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Improvements in small scale soil less-systems: testing growing media and nutrient solution

Presentata da: **Dr. Marco Fecondini**

Coordinatore Dottorato **Relatore**

Prof. Giovanni Dinelli **Prof. Giorgio Prosdocimi Gianquinto**

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1. Introduction

This PhD thesis is composed by three different experiment, two of them (see chapter 2 and 4) were carried out in the Controlled Environment Systems Research (University of Guelph, Ontario, Canada). The third was carried out in the University of Bologna (chapter 3). Experiments are aimed to a better knowledge of different aspects of soil less crop production. This knowledge can be either applied to different field and application of soil less crop production (see 1.1.1 to 1.1.3), from the most common ones to the ones that might play an important function in the future.

Soil less culture is a technology for growing plants out of soil. Plants are fed by a nutrient solutions (water containing fertilizers) with or without the use of a growing media (as sand, gravel, vermiculite, rockwool, perlite, peatmoss. coir, or sawdust) to provide mechanical support. Water solution systems have no other supporting medium for the plant roots: aggregate systems have a solid medium of support. Soil less systems are further categorized as open (i.e., once the nutrient solution is delivered to the plant roots, it is not reused) or closed (i.e., surplus solution is recovered, replenished, and recycled). In many cases the word hydroponics is used to describe some kind of soil less cultures and the difference between hydroponics and a soilless culture is often blurred.

Soil-less is usually used in combination with greenhouses, it is high technology and capital-intensive. It is also highly productive, conservative of water and land, and also protective of the environment if a closed cycle is used. It requires more advanced skills than field agriculture. Since regulating the aerial and root environment is a major concern in such agricultural systems, production takes place inside enclosures designed to control air and root temperatures, light, water, plant nutrition, and adverse climate.

The development of soil less has not been rapid. Jensen (1997) underlined that although the first use of soil less and controlled environment was the growing of off-season cucumbers under "transparent stone" (mica) for the Roman Emperor Tiberius during the 1st century, the technology is believed to have been used little, if at all, for the following 1500 years.

Greenhouses (and experimental hydroponics) appeared in France and England during the 17th century; Woodward grew mint plants without soil in England in the year 1699. The basic laboratory techniques of nutrient solution culture were developed (independently) by Sachs and Knap in Germany about 1860 (Hoagland and Arnon, 1938).

In the United States, interest began to develop in the possible use of complete nutrient solutions for large-scale crop production about 1925. Greenhouse soils had to be replaced at frequent intervals or else be maintained in good condition from year to year by adding large quantities of commercial fertilizers. As a result of these difficulties, research workers in certain U.S. agricultural experiment stations turned to nutrient solution culture methods as a means of replacing the natural soil system with either an aerated nutrient solution or an artificial soil composed of chemically inert aggregates moistened with nutrient solutions (Withrow and Withrow, 1948).

Between 1925 and 1935, extensive development took place in modifying the methods of the plant physiologists to large-scale crop production. Workers at the New Jersey Agricultural Experiment Station improved the sand culture method (Shive and Robbins, 1937). The water and sand culture methods were used for large-scale production by investigators at the California Agricultural Experiment Station (Hoagland and Arnon, 1938). Each of these two methods involved certain fundamental limitations for commercial crop production, which partially were overcome with the introduction of the subirrigation system initiated in 1934 at the New Jersey and Indiana Agricultural Experiment Stations (Withrow and Withrow, 1948). Gericke (1940) published a description of a quasi-commercial use of the liquid technique and apparently coined the word hydroponics in passing. The technology was used in a few limited applications on Pacific islands during World War II. After the war, Purdue Univ. popularized hydroponics (called nutriculture) in a classic series of extension service bulletins (Withrow and Withrow, 1948) describing the precise delivery of nutrient solution to plant roots in either liquid or aggregate systems. While there was commercial interest in the use of such systems, hydroponics or nutriculture

was not widely accepted because of the high cost in construction of the concrete growing beds.

After a period of ~20 years, interest in hydroponics was renewed with the advent of plastics (Jensen, 1997). Plastics were used not only in the glazing of greenhouses, but also in place of concrete in lining the growing beds. Plastics were also important in the introduction of drip irrigation. Numerous promotional schemes involving hydroponics became common with huge investments made in growing systems.

Greenhouse areas began to expand significantly in Europe and Asia during the 1950s and 1960s, and large hydroponic systems were developed in the deserts of California, Arizona, Abu Dhabi, and Iran about 1970 (Fontes, 1973; Jensen and Teran, 1971). In these desert locations, the advantages of the technology were augmented by the duration and interest of the solar radiation, which maximized photosynthetic production.

Unfortunately, escalating oil prices, starting in 1973, substantially increased the costs of controlled environment agriculture heating and cooling by one or two orders of magnitude. This, along with fewer chemicals registered for pest control, caused many bankruptcies and a decreasing interest in hydroponics, especially in the United States (Jensen, 1997).

Since the inception of hydroponics, research to refine the methodology has continued. In the late 1960s researchers at the Glasshouse Crops Research Institute (GCRI), Littlehampton, England developed the nutrient film technique along with a number of subsequent refinements (Graves, 1983). This research gave rise to the hydroponic systems used today. Jensen and Collins (1985) published a complete review of hydroponics highlighting many new cultural systems developed in Europe and the United States.

Almost 40 years have passed since the last real commercial interest in hydroponics, but today there is renewed interest among growers establishing controlled environment soil-less agriculture. This is especially true in regions where there is concern about controlling pollution of ground water with nutrient wastes or soil sterilants. Today growers

appear to be much more critical in regard to site selection, structures, the growing system, pest control, and markets.

From its origins in academic research, to its utilization in industry and government, hydroponics has found many new applications. It is a versatile technology, appropriate for both developing countries and high-tech space stations (Jensen, 1997). Hydroponic technology can efficiently generate food crops from barren desert sand and desalinated ocean water, in mountainous regions too steep to farm, on city rooftops and concrete schoolyards and in arctic communities. In highly populated tourist areas where skyrocketing land prices have driven out traditional agriculture, hydroponics can provide locally grown high-value specialty crops such as fresh salad greens, herbs and cut flowers. It can be an important tool for feeding the city of the future.

1.1 Soil-less for biorigeneretive life support systems

Future Space stations and Space habitats/outposts should be envisioned as self-sufficient ecological closed or semi-closed systems. NASA and ESA (European Space Agency) are carrying out several projects that involve many research groups from complementary areas with a final goal of designing a facility for plant cultivation in Space (De Micco et al., 2009). Currently, spacecraft life support systems rely on open-loop (nonrecycling) technologies. These are simple and sufficiently reliable for human space flight mission of relatively short duration, small crew sizes and limited power availability (Schwartzkopf, 1992). Life support technologies for the coming era of exploration, however, must address a different set of requirements. Longer duration mission, larger crew sizes, and changes in crew components during the mission will require maximizing crew safety by increasing the degree of self-sufficiency of the life support systems, minimizing the economic cost associated with resupply and the accompanying complexity of logistic, and maintaining a familiar, Earthlike environment to promote human productivity and psychological well-being (Schwartzkopf, 1992).

De Micco et al. (2009) underline that It has been critical to (i) identify, for this particular environment, highly productive species able to optimize O2 production/CO2 consumption, and (ii) develop high-tech controlled environments. Research activities have included seedling production in simulated microgravity and in Space with the two-fold objective of (i) integrating the crew diet with fresh food, and (ii) studying specific biological phenomena.

The launch needs (propellant) and consequently the costs of a space mission is determined by the amount of total mass that have to be put into Earth orbit, propelled towards the targeted planet, enter into orbit again and finally transported back to Earth. A single crew needs on average 3 Kg of combined food and water a day, so for a typical Mars mission of minimum 2 years (730–1000 days) about 12 tonnes of 'food' (consumables) would be needed. The frequent consumable re-supply is costly and it becomes logistically difficult as missions extend further. To keep the weight down, foods are eaten in their packaging (plastic pouches). Another issue is the food storage: many processed foods and reconstituted beverages do not retain their nutrition or palatability for even a year, turning off-colour or becoming mushy or tasteless.

Why plants? Plant based life support processes could be the optimal tool for CO2 reduction and O2 production (Myers, 1954). In addition to the atmospheric regeneration, plants could be used to produce food and purify waste water (trough transpiration); this concept has been called “Bioregenerative Life Support System”, or “Controlled Ecological Life Support System” (CELSS) or “Environmental Closed Life Support System” (ECLSS). These terms have generally been used in a more inclusive sense for both biological and physical-chemical approaches.

By growing enough plants to cover around 40% of what the crew eats, humans could get 'for free' the oxygen and water needed to live. Although still on the drawing board, ESA has already started research to see what could be grown on other planets (and what a self-supporting eco-system might look like on Mars) (www.esa.int).

1.2 Simplified hydroponics for food security in developing countries

According to the UN Food and Agriculture Organization, in 2009 an estimated 950 million people live a day to day existence of not having enough to eat. There is a present need for more food production, or food production that reaches the people who need it. Many of the 800 million people in need live in areas where solar resources and annual rainfall are sufficient to produce vegetable crops. The majority of humans requiring food live in the tropics, often in areas with solar resources sufficient to grow food year round (Bradley and Marulanda, 2000). Simplified hydroponics is a technology incorporating soilless culture techniques without the use of mechanical devices or testing equipment. All hydroponic nutrient is hand poured over plants once in the morning, or supplied in floating beds that are hand aerated twice a day. The only energy requirements for garden operation are natural sunlight and human labor. Main differences between high technology hydroponics (HTH) and Simplified Hydroponics (SH) are resumed in Table 1-1.

Table 1-1 High technology hydroponic (HTH) compared with simplified hydroponic (SH)

HTH	SH
Market oriented	Feed family + small income
High tech	Low cost, simple technology
Little labour	Family labour
Rural areas	Urban and peri urban areas

Conceptually SH is a low input branch of Hydroponics, developed in Latin America. It uses the general concepts of hydroponics but differs from HTH used in the U.S.A., Europe and Australia as follows (Caldeyro Stajano et al., 2003):

• HTH: it is oriented to the market to maximize the cost/benefit ratio for the enterprise. It uses high technology and little labour. It is located in rural areas.

• SH: its main aim is for the family to be able to feed itself and to produce a small income. It is appropriate for low-resource populations. SH uses very low cost, simple technology; requires almost no investment; and uses family labour. SH can be generally located in the urban or peri-urban areas although also is suited to rural conditions.

Moreover SH present several advantages (Caldeyro Stajano et al., 2003):

• It is a low cost and easy-to-learn technique.

• It allows the production of vegetables “without soil” in containers with water or in low-cost natural substrates (sand, rice skulls, pumice stone, etc.). It allows to grow a wide variety of vegetables, such as lettuce, tomatoes, carrots, garlic, watercress, aubergines, beans, parsley, radish, leek, strawberries, melons, flowers, aromatic and medicinal plants, etc.

• It allows the use of recycled materials to build the containers, thus making low-cost materials such as wood and disposable containers.

• It is ideal for food production in urban and suburban areas (Urban Agriculture). It offers the advantage of using places that have not previously been thought appropriate for food production (courtyards, small gardens, walls, balconies, rooftops).

• High efficiency of the use of water, but requires uncontaminated water availability.

• Generation of direct income for family or community micro enterprises.

• It allows the production of high quality, harmless food. The fruits and vegetables have a very high biological and nutritional value. Since they are grown by the family, they are harvested immediately before their use, thus, the products are fresh and they keep their nutritional and medicinal qualities intact. Another advantage for the settlements is that it allows cultivation out of the ground, harmless and uncontaminated. In order to assure the harmlessness of the final product, it is essential to use drinking water and / or clean rainwater.

1.3 Soilless for feeding the city of the future

In 2007 for the first time in human history, more than half of the world population was living in the cities. The trend for the future is clearly toward urban migration (Fig. 1-1).

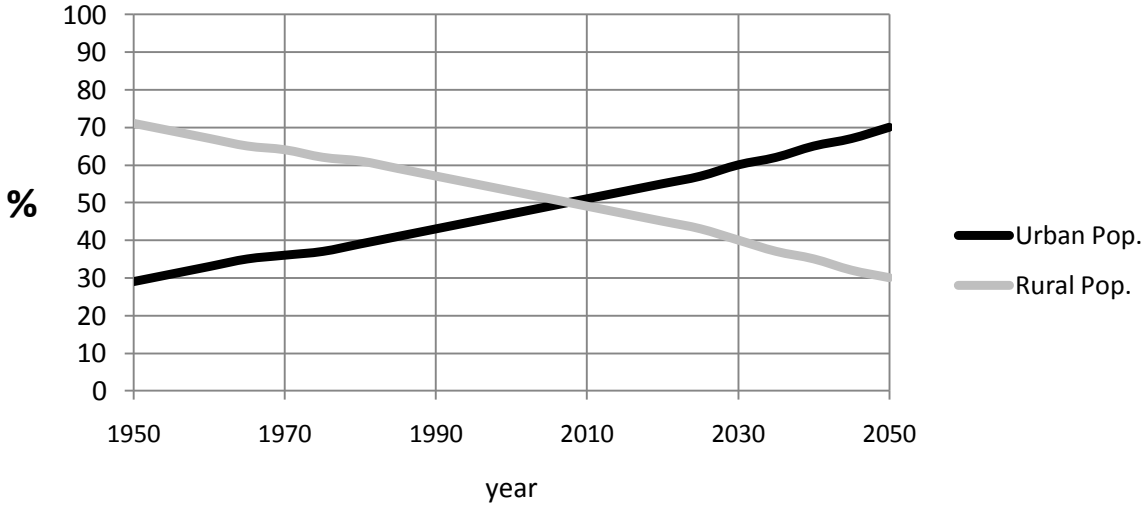


Fig. 1-1 Balance between world urban and rural population (%) from 1950 to 2050 (FAO STAT, 2009)

By the year 2050, nearly 80% of the earth's population will reside in urban centers. Applying the most conservative estimates to current demographic trends, the human population will increase by about 3 billion people during the interim. An estimated 109 hectares of new land (about 20% more land than is represented by the country of Brazil) will be needed to grow enough food to feed them, if traditional farming practices continue as they are practiced today (Vogel, 2008). At present, throughout the world, over 80% of the land that is suitable for raising crops is in use (FAO STAT). Historically, some 15% of that has been laid waste by poor management practices (Vogel, 2008). How to feed with fresh products in a sustainable way these cities will be a world issue.

Some futuristic project took place to study a solution for this issue. Despommier (2009) proposed as solution the “Vertical Farm”- He predicted that future cities could grow most of their food inside city limits, in ultraefficient greenhouses. “Vertical farms,” proponents say, could produce more food using a fraction of the resources that traditional farms consume. The lives of millions of people may depend on it. Despommier, a

parasitologist at Columbia University calculated that with projected population increases, the world will need 1 billion more hectares of arable land by 2050 (Despommier, 2009a)—roughly the area of Brazil and far more land than will be available. Researchers are now putting prototypes of intensive urban farms to a real-world test. The basic concept (Despommier, 2009, Vogel, 2008) is an evolution, not a revolution, of greenhouse technology. Well-designed greenhouses can improve water use efficiency and use little area compared with farm fields. Upper floors would grow hydroponic crops; lower floors would house chickens and fish that consume plant waste. Heat and lighting would be powered by geothermal, tidal, solar, or other renewable energy sources. Nitrogen and other nutrients would be sieved from animal waste and perhaps from the city sewage system

Another approach that could be implemented quickly is rooftop greenhouses. In a demonstration of what can be grown on New York City's roofs, says Theodore Caplow (New York Sun Works, http://nysunworks.org/), executive director of this engineering company that last summer built and operated the Science Barge, a floating greenhouse on the Hudson River that used solar power and recycled water to grow fruits and vegetables. New Yorkers eat 100 kilograms of fresh vegetables on average per year, and the rooftops of New York City would provide roughly twice the needed space to supply the entire city. New York Sun Works is now installing a demonstration greenhouse on top of a New York City school that would serve as a teaching area and supply produce to its cafeteria. A more ambitious concept is farming the facades of office buildings. Double-glass facades are already popular among architects as an energy-saver, allowing winter sun in while insulating against noise and heat loss. In the summer, most double facades have built-in shades to keep the interior cool. Hydroponic gardens could provide that shade.

Concluding, It's clear that a large technological breakthroughs is needed, and it is important to underline that vertical farm and other kind of similar projects could be a resource for the future, but at the same time there is a lack of knowledge and details about them, in particular opponents question the potential profitability of vertical farming. Bomford (2010) underlined that a detailed cost analysis of start-up costs, operation costs,

and revenue has not been done. The extra cost of lighting, heating, and powering the vertical farm may negate any of the cost benefits received by the decrease in transportation expenses. The economic and environmental benefits of vertical farming rest partly on the concept of minimizing food miles, the distance that food travels from farm to consumer. Anyway in hydroponics seemed to be present in most of these futuristic project.

2 Testing hydroponic systems, nutrient solutions on soybean for space missions.

2.1 Introduction

Urea is one of the most important nitrogen fertilizers used for vegetable production in the field (Vavrina and Obreza, 1993). It is seldom used in hydroponic cultivation for vegetable production, although a few successes have been reported in reducing nitrate accumulation in leafy vegetables by partial replacement of nitrate with urea in the nutrient solution (Gunes et al., 1994). In recent years much attention has been focused on whether urea should be used as the sole hydroponic N source for vegetables, especially for the leafy vegetables (Luo et al., 1993; Khan et al., 1997; Zhu et al., 1997). Studies of the utilization of hydroponically applied urea by fruit vegetables have been limited at seedling stage (Kirkby and Mengel, 1967; Gerendas and Sattelmacher, 1997). According to their findings, urea was not a suitable hydroponic N source compared to nitrate. Similar results were also obtained in experiment with tomatoes at seedling stage (Ikeda and Tan, 1998). The response of fruit vegetables at different growth stages to the utilization of urea in hydroponic culture has received much less attention.

