

**DOCTORAL SCHOOL  
TECHNICAL UNIVERSITY OF CONSTRUCTION  
FROM BUCHAREST**

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**THESIS TITLE :**  
**Energy Aspects of Microclimate Factors in Protected  
Cultivation Systems**  
***- Applications At Healing And Acclimatization  
Chambers***

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**RESEARCH RAPORT NR.1 :**

***State of the Art of Researches regarding healing and acclimatization  
chamber for grafted vegetables***

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# 1. Introduction

The name of *Protected Cultivation Systems* emerged from the need to be able to cultivate agricultural products in climatically unfavorable areas, particularly in areas where the light intensity is too low or too high, where the temperatures are too high or too low, with moisture excess or no presence of it, soil with no nutrient or with a high percentage of pest rate, areas where extreme atmospheric phenomena such as strong winds or inappropriate carbon dioxide levels occurs.

*Protected Cultivation Systems* is a specialized system known in agricultural activities where plant growing conditions are modified in order to extend the harvesting period, to modify production cycles, increase the quality and quantity of production, while open field cultures is limited (Castilla, 2013).

For its development each cultivar has an optimal set of environmental factors. If this regime is altered, the plant reacts by generating certain processes between it and the microclimate in the surrounding area. For example, by placing a shield near a plant, changes to parts of the plant or the entire plant can be generated. These shields can be placed sideways to the plant, under the plant, directly on the ground or above the plant, forming a roof.

The shields placed above the plant can be: (i) tunnels with a height of about 1m dedicated to small cultivars; (ii) solariums and greenhouses, stronger, higher structures, which are used for large-scale crops, for shrubs and trees.

These structures not only modify the natural conditions of the microclimate in order to achieve optimal crop productivity, but also they help to efficiently use local resources such as soil, water, energy, nutrients, space for culture, climate resources such as solar radiation, humidity and CO<sub>2</sub> concentration (Wittwer & Castilla, 1995).

The costs invested in greenhouse, such as labour and energy costs, are much higher compared to conventional open-field plant production. In order to improve the production in *Protected Cultivation Systems*, it has to be followed a good use of plant production potential through increased labor productivity and high energy efficiency (Druma, 1998).

In these *Protected Cultivation Systems* complex thermal phenomena develop as a result of the interaction between the temperatures inside the greenhouse and the one used for ventilation. Also, solar energy which enters in the greenhouses undergoes significant changes, occurring when the radiation is passing through the material that covers the building.

From the thermal point of view, the atmosphere inside the greenhouse generates a series of complex phenomena related to temperature and humidity, evaporation and condensation, plants, soil, the design of the greenhouse, and the heating system

The transfer and heat exchange is achieved through three distinct mechanisms:

- Thermal conductivity, characterized mainly by the thermal conductivity coefficient  $\lambda$ , expressed in W / m K;
- Convection, or the process of heat transfer between constructive elements and atmosphere, this being:
  - Spontaneous (natural and free) convection due to natural causes, such as temperature difference that causes air density differences that generate its movement;
  - Forced convection caused by external causes;
- Radiation, heat transfer takes place between different temperatures areas due to electromagnetic waves (Bodolan & Bratucu, 2014).

## **2. Protected Cultivation Systems: short history**

The first attestation of a porous culture, according to the historian Columella, dates back to the Roman Emperor Tiberius Caesar (14-37 AD) when transparent glass plates or mica or alabaster sheets were used to cover cucumber cultures (Wittwer & Castilla, 1995).

However, this method of culture seems to have disappeared with the fall of the Roman Empire (Dalrymple, 1973). Until the renaissance period, there are no more documentary attestations on greenhouses and solariums. From the end of the 16th century to the 19th century, in England and France, a series of seedling bed structures similar to those used by Romanian and Bulgarian farmers to the north and south of the Danube appear. These seedlings beds were opened during the day so the sun to warm the seedlings, and in the evening they were closed to protect them from the night's colds. At the end of the 17th century it was mentioned for the first time the use of glass frames and bells, similar to those shown in Figure 1, to cover hot beds where a wide variety of vegetables were grown (Woolf, 1997). The plants were placed on beds of natural manure that fed and heated them at the same time.

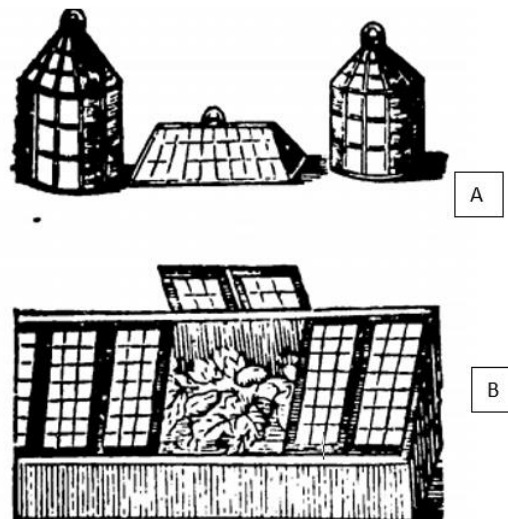


Fig. 1: A - Glass bells used in the 1600s; B - An old drawing published in 1670 in the Netherlands illustrating a covered vegetable growing

At first, the glass was used only to build the sloping roof of the greenhouse while the other walls being build from other materials such as wood, bamboo or other building materials. Later in the 1800s, glass was used as well in building the side walls. The two-sided glassed-glass roof with triangular section appeared at the end of the 1800s (Wittwer & Castilla, 1995). The first countries which developed the building of this type of greenhouse were England, the Netherlands, France and the Scandinavian countries, and then greenhouse culture will also develop in America and Asia. In the 20th century, especially after the Second World War, with the world economic revolution, agricultural production in greenhouses was also developed. The appearance of plastic foils has facilitated the growth of crops in *Protected Cultivation Systems*, especially in solarium in Asia (mainly in Japan, Korea and China) but also in Europe, followed by the Netherlands, Spain, Italy and France. The low vegetable production price obtained in solariums during the off season has made that this type of crop in *Protected Cultivation Systems* to take a very large scale in the energy crisis in Europe.

Greenhouses and solariums have evolved in two directions (Enoch, 1985): (i) a first concept aims to achieve an exact control of microclimate factors so as to maximize production, objective which involves sophisticated constructions; (ii) the second concept involves minimal control of microclimate factors with minimal production costs. The first concept is met in the northern European countries the second being applied in the Mediterranean countries. In our days many constructive greenhouses are being used: (i) Traditional English greenhouses built on vertical single-aperture or multi-aperture openings of 6,7m height; (ii) Dutch

greenhouses with multi-apertures of height 3.2 m and poles openings between 3.4m 6.4m or 9.6m; or with a single opening of 22 m high, with high lateral walls of 3.5 m or 4.5 m and roof tilts of 22<sup>0</sup>-26<sup>0</sup> (Critten & Bailey, 2002).

Production costs are generally influenced by additional lighting requirements, heat losses during cold periods or the cost of ventilation during periods of high temperatures.

### **3. Energy aspects in Protected Cultivation Systems**

Greenhouses are complex systems where physical, chemical and biological processes occur simultaneously. Therefore, the growing conditions of crops can be modified by intervening on some subsystems, more precisely interfering on the heating and air filtration system, the ventilation system, the cooling and shading system, the humidification, irrigation and fermentation system, the CO<sub>2</sub> concentration correction system, on sensors and on the automation system. The main problem in controlling microclimate conditions in crop *Protected Cultivation Systems* is that sudden temperature fluctuations may occur, changes that depend on solar radiation, on outdoor temperature, relative humidity, greenhouse pattern, etc. This microclimate is not evenly distributed in the greenhouse, the parameters ranging from the center to the edge of the greenhouse, from the ground to the roof or from the limit of the mass of the plants towards the interior.

#### **3.1. Solar radiation**

Solar radiation reaching 99% of earth's surface have wavelengths ranging from 300 nm to 2500 nm and depending on the ranges of characteristic wavelengths, three categories of radiation are distinguished:

- Ultraviolet (UV) radiation below 380 nm; these radiation are low at low altitudes and when the sun's height is low (sunrise and sunset). This type of radiation has an aging effect on plastic materials and has an important role in plant morphogenesis (Raviv, 1988). It represents about 2-4% of total global solar radiation. Three types of UV radiation are distinguished: (i) UV-A (wavelengths greater than 320 nm), the radiation that tans the skin; (ii) UV-B (with wavelengths ranging from 290 nm to 320 nm), which generates skin cancer; (iii) UV-C (with wavelengths from 200 nm to 290 nm) which are potentially dangerous for humans but are completely absorbed by the ozone layer (Monteith & Unsworth, 2007).

- Visible radiation to the human eye, from 380 nm (violet-blue) to 780 nm (red). This range includes PAR radiation (active photosynthetic or photoactive radiation) between 400nm and 700nm, representing 45-50% of global radiation.
- Infrared (IR) radiation from 780 nm to 5000 nm. It represents nearly 50% of global radiation energy. The energy fraction in the range 2500 nm to 5000 nm is very low. In IR, NIR (near IR) is the band between 760 nm and 2500 nm (Castilla, 2013). Figure 2 is a representation of the solar radiation spectrum.

