uptake kinetics in terms of I_{\max} and K_m did not show any significant difference in response to NaCl treatments (Table 6.2). It should be noted that for HC nutrient solution, non-linear statistic procedure was not able to estimate M-M parameters because of an incomplete nutrient depletion by the end of the experiment. This was caused by too large a quantity of K^+ used in the initial solution. The initial 24-hr K^+ uptake rate did not vary in response to NaCl as previously discussed in this paper (Figure 6.2b).

6.4.3 Model development and analysis

Increasing NaCl concentration produced a hyperbolic decrease in I_{\max} value for NO_3^- uptake which suggested a non-competitive equation (Tab. 6.1) as the best solution for fitting data and explanation of variance. The parameterization of the proposed equation was accomplished by non-linear regression and applied for all collected data (n=144). The results of this analysis are summarized in Table 6.3. In this first equation we assumed efflux was negligible at nutrient concentrations approaching 0; however several studies point out that incorporation of net uptake flux can greatly improve M-M kinetics in nutrient uptake simulation (Barber, 1995; Le Bot et al., 1998; Hogh-Jensen et al., 1997; Silberbush and Lieth, 2004; Mattson et al., 2006). To this end NCM-M equation was modified and rearranged as follow:

$$I_{\text{NO}_3} = \left(\frac{[\text{NO}_3^-] - [\text{NO}_3^-]_{\min}}{[\text{NO}_3^-] - [\text{NO}_3^-]_{\min} + K_m} \right) \left(I_{\max} - I_{\max} \left(\frac{[\text{NaCl}]}{[\text{NaCl}] + K_i} \right) \right) \quad (6.1)$$

where I_{NO_3} is the net instantaneous flux of nutrient expressed as ($\mu\text{mol gDW}^{-1} \text{h}^{-1}$), K_m and K_i are the M-M affinity constants respectively for nutrient and salt (mol m^{-3}) and $[\text{NO}_3^-]_{\min}$ (mol m^{-3}) is the minimum nitrate concentration below which nitrate effluxes occur. However, both R^2 and absolute error (MAE) were little improved by this procedure. Moreover, the resulting distribution of the residuals was still slightly skewed, while the value of the median was negative (data not shown). Non-competitive inhibition represented by NCM-M equation (Table 6.1) implies that as inhibitor concentration approaches infinity I_{\max} approaches zero as in the equation:

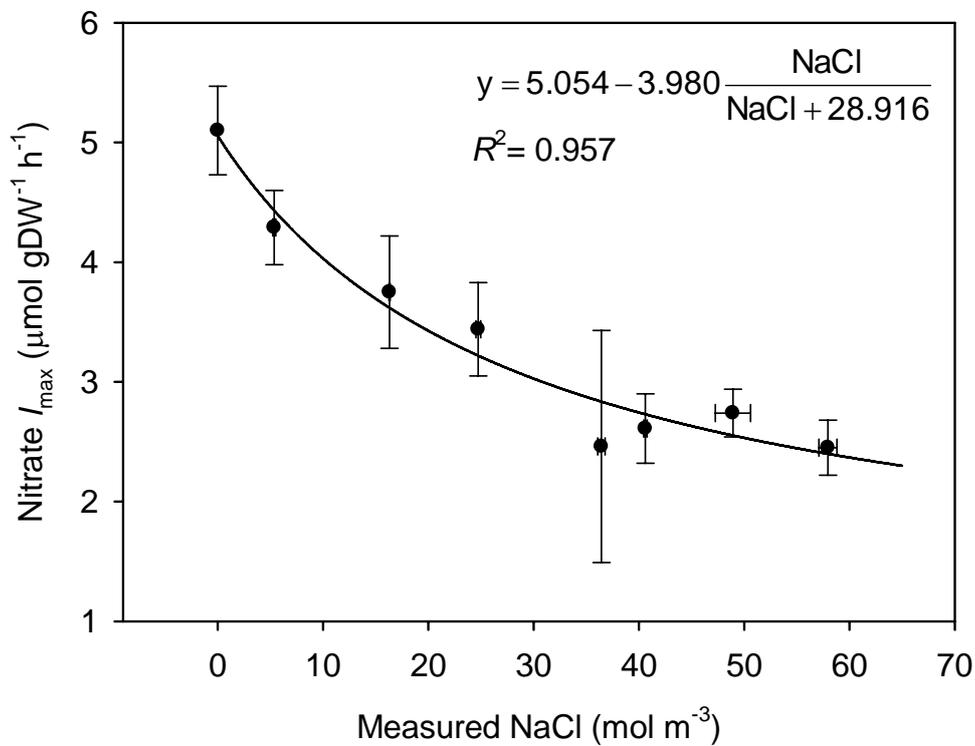
$$\lim_{[\text{Ir}] \rightarrow \infty} I_{\max} - I_{\max} \left(\frac{[\text{Ir}]}{[\text{Ir}] + K_i} \right) = 0 \quad (6.2)$$