In a life support system, urea is about 85% of the recycled nitrogen available for plant growth, (Wydeven and Golub, 1992). Water purification is particularly relevant in a regenerative system since liquid wastes (urine and wash-water) will be the dominant waste streams. Estimates of urine production range from 1.3-2.1 L person-1 day-1, while gray water production (e.g., liquid waste from the shower, clothes washer and dishwasher) is estimated in the range of 25 L person-1 day-1 (Wydeven and Golub 1992). Combined, the mass of liquid wastes will be 50 times greater than inedible plant biomass and 1000 times greater than faecal dry matter. A biomass production system scaled to meet food requirements for one human would produce approximately 40 L of atmospheric condensate per day (Muhlestein et al., 1999).

Results from plant growth studies indicated that the costs of storage or re-supply to provide plant nutrient requirements would be significant. Mackowiak et al. (1996) estimated that the mass of reagent-grade salts required to support plant growth would be equivalent to 30% of the mass of human food requirements. The high nutrient demand of the system appears to be partially a result of significantly higher accumulation of nutrients in hydroponically-grown plants relative to field-grown crops (McKeehen et al., 1996a). Decreasing nutrient levels in the hydroponic solutions may reduce excessive nutrient uptake, but may also increase the potential risk of nutrient deficiency. Even if plant uptake can be minimized, inorganic nutrient requirements will remain a significant mass flux in the system.

Urine recycling is desiderable because it represents a significant source of water and nitrogen within the system. Based on an average urea content of 7000 mg L-1 (Putnam, 1971), urine production would contain nearly 900 mmol of N per person per day. Average N use for crops such as wheat and potato used in previous experiments equals approximately 35 mmol per square meter per day (Mackowiak et al., 1996a). Assuming a crop growth area requirement of 40 m2 per person, the estimated N flux in urine is over half of the plant N requirement in the system. The simplest approach for recycling the nitrogen and water within urine would be direct incorporation into the plant growth system. The major problems with direct recycling are potential phytotoxic effects of ammonium as N source (urea can be readily converted to ammonium by micro-organisms), and NaCl accumulation. NH4+ is also toxic to plants, but this may be not true if pH is rigorously controlled (Lahav et al., 1976). Physical-chemical methods for removal of NaCl from the urine prior to recycling, and microbiological conversion of ammonium to nitrate (nitrification) could eliminate potential phytotoxicity. (Garland et al., 1997)

Results from our previous experiment confirmed the highest acid requirement for the hydroponic cultivation of crops for a CELLS, due to the preferential uptake of anions over cations from a nitrate-based nutrient solution. On the other hand the need of minimize the weight of fertilizers brought from Earth pushes forward the potential employment of urea

as N source. The following experiment aimed to assess the effects of urea as the sole nitrogen source on productive behaviour and N nutrition of soybean in hydroponic cultivation. Moreover as urea, compared to nitrate, does not inhibit the nodulation (Vigue et al., 1977; Imsande, 1988), the interaction between nitrogen source and inoculation with a Rhizobium strain was tested, both on a solid growth medium and NFT.

2.2 Materials and methods

The experiment was carried out at University of Guelph, Ontario, Canada. Soybean plants (Glycine max L. Merr. cultivar 'OT9814') were grown in a walk in growth chamber using metallic gullies equipped with a recirculating hydroponic system. Growth conditions were kept constant during the cycle: the temperature regime was 26/18 °C (light/dark) and the relative humidity (RH) was kept within the range of 70-85%. Light was provided by fluorescent lamps setting photosynthetic active radiation (PAR) at 750 µmol cm^{-2} s^{-1} according to a day/night regime of 16/8 hours. Lamps could be moved towards above in order to keep the PAR constant at the canopy level during the cycle. Plant density was 50 plants m^{-2}.

The influences of the nutrient solution, the inoculation with Bradyrhizobium japonicum and the growing medium on plant growth and yield were tested.

Two different nutrient solutions were compared (Table 2-1), differing in the source of nitrogen. The first one (W) was based on the standard Hoagland solution 1/2 strength (Hoagland and Arnon, 1950), modified by Wheeler et al. (2008), according to specific requirement of soybean and containing nitrogen as nitrate. The second one (U) was obtained from the first by replacing the nitrate with urea; consequently its salt composition was slightly different, in order to obtain the same concentration of the nutrients (Table 2-2); moreover, in the solution containing urea, the concentration of sulphur and molybdenum were slightly increased, in order to support the bacteria infection. In both the nutrient solutions, the 2(N-Morpholino)ethanesulfonic acid (MES) was added as a buffering agent, in order to stabilize the solutions pH. Since urea hardly dissociate in a water solution (in absence of specific enzymes), causing a delay in plant growth at least in the early

developmental stage, the effect of inoculation with a Bradirhizobium japonicum strain BUS-2 was also tested.

Table 2-1 Wheeler and Urea nutrient solutions recipes. Nutrient solution was used only at the beginning of the experiment, then only stock solution was added to the tank mixed with water in the right amount to reach desired EC.

	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B	Mo	MES
Wheeler sol.				mM						µM			mM
Nutrient solution	7.5	0.5	3	2.5	1	1	60	7.4	0.96	1.04	7.13	0.01	2
Stock solution	70	10	56	12	10	10	134	96	12.5	13.5	93	0.13	-
Urea sol.				mM						µM			mM
Nutrient solution	12	0.25	4.3	3	1	3	54	7.4	0.96	1.04	7.13	0.05	2
Stock solution	70	10	56	12	10	31	134	96	12.5	13.5	93	0.65	-

Table 2-2 Salt composition of Wheeler and Urea nutrient solutions.

Urea solution	Wheeler solution
Urea	
Calcium chloride	Calcium nitrate
Monopotassium phosphate	Monopotassium phosphate
Dipotassium phosphate	
Magnesium sulphate	Magnesium sulphate
Potassium sulphate	Potassium nitrate
Iron chelate (Fe-EDTA-7% Fe)	Iron chelate (Fe-EDTA-7% Fe)
Boric acid	Boric acid
Manganese chloride	Manganese chloride
Zinc sulphate	Zinc sulphate
Copper sulphate	Copper sulphate
Ammonium molybdate	Ammonium molybdate

Concerning the substrates, previous studies about hydroponic cultivation of crops for space explorations have been usually performed using the NFT system. In this experiment, we compared the NFT system with the cultivation in rockwool (Grodan©) on inoculated plants, to evaluate their effects on plant development and plant-bacterium interactions (as Rhizobium usually lives in the soil).

Resuming, the following 6 treatments were tested, in a two factor incomplete randomized block design with 3 replicates:

- inoculated plant grown in NFT with Wheeler solution (INW);
- inoculated plant grown in NFT with Urea solution (INU);
- not inoculated plant grown in NFT with Wheeler solution (NNW);
- not inoculated plant grown in NFT with Urea solution (NNU);
- inoculated plant grown in rockwool with Wheeler solution (IRW);
- inoculated plant grown in rockwool with Urea solution (IRU).

Sowing was preceded by a sterilization (Somasegaran and Hoben, 1994) and inoculation procedure (Vincent, 1970): seeds were rinsed in 95% alcohol for 20 seconds to remove waxy materials, then they were completely immersed in a sodium hypochlorite solution (2.5%) and gently swirled to bring the seeds and the disinfectant in contact. After 5 minutes, sodium hypochlorite was drained off and seeds were rinsed 6 times in sterile H_2O. All seeds were incubated overnight in sterile H_2O at room temperature in darkness. Inoculation was performed on a part of seeds using 200 ml of deionised water containing 0.5 g of peat carrier, then all seeds were placed into the Petri dishes, with agar as growing medium, at 27 °C in the darkness.

After two days, the seedlings to be grown in NFT were moved to autoclaved glass tubes, wrapped in aluminium foil to protect roots from light, with seedling agar as growing medium (Table 2-3). The plants to be grown in rockwool were directly transplanted in rockwool cubes and constantly irrigated with nutrient solution containing the same salts of the seedling agar. A 12 h photoperiod was provided until 1 week after germination, when the plantlets were moved to the growth chamber. Before the transplant, the roots of inoculated seedlings were infected again dipping them in 200 ml of deionised water containing 10% sucrose and 0.5 of peat carrier. On not-inoculated plants only a 10% sugar solution was used on roots.

Table 2-3 Seedling agar recipe.

	g l^{-1}
Agar	10
$CaHPO_4$	1
K_2HPO_4	0.2
$MgSO_4$ $7H_2O$	0.2
NaCl	0.2
FeCl	0.1

Gullies were sealed using a two sided polyethylene film, with the black inside, to reduce the lighting to the roots, and the white one outside, to reflect light.

Fertigation was performed with one separate nutrient solution reservoir per each treatment. Each reservoir was equipped with its own submerged pump, in order to work independently. Nutrient solution returned to the reservoir by gravity dependent flow.

EC and pH target were 1.2±0.1 dS m^{-1} and 5.8±0.2, respectively and they were controlled manually and adjusted every day by adding HNO_3 0.5M (Wheeler solution) or H_3PO_4 0.5M (Urea solution) or KOH 0.5M respectively in the storage tanks. At the beginning of the experiment, reservoirs were filled with nutrient solution (Table 2-1), while the volume of the nutrient solution was kept at a constant level by adding deionised water and stock solution. Water depletion in each reservoir was measured twice a week.

2.2.1 Data Collection

Plant growth and yield

The plant growth and development were measured at 7-day intervals until the beginning of pods filling, and then were measured at 21-day intervals. Growth analysis was based on non-destructive measurements of plant height, number of nodes and leaves carried out on 6 plants per treatment.

Additionally, destructive measurements (height and diameter of stem, number of leaves, fresh and dry weight) were performed on 30, 52 and 80 DAS (at the full vegetative

growth, at the beginning of pods formation and at the beginning of pods filling). Plant LA was measured by using a leaf area meter (LI-3100, Li-Cor, Lincoln, USA). Fresh weight, dry weight (after oven-drying at 50°C), % of DM and DM partitioning were measured. On the basis of the collected data, the following growth indexes were Specific Leaf Area (SLA), as the Leaf Area/ DM of leaves ratio; Leaf Area Ratio (LAR), as the Leaf Area/total DM ratio, to assess how the plant's total stock of organic material is divided between photosynthetic organs (leaves) and the rest of plant parts.

The chlorophyll content was estimated on 22, 40 and 54 DAS using a colorimetric method (CCM-200 chlorophyll meter, Opti-Sciences, Inc.), on the middle leaflet of the second and third fully expanded trifoliate leaves from the top of 2 plants per each treatment replicate (2 measurements per leaf).

At harvest, yield was measured on a unit area basis for the different treatments compared and the harvest index (HI) was calculated by dividing the edible DM by the total DM in order to evaluate the plant yield performances. Moreover, 100 seeds weight (14% water content) was calculated.

Data were analyzed with ANOVA and means were compared by the LSD test.

Nodules

Trend of number and dry weight of nodules per plant was determined on 30 and 52 DAS and at harvest (122 DAS). ANOVA was performed on number and dry weight of nodules per plant and on the mean nodule dry weight on 52 DAS; means were compared by the LSD test.

Gas exchanges

Gas exchange measurements were performed using an open flow gas exchange system (Li-6400; Licor, Lincoln, NB, USA) on the middle leaflet of the second and third fully expanded trifoliate leaves from the top of 3 plants per each treatment, on 33, 47 and 66 DAS. Net photosynthesis rate (NP), transpiration rate (Tr), stomatal conductance (gs),

intercellular CO2 concentration (Ci), and leaf temperature (Tleaf) were determined at CO2 concentration of 400 µmol mol^{-1} and 700 µmol m^{-2} s^{-1} of PAR.

Data were analyzed with ANOVA and means were compared by the LSD test.

Water use and biomass efficiency indexes

Crop water consumption was calculated throughout the growth cycle by measuring the volume of the supplied and the drained nutrient solution, assuming the water consume unaffected by evaporation (due to the plastic covering). At the end of the growing cycle, the cumulative water consumption was calculated and the following efficiency indexes were estimated: Water Use Efficiency (WUE), expressed as g of edible DM per kg of nutrient solution; Radiation Use Efficiency (RUE), by dividing edible productivities by daily PAR; moreover, considering that the control of pH was performed by adding acid or base during the whole experiment, and as the acid (and base) budget for a hydroponic cultivation has to be considered for space missions, Acid Use Efficiency (AUE), as g of edible DM per mmole of H^+, and Base Use Efficiency (BUE), as g of edible DM per mmole of OH^-, were estimated.

Data were analyzed with ANOVA and means were compared by the LSD test.

2.3 Results

Plant growth and yield

A comparison of main growth parameters is shown in Table 2-4 and Fig. 2-1. The highest values of stem length and diameter, maximum number of leaves (before leaf fall) and maximum leaf area (before leaf fall) occurred in plants grown in the nutrient solution containing nitrate (W). Nutrient solution containing urea (U) decreased all the biometric parameters; these plants also were the smallest in terms of total dry weight (Table 2-7 to Table 2-9). Thus, stem lengths in U plants were probably shorter because of slightly delayed development. Wheeler solution increased the number of leaves, even though rockwool enhanced the number of leaves (and consequently the leaf area) in both the nutrient solutions; values obtained in inoculated plants grown in rockwool fed urea (IRU) were comparable to those obtained in NFT fed nitrate (both inoculated – INW - and not inoculated - NNW) (Table 2-4 and Fig. 2-1).

Table 2-4 Main biometric characteristics of soybean plants in response to nutrient solution, growth medium and inoculation (Mean values; ns = not significant; * = significant at P≤0.05) ([1] lsd).

	Plant height (cm)	Number of nodes	Stem diameter (mm)	Maximum number of leaves	Maximum leaf area (cm^2)	LA/leaf (cm^2)
Nutrient solution						
U	98.3 b	13.4 b	3.2 b	22.0 b	1242 b	56.5 b
W	118.0 a	16.2 a	4.1 a	43.6 a	3837 a	88.0 a
Treatments						
NN	106.8	15.5	3.4	28.2 b	1629 b	57.8 b
IN	108.4	14.8	3.8	27.8 b	1726 b	62.1 b
IR	109.2	14.2	3.8	42.3 a	3715 a	87.8 a
Significance						
Nutrient solution	*	*	*	*	*	*
Treatments	n.s.	n.s.	n.s.	* (9.47[1])	* -552.1	* -16.8
Interaction	n.s.	n.s.	n.s.	* -13.4	n.s.	n.s.

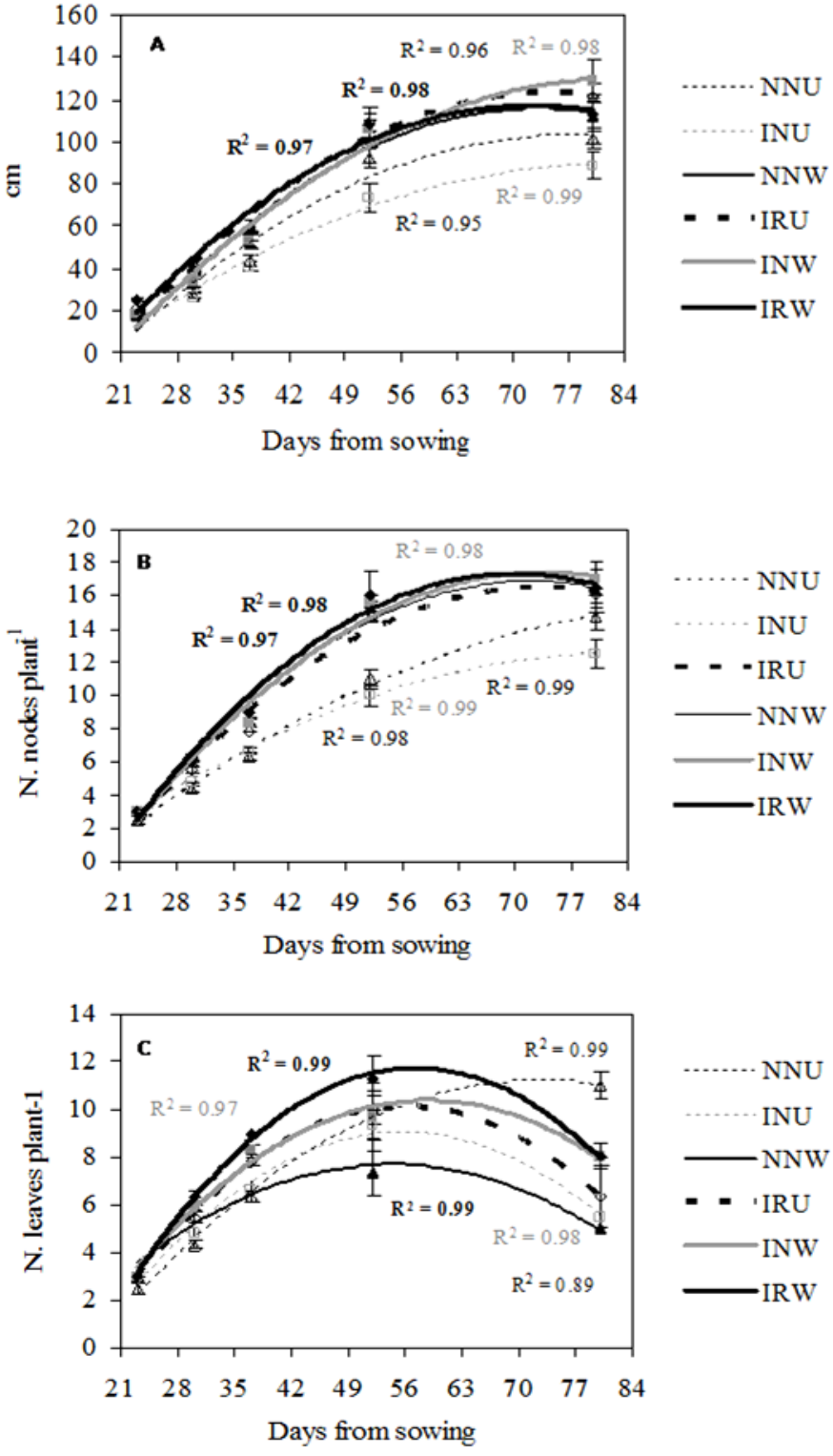


Fig. 2-1 Trend of soybean plant height (A), number of nodes (B) and number of trifoliate leaves (C) throughout the growing cycle for the different treatments.