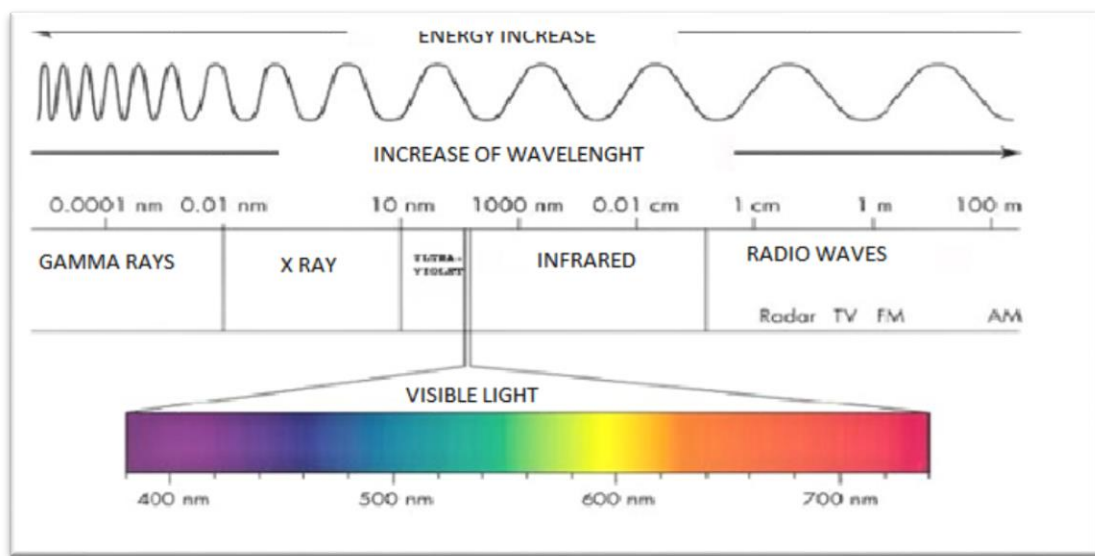


Fig. 2: The spectrum of solar radiation

The nature of the radiation changes according to the position of the sun and the opacity of the atmosphere. When the sun is towards sunset or sunrise, short wavelengths are reduced (so there is less UV and more red). The clouds reduce the amount of energy of solar radiation, by reducing NIR. PAR in relation to global radiation increases with the increase of dispersion (diffusion) and decreases when the sky is clear, especially in the summer (it reaches about 45-48% of the global radiation). During the day, if the sky is clear, the intensity of the radiation develops in regularly depending on the position of the sun, but if it's cloudy, it could vary a lot and very irregularly.

Also, solar radiation varies a lot depending on the latitude and season, when high differences between winter and summer register, due to the difference in the duration of solar diurnal activity.

The materials which cover the greenhouses are transparent for solar radiation. During the day, solar radiation passes through the greenhouse tile and is absorbed

by plants and soil. The plants and the soil are heated and release energy, mostly with wavelengths of  $10\text{ }\mu\text{m}$ , but can vary between  $2.5\text{ }\mu\text{m}$  and  $25\text{ }\mu\text{m}$  (remote IR). This energy released by plants and soil is intercepted by the greenhouse envelope is usually opaque to IR radiation, so it is reheated and it releases energy inside and outside in similar proportions (Figure 3). In this way, the greenhouse is heated because the premise is closed and no fresh air ventilation from the outside exists. These phenomena generate an increase in temperature that is very obvious during the day, in relation with the temperature from outside. This effect may vary depending on the specific radiation transmission and absorption conditions of the greenhouse tile, depending on the ventilation and the sealing of the greenhouse. This is called the Greenhouse Effect.

During the night, the temperature gradient with the exterior depends on a complex energy balance which is influenced especially by the difference between the sky temperature and the temperature inside the greenhouse as well as the sealing of the greenhouse. Thus, if the material used as sealing is permeable to IR radiation, on nights without wind and no air circulation inside the greenhouse, energy losses in IR radiation are very high, generating a decrease in the greenhouse temperature which is below the one outside, and so it will appear the so-called thermal reversal phenomenon. In the past, the greenhouse effect was considered responsible for the microclimate changes inside the greenhouse, but now it was demonstrated that the convective effect in the greenhouse sealing is very important so the use of the expression "greenhouse effect" should refer to both the radiation and convective processes (Papadakis et al., 2000)

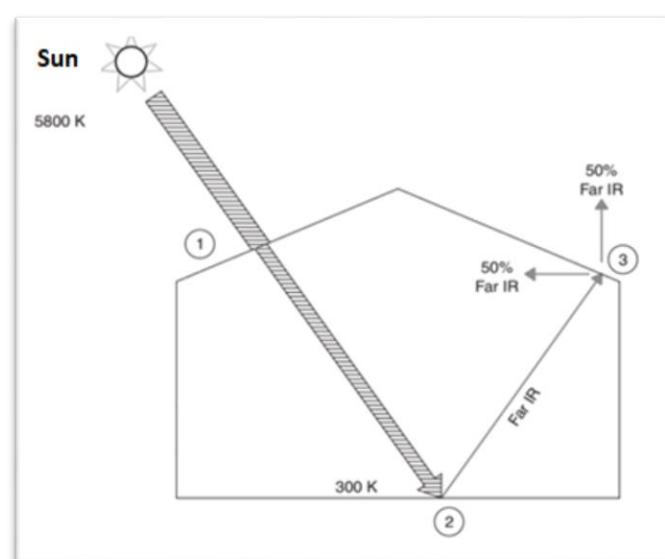


Fig. 3: Producing the greenhouse effect



### 3.2. Energy Balance

Heating in the greenhouse is produced by the heating system ( $E_i$ ) and the incident solar radiation ( $E_s$ ), while the losses are caused by the ventilation ( $E_v$ ), the sealing of the greenhouse ( $E_e$ ) and the heat transfer through the greenhouse. Part of the energy which enters the greenhouse is stored in the sealing, in soil and various components of the greenhouse ( $E_{stoc}$ ). The energy balance equation can be written as follows (Fernandez & Bailey, 1992):

$$E_i + E_s = E_v + E_e + E_{anv} + E_{stoc} \quad (1)$$

Generally heat transfer is made through conduction, convection and radiation or combinations of these processes. Figure 4 schematically shows the complex of thermal processes taking place in a greenhouse.

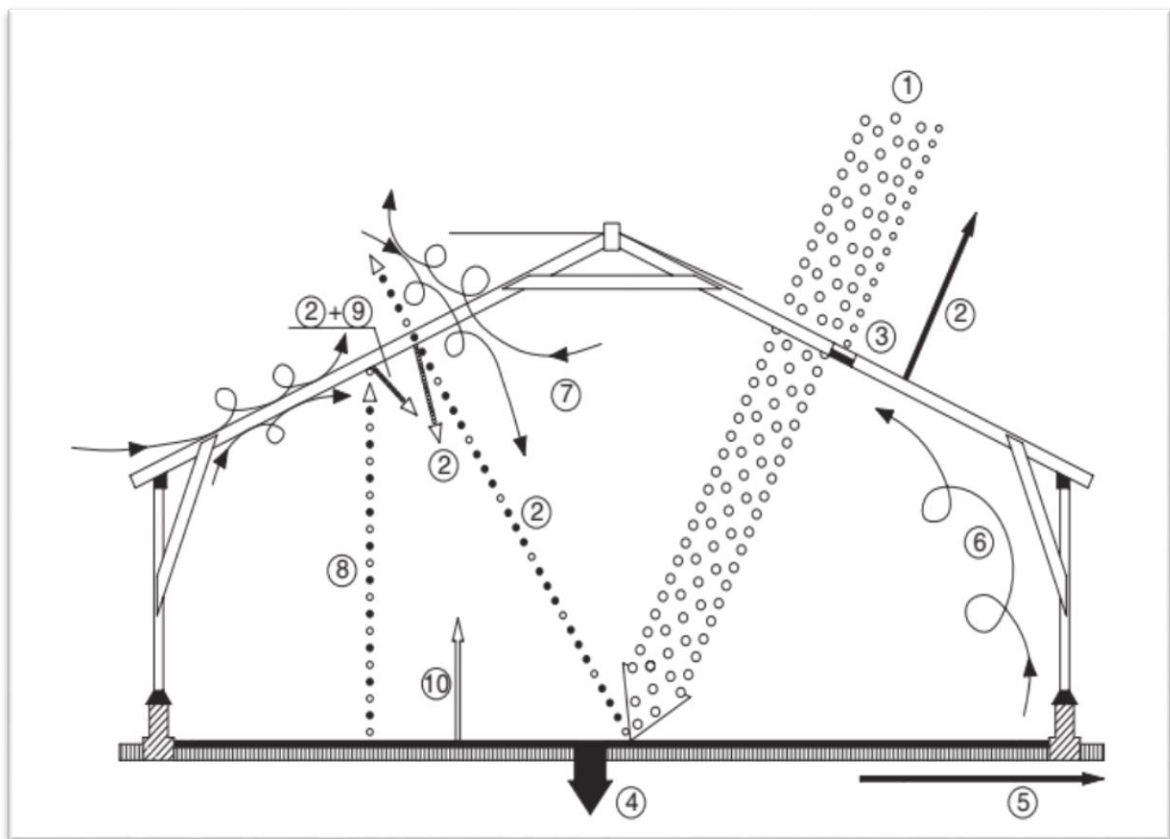


Fig. 4: Thermal balance and radiation flux in greenhouses with: 1 - solar radiation, 2 - reflected radiation, 3 - absorbed radiation, 4 - thermal flow in the soil, 5 - horizontal thermal flow, 6 - ventilation, indoor / outdoor heat exchange, 8-soil radiation, 9-sealing radiation, 10- evapo transpiration.

The amount of heat  $Q$  [W] that is lost through the greenhouse and the soil due to the processes of conduction, convection and radiation can be expressed by the following formula:

$$Q = K \cdot (T_i - T_e) \cdot S_a \quad (2)$$

where  $Q$  = Heat flux changed from inside to outside [W];  $T_i$  = Indoor temperature [K];  $T_e$  = outside emperature [K];  $S_a$  = sealing area [m<sup>2</sup>];  $K$  = global heat transfer coefficient of the greenhouse coating material characteristic for any material [W/(m<sup>2</sup>·K)] (Maslak, 2015). The global transmissivity of the greenhouse must be understood as an average daily transmission (the average amount of radiation which is entering the greenhouse and which is accumulated by all elements in the enclosure), value that is different from the instantaneous values.

Transmissivity depends on a number of factors such as: (i) climatic conditions (atmospheric nebulosity that has a direct influence on diffuse and direct radiations); (ii) the sun position (which will depend on the date and time of the day and the latitude); (iii) the geometry of the greenhouse's roof; (iv) orientation of the greenhouse (east-west, north-south); (v) coating material (radiometric characteristics, cleanliness, condensation behaviour inside the surface); and (vi) greenhouse structure and equipment inside it, which, due to the shadows, limit the available indoor radiation (Bot, 1983, Soriano et al., 2004, Von Zabeltitz, 1999).

The transmission of direct solar radiation will vary depending on the incidence angle (formed by the sun's radius and perpendicular to the incidence surface), transmissivity being higher when the incidence angle is lower (i.e., when the radiations are perpendicular to the surface of the glass roof). Transmissivity to diffuse radiation, which does not have a precise direction, is less influenced by the geometry of the greenhouse's roof (Baille, 1999).

