

## PLANT RESPONSES TO IMITATED MICROGRAVITY WATER DISTRIBUTION IN SUBSTRATE MEDIUM ON EARTH\*

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**Key words:** *pea, waterlogging, oxygen deficiency stress, phenological characteristics, microgravity*

### Abstract

*The problems with air and water control in the root zone often lead to different types of plant stress in microgravity. The insufficient substrate moisture leads to nutrient and water deficiency stress and the increased substrate moisture to oxygen deficiency stress.*

*In a ground-based experiment carried out in the laboratory prototype of the SVET Space Greenhouse the impact of the increased water content in the substrate medium on height and phenological characteristics of pea (*Pisum sativum* L. cv "Ran-1") was studied. Plants grown in conditions with high water content (waterlogging) showed typical visual signs of oxygen deficiency stress – chlorosis and stunted leaf growth, plants stopped to develop in height and died. Unusual leaf wither and drop was observed before the waterlogging stress in the test plants and during the whole vegetative cycle in the control plants. To find the reasons for the observed phenomena and to study the effect of the environmental factors on pea growth a second experiment repeating only the cultivation procedures with the control plants was carried out. Pea plants showed the same visual signs of leaf wither as before although the environmental factors were within the optimal range. Analyses of the moisture distribution in the substrate volume reveal that a zone with increased water content had formed at one half of the Vegetation Vessel and probably changed the substrate medium characteristics, which led to the unusual leaf wither and drop.*

### INTRODUCTION

One of the main problems connected with the use of substrate media for space plant growth application is the control of substrate moisture and oxygen content. Microgravity changes the behavior of fluids and gases - the water distribution in the substrate medium is due to capillary forces and the gas exchange is carried out by the gravity independent process diffusion. Water initially fills in all the pores in the substrate medium around the water-source, displacing the air from the substrate volume and slowly distributing in the substrate medium. Oxygen content in the root zone depends on the amount of water that the substrate can hold. The oxygen content can further decrease because water does not drain in microgravity and the diffusion of gases in water is 10 000 fold less than in free air [1]. The high water content may lead to oxygen deficiency and plants grown in different space plant growth facilities have shown signs of oxygen deficiency stress [2,3]. Basically these hypoxic signs are related to problems with moisture control in the root zone.

The pattern of water distribution in substrate medium in microgravity cannot be repeated on earth but separate events could be imitated. The high water content in the substrate medium and the subsequent oxygen deficiency could be replaced with the waterlogging process.

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\* The research was funded by the Bulgarian Ministry of Science and Education – Grant КИ-1-01/03

There is not much information about the tolerance of plants candidates for growth in the future Biological Life Support Systems to increased moisture and lack of oxygen in the root zone. To study the impact of waterlogging and the environmental factors on height and phenological characteristics of pea (*Pisum sativum* L. cv "Ran-1") we carried out two ground experiments.

### **Experiment 1 (04 March – 03 May 2004)**

The aim of this experiment was to study the impact of high moisture content and consequent lack of oxygen – processes that occurred in microgravity, on plant heights and phenological characteristics.

## **MATERIALS AND METHODS**

### **Hardware**

The experiment was conducted in the laboratory prototype of the flown on the MIR Orbital Station SVET Space Greenhouse (SVET SG). SVET SG consists of Plant Growth Chamber (PGC) of an open type that contains Light Unit (LU) and Root Module (RM). The LU provides  $400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  PAR at the top of the canopy. A new Root Module was developed for the experiments to maintain substrate moisture in a range from zero (air-dry substrate) to saturation. The RM has  $0,1 \text{ m}^2$  growing area and is divided into two  $5,2 \text{ L}$  Vegetation Vessels (VV1,2) each with two flowerbeds. A moisture sensor placed at 3 cm depth below the surface maintains the moisture control in each VV. The two VVs were filled with 1,0-1,5 mm particle sized substrate Balkanine.

### **Plant culture and cultivation procedures**

Pea (*Pisum sativum* L. cv "Ran-1") was cultivated for 56 days. Plants were sown at 2,5 cm depth after the initial substrate moistening. Initially, all plants were grown at one and the same conditions - 16/8 hours light/dark period, substrate moisture 1/3 from saturation, air temperature varying between 23-25°C and upward air flow (from the laboratory through the PGC and out of it) at 0,2 m/s speed. On the 9<sup>th</sup> day the moisture in the two VV was raised for 24 hours by increasing the moisture thresholds with 5%. On the 17<sup>th</sup> day of plant development – waterlogging stress was applied in VV1 (test) by raising the moisture threshold for 1 week and slowly submerging the substrate. At the same time, the Light Period (LP) was daily increased with 1-hour step to 23 hour light / 1 hour dark. Plants in VV2 (controls) continued to grow at the same substrate moisture as before and the new LP as for VV1. During the stress treatment the air temperature varied between 16-23°C for the test and control plants.