Growth indexes (SLA, LAR), calculated on the basis of values on 30, 52 and 80 DAS varied during the growth cycle and were influenced by the nutrient solution, while were unaffected by the other treatments (Table 2-5). Considering the SLA, during the growing cycle, as plants become bigger and heavier their leaves become larger, and a necessary part of the architecture of these larger leaves is represented by the midrib and the main veins, whose presence naturally lowers the leaf area per unit leaf dry weight. Also, individual leaves, once they have ceased to expand, tend to become gradually heavier, and this reinforces the fall in SLA (Evans, 1972). On 30 and 52 DAS, SLA was higher in W compared to U, but there were no differences on 80 DAS. LAR decreased during the plant growth, depending on SLA and on the proportion of dry matter going to leaves during the lifecycle of the plant (Evans, 1972). The index was significantly affected by nutrient solution on 30 DAS showing the highest values with W; an interaction was found on 52 DAS, when the lowest LAR values were recorded in inoculated plants grown in urea and plants grown in NFT supply with nitrate, with no differences among the treatments; the highest values were recorded in NNU and IRW (Fig. 2-2). All the differences disappeared during the following stages of growth.

Table 2-5 Growth indexes of soybean plants calculated on 30, 52 and 80 DAS in response to nutrient solution, growth medium and inoculation (Mean values; ns = not significant; * = significant at P≤0.05) ([1] lsd).

	30 DAS		52 DAS		80 DAS	
	LAR ($cm^2\ g^{-1}$)	SLA ($cm^2\ g^{-1}$)	LAR ($cm^2\ g^{-1}$)	SLA ($cm^2\ g^{-1}$)	LAR ($cm^2\ g^{-1}$)	SLA ($cm^2\ g^{-1}$)
Nutrient solution						
U	180.6 b	229.6 b	163.8	247.5 b	69.2	213
W	223.0 a	292.7 a	176.8	320.6 a	61	235
Treatments						
NN	206.3	269	178.8	303	67.3	215.7
IN	206.5	267.7	154.2	255.5	63.5	206.7
IR	192.5	246.8	177.9	293.7	64.7	249.7
Significance						
Nutrient solution	*	*	n.s.	*	n.s.	n.s.
Treatments	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Interaction	n.s.	n.s.	* (30.87[1])	n.s.	n.s.	n.s.

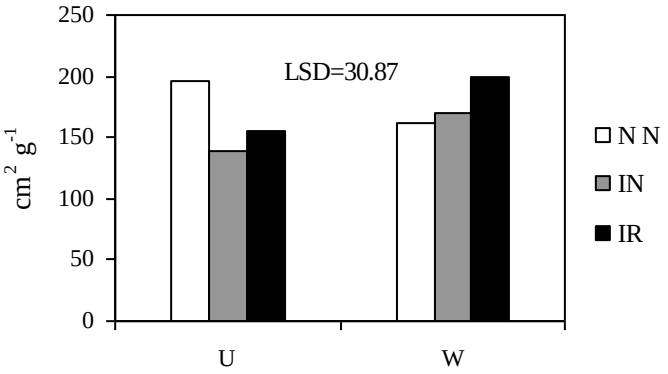


Fig. 2-2 Nutrient solution x growth medium x inoculation interaction on LAR on 52 DAS.

The estimated chlorophyll content (measured at 22, 40 and 54 DAS) showed an interaction only in the first measurement, when NNU had the lowest value (18.4 CCI units), while there were no differences among the other treatments (20.2 CCI units on average) (Table 2-6). In the following measurements, the difference disappeared. This result seems to indicate a difficulty of not inoculated plants in uptaking nitrogen from urea during the early growth.

Table 2-6 Estimated chlorophyll content in soybean leaves on 22, 40 and 54 DAS in response to nutrient solution, growth medium and inoculation (Mean values; ns = not significant; * = significant at P≤0.05) ([1] lsd).

	22 DAS	40 DAS	54 DAS
Nutrient solution			
U	18.8	24.7	36.2
W	20.8	25.2	41
Treatments			
NN	18.4	25.2	38
IN	20.4	25.1	37.8
IR	20.7	24.5	40.1
Significance			
Nutrient solution	*	n.s.	n.s.
Treatments	* (1.8[1])	n.s.	n.s.
Interaction	* -2.76	n.s.	n.s.

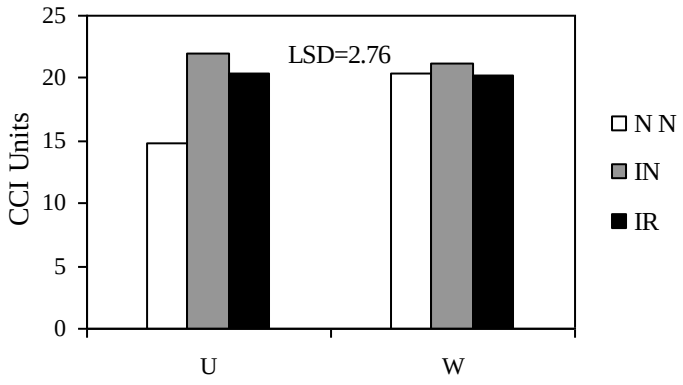


Fig. 2-3 . Nutrient solution x growth medium x inoculation interaction on chlorophyll content on 22 DAS.

Data about the dry mass are shown in Table 2-7 to Table 2-9. Considering the leaves, the dry weight decreased during the pod-filling (80 DAS, Table 2-9). On 30 days from sowing, plants supply with nitrate accumulated more biomass than the ones fed with urea, while the percentage of dry mass was always higher in plant U. Moreover, rockwool increased the plant biomass compared to NFT (Table 2-7). On 52 DAS, nutrient solution and other treatments still had an influence on the measured parameters: considering DM content in pods, Wheeler nutrient solution had a negative effect on inoculated plants compared to not inoculated ones, while in Urea plants there were no differences between inoculated and not inoculated (Fig. 2-4a). Moreover, the growth in a substrate significantly increased the DM content in pods when plants were fed urea and showed a trend of higher values in W plants (Fig. 2-4a). The total DM percentage also showed an interaction among the treatments: U plants tended to reach the highest values compared to W ones, especially when inoculated and grown in NFT (Fig. 2-4b). These differences were particularly evident observing the DM percentage in leaves and pods (Fig. 2-4cd). Data reported seemed to indicate that Wheeler solution is able to feed plants in a better way than Urea solution that produces plants with reduced water content. NFT in association with urea and inoculation does not allow the bacteria using efficiently the urea, while the rockwool enhances the dry matter accumulation in plants fed urea and seemed to reduce the inhibitory effect of

nitrate on nodulation in plants supplied with Wheeler solution. Besides, rockwool in general seemed to anticipate plant development, allocating more biomass in pods compared to the other treatments.

On 80 DAS Wheeler solution still recorded higher values of biomass; there were no differences between the nutrient solutions in terms of total DM percentage even though U showed higher values of DM percentage in pods and seeds (Table 2-9); dry matter content in stem and leaves were similar between the nutrient solutions, probably because the senescence of these plant organs began, as the photosynthetates were moving towards pods and seeds. The other treatments did not have any effects in this time of growing cycle, indicating that the rockwool earliness in biomass accumulation in pods was made up from the other treatments.

Table 2-7 Total DM and DM of different organs on 30 DAS of soybean plants in response to nutrient solution, growth medium and inoculation (values per plant) (Mean values; ns = not significant; * = significant at P≤0.05) ([1] lsd).

	Total DM (g)	DM stem (g)	DM leaves (g)	Total DM (g 100 g^{-1} FW)	DM stem (g 100 g^{-1} FW)	DM leaves (g 100 g^{-1} FW)
Nutrient solution						
U	2.2 b	0.5 b	1.7 b	16.9 a	14.5 a	17.9 a
W	4.0 a	1.0 a	3.1 a	14.0 b	11.6 b	15.0 b
Treatments						
NN	2.7 b	0.6 b	2.0 b	15.6	13.1	16.5
IN	2.8 b	0.7 b	2.1 b	15.4	12.9	16.4
IR	3.9 a	0.9 a	3.1 a	15.4	13.1	16.4
Significance						
Nutrient solution	*	*	*	*	*	*
Treatments	* (0.76[1])	* -0.2	* -0.58	n.s.	n.s.	n.s.
Interaction	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 2-8 Total DM and DM of different organs on 52 DAS of soybean plants in response to nutrient solution, growth medium and inoculation (values per plant) (Mean values; ns = not significant; * = significant at P≤0.05) ([1] lsd).

	DM total (g)	DM stem (g)	DM leaves (g)	DM pods (g)	Total DM (g 100 g^{-1} FW)	DM stem (g 100 g^{-1} FW)	DM leaves (g 100 g^{-1} FW)	DM pods (g 100 g^{-1} FW)
Nutrient solution								
U	10.3 b	3.3 b	6.7 b	0.29	18.5 a	16.4	20.2 a	17.3 a
W	25.5 a	10.4 a	15.0 a	0.2	13.5 b	14.8	13.4 b	8.9 b
Treatments								
NN	17.6	6.9	10.6	0.23 b	15.5	15.7	16	11.9
IN	13.4	5.3	8.1	0.02 b	16.5	14.5	18.3	12
IR	22.7	8.3	13.9	0.49 a	15.9	16.5	16	15.3
Significance								
Nutrient solution	*	*	*	n.s.	*	n.s.	*	*
Treatments	n.s.	n.s.	n.s.	* (0.21[1])	n.s.	n.s.	n.s.	n.s.
Interaction	n.s.	n.s.	n.s.	* -0.31	* -3.85	n.s.	* -5.27	* -7

Table 2-9 Total DM and DM of different organs on 80 DAS of soybean plants in response to nutrient solution, growth medium and inoculation (values per plant). (Mean values; ns = not significant; * = significant at P≤0.05).

	DM Total (g)	DM stem (g)	DM leaves (g)	DM pods (g)	DM seeds (g)	DM total (g 100 g^{-1} FW)	DM stem (g 100 g^{-1} FW)	DM leaves (g 100 g^{-1} FW)	DM pods (g 100 g^{-1} FW)	DM seeds (g 100 g^{-1} FW)
Nutrient solution										
U	14.4 b	3.8 b	4.8 b	2.7 b	3.2	15.8	19.6	18.7	17.7 a	27.2 a
W	47.1 a	18.4 a	12.9 a	10.4 a	5.4	14.7	17.9	17.2	14.3 b	17.7 b
Treatments										
NN	28.7	10.4	8.6	5.9	3.8	15.8	19.5	19.5	16	20.8
IN	29.9	11.5	9.3	6.1	3	14.5	17.9	14.9	15.7	21.7
IR	33.7	11.5	8.6	7.6	6.1	15.5	18.9	19.6	16.2	24.9
Significance										
Nutrient solution	*	*	*	*	n.s.	n.s.	n.s.	n.s.	*	*
Treatments	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Interaction	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

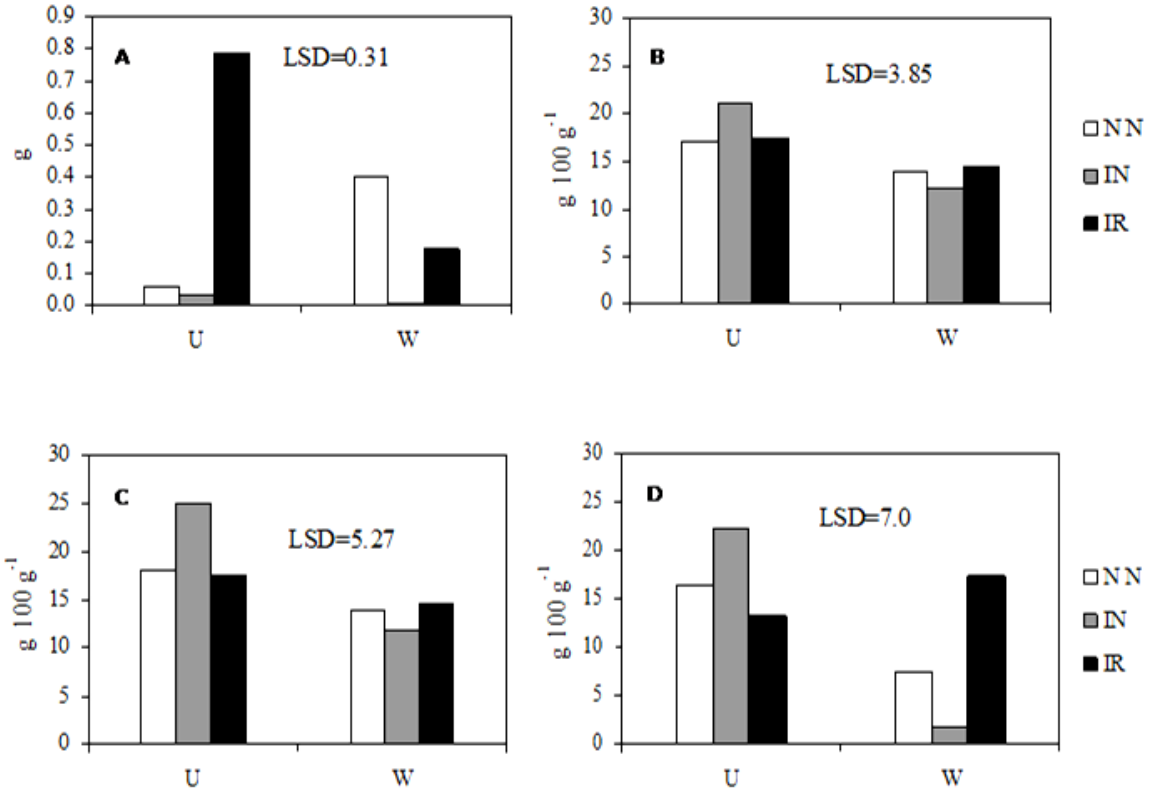


Fig. 2-4 . Nutrient solution x growth medium x inoculation interaction on pods DM (A), total DM percentage (B), DM percentage in leaves (C) and DM percentage in pods (D) on 52 DAS.

Considering the DM partitioning, as expected (Evans, 1972) the fraction of DM in leaves decreased with plant age, because as the dry weight increased, the proportion of dry matter going to new leaves steadily declines throughout the life of the plant. Moreover, on 30 DAS U had higher DM percentage in leaves and W in the stem (Table 2-10), while the other treatments did not show any differences; in the following stages of growth, this trend was kept, even though an interaction was found in pods on 52 DAS (Fig. 2-5), when the values were similar to the DM content in pods (see Fig. 2-4a). These results seemed to indicate again an early in biomass allocation in pods when plants are grown in rockwool. In addition, on 80 DAS, IR treatment showed higher DM percentage in seeds compared to the others, and IN showed the lowest (Table 2-10), even though the differences between NN and IN disappeared at harvest (Table 2-11).

Table 2-10 DM partitioning in the different organs on 30, 52 and 80 DAS in response to nutrient solution, growth medium and inoculation (Mean values; ns = not significant; * = significant at P≤0.05) ([1] lsd).

	30 DAS		52 DAS			80 DAS			
	stem	leaves	stem	leaves	pods	stem	leaves	pods	seeds
Nutrient solution									
U	21.3 b	78.7 a	31.7 b	66.4 a	1.9	34.8 b	44.2 a	21	20.7 a
W	23.8 a	76.2 b	43.6 a	56.0 b	0.4	47.0 a	29.6 b	23.4	11.1 b
Treatments									
NN	23.1	76.9	39.1	60.4	0.7	37.9	38.3	23.9	15.9 b
IN	22.6	77.4	37.9	61.9	0.3	45.7	36.5	17.8	11.2 c
IR	21.9	78.1	36.1	61.5	2.5	39.1	36	24.9	20.7 a
Significance									
Nutrient solution	*	*	*	*	*	*	*		*
Treatments	n.s.	n.s.	n.s.	n.s.	* (0.74[1])	n.s.	n.s.	n.s.	* -4.04
Interaction	n.s.	n.s.	n.s.	n.s.	* -1.1	n.s.	n.s.	n.s.	n.s.

