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Research Article

Experimental research on the variation of microclimate factors in healing tunnels designed for grafted vegetable seedlings

Ionel L. DUMITRESCU[1*], Marian BOGOESCU[1,2]

[1]*Research and Development Institute for Processing and Marketing of Horticultural Products - HORTING, 5N Drumul Gilaului Street, District 4,041715, Bucharest, Romania; lucian.dumitrescu@horting.ro (*corresponding author)*
[2]*Academy of Agricultural and Forestry Sciences, 61 B-dul Marasti, District 1 011464, Bucharest, Romania; marianbogoescu@gmail.com*

During the healing process, the scion and the rootstock must establish vascular connection, which is considered the most critical process in the production of grafted vegetables. Therefore, the healing process must be carried out in a monitored and controlled environment, in special healing areas, where parameters characterizing the microclimate must be maintained at the optimal values, ideally with great precision. The main objective of the current experiment was to analyse the variation of microclimate factors in the curing and acclimatization chambers of grafted vegetable seedlings during specific technological phase. The measurements of different abiotic factors such as relative humidity, temperature, light radiation intensity and CO_2 concentration were carried out inside an experimental research healing tunnel situated inside a greenhouse. After grafting, the grafted seedlings were placed in specialized enclosures for their healing and the results shown that variations of the plants density and determined microclimate factors significantly affect the quality of seedlings. Because the stages of obtaining grafted seedlings are relatively short, fluctuations in environmental factors can have dramatic effects on the quality and quantity of grafted seedling production. Such a study of the prediction of microclimate factors must lead to the development of a versatile prediction model that can be used in any climatic conditions, at any time of the year and in any geographical location.

Keywords: grafted vegetables; greenhouse; healing tunnels; microclimate

Introduction

Vegetable grafting is a horticultural technology that combines parts of two plants. Through this procedure, it is aimed that the newly created plant will be resistant to diseases and nematodes from the soil and, at the same time, has the highest possible productivity and quality of the fruits. The new plant created by this process borrows qualities from the plants of origin of the two elements that are joined. Respectively, the graft will produce fruits with the characteristics of the plant from which it comes and the rootstock will offer resistance against diseases and/or soil nematodes (Kubota *et al*., 2008; Maurya *et al*., 2019; Thies, 2021). Cultivation of grafted vegetables was first used in Japan and Korea in the late 1920's. Initially, watermelons were grafted onto a pumpkin rootstock (Yamakawa, 1983; Malik *et al*., 2021). Since the late 1950's, a rapid increase in the use of grafted seedlings was observed throughout the world. During this period, the purpose of

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grafting was also expanded. It was initially started to reduce infection by soil-borne diseases caused by pathogens such as *Fusarium oxysporum* Schlecht (Marukawa and Takatsu, 1969; Ryu *et al*., 1973; Hirata, 1975; Yamakawa, 1983; Itagi, 1992; Lee, 1994). Other research has shown that the use of certain rootstocks can increase the tolerance of grafted seedlings to low-temperatures of soil, a situation that farmers may encounter in greenhouses covered with plastic film (Rivero *et al*., 2003; Edelstein, 2004; Aidoo *et al*., 2017), to improve salt and wet/dry soil tolerance (Park, 1987), to improve water and nutrient uptake (Marukawa and Takatsu, 1969; Gomi and Masuda, 1981; Masuda and Gomi, 1982; Kate and Lou, 1989; Kim and Lee, 1989; Heo, 1991; Jang, 1992) and to provide a vigorous plants growth. Among other purposes of using grafted seedlings, it was also aimed to extend the duration of the economic period of plant production (Itagi, 1992; Ito, 1992; Malik *et al*., 2021).