### **3.3. Concentration of CO<sub>2</sub>**

The level of CO<sub>2</sub> present in the greenhouse is determined by ventilation, by photosynthesis and plant breeding and by the amount of CO<sub>2</sub> generated by the soil. During the night due to plant breeding, the CO<sub>2</sub> concentration exceeds the external concentration value of approximate 360 ppm.

During the day this concentration decreases due to increase of photosynthesis process. For a biologically active process, the CO<sub>2</sub> concentration should not drop under 30 ppm. This can be done either by ventilation (at least 20-

30 air cycles per hour to be changed in the greenhouse surface) or through CO<sub>2</sub> injection were the greenhouses run in a closed system.

Figure 5 illustrates the variation of CO<sub>2</sub> concentration in a greenhouse.

### 3.4. Air humidity

In greenhouses the evapotranspiration of crops, the evaporation of water from the soil and the condensation of water vapour on different surfaces, influence the relative humidity (RH) of the air inside the greenhouses (Day & Bailey, 1999). During the day, in greenhouses, RH decreases when the temperature rises (see figure 6), although absolute humidity increases due to evapotranspiration. The cool and dry air presented outside and which is introduced in the greenhouse during ventilation causes RH to decrease as it has a heat exchange faster than moisture exchange and thus relative humidity decreases.

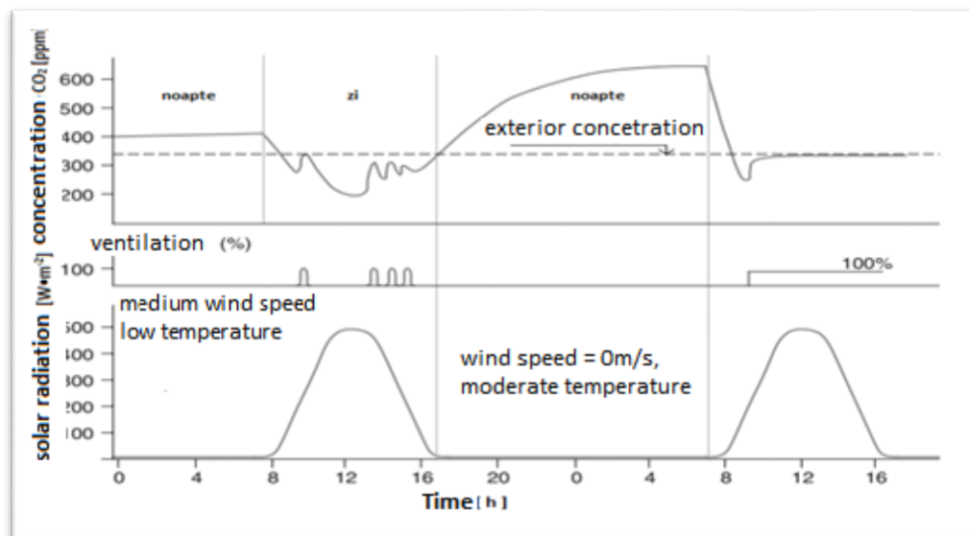


Fig. 5: The influence of outdoor ventilation and global solar radiation on CO<sub>2</sub> concentration in two autumn days in a greenhouse without CO<sub>2</sub> enrichment and in which tomatoes are grown

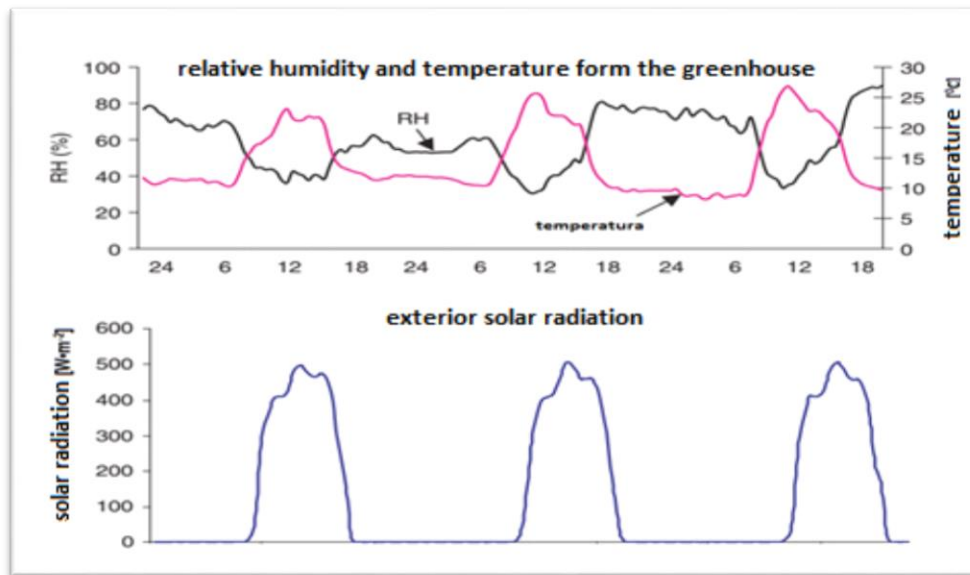


Fig. 6: Temperature and RH evolution depending on the intensity of solar radiation at the end of summer in an unheated greenhouse in Granada-Spain

During the night, as the air in the greenhouses becomes colder, RH rises and can reach its saturation level so condensation can occur on the cooler surfaces of the greenhouse, especially on the roof. If the roof has a sufficient inclination, the drops of water slide on it and then on the side walls. If the roof is not tilted the drops fall on the crop, which is not desirable. If the greenhouse is covered with two rows of foil, the inner foil will be warmer and the possible condensation time will be longer.

## 4. Research on healing and/or acclimatization chambers

### 4.1. *The healing process*

Farmers, when setting up crops, are generally looking for the most cost-effective solutions for materials and manual labour.

For this they need healthy seedlings at a certain date which is usually established before. These seedlings are most often obtained in specialized enclosures located inside the greenhouses or in other *Protected Cultivation Systems* and thus the external weather conditions cannot influence the micro-climate factors inside the seedlings, ensuring optimum conditions for high germination and a high percentage of seedling surviving.

Advanced technologies on controlling microclimate factors and the equipment that can modify these factors according to crop requirements can completely transform a seedling into a completely independent enclosure outside weather conditions.

Researches on complex actions in order to control environmental factors in this type of premises have been made since 1924, and were carried out in so-called Phytotrons (Crocker, 1948), but applications in commercial greenhouses appeared only towards the end of the nineteenth century. This type of premises is also called Growing Chambers and can have relatively small size (0.47 m x 0.36 m x 0.61 m as experienced by van Israel in 2000) and complex forces generally used for various specific research, or they can have big dimensions (such as the one developed by W. Dong, which had a 15 m<sup>2</sup> height and a height of 1.9 m) commonly used for commercial and seedling production activities.

The first prototypes of this type of chambers were developed in England and in the Netherlands in the early 1960s (Germin, 1963). These chambers are mainly used for the production of seedlings, but they could also be used to grow plants up to the maturity stage only for valuable crops or for growing plants in areas with extreme atmospheric conditions due to high operating costs. Also, this type of premises is used successfully in obtaining grafted seedlings when the internal microclimate conditions must be very strict.

Vegetable grafting is a horticultural technique by which two plants are joined together in the seedling stage to create a plant with improved characteristic to resistance to soil diseases and/or to soil nematode which corresponds to the farmers' requirements in terms of quality and productivity. Practically, the top of a plant (graft) with good fruit quality and good yield is attached to the root of a tolerant or disease resistant plant, nematodes or other extreme conditions such as soil salinity, drought, excessive humidity, etc. (Kubota et al., 2008). Grafting techniques appeared for the first time in Europe and North America since the 1950s, and they expanded a lot after the 1990s. The first written document regarding grafting technique appears in China in fifth-century and it refers to a self-grafting technique of a gourd plant. In Korea written testimonies about the use of grafting on melon seedlings exists since the XVII century.

Since January the 1<sup>st</sup>, 2008, the use of methyl bromide (BM) for soil disinfection in greenhouses and solariums (Ristaino & Thomas, 1997; the United Nations Environment Program, Ozone Secretariat, 2006) was banned in Italy and immediately across Europe. BM was used to prevent the soils from pathogens, nematodes and a wide range of weeds. But being considered a gas with high ozone

depletion potential, the Montreal Protocol was forbidden. As a replacement for this gas, a number of other chemical or non-chemical treatment methods have been developed in greenhouses and solariums. A non-chemical method of preventing diseases and pests from soil that can affect greenhouse and sunflower crops is the use of grafted seedlings.

The entire process of producing grafted seedlings, from grafting and rootstock sowing, to grafting, healing and acclimatization before planting in field / greenhouse, takes about 5 weeks. The restoration of vascular connections between grafts and rootstocks, such as calving or healing, takes about 5 days. Figure 7 shows the chronology of this process (Rivard & Louws, 2006).