Flowering started on 35 DAS in all treatments; the entire growth chamber was harvested in a single day, on 122 DAS. Yield was affected by all the treatments, particularly W produced more than U and plants in rockwool had the highest value; the weight of 100 seeds did not vary among the treatments (25.4 g on average) and was higher than that obtained from cultivation trial in open field (Table 2-11). The number of seeds per pod did not vary among the treatments (2.4 seeds pods-1), so it is thanks to the number of pods that yield was different (data not shown). The Harvest Index (HI) was lower in U plants compared to W; the growth in rockwool increased the HI compared to the growth in NFT. Nevertheless, the results compared favorably to those obtained in previous experiments on hydroponically-grown soybean (Wheeler et al. 2003 and 2008).

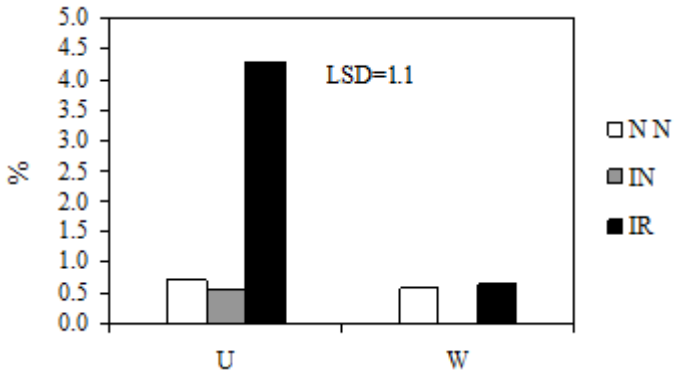


Fig. 2-5 Nutrient solution x growth medium x inoculation interaction in DM partitioning in pods on 52 DAS

Table 2-11 . Yield, 100 seeds weight and Harvest Index of soybean plants in response to nutrient solution, growth medium and inoculation (Mean values; ns = not significant; * = significant at P≤0.05) ([1] lsd).

	Yield ($g\ m^{-2}$)	100 seeds weight (g)	HI
Nutrient solution			
U	183.8 b	24	0.30 b
W	1000.7 a	27	0.41 a
Treatments			
NN	315.6 b	32	0.29 b
IN	291.8 b	21	0.33 b
IR	1169.3 a	23	0.44 a
Significance			
Nutrient solution	*	n.s.	*
Treatments	*	n.s.	*
	(250.6[1])		-0.09
Interaction	n.s.	n.s.	n.s.

Results from the growth indexes indicated a delay in leaf biomass production during the early phases of growth; this gap seemed to be only partially closed in the following developmental stages, because both total biomass production and yield were affected. A possible explanation of increased seed yield for nitrate-fertilized soybeans may be that such plants received a better start, since nodules are not visible on soybean seedlings until about 9 days after planting and N_2 fixation under favorable moisture and temperature begins at about 14 days (Ham et al., 1975).

Nodules

Nodule count reflected the efficiency of rhizobia. As expected, NN plants were poorly nodulated; the other treatments showed an increasing in number of nodules, followed by a reduction during the pod-filling (Fig. 2-6). A decline in the rate of nitrogen fixation per plant with the onset of pod filling has been reported for peas grown under field conditions (Dean and Clark, 1980) and under controlled-environment conditions (Bethlenfalvay and Phillips, 1977; LaRue and Kurz, 1973). There are similar reports for soybeans and common beans (Bethlenfalvay and Phillips, 1977; Quebedeaux et al., 1975).

On 52 DAS, inoculated plants in urea reached the highest nodule number (Fig. 2-6a); in plants fed nitrate there were no differences between inoculated and not inoculated. Colonization was greater for IR plants respect to IN when fed nitrate and comparable to inoculated plants fed urea (Fig. 2-6a). Urea increased the total dry weight of nodules per plant; rockwool also had a positive effect on this parameter, while the lowest values were obtained in not inoculated plants (Table 2-12).

Dry weight per nodule showed a statistical interaction among the treatments: urea increased the weight in association to all the other treatments, with highest values in NN plants; IR only increased the weight in plants fed urea compared to IN, while there were no differences among the treatments in plants fed nitrate, showing the lowest values (Fig. 2-6b). Considering the data obtained, urea seemed to promote or at least to not inhibit the nodulation compared to nitrate, that decreased the rate of nodule formation and also reduced the dry mass of nodules. These results are in agreement to that reported in previous studies (Vigue et al., 1977; Imsande, 1988). The growth in rockwool seemed to reduce the inhibitory effect of nitrate on nodulation even though it did not allow the nodule biomass accumulation in presence of nitrate.

Well nodulated plants did not have more dry matter than the poorly nodulated (see Table 2-7 to Table 2-9), probably because the lower energy requirement for deriving organic N from NH^{4+} than from N_2 balanced advantage of inoculation in terms of nitrogen uptake. These results are in agreement with those reported by Bethlenfalvay et al. (1978) on pea.

Table 2-12 Number and DM of nodules per plant and nodule average DM on 52 DAS in response to nutrient solution, growth medium and inoculation (Mean values; ns = not significant; * = significant at P≤0.05) ([1] lsd).

	Number nodules plant $^{-1}$	Total nodule DM (mg plant $^{-1}$)	Dry weight (mg nodule^{-1})
Nutrient solution			
U	144.8	256.8 a	2.53
W	107.9	111.4 b	0.97
Treatments			
NN	19	48.3 c	2.7
IN	139	189.0 b	1.13
IR	221	315.0 a	1.42
Significance			
Nutrient solution	n.s.	*	*
Treatments	*	*	*
	(67.07[1])	-110	-0.56
Interaction	*	n.s.	*
	-94.8		-0.79

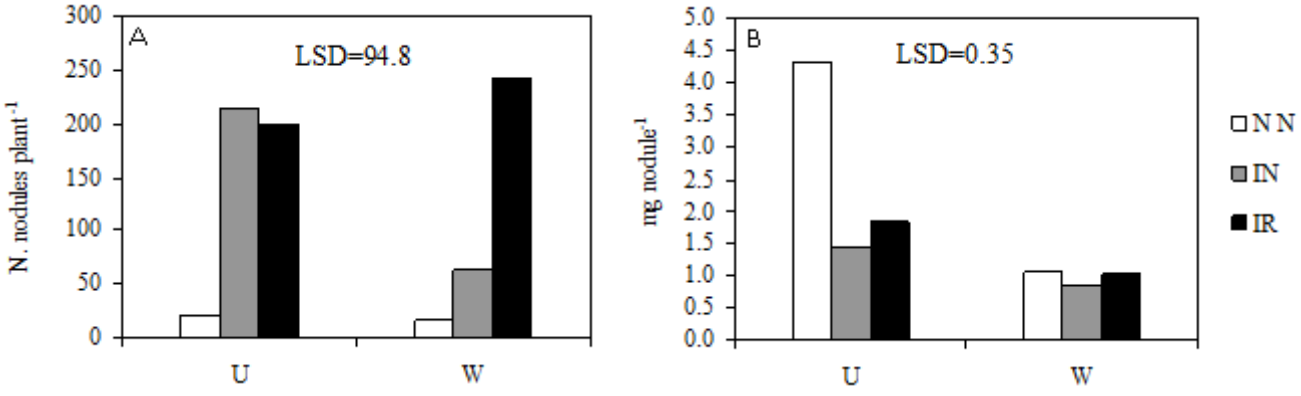


Fig. 2-6 Nutrient solution x growth medium x inoculation interaction on number of nodules per plant (A) and nodule average DM (B).

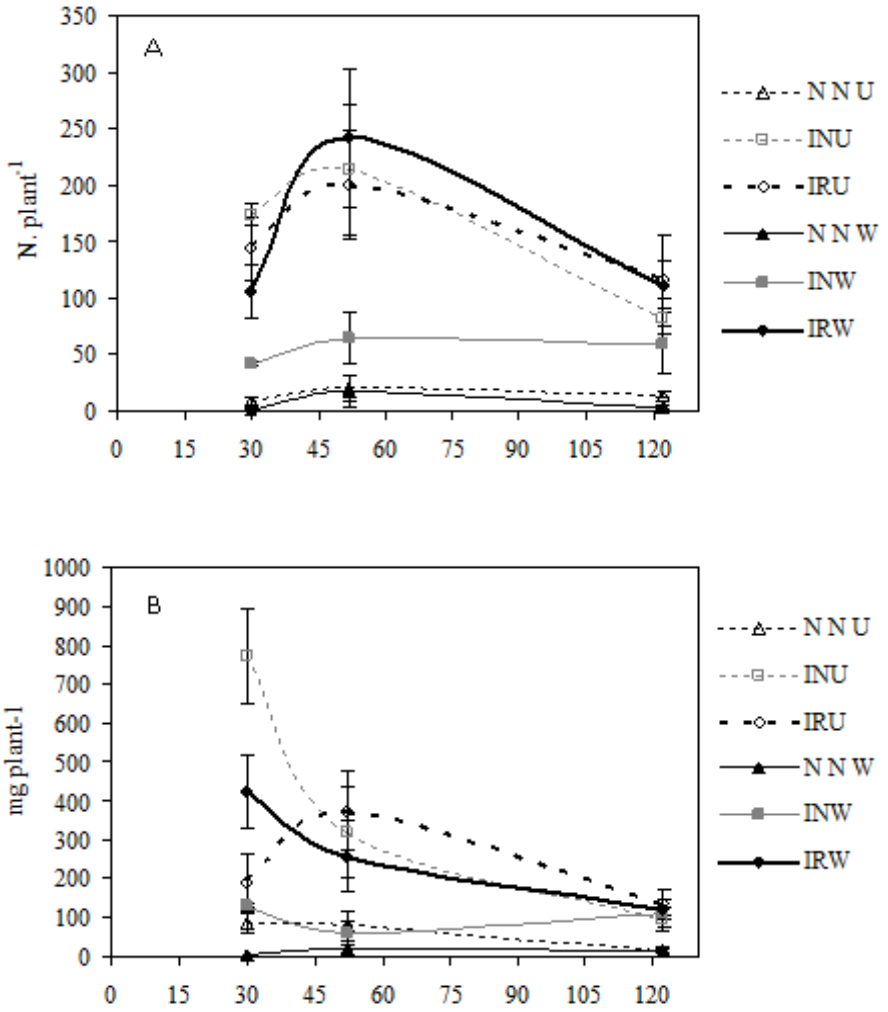


Fig. 2-7 Trend of number (A) and DM (B) of nodules per plant throughout the growing cycle in response to nutrient solution, growth medium and inoculation (Mean±St.Err).

Gas exchanges

Data on net photosynthesis (NP), stomatal conductance (gs), transpiration rate (Tr), CO2 concentration (Ci) and leaf temperature (Tleaf) are shown in Table 2-13 and Table 2-14. Net photosynthesis was always included in the values considered optimal for soybean (Salisbury and Ross, 1992) and ranged from 16.8 µmol CO2 m^{-2} s^{-1} (33 DAS) to 13.8 µmol CO2 m^{-2} s^{-1} (66 DAS) on the average among the treatments, showing the higher values in plants fed nitrate compared to urea.

Stomatal conductance and transpiration rate followed a similar trend during plant growth; IN showed lower values in the average of all measurement dates, compared to the

other treatments. CO_2 concentration also was lower for IN on the average, indicating a probable difficulty for inoculated plants grown in NFT, where roots are submerged during the whole cultivation cycle.

Table 2-13 Net photosynthetic rate (NP), stomatal conductance (gs) and transpiration rate (Tr), in response to nutrient solution, growth medium and inoculation (Mean values; ns = not significant; * = significant at P≤0.05) ([1] lsd).

	NP (µmol CO_2 m^{-2} s^{-1})			g_s (mol m^{-2} s^{-1})			Tr (mmol m^{-2} s^{-1})		
	33 DAS	47 DAS	66 DAS	33 DAS	47 DAS	66 DAS	33 DAS	47 DAS	66 DAS
Nutrient solution									
U	16.2 b	14.0 b	12.3	0.4	0.5	0.2	4.8	5.2	3.5
W	17.4 a	15.3 a	15.4	0.4	0.5	0.4	5.3	6.4	5.6
Treatments									
NN	17	13.4 b	13.7	0.5	0.5	0.4	6.5	6	5.5
IN	17.1	16.1 a	12.6	0.3	0.5	0.2	4.1	5.4	2.7
IR	16.2	14.6 ab	15.2	0.4	0.5	0.4	4.7	6	5.5
Significance									
Nutrient solution	*	*	*	n.s.	n.s.	*	*	*	*
Treatments	n.s.	* (1.67[1])	n.s.	* -0.09	n.s.	*	* -0.6	n.s.	* -0.64
Interaction	n.s.	n.s.	* -3.47	* -0.12	* -0.12	* -0.11	* -0.73	* -1.02	* -0.91

Table 2-14 Intercellular CO_2 concentration (Ci) and leaf temperature (Tleaf) in response to nutrient solution, growth medium and inoculation (Mean values; ns = not significant; * = significant at P≤0.05) ([1] lsd).

	C_i (µmol m^{-2} s^{-1})			T_{leaf} (°C)		
	33 DAS	47 DAS	66 DAS	33 DAS	47 DAS	66 DAS
Nutrient solution						
U	292	310	259.4	24.5	23.5	24.7
W	293.4	312.5	283.7	24.5	23.4	24.2
Treatments						
NN	307	321.1	312.3	23.9	23.4	23.9
IN	280.8	309.4	231.5	25.2	23.2	25.1
IR	290.2	303.3	270.9	24.6	23.8	24.3
Significance						
Nutrient solution	n.s.	n.s.	*	n.s.	n.s.	*
Treatments	* (12.17[1])	* -16.47	* -19.41	* -0.51	* -0.34	* -0.41
Interaction	* -17.98	* -23.29	* -27.46	n.s.	* -0.52	* -0.58

Water use and efficiency indexes

Rate and trend of nutrient solution uptake during the growing cycle varied among the treatments: in plants fed nitrate and in plants grown in rockwool with urea (IRU) it increased rapidly during early growth as the total evaporating surface of foliage increased, reached the maximum on about 60 DAS, and then declined with age. In NNU and INU, however, the water consumption was almost constant during the entire growing cycle (3 litres m^{-2} on average) (Fig. 2-8a).

Total water use throughout the growth ranged from 89 (INU) to 599 litres m^{-2} (IRW) (Fig. 2-8b). The lower values of nutrient solution uptake in NNU and INU were related to the reduced number and surface of foliage. These two treatments also showed a reduced difference between the EC before and after the correction (by adding deionised water and fresh nutrient solution), indicating a difficulty in uptaking nutrients during the growth (Table 2-15), probably due to the reduced urea hydrolysis in the nutrient solution. However, IRU plants showed a slightly greater water use compared to the other urea treatments, cushioning the negative effects of nutrient unavailability. The water use calculated on a per area and per day basis was consistent with date reported above (Table 2-15) and were comparable to those obtained by Wheeler et al. in 2008.

Solution pH tended to rise in Wheeler treatments, requiring acid for pH control (44.5 mmol H^+ m^{-2} d^{-1}, on average), while a reduced amount of acid was used in Urea treatments (7.6 mmol H^+ m^{-2} d^{-1}, on average) (Table 2-16). The maximum acid use was recorded in plants IRW. pH tended to decrease in Urea treatments, so a greater amount of KOH was used to control it, compared to Wheeler solution (12.9 vs 3.1 mmol OH^- m^{-2} d^{-1}, on average) (Table 2-15). Moreover, plants grown in rockwool had a trend of greater acid requirement, in both Wheeler and Urea solution, and a higher base requirement in plants fed urea, compared to plant grown in NFT.

These results are consistent with previous experimental evidence, indicating a preferential uptake of anions over cations from a nitrate-based nutrient solution throughout the growing cycle (Willumsen, 1980), while for plants assimilating NH_4^+ and

urea, in the absence of the readily permeable NO^{3-} anion, inorganic cation uptake exceeds inorganic anion uptake substantially, with a resulting acidification of the rooting medium (Lahav et al., 1976; Breteler, 1973; Houba et al., 1971; Kirkby and Mengel, 1967).

Data on biomass efficiency indexes are shown in Table 2-17. Plants grown in NFT fed urea had lowest values of Water use Efficiency (WUE) (0.33 g of seed per litre of nutrient solution, on average); the index was a little higher for U plants grown in rockwool and was the highest in W plants (Table 2-17). Thus, apart from NNU and INU plants, the results compared favourably to those obtained in previous experiments (Wheeler et al. 2003 and 2008).

In experiments on hydroponically-grown soybean, Wheeler et al. (2008) reported a value of Radiation Use efficiency (RUE) of 0.19 g seeds per mole of PAR and Dougher and Bugbee (1997) a RUE of 0.25-0.28 g seeds per mole of PAR. U plants showed lower RUE, particularly in NNU and INU (0.02 g seeds mol^{-1} PAR, on average). Plants fed nitrate recorded a higher RUE, particularly in IR (Table 2-17). Acid Use Efficiency (AUE) was very low in NNU and INU plants (0.12 g seeds $mmol^{-1}$ H^+ on average) but was the highest in IRU plants; nutrient solution containing nitrate showed an average value of 0.35 g seeds $mmol^{-1}$ H^+, comparable to that obtained by Wheeler et al. in 2003. NNU and INU also had low Base Use Efficiency (BUE), indicating a reduced uptake of NH4-N (Table 2-17).

