



# Comparative study on lettuce cultivation within Fieldlab Vertical Farming

Comparative study on the cultivation of lettuce in indoor farms of WUR, Delphy, Logiqs, Vertify and Philips Horticulture LED Solutions

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Report WPR-1300



Logiqs

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## Referaat

Binnen het project Fieldlab Vertical Farming is een vergelijkend onderzoek uitgevoerd naar de teelt van slasoort Caipira in de vertical farm faciliteiten van deelnemers Delphy Improvement Centre, Philips Horticulture LED Solutions, Vertify, Logiqs en WUR Glastuinbouw. De faciliteiten verschillen in omvang en technische opzet. Dezelfde inputs en instellingen werden gebruikt, b.v. de klimaatinstellingen. Bij het analyseren van het met sensoren verzamelde klimaatdata constateerden we dat de ingestelde klimaatomstandigheden bij de meeste bedrijven op celniveau werden bereikt, wat aangeeft dat de cellen presteerden zoals was ingesteld. Wanneer we echter kijken naar het klimaat gemeten rond het gewas, weken de klimaatomstandigheden af van de streefwaarden en waren ze significant verschillend tussen de partners. Dit was het resultaat van de locatie waar het klimaat werd gemeten, en de manier waarop het klimaat werd gestuurd, bijvoorbeeld door windsnelheid en vochtigheidscontrole. Slakroppen uit de cel met de lichtste kroppen hadden een 70% lager versgewicht vergeleken met de cel met de zwaarste kroppen. De gewichtsvermindering was onder meer te danken aan een 37% lagere DLI. De andere belangrijke factor die bijdroeg aan de verschillen in het verse kroggewicht was een verschil in transpiratie als gevolg van variatie in de klimaatrealisatie rondom het gewas.

## Abstract

Within the Fieldlab Vertical Farming project, a comparison study on the cultivation of Caipira lettuce variety was conducted in the indoor facilities of participants Delphy Improvement Centre, Philips Horticulture LED Solutions, Vertify, Logiqs and WUR Greenhouse Horticulture. The facilities differ in size and technical set-up. The same inputs were used, e.g. the climate settings. When analysing the climate collected by the sensors, we observed that the programmed climate conditions were achieved at room level for most of the companies, indicating that farms performed as programmed. However, when looking at climate measured around the crop, climate conditions deviated from the setpoints and were significantly different among the partners. This was the result of the location where the climate was measured, and the way the climate was controlled, for example by wind speed and humidity control. Lettuce heads from the cell with the lightest heads had a 70% lower fresh weight compared to the cell with the heaviest heads. The reduction in weight was due, among other reasons, to 37% lower DLI. The other main factor contributing to differences in fresh head weight was a difference in transpiration due to variation in climate realization around the crop.

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# 1 Introduction

## 1.1 Emergence of vertical farm

Aided by substantial technological advances, agricultural innovations are emerging rapidly. One concept that has sparked interest among investors in the latest years is vertical farming. In this production system, crops are grown with high space use efficiency mainly in multiple stacked tiers. The closed system allows for location- and weather-independent production, from tundra to desert and from outer space to heavily urbanized regions, reducing land use and water consumption while eliminating the need for long-distance transportation <sup>[1]</sup>. The latter feature is essential in the creation of a sustainable food system since 24% of all food never reaches consumers, due to quality loss during transport. In addition, growing crops in closed systems enable year-round production, thereby guaranteeing quantity and quality independent of solar light and other outdoor conditions. On the other hand, several drawbacks have hampered the widespread implementation of vertical farm systems. Firstly, limited growing space requires plants to be small and compact, enabling them to be planted at a high density. Secondly, the final product must have a substantial economic value to compensate for the high initial investment and operational costs, have short production cycles and high harvest index <sup>[2]</sup>. As a result, no high-calorie crops are grown commercially in vertical farming systems. Commercial production involves mainly leafy vegetables (lettuce and herbs), and, in few cases, tomatoes and strawberries <sup>[1]</sup>. Interesting is also the application of vertical farming for the production of starting material (young plants production).

## 1.2 Effect of environmental factor on lettuce growth, development and quality

Lettuce is one of the most popular leafy vegetables produced in vertical farms (VF). Five key environmental factors influencing crop performance in Controlled-Environment Agriculture (CEA) are often presented in literature: light, air velocity, temperature, air humidity and CO<sub>2</sub> concentration.

Light is the primary source of energy required for the photosynthetic process and many other physiological processes related to plant growth and development <sup>[5]</sup>. Plant absorbs the light in the wavelength range of 400–700 nm, defined as photosynthetically active radiation (PAR). The most important characteristics of light for plant growth and development are light quantity (intensity), light quality (spectral composition), and light duration (photoperiod) <sup>[12]</sup>.

Light intensity affects many physiological processes related to plant growth and photochemical reactions that convert CO<sub>2</sub> into carbohydrates and are considered the key factor in regulating biosynthesis in plants <sup>[5]</sup>.

Different studies have found that the optimal light intensity for lettuce ranges from 200 to 250  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . LED lamps are the most common artificial light source used in VFs due to its energy-efficiency and low heat generation compared to other lighting sources, promoting a cooler environment and reducing the risk of heat stress for the plants. LEDs can be dynamically modified in spectrum composition which can be used to steer plant morphology, yield, and quality <sup>[6-10]</sup>. Red and blue LED's, at their combination of 90% red and 10% blue <sup>[11-13]</sup>, have been found to be the optimal light mix, as these two wavebands effectively promote plant growth, photomorphogenesis, photosynthetic rate, biomass accumulation, pigment content (chlorophyll a and b, anthocyanins) and antioxidant capacity <sup>[14]</sup>. Light quality also affects physiological processes such as photosynthetic and transpiration rates by controlling stomatal conductance <sup>[15]</sup>.