There are several factors that can contribute to an optimal grafting, such as genetic compatibility, the diameters of the stems of the two plants to be joined by grafting- to be relatively equal, the healing process of the new grafted plant which should take place in optimal environment conditions (Maurya *et al*., 2019). Even more, successful vegetable grafting requires high relative humidity (RH) and optimum temperature for one week after grafting, to reduce scion water loss through evapo-transpiration. This phenomenon occurs because the vascular tissue between the scion and the rootstock is not restored and therefore there is no transport of water between the two parts. Generally, in the healing room, according to the seedlings' cultivar, the adjustable temperature ranges from 15 to 27 °C, with a control precision of ±0.5 °C, whereas relative humidity (RH) must be in the range of 60-95%, with a precision of ±3% and the light in the range of 0 to 5,000 lx (Hu *et al*., 2011).

The stages of the grafting process are relatively short, but even so, fluctuations in environmental factors can have dramatic effects on the quality and quantity of grafted seedlings production. Monitoring microclimate factors during grafted seedlings' healing, development and acclimation is essential for vegetable seedlings' survival (Lee and Oda, 2010; Maurya *et al*., 2019). However, it is quite difficult to control and create an optimal environment for healing and acclimatization of grafted seedlings under natural conditions. The variation of microclimate factors in healing and/or acclimatization enclosures can be predicted either by experimental measurements or by numerical modelling.

Healing rooms are very varied from a constructive point of view. Depending on the volume of seedling production, they can be small and extremely simple, like plastic bags, or they can be complex structures like healing tunnels. Curing chambers can be manually controlled environmental factors, enclosures typically found on small farmers, or, for large and specialized farms, environmental factors can be managed by intelligent environmental control systems. The experimental measurements presented in the hereby study undoubtedly provide real, reliable, specific and valid results for the model healing/acclimation enclosures in which they were performed. Monitoring microclimate factors in interaction with external climate factors, as well as their interaction with plants, can increase the understanding of the physical phenomena that occur in these enclosures and can be used to test any design concept for such enclosures, to optimize the healing processes of vegetable seedlings and to manage the variation of microclimate factors.

Materials and Methods

Experimental conditions

The experiment was carried out in a healing tunnel prototype placed in the research greenhouse at the HORTING Institute in Bucharest, Romania. The biological material used in the experimental determinations was a grafted seedling consisting of a Romanian eggplant hybrid 'Andra' F1 as scion and for rootstocks was used a Dutch tomato hybrid 'Emperador' F1.

Measurements of the microclimate indicators were performed in 4 independent zones, built on the structure of the healing tunnel commonly used in HORTING research greenhouse. Each compartment has the following dimensions: 1,8 m length, 1,5 m width and 1 m height. The volume inside each of these compartments is 1.89 m^3. Figure 1 shows an overview of healing tunnels situated in the grafting dedicated area and Figure 2 presents a schematic diagram of a 5-section tunnel.

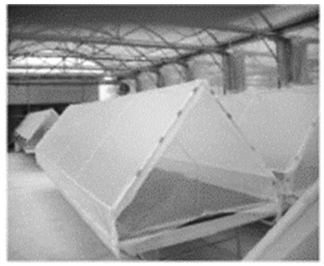

Figure 1. Healing tunnels at HORTING Institute

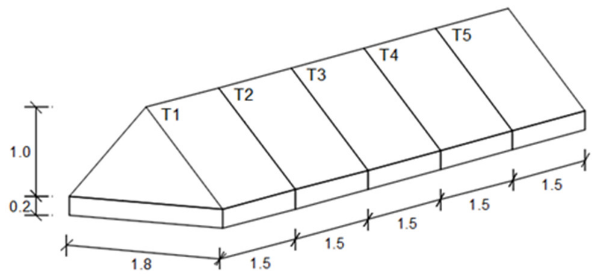

Figure 2. Schematic outline of a healing tunnel with 5 compartments

After grafting, the seedlings are placed for 5 days in the healing tunnels. In each compartment were placed 9 alveolar pallets with 104 nutritive cubes capacity, but with a different grafted seedlings density, as follows: 1) 74 seedlings/m^2 were placed in T1 compartment; 2) 167 seedlings/m^2 were uniformly placed in T2 compartment; 3) 347 seedlings/m^2 were placed in T3 compartment; 4) 694 seedlings/m^2 were placed in T4 compartment.