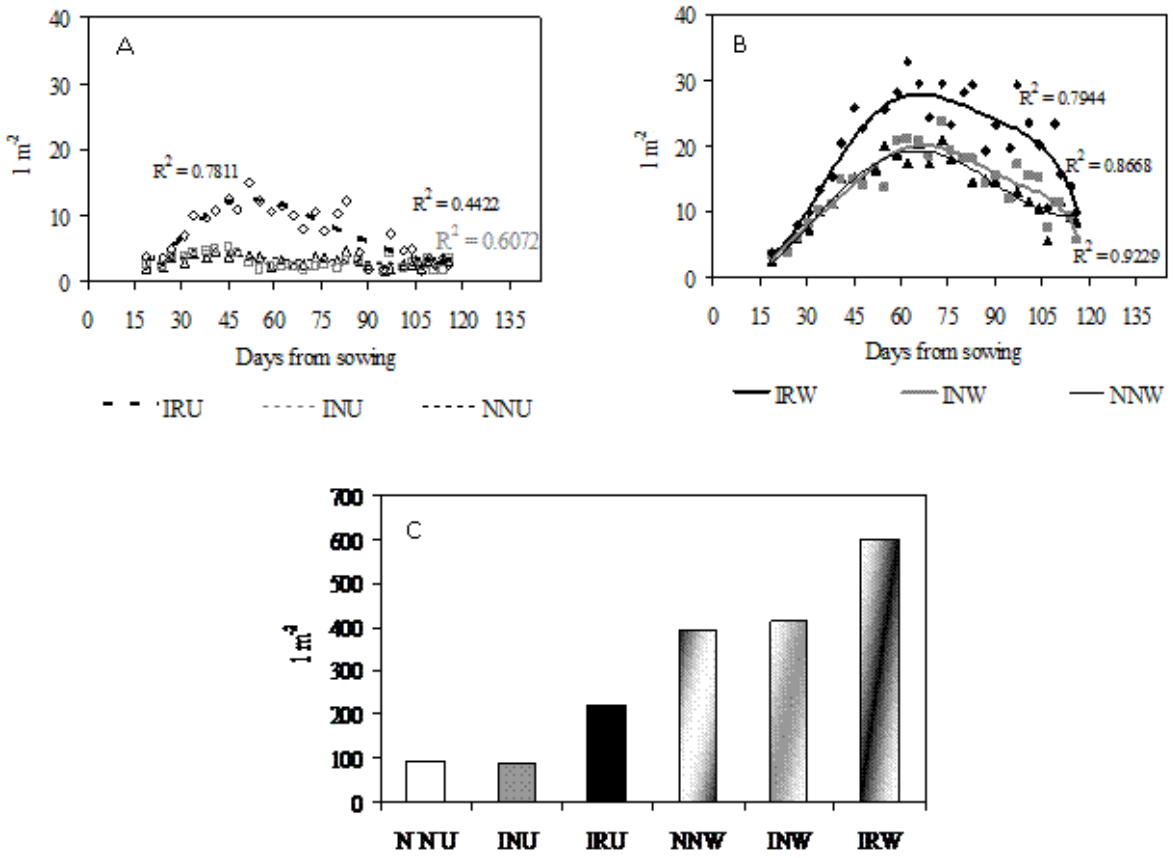


Fig. 2-8 Trend of water use throughout plant growth in Urea (A) and Wheeler (B) solutions and cumulative water use at the end of the experiment (C) for the different treatments.

Table 2-15 . Average fluctuations of nutrient solution EC (with their coefficients of variation) and plant water use on a per area and per day basis for the different treatments.

	EC before adjustment		EC after adjustment		Water consumption
	(dS m^{-1})	CV (%)	(dS m^{-1})	CV (%)	(l m^{-2} d^{-1})
NNU	1.73 ± 0.04	18.2	1.72 ± 0.06	17.4	2.6
INU	1.67 ± 0.04	23	1.56 ± 0.07	24.7	2.5
IRU	1.73 ± 0.04	19.5	1.45 ± 0.04	16	6.3
NNW	1.28 ± 0.02	12	1.15 ± 0.03	14.4	10.9
INW	1.34 ± 0.02	12.8	1.16 ± 0.03	14.3	11.4
IRW	1.42 ± 0.04	22.3	1.20 ± 0.04	18	16.5

Table 2-16 Average fluctuations of nutrient solution pH (with their coefficients of variation) and plant acid and base use on a per area and per day basis for the different treatments.

	pH before correction	CV (%)	pH after correction	CV (%)	Acid (mmol H^+ m^{-2} d^{-1})	Base (mmol OH^- m^{-2} d^{-1})
NNU	5.76±0.05	7.3	6.06±0.02	2.2	6.8	12.7
INU	5.90±0.05	7.1	6.04±0.03	3.9	7.4	7.4
IRU	5.75±0.05	8.4	6.07±0.02	3	8.5	18.6
NNW	6.42±0.06	8.9	5.92±0.02	3.4	38.7	4.3
INW	6.53±0.05	7.4	5.95±0.03	4.2	40.3	2.7
IRW	6.87±0.05	7.1	5.93±0.03	4.1	54.4	2.3

Table 2-17 Biomass efficiency indexes for the different treatments.

	Growth cycle (days)	WUE seeds (g l^{-1})	RUE seeds (g mol^{-1})	AUE seeds (g $mmol^{-1}$)	BUE seeds (g $mmol^{-1}$)
NNU	122	0.33	0.02	0.13	0.07
INU	122	0.33	0.02	0.1	0.1
IRU	122	0.88	0.12	0.63	0.29
NNW	122	1.18	0.29	0.33	2.91
INW	122	1.12	0.29	0.31	4.64
IRW	122	1.29	0.51	0.4	9.59

2.4 Conclusions

The realization of CELSS based on higher plants is a complex objective as it involves the development of new technologies and the understanding of the effects of space factors both on the behaviour of biological systems and on the functioning of physical principles (De Micco *et al*., 2009).

Apart from challenges in engineering research and medical aspects to face the constraints to the long permanence of the human body under space conditions, there are also many technical problems regarding the supplying of resources.

To bring down the materials to be brought in space for plant cultivation, a reduction in the amount of fertilizer or growth media can be supposed.

In our experiment, results from chemical and proximate analyses on seeds showed a higher mineral content compared to reference values, which could have been a result of the luxuriant nutrient uptake by the hydroponically-grown plants. Methods for controlling excessive nutrient uptake by the plant might be explored. This could include selection of low nutrient accumulating cultivars and/or the use of less nutrient-rich solutions for growing plants, that would allow the reduction of fertilizers to be brought in space for plant cultivation.

The use of urea as nitrogen source for plant growth could be a good way to recycle human waste (urine) in a CELLS, but our experiment confirmed that is not able to provide a sufficient amount of nitrogen at least in the early stages of plant growth. This early disadvantage was not recovered by plant in the following phases of lifecycle, consequently, water use was very low and both growth and yield were reduced in plants fed urea as sole source of nitrogen; HI also was lower, indicating a higher production of inedible tissues (waste) in relation to the edible ones; however, the quality of seeds was enhanced thanks to the concentration of nitrogen in a reduced amount of seeds.

The inoculation with *Rhizobium* did not improve plant performances when fed urea, because symbiotic N fixation starts after nodule formation and is much slower in plant early stage (Brun, 1978), so plants were not provided with nitrogen at seedling stages (due to the reduced urea hydrolysis in nutrient solution); the positive effect of urea on nodulation was not sufficient to obtain good results in terms of plant yield. Failed bacteria positive effects on plants seemed to be related to the growth in NFT (most of roots are submerged during the whole cultivation cycle), that in plants fed Wheeler solution are gathered to the nitrate inhibitory effect on bacteria.

Growth in substrate enhances the dry matter accumulation in plants fed urea and seemed to reduce the inhibitory effect of nitrate on nodulation in plants supplied with

Wheeler solution. Moreover, the growth in rockwool increased the HI compared to the growth in NFT and improved the plant efficiency in both Urea and Wheeler solutions.

Considering the results obtained in this experiment, further research could be performed using a nutrient solution containing both urea and nitrate as sources of nitrogen, at least in early plant growth; moreover, the inoculation with *Rhizobium* could be involved, as during early developmental stages plants could use nitrate from the nutrient solution while bacteria are becoming active on root surface; urea could limit the inhibiting effect of nitrate on nodules. In following plant growth stages, when plants are well nodulated and nitrogen fixation has begun, nitrate supplying could be interrupted and urea could be used as sole nitrogen source.

Furthermore, as solid substrate seemed to have some positive effects on plant performances, even when nitrogen availability is limited (urea), further testing could be performer in order to evaluate the effect of other solid substrates, even having a reduced weight or being recyclables, and the use of enzymes (ureases) in the nutrient solution could be tested, in order to improve the efficiency of urea in plant nutrition.

The Martian surface composition measured by Viking can be represented by several combinations of minerals incorporating major fractions of zeolites known to occur in altered mafic rocks and polar soils on Earth (Gaffey et al., 1985). In the next chapter zeolites are tested as growing media. These silicates are present either on the earth and on mars (Tokano and Bish, 2005). Rockwool was used to test growing media respect NFT, but local growing media are obviously cost saving if they suitable for growing plants.

3 Zeolites in media mixes for soilless production: first results on tomato

3.1 Introduction

Soilless crop production expanded rapidly from 1970s because of the advantages in controlling environmental factors and controlling plant's watering and mineral nutrition. Greenhouse crop surfaces in Europe reach approximately 50,000 ha out of which 20,000 ha are grown in soilless systems (Bougoul et al., 2005). Among various soilless practices, the use of substrates is the most spread among growers. A number of materials such as gravel, sand, peat, sawdust, pumice (tuff), coir, vermiculite, perlite and rockwool pure or in mixture have been used as growing media. The selection of a particular material for substrate use depends on its availability, cost, properties and local experience on its use (Klougart 1983, Verdonck et al., 1983). All horticulture substrates are acting as hydraulic and mineral reservoir and mechanical supports for plants but with physical and chemicals characteristics which can widely vary. Water and nutrient managements are strictly correlated with the kind of substrate and its physical and chemicals properties.

Zeolites are silicates characterised by high cationic exchange capacity (CEC) (Pabalan and Bertetti, 2001). Their particular crystalline structure leaves the water and ions to easily circulate in and out of the crystal (Passaglia and Scheppard, 2001). Moreover they show low density, reversible dehydration and high water retention (Mumpton, 1999). Zeolite minerals are widely used in several activities such as water and wastewater treatment, in pouzzolane cement as lightweight aggregate, in the drying of acid-gases, in the separation of oxygen from air, in the extraction of Cs and Sr from nuclear wastes and the mitigation of radioactive fallout, but also as dietary supplement to improve animal production. With respect to agriculture, zeolites may be used as a soil amendment to improve CEC and water sorption capacities. Considering this property. zeolites are natural own slow release fertiliser. Natural zeolites can be treated with ammonium (Lewis et al, 1984) and potassium (Hershey et al, 1980) which are then held within the 3-dimensional structure of the zeolite crystal, and released only gradually as they are used up from the soil. This prevents the leaching of

excess nutrients into watercourses (Petrovic, 1990, Huang, 1992), thus reducing problems such as nitrogen pollution and eutrophication, as well as reducing the quantity of fertiliser required. Zeolites were tested in several crops in Japan by Torii (1978) adding 10-20 t/Ha where they showed significant increases in yields of wheat (13-15%), eggplant (19-55%), apples (13-38%), and carrots (63%). Similar results were obtained in Ukraina with barley, potato, clover and wheat adding 15 t/Ha of zeolites to sandy loams (Mazur et al., 1984).

Concerning vegetables crops Lewies et al. (1984) showed an increase in root weight of radish of 53-59% using NH4+ enriched clinoptilotite (a natural zeolite) as amendment in greenhouse experiments. The use of clinoptilolite as the principal constituent of artificial soil was developed in Bulgaria in the late 1970s (Mumpton, 1999), where nutrient-treated zeoponic substrate used for growing crops produced greater development of root-system and larger yields of strawberries, tomatoes and peppers (Petrov et al., 1982).

Zeolites were also tested in soilless systems, mainly in mixtures with perlite. Kanarziska et al. (1997) reported highest yield and improved quality (sugars) with cucumber grown in a perlite-zeolite (clinoptilite) mixture 2:1. Djedidi et al. (1997) cultivated tomato on perlite and mixtures of perlite and zeolite in 1:1, 2:1 and 1:2 ratios. In their experiment the ratio of 2:1 increased yield, soluble solid content and quality. Soilless culture of gerbera gave higher yield on perlite/zeolite (P/Z 1:1 ratio) than other mixtures, due to sufficient aeration and improved water retention capacity (Issa et al., 1997). Turhan and Atilla (2004) studied the effect of perlite alone and mixture of P/Z (1:1 ratio) on ionic composition in "camarosa" strawberry plantlets during plant vegetative phase. They found that using P/Z mixtures as substrate to grow strawberry may be beneficial. Similar results were obtained by Fotouhi Ghazvini et al. (2007) with strawberries.

3.2 Materials and methods

The experiment was carried out in a commercial plastic greenhouse near Bologna (North Italy, 44°33’ N, 11°28’ E). Five substrates, rockwool (RW), perlite (P), coconut fiber (F), perlite - zeolite (70:30 v/v, PZ), coconut fiber - zeolite (70:30 v/v, FZ) and three tomato cultivars (Idoll, Secolo, Grandella). The experimental design was a split plot with 4

replications and substrates as main plots. Each plot was composed by 3 slabs of substrate, and all measurements were taken on the central one.

Plants were transplanted on March 5th, 2008 (3.12 plants m^{-2}, 1.6 m between rows and 20 cm on the row). Crop management followed normal greenhouse practices in northern Italy. A V-training system was adopted, and water and nutrient management was done according with Grodan® standards.

3.2.1 Data Collection

Phenology

Data were collected 28, 8, 8, 23, 5, 7, 7 days after transplanting using the BBCH-scale (Feller et al., 1995).

Chlorophyll content

Measurements were taken weekly from 28 DAT to 180 DAT using Yara N-Tester (manufactured by Yara International ASA, Norway). The value determined by the instrument provides an indication of the relative amount of chlorophyll present in plant leaves, by measuring the light transmittance of a leaf at two wavelength regions (peaks: approx. 650 and 950 nm). The first one matches with the peak of absorbance area of chlorophyll where absorbance is unaffected by carotenoids. The second one is a reference wavelength where the absorbance of chlorophyll is extremely low (for further information see Gianquinto et al., 2004). Measurements were carried out on the first completely unfolded leaf. The instrument automatically calculates the mean of thirty independent readings therefore about 6 measurements were taken for each of the five plants in the central slab of each plot.

Yield

Harvest started 92 DAT. Data were recorded from the first to sixth truss and then from the tenth to the twelfth truss. Commercial size fruits were weighted and counted.

3.3 Results

Phenology

Phenological data (not shown) showed differences in the cultivars behavior, but there was no evidence of influence of substrates on phenology.

N-Tester

N-Tester readings curves (Fig. 3-1 and Fig. 3-2) are useful to compare substrate's performance during the season. RW shown more constant values than other substrates. The influence of zeolites on the N-Tester readings was similar in F and P. It seemed to reduce both peaks, the highest and the lowest given by the instrument. We should consider that a strong reduction of N-Tester values can be a consequence of a not optimal status of the plants (prolonged stresses, nutritional disorders). At the same time, higher peaks can be the consequence of a water shock because it results in an increase of the chlorophyll concentration per leaf area (Gianquinto et al., 2004). N-Tester values in substrates mixed with zeolites are more constant and therein suggest that plants grown on those substrates are less susceptible to environmental stresses and nutritional disorder.

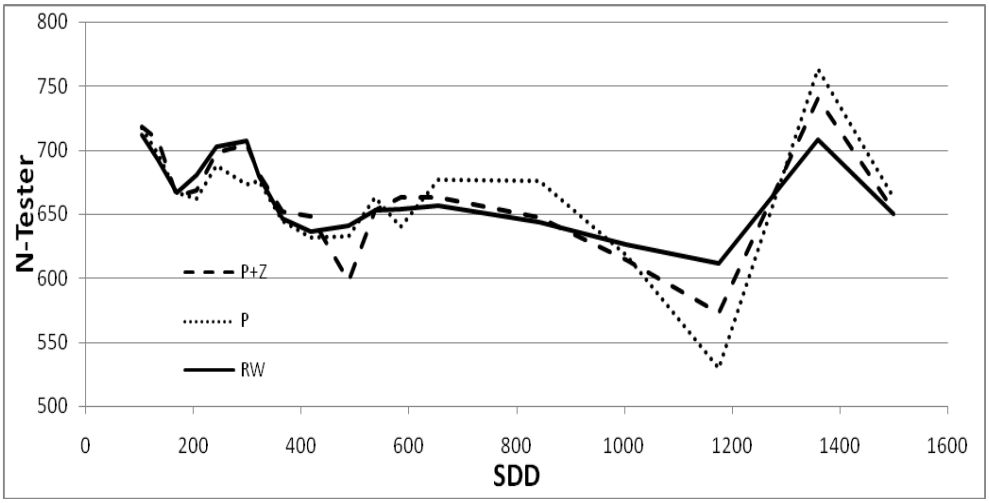


Fig. 3-1 Changes in Yara N-Tester values during the growing season (Sum of Degree Days) in rockwool (RW), perlite (P), perlite mixed with zeolites (PZ).

