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SUBSTRATE MEDIA SELECTION FOR USE IN SPACE GREENHOUSES: LABORATORY APPARATUS FOR ASSESSMENT OF SEED GERMINATION RESPONSES TO GRADUATED SUBSTRATE SURFACE MOISTURE

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Abstract

The study proposed is a part of research on the development of an algorithm for automatic control of plant growth environment in space greenhouses in order to maintain optimal conditions in chosen substrate at each plant development stage. In particular the dependence of seed germination on the substrate moisture level was investigated. For the purpose, a seed germination and plant growth-testing apparatus was developed to evaluate substrate agro-properties at different constant moisture, light intensity and temperature levels. The effect of Balkanine substrate moisture on the germination percentage and rate for seeds of a Lettuce crop was evaluated. Highest seed germination (82-84 %) at moisture levels between the water holding capacity and saturation and delay in passing to the activation phase at lowering water potentials was observed and discussed. A critical level of about 22% volumetric water content (VWC) for radical emergence was determined. Initial substrate moisture of 0.35-0.5 cm^3/cm^3 *VWC in the sowing substrate layer is recommended for the experimental salad crop. Discussions about the number and heights of experimental dishes necessary to achieve the desired gradient of moisture levels on the substrate surface and about the possibilities provided by the apparatus to record experimentally profiles of the germination–environmental parameters relations for Balkanine substrate are drawn.*

Introduction

The key to successful plant development in space is to provide suitable root environment for the growing plants. Systems for controlling root environment are required to provide steady substrate moistening, non-stop and balanced transfer of nutrients to plant roots and good gas exchange.

A range of factors, conflicting and inadequate requirements have raised problems on the development of root modules for space application. Mass, volume and power constraints have been caused by the requirement to get maximum yield from minimum plant area, power and time. This imposes hard power and space restrictions on the equipment and reduces the root module volume which leads to higher density of components in the root area. The problems of water containment and liquid and gas phase separation in microgravity, discussed in the NASA Technical Memorandum, presented by Steinberg S.L. et al. [1], are of great importance for providing adequate water-air balance in small volume root environment. The separate-phase systems based on porous solids have established themselves for space utilization in most plant growth facilities. Substrates of solid particles are considered to be the most appropriate medium for plant growth in long term space experiments because of their longevity, repeated use and repeated crops in the same substrate.

Microgravity affects heat transfer, mass exchange processes, fluid behavior, gradients of nutritive concentration in the substrates, and capillary properties of artificial soils. The substrate media selected for plant growth research purposes should have defined physical and hydraulic characteristics. The problems with selection of a substrate capable to support suitable root environment in small volume containers on earth and in space are due to the impossibility to comply with all the requirements which are quite conflicting and sometimes mutually incompatible. In addition the process of selection involves a lot of long, hard and expensive analysis in specialized laboratories.

A lot of researches have been directed at study and control of fluid movement in a multi-porous medium. Another important issue is related to seed germination and plant growth responses to various conditions in such medium. There is a need of measurements of the substrate agro-properties that contribute to reveal its productivity. This corresponds with the research work of Kostov et al. [2] on the development of the Svet-3 SG equipment - a space greenhouse (SG) of third generation which allows evaluating plant status and optimizing growth conditions during the experiment in order to provide most favorable conditions at each stage of plant development. Such adaptive control of the plant environmental parameters requires development of an algorithm for automatic control of substrate moisture so that optimal conditions are maintained in the rhizosphere. Therefore there is a need of experiments to show what different moisture levels are necessary to be maintained in the chosen substrate at different plant development stages - from sowing to harvest.

Seed germination is strongly dependent on a variety of factors such as substrate temperature and moisture, physical properties and chemical substrate composition, quantity and quality of light, depth of sowing, plant variety, preliminary seed treatment etc. Such an experiment requires available equipment to maintain different constant light intensity, temperature and substrate moisture levels. Soil moisture is critical as it affects how quickly water penetrates into the seed. Moisture in appropriate amount for fast, uniform and complete germination of the seeds is very important.

With these considerations in mind, a seed germination and plant growth-testing apparatus was developed to study some critical problems on providing adequate water and air supply to the plant roots. It allows testing a variety of substrate media in order to evaluate substrate agro-properties at different constant moisture levels. Adjusting light intensity and constant temperature maintained by inverter air conditioner allow recording profiles of the germination–environmental parameters relations.

