ZR-ACREO-WP2\_D2.2\_Requirements for the new sensors for shoot portions and for soil-root portions Grant Agreement n° 308313

### **THEME [ENV.2012.6.3-1] Innovative resource efficient technologies, processes and services**



# **ZEPHYR project – Deliverable D2.2**

# **Requirements for the New Sensors for Shoot Portions and for Soil-Root Portions**

Funding scheme: **Collaborative Project**

Project Acronym: **ZEPHYR** 

Project Coordinator: **TUSCIA UNIVERSITY** 

Proposal full title: **Zero-impact innovative technology in forest plant production** 

Grant Agreement n°: **308313** 

Author: **Cristina Rusu**

Summary: **This document provides the basic requirements for the development of new sensors for shoot portions and for soil-root portions. These will be further worked out towards the final design which will be described in D2.3 "Design of the new sensors".** 

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### **1. Scope of the document**

### **1.1 Purpose**

This document provides the basic requirements for the development of the new sensors able to measure growth rate in aboveground- and below-ground plant portions. These requirements will be further evaluated and considered in order to achieve a final design which will be described in D2.3 "Design of the new sensors".

### **1.2 Overview of the document**

Zephyr project aims to introduce an innovative technology to pre-cultivate forest stock to be used for forestation regeneration materials by means of a zero-impact and cost-friendly growth chamber unit. Thus, the project will develop a highly automated production unit able to recycle water and fertilizers and to avoid pesticide use, through a combined action of:

- optimal environmental conditions;
- sensor-monitoring of soil parameters and plant traits;
- automated tray movement to reduce unit dimension and to equalize nursery treatments;

Zephyr project will deliver a family of new specific wireless sensors to detect morphological and physiological seedling traits during their initial development and soil characteristics. These sensors will enable a cost effective control of nursery operations for forest management by allowing precise and punctual interventions (watering, fertilizing, temperature and humidity regulation, etc.) only if and when they are needed, with a considerable reduction of energy and expenses.

The deliverable description continues in Chapter 2, with an Introduction to the current situation (see Section 2.1) regarding sensors utilized at present by our partners in their nurseries. Section 2.2 describes some of the commercially available sensors to be used for measuring soil parameters with a particular focus for those characterized by a small dimension 'mini-plugs' dimensions. Section 2.3 describes some of the commercial sensors to be used for measuring the seedlings traits such as, shoot height / mass, 'greenness' (leaves number, colours, etc.).

Chapter 3 presents an overview of Zephyr project requirements regarding soil and seedlings traits to be measured by the new sensors. In particular, Zephyr project requirements are described in Section 3.1 whereas parameters to be measured and monitor are described in section 3.2.

Chapter 4 describes the general requirements for soil parameter sensors, with first results obtained by simulations presented in Section 4.1 and experiments presented in Section 4.2.

Chapter 5 describes the general requirements necessary for development of sensors able to measure seedlings growth rate by means of above-ground morphological and physiological parameters. In particular, requirements for the choice of optical sensors for detection in visible light are presented in Section 5.1 while those for fluorescence are presented in Section 5.2.

Chapter 6 summarizes the overall requirements for developing the final sensors.

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## **2. Sensors for soil & plant parameters - current status**

### **2.1 Introduction**

Plant growth chambers currently on the market are produced in many places around the world and come in a variety of standard sizes. Reach-in units (growth chambers) start from the dimension of a little refrigerator, while walk-in units (growth rooms) reach a growth area up to 25 square meters. The standard chamber models have climate control e.g. ambient temperature, humidity, light and sometimes CO2. Specific for R&D and/or hydroponic & aeroponic growing systems are produced by only a dozen of companies in Europe, USA $^1$  / Canada and Australia<sup>2</sup>. These make use of big and complex system (Figure 2-1) featuring Nutrient injection, sampling, and control; Photosynthesis and respiration measurement; Realtime pH measurement and control, Transpiration rate measurement, Leaf/canopy temperature measurement and control, etc.



Figure 2-1 (a) Less complex systems for irrigation control using soil moisture sensors; (B) 'Feed on Demand' system is an example of real-time on-line measurement and control of multiple process variables (from [1])

However, specific chambers for pre-cultivation of forest material do not exist on the market; while researchers may use standard products, the end users do not have any option for an actual production.

In regular nurseries / cabinets for pre-cultivation of forest material (Figure 2-2), measurement of soil parameters and evaluation of seedling growth rate are generally done manually and in particular cases it is necessary to send sample to be measured in specific laboratories. For example, the soil temperature is generally measured by means of a normal thermometer placed in the pot or in the growth chamber. In the case of pH and ions concentration measurements these are not normally measured in real-time during permanence of

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<sup>1</sup> http://www.egc.com/prod\_hydroponics.php

<sup>2</sup> http://www.plantaccelerator.org.au/

seedlings in growth chamber but if these parameters are needed then normally a sample of soil is collected and sent to the laboratory to be evaluated. In regard to seedlings morphological traits to evaluate growth rate, normally destructive methods are used with a sub-sample. In this way, it is possible to evaluate dry-weight at different time intervals, or morphometric traits regarding leaves, stem, or roots. In the case that destructive methods are not used then visual inspections and photographs are used in a comparative analysis. Chlorophyll concentration is normally measured after its biochemical extraction, and alternatively an automated measurement is possible by means of expensive and time consuming devices (normally fluorometers).



Figure 2-2. Open-shelf cabinets located in an air-conditioned room for pre-cultivation of forest material (courtesy of Univ. of Thrace)

### **2.2 Commercial sensors for soil parameters**

There are various companies selling sensors for measuring basic soil parameters such as soil humidity (e.g. soil water content, Figure 2-3), temperature, and electrical conductivity.

Soil water content can be determined by

- Direct methods: removal/separation water from soil/substrate matrix by heating/extraction/ replacement by a solvent or chemical reaction. The most common is gravimetric method which is based upon water removal by heating.
- Indirect methods: measuring physical soil/substrate properties (e.g. dielectric constant) dependent upon water content in the substrate:
	- o nuclear techniques (e.g. neutron thermalization, nuclear magnetic resonance, gamma ray attenuation)
	- o electromagnetic techniques:
		- time / frequency / amplitude domain reflectometry (TDR / FDR / ADR)
		- time domain transmission (TDT)
		- phase transmission and ground penetrating radar (GPR)
		- active / passive microwave, electromagnetic induction (EMI),
		- capacitance



Figure 2-3 General schematic of water availability for plants

The drawback of TDR and GPR parameters is the cost of the equipment and the level of skill needed to operate such equipment. The TDR, FDR, TDT sensors are typically not wireless, which limits the range of their possible deployment. The other methodologies mentioned above are really only applicable to large-scale environments because of their costs.