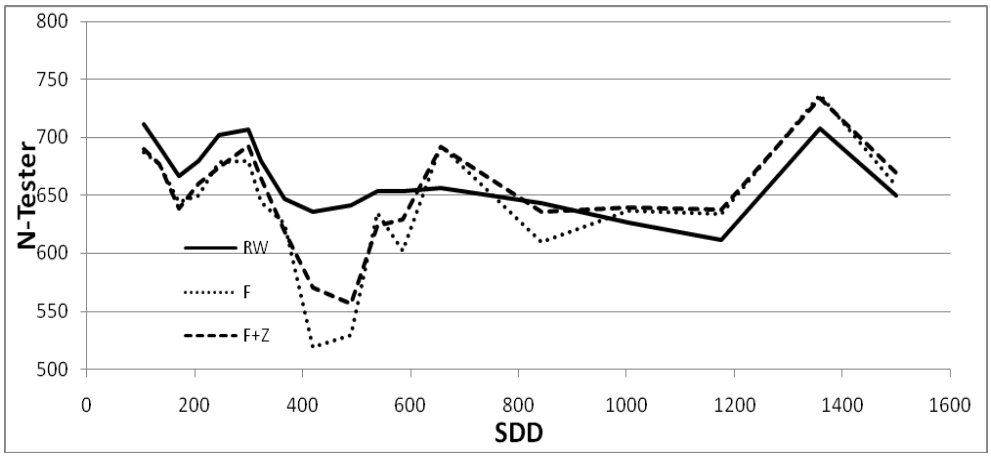


Fig. 3-2 Changes in Yara N-Tester values during the growing season (Sum of Degree Days) in rockwool (RW), coconut fiber (F), coconut fiber mixed with zeolites (FZ).

Yield

Data analysis (Table 3-2) shown different behaviours of cultivars (P<<0.001), growing media (P=0.007) and a slight significant interaction (P=0.043). Idoll was the cultivar with the highest yield (Table 3-1) followed by Grandella and then Secolo. Grandella showed better yield with FZ than F and no differences between P and PZ. Idoll presented higher yield on PZ than on P and no differences on F and FZ. The highest yield was obtained with Idoll on PZ that performed even better than on RW, whilst there was not statistical difference (Duncan's test P=0.005) between RW and F, FZ, P, PZ with Grandella.

Table 3-1 weight (g) of the 9 trusses harvested from the three cultivars. Small letters for mean separation at P=0.05, Duncan's Test.

Grandella	761.65	b
Secolo	608.23	c
Idoll	780.24	a

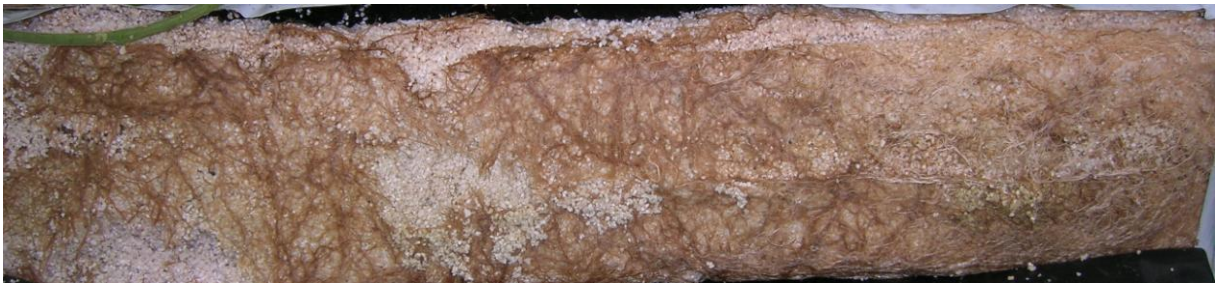
Table 3-2 Mean weight (g) of the 9 trusses harvested from the three cultivars (Idoll, Secolo, Grandella) grown on rockwool (RW), perlite (P), coconut fiber (F), perlite - zeolite (PZ), coconut fiber - zeolite (FZ). *,,***, ns represent significance at P<0.05, P<0.01, P<0.001 and not significant. Small letters for mean separation at P=0.05, Duncan's Test.**

Growing Media	Cultivar (C)	Weight (g)	
RW	Grandella	758.62	bc
	Secolo	574.54	f
	Idoll	771.28	bc
F	Grandella	746.28	c
	Secolo	617.36	de
	Idoll	786.48	abc
F+Z	Grandella	789.56	ab
	Secolo	641.52	d
	Idoll	781.76	abc
P	Grandella	764.47	bc
	Secolo	590.77	ef
	Idoll	758.73	bc
P+Z	Grandella	749.34	c
	Secolo	616.98	de
	Idoll	802.95	a
Growing Media		**	
Cultivar		***	
Interaction GM*C		*	

Roots development

At the end of the growing cycle growing media slides were opened to take pictures of the roots. No measurement were done concerning roots, but the influence of zeolites was clear on P (Fig. 3-3) and on F (Fig. 3-4).

A



B

Fig. 3-3 Picture A represent the lower side of a perlite (P) slide. B is perlite mixed with zeolites (P+Z). Pictures were taken at the end of the growing cycle.

A

B

Fig. 3-4 Picture A represent the lower side of a coconut fiber (F) slide. B is coconufiber mixed with zeolites (F+Z). Pictures were taken at the end of the growing cycle.

3.4 Discussion

Plants showed good growth with growing medium adopted. Considering that this experiment was a preliminary work on the use of zeolites in growing media for commercial soilless crop production the purpose is to understand if it would be useful to consider further tests. In the literature there is several work about P and Z mixtures (Kanarziska et al., 1997, Djedidi et al., 1997, Issa et al., 1997, Turhan and Atilla, 2004, Fotouhi Ghazvini et al., 2007) while no previous experiments on F and Z mixtures are presented. Probably this is due to the complementary proprieties of P and Z where the latter one improves the CSC and the water retention relative to the former component. Therefore it was expected to find that zeolites are better substrate improvers if added to P than F, whilst good results were obtained in both cases. Therefore further tests should take into account also F, with different ratios of Z. Moreover, further experiments would analyze the influence of zeolites added to growing media on roots behavior. Root status is one of the key point of good performance on hydroponic crop production, field tests shown better development in roots systems is zeolites were applied in soils (Mumpton, 1999, Petrov et al., 1982).

In conclusion, substrate mixes allow to adapt growing media to different plant needs and fertigation managements, this is due to the possibility to obtain a wide range of chemical and physical proprieties.

4 Growing pepper with vermicasting, effect of improved water retention on stem water potential

4.1 Introduction

4.1.1 Which techniques are used to measure water in soilless substrates?

Because of the difficulties associated with measuring plant-based parameters, usual approach has been to utilize soil-based approaches that can either measure soil *water activity* (potential measurement), and/or *soil water quantity* (volumetric or gravimetric measurement). Since more than 60% of ornamental plants are now grown in containers in the United States (USDA, 2007) whose production utilize soilless substrates rather than soils, we can consider soil and/or substrates as interchangeable terms when we discuss the measurement of water in the root zone of plants.

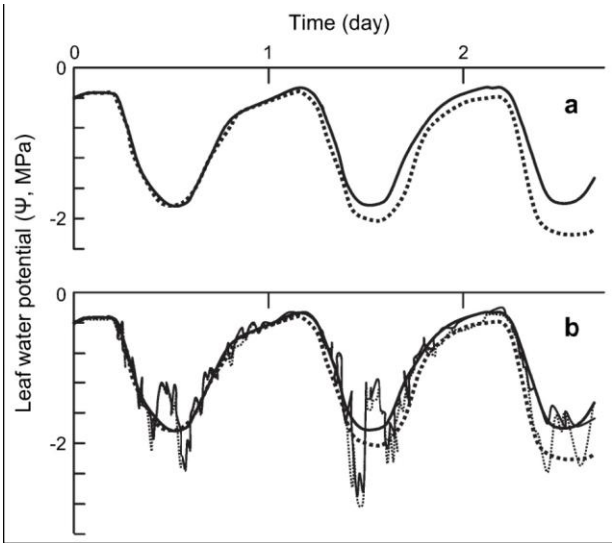


Fig. 4-1 A Schematic illustration of expected time-courses of leaf water potential over a period of 3 days, following withholding of irrigation at time 0 (dashed lines) and for corresponding irrigated controls (continuous lines). (a) Typical diurnal trends of ψleaf smoothed by taking 3 h running means. In (b), the expected magnitude of instantaneous variation in ψleaf, as measured with a pressure chamber, is shown (Jones, 2007).

The term substrate will be used to refer to the media that supplies a plant in a container with water, nutrients, and physical anchoring (Raviv and Lieth, 2008). Soil or substrate water potential is defined as the amount of work per unit of pure water (i.e. cm_3· mL or m_3) that must be done by external forces (i.e. suction or pressure) to reversibly transfer a measurable amount of water from the standard state to the point under consideration, at constant temperature and pressure (Hopmans and Rottson, 2002). Total soil water potential can be described by the equation:

$$\Psi t = \Psi p + \Psi z + \Psi s + \Psi a$$

Where Ψp, Ψz, Ψs, and Ψa are the pressure, gravitational, solute (osmotic) and air pressure potentials, respectively.

Since water in a substrate has various forces acting upon it, potential energy usually differs from point to point, and hence its potential energy is variable as well. It is important to note that two regions of substrates may hold different amounts of water at the same potential energy status and would not experience a flow of water between regions. Substrate water quantity (equivalent to soil water content) is defined as either the ratio of the mass of water present in the sample before drying, divided by the mass of the sample after it has been dried (gravimetric; kg kg^{-1}), or alternatively, as the volume of water present in a unit volume of soil or substrate (volumetric; cm3 cm^{-3}).

4.1.2 Stem Psychrometer

Since the fifties thermocouple hydrometer were used in the field of water relations research (Spanner, 1951). Most in situ hygrometers have been designed for attachment to leaves, but there are advantages in using stem hygrometers. Less significance energy balance disruptions and ease attachment of the stem hydrometer favor its use over that of the leaf hygrometer. The thermocouple hygrometer relies, for its success, on the accurate determination of very small differential temperature (0.01-0.5 °C), and also on the

assumption that the initial measuring junction and sample temperature are identical. It has long been realized that failure to achieve the latter is a major source of error in hygrometry (Dixon and Tyree, 1984).

Michel (1979) addressed the problem of temperature gradients in stem hygrometer but stopped short of actually measuring the error; the error inducing gradient is that between the sample and the measuring junction, not the measuring junction and the reference (ie instrument temperature). Dixon and Tyree (1984) measured this error and obtained a good validation of the psychrometer when applied to the stem of the plant using a correction factor.

The stem psychrometer is constructed of chromium plated brass. The chamber houses two chromel/constantan thermocouples in series (Fig. 4-2); one in the chamber air (C) and the other extending to contact the sample surface (S). The differential output from these two junctions is a measure of the temperature gradient between the sample and the measuring junction (i.e. the thermocouple in the chamber air). The PSY-1 automatically performs a psychrometric (wet-bulb) reading, with automatic correction for the temperature gradient, resulting in the precise measurement of the samples water potential.

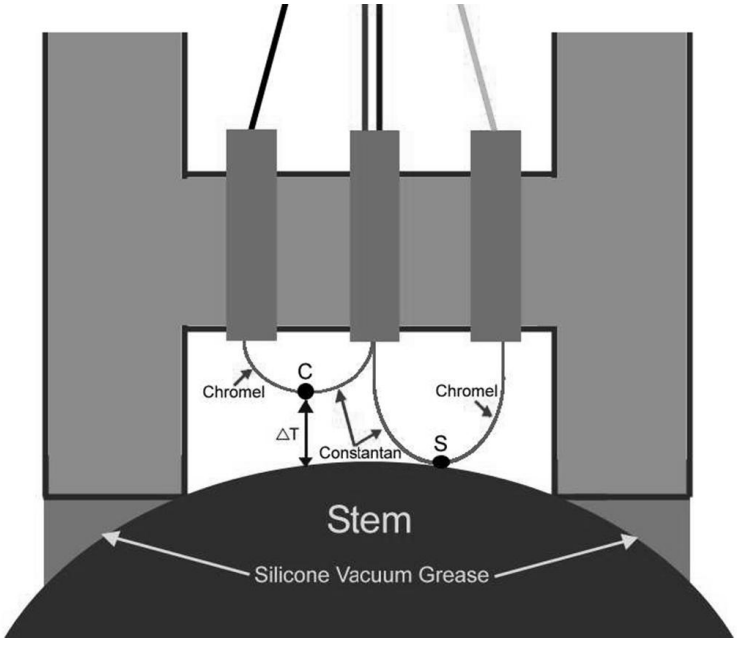


Fig. 4-2 Stem psychrometer components.

A copper/constantan thermocouple is embedded in the chamber body to allow measurement of chamber temperature. This temperature value is recorded and used to compensate the measured water potential values.

The stem psychrometer provides the means to measure in situ water potential over a wide range with accuracy and repeatability. However, certain precautions must be adhered to if meaningful results are to be achieved (Dixon and Tyree, 1984).

4.1.3 Vermicasting as pepper substrate

Peppers (Capsicum annum L.), which belong to the family Solanaceae, are known for their versatility as a vegetable crop and are consumed both as fresh vegetables or dehydrated for spices. As with other vegetable crops, peppers are still usually grown using conventional applications of inorganic fertilizers and pesticides (Bosland and Vostava, 2000). However a growing awareness of some of the adverse economic and environmental impacts of agrochemicals in crop production, has stimulated greater interest in the utilization of organic amendments such as composts or vermicasting for crop production (Follet et al., 1981). The use of organic amendments, such as traditional thermophilic composts, has been used to increase crop productivity and yields (Johnston et al., 1995; Maynard, 1993), and their use is usually associated with improved soil structure and enhanced soil fertility (Follet et al., 1981), increased soil microbial populations (Barakan et al., 1995) and activity (Zink and Allen, 1998), and an improved moisture-holding capacity of the soil. Recently, there is increasing interest in the potential of vermicasting, which are products of a non-thermophilic biodegradation of organic materials through interactions between earthworms and microorganisms, as plant growth media and soil amendments. Vermicasting are finely divided peat-like materials with high porosity, aeration, drainage, water-holding capacity and microbial activity, which make them excellent soil amendments or conditioners (Atiyeh et al., 1999, 2000; Edwards, 1998). Greenhouse experiments conducted by Arancon et al. (2004) have demonstrated that vermicasting contain plant growth-regulating materials, such as plant growth hormones and humic acids, which are

probably responsible, at least in part, for the increased germination, growth, and yields of plants in response to vermicasting application or substitution (Atiyeh et al., 2002).

The objectives of the research reported here was to assess the effects of a commercial vermicasting on the growth and yields of greenhouse peppers grown in a soil-less system. Vermicasting effect was also measured on the physical characteristics of the growing media and his effect on the stem water potential of the plants.

4.2 Materials and methods

Pepper plants (*Capsicum annuum* L.) were grown in a glass greenhouse in the Controlled Environment Systems Research of the University of Guelph (N43° 33.000, W80° 15.000) from 15th of May 2010 till 20th of September 2010. Seedling were transplanted in 15 liters pot on the June the 1st using four different growing media based on the mix of vermicasting with a normal commercial mix of perlite (10% v/v) and peat (90% v/v) that was also used as control. Ratios tested were 10%, 20%, 30% v/v of vermicasting. Plants were trained with a double stem training system with a density of 2.8 plants/m^{-2}. Nutrient solution (Table 4-1) was given by drip irrigation three times a day, pore thru analysis was done to correct the nutrient solution EC and pH during the cycle.

	pH	EC	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B	Mo
		dS/m	mM						µM					
Nutrient solution	5.6	1.9	10.8	0.7	3.4	3	1.2	1.5	26.2	8.0	0.96	2.1	7.13	0.01

Table 4-1 Nutrient solution composition

Growth analysis was based on non-destructive measurements of plant height, number of leaves, number of flowers and fruits and stem diameter. These measurement were carried out on each plant of the experiment every two weeks. The chlorophyll content was estimated using a colorimetric method (CCM-200 chlorophyll meter, Opti-Sciences, Inc.), on three fully expanded leaves from the top of each plant (3 measurement per leaves).

At harvest, yield was measured on a unit area basis for the different treatments compared and destructive measurements were done on each plant (leaf area index, leaves dry matter, stem dry matter).

Growing media pH and EC were measured using 1:1 diluition (D.W. Reed, 1996). Physical characteristics were measured using a Porometer developed by North Carolina State University (Bilderback et al., 1982, Fonteno and Harden, 2003).

The pour-through technique was used for monitoring nutrient solution leachate. When the potting media moisture was at or near saturation a short irrigation was performed to collect leachate from the bottom holes of the container. The basic principle is to collect enough leachate for analysis without completely diluting the sample. After collecting the leachate it was poured and filtered into a suitable container for analysis of pH and EC.

Stem Psychrometer - Calibration

Calibration is a necessary procedure to develop a relationship between uV (microvolt, psychrometer output) and bars (or MPa) of water potential and it was accomplished by preparing a range of standard NaCl (or KCL) solutions of known molality (eg. 0.1, 0.2, 0.3, 0.4, 0.5 molal). Saturated filter paper disks (Watman's No.1) were used for calibration. Measurement of thermocouple output in the suggested range is essentially linear at a given temperature (Fig. 4-3) .