Materials and Methods

SUBSTRATE MATERIAL

Balkanine™ (Stoilov, G., I. Petkov, D. Dimitrov, (1979), Bulgarian Patent # 40343) was used as a root medium during the experiments.

After determining the water holding capacities for four Particle Size Distributions (PSD) of Balkanine, the 1.5-2.0 mm fraction was selected to be used for seed experiments.

PLANT MATERIAL

Lettuce (*Lactuca Sativa* L.), "Yellow beauty" variety, seeds s/n 8711989*171 (SORTOVI SEMENA I RASTITELNA ZASHTITA LTD. Sofia, Bulgaria) were used for the experiments.

LABORATORY APPARATUS

An apparatus for assessment of the agro-physical properties of substrates was developed.

Description of the apparatus

An apparatus, designed for these experiments, was specially developed and produced. It is an advanced modification of the device described by Xiao-Chun Wan et al. [3]. The apparatus is composed of a plastic water container with storage capacity of about 25 l, twelve plastic pots of different heights filled with the substrate subjected to test and a water reservoir (Mariotte siphon arrangement) which represents a 20 l polyvinyl chloride (PVC) bottle (Fig. 1).

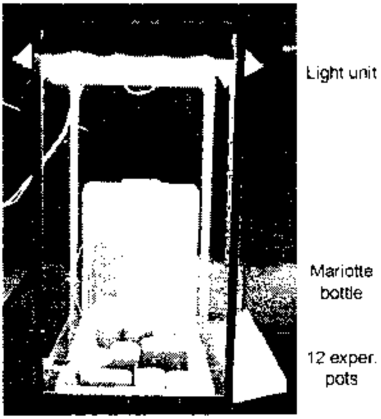

Fig. 1. A view of the experimental apparatus

The water reservoir and the container are connected by a polyethylene tubing (6 mm OD), which siphons water from the reservoir to the water container in which the pots are placed.

The water container is surrounded by a light aluminum mechanical structure, 1.2 m high, which holds fixed a movable light unit. The light unit can be positioned horizontally or at some angle and provides adjusting distance from the lighted container surface.

A horizontal shelf 60 cm long, 13 cm wide and with a slope of 15° of the narrow side is mounted on the aluminum structure. Five Petri dishes are placed there and used for a germination test experiment performed by standard method (Fig. 2).

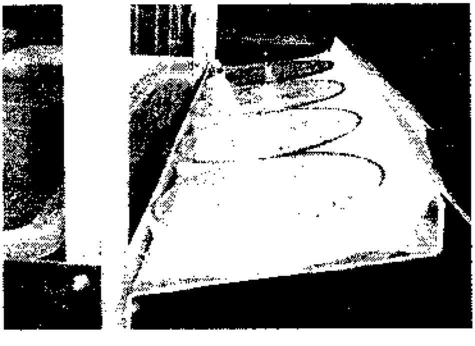

Fig. 2. Five Petri dishes are placed on the shelf

Mariotte siphon (bottle)

The water reservoir (20 liters PVC bottle) is equipped with a vertically inserted stainless steel pipe (6 mm OD) allowing ambient air to enter the reservoir through the water in the form of air bubbles. The water pressure in the bottom end of the pipe is equal to the atmospheric pressure and the water level in the container is maintained constant at the height of this end of the pipe. Another pipe is inserted below the air-entry pipe to siphon the water from the water reservoir through a flexible hose to the water container. The Mariotte bottle can be positioned at different heights to allow adjusting the desirable water table in the container. The Mariotte reservoir is filled with sufficient water amount to support a two-month plant experiment without need of recharging.

Water container and experimental pots (EP) for substrate

The water container has internal dimensions L=55 x D=35 x H=13 cm and allows disposing the 12 PVC pots filled with substrate. The container is placed on a levelled laboratory table next to the Mariotte bottle. The container has a firm bottom which bears the load of the 12 pots with substrate and the water without deformation providing good levelling and the same height of the water table in each pot with substrate.

Twelve pots, respectively 3.5, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18 and 20 cm high and with sowing area of about 86.5 cm^2 allow testing the agro-physical properties of particulate substrates at different substrate surface moisture levels and the same other environmental conditions. The pots are filled to the brim with substrate which has been previously packed to the desirable bulk density. 27 small holes, uniformly situated on the bottom pot wall, permit the water to enter the substrate from the container.