Instrumentation and experimental procedures

The measurements of the microclimate parameters were made with the following devices: two types of USB-data logger models to measure relative humidity and the temperature, MicroLite model LITE5032P-RH and EXTECH model RHT10, respectively. For the measurement of CO_2 concentration was used a Testo 535 portable analyser with infrared absorption on 2 channels. Measurements in the enclosures were made 1 hour after sunrise, at noon (13:00) and 1 hour before sunset; the CO_2 enrichment of the enclosures was made after the noon measurement, by opening the front sides; the sides were raised as long as was necessary to equalize the value of the concentrations inside and outside; the measured values were individually recorded because the device is not provided with a data acquisition system. For measuring the light intensity in the enclosure of healing/acclimation tunnel, a photo-radiometer HD model 2102.2 data logger + measuring probe LP 471 PAR was used. For measuring the solar radiation intensity, outside and inside of the research greenhouse, a multifunctional device LM-8102 was used.

Data-Logger-USB devices for measuring temperature (T) and relative humidity (φ) outside the research greenhouse module, inside the greenhouse and in the premises where the experimental research was carried out, were placed at the measurement points area shown in Figure 3. Also, in Figure 3 is marked the positioning where the PAR sensor of the Data-Logger was placed to measure the value of Photosynthetic Photon Flux Radiation in the healing enclosure. It was hypothesized that in all 4 healing enclosures this light radiation indicator has the same value, because in the area there are no elements which does not disturb the value of the Daily Light Radiation.

The area where the CO_2 concentration and light radiation intensity (PPFD) measurements were made in each section is illustrated in Figure 4.