Molality	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*1*
NaCl Solution Water Potential (MPa)	*-0.462*	*-0.915*	*-1.368*	*-1.823*	*-2.281*	*-4.640*
Chamber Temperature (°C)	25.0	25.0	25.0	25.0	25.0	25.0
Wet Bulb Depression (µV)	2.9	4.9	6.9	8.4	9.9	18.6
Corrected Wet Bulb Depression (µV)	2.9	4.9	6.9	8.4	9.9	18.6

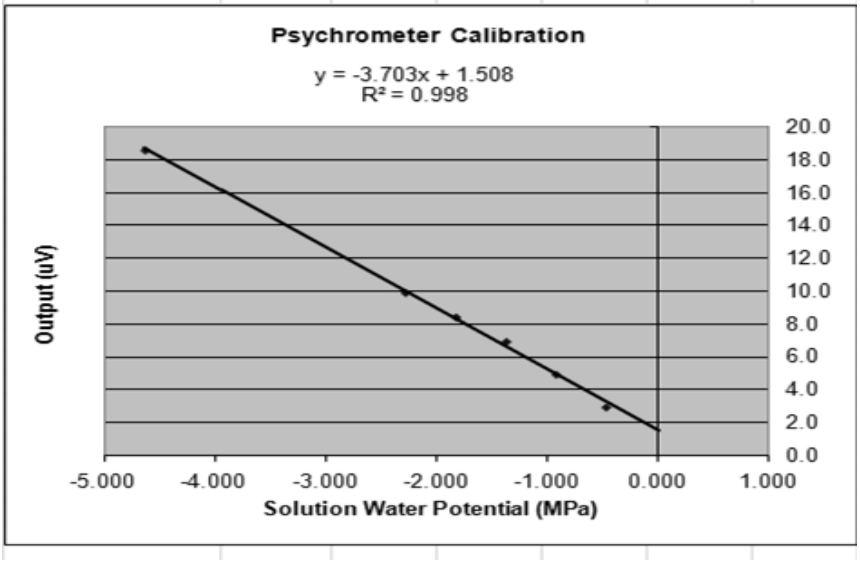


Fig. 4-3 Linear relationship between uV output and NaCl concentration in the calibration solution

The vapour seal between the faces of the chamber and the calibration disk holder was enhanced with a small amount of silicon grease. Calibration was carried out in a temperature controlled chamber at 25 C. Little or no correction is needed when using the dew point mode. Temperature correction for psychrometric measurements is given by:

Corrected Reading = Reading/(0.027T + 0.325)

Where: T is chamber temperature in C.

The temperature controlled chamber consisted in a good quality circulating water bath, with proportional control of water temperature. This eliminates the typical "sawtooth" temperature control of less sophisticated baths. The water circulates through a flexible copper tube (1-2 cm diameter and 2-3 m long) which is then coiled and placed

inside a box close to the inside edges. 30 minuts were considered sufficient as calibration time.

Calibration was done on 15 different thermocouples, and twelve were used on the experiment (threshold R^2>0.90).

Stem Psychrometer – Sample preparation and instrument attachment

The stem psychrometer (Fig. 4-4) was prepared ensuring that thermocouple junction (S), which is to be in contact with the sample, is extended beyond the edge of the chamber well by gently pushing it from one side or the other. It needs to be just at the surface and not extended too far.

Fig. 4-4 Stem Psychrometer picture with indication of the position of the two chamber and of the calibration disk holder.

A section of sapwood (approximately 3 cm x 1 cm) was exposed by cutting or peeling away the bark, phloem and cambium layers. The area was rinsed with distilled water. Stem psychrometer was mounted on the exposed area ensuring that the chamber is completely covered by the sample. Only enough tightening pressure to ensure a vapour seal between the sample and chamber well is necessary. Then remaining exposed sapwood was greased

to reduce local evaporation and possible induced water potential gradients in the tissue. Also, some grease (eg. Vacuum grease) was applied around the junction of the sample and stem psychrometer. An imperfect vapour seal can be detected by directing an airstream at the sample_psychrometer junction and watching the mirovoltmeter needle for erratic deflections. At this point, the attached instrument and portion of stem was insulated with cotton wool and a temperature control jacket was fitted around the installation.

Stem Psychrometer – Measurements

Measurement were taken every hour using a Wescor HR-33T microvoltomer (Wescor, Inc., Logan, Utah, U.S.A). For each measurement it was necessary to note down the wet bulb reading (WB), body temperature (BT), and delta temperature (DT, or difference in temperature between the S and C thermocouple). The latter should be small +/- 1 uV to have trustable results.

To figure out stem water potential:

$$\text{Water Potential} = \frac{[\text{WB reading} / (0.325+0.027*\text{BT}) - \text{Calib. Int.}]}{\text{Calib. Coefficient}} + \text{DT}/61 * 80$$

Calib. Int. and Calib. Coefficient are the intercept and the coefficient corresponding to that psychrometers' calibration. Results is in bar.

Values obtained from stem psychrometer were corrected using the formula above and compared with those obtained using a Scholander-Hammel pressure bomb (Sholander et al., 1965) as described by Dixon and Tyree (1984) and adapted to vegetable plant.

Three plants per treatment were used in the dehydration test. The pot was sealed with aluminum foil to avoid loss of water by evaporation. Experiment was performed during four sunny days. No water was given to the plants during these days, therefore the growing media was dehydrated by the root's uptake. Stem psychrometer was then covered with

cotton and aluminum foil to get the best insulation and avoid temperature gradient between the device and the plant sap.

4.3 Results

Growing media analysis

Vermicasting shown a strong effect on the pH and EC of the growing media (Table 4-2) enhancing both salinity and pH linearly. In particular 30% of vermicasting causes high level of both EC and pH for standards growing media, with the consequence that the nutrient solution and the watering must be adapted to this.

Table 4-2 Growing media Electrical Conductivity (EC) and pH Growing measured using 1:1 dilution. Means were obtained from nine replicates. Means followed by the same letter are not significant for P≤0.05.

	pH		EC (uS/cm)	
C	5,03	a	92,1	d
10%	5,33	b	358,7	c
20%	6,07	c	792,3	b
30%	6,39	d	1126,0	a

Consequence of this was found also in the value obtained from the pour-through pH and EC analysis in particular in the first part of the growing cycle (Table 4-4). EC was in several measurements too high for 20% and 30% of vermicasting.

Significant differences in total porosity, container capacity, air space and bulk density were observed (Table 4-3). Bulk density shown that vermicasting has high density respect most of growing media, therefore it's important to consider shipping costs in case that production is not close to growing facilities. Growing media with higher container capacity are able to attain higher volumetric water contents, which could potentially increase the amount of water readily available for plants. Growing media with increased volumetric water contents have been shown to increase tomato yields. Ismail et al. (2008) showed that increased soil water contents resulting from multiple water applications produced a higher

tomato yield. Marouelli and Silva (2007) demonstrated that the number of marketable pepper fruit was linearly increased as the soil water tension decreased. Vermicasting decreased, with significant difference, total porosity and air space.

Table 4-3 Physical properties of the growing substrates. Means were obtained from three replicates. Means followed by the same letter are not significant for P≤0.05.

	Total Porosity (%)	Container Capacity (%)	Air Space (%)	Bulk Density (g/L)
C	90.1 a	74.3 c	15.7 a	73.0 d
10%	88.9 ab	78.5 b	12.2 a	108.8 c
20%	86.2 b	80.7 ab	7.4 b	172.8 b
30%	84.1 bc	82.5 a	4.6 c	209.4 a

Table 4-4 Results of pour-through pH and EC analysis ten days after transplant. Vermicasting caused too high EC in the leaching and therefore nutrient solution had to be corrected. Means were obtained from nine replicates. Means followed by the same letter are not significant for P≤0.05.

	pH		EC (uS/cm)	
C	5.14	c	1975.4	a
10%	5.5	ab	2411.0	b
20%	5.76	a	2602.1	c
30%	5.85	a	2785.2	d

Yield and plant growth

Height of pepper plants differed significantly between C and vermicasting treatments (10%, 20%, 30%) only at the end of the growing cycle (Fig. 4-5) where the vermicasting effect consisted of higher plants. No differences were present within the different ratios of vermicasting. Number of leaves shown no statistical differences between treatments at the end of the growing cycle (Fig. 4-6). Anyway during July (43-57 DAT) there were significant differences between C and 30%.

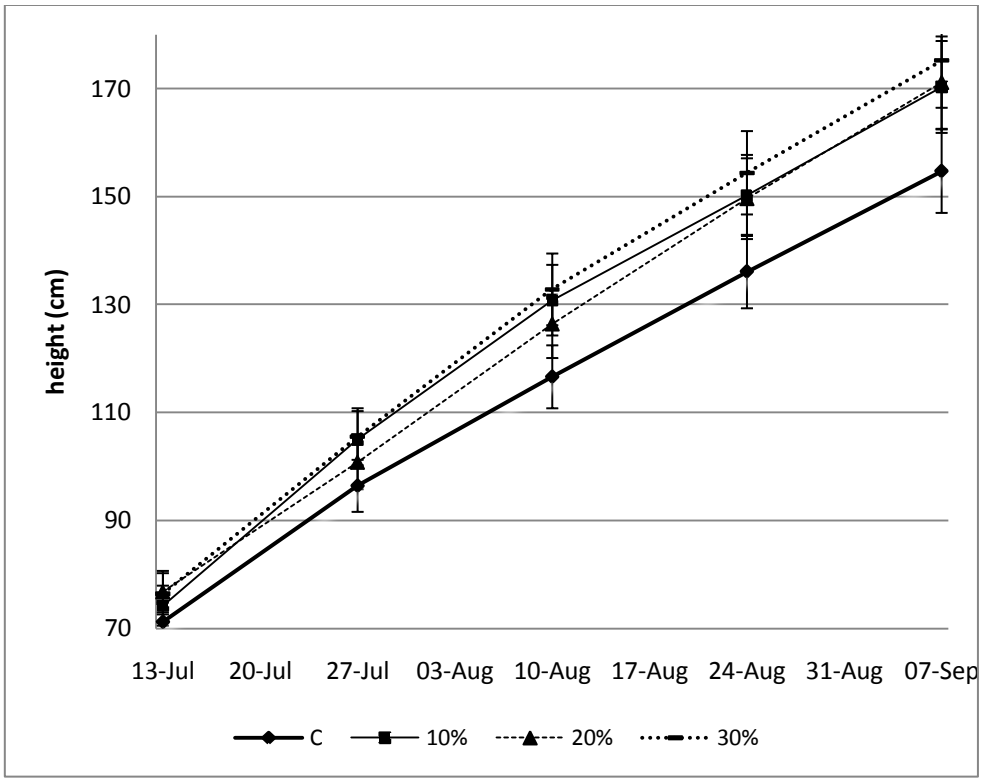


Fig. 4-5 Plant height during the growing cycle for the four treatments.

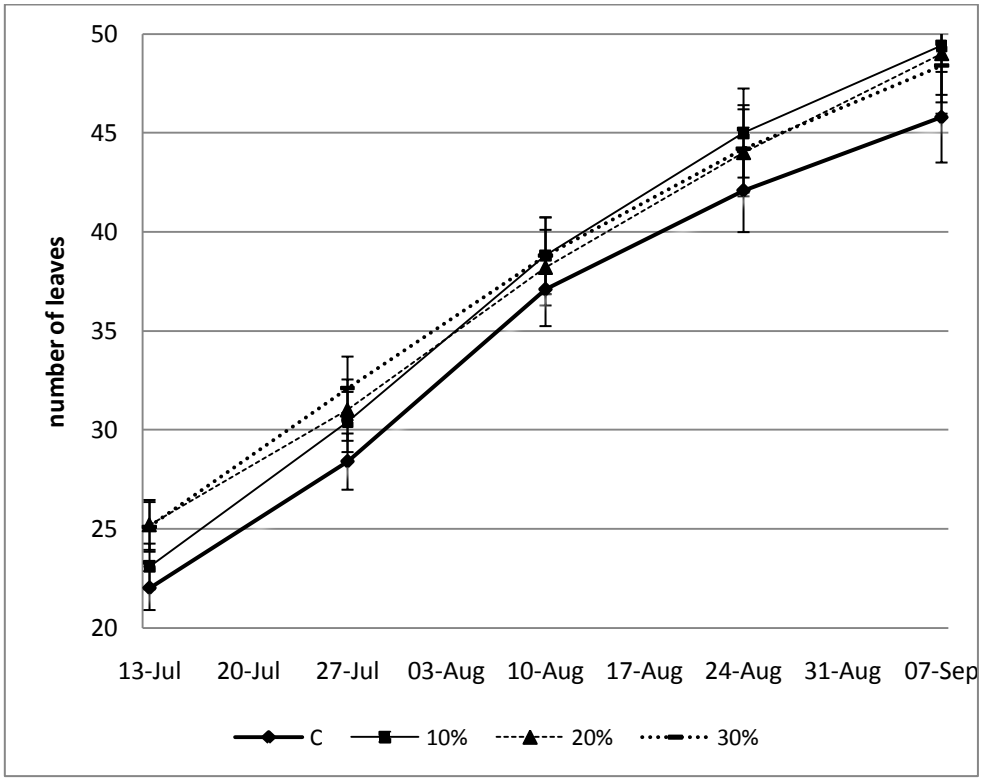


Fig. 4-6 Number of leaves during the growing cycle for the 4 treatments

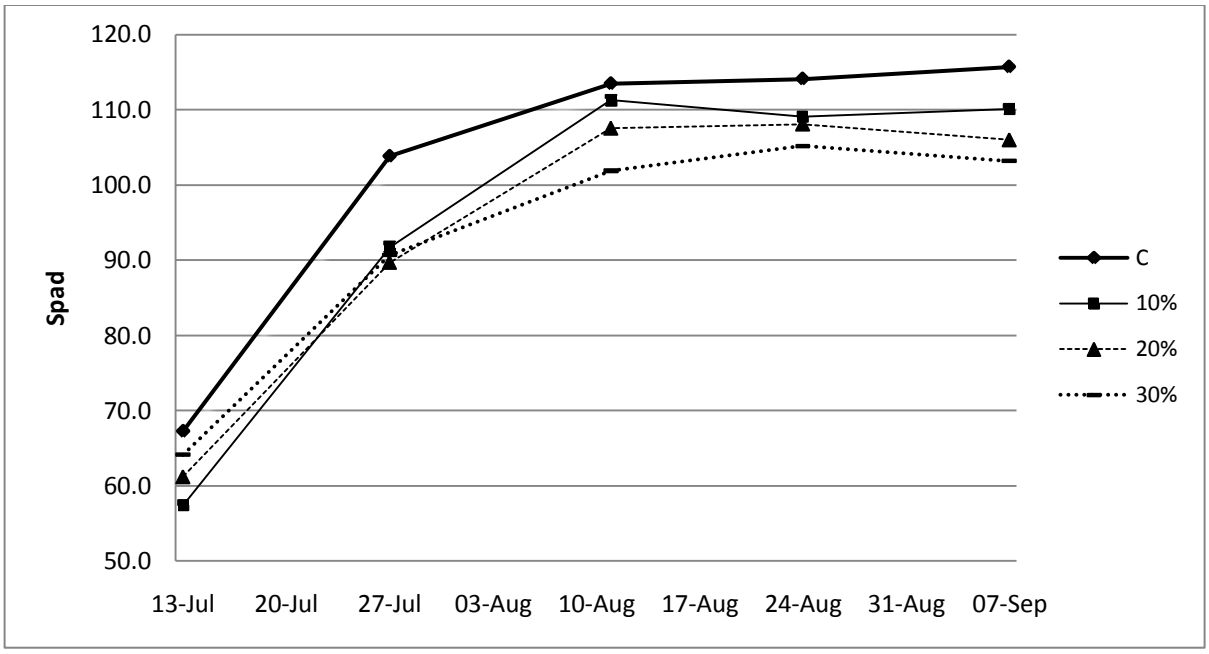
Vermicasting had a positive effect on pepper yield. Statistical analysis shown differences only between vermicasting treatments and control (Table 4-5). Results shown also an higher LAI whereas there are no differences concerning the DM of leaves and stem. Numerous researcher have found increase yield for vermicasting on other vegetables crop (Arancon et al. 2004, Atiyeh et al. 2000, Zaller, 2007) grown in open field or in greenhouses. LAI had a similar behavior with yield and plant growth, with higher values for vermicasting and statistical differences between C and 10%-30%, whereas there were no differences with 20% treatment.

Table 4-5 Marketable yield, leaf area index (LAI), leaves and stem dry matter (%)

Treatment	Yield ($g*m^{-2}$)		LAI (cm^2)		Leaves DW/FW		Stem DW/FW	
C	4187.2	*b*	7639.2	*b*	12.34%	*a*	14.51%	*a*
10%	4510.4	*a*	8247.6	*a*	12.56%	*a*	15.53%	*a*
20%	4972.8	*a*	8001.3	*ab*	12.10%	*a*	15.09%	*a*
30%	4711.3	*a*	8181.9	*a*	12.79%	*a*	15.57%	*a*

Chlorophyll content

Chlorophyll measurements shown higher values for C, and this is probably a consequence of reduced growth. Spad values can be influenced by several aspects, first of all water stress can enhance the measured value by incising the concentration of chlorophyll in partially dehydrated tissues, and then, also plants that have slower growth usually have highest concentration of nitrogen in the leaves. Therefore higher values obtained for C despite its reduced growth should be a direct consequence of the latter.



Stem Psychrometer – water pressure measurements

Stem psychrometer shown excellent agreement with values obtained by the pressure bomb, as long as the temperature gradients remained stable, the relationship between ψpb and ψhyg was linear with R^2=0.974 (Fig. 4-7).