Plant heights were measured on the 4, 7, 10, 13, 17, 21, 25, 29, 56 day of development and during this period phenological observation was made.

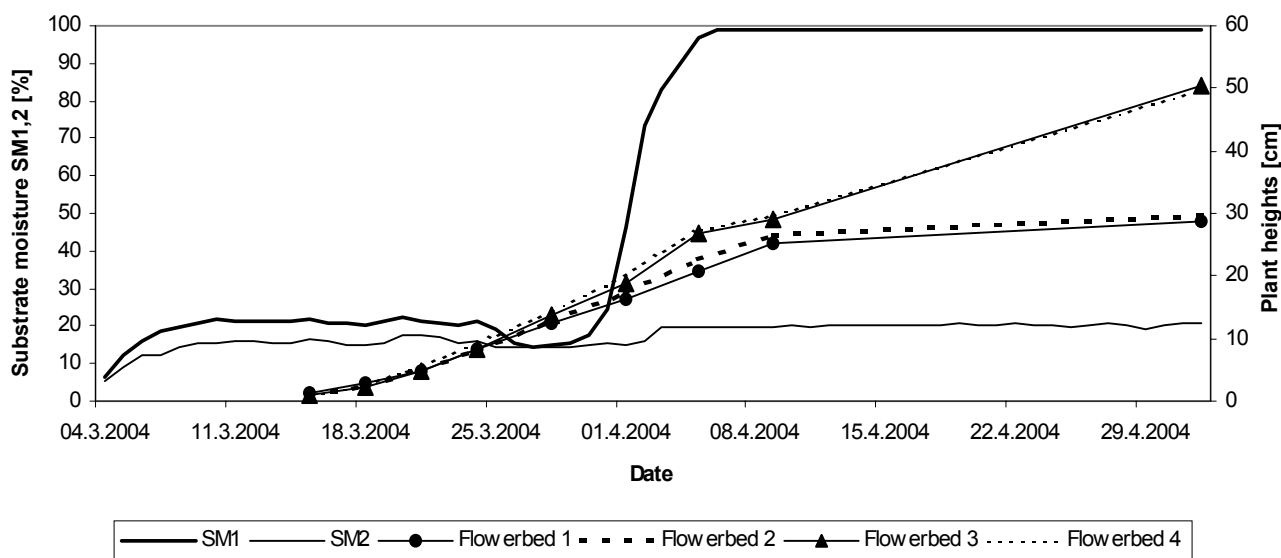
## **RESULTS**

Seeds started to germinate 4 days after planting. It is a routine procedure to increase the substrate moisture by raising the moisture threshold during plant growth. But three days after we raised the moisture threshold yellow-brown-red spots were noticed on the lower leaves. The affected leaves subsequently withered and dropped.

### **Vegetation Vessel 1**

During the waterlogging stress test plants showed typical visual responses to oxygen deficiency stress. They stopped to develop in height and at the end of the experiment were by 57% lower in height than the control plants grown at moisture 1/3 from saturation "Fig.

1". On the 3<sup>rd</sup> day after treatment chlorosis was observed on plant leaves. A few new leaves developed but they were small and stunted. The leaf wither and senescence continued during the treatment and plants died before reaching maturity. On the 10<sup>th</sup> day of the waterlogging stress new shoot growth was observed from the stems of the withered plants.