Some of electromagnetic techniques are also utilized for quantitative measurements of nutrient concentration in the soil. The nutrients are taken up in an ionic form and soil electrical conductivity provides clues to the amount of ions dissolved in water (or total dissolved salts, TDS). The electrical conductivity of the bulk soil is a function of both soil water content and the pore water conductivity.

Dielectric constant is a measure of how easy a medium can conduct electrical signal; therefore dry soil presents a lower dielectric constant than moist soil. In the last 10 years, new soil moisture sensors have become available which measure one-parameter only (e.g. volumetric moisture) or multi-parameters (temperature, water content and conductivity) such as GS3 by Decagon<sup>3</sup> (USA), WET-2 by Delta-T Devices<sup>4</sup> (UK), TrimePico32 by Imko<sup>5</sup> (D) (Figure 2-4). Their working principle is based on measuring the apparent dielectric constant of the growing medium. This technique is relatively simple (compared neutron / TDR, etc.) and highly reproducible. These sensors can be connected to data loggers, if required, and can be used as a hand-held sensor for measuring moisture content in the substrate. This facility allows for spot checks of substrate moisture levels throughout a greenhouse, and/or enable sensors to be buried in the underneath soil / substrate for long-term consecutive measurements. These sensors can be interfaced with a greenhouse/nursery climate computerized controller unit and used for controlled irrigation systems automation according to user-supplied set points. However, dielectric sensor performance requires substratespecific calibration because each soil/substrate presents a typical dielectric property.

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<sup>3</sup> http://www.decagon.com

<sup>4</sup> http://www.delta-t.co.uk

<sup>5</sup> http://www.imko.de



Figure 2-4. Soil parameters sensor for R&D labs and greenhouses / nurseries measuring water content, electrical conductivity, temperature; ( a) Decagon GS3 (USA), 93 mm x 24 mm x 65 mm; (b) Delta-T Devices WET-1 (UK), probes 68 mm  $x \oslash \oslash 3$ mm, housing: 55 x 45 x 12mm; (c) Imko TrimePico32 (D), 155 x Ø32mm and probes 110 mm  $x \varnothing$  32mm.





On the consumer market, some soil parameters sensors start to appear, with focus on wireless and autonomy sensors such as the Wi-Fi Plant Sensor from Koubachi AG $^6$  launched in 2012 or the 2013 press release of Parrot Flower Power from Parrot<sup>7</sup> (Figure 2-5).

The commercial sensors have the sensor itself (without electronics and power supply) of big size ( $>10$  cm x 3.2 cm x 0.7 cm) and quite expensive ( $>1000$  euro).

6 http://www.koubachi.com

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<sup>7</sup> http://www.parrot.com/flower-power/

### **2.3 Commercial sensors for above-ground seedling growth parameters**

#### **2.3.1 Introduction**

In the Zephyr project, we are interested in getting information on above-ground seedling growth parameters, as well. The image analysis is normally used to evaluate the relative growth rate (RGR) of seedlings by measuring the following morphological and physiological parameters:

- 1. Shoot height at different time-intervals (named RSGR);
- 2. Variation of the total leaf area versus soil at different time-intervals (named RPGAVS);
- 3. Variation of chlorophyll content by fluorescence at different time-intervals (named RChF);
- 4. Variation of (oxidative phosphorylation) NADH fluorescence at different time-intervals (named RMiMet)

The focus is primarily on the first three main parameters, while the fourth one is ' a nice to have' parameter.

#### **2.3.2 Image analysis - commercial software**

Plant development can be monitored over time by using non-destructive screening by means of image acquisition techniques. During such screening different images of each plant are recorded that are analysed by applying sophisticated image analysis algorithms.

Image analysis is the extraction of meaningful information from images; mainly from digital images by means of digital image processing techniques. Image analysis tasks can be as simple as reading bar coded tags or as sophisticated as identifying a person from their face.

A large number of commercial software for image analysis is available on the market. These are typical developed for specific areas such as:

- Medicine; such as detecting cancer in an MRI scan
- Microscopy; such as counting the germs in a swab
- Materials science; such as determining cracks in weld
- Security; such as detecting a person's eye colour
- Etc.

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It also exist some dedicated software or plug-ins to open-source software for analysis of plants, which are presented below. On the other hand, most of the image analysis which needs to be done could be done with basic image analysis tools which are available in all image analysis software. For instance, Matlab together with its image processing could be used as a toolbox or the open-source image processing software, ImageJ.

• HTPheno<sup>8</sup> is an image analysis pipeline for high-throughput plant phenotyping. It is implemented as a plugin for ImageJ, an open source image processing software. It provides the possibility to analyse colour images of plants which are taken in two different views (top view and side view) during a screening. Within the analysis different phenotypical parameters for each plant such as height, width and projected shoot area

<sup>&</sup>lt;sup>8</sup> Hartmann et al. BMC Bioinformatics 2011, 12:148, <u>http://www.biomedcentral.com/1471-2105/12/148</u>

of the plants are calculated for the duration of the screening. HTPheno is applied to analyse two barley cultivars.

- PlantScan<sup>9</sup> is the next generation phenotyping platform to capture information on plant structure and function on an industrial scale enabling the discovery of new traits and the selection of varieties for tomorrow's agriculture. The PlantScan software is quasiopen source (requires written approval from the Australian Plant Phenomics Facility). They use hardware from LemnaTec a scan analyzer.
- LemnaTec<sup>10</sup> scan analyser for automated imaging: they use conveyer belts for moving the plants into measurement cabinets for visible, near infer-red and fluorescence analyse. In this way, all images have the same conditions, such as background, illumination, etc. (Figure 2-6). For small seedlings, a scanner moves on top of the seedlings for imaging (Figure 2-7).



Figure 2-6. Automated imaging system for big plants (LemnaTech).



Figure 2-7. Automated scanner for small plants and seedlings (LemnaTec).

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<sup>9</sup> http://www.plantphenomics.org/node/157

<sup>&</sup>lt;sup>10</sup> http://www.lemnatec.com/about-us

• IMAGING-PAM M-Series: portable Chlorophyll Fluorometer



- Assess 2.0: Image Analysis Software for Plant Disease Quantification is an image analysis software for quick and easy quantifying of disease symptoms on plants. Rapid measurement of leaf area, percent disease, root length, lesion count, percent ground cover, and applications could be done with this software. Automatic measurement (user-independent) is possible of many plant diseases and ground cover, to make disease quantification fast, easy, reliable, and reproducible. The software can also be used as an interactive laboratory tool for real-time length and area measurements of anything that can be imaged (scanned, digital photographs, microscopy, etc.).
- The LEAF GUI software is designed for plant biologists and ecologists who wish to analyse the macroscopic structure of veins in leaves. The software allows users to extract descriptive statistics on the dimensions and positions of leaf veins and the areoles they surround by following a series of threshold, cleaning and segmentation algorithms given images of leaves where veins have been enhanced relative to the background. The LEAF GUI was designed to be free for academic use.
- Qubit Systems<sup>11</sup> offers a wide range of instruments for research and teaching in plant and soil biology, e.g Z210 Transect FluorCam. Their instrumentation are sophisticated fluorimeters, quite large and expensive, hard to miniaturized and integrate in our project. Thier software comes with the instrumentation.