where $[\text{Ir}]$ represent the concentration of the inhibitor (NaCl concentration in our experiment). However, our results show a strong asymptotic pattern for I_{\max} depending on NaCl concentration (Figure 6.3). Therefore, as suggested by Walsh et al. 2007, we introduced an asymptote equal to $I_{\max 2}$ which represents the minimum values achievable by I_{\max} when the inhibitor concentration approaches infinity as follows:

$$\lim_{I_r \rightarrow \infty} I_{\max} - \Delta I_{\max} \left(\frac{[I_r]}{[I_r] + K_i} \right) = I_{\max 2} \quad (6.3)$$

where $\Delta I_{\max} = I_{\max} - I_{\max 2}$.

Figure 6.3. Effects of NaCl treatments on the estimated maximum nitrate uptake rate (I_{\max}). I_{\max} values, determined through non-linear regression to the M-M equation, were plotted against the measured NaCl concentration. Data are estimated parameter \pm model standard error (Y axis) and experimental data means ($n=3$) \pm standard error (X axis).



Then by rearranging the Equation 6.1 we obtained the following equation.

$$I_{\text{NO}_3} = \left(\frac{[\text{NO}_3^-] - [\text{NO}_3^-]_{\min}}{[\text{NO}_3^-] - [\text{NO}_3^-]_{\min} + K_m} \right) \left(I_{\max} - \Delta I_{\max} \left(\frac{[\text{NaCl}]}{[\text{NaCl}] + K_i} \right) \right) \quad (6.4)$$

Table 6.3. Parameters estimated for the different proposed equations. Non-linear regression was used for fitting data collected during the experiment and the different parameters were estimated respectively for each proposed model. I_{\max} ($\mu\text{mol gDW}^{-1} \text{h}^{-1}$ where gDW correspond to grams of total plant dry weight) represent the maximum influx of nutrients detectable when NaCl is not present in the nutrient solution while $I_{\max 2}$ represent the minimum value of I_{\max} for NaCl concentration approaching infinity. K_m and K_i (mol m^{-3}) are M-M kinetic affinity constants estimated for the nutrient and the inhibitor, respectively; while N_{\min} (mol m^{-3}) is the minimum nitrogen concentration below which no net nitrogen influx occurs. Model R^2 and MAE (Mean Absolute Error) are also reported for each equation.

Equation number	I_{\max}	$I_{\max 2}$	K_m	K_i	$[N_{\min}]$ ($\times 10^{-3}$)	MAE	R^2 (%)
Nitrate uptake proposed model							
(NCM-M)	4.69		0.70	58.24		0.26	91.57
(6.1)	4.65		0.68	58.41	9.26	0.24	91.60
(6.4)	4.74	0.78	0.68	39.73	9.30	0.23	91.66
Potassium uptake proposed model							
(M-M)	0.65		0.25			0.08	80.01

Having a minimum I_{\max} implies that the salt inhibition is not complete even though a very high concentration (in a reasonable range) of salt is present. Although this last equation gave little improvements, with regard to mean absolute error and R^2 value (Table 6.3), the resulting distribution of residual errors was the most suitable among the three fitted equation (Figure 6.5). Eq. 6.4 was able to simulate nitrate uptake in a suitable manner optimizing the interpolation of experimental data (Figure 6.5) and showing the best statistic performance compared to the other tested equations. In particular Figure 6.5a shows the distribution of predicted data plotted against measured data. The resulting graph confirms a regular spread of predicted data which are proportionally dispersed along the regression line. Nevertheless, the intercept value is somewhat higher than 0 (intercept = 0.124) denoting a slight overestimation of predicted. Figure 6.5d reports the surface of simulated data showing a visual interpolation of measured data. Overall the proposed model resulted in a P -value < 0.0001 which denoted a high capability in estimating nutrient uptake depending on sodium chloride and nitrate external concentration.