**Fig. 1.** Plant heights during Experiment 1

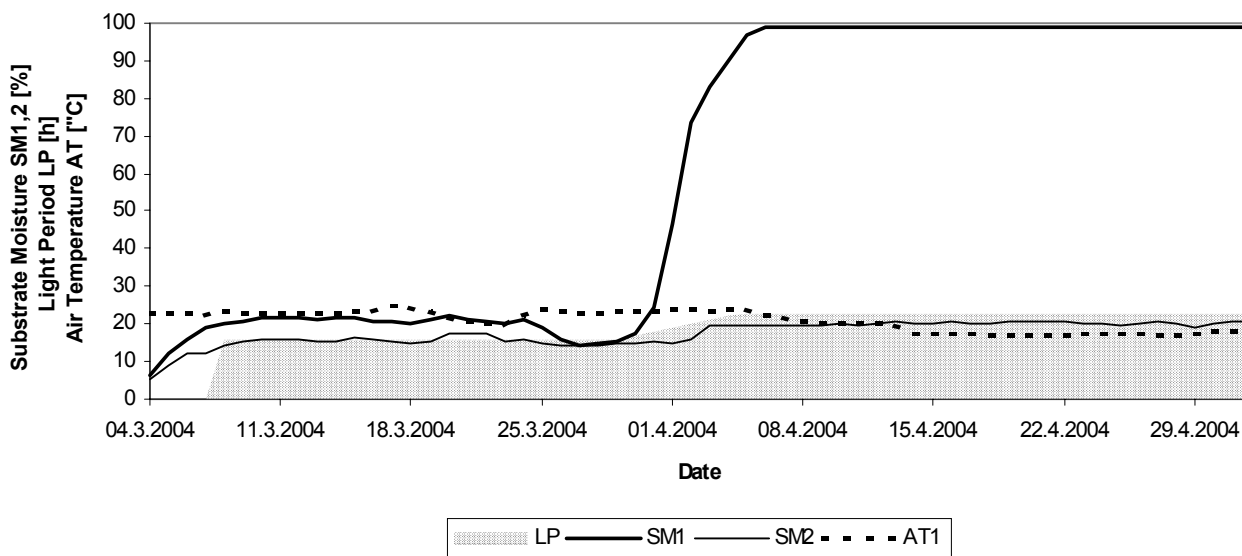
## Vegetation Vessel 2

Extra elongation of the vegetative growth and delay in the reproductive phase was observed in the control plants grown at moisture 1/3 from saturation. The induced processes of leaf wither and drop continued during the whole vegetative cycle and plants lost 2/3 of their leaves before reaching reproductive phase. The first flower buds were formed on phase 18<sup>th</sup>–20<sup>th</sup> leaf (39<sup>th</sup> day) at normal for that variety pea up to 11<sup>th</sup> leaf. The first fruits were obtained on the 41<sup>st</sup> day.

After the experiment analysis of the root systems in both VVs was made. Considerable differences were observed - the root systems of the test plants (in VV1) were shallow with poorly developed main root and with many secondary roots growing upwards in the substrate and out of it. In VV2 due to insufficient water conductive properties of substrate Balkanine, a zone with higher moisture content had formed at nearly one half of the substrate volume and prevented root development in depth. For this reason the root systems of control plants were situated in layer of 4,5 cm in depth. The root systems were with well-developed main and secondary roots.

## DISCUSSION

The observed earlier leaf wither and drop, which occurred before the waterlogging stress in VV1 and during the whole vegetative cycle in VV2, is supposed to be due to inappropriate environmental factors - the interaction between high air temperatures, low relative air humidity and dry air with 0,2 m/s speed in the PGC. As "Fig. 2" shows the air temperature in the laboratory from the beginning of the experiment, during the moisture raise and until the beginning of the waterlogging stress in VV1 was higher than the required optimum for pea growth. During Experiment 2 we studied the effect of the environmental factors on pea growth.



**Fig. 2.** Environmental parameters maintained in SVET SG during Experiment 1

### **Experiment 2 (08.07-06.08.2004)**

The objective of this experiment was to study the impact of the environmental factors on plant growth. Experiment 2 repeated the course of Experiment 1 from the beginning until the 5% - 24 hour raise of moisture and with the addition of the same amount of water but at lower air temperature and higher relative humidity in the laboratory.

## **MATERIALS AND METHODS**

### **Hardware**

In Experiment 2 only VV2 of the new RM was used but equipped with two sensors (Sensor 2- the original for VV2 + Sensor 1 from VV1) placed respectively at 7,8 cm and at 3,8 cm from the bottom of the VV2. Sensor 1 or Sensor 2, depending on the aim, maintained the monitoring and the control of the substrate moisture. VV2 was filled again with 1,0-1,5 mm particle sized substrate Balkanine.

A new sensor to measure temperature and air humidity was added one in the PGC and one in the laboratory.

### **Plant culture and cultivation procedures**

Pea (*Pisum sativum* L. vr "Ran-1") was grown for 28 days. The seeds were planted along with the filling up of VV2 with substrate at 2,5 cm depth before initial substrate moistening.