 $\overline{a}$ <sup>11</sup> http://qubitsystems.com/featured/plant-soil/



Z210 Transect FluorCam from Qubit Systems

Most of the quasi / open source software makes use of relatively simple tools, and we may use it under some of the following conditions:

- · It processes one plant at a time, using a conveyor belt.
- · It has very strict calibration requirements, such as lighting and background (idealized conditions)
- · It requires unchanging parts of the plant background, such as the colour of the plant holder.

Our approach, of stereoscopic analysis that is described in Chapter 5, is meant to be of much smaller size, more flexible and circumvent some of the above restrictions.

### **3. Zephyr sensors - general**

### **3.1 Introduction**

A schematic of the Zephyr working principle is shown in Figure 3-1 and Figure 3-2. Figure 3-1 shows seedlings growing in 'mini-plugs' trays upon a multiple-floor rotating system and Figure 3-2 shows how soil parameters and morphological/physiological growth parameters will be measured by sensors and sent to a control system for optimization of seedling growth performance.



Figure 3-1. Image of a production prototype based on a multiple floor production unit for the precultivation of forest seedlings, adopting a technology for automated movement of trays with "miniplugs" in a controlled environment

The new design of the soil parameters sensors allows for efficient integration into the wireless network (WN) such that the environmental impact is minimum during manufacturing (e.g. electrical cables). In addition dedicated data analysis gives the ability to diagnose and quantify the different parameters in real-time and become automatic. In this way, the water amount, nutrients and light will be effectively used according to the need. Pesticides will be fully avoided through a combined action of the optimal environmental conditions, the movement of the trays and the use of a bactericide lamp in the recycled water, as already experimented in the previous Pre-Forest project.

For any new technology to penetrate the market place it either must be significantly less expensive than the existing technology or it must have additional features that provide a competitive advantage and justify the same cost as the technology to be replaced.

Advantages of Wireless Sensors

− Much cheaper to deploy than wired sensors

− The number and position of sensors can be modified easily, depending upon cultivation protocol

− No rewiring is needed during variation of sensor arrangement.

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Within Zephyr, the water will be recycled within the system unit so water being initially rich in salts (especially if tap water is used) will become progressively poorer in consequence of seedling absorption (after a cycle of irrigation). The system unit will add salts in order to replace optimal values of salt concentrations and to reuse the same water with the correct concentration of microelements and minerals. Moreover, essential for optimal seedling growth is not only the occurrence in water of minimum salt concentration but also the avoidance that maximum salt concentration is reached because too much salts or minerals can have a toxic effect upon seedlings. The sensor to be applied to the system unit will help to maintain salt at their optimal concentration.



Figure 3-2. Sketch of the wireless soil parameters stick sensors and the closed loop information system for the water amount and nutrients.

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The general idea is to use within Zephyr project both the new non-destructive methods and the old methods in order to make a comparison and to confirm the efficiency of your wireless or non-wireless sensors.

### **3.2 Parameters of interest**

### **3.2.1 Sensors parameters**

The most important parameters to measure during the 5-6 weeks when seeds germinate and young seedlings form are:

- > below-ground soil parameters measured by the 'sensor stick':
	- soil humidity ,
	- wetness / porosity of the soil
	- ions concentration: the total concentration (first priority), and for each ions (second priority).
- > Above-ground seedlings parameters measured by optical camera (not to be placed on 'sensor stick'):
	- 'Greenness': as an indirect measurement of seedling growth (the most important parameter to be evaluated as first step)
	- Seedlings height, biomass
	- fluorescence measurement (feasibility to be evaluated later on):
		- o amount of chlorophyll as indicator of leaf-biomass increase
			- o oxidative phosphorylation (NADH) as indicator of optimal physiological activity.

Image analysis will be used for estimation of green biomass increase in order to follow the growth of seedlings (including shoot growth). This will allow inter-, as well as intra-detection of tray variation in growth that can indicate uneven growth conditions or differences in genetic background of seeds

#### **3.2.2 Sensor stick**

- $\triangleright$  Number of 'sensor stick': total of 4 6, which corresponds to 1 sensor stick to ca. 30 pots.
- $\triangleright$  The 'sensor stick' size will be of about 10 mm wide x 2 mm thick x 50 mm long

### **3.2.3 Data reading & communication**

- > Measurements will be collected 2 3 times in 24 hours.
- > Two reading & communication methods will be considered
	- active (with battery) via '802' communication method passive (first priority)
	- (RFID-based) method using a mobile Reader (which sends energy to the 'sensor stick' and reads the sensors' data (second priority)

For the first trials, flexible connectors will be used to connect to the 'sensor stick' to the communication module (Figure 4-17) because at the present the size of the data reading & communication unit is too big compared to pot dimension. The data reading & communication unit will be placed in a pot adjacent to that containing the 'sensor stick'.

#### **3.2.4 Jiffy soil parameters**

After discussion among all partners, it has been decided to use the following soilless substrate: JIFFY Preforma.

Preforma is a peat based propagation material designed to work with both seed and cutting based crops. It allows the crop to grow at an optimal rate with minimal plant losses.

The following table summarizes Preforma main physical and hydraulic properties; general information about physical and hydraulic properties of soilless substrates is provided in chapter 4, sections 4.1 and 4.2.

#### **Table 1. Information provided by the manufacturer**

**Results Physical research** 



Cocopeat Peat



#### Data at different pressure heights:





### **4. Sensors for soil parameters - Requirements**

### **4.1 Advantages of soilless growing media**

Overall profitability of intensive crops (especially greenhouse), grown in soilless substrates, is higher than overall profitability obtained by intensive crops grown in soil. This is due to the following aspects:

- their superior physical and chemical properties;
- their initial low infestation rate with pathogenic pests;
- their ease of disinfestations among growing cycles.

The result is a worldwide rapid expansion of the use of such substrates during the last decades. The most important physical requirements for container media in order to achieve optimal growth are:

- high content of available water:
- an adequate air supply.

Water availability for plant roots is strongly related to the hydraulic conductivity characteristics of the medium. In porous materials, for example, it drops dramatically with reduced water content.

The main advantage of soilless over soil cultivation is its ability to provide simultaneously sufficient levels of oxygen and water to the roots. The physical properties of porous substrates are more suitable than those of soils used for the production of most horticultural crops. Much weaker matric forces in substrates, compared to soil, hold the water. Consequently, plants grown in porous media require less energy to extract water than those grown in soil and have a lower risk of oxygen deficiency. The above mentioned factors lead to improved yields in terms of quality and quantity.