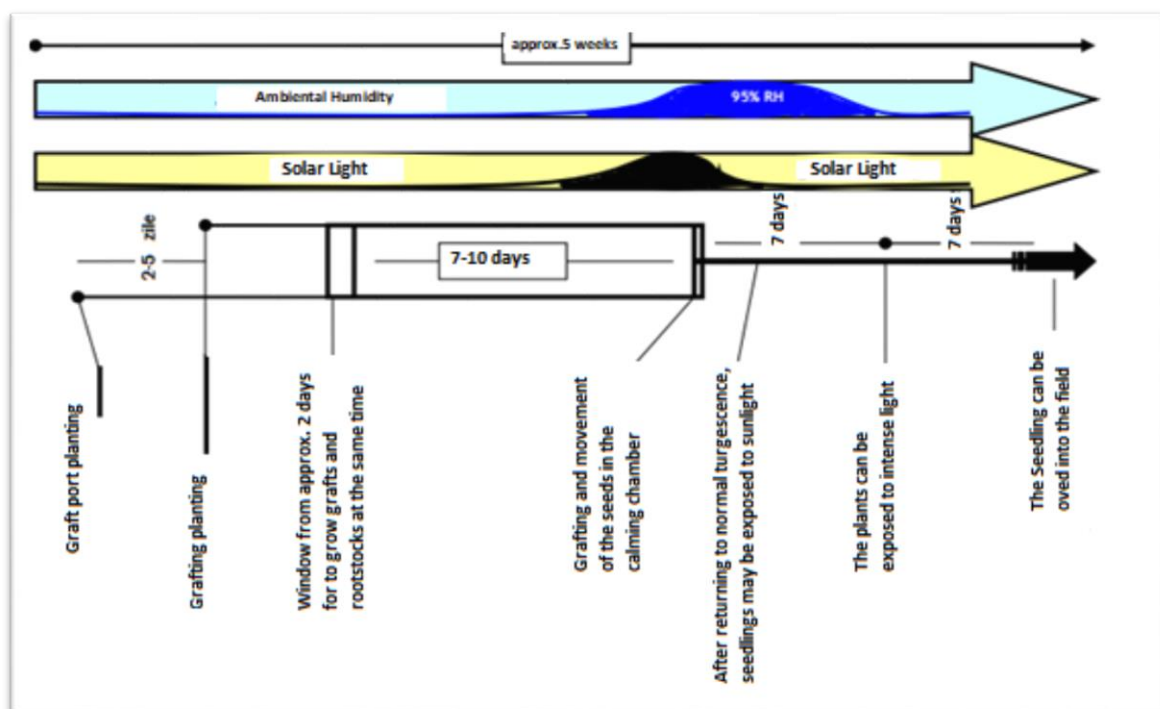


Fig. 7: Chronology of the healing process

#### 4.2. *Healing chambers with manual control of microclimate factors*

In 2006 at North Carolina State University (NCSU) - Department of Plant Pathology, C. Rivard and collaborators (Rivard & Louws, 2006) conducted an experiment to demonstrate that the graft process can be quite affordable financial speaking, for small, family farms. In grafting process, immediately after the joining of the two plants, it is necessary to restore the graft and graft port as the graft does not yet have a biological root connection and cannot be fed with water and nutrients. So the healing chamber has to reduce the stress that might occur due to

lack of water to which the graft is subjected. The best way to do this is to increase the humidity inside the premises, decrease the intensity of light radiation and reduce the temperature. In this way, the seedling evapotranspiration process is diminished. Thus, a healing chamber must ensure during the healing period high humidity, low light intensity and moderate temperature. Also, these indicators, humidity, luminous intensity and temperature must be as constant as possible. The humidity should be about 80% -95%, the temperature between 21°C and 26°C and the solar radiation intensity 0 (it is preferable to use artificial light in the first 5-7 days after grafting).

Figure 8 shows the healing chamber built by NCSU researchers, an extremely simple and cheap construction.



Fig. 8: NCSU healing chamber

The chamber is placed inside a greenhouse on a working table under a foil which can assure shade in the room. The room was built on a foil with which, by lifting the edges, a small reservoir of water will be formed, which will be the water source to maintain humidity. The skeleton was built of 1" PVC pipes. The whole structure was wrapped with plastic foil, and the access inside being very easy, and for the shading, were used black plastic bags which were cut longitudinally.

A healing chamber can be built in using different methods by using wooden frames or plastic-coated metal frames. The main purpose is to create an environment where the humidity and temperature can be controlled (Wilson et al., 2010). In general, the materials from which these healing chambers are built are chosen from the local resources such as: bamboo, PVC pipes, bricks, nylon nets, wood boards, black and transparent polyethylene film, silver/black / green shades, plastic clamps, ropes, wire or nails.



In 2011, Sacha Johnson of Washington State University (Sacha Johnson et al., 2011) proposes a new model of a callus chambers. He pointed out that the room size is very important in order to control the humidity inside, because in a high room it will be difficult to maintain a constant level of high humidity. Also, the length and width of the chamber must ensure that when it is closed or opened, a low variation of humidity inside. Nowadays, especially for healing chambers used by farmers, there is little information available on the design and use of these rooms.

For this reason, the construction and management of such healing chambers should be analysed individually. Figure 9 shows a healing chamber.



Fig. 9: Healing chamber with low height

To control the humidity inside this premises a spray hose or a spray-type manual container can be used to moisten the entire premises as well as the seedling trays before being inserted into the chamber. Immediately after the seedlings were introduced inside, fully cover the chamber and seal it as well as possible. High moisture variations in the critical 5-day period can cause inappropriate seedlings, or even survival rates may drop dramatically. The healing chambers may be the simplest as those described above or as shown in Figures 10 and 11.





Fig. 10: Grafted plant in a resealed plastic bag

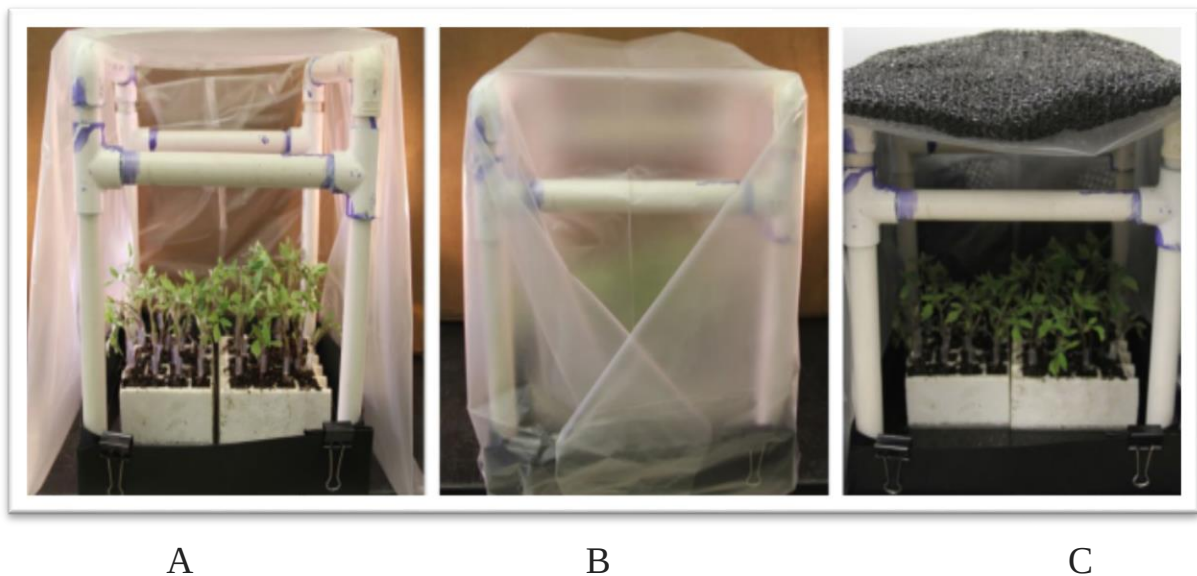


Fig. 11: Healing chamber made of PVC pipes  
(A - open, B - closed with plastic foil, C - covered with shadow web)

#### **4.3. *Automated healing chambers used in research***

In 2011, Johnson and Miles (Johnson & Miles, 2011) conducted a comparative study on the effect of the constructive solution of the healing chambers on the survival rate of grafted seedlings of eggplants, tomatoes and melons. Three types of healing chambers were selected: (i) industrial model; (ii) model for researches; (iii) Simple model used in family farms.

All three rooms were built of PVC pipes and were assembled on the growing tables. Floors were covered with transparent film for creation of humidification water tank.

A PVC film of 0.15 mm thick with a transmittance for PAR (90% active photosynthetic radiation) was used in chambers coating. A very heavy polypropylene mesh with 27% PAR transmittance was used for shading. The rooms were placed inside a greenhouse where the temperature was between 20.2°C and 24.8°C, and relative humidity was maintained within the 29% and 68% range. The intensity of the light radiation was artificially supplemented so as to provide a 12-hour lighting interval similar to diurnal illumination. The materials used for covering the rooms, the humidification method, the dimensions and the volume of the three rooms are summarized in Table 1.

Table 1: Material, humidification, size and volume description for the three room types

| Healing chamber model | Materials used for coating and shading | Moisture mode                                             | Dimensions (m)  | Volume (m <sup>3</sup> ) |
|-----------------------|----------------------------------------|-----------------------------------------------------------|-----------------|--------------------------|
| Research              | Shading mesh, plastic, humidifier      | Plants were wet every 5 minutes for 20 seconds            | 3.0 x 1.2 x 0.8 | 2.88                     |
| Industry              | Plastic shading mesh                   | Interior surfaces manually sprinkled on day 1, 3, 5 and 6 | 2.2 x 0.9 x 0.6 | 1.19                     |
| Family farms          | Shading mesh                           | Plants sprinkled manually twice a day                     | 1.7 x 0.8 x 0.5 | 0.68                     |

Relative temperature and humidity were measured at 5 minute intervals. The plants stayed in these rooms for 7 days and then removed in the greenhouse for another 7 days. The survival rate was observed from day 6 after grafting till day 14. The recordings show that daily temperature fluctuations were made in all three models of healing chambers, the minimum values of 22.2 °C being recorded between 02:00 and 04:00 and the maximum values of 25.6 °C between 14:00 to 16:00 (see Figure 12). The temperature variation in the three room models was similar to that of the greenhouse temperature in which the experiments were made. The relative humidity variation (RH) had the same aspect in all three rooms when they were closed on day 2, 3 and 4, with maximum values between 02:00-04:00 and with minimum values around 16:00. Only the family farm model has a daily

decrease in RH because it has not been covered and retained moisture. The industrial model room had the highest RH value on day 2 and 3, the research model room reached its maximum value on day 4 and both had similar RH values on days 5 and 7.