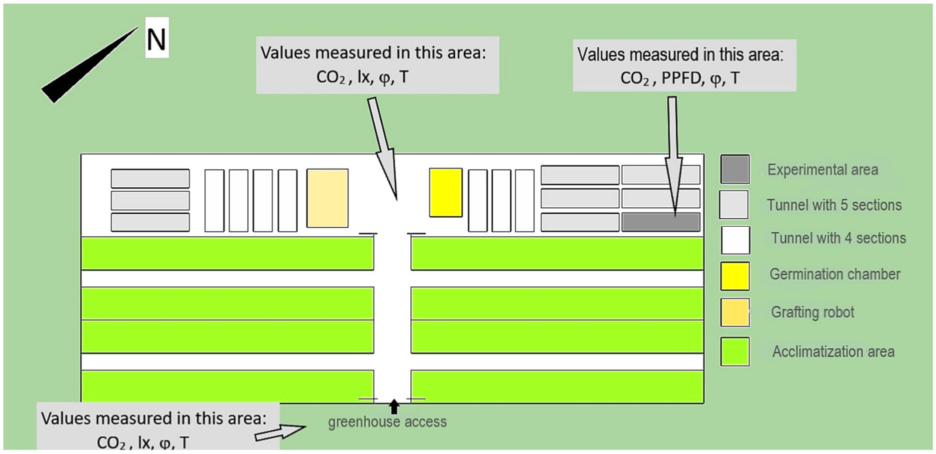

Figure 3. Areas where the experimental measurements were made

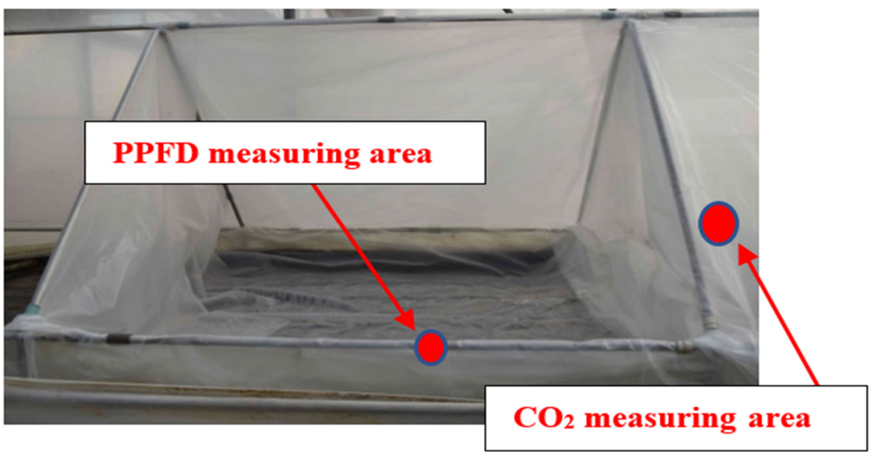

Figure 4. Measuring area for CO_2 and PPFD in a healing zone

Results

The variation of temperature and relative humidity inside the healing tunnel

The values of temperature and relative humidity, measured with Data-Logger-USB devices placed in each of in sections 3 and 4, inside the healing tunnel from the greenhouse, are illustrated in Figures 5 and 6 respectively. Within the experiment, the greenhouse was to be considered as an external environment of the healing enclosures, while also taking into account the outside of the greenhouse.

The graph shown in Figure 6 shows that the temperatures in sections 3 and 4, as well as inside the series, had the same variation curve as the outside temperature. From the point of view of thermodynamic processes, this was to be expected. But, from the point of view of the microclimate factors management inside the greenhouse and in the healing tunnels, it is found difficult to determine with no system for monitoring the microclimate conditions. For example, between 15:00 on the second day of measurements and 03:00 on the

3rd day, there was a temperature difference of about 30 °C, a difference that can have a significant negative influence on the seedlings' survival rate.

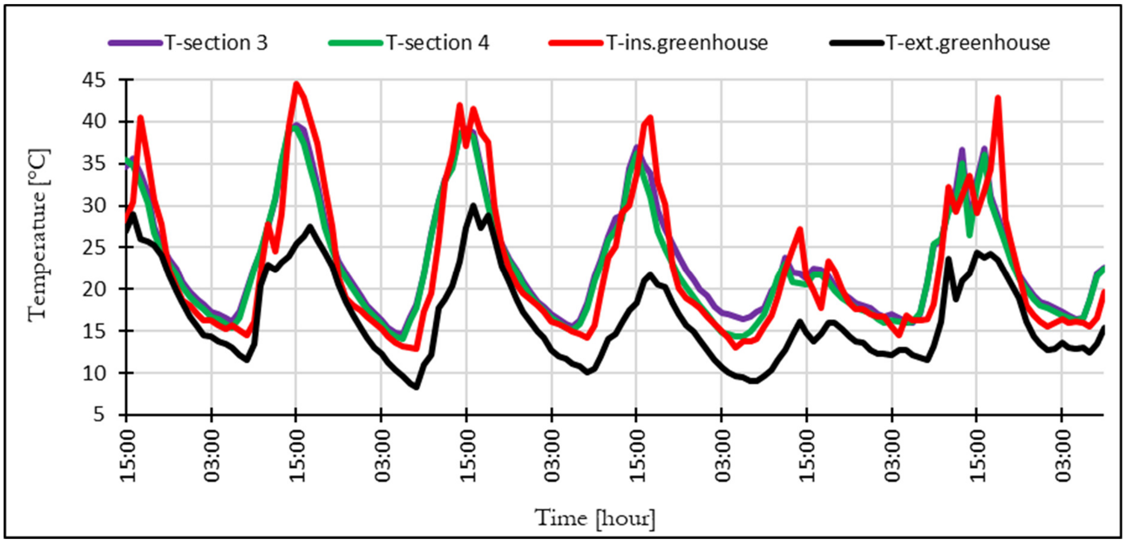

Figure 5. Temperature variation in tunnel sections, inside and outside the greenhouse