Most media-grown plants are grown in greenhouses under supposedly near-optimal production conditions. An inherent drawback of soilless vs. soil cultivation is the fact that in the latter the root volume is unrestricted while in in-container cultivation the root volume is restricted. This restricted volume has several important effects, especially a limited supply of nutrients. The limited root volume also increases root-to-root competition since there are more roots per unit volume of medium.

### **4.1.1 Physical and hydraulic properties of soilless substrates**

Bulk density (BD); it is defined as the dry mass per unit of volume (in a moist status). As many media are composed of more than one ingredient, the characteristics of each ingredient contribute to the total BD. In particular, media components that differ significantly in particle sizes have higher BDs as a mix. Similarly, they have lower total porosity (TP), water-holding capacity and air-filled porosity (AFP) than media composed of similar particle sizes.

Total porosity (TP): TP and its components are expressed as a percentage of the total volume of the medium. The combined volume of the aqueous and the gaseous phases of the medium are defined as its *total pore space* or *total porosity*. TP is related to the shape, size and arrangement of media particles. It can be divided into air-filled porosity and water-

holding capacity. The volumetric amount of water, θ, which saturates a given volume of a substrate, is defined as its *effective pore space*. The difference between total pore space and effective pore space constitutes the volume of closed pores not accessible to water.

Air-filled porosity **(**AFP): it is defined as the volumetric percentage of the medium filled with air at the end of the free gravitational drainage. This value varies greatly in accordance with the height and shape of the container, so it usually determines that AFP is the volumetric percentage occupied by air at a pressure head of 10 cm (or water suction of 1 kPa). Most media and mixes have an AFP of 10% to 30%. Optimal AFP may vary greatly according to the size of the container and the irrigation frequency.

Container capacity (CC): it is defined as the amount of water which remains in the container after water has stopped draining.

Easy available water (EAW): EAW in a medium is defined as the difference in water content between *container capacity* (usually defined at a water suction of 1 kPa) and water content at 5 kPa. Scientific horticultural literature, suggests that volumetric EAW should be in the range of 20-30%.

Water buffering capacity: it is defined as the water content between 5 kPa and 10 kPa. Water held by media at tensions higher than 10 kPa is usually considered as unavailable. The capacity of a medium to store water and air, as well as its ability to provide them to the plant (via its hydraulic conductivity and rate of gas exchange) are determined by its TP and porosity characteristics, namely pore size distribution, tortuosity and pore continuity. Water is mainly held by the micropore space of a growth medium, while rapid drainage and air entry is facilitated by macropores. Therefore, an adequate distribution of large and small pores is essential for a good medium. Since pore size and distribution determine the rate of water drainage and gas exchange, these factors are critical in defining a growing medium with optimum physical characteristics.

Most soilless growth media contain 60% to 90% total pore space.

Hydraulic conductivity: the conductive properties of unsaturated media are markedly dependent on their texture and structure. At saturation, all pores are water-filled and conductive. The most conductive media are those in which large and continuous pores constitute most of the overall pore volume, whereas the least conductive are media in which the pore volume consists of numerous micropores. When the growing medium de-saturates, some of the pores become air-filled so that the conductive portion of the cross-sectional area diminishes. Furthermore, as suction develops, the largest pores are the first to empty and become nonconductive. Thus, a steep decrease in the initially high hydraulic conductivity is expected in soilless container media with high percentage of large pores.

Water retention curve: the relationship of soil water content to water suction is of fundamental importance to understand soil water status and water availability to the plants. Owing to the characteristic texture of container media and soil substrates, unsaturated conditions occur soon after - or even during - irrigation. Therefore, the water holding capacity of the media at different suctions is a vital tool to analyse water flow, water availability and irrigation management.

Water availability to roots is largely determined by how tightly water is held by the solid phase of the medium. The closer a water molecule approaches a solid, the more tightly it is held through the forces of adhesion and cohesion. The water suction combines adhesion forces between solid soil surfaces and water and cohesion forces among water molecules. It

can be measured using a tensiometer. Water suction is measured as a pressure signal according to the definition of the SI-unit system, so it can be expressed in different units (Table 2).



### **Table 2. Various soil parameters and their measurements units**

pF is the base 10 logarithm of the water potential in cm and frequently water retention curves are presented as pF curves.

When suction is applied incrementally to a saturated soil, the first pores to be emptied are the relatively large ones, unable to retain water against the suction applied. A gradual increase in suction will result in the emptying of progressively smaller pores, until, at high suction values, only the very narrow pores retain water. Similarly, an increase in media-water suction is associated with decreasing thickness of the hydration envelopes adsorbed to the soil particle surfaces. Increasing suction is thus associated with decreasing media wetness.

The amount of water remaining in the media at equilibrium is a function of the size and volume of the water-filled pores and the amount of water adsorbed to the particles; hence it is a function of water suction.

This function is measured experimentally and is represented graphically by a curve called the moisture retention curve (RC), also known as the moisture release curve or the mediamoisture characteristic.

Owing to its unique texture, the retention curve of a container medium is usually determined for very low suctions: -10 cm (pF 1.0), -20 cm (pF 1.3), -32 cm (pF 1.5), -50 cm (pF 1.7) and - 100 cm (pF 2.0), rarely lower than -100 cm (pF 2.0) as already this condition is very dry for plants in greenhouse conditions.

Retention curves of container growing media have an intrinsically different shape than field soils, mainly due to the low suction range that usually exists in container growing media.

The rate of moisture content decrease per unit suction increase differs significantly between container media and soils, being steeper in the former. The moisture content decrease occurs over a very narrow suction range (up to 25 cm) and subsequently reaches a constant value.

#### **4.1.2 Jiffy Preforma hydraulic properties**

According to Preforma pF curve provided by Jiffy (Table 1), this substrate is characterized by 5% of the total volume represented by dry matter and 95% by pores. When the pores are totally filled with water the substrate reaches the "saturation point" (95% water 0% air). Gravity will pull some of this water down through the soil below the crop's root zone. The water redistributed below the root zone because of the force of gravity is called gravitational water. In general, gravitational water is not available to plants, because the redistribution process occurs quickly (in two days or less). After the redistribution process is complete, the substrate contains the greatest amount of water potentially available to plants, the so-called "easy available water (EAW)".

Preforma soil EAW is about 10%: water content at 1 kPa  $(-10cm)$ – water content at 5 kPa  $(-50cm)$  = 52% - 42%

As EAW corresponds to half of total available water, it is possible to conclude that plants are able to easily absorb water from Preforma plugs until they reach moisture of  $\sim$  32%. When all the total available water has been used, the plants cannot easily extract further water. This stage is generally considered as the time to irrigate.

### **4.2 Analysis of water retention properties of Preforma soil**

In order to establish watering frequencies and quantities to apply in the growth chamber, a test has been carried out by the Unitus working group (DAFNE Department) to analyze water retention properties of Preforma soil, under LED or FLUO lamps, without growing plants.