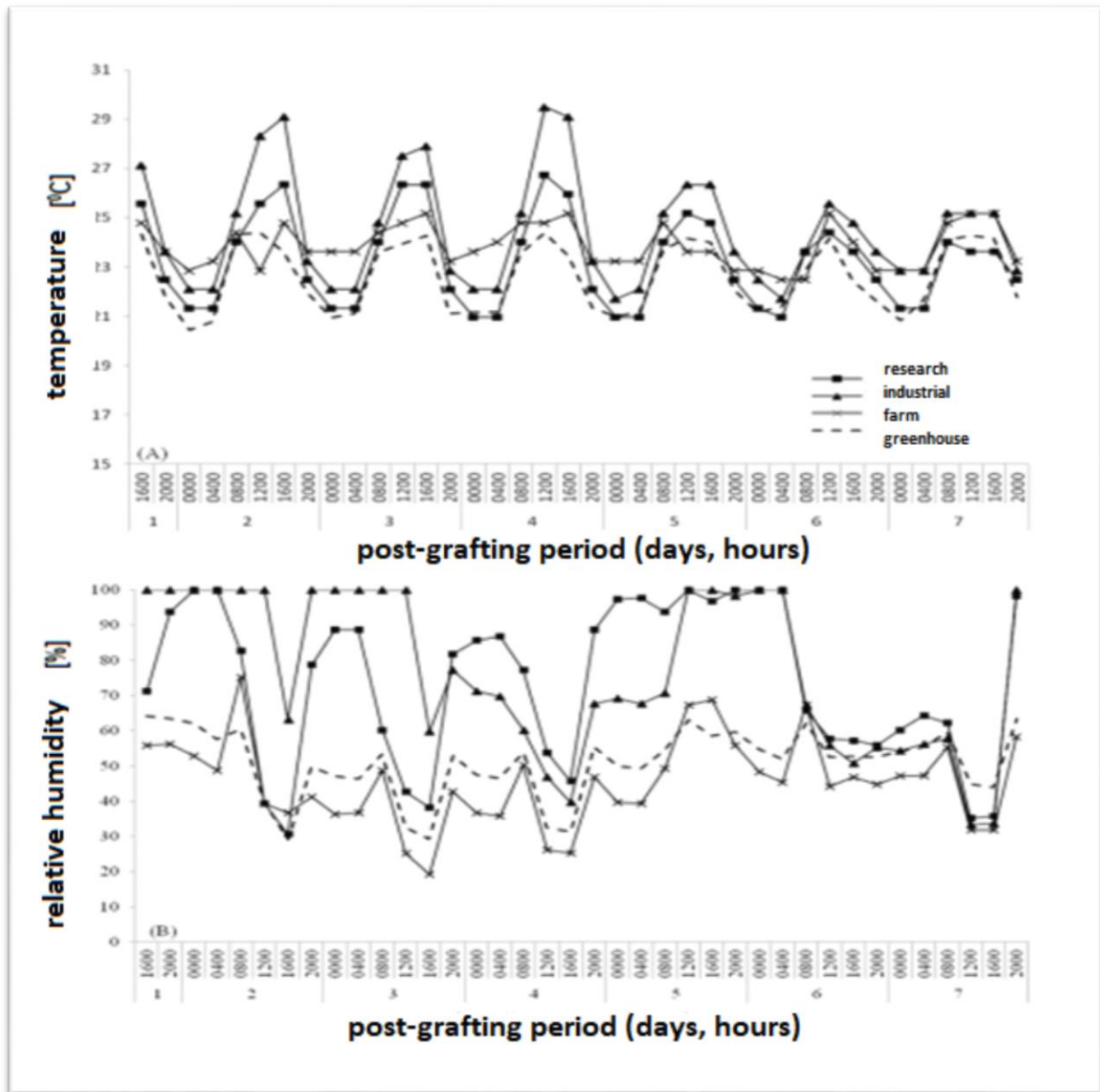


Fig. 12: Relative temperature and humidity variation in the three models of healing chambers and in the greenhouse where experiments were conducted

In the industrial and research room, there was found a vapour pressure deficiency of 0.06 kPa and 0.51 kPa, respectively. This vapour pressure deficiency, calculated as the difference between vapour saturation pressure  $p_s$  and partial vapour pressure  $p_v$  in a given volume of air having values close to 0 kPa shows that

relative humidity  $\phi$  is, in that volume, the highest value. Both rooms were closed-type.

Evaporation in this type of system occurs until all available moisture is consumed or until the air in the premises becomes saturated moist air, i.e. the water vapour pressure reaches the saturation value. The speed at which pressure is reached depends on the temperature and the ratio between the total air volume and volume of the water vapour  $V / V_v$ .

High temperatures lead to an acceleration of the evaporation process and a decrease in relative humidity, although the evaporation rate decreases as the pressure increases towards saturation pressure (Giancoli, 2005). Since the volume of the healing chamber has about 58% less volume than that of the research room, and by calculating the value of the vapour pressure in the industrial type chamber for a certain temperature, it is found that the volume of the water vapour in the premises is sufficient for the saturation pressure and thus higher relative humidity could be reached more rapidly than in the research room. Thus, in the industrial room, relatively higher moistures were obtained during the 7<sup>th</sup> day of healing periods, even if the average temperatures were higher than in the research room. So, a very important and constructive result of the healing chambers is that the size must be reduced to the minimum so the relative humidity to be close to saturation, and the process of leaf-evapotranspiration to be as small as possible.

A research model similar to the one used in Johnson attempts was also used by Mun B. and colleagues (Mun, Jang, Goto, Ishigami, & Chun, 2011) to test the CO<sub>2</sub> exchange rate measurement system that has place in the healing chambers during the healing and acclimatization of vegetable seedling and cucumber seedlings. The CO<sub>2</sub> exchange rate gives accurate information on the intensity of the photosynthesis process of the seedlings. An intense photosynthesis process with increases in CO<sub>2</sub> concentration shows that the connection between the graft and graft port has been achieved, so the seed has been cured / healed. This enclosure is shown in Figure 13.

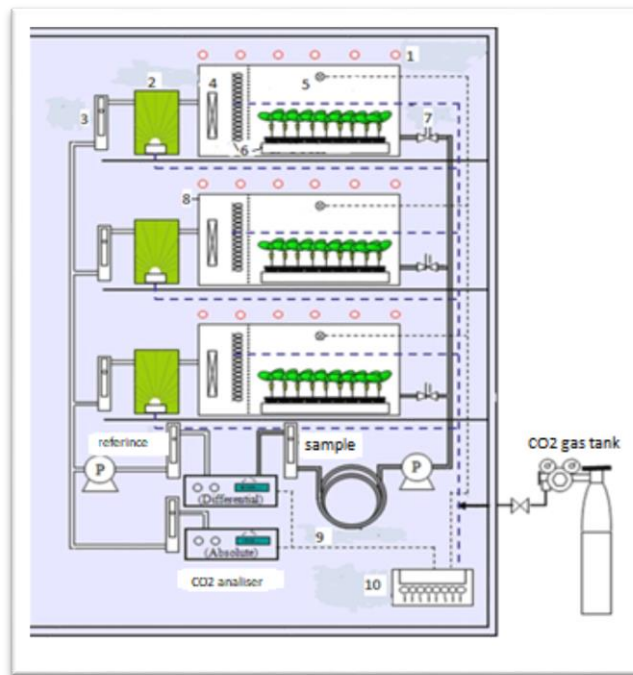


Fig. 13: Scheme of semi-open system with 3 healing chambers used at Chiba University-Japan for continuous measurement of CO<sub>2</sub> exchange rate.

Legend: 1 - florescent lamp; 2 - humidifier; 3 - flowmeter; 4 fan; 5 - temperature / humidity sensor; 6 - heater / cooler; 7 - 3-way solenoid valve; 8 - healing room; 9 - Dehumidifier; 10 - process calculator - data logger and controller

The microclimate factors inside the healing chambers were monitored every 3 minutes during the experiments. The humidifier, heater and cooler were monitored by a process computer (PC). Different intensities of photosynthetic photon flux density (PPF) at different relative humidity RH values were applied in each room. The three values of the microclimate conditions were: (i) 120  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$  and 85% RH, (ii) 200  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$  and 80% RH, (iii) 200  $\mu\text{mol}/(\text{m}^2\cdot\text{s})$  and 70% RH. The lighting period was 14 hours a day. During the darkness RH was not controlled but was maintained around 95% - 98% by permanent humidification. The three levels of light intensity PPF were achieved by changing the number of light sources. To keep the temperature around 27°C, electrical resistors and a cold water heat exchanger were used. For the control of relative humidity, humidifiers were used, the flow of air introduced into the enclosures first passing through them. Correction of the CO<sub>2</sub> level was done by injection of liquid CO<sub>2</sub>. The CO<sub>2</sub> concentration at the entrance of the room was measured with a total gas analyser, and the CO<sub>2</sub> exchange rate, basically its concentration in the air at room exit, was measured with a diffuse gas analyser after the air was first dehumidified. According to Mitchell (Mitchell, 1992) the parameters of the photosynthesis process are calculated with the formula:

$$P_{total} = \frac{f \cdot \Delta CO_2}{A} \dots \left[ \frac{\mu mol}{m^2 \cdot s} \right] \quad (3)$$

where:  $f$  = the flow of air through the enclosure in mol/s,  $\Delta CO_2$  = the difference in concentration of  $CO_2$  in air which enters and in the air leaving the chamber in  $\mu mol/ mol$ ,  $A$  = the area of the seedlings in  $m^2$ .

The  $CO_2$  exchange rate for grafted cucumber seedlings during the healing and acclimatization period for the three microclimate conditions is shown in Figure 14.

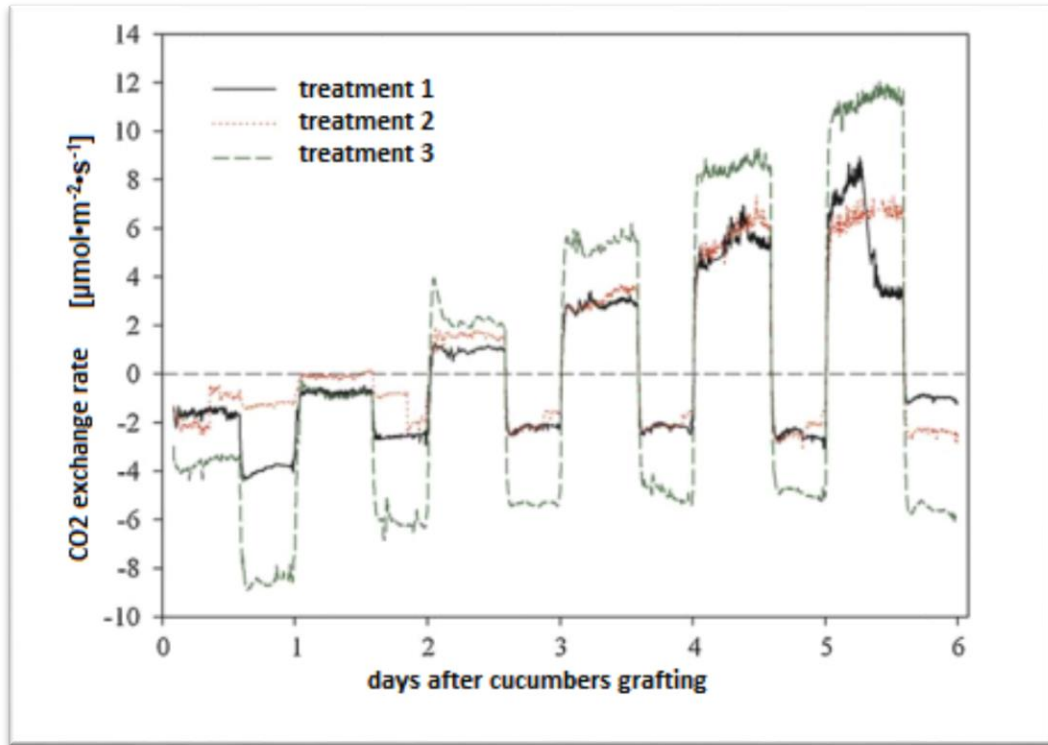


Fig. 14:  $CO_2$  exchange rate during calving and acclimatization of cucumber seedlings grafted for 3 variants of microclimate

In Figure 15 one can actually see such a system of healing chambers used in many experiments on grafted seedlings and not only.