Light unit

The substrate surface is lighted by four 15 W fluorescent lamps, 60 cm long and with "Cool white" light characteristic. A 6 W fluorescent lamp of the same light characteristic is used to ensure the minimal lighting necessary for the operation of a photo camera during the "night". A timer controls the light unit operation and adjusts the photoperiod from 0 to 24 hours in steps of 15 min. A simplified hanging system provides a possibility to adjust the distance to the experimental surface at 5 to 100 cm in steps of 2 cm.

Petri controls

A shelf for the five Petri dishes (D=120 mm, H=20 mm) is mounted on the long side of the metal structure. The percentage germination of the seeds selected for the main experiment is determined in a control experiment with 100 seeds sown in each Petri dish. Both experiments are simultaneously conducted. The Petri dishes are positioned at the mean height of the experimental pots with substrate (about 12 cm) so that all the seeds for both, the control and main experiment, are placed under comparable environmental conditions.

Observation system and data collection

Although the apparatus maintains automatically the water level in the substrate and the air-controlled system takes care of the environment, a set of sensors provide continuous monitoring of the changes in some environmental parameters. Four temperature sensors SMT160-30 (SMARTEC B.V, Delpratsingel 26, 4811 AP Breda, Netherlands) and a relative air humidity sensor SMT RH05 (SMARTEC) are located in places of interest. A photo camera Konica Q-M100 takes pictures automatically at previously set time intervals. Data obtained from the sensors and the photo camera are collected and saved in a computer. The computer operates in a mode of a Dial-Up Server which permits remote access to the data collected by a modem connection. A Web camera is connected to another computer operating as an IP Server and provides user access through the Web for visual observation in real time during the experiment. A data collection system allows inclusion of other sensors in order to expand the range of the environmental variables monitored.

Description of the experiments

The apparatus described above provides a possibility for testing all kinds of substrates (Balkanine in our case) at different moisture levels. Adjusting light intensity and constant temperature maintained by an inverter air conditioner allow recording the profiles of the germination–environmental parameters relations.

Experiment objective – evaluation of the effect of Balkanine moisture on the germination time and percentage for seeds of a Lettuce crop.

Initial conditions for the experiment:

- Balkanine substrate, 1.5-2.0 mm PSD;
- Twelve pots of different heights, filled and compacted with substrate and providing 12 different water tables above and below the sowing substrate surface: $h_1 = +1.5$ cm, $h_2 = 0$ cm, $h_3 = -1$ cm, $h_4 = -2$ cm, $h_5 = -3$ cm, $h_6 = -4$ cm, $h_7 = -5$ cm, $h_8 = -7$ cm; $h_9 = -9$ cm, $h_{10} = -11$ cm, $h_{11} = -13$ cm and $h_{12} = -15$ cm;
- Seeds of Lettuce (*Lactuca Sativa L.*), "Yellow beauty" variety; 12 pots with 100 seeds sown in each one;
- Water – from the water-main, purified by CFS-SOLVO®, coagulant for potable water treatment;

- Lighting period – 16 h day/8 h night;
- Air temperature in the laboratory - controlled at (25±2)°C - a typical ambient temperature level for the living cabin environment onboard space vehicles;
- Relative air humidity - (60-75) %, uncontrolled;
- Petri control experiment – 5 Petri dishes (D120 mm x H20 mm) with 100 seeds sown in each one;
- Duration of the experiment – 1+2 weeks.

The experiment was performed in two stages – (1) initial capillary substrate moistening in every EP till equilibrium between moistening and evaporation from the substrate surface was reached (in the first week), and (2) 100 seeds were placed among the particles of the surface substrate layer in each of the 12 EPs and 100 seeds in each of the Petri dishes (Petri control) for the next two weeks (see Fig. 3).