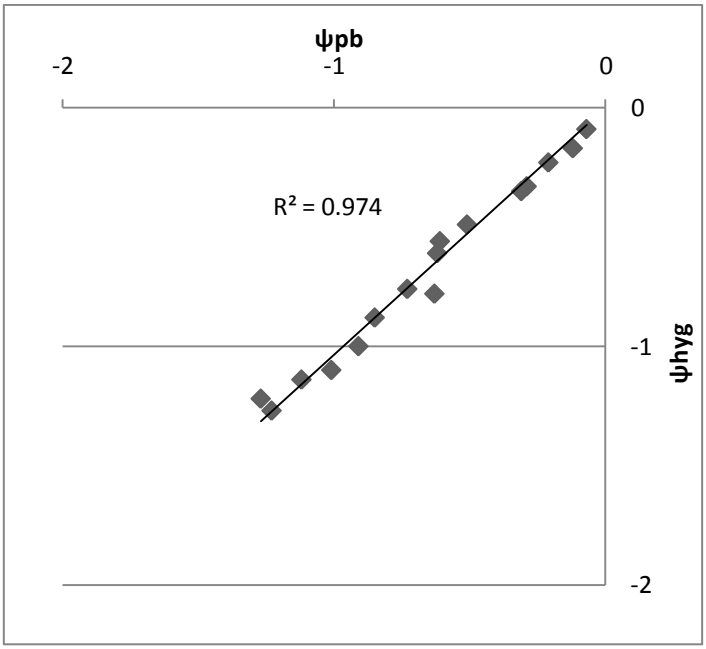
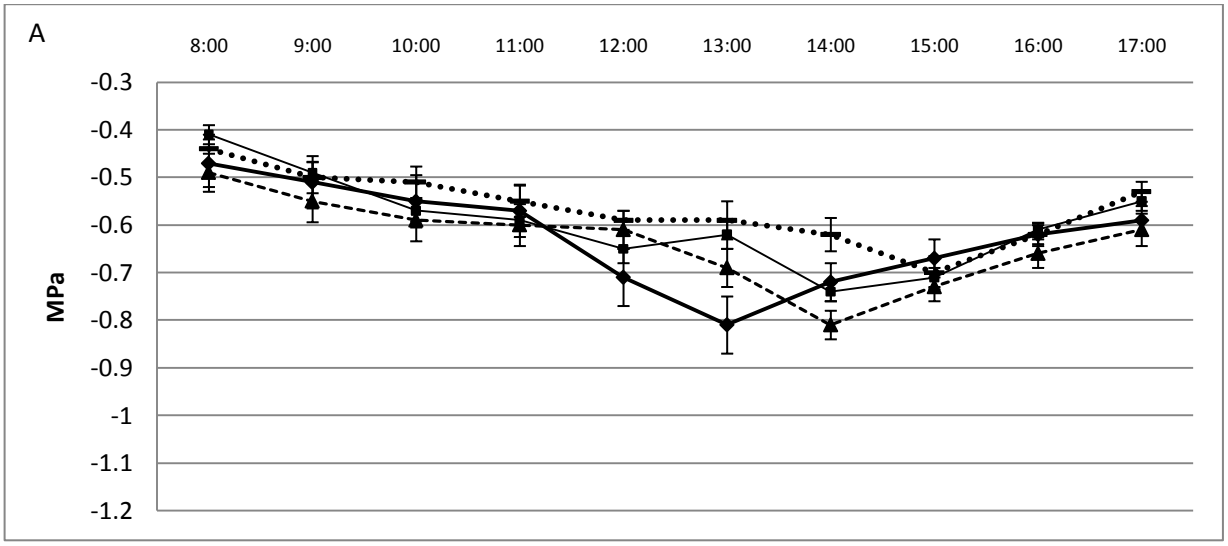


Fig. 4-7 The relationship between ψpb (bomb pressure) and ψhyg (stem psychrometer water pressure, after correction for temperature gradients). The dashed lines represents a 1:1 correlation.

Values recorded at hourly intervals from 8 am to 5 pm during three days are shown in Fig. 4-8. Data shown slight differences between treatments from the first day (Fig. 4-8a), but just in the warmest hours of the day (1 pm). In the following days (Fig. 4-8cb) differences were increasing with dehydration of the growing media, but anyway the gap was much lower than expected (water retention is strongly improved adding vermicasting, see Table 4-3). In literature there are not similar data to compare, *in situ* thermocouple psychrometers were previously used on field crop (cotton, corn, soybean, Wullschleger et al. 1987), trees (Edwards and Dixon, 1993), potatoes (Coffey et al. 1997) and tomatoes (Lee et al. 1988, Johnson et al, 1992), but most of the test were for physiological studies or for the validation of the instrument. There are no similar test done for growing media comparison. Johnson et al. (1992) used in situ thermocouple psychrometer for the study of tomato's water relation, the test aimed to describe different behaviors of water potential within the fruit and the stem. Stem water potential reached -1.2 MPa with strong radiation applied on the plants (400 W m^{-2}) whilst it reached a lowest value of 0.6 MPa at 80 W m^{-2}. Lowest value shown in C were -1.1 MPa in this experiment. Water potential values are also influenced from the LAI of each plant. Every treatment have the same pot volume, every pot contains different amount of water depending on the container capacity of the growing media (see Table 4-3). But even if some treatments have highest amount of available water the influence of LAI (if these plants are more developed) could reduce the stem water potential. Plants grown on the C had lower LAI of other treatment (see Table 4-5), and so water consumption is reduced, less water in the growing media doesn't mean a strong effect on the stem water potential recorded by the instrument. Therefore results were also figured out as MPA m^{-2} (ψhyg/LAI, LAI was measured after the experiment) to normalize stem water potential data on the LAI of the plants (Fig. 4-9). These data shown a strong influence of the growing media, since the first day (Fig. 4-9a) of experiment values recorded on C are much lower than other treatments. In the following days (Fig. 4-9bc) the gap is increasing, and even when solar radiation is decreasing (5 pm) plants grown on C are not able to restore a similar water potential. In the third day even 10% during several hours of the day have different values than 20% and 30% that keep for most of the day very similar values.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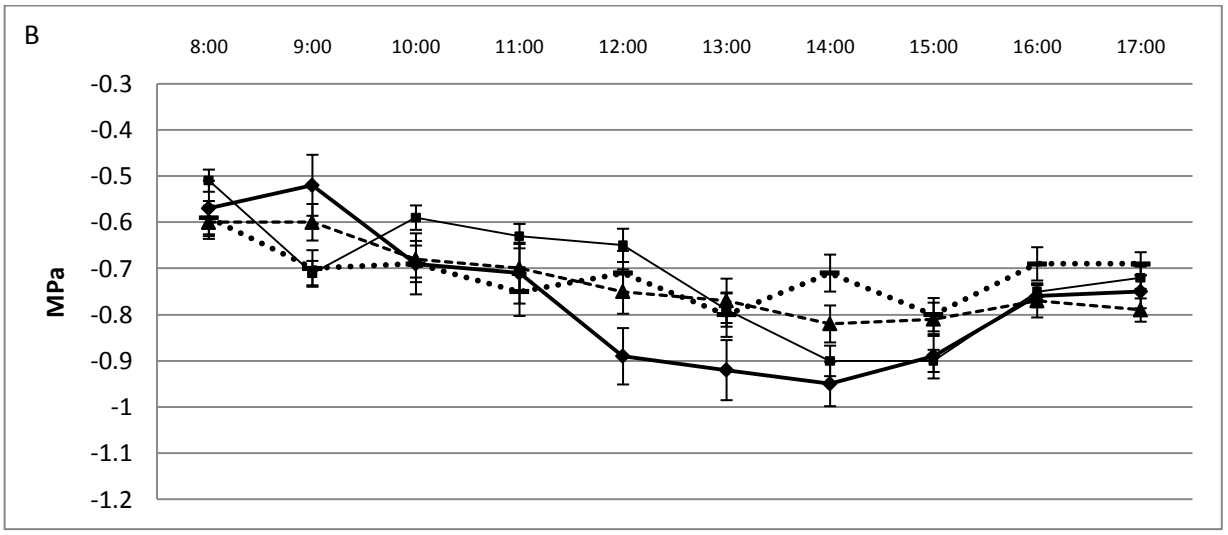
-0.9
-1
-1.1
-1.2
MPa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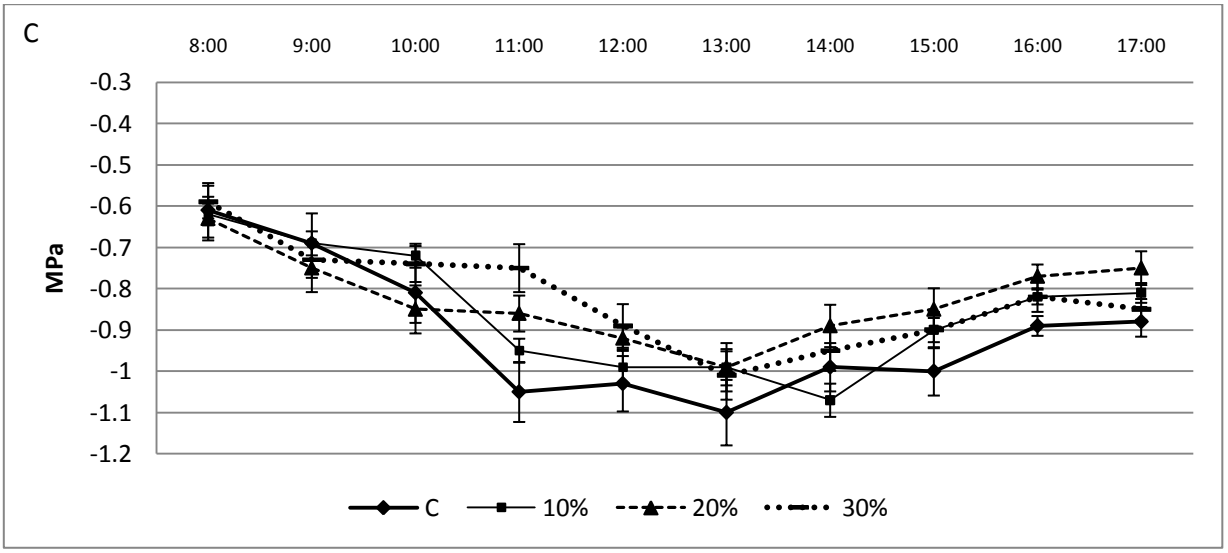
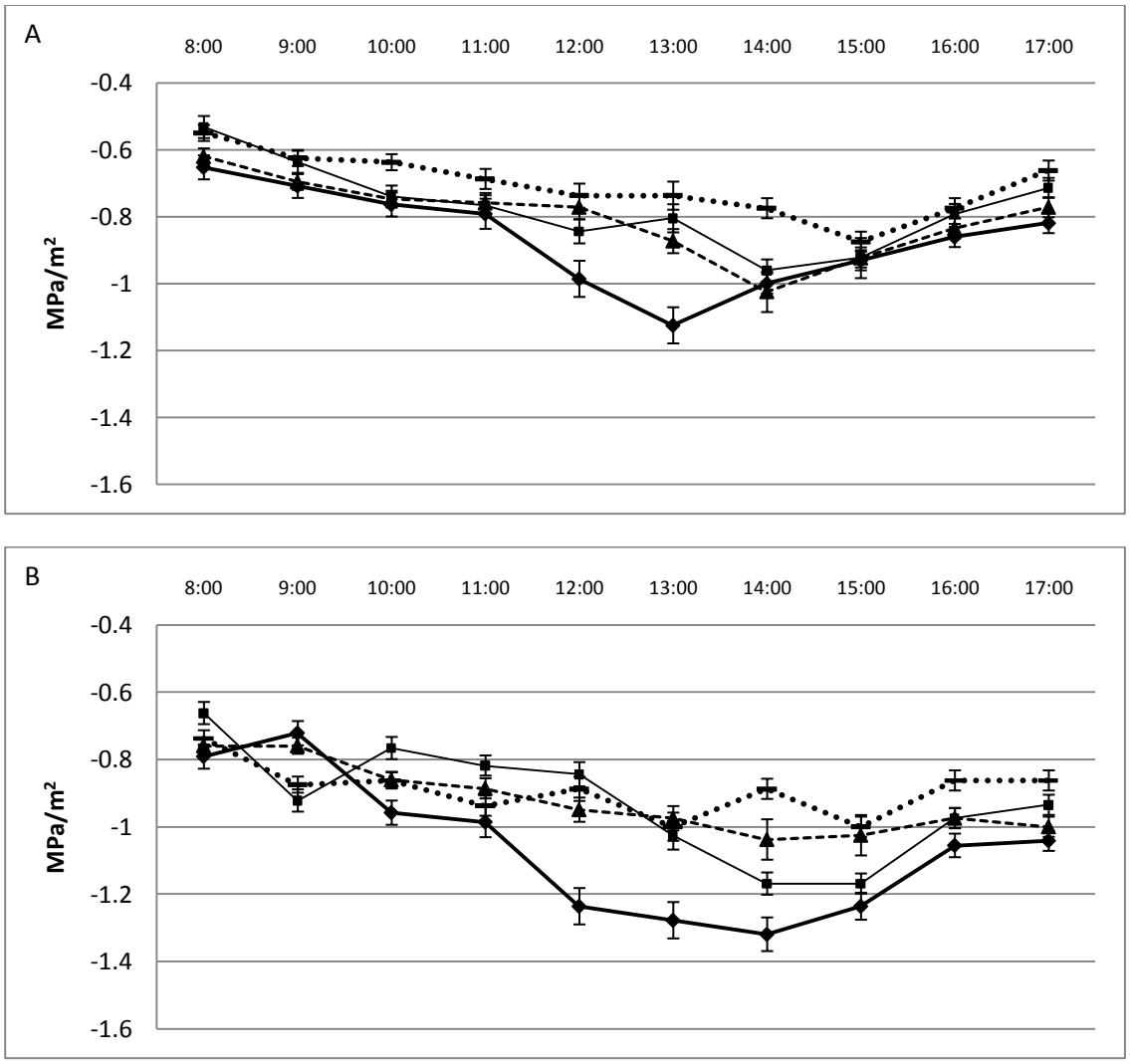


Fig. 4-8 Mean values and their standard deviation of stem water potential recorded during the first (A), the second (B) and the third (C) day of the experiment.



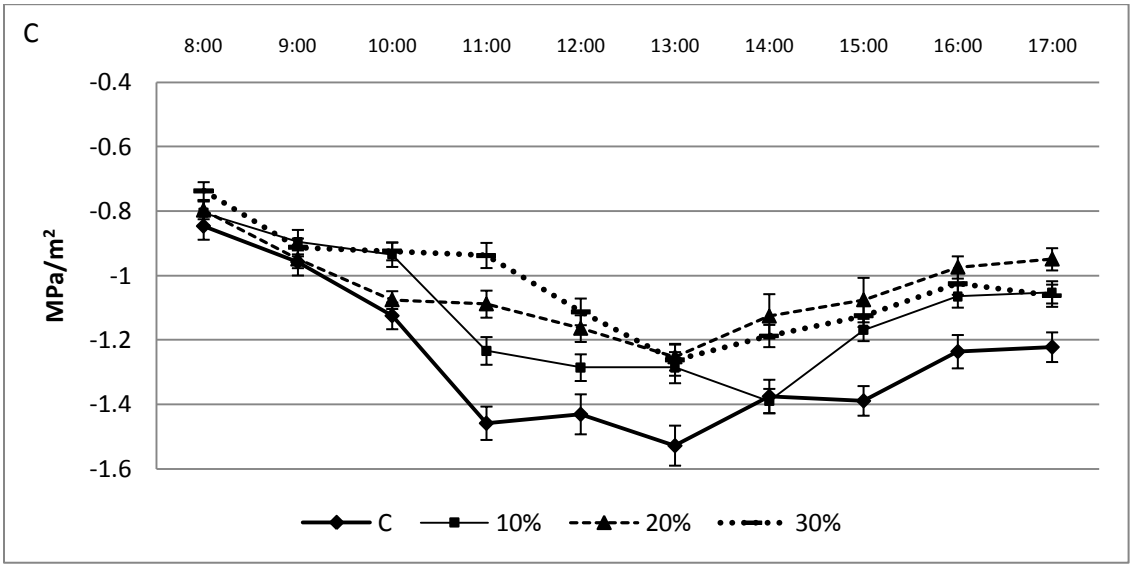


Fig. 4-9 Means of stem water potential per m^2 of leaf area and its standard deviation for the control and the three vermicasting treatments from 8 am to 5 pm of the first (A), second (B) and third (C) day of the experiment.

4.4 Discussion

In conclusion significant differences were observed on growing media physical characteristics, plant growth, yield and stem water potential. Growing media with higher container capacity are able to attain higher volumetric water contents, which could potentially increase the amount of water readily available for plants. Growing media with increased volumetric water contents have been shown to increase yield of vegetables crop. For instance Ismail et al. (2008) showed that increased soil water content resulting from multiple applications produced higher tomato yield. Marouelli and Silva (2007) demonstrated that the number of marketable fruit was linearly increased as the soil water tension decreased. Vermicasting was beneficial for pepper production, but the increment was not linear with the amount of it. Increasing its amount from 10% to 30% influenced growing media physical characteristic, pH, EC more than plant growth parameters. Growers should take into account the strong changes in pH and EC, in particular if its amount is more than 20%, because a different management of water and nutrients is needed. Vermicasting is an interesting growing media also because of its waste recycle origins, it can also be used in organic farming. It presents high bulk density and therefore its use have high shipping costs. Further studies should take into account the influence of vermicasting on growing media's microbiological life.

Stem psychrometer is an interesting tool for research purpose on plant's water relations. Concerning soil-less crop growing it can be used for a better characterization of the effect of physical growing media characteristics on plant's water status. Non destructive continues measurement can drive a better understanding of water management in different fields of soil less agriculture.

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