Fig. 15: Image from the interior of the healing system used in research

This system can be used to investigate how the healing of grafted seedlings is influenced by light quality, temperature variation, air composition, relative air humidity, air velocity in the enclosure, etc.

However, similar equipment with 10 distinct rooms was used by M.W. Van Iersel (Van Iersel & Bugbee, 2000) from the Department of Horticulture at the University of Georgia, USA. This multi-chamber system allows the installation of multiple experimental variants with different microclimatic and / or biological parameters. The scheme of such an installation is shown in Figure 16.

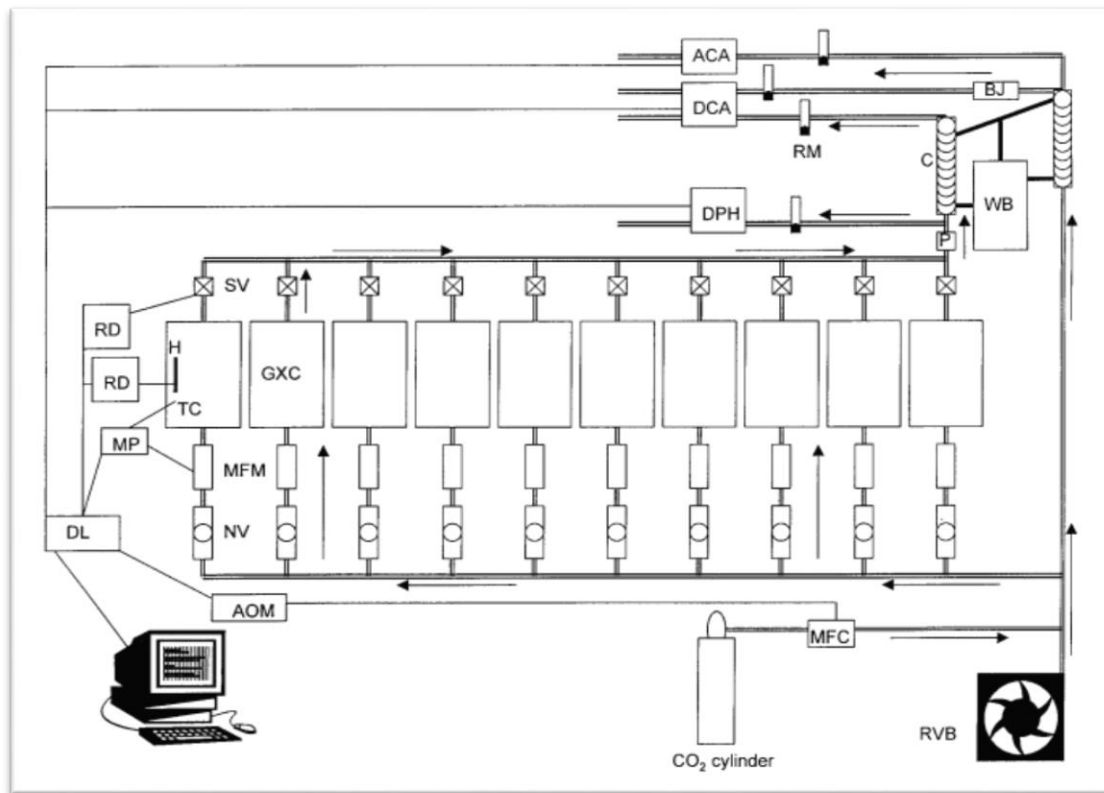


Fig. 16: Schematics of semi-open system with 10 healing enclosures.

The notes made in Figure 9 are: ACA (absolute CO<sub>2</sub> analyser) = Total CO<sub>2</sub> Analyser; AOM (analogue output module) = Analogue output module; BJ (buffer jar) = Vas swab; C (condenser) = condenser for H<sub>2</sub>O; DCA (Differential CO<sub>2</sub> Analyser) = Differential CO<sub>2</sub> Analyser; DL (data logger) = data storage mode; VAT (dew point hygrometer) = Hygrometer, GXC (gas exchange chamber) = Calling room; H (heater) = heater; MFC (mass flow controller) = mass flow controller; MFM (mass flow meter) = mass flow meter; MP (multiplexer) = Multiplexer; NV (needle valve) = Needle electrodes; P (pump) = Pump; RM (rota meter) = Flow meter; RVB (rotary vane blower) = Ventilator; SV (solenoid valve) = Electro valve; WB (water bath) = Water basin; RD (relay driver) = Relay; TC (thermocouple) = Thermocouple

The operating principle is as follows:

- the RVB fan introduces air through separate pipes in the 10 chambers;
- the CO<sub>2</sub> concentration is measured with the ACA infrared analyser; infrared analyser measures how much infrared radiation absorbs a gas - an infrared light beam is emitted through a volume of air and, depending on the concentration of a particular component, the gas heats up, increases the



pressure inside it and compares to the standard pressure can determine the concentration of that component;

- At the entrance to each room is measured the air mass entering a MFM table counter; mass measurement is preferred for measuring volumetric flow because volumetric measuring devices cannot distinguish temperature or pressure changes this results in errors while mass flow meters automatically correct the flow rate; Figure 17 illustrates that the same mass may occupy a different volume if the pressure varies; they are linked via MP multi switches to the DL data acquisition module.

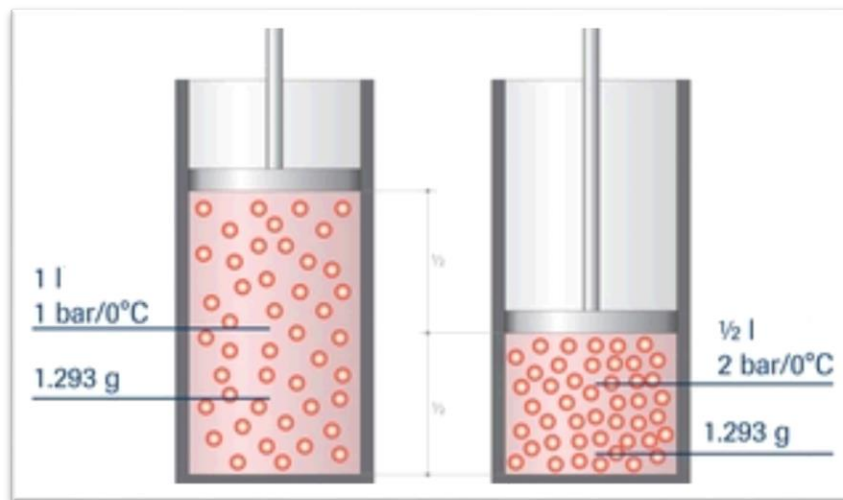


Fig. 17: Volume variation with constant mass variation

- the SV ventilators control how the air exits the rooms; they are opened independently, at intervals of 16 second so the measurements can be conducted for each chamber
- the air presented in each chamber is pushed by pump P through a DHP hygrometer and then through the condenser C to the differential infrared gas analyser; the air is passed through the condenser to remove water vapour that may interfere with the CO<sub>2</sub> gas analyser (Bugbee, 1992); the DCA gas analyser measures the difference of CO<sub>2</sub> concentration from the entrance in the chamber and the exit from the chamber. the amount of CO<sub>2</sub> that is introduced in each chamber is adjusted with mass flow controller MFC, which is managed by the DL data storage module and an AOM analogue output module; in case of CO<sub>2</sub> concentration variation it can be adjusted by NV valves;
- for the control of humidity inside each chamber a radiator heat exchangers is mounted with a fan through which cold water circulates; the humidity is

adjusted by adjusting the temperature of the water which circulates through this heat exchanger; the dew point is permanently measured with the DHP hygrometer, which is linked to the DL data storage module; the dew point temperature is close to the temperature of the chiller; thus the relative humidity in the chamber can be adjusted with help of the cooler's temperature and ambient air temperature; the condensed water is pre-drilled to the water tank WB

- For heating, it is used a 50/100W resistor mounted in each chamber near the fan which is used also for cooling; the DL data acquisition module is in charge of turning on or turning off this resistor depending on the setted temperature;

Figure 18 shows a schematic diagram of a healing having the following size: of 0.47 m in length x 0.36 m in width x 0.61 m in height. These chambers are designed in an open system, so they can be used in various healing researches. Inside them, smaller junctions can be added for use in various research, and the control of microclimate conditions inside the chambers is basically the control of microclimate factors for research inside them.