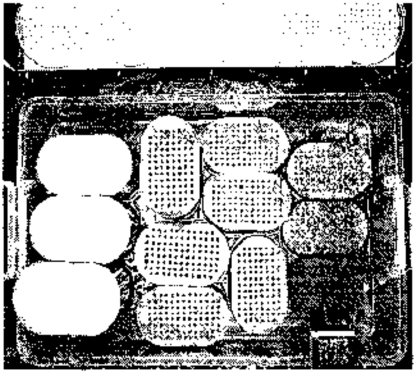

Fig. 3. Seeds are sown in the Petri controls and experimental pots using templates

The seeds in EP No. 1 were covered by a glass plate and the pot was dipped 1.5 cm below the water surface. A seed was counted germinated when the shoot reached a height of 10-15 mm. Then it was removed. As an exception to the procedure described above some plant shoots were left to the end of the experiment for visual evaluation of the germination uniformity in each EP.

A Petri control experiment was performed in 5 Petri dishes simultaneously with the main experiment in order to evaluate the seed germination for the used batch of seeds. Two sheets of filtering paper were placed in each dish and damped with 5 ml of purified water. 100 seeds were arranged on the paper using a sowing template. the dishes were covered and placed on the apparatus shelf. Both experiments (the main one and the Petri control) were performed under the same environmental conditions. The shoots (10-15 mm) were counted and removed from the Petri dishes every day. Data about the seed germination in the Petri controls were statistically processed and the results about the germination time and percentage in the EPs were presented graphically.

A surface substrate layer of about 1 cm in height was removed in each EP and the absolute water content was determined. Data obtained were added to the data set obtained during measurements of the physical and hydro-dynamic properties of Balkanine in Russia, USA and Bulgaria (Zakharov [4]; Bingham at al., [5, 6]; Jones and Or, [7]; Ivanova at al. [9, 10]).

Substrate-water characteristic curve (SWCC) gives matric head (h) - volumetric water content (θ) relation. Fitted SWCC of Balkanine (1-2 mm PSD) is determined by Jones and Or [8] using the van Genuchten [11] nonlinear model, defined as -

$$\theta = \theta_s + (\theta_s - \theta_r)/[1 + (\alpha.|h|)^n]^m$$

where θ is current volumetric water content, θ_s is saturated volumetric water content, θ_r is residual volumetric water content, α, n, m are fitting parameters, and h is matric head (cm).

Total daily evaporation and evapo-transpiration during the experiment were determined by measuring the weight of the Mariotte reservoir with water.

The experimental apparatus ability to maintain relatively constant substrate moisture content and water gradient in each EP during the experiment was analyzed. Some suggestions for additional evaporation and transpiration measurements were made.

Results

The picture shown in Fig. 4 gives an idea of the reducing number of germinated seeds in the higher seedling pots.

Fig. 4. A view of the experiment

The germination percentage for the used batch of seeds selected for the main experiment, determined in the Petri control, is 81%±1.58 SD. The seed germination percentage distribution depending on water table in the 12 EPs is shown in Fig. 5.

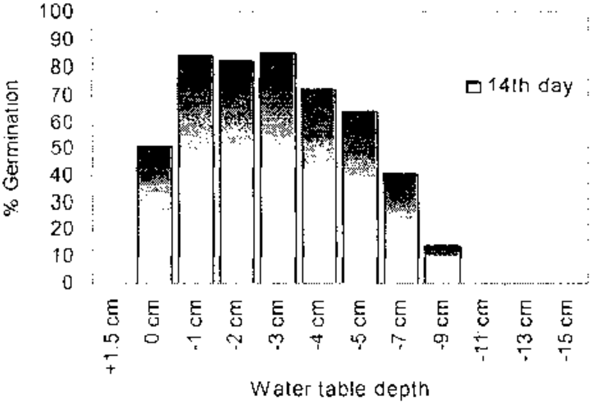

Fig. 5. Seed germination percentages in EP at the 14th DAP depending on the water table depth

Highest seed germination of (82-84) % is observed in the EPs No. 3, 4 and 5 where the substrate moisture in the sowing layer is between the water holding capacity and saturation. Water imhibitions in the lowering water potential from EP No. 6 towards EP No. 12 reduces the seed water content. postpones passing to the activation phase and delays entering the radical growth phase. Radical emergence and growth occurs when the water content exceeds a critical level. For our experiment this critical level is about 22% volumetric water content.