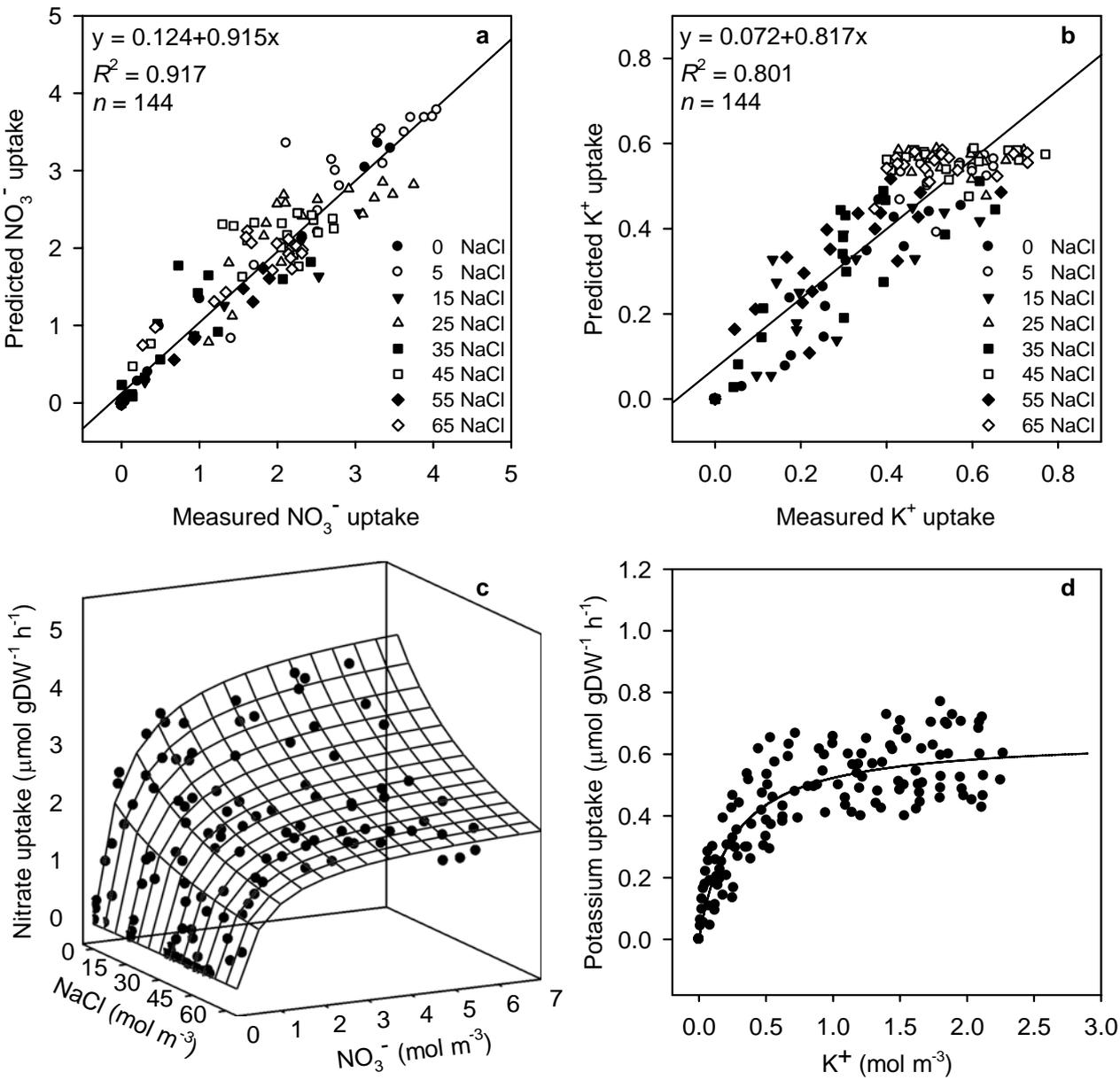
As discussed earlier in the paper, NaCl treatments did not have any influence on potassium uptake kinetics. This suggested that uptake of the nutrient should be simulated only as a function external K^+ concentration (Eq. M-M, Table 6.1). Non-linear regression was used for fitting