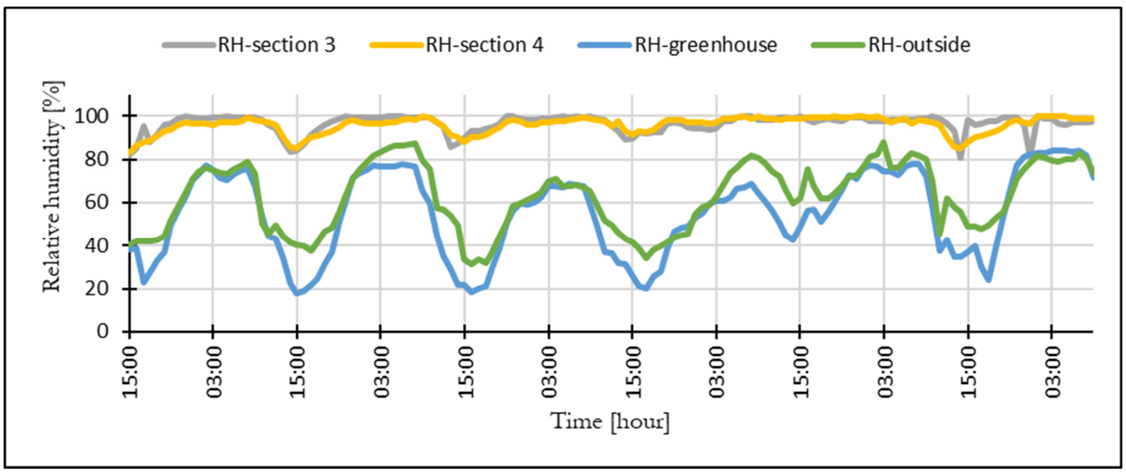

Figure 6. Relative humidity variation in tunnel sections 3 and 4, inside and outside the greenhouse

The relative humidity in sections 3 and 4 was more or less the same, with the values between 80% and 99%, without variations depending on the relative humidity in the greenhouse or in the external environment.

Also, as can be seen in the graph in Figure 7, the variation of the temperature in the research micro greenhouse and the external environment had no significant effects on the relative humidity in the healing enclosures. This is mainly due to the intense evapotranspiration process that normally takes place in the healing premises. It was chosen to investigate the variations of the relative humidity in sections 3 and 4 and thus was represented in the graph, because of the usual density of grafted vegetables used in seedling farms and that they simulate.

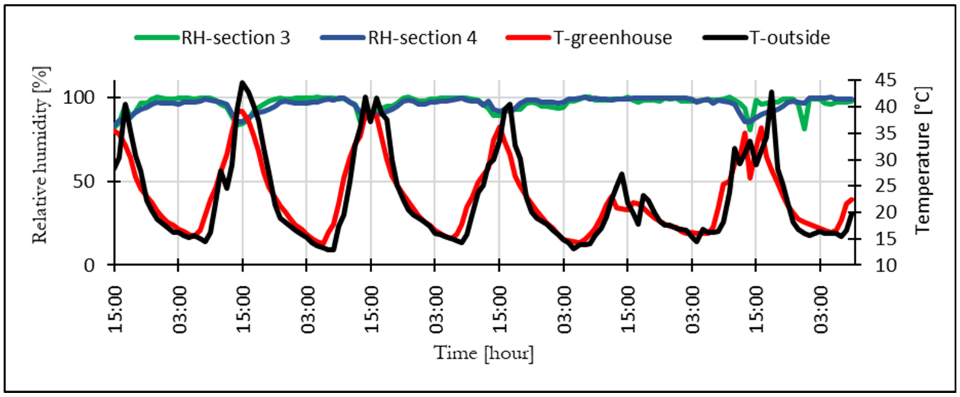

Figure 7. Variation of relative humidity in sections 3 and 4 of the healing tunnel, depending on greenhouse and outdoor temperatures

The variation of CO_2 concentration and photosynthetically photon flux density (PPFD) inside the healing tunnel

The CO_2 concentration measurements were performed 3 times a day:

- at 08:00, i.e. about 1 hour after sunrise, when is considered that the photosynthetic activity is very low and the CO_2 concentrations in the sections are high;

- at 13:00, i.e. at noon when it can be considered that the photosynthesis activity is intense, so the CO_2 concentrations are low; after the measurements, the sections were ventilated for about 15 minutes to increase the CO_2 concentration in the enclosures to at least the level of the one within the greenhouse;

- at 19:00, i.e. about 1 hour before sunset, so in the final phase of photosynthetic activity of seedlings.