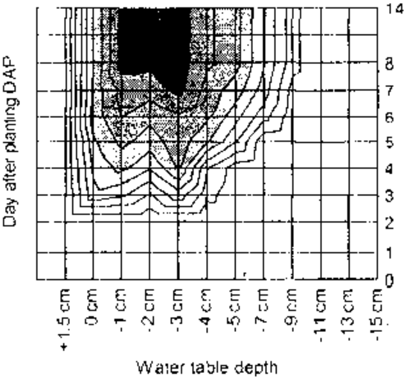

Fig. 6. A 3-dimensional plot of seed germination rates versus the water table depth in the 14-day germination period

From EP No. 6 to EP No. 9 the germination time is prolonged by 1 to 5 days respectively (Fig. 6).

Discussions

Soil moisture is critical as it affects the rate of water penetration into the seed. The germination experiment results suggest clearly a strong dependence of the seed germination percentage and rate on substrate moisture. Highest germination was observed at high moisture levels (0.35-0.5 cm^3/cm^3) between water holding capacity and saturation (Fig. 5). A few centimeters lower water table in the next EPs (matric suction at inner-particle moisture) reduces germination to zero. At the same time the germination time prolongs by 3÷8 days. Hence the automatic system for initial Balkanine wetting should provide higher moisture in the sowing substrate layer what was fixed to 0.35-0.5 cm^3/cm^3 volumetric water content or 60-85% of saturation for the experimental salad crop.

The water status of the surface substrate layers was determined for the ten EPs (No 3-12) (Fig. 7).

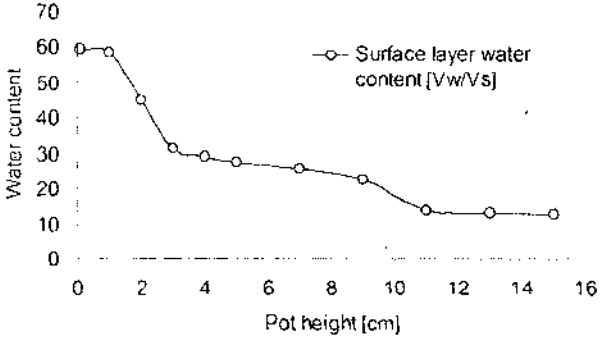

Fig. 7. Absolute volumetric water content in the surface substrate layer against the experimental pot height at the end of the experiment

The zeolite used for Balkanine production has double porosity - inner-particle and inter-particle. Zakharov [4], and Jones (in Bingham et al., [5, 6]) measure the basic hydro-physical characteristics and report that the sharp drop in the matric potential at 22% volumetric water content, due to full macro-pore water draining, leads to drastic decrease of water conductivity (Fig. 8).

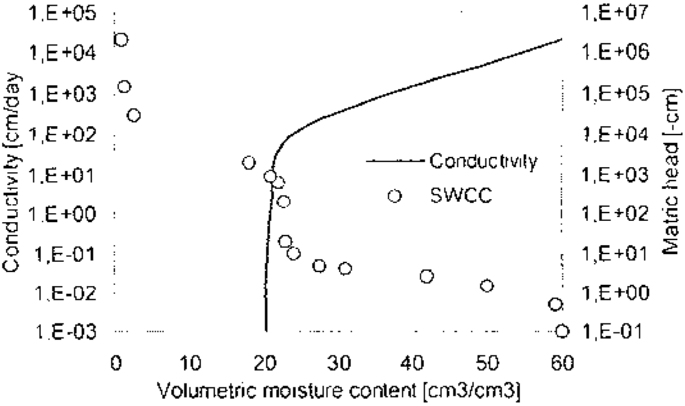

Fig. 8. Matric head and water conductivity of 1-2 mm Balkanine versus volumetric moisture content. (Jones and Or, 1999)

The total daily evaporation and evapo-transpiration during the experiment were determined by measuring the weight of the Mariotte reservoir with water. With some simplifications a mean daily evaporation of 0.15 cm^3/cm^2.day (without plants) was calculated. Shaidorov [12] reports about a mean daily evaporation of 0.24 cm^3/cm^2.day in plant experiments with Balkanine and at air flow rate of about 0.3 m/sec.

Measured and plotted values of surface layer water contents (Fig. 9) in EPs shows close congruence with SWCC plotted for a similar dish. Using the RETC program (van Genuchten et al., [13]) the retention and conductivity characteristics were modelled where θ_s=0.58 cm^3/cm^3 (saturation) and θ_r=0.23 cm^3/cm^3 (pot No. 9) were enclosed in model.