experimental data. Estimated parameters are summarized in Table 6.3. No significant value was found for $[K_{\min}^+]$ during experimental data fitting denoting a negligible potassium efflux phenomenon. Model-derived predicted data are plotted against measured data in Figure 6.5b which show a uniform data spread along the regression line even though the intercept value was found slightly higher than 0 coupled with a slope less than 1. This denotes a slight overestimation of uptake values at low concentrations which tend to diminish at higher concentrations. Once again the proposed model resulted in a P -value < 0.0001 which denoted a high capability in estimating nutrient uptake depending on external potassium concentration.

6.5 Conclusions

Nowadays cut-rose production is normally conducted in soilless systems (Cabrera and Perdeomo, 2003) where an accumulation of salts may occur due to both the low leaching fraction (closed systems) and the use of poor quality water (closed and open systems). Raviv et al. (1998) reports using wastewater for rose irrigation may improve nutrient solution management without significant yield losses. However, under saline conditions, even in the short period, plant nutrition can change as influenced by inhibitor ions such as Na^+ and Cl^- . Using our model, we can determine the influence of NaCl present in the irrigation water on NO_3^- uptake. For example at a commonly used NO_3^- concentration for roses (12 mM) when NaCl is not present NO_3^- uptake rates are $4.48 \mu\text{mol gDW}^{-1} \text{h}^{-1}$, when NaCl is present at 8 mM, NO_3^- uptake rates is $3.86 \mu\text{mol gDW}^{-1} \text{h}^{-1}$ which is a decrease of $0.62 \mu\text{mol gDW}^{-1} \text{h}^{-1}$. Our model could be used by rose producers to determine the fertilizer concentration required to counteract NaCl inhibition. However, more work is required to calibrate the model for other rootstocks/cultivar, and test the model under long-term production. If short-term response relates to long-term salinity tolerance, our model might be used to quickly screen rootstock cultivars for their salt tolerance in regards to NO_3^- absorption.

Figure 6.5. Predicted versus measured data for nitrate (a) and potassium (b) uptake rates. Symbols represent daily measured and predicted uptake events for each plant in each of the different NaCl treatments. The relationship between of NaCl and NO_3^- concentration on NO_3^- uptake as represented by Eq. 6.4 (c) where the surface represents simulated data and symbols represent measured data. Potassium uptake was not influenced by Na concentration. Global fitting of K^+ uptake as a function of K^+ solution concentration (d); the line represents simulated data whereas symbols represent measured data. Equations representing Figure 6.5c and 6.5d are reported in Table 6.3.



6.6 References

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7 GENERAL DISCUSSION

7.1 The management of soilless culture under saline conditions

High technologies coupled with intensive cultivation systems, such as hydroponic culture, are the main factors that have contributed to high greenhouse production in the last years. Nowadays, greenhouses are part of a complex production process that includes also post-harvest processing and distribution (chapter 1). In this scenario, the management of irrigation and fertilization and, in general, the use of water resources remain focal points (chapters 5 and 6). Massive fertilization leads to soil toxicity, increased salinity of groundwater and eutrophication of surface water (chapter 6). However, a high leaching fraction is required for many cultures when the use of saline water is imposed (chapter 2). Because in many cultural systems, and particularly under greenhouse conditions, the supply of water and fertilizers are coupled, a high leaching fraction may turn in waste of water and nutrients (chapters 2 and 4).

On the other hand, the insufficient delivery of nutrients and water causes crop damages that may lead to limited yield and produce quality. In this regard, several models are used for irrigation and fertilization scheduling (chapter 1). Under saline environment, plant nutrition may change affecting nutrient solution management in soilless systems (chapters 1, 2 and 5). Such changes regard nutrient absorption in the long period (chapter 5) as well as in the short term (chapter 6). Furthermore the presence of poor quality water may influence the kinetics of ballast ion accumulation depending on salinity level (chapter 4).