In absence of growing plants, daily loss of water (corresponding to fresh weight loss) in the soil is caused only by evaporation. So, in order to evaluate daily evaporation of soil plugs exposed to LED/fluorescent lamps in the growth chamber, fresh weight of 12 saturated ( $\approx$ 80% of moisture) Jiffy's soil plugs (27 cc) have been recorded at time 0 and after  $3 - 5 6 - 7 - 10$  days.



Growth chamber parameters:

#### **Results:**



#### **Table 3: Fresh weight variation**



Figure 4-1. Variation of average fresh weight of plugs under fluorescent lamp and LED



### **Table 4: Average volume % water variation**

\*average air dry weight of a plug (27cc): 2,5 g



Figure 4-2. Variation of average water content under fluorescent lamp and LED

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### **Interpretation of data**

According to Jiffy's pF curve, Preforma substrate shows a very narrow EAW range.



At 10 cm pressure height, the substrate contains 52% of water while at 50 cm pressure height it contains 42% of water. The EAW for this material is 10% (52% - 42%). This means there is 100 ml of water available per litre /plug (2,7mL of water per 27cc pot).

For a good distribution of moisture in the entire pot, it is important that water permeability is high. If it is high, the water will be quickly transported to all the parts of the pot thus creating homogenous moisture content. The water permeability is high if the pot is wet, but, as it gets dryer, the difference between the different potting soil become more clear. Some materials are easy to re-wet (sand, pumice and coco grinds), others are not (peat, as Jiffy's soil). The main reason is that peat hardly takes up water after it has dried. Moreover, relevant differences within the same group of peat have been noticed, i.e. mostly, light peats are less sensitive then the dark peats.

According to above mentioned data:

- Starting with a seeded pot with almost 50% of volume water, we have observed a fast decrease in available water content in 2 days (EAW is ½ of total available water, whose range varies from  $\sim$  50% to  $\sim$ 30% volume water).
- The fast decrease in moisture content lasts a few days, until plug reaches  $\frac{1}{2}$  of its initial fresh weight  $($   $\sim$  after 5 days of free evaporation). Remaining water is strongly retained, probably thanks to the great amount of starch, and daily fresh weight change becomes lower. The starch polymer is useful to stabilize soil mixture and it is a valid tool for an effective water management for all aspects of the horticultural market. The polymer will absorb over two hundred times its weight in tap water.

When applied at the recommended application rates, the polymer can reduce plant watering by 50%.

- To keep soil moisture constant in EAW range, avoiding saturation and dry suboptimal condition, an irrigation protocol based on small amounts of water added frequently to plugs is required  $(2.7 \text{ ml of water per pot when soil moisture is about}$  $40\%$ ).
- The faster loss in water showed by plugs exposed to LED lamps than the loss in water of those exposed to Fluorescent lamps is only due to the presence of an independent aeration system (based on fans) for each shelf, which increases the speed of the drying process, especially in upper layers of soil.

### **4.3 Simulations for detection method**

We have chosen to focus on impedance<sup>12</sup> sensors, which are relatively cheap, rugged, portable and can be easily connected to newly developed data-loggers able of wireless data transmission. Moreover, as miniaturization of sensors electrodes and electronics is also possible, impedance sensors are ideally suited to measuring substrate moisture in small containers.

Preliminary simulations have been performed –using COMSOL Multiphysics. The simulations were set up according to:

- 2D geometry (out-of-plane direction = electrode length direction)
- 4 different electrode configurations
- Electrostatic analysis  $(DC)$  To study effect of water content
- Frequency analysis  $(AC)$  To study effect of conductivity in soil and water content

and such that by finding the most suitable working frequency range, the design of the sensors and power consumption can be assessed.

The permittivity of the soil surrounding the electrodes was simulated as a mixture of the permittivity of water,  $\varepsilon_{water}$ , and the permittivity of the soil particles (soil matrix),  $\varepsilon_{soil\ particle} = 3$ , according to the following relation:

$$
\varepsilon_{\text{solid}} = 3(1 - \alpha) + \alpha \varepsilon_{\text{water}} , \quad \alpha \in [0, 1]
$$

where α factor describing the water content, between 0 and 1.

For the glass (quartz) substrate the permittivity was set to 4.2.

The results from one of the simulations are presented in Figure 4-3 and Figure 4-4 with the following parameters, utilized also for all other simulations:

- **Electrodes**: Cu, dimensions 3 x 0.200 mm,
- **Soil**:  $\varepsilon_{\text{solid}} = 3(1 \alpha) + \alpha \varepsilon_{\text{water}}$ , where  $\alpha = [0 1]$ ;  $\sigma_{\text{solid}} = [0.1 1]$
- **Substrate**: (Quartz glass) Dim. 15 x 2 mm,  $\varepsilon_{\text{quartz}} = 4.2$ ,  $\sigma = 10^{-14}$  [S/m]
- **Length**: 50 mm (out-of-plane)

 $\ddot{\phantom{a}}$ 

 $12$  J.R Macdonald, Impedance speciroscopy and its use in analyzing the steady-state ac response of solid and liquid electrolytes, J. Electroanal. Chem, 223 (1987) 25-50



Figure 4-3. Geometrical layout (left) and electrical field from Comsol simulation (right) of two metal, flat electrodes on quartz substrate into soil



Figure 4-4. DC-analysis: Capacitance vs. water content for the electrode geometry from Figure 4-1.



Figure 4-5. AC-analysis: Water content in soil ( $\alpha$ ) for electrodes geometry from Figure 4-3To the left: Impedance (ohm) between the electrodes as a function of frequency. To the right: Phase (rad) as a function of frequency. The different curves represent different values of α, blue: α=0.1, pink: α=1.



Figure 4-6. AC-analysis: Conductivity in soil (σ) for electrode geometry from Figure 4-3; To the left: Impedance (ohm) between the electrodes as a function of frequency. To the right: Phase (rad) as a function of frequency. The different curves represent different values of the conductivity, blue: σ=0.1, pink:  $\sigma = 1$ .

All other tested geometries as in Figure 4-7 and Figure 4-8 show similar behaviour as in Figure 4-4, Figure 4-5 and Figure 4-6. The electrode configuration seems to only have minor effects on the initial value of the capacitance and the capacitance shifts (water sensitivity).

We have quickly looked also into:

- effects of parasitic capacitance due to substrate thickness (Figure 4-7)
- effects of isolation layer on top of the metal electrodes (e.g. electrical insulation to protect the metal from corrosive effects, or /and air layer due to not good contact between soil and electrode substrate)

The substrate and isolation layer will add parasitic capacitance which will reduced relative signal shifts and thus sensors sensitivity.