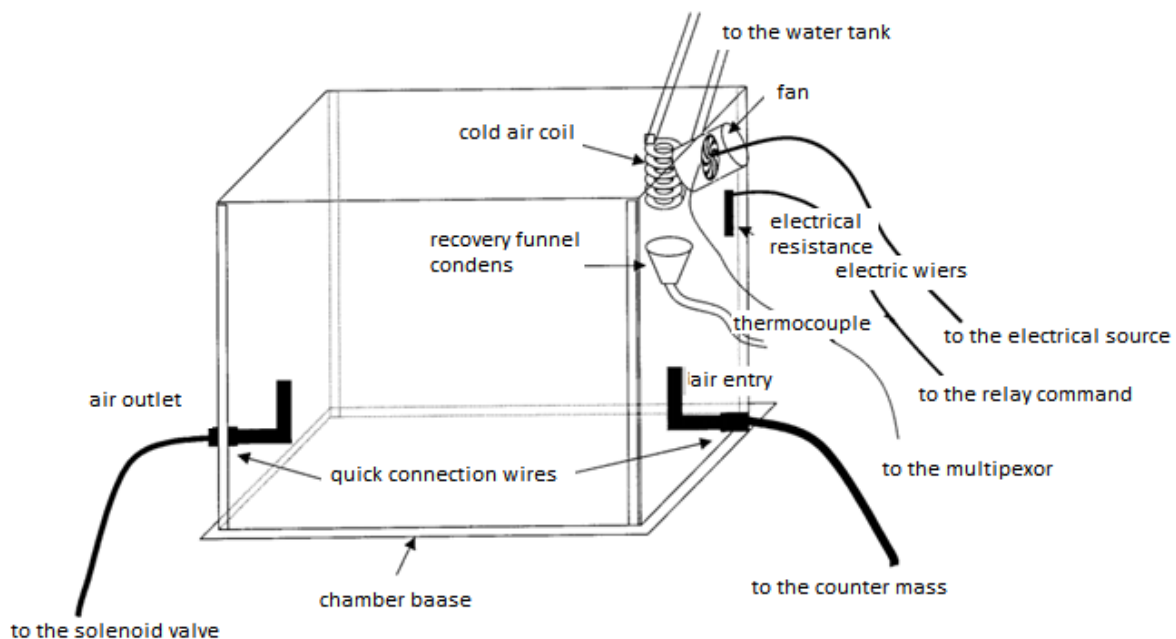


Fig. 18: Drowing of the healing chamber

As a result of the researches conducted with this type of chamber system, it can be concluded that these multi-compartment systems offer the possibility of

simultaneous investigation of several parameters which characterise the processes that occur during the callming process, which represents is a great advantage because the interaction between these parameters can be decisive in the healing process.

In the post-grafting period, the microclimate conditions in which the capillary ties of the graft seedlings are restored, must be managed with great care. The researches conducted in such systems have as main purpose, for different vegetable varieties, to determine with accuracy the optimal values of the microclimate factors for the period of healing process and acclimatization of grafted seedlings. Not only, by using these research devices, it was possible to determine the optimum conditions for the development of the two components of grafted seedlings, graft and rootstock so that they are physiologically compatible.

#### ***4.4. High productivity automated tapping chambers***

The healing process of the grafted seedlings can significantly affect the survival rate of the seedlings. Inappropriate microclimate causes uneven development of the two components, grafts and rootstocks, or even their searing. The sudden change in external climate factors can dramatically affect the microclimate in the sealing and / or acclimatizing areas of the seedlings. In such cases, farm workers must observe and intervene to maintain optimal development conditions in these enclosures by ventilating, covering, shading, watering, etc.them. So the work of farm workers must be highly qualified, very precise, but also very intense (Chiu, Chen, Chang, & Chou, 2007).

As the use of grafted seedlings is developing more and more in the world, the necessity of replacing manual control of microclimate conditions in healing chamberswith automation systems is obvious. There is also a specialization of farms in exclusive seedlings production. This specialization generates qualitative, quantitative and obvious benefits for both seedlings producers and farmers who use these seedlings. This specialization of industrial type required the design and development of healing / acclimatization chambers that could provide plants in real time, with optimal microclimate conditions.

Figure 19 presents the general principle of monitoring and controlling the microclimate factors in industrial healing / acclimatization chambers.

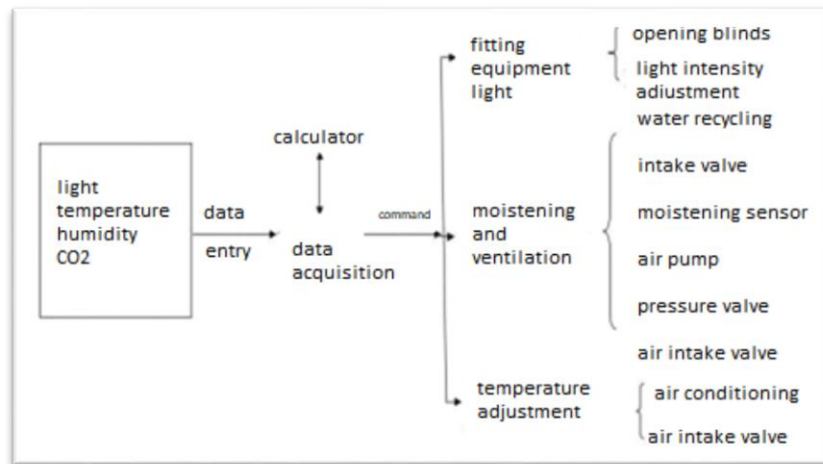


Figure 19: Schematic diagram of a system for controlling environmental conditions from a healing room

In this regard, in 1999, Chiu and collaborators (Chiu, Jou & Chen, 1999) developed a closed automated callming room at Lan Yang Institute of Technology in Taiwan. The room dimensions were 3520 mm x1800 mm x 2100 mm, where the isolating walls have a thickness of 50 mm and the internal height is 1850 mm.

The transfer of heat between the inside and the outside is negligible because the room is well thermal isolated. The room heating depends on the heat generated by the lighting system, fans, and the heat generated by the breeding of the seedlings. So the temperature will increase as long as the system is operating, being less during the cooling of the enclosure. An experiment has been conducted to assess the rate of temperature increase as lights and fans are operating, and so it can be determined whether additional heating or cooling equipment is needed.

In the room, 5760 seeders can be placed simultaneously on 5 trolleys with 5 shelves each, and on each shelf there are 4 trays with 72 cubes of nutritive seedlings. Figure 20 illustrates a schematic representation of the acclimatization chamber developed by Chiu. For this experiment, it was intended to keep the microclimate conditions for melon seedling constant and uniform, i.e. the temperature between 25°C and 28°C, the relative humidity at 95% and 12 hours per day of illumination.

In order to verify the uniformity of microclimate factors inside the chamber, Chiu placed sensors for measuring them in 27 points as can be seen in Figure 21.

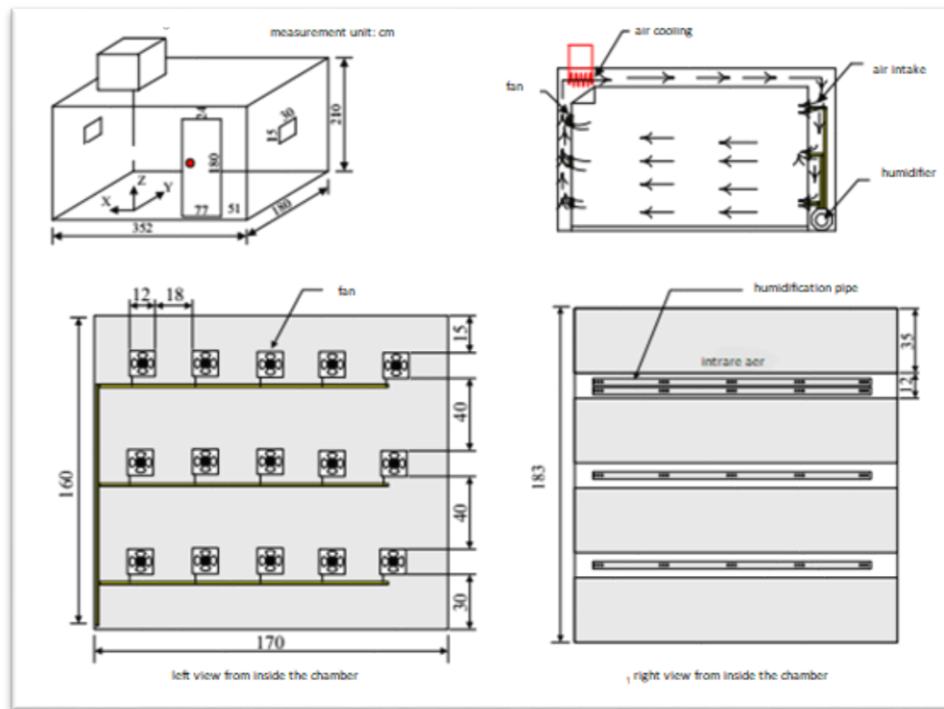


Fig. 20: Scheme of the acclimatization room

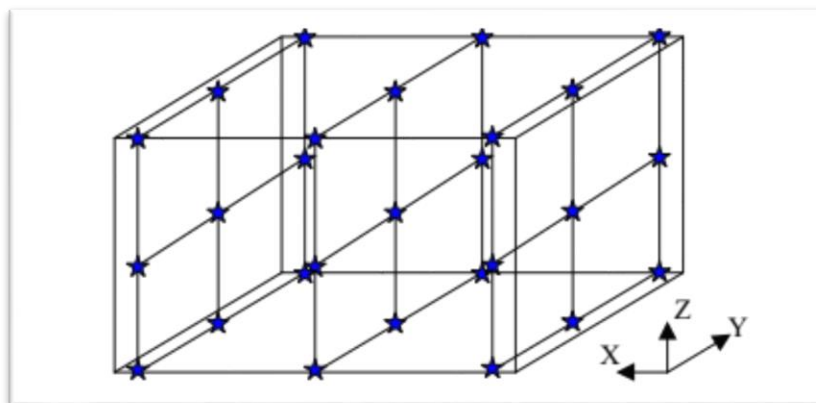


Fig. 21: Plan of location of sensors for measuring microclimate parameters inside the room

For monitoring the microclimate factors, the nonlinear sequential control method was used. The implementation of sequential control involves using both the method of setting an artificial microclimate and the method of using existing microclimate factors outside the room. Thus, if the external weather conditions are close to the healing conditions required for the seedlings, the outside air is directed to the chamber. Under other conditions, the system will close the air outlets and like this will form a closed system in which the microclimate factors will be controlled by specialized equipment.

The control of microclimate factors was done following the following strategy:

- i. If the indoor temperature exceeds the maximum setted temperature, the air conditioning system will start up. It will stop if the interior temperature drops below the minimum;
- ii. If the humidity drops below the setted minimum, the humidifier will start to operate and will stops when it reaches the maximum set value;
- iii. If the weather conditions are within the setted limits, the ventilation system of the chamber is activated and allows the ambient air to enter inside the premises. In this case the conditioning and humidification systems will be switched off if they were turned on
- iv. The lighting system and the ventilation windows are periodically activated by timing relays. All equipment can be turned on or off manually.