As evident from Fig. 9, the unsaturated hydraulic conductivity curve is considerably smoother than the one determined during hydro-dynamical measurements (Fig. 8) because of the evaporation. Evaporation from surface substrate layer was maintained relatively constant by controlling the ambient temperature and the streamlining air flow. Moistures of the surface substrate layers measured for EP No. 10, 11 and 12 were 0.13-0.14 cm^3/cm^3 and corresponded to the Balkanine maximum hygroscopicity.

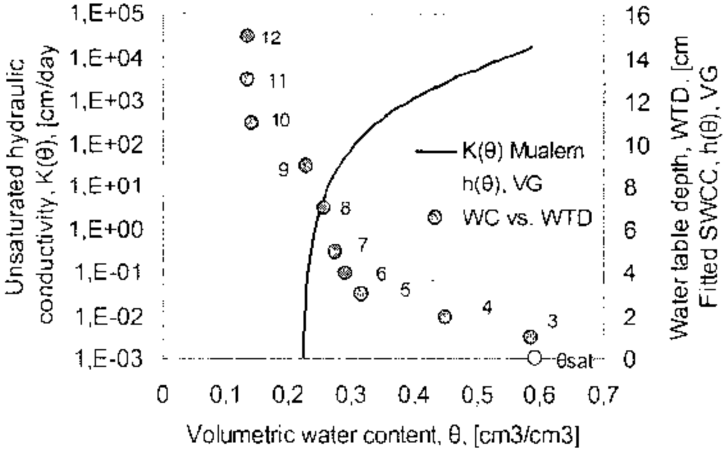

Fig. 9. Measured water contents in 1-cm surface layer for each EP (filled circlets with the EP number), fitted "SWCC" (VG model), and modelled unsaturated hydraulic conductivity versus volumetric water content (Mualem model)

Conclusions

Summarizing the results we can conclude the following: (1) using measurement data about the physical, hydrostatical and hydrodynamical properties of a particular substrate, the number and heights of experimental dishes necessary to achieve desired gradient of moisture levels on the substrate surface when placed in water container for capillary moistening can be determined; (2) using the apparatus described above the germination percentage and norm for seeds of experimental plants can be precisely determined; (3) series of plant experiments can be carried out at different temperature and light intensity gradients, substrate water control algorithm for optimal seed germination can be developed.

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ПОДБОР НА СУБСТРАТНИ СРЕДИ ЗА ПРИЛОЖЕНИЕ В КОСМИЧЕСКИ ОРАНЖЕРИИ: ЛАБОРАТОРЕН АПАРАТ ЗА ОЦЕНКА НА КЪЛНЯЕМОСТТА НА СЕМЕНА В УСЛОВИЯ НА ПОВЪРХНОСТНИ СУБСТРАТНИ СЛОЕВЕ С ГРАДИРАНИ ВЛАЖНОСТИ

П. Костов, И. Стоянов, С. Сапунова

Резюме

Предложеното изследване е част от научноизследователска работа по разработването на алгоритъм за автоматично управление на космически оранжерии с цел поддържане на оптимални условия във всеки етап от развитието на растенията. В частност беше изследвана зависимостта на кълняемостта на семена от почвената (субстратна) влажност. За целта беше разработен апарат за тестване на процеса на развитие на растенията, предназначен за оценка на качествата на субстрати при различни, постоянни нива на влажност, интензивност на светлината и температура. Беше оценено влиянието на влажността на субстрат "Балканин" върху процента на кълняемост и нормата на покълване на семена от салата (*Lactuca Sativa* L.). Най-висока кълняемост на семената (82-84 %) беше наблюдавана при влажност на субстрата между пределната почвена влагоемност и насищане. При намаляващи водни потенциали беше отчетено забавяне на преминаването към активационна фаза. Критична обемна влажност на субстрата от 22% беше установена за покълването на минимален процент семена. За конкретната експериментална култура беше препоръчано първоначално овлажняване на субстрата до 0.35-0.5 cm^3/cm^3 обемно влагосъдържание в посевния субстратен слой. Дискутиран е метод за определяне на броя и височината на експерименталните съдове, необходими за постигане на желан градиент на нивата на влажност в повърхностния слой на субстрата, както и възможностите, предоставяни от апарата, за експериментално снемане на профили на зависимостите кълняемост – параметри на средата за субстрат "Балканин".