Figure 4-7. Various substrate thicknesses

The best sensitivity for water content has been seen when only two metal electrodes as circular wires were placed in the soil instead of plate electrodes (Figure 4-8e). Configurations with electrodes facing each other (Figure 4-8c) show a slightly better sensitivity compared to the configuration where both electrodes pointing upwards (Figure 4-8a).

As can be seen in the impedance plots the soil conductivity can be measured at low frequencies, where the circuit is more or less resistive. To measure the water content we have to go up to higher frequencies, typically above 1 MHz or higher. This is also confirmed in the real measurements.





Figure 4-8. Three other tested geometries together with their resulting electric potential and electric field lines from COMSOL.

We also believe that changing the electrode area / length will not affect the cut-off frequency (where impedance has dropped a factor of  $\sqrt{2}$ ),  $\sqrt{c} \frac{2\pi RC}{2\pi RC}$ , because it depends on resistance and capacitance, which both will change due to the geometry and the effect will be cancelled out . This means that it is mainly the soil properties, dielectric constant and electrical permittivity, which plays an important role.

Page *26* of *44* It can be seen from all simulations that a big distance between electrodes is needed in order to have information of the water content in the soil; with other words to have information on the soil bulk. Interdigital electrodes configuration is not a real option because the distance

between the finger electrodes is too small meaning that it will be a local measurement (eventually only air is measured) and not a bulk characteristic of the soil.

### **4.4 First measurements for detection technique**

### **4.4.1 Preliminary testing**

For quick, preliminary tests two sizes of PCB strips with Cu on one side were manufactured for 50 x 3 mm in soil at ca. 40 mm distance and 50 x 10 mm in soil at ca. 10 mm distance. Impedance between these electrodes was measured with HP LCR meter and Network Analyzer, respectively (Figure 4-9) by sweeping the frequency between 20 Hz to 1 MHz for LCR meter and from 1 kHz up to 100MHz for network analyzer. Standard potting soil was utilized.



Figure 4-9. Photo of measurement set-up, baker with soil and Cu electrodes on PCB substrate and network analyser.

The impedance changes its values depending on the amount of water into the soil, as shown in Figure 4-10. It can be clearly seen how the impedance amplitude, abs(Z), changes with amount of water (below 10<sup>7</sup> Hz). For the impedance phase, above 10<sup>7</sup> Hz, the signal changes with water amount. The more water into soil, more ions from the soil dissolve into the water and thus the impedance signal shows contribution from both water amount and conductivity, as predicted by simulation. However, it is not easy to separate the two effects.

### **4.4.2 Impedance at high and low water content**

These measurements are intend to give a better understanding of

- To determine the influence of the soil on the sensor response
- To establish whether high frequency measurements are reliable
- Responses at extreme ionic strengths (high conductivity)



**Zephyr magnitude Impedans**

Figure 4-10. Impedance behaviour dependence on the amount of water into the soil.

#### Methods and measurements

- Use MilliQ water and two buffers with mixtures of sodium phosphate and di-sodium phosphate at pH=6.85 (slight adjustment with NaOH) having ionic strengths of 0.1M (Buf1) and 1M (Buf2), respectively.

- Conductivity measured using commercial conductometer from Radiometer; Sensor responses measured using HP analyzer.

- Soil was ordinary soil, pretty dry at start.

Measured 3 different soil samples and fluids without soil, respectively.

The soil volume was 150mL in all three cases. Weighing soil samples allowed us to determine effective soil density,  $\rho_{\text{eff}} \approx 0.32 \pm 0.03$  g/mL.

When mixing soil with the three fluids added 10 mL of fluid and measured; then added to a total volume of fluid = 30mL and measured, and finally added to a total fluid volume of 50mL and measured. 50mL resulted in a very wet soil.

Next day the three soil samples were centrifuged to elute the fluid.

Conductivities: MilliQ ≈ 0.07mS/cm (21 °C); Buf1 ≈ 10mS/cm (19 °C); Buf2 ≈ 51mS/cm (21 °C)

### Results & short discussion



Figure 4-11. Amplitude and phase of the dry samples.

The data from Figure 4-11 is meant to illustrate two main issues:

- the contribution of the "semi"-dry soil to the signal, and, more importantly,
- typical spread between the 3 different samples of one and the same soil is relatively small, i.e., effects of variations in ,  $\rho_{\text{eff}}$  are small.

Note that the signal amplitude at low frequency is here much higher than what is the case after liquid addition,  $\approx 6$  K $\Omega$ . This can have to do with the fact that the effective contact area of the "dry soil" grains and the electrode is small, or it can also be due to a finite resistance between the soil grains and the electrode, or both. Two other possibilities include (i) small conductivity of the soil itself, and (ii) low connectivity (long resistive path) between the grains at these low packing densities.

Experiments on the soil response at different packing densities can help to partly resolve this issue by helping to determine which of the possibilities the most plausible one is.



Figure 4-12. Response from the soil soaked with milliQ and buffers, respectively, at two different liquid doses. Note that at the highest MilliQ level there is a feature resembling a resonance as shown in Figure 4-13.

Note that the absolute response magnitude in pure liquids is not too different from the responses of samples with soil, at least when proportion of liquid to soil is 1:3 (v/v), i.e., at high liquid additions. This suggests that initially the soil influence was negligible, at least for measurements immediately after liquid addition as was the case here. Note also that MilliQ response results in a sharp resonance, Figure 4-13, at about 33 MHz. The resonance smudges out and shifts to a slightly lower frequency in a buffer. Similar trends are seen in

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Figure 4-12, at least at high liquid content. Note that soil addition shifts resonance due to milliQ and the responses from buffers as well to higher frequencies.



Figure 4-13. Electrode responses from liquids without any soil. Comparison between as prepared liquids and those eluted after 24h in soil.

After elution liquids behave differently. For example, both buffers have **higher** amplitude at low frequencies compared to as prepared fluids. That indicates lower conductivities, i.e., ion transfer from **buffer** to the soil. MilliQ on the other hand contains a lot of ions transported from the **soil** to the liquid which decreased its amplitude and increase its conductivity. This is also mirrored by the conductivity data as measured using commercial conductometer: eluted

MilliQ ≈ 2,75 mS/cm (instead of 0.07mS/cm) while Buf1 ≈ 5,20 mS/cm (instead of 10mS/cm) and Buf2  $\approx$  33.60 mS/cm (instead of 48 mS/cm)

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Finally the data in Figures 4-11 to 4-13 suggests that measurements at high frequencies are still reliable.

### **4.5 Substrate and electrodes for sensors for soil parameters**

As seen in section 4.3 Simulations, the sensors mainly consists of metal electrodes (e.g. Figure 4-3), insulator substrate and of course electronics. The suitable material combination for the electrodes and substrate is briefly described in this section.

Gold, silver and platinum are metals of choice in electrochemical and impedance measurements in liquids due to their well-studied and known behaviour, thus less 'influence' on the signal.