It was observed that large variations in CO_2 concentration were manifested in sections 3 and 4. This might be due to the high density of the plants. In sections 1 and 2 where plant densities were low, consumption and generation of CO_2 varied within relatively small limits. The variation of the CO_2 concentration in the 3 and 4 experimental sections is illustrated in Figure 8.

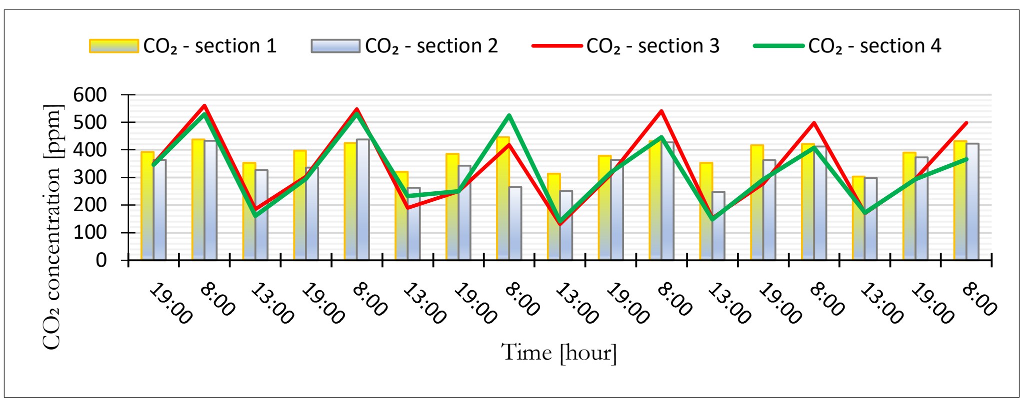

Figure 8. Variation of CO_2 concentration inside the healing tunnel

It was also observed that during the day, the concentration of CO_2 dropped significantly in the healing sections. This is unfavourable for seedling growth. There are studies that show that by increasing the concentration of CO_2 to values above 700-1,000 ppm, there is an increase in the net rate of photosynthesis (Stulen and Den Hertog, 1993; Sánchez-Guerrero *et al.*, 2005). Even if after the measurements at 1:00 p.m. the CO_2 concentration in the sections was brought to the level of that in the greenhouse, the values obtained hereby of around 450-500 ppm are lower than the recommendations made by researchers in the field.

The variation of CO_2 concentration in sections 3 and 4 according to the PPFD value is illustrated in Figure 10. It can be seen that at higher values of the intensity of the light radiation, the CO_2 concentration decreased as a result of the increase of photosynthesis process, which is considered a "consumer" of CO_2.