Figure 22 illustrates how the room temperature has increased with the lights and fans running. From the initial temperature of 19.5°C after 24 hours the temperature graduallyit reached about 45°C. The temperature grew faster in regions with lower values and grew slower in areas with higher values. Knowing that the temperature in thepremises should be in the range of 25°C to 30°C, it was wanted to know how long these set values had been reached since the system started. Figure 3 shows that in about 58 minutes from the start of the system the temperature reaches from 19.5°C to 25°C and in about 90 minutes from 19.5°C to 30°C.

The results of this research have shown that such a healing / acclimatization chamber can fulfill the optimal microclimate conditions for the development of grafted seedlings in the post-graft period.

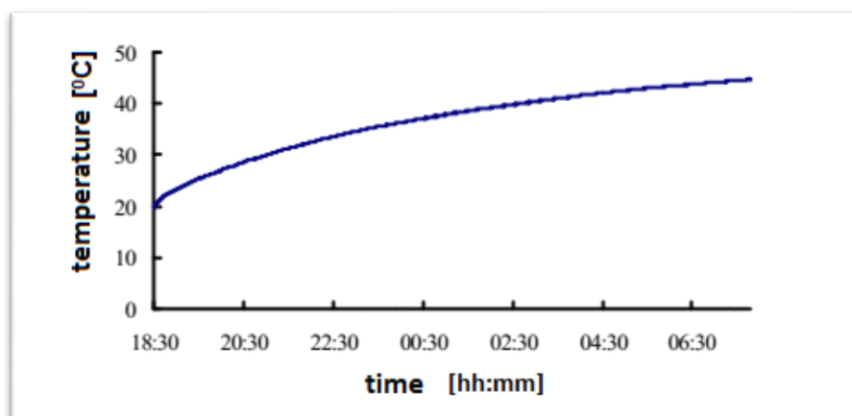


Fig. 22: Increasing the temperature in the tapping room under the effect of the system lighting, ventilators and plant breathers

In addition, Chiu and a team of collaborators, used in 2007 (Chiu et al., 2007) the same healing chamber in a research which aim was to to fully automate the grafting process. The aim was the development of a robot to help in grafting of vegetables process, in addition to a healing chamber. The developed room was larger than the previous one and could accommodate 12.960 seedlings during the healing period. The same microclimate control strategy was also used in this enclosure.

As in the previous experiment, the developed chamber shown in Figure 23 provided optimal microclimate conditions for the healing of altiotic seedlings. Temperature variations were about 1°C, relative humidity variations of about 3.6% and wind speed was less than 0.21 m /s.



Fig. 23: Snapshot inside the healing chamber

Japan has invested heavily in the development of complete vegetable grafting technologies, so Mitsubishi has built a healing chamber that can automatically provide optimum microclimate conditions for seedlings. Also in China, Beijing Jingpeng International Hi-Tech Corporation made researches and developed healing chamber owned by the industrial production of grafted vegetable seedlings (Dong, 2015). Starting from the general principle shown in Figure 10 taking into account the fact that we have a perfect gas in the enclosure for precise and real-time control of temperature, humidity, light, CO<sub>2</sub> concentration and other factors, it is necessary to design hardware and software applications.



Chinese researchers have proposed that healing chamber to be developed where the temperature can be adjusted between 15°C and 30°C with an accuracy of  $\pm 0.5^{\circ}\text{C}$  with a relative humidity that can vary within 60% and 90% with an adjustment accuracy of  $\pm 3\%$  and a light intensity which can be made between 0 and 5000 lx.

The chamber developed by Chinese researchers has the following structure: a smart microclimate control system, a humidification system, fertilization equipment, ventilation equipment, CO<sub>2</sub> concentration control equipment, temperature and light control systems, a sealing and protection against the external environment.

The Intelligent Environment Control System (IECS) is made of an intelligent control box, an acquisition module and a data control module, an analog input module, a output module and control module.

The humidification system used can produce fog with droplets of less than 20 $\mu\text{m}$  diameter. This can quickly increase humidity and temperature decrease.

The lighting system, by providing variation in intensity, quality and lighting time, can influence the photosynthesis process. Controlling this parameter can reduce the evapotranspiration of seedlings and in this way wilting them during the healing period.

After designing and execution of this healing chamber, grafted cucumber seedlings were introduced into the enclosure and the microclimate monitoring systems were checked. Figure 24 shows the chamber which was ready for experimentation.



Fig. 24: Healing chamber developed in China



The measurements made have shown that the microclimate parameters in the enclosure can be controlled and modified with precision. The development of such fully automated chamber can meet the needs of farmers regardless of the existing natural conditions.

On a grafting plant in Yu-chia, Taiwan, about 1 million seedlings are produced per year. Healing is done in healing chamber equipped with ultrasonic humidifiers with temperature control and floating lamps as it can be seen in Figure 25.



Fig. 25: Closed room in Yu-Chia Nursery, Taiwan

In Japan, Jardin Co., Ltd. of Chiba Prefecture specialized in the production of vegetable and ornamental plants produces about 15 million seedlings annually. For the growth and healing they use closed enclosures, fully automated. Such as a room is shown in Figure 26, with automatic lighting control (12-14 hours / day) of temperature and humidity (about 70%), with variable flow irrigation system and water recovery system and with a CO<sub>2</sub> enrichment system while maintaining it at about 1000 ppm.



Fig. 26: Healing chamber in Jardin Co., Japan,

Figure 27 illustrates how these chambers are placed in specialized spaces. To produce about 6 million grafted seedlings per year, the farm has 20 permanent workers. The automated healing chambers are also equipped with water recirculating systems used for humidifying rooms and irrigating seedlings. Ohyama et al. (2003) states that more than 90% of water used during the healing period can be recycled. The principle of water recycling is shown in Figure 28.



Fig. 27: Healing chambers in a seedling farm

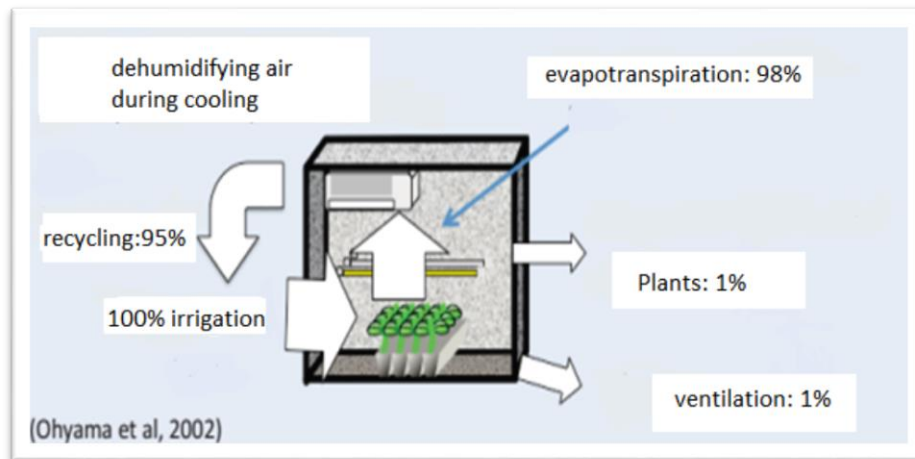


Fig. 28: Basic scheme of efficient water use

Nowadays more and more industrial seedlings use closed systems of healing chambers called CPOS (Closed-type Plant Production System). Mirai Co., Ltd. of Japan has implemented such a system for producing especially leafy vegetables.

Such a "plant factory" has an area of 500m<sup>2</sup> and a height of 6 m. By controlling the temperature, the light (using fluorescent lamps and LED light) and the air flow in a closed and sterile system, the danger of diseases and pests is minimal and pesticides are not used at all.

Due to the fact that drinkable water is used for irrigation and humidification, vegetables are "ready to eat" and packed immediately after being harvested in the same room without rinsing. Figure 29 shows a fully automated closed-type farm.



Fig. 29: Fully automated closed-door chamber for the industrial production of "ready-to-eat" seedlings and / or vegetables

## 5. Conclusions

As a result of the use of grafted seedlings, there is a rapid increase in the demand for such high-quality seedlings by farmers. The obtaining of grafted seedlings thus becomes an important branch of horticultural production.

A very important element in obtaining the quality and quantity required is the microclimate factors in the premises where the seedlings are grown. The stages to obtain grafted seedlings are relatively short, fluctuations in environmental factors may have dramatic effects on the quality and quantity of grafting seedlings production. For this reason, the use of artificial environments for grafting grafted seedlings is a very useful method. The advantages of using an artificial environment are mainly the predictability of the production indicators and the standardization of the seedlings quality as a result of the constancy of the microclimate conditions in the healing chambers.

The research on the processes taking place in the *Protected Cultivation Systems* of the crops was oriented, especially to the analysis of the microclimate factors, their interaction and the influence of the constructive elements on them in greenhouses and solariums. As far as the microclimate conditions of the healing installations are concerned, their management was *a priori* considered that the same processes are being carried out here.

The healing and acclimatization chambers, from a technological point of view, have developed a lot. The development and their automation was done from a strictly technological horticultural requirements. Even though the processes on the premises seem to be similar to those in greenhouses / solariums, these processes are different in healing rooms. Healing chambers are generally either small and very sophisticated premises generally used in research, or large, less complex enclosures, especially used for industrial production of grafted seedlings.

The growth in demand for grafted seedlings makes that their traditionally producing in greenhouses and solariums to become an obsolete method at a given time. At the same time, this may lead to the development of interdisciplinary researches to analyze the dynamics of microclimate factors and other phenomena that occur in healing chambers, so that within them can be achieved optimal conditions of development and callusation of aliot seedlings, irrespective of environmental conditions from outside.

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Through the researches and experiments that will continue to be carried out in this project, the monitoring of the microclimate conditions required to be met in the specified callming chambers for each type of plant analyzed, the monitoring of the acclimatization dynamics and of the other phenomena that occur in the healing chambers. These experiments will create the premises for the development of a call tunnel that can provide maximum productivity or more accurately a high survival rate of grafted vegetables and high seedling quality through an optimal plant growth control environment.

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