Following the cultivation procedures of Experiment 1 equivalent amount of water was daily added automatically by Sensor 1 or Sensor 2 or manually adding the lacking amount of water. When the plants reached the same stage of development the moisture threshold of Sensor 2 was raised with 5% for 24 hours.

The temperature in the laboratory was 20-23°C, the relative air humidity was 65-85% and the photoperiod was 16 / 8 hours day / night.

## **RESULTS**

Change in the orientation of the emerging plants was observed. The plants emerged two by two from one and the same place in the flowerbed. The plants developed normally

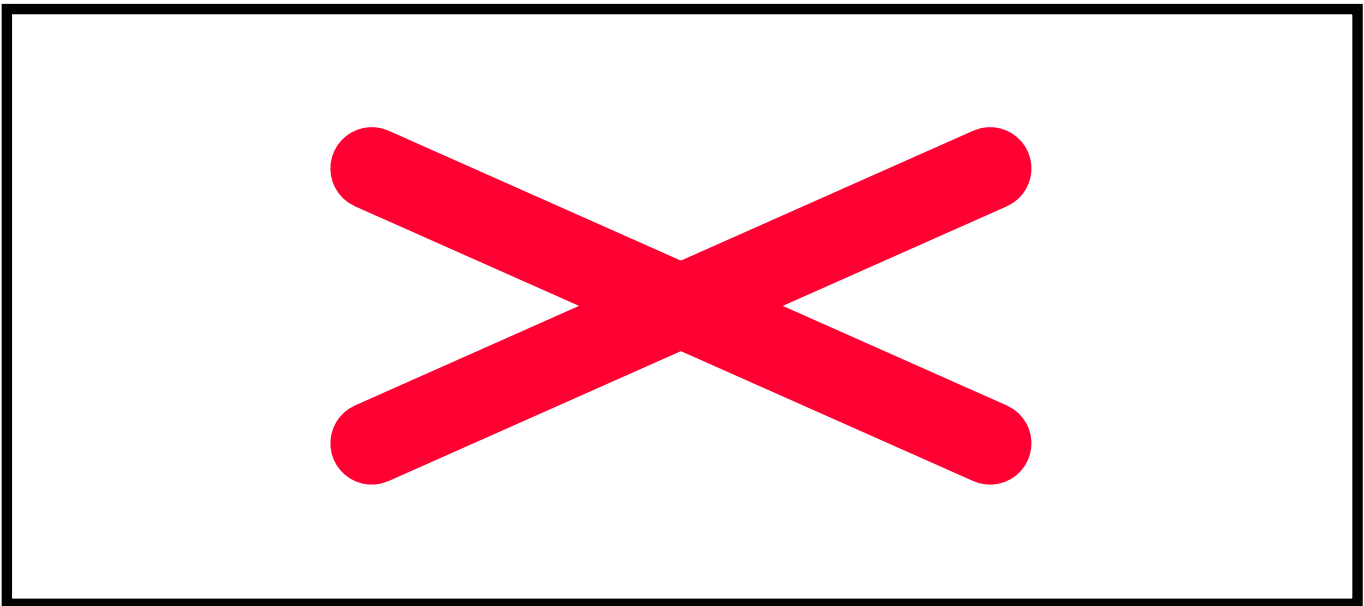
till the moisture threshold was raised with 5% for 24 hours. Two days later the same yellow-brown-red spots on the lower leaves were observed as in Experiment 1.

At the end of the experiment analysis of root system development was made. The root system was well developed but again situated in a layer of 4,5 cm in depth in the substrate volume.

## DISCUSSION

The change in the orientation of the emerging plants is probably due to the preliminary seed sowing before the initial substrate moistening.

Experiment 2 was carried out in environment optimal for pea growth (Fig. 3) therefore the leaf illness was not due to climatic factors but due to processes occurring in the substrate medium induced before or during the moisture raise. The experiment design and the use of the two sensors allowed monitoring of the moisture dynamics vertically in the substrate volume. The moisture control during the process of initial moistening of air-dry substrate Balkanine led to saturation of the layers at the bottom of the Vegetation Vessel. The zone of saturation has limited root development in depth and practically made half of the VV's volume unusable. This is the reason why roots grew at 4,5 cm depth in both experiments. The raise of moisture in substrate Balkanine before it is well moistured to have good water conductivity leads also to moisture increase in the zone where roots develop at the moment. Although for a short time the higher water content in that zone is fatal for plants and obviously the reason for the leaf wither and drop.



**Fig. 3.** Environmental parameters maintained in SVET SG during Experiment 2

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