Managing closed systems is much more complicated in the presence of saline water. Under these conditions, there is a more or less rapid accumulation of non-essential (ballast) ions (such as Na^+ and Cl^-) in the recirculating nutrient solution, which results in a concomitant increase in the total salinity, namely in electrical conductivity, if the management strategy aims to maintain a constant nutrient concentration in the root zone (chapter 2); alternatively, there may be a parallel depletion of nutrients, if the nutrient replenishment is based on a feedback control of EC (chapter 2). In both cases, the nutrient solution has to be replaced more or less frequently (semi-closed system). The procedure of nutrient solution replacement (flushing) may be partial or total and may involve different strategies of substrate washing (substrate culture). Nutrient solution is a sort of ballast ions “garbage” where all chemicals not absorbed by the culture are accumulated.

As another example, salts can be accumulated in the substrate; this is the case of sub-irrigation strategies. Sub-irrigation may reduce salt accumulation in the recirculating nutrient solution, since the upward water movement (by capillary force and evapotranspiration-driven mass flow)

makes soluble salts to accumulate in the growing medium. This technique can be adopted successfully with annual plant (tomato) where, at end of cycle, the substrate can be reused. On the contrary it could induce several saline stresses in perennial plants such as roses.

Complications reported above not only determine environmental pollution and plant stresses, but bring on high production costs (chapter 3). In a productive cycle the optimization of costs is compulsory to maximize profits. To this extent economic balances are object of off-line simulations able to predict data also by comparing different technical strategies (chapters 2 and 3). As discussed in the chapter 2 the use of models can be applied for simulating different nutrient solution management strategies, also depending on different cultivation systems (substrate or liquid cultures) or species. Such models not only enable on-line nutrient solution control, furthermore they are useful tools for predicting data whose use is suitable for making decision for the long period.

7.2 Models for crop mineral nutrition

In the previous chapters a heterogeneous overview of different models has been given. Most of them show high capability in predicting experimental observation, while other models need further studies to be applied for operational purposes. Such models are occasionally characterized by empirical approach. In regression models, mathematics is the only support that can justify their frameworks. In other cases, the models are based on mechanistic approaches that involve more general physical and/or biological laws. Their plurality gives an idea on the enormous possibility and benefits which can arise by their application.

Crop modelling follows general rules that mainly deal with model framework and modelling process (chapter 1): i) model subject must be observed and its simulation environment must be defined; ii) model assemblers must base their conclusions on experimental data; iii) model goodness must be appropriately validated. Nevertheless, there is no limit to constrain modelling approaches. In classic modelling literature different model classifications have been reported (chapter 1). Most of them remarks the differences between empirical (e.g. chapters 4 and 5) and mechanistic (e.g. chapter 6) model, others regard the ability in predicting data variability, and others again refer to the different use of mathematics or statistics (e.g. chapter 5 and 6).

These varieties of approaches furnish the base to accomplish many possible targets in different ways. During this work different modelling approaches and methods have been tested. While in the chapter 6 an example is given on how different patterns arise from the uptake kinetics of different ions under the same stress conditions, on the contrary in the chapter 2 it is reported that uptake concentration concept is a suitable parameter to simulate nutrient absorption for many

ions under (semi-) commercial conditions. Instead, chapter 4 and 5 report an interesting example on the ability of empirical models to simulate nutrient and non-nutrient absorption. In these examples the use of statistics gave good results showing high capability in predicting data; nevertheless, these meaningful results were observed with regard to cation absorption, while anion absorption was poorly estimated, thus implying that multiple regression was not able to describe adequately the absorption of such ions.

In the practice it is very difficult to understand the limit between different approaches. Although the appearance of models can be representative of their goodness, this must not represent only a mere exercise of style. The secret of a good model lies into its robustness and application flexibility. Furthermore, modelers should focus on easy-to-use models; therefore they must know the final users. In fact, models are projected for researchers and growers as well. Although this aspect does not deal with the validity of the model, nevertheless it is very important for model packaging. Researchers have the possibility to use simulation models under experimental condition not reproducible in commercial greenhouses. Growers need easy-to-use models, possibly enabling on-site calibrations. In effect on-site calibration represents an important model characteristic that may greatly improve their application for specific cultivation systems. To accomplish this task many modelers use to put in their model package, which is substantially a computer program, a section that can be updated by users. This kind of procedure sometimes is simple and involves few and easy-to-estimate variables such as number of days, mean air temperature or phenologic phases; sometimes it is unrealizable because it involves variables that are either not easy to determine, especially under the typical conditions of commercial culture.