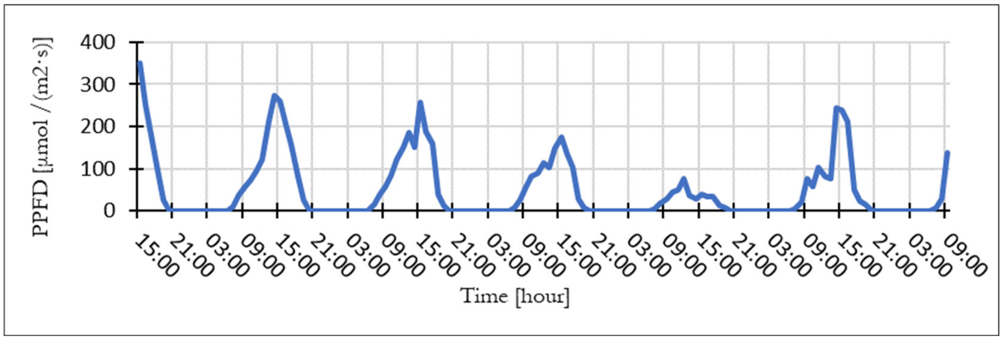

Figure 9. Photosynthetically photon flux density variation

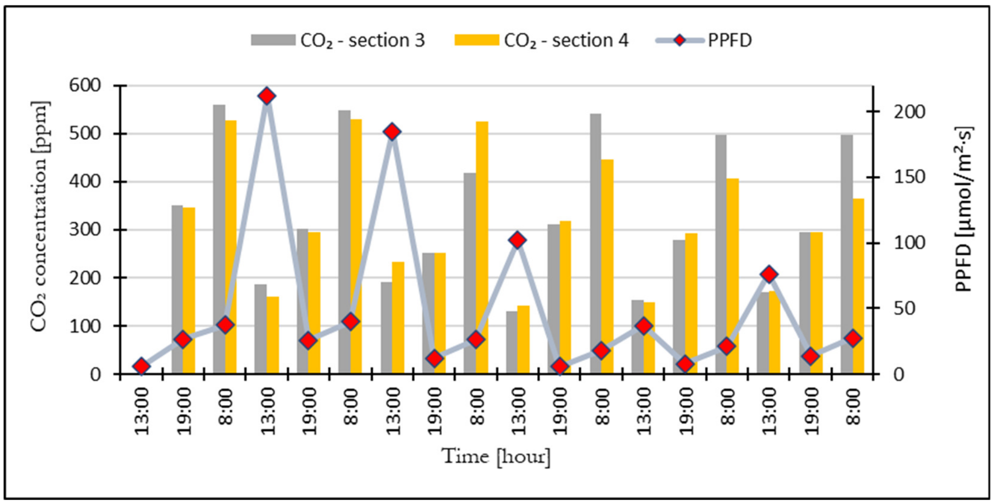

Figure 10. Variation of CO_2 concentration and photosynthetic photon flux density

During the day, when the light radiation is more intense, the concentration of CO_2 decreased (Figure 9) because the process of photosynthesis is much more intense. In Figure 10 it can be seen that at higher values of light radiation intensity, the concentration of CO_2 decreased, as a result of the intensification of the photosynthesis process. An intense photosynthesis process with decreases in CO_2 concentration shows that the connection between the scion and rootstock has been achieved, so the grafted plants are healed.

Discussion

The use of grafted vegetables of resistant genotypes is now widespread in the world. As a result of the increasing using of grafted vegetable seedlings, there is a high demand for such high-quality seedlings. Obtaining grafted seedlings thus becomes an important branch of horticultural production (Kyriacou *et al.*, 2017; Maurya *et al.*, 2019; Malik *et al.*, 2021). An essential element for obtaining grafted vegetable seedlings with good quality and a high survival rate, as a result of the relatively short duration of each specific technological step, is the fluctuation of the microclimate conditions in the healing enclosures, fluctuations that can often compromise the technological process of obtaining grafted seedlings (Niu *et al*., 2018; Ilić *et al*., 2022).

Research into the physical and biophysical processes that take place in protected vegetable cultivation areas has generally been oriented towards the analysis of microclimate factors, their interaction and the influence of the constructive elements in greenhouses on them. Regarding the microclimate conditions in the premises intended for healing of grafted seedlings, it was considered "*a priori"* that they can be analysed in a similar way, that the processes developed in these specialized premises are similar to those in greenhouses. But even if the processes in the healing enclosure seem similar to those in the greenhouses, these are very different. First of all, the air volumes of these enclosures are relatively small compared to the leaf surface. For this reason, the mode of interaction between the biological growth rate of plants and microclimate factors is extremely dynamic (Niu *et al*., 2018). E.g., in general, 75-85% relative humidity accelerates the rate of photosynthesis, although relative humidity may influence photosynthesis differently under other environmental conditions. Thus, the high relative humidity, around 100%, decreases the rate of photosynthesis due to the reduced opening of the stomata. Under conditions of low relative humidity, photosynthesis is likely to decrease due to water stress induced by excess transpiration (Kitaya, 2005). The components of the healing enclosures, depending on their design and complexity, can ensure more or less control over the thermal processes that take place in these spaces, with direct consequences on the healing and development of the grafted vegetable seedlings. Healing chambers are generally either small and highly sophisticated spaces, generally used in research, or large, used specifically for the industrial production of grafted seedlings, and can provide a suitable environment for newly grafted vegetable seedlings (Dong *et al*., 2015; Maurya *et al*., 2019).