Printing tests of silver and gold structures on glass and ceramic substrates were carried out in order to investigate the application process and the material properties. Goal is to fabricate high-purity metal structures with good adhesion to the substrate and good electrical conductivity.

Gold structures were produced using inkjet- and aerosol printing (Figure 4-14). Nanoscaled gold dispensions (so-called "functional gold inks") were deposited without using any masks on glass and alumina  $(A_2O_3)$  substrates with different porosity and partly coating. After printing the test structures were activated using oven sintering processes at 400°C.

The printed and sintered test structures were investigated regarding electrical conductivity and structure morphology.



Figure 4-14. Aerosol printed gold test structure (1x1 mm) on glass substrate (left) Inkjet printed and sintered test structure (1x1 mm) on Al2O3 substrate (right)

Silver structures were applied using screen printing technology. Silver powder of micrometrescale was mixed-up with a suitable binder to a viscous paste. By usage of a screen the paste was applied on quartz glass and different alumina substrates. Following the printing process, the test structures were heat-treated at temperatures from 825-915°C under nitrogen or a nitrogen / hydrogen mixture. Figure 4-15 shows different screen printed silver structures after printing.



Figure 4-15. Enlargement of screen printed silver test structure on porous Al2O3 substrate (left) and dense Al2O3 substrate (right) after printing.

Because of differences in the fabrication routes of the silver and gold structures as well as to guarantee maximum flexibility concerning the choice of substrate types and their arrangement, a new design of the sensor stick is proposed (see Figure 4-16).



Figure 4-16. Flexible sensor stick - new proposed design

Single rods of the same or different materials are now used as substrates. The electrodes will be printed on these rods and heat-treated. Afterwards the rods are arranged and will be fixed with a non-conductive resin. So different substrates can mixed in on device and possible add-on functions / sensors could be integrated in further development steps.

### **4.6 Electronics for sensors for soil parameters**

To determine water amount and conductivity in the soil an electronic circuit must be designed, that is able to measure the complex impedance of the soil. This section gives an overview of possible circuit topologies. While some circuits are more flexible, others are more suited for miniaturization.

The first design and testing for the sensor interface electronics and communication modules is sketched in Figure 4-17. The improved design will be obtained by miniaturizing the sensor electronics module and the communication module to be inserted both on the sensor stick. The final result will be to have all on the same stick integrated sensor/s, plus electronics, and plus communication.



Figure 4-17. Schematic of the first sensor stick with its electronics and communication modules

All the preliminary simulations and measurements have been done on 'standard' soil (potting soil). The soil for Zephy project is peat, that is not a soil and Preforma peat is not a simple peat; it is rich in nutrients so that our cultures are not traditional cultures.

Therefore more tests will be done for deciding the best electronic scheme from the following alternatives, spectrum vector analyser, Single frequency vector analyser, Multiple frequency scalar analyser, LC-oscillator.

### Spectrum vector analyser

A circuit like this makes it possible to analyze any complex impedance over a large frequency range. Very detailed information can be retrieved, but the circuit is not easily miniaturized to fit on a sensor stick.

There is on the market a chip from Analog Devices containing a complete spectrum impedance analyzer. The frequency range is however limited to 100 kHz and the chip is because of this most likely not suitable for Zephyr.



Single frequency vector analyser



This circuit will allow measurement of the complex impedance at one single frequency. The frequency has to be well chosen in respect to the measured object. In the Zephyr case the system must be calibrated for the specific soil type. There may be soils that cannot be measured without changing the operation frequency. This circuit can be miniaturized, maybe to the degree that it fits on a sensor stick.

Multiple frequency scalar analyser



This simple circuit does not measure complex impedance. However, knowing the physics of the measured object, it may still be possible to extract the required parameters. It is not yet

known if this circuit fulfills the needs of the Zephyr application, but it is clearly very suitable for miniaturization.

A design based on this circuit could be based on filtered square wave oscillators. These can be found in 3x3 mm packages. Microcontrollers with 16-bit Analog-to-Digital converters can be found in 4x4 mm packages. It is thus likely that a design of this circuit could be made to fit on a sensor stick.

LC-oscillator



An oscillator circuit is formed by connecting an inductor in parallel with the measured object. The circuit's resonant frequency is dependent on the detected capacitance. This circuit may be very small but a circuit for measuring the conductivity must be added. The losses in the soil might make the resonance so flat that stable oscillation cannot be achieved.

## **5. Plant growth sensors requirements**

### **5.1 Image processing**

### **5.1.1 Extraction of "plant green"**

In this section, we discuss a simplified method to extract the green colors related to plants in a digital photo. It is based on hue-saturation-value (HSV) analysis of the image data, The repeatability of lighting conditions is rather important, and the light source should not be predominantly green; preferably a bluish light would help increase the contrast between grayish background and the green plants. An example is shown in Figure 5-1, and the result from the digital filtering process is shown in Figure 5-2.



Figure 5-1 An arbitrary scene with a tray of plant shoots.



Figure 5-2 Green information extracted from the same scene.

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### **5.1.2 Basic stereographic analysis**

A computer-generated scene containing various artificial objects can be recorded stereographically using a ray-tracing software. This provides for idealized lighting, optics, camera sensor response, alignment, simultaneity, etc.

Figure 5-3 represents such an ideal pair of images; they are placed in such a way so that if the right eye of the observer looks at the left-hand image, and vice versa, the depth of the scene becomes apparent to the viewer (cross-eye method). We would like to build an algorithm that extracts the depth information from the original pair of images by correlating corresponding pixels.



Figure 5-3. A pair of digital images generated by a computer.



Figure 5-4. Extracted depth information. The deep-red represents the background of the scene, while the blue represents the foremost object.

The result from the correlation algorithm will provide a depth-map whereby each pixel will be assigned a number. A new image pair represents the color-coded form of this mapping in Figure 5-4; it can also be viewed using the cross-eye method.

### **5.1.3 Single-camera tests**

The initial tests were performed using a single camera which was moved to the side by 15 mm in order to produce the pair of images in Figure 5-5, Figure 5-6 and Figure 5-7 .



Figure 5-5. Original scene at a distance of 1m from the camera.



Figure 5-6. Green plant information.



Figure 5-7. Depth map of the green pixels only.

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It is important to reduce the "green" in the background, so that the information regarding the plants is properly extracted. This suggests that the light source should not be predominantly green (everything would appear to be a plant to the algorithm).

We would expect that the "back" portion of the scene will not be of interest since we would like to extract the tip of the plants, observed from above. Such tests have not been performed yet.

#### **5.1.4 Dual-camera tests**

The single-camera method is vulnerable to the changes in the scene between the shots. It is desirable to take the stereographic images simultaneously, using two almost identical cameras. The physical size of these cameras puts a lower limit on the baseline, hence we expect to use as small cameras as possible.