For instance, Michaelis-Menten-based models (chapter 6) are calibrated by means of the nutrient depletion technique, which necessitates of sophisticated experimental apparatus, laboratory analysis and statistics. Another example is given in the chapter 5, where an easy-to-measure dependent variable, such as crop water uptake, needs, for model parameterization, the use complex mathematics and statistics. Finally, in the chapter 2 we report a nutrient absorption model that, despite its complexity, is based on the concept of uptake concentration that does not require much sophisticated analysis or laboratory instruments to be estimated. As matter of fact, the latter would show more flexibility in commercial applications where it is supposed grower's education does not imply the knowledge of advanced statistics techniques.

7.3 Integrated models

Models can run by themselves or take place in a DSS. The necessity of developing DSSs arises from the need of simulating complex systems that involves a large quantity of information. An

example of the many targets achievable by using a DSS is given in the chapter 3, where a wide discussion on the use of water in closed-loop soilless systems is reported. The fact that DSSs provide for simulated data implies that they can be used as making decision tool. These possibilities have encouraged modelers to produce a number of different DSSs for horticultural crops (chapter 3). Most of them provide for information on plant development, water uptake and crop timing (chapter 3). However, only few works are functional for nutrient absorption and especially under saline conditions. In this sense, this thesis provides for experimental data that can be handled for reassessing the use of poor quality water in rose and tomato plant.

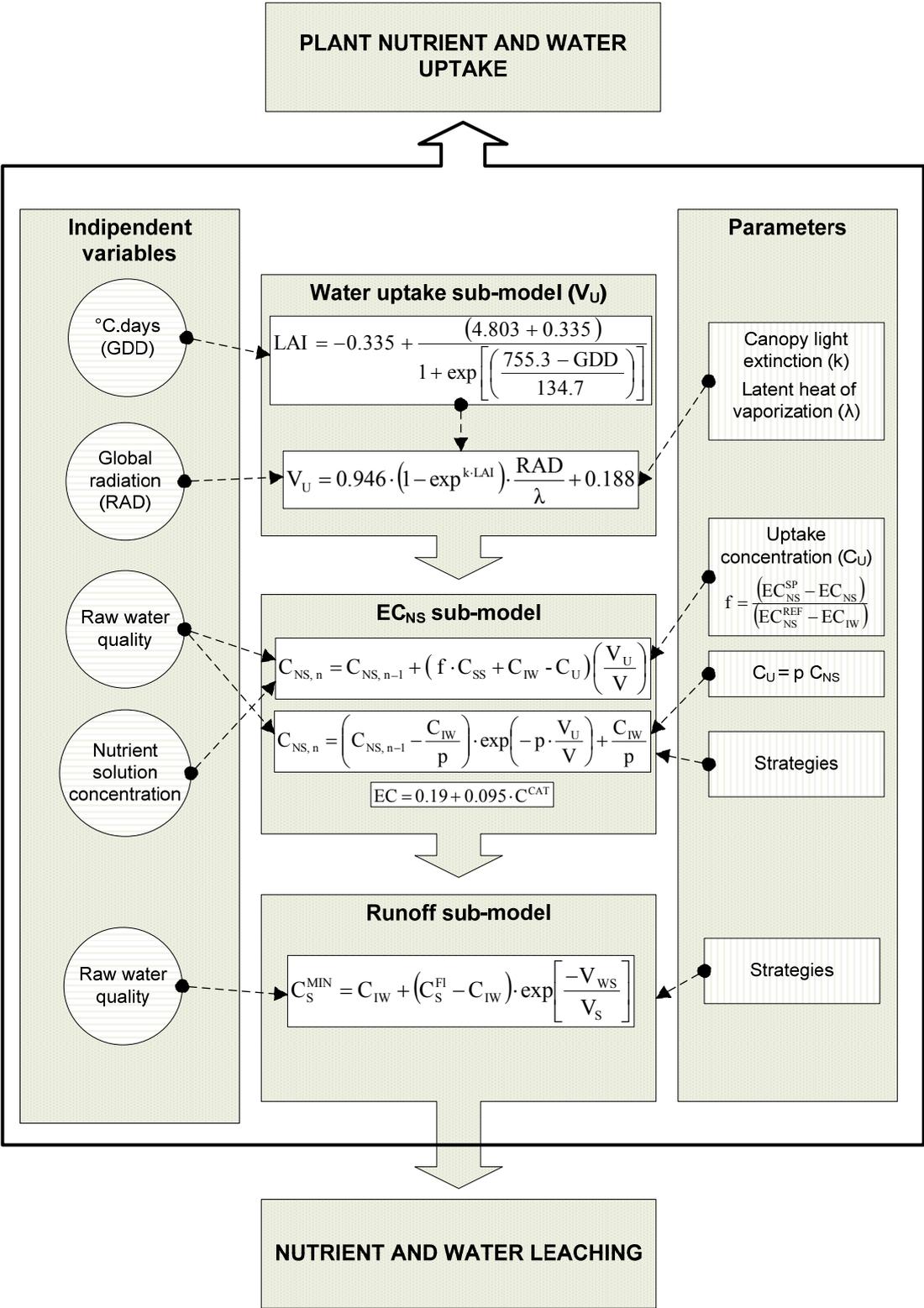
As mentioned above model integration can occur in order to accomplish different tasks that either are linked or are not linked each other. Moreover, in the same DSS, a number of different models may depend each other by successive steps (series) or they may work coupled to accomplish the same task (parallel).