A very important element in obtaining the quality and quantity required for grafted seedlings is the microclimate factors in the premises where the seedlings are grown. Because the stages of obtaining grafted seedlings are relatively short, fluctuations in environmental factors can have dramatic effects on the quality and quantity of grafted seedling production (Maurya *et al*., 2019). Light intensity, relative humidity, and temperature are the key environmental factors influencing the healing a of grafted seedlings. Management of microclimate factors during healing is usually done through a grower's empirical knowledge of the season or weather. To prevent wilting of grafted plants through excessive transpiration and to promote healing, the healing enclosure should be closed during the three-to-four-day healing period. The opening and closing of the tunnel is controlled according to the condition of the grafted plants and the weather. When the tunnel is closed, the air in the tunnel is saturated (RH > 90%) and the light intensity is slightly higher than the light compensation point (below the photosynthetic photon flux (PPF) of 50 $\mu mol/(m^2 \cdot s)$ (Kim and Park, 2001; Lee and Oda, 2010). Under these environmental conditions, grafted seedlings grow poorly and are at risk of heat stress, pathogen infection, and overgrowth in which roots emerge from scion hypocotyls. For this reason, the usability of artificial environment for grafted seedlings is a very useful and efficient approach. The advantages of handling grafted seedlings inside an artificial environment are mainly the predictability of production indicators and the standardization of seedlings' quality, as a result of the constant microclimate conditions in the curing areas (Dumitrescu and Ghiaus, 2019; Dumitrescu *et al*., 2019).

In order to control and monitor the thermophysical parameters in the healing rooms, it is necessary to understand the physiology of the plants and their interaction with the microclimate factors in the curing enclosures. The analysis of the dynamics of thermophysical parameters and their numerical modelling can help to simulate and predict their evolution. In this way, several advantaged can be noted: the management system of the microclimate conditions in the healing enclosures can be more easily designed, the performance of already existing enclosures can be evaluated in order to modify them to meet the needs of the farmers, thus to improve the micro-climate, so that on the end the seedlings benefit of the optimal conditions (Maurya *et al*., 2019).

For this reason, especially in seedling farms, a study that accurately assesses the variation in microclimate conditions becomes a necessity. Such a study of the prediction of microclimate factors must lead to the development of a versatile prediction model that can be used in any climatic conditions, at any time of the year and in any geographical location. At the same time, this can lead to the development of interdisciplinary research, to analyse the dynamics of microclimate factors and their interaction with the development stage of the seedlings, so that depending on the genotype of the scion-rootstock combination, optimal microclimate conditions for development and healing can be established. Also, the analysis of the evolution of microclimate factors must lead to the development of a versatile numerical prediction model that can be used in any climatic conditions, at any time and in any location.

Further research and experiments must establish precisely the microclimate conditions that must be met in the healing chambers, conditions specified for each genotype used in the production of grafted vegetable seedlings. It is also necessary to determine how to monitor the other phenomena that occur in healing rooms. For this, interdisciplinary research must be developed that will be able to specify the optimal conditions for the development and healing of grafted seedlings, regardless of the external environment.

Conclusions

The components of healing enclosures can ensure more or less control of the micro-climate, with direct consequences on the healing and development of the grafted vegetable seedlings. Because the stages of obtaining grafted seedlings are relatively short, fluctuations in environmental factors can have dramatic effects on the quality and quantity of grafted seedling production. Such a study of the prediction of microclimate factors must lead to the development of a versatile prediction model that can be used in any climatic conditions, at any time of the year and in any geographical location. At the same time, this can lead to the development of interdisciplinary research to analyse the dynamics of microclimate factors and their interaction with the development stage of the seedlings, so that depending on the genotype of the scion-rootstock combination, optimal microclimate conditions for development and healing can be established. The advantages of using a fully monitored and automated artificial environment will facilitate a predictable production process and the achievement of a standardized quality of vegetable seedlings. Also, the production of scions and rootstocks of a standardized quality will facilitate, especially in the case of farms specialized in the production of grafted seedlings, the use of automation in the grafting process.

Authors' Contributions

Both authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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