Alignment is very important: if the two cameras are rotated with respect to one another, the resulting depth maps will need to be corrected.

#### **5.1.5 Stereoscopic measurement**

The stereoscopic measurement requires two images of the scene shot from a few centimetres apart. In our case the tray of plants will be photographed from above.

- The actual shooting of the images could be done in one of three different ways:
	- Two cameras placed a few centimetres apart
	- One camera which is moved a few centimetres between the exposures
	- One fixed camera where the object is moved a few centimetres between the exposures

The following equipment is needed:

- Digital camera(s)
- Lens
- Lightning
- Mechanics
- Computer and software for image processing.

For a good stereoscopic analysis of the plant height, we need images which are well focused over the whole height of the plants. The depth of focus of the image is a combination of the size of the sensor, the focal length of the lens, lens aperture and the distance between camera and object. At the same time, these parameters will also affect the images in other ways.

- For an image with a large depth of focus we need a small aperture. But, this will require longer shutter speed or more light for a well exposed image. A long exposure time might result in non-sharpness due to movements.
- The focal length will also affect the field of view. The shorter focal length the larger depth of focus and larger field of view.
- The smaller sensor the larger depth of focus but also fewer pixels and lower spatial resolution.

#### Lens

The choice of lens will primarily be done dependent on required field of view and suitable placement of the camera. As a starting point we are using a 1" sensor, 12,8 \* 9,6 mm, and a

12,5 mm lens. The angular field of view is 55,6°. Thus, the field of view is approx. 0,5 m from a distance of 0,5 m.

Using aperture 4 we will have a depth of focus exceeding 15 cm which we believe is sufficient for the application. In order to have well exposed images we have to combine lighting and shutter speed accordingly. It is an advantage to have a short shutter speed in order to avoid any non-sharpness due to possible movement in the plants.

### **Sensor**

The actual number of pixels needed for a sufficient resolution has to be tested. Our first tests will be performed with an 1" sensor with 6 Mpixels. The fewer number of pixels the lower cost for the camera. Fewer pixels will also give a smaller sensor which also is an advantage with respect to the cost of the lens. Besides, a smaller sensor is also an advantage with respect to the depth of focus, and could thus help us with respect to lightning.

### **Lightning**

The lightning is crucial for the analysis. First, we need well exposed images. The amount of light needed has to be tested as this depends on aperture, shutter speed and sensitivity of the sensor and the colour and texture of the actual objects. As described above, aperture 4 for a 1" sensor is most likely a suitable setting. As we also would like a relatively short shutter speed, no longer that 1/50 sec., we need to arrange the lighting in order to have wellexposed images.

Secondly, the light should not be dominated by green wavelength. Best is most probably to have a white light, but maybe the light used for growing is suitable, as this is dominated by blue and red wavelengths.

### **5.1.6 Extraction of "plant green"**

The same hardware could be used for the extraction of plant green as for the stereoscopic analysis. Even more important is to have a light source with suitable colour.

### **5.2 Fluorescent imaging of shoots**

### **5.2.1 "Kautsky Induction"**

The shoots are to be evaluated after dark adaptation regarding the initial level "F0" at time t=0 and the maximum fluorescence "Fm" from which the plant photosynthetic performance can be calculated. The theories and method is well established and based on work published by Kautsky and Hirsch back in 1931.

In this project the aim is to assemble a small system, with as few components as possible, designed to measure only a few parameters and under the dark part of the 24 hrs cycle when the plants are dark adapted. We will design an illumination system to excite chlorophyll 'a' (around its excitation optima of 420 nm, Figure 5-8), for this we will use LED in combination with band pass filter. The illumination should be able to excite chlorophyll using pulses of different length. The emitted light from chlorophyll 'a' will be collected (around its optimum of 670nm) using a CCD chip in combination with a long band pass filter.



Figure 5-8. Excitation and fluorescence wavelength of chlorophyll a and b.

It would be possible to use a more complicated system where the measurements could be performed with the growth lights on. Using this strategy we would get additional data on a range of parameters. Also for this type of measurements there are a number of commercially available instruments on the market. However, this would require a pulse-modulated excitation light and additional data processing. Since this is not totally necessary for the project and would require additional complexity to the system we have chosen not to pursue this direction.

### **5.2.2 Chlorophyll content in leafs**

 $\ddot{\phantom{a}}$ 

By measuring chlorophyll fluorescence from leafs at 735nm and the interval 700-710 respectively, the quote F735 / F700 can be calculated. This quote is linearly proportional to chlorophyll content and can be a good indicator of chlorophyll content in plant leaves. For this we need the same excitation light as described above, in combination with a CCD chip with two filter sets, one for 35 nm and the other band-pass filter for 700-710 nm<sup>13</sup>

For both fluorescent image analyses, we will be able to use similar data processing as for the visible light analyses of shot height and green mass as has been described in previous section.

 $13$  Gitelson et al., The chlorophyll fluorescence ratio F735 / F700 as an accurate measure of the chlorophyll content in plants, Remote sensing of environment 69 (3):296, (1999.)

### **6. Summary**

1) The main parameters to measure and monitor are shown in Table 1.



\* image analysis by comparing images at different time-intervals (interference between leaves and soil). Beside comparative analysis of colour (or Black & White) images (taken by camera or video-camera) also IR images will be collected in order to evaluate if IR images of growing seedlings are better suited to evaluate their growth rate. In this way we could probably make an evaluation in order to decide if a normal or IR camera (or video-camera) should be placed on the unit system.

- 2) Impedance will be utilized as detection technique for the measurement of soil parameters such as water humidity / content and conductivity.
- 3) Few electronic scheme are proposed, spectrum vector analyser, single frequency vector analyser, multiple frequency scalar analyser, LC-oscillator, and the best alternative will be chosen such that miniaturisation and reliable signal for the impedance detection technique are obtained.
- 4) According to Preforma pF curve, sensors for soil parameters have to measure and detect when soil moisture reaches the lower limit of EAW range (42 % of water content), in order to activate the irrigation system before substrate reaches the lower limit of total available water (32%), and to restore the moisture to the upper limit of EAW range (52%).
- 5) The method of depositing silver and gold metal electrodes on various substrate materials and geometries is flexible and promising.
- 6) Stereoscopic analysis will be further developed for above-ground seedling growth measurements.
- 7) An attempt to collect fluorescence images will be done experimentally in order to understand if physiological parameters related to photosynthetic and

metabolic activities are needed to support indications about seedling growth rate collected by morphological traits measurements.

8) Tap water will be used for watering / irrigation in the unit system but its properties will be preliminarily tested in order to know if these fall within the range approved by European regulation (Table 2) and measured by our partner Univ. of Thrace (Table 3).



 $1$  dS/m = deciSienen/netre in SJ, units (equivalent to 1 mmho/cm = 1 millinmho/centi-netre)