In the Figure 7.1 a flow-chart shows the framework of the DSS presented in the chapter 3 (SIMULHYDRO). In this example, different models (or sub-models) are integrated to accomplish the task of calculating nutrient and water absorption and leaching. Such models depend on each other by successive steps. Nevertheless, no model used in each step is fundamental for running the simulation, because it may be replaced by direct input of data or by analogous models. For instance, the water uptake model (V_U sub-model) could be replaced by the direct measurement of crop water demand provided by the water meters implemented in the growing systems or using particular device such as Prodrain® (HORTIMAX). It is a device that continuously measures the substrate weight and giving information on the substrate's water content, the drain quantity and the condition of the crop. Hence irrigation can be automated basing on this information.

The same happens for the model that simulates nutrient absorption and then the ion concentration in the recycling water (EC_{NS} sub-model). The model proposed in this example does not take into account what occurs at very low nutrient concentration under saline condition (chapter 2). To this purpose, such model could be replaced by the model discussed in the chapter 6 with further improvements for nitrate uptake simulation.

Flexibility is one of the most important characteristics that a model should have. However, some modelling approaches such as black boxes or neural networks are a sort of packs from which data come out. Although these models offer great capability in predicting data, they do not allow easy framework modification.

Figure 7.1. The chart shows the integration of different models as an example of DSS framework.



7.4 Conclusions

Modelling is not the panacea for all plant cultivations problems. With regard to saline environment, and especially for plants grown in soilless systems, several strategies have been proposed for improving water quality. However modelling plant nutrition has given proof of being a profitable approach for making decision in the long and short period (chapter 3). The application of models shows high capability in nutrient solution managing. Models are easy-to-use tool that does not require any specific grower's education.

Moreover, model application can decrease cost production compared to other approaches (chapter 3). In effect models that involve different strategies to manage cultivations under saline conditions are more efficient, in terms of costs, compared to water depuration and collection. In this sense DSSs give a great example of profitable culture managements.

DSSs and models in general should be flexible systems that enable on-site calibration. Nevertheless there are not peremptory constraints in modelling approaches. The goodness of a model or DSS is detectable by its robustness, ability in predicting data and its capacity to be integrated and applied to many different cultural systems.

This PhD thesis focuses on nutrient absorption under saline conditions. Such area has been not much investigated by modelers that have widely focused on water uptake and plant development models in the past (chapters 1 and 3). As matter of fact, groundwater and soil salinization is increasing in large costal and semi-arid regions such as Mediterranean area (chapter 1). In this sense experimental results collected and reassumed in this manuscript: i) furnish insights into plant nutrition under saline conditions, ii) give proof that model application can really improve greenhouse management and iii) nonetheless offer an meaningful base for further research studies.

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SUMMARY

Horticultural crops are widely diffused in the world since the demand of fruits, vegetables and ornamentals as well, is fundamental for the life of human beings and their health.

This study is characterized by horticultural plant cultivation in soilless systems. Nowadays soilless is diffused and normally applied to many industrial cultures (tomato, cucumber, rose and others), especially in floriculture production. In hydroponic horticultural production, beside the risk of root pathogen infections, nutrient solution management remains the primary difficulty, especially when poor quality water is used (saline water). In fact under saline conditions ballast ions, such as Na and Cl, build up in the recirculating nutrient solution (closed-loop systems) determining high EC (electrical conductivity) levels. Such conditions affect plant nutrition through ion competition and osmotic and toxic stress.

As remarked by many authors, simulation models represent, among others, valuable tools for managing nutrient solution and plant nutrition, even in saline environments. This thesis focuses on plant nutrition modelling with particular emphasis towards soilless cultivation under saline conditions. To this purpose a certain number of models have been developed and their application has been statistically and experimentally (greenhouse cultivation) evaluated. Such models are able to predict, with satisfactory accuracy: i) the variation of the concentration of both nutrients and non-essential elements (such as Na⁺ and Cl⁻) in the recirculating water of closed growing systems; ii) the uptake of nutrient and non-essential elements depending on the external salt concentrations.

Although the structure of the thesis involves several concepts of mathematics, statistics and computer programming, nevertheless most of the work is based on physiological and biological assumptions derived from experimental observations. Such experiments have been carried out on roses (*Rosa hybrida*) and tomato (*Solanum lycopersicum*) plant during the period since 2005 to 2007.

Finally, this work shows in which way modelling and simulation studies can be applied to plant fertilization and nutrient solution management giving actual examples of possible commercial applications.

RIASSUNTO

Le coltivazioni ortofloricole sono ampiamente diffuse in tutto il mondo poiché l'uso e il consumo di frutta, verdura e fiori sono fondamentali per la vita degli esseri umani e per la loro salute.

Questo studio è caratterizzato dalla coltivazione di specie orticole in sistemi fuori suolo. Oggigiorno il fuori suolo è diffuso e normalmente applicato a molte colture industriali (pomodoro, cetriolo, rosa ecc.), specialmente in floricoltura. Nelle colture ortofloricole idroponiche, insieme ai rischi dovuti ai patogeni radicali, la gestione della soluzione nutritiva resta una delle principali difficoltà, specialmente quando si è costretti a utilizzare acqua di scarsa qualità (acque saline). In fatti in condizioni saline gli ioni non assorbiti dalla pianta (Na, Cl, ecc) si accumulano nella soluzione nutritiva ricircolante (sistemi a ciclo chiuso) determinando alti livelli di CE (conducibilità elettrica). Tali condizioni influenzano la nutrizione della pianta attraverso competizione ionica e condizioni di stress osmotico e tossico.

Come evidenziato da diversi autori, i modelli di simulazione rappresentano, tra gli altri, validi strumenti per la gestione della soluzione nutritiva e della fertilizzazione in generale, anche nelle coltivazioni in ambiente salino. Questa tesi è rivolta alla modellizzazione dei fenomeni implicati nella nutrizione delle piante con particolare enfasi verso le coltivazioni fuori suolo in condizioni saline. A questo proposito sono stati sviluppati una serie di modelli e la loro applicazione è stata testata sperimentalmente (coltivazione in serra) e statisticamente. Tali modelli sono in grado di predire, con soddisfacente accuratezza, la variazione della concentrazione dei nutrienti e non nutrienti (come Na e Cl) nella soluzione nutritiva ricircolante di sistemi di crescita a ciclo chiuso e quindi il tasso di assorbimento di nutrienti e non nutrienti da parte della pianta.

Sebbene la struttura stessa di questa tesi comprenda concetti di matematica, statistica e linguaggio macchina, l'intero lavoro si basa su assunti di natura fisiologica e biologica, derivati da osservazioni sperimentali. Tali esperimenti sono stati condotti su piante di rosa (*Rosa hybrida*) e pomodoro (*Solanum lycopersicum*) durante il periodo dal 2005 al 2007.

In fine questo lavoro mostra in quale modo la modellizzazione e gli studi in simulazione possono essere utilizzati per la fertilizzazione delle piante e per la gestione delle soluzioni nutritive dando reali esempi di possibili applicazioni commerciali.