In terms of photoperiod, a length between 16 and 18 h d<sup>-1</sup> has been to improve lettuce plant growth and light use efficiency <sup>[11]</sup>.

Temperature is one of the primary environmental factors affecting plant growth and development <sup>[38]</sup>, as it affects plant growth both directly (leaf and air temperature) and indirectly (absorption and transport of water and fertilizer through plant roots) <sup>[40]</sup>. Leaf temperature determines the growth and development rates of the plant more than air temperature <sup>[39]</sup> while air temperature affects the leaf development rate and metabolism <sup>[41]</sup>. The optimal temperature is essential for fast plant growth and high accumulation of organic matter <sup>[40]</sup>.

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According to different studies, the highest photosynthetic and growth rates in lettuce were observed when air temperature reached 22 to 26 °C and 15 to 20 °C, during the light and dark periods respectively, in the initial and middle growth stages. Whereas for the later stage, that ranged from 20 to 24 °C and from 15 to 20 °C during the light and dark period, respectively. Leaf to air temperature difference is related to the air velocity and uniformity. At high air velocity, the leaf boundary layer resistance decreases causing a smaller leaf to air temperature difference which impacts transpiration and, in extreme conditions, photosynthesis [38]. Air velocity is an important environmental factor affecting plant growth in VFs [16] and is considered the main challenge in VFs design for plant production [17]. The air velocity regulates air temperature, relative air humidity and CO<sub>2</sub> concentration within the plant canopy [18]. Controlling the airflow in a VF is essential to enhance the exchange of CO<sub>2</sub> (via photosynthesis) and H<sub>2</sub>O (via transpiration) between leaves and surrounding air [19-33] and thus promoting plant growth. Additionally, CO<sub>2</sub> utilization efficiency during the light period is strongly related to the air velocity [20]. Air velocity affects plant growth and development directly and indirectly. The direct effect of air velocity is through energy and mass transfer between leaves and surrounding air. The heat exchange takes place in the form of sensible heat by convection and latent heat by transpiration, affecting leaf temperature, and thereby the growth and development of plants. However, the relationship between the air velocity and plant growth rate is not linear but tends toward the maximum or optimum [18]. The optimal air velocity should be determined based on plant species, plant structure, plant canopy depth and airflow direction [19]. An optimum air velocity for plant growth was reported to be in the range of 0.3–0.7 m s<sup>-1</sup> [19].

Selecting the appropriate airflow system is essential to control the environmental conditions in an homogeneous way throughout the cultivation area. The structure of VFs and the density of plant cultivation are the most important criteria based on which a suitable airflow system is chosen [19]. Airflow can be applied both vertically or horizontally [22]. In the case of VF, the structure affects the uniformity of air velocity distribution within the multi-layers. Air velocity along the layers close to the ventilation system is usually higher than those located further away. Different studies [23] compared the effect of airflow direction and air velocity on CO<sub>2</sub> exchange and growth rate of tomato seedlings. They reported that the upward and downward forced airflow enhanced CO<sub>2</sub> exchange and increased dry mass production by 1.5 and 1.3 times, respectively, compared with the conventional horizontal air flowing system.

CO<sub>2</sub> represents the raw material for photosynthesis and is involved in several physiological processes related to plant growth. Photosynthetic rate, plant dry weight, soluble sugar, and chlorophyll content, increase with an increase in CO<sub>2</sub> concentration [42]. Plant structural characteristics, such as the shoot to root ratio, leaf thickness and stomatal density, can be modified at high CO<sub>2</sub> levels [43]. CO<sub>2</sub> uptake by the plant is dependent on the growth stage, air velocity, light intensity, and air temperature [44]. The photosynthetic rate of lettuce grown under red, blue, and white light significantly increased when the CO<sub>2</sub> concentration increased from 350 to 1000 ppm. Optimal CO<sub>2</sub> concentration should be determined by considering plant species, growth stage, cultivation conditions and other environmental factors [46].

Air humidity is a key factor influencing stomata functioning and its effect on plant growth varies according to the species, growing conditions, and length of the growing period [48]. Stomatal conductance responds to the vapor pressure deficit (VPD) in the air [40]. For example, at low relative air humidity (high VPD), the rate of evaporation from leaves increases, leading to excessive loss of water. As a result, the stomata closes and no further CO<sub>2</sub> can be absorbed; consequently, photosynthesis is hampered.

The transport of CO<sub>2</sub> from the air into chloroplasts (photosynthesis) and that of H<sub>2</sub>O from the stomatal cavity to the air (transpiration) are controlled by two resistances in series [24]. The first resistance is known as leaf boundary layer resistance, and the second resistance is known as stomatal resistance [25]. The leaf boundary layer resistance involves the movement of a thin layer of air very close to the leaf surface [25]. Leaf morphological characteristics such as leaf size, shape, and texture as well as the air velocity are the main factors influencing the boundary layer resistance [24]. Studies reported that the leaf boundary layer resistance decreased significantly with the increase in air velocity from 0.005 to 0.1 m s<sup>-1</sup>, breaking the leaf boundary layer resistance to enhance CO<sub>2</sub> and H<sub>2</sub>O transport. Also mentioned that the optimum air velocity is essential to promote gas exchange between plant leaves and air as well as to reduce the resistance of gas transport in the boundary layer [18]. Stomatal resistance is an important parameter for the physiological control of transpiration and its regulation for gas exchange is significant to plant growth [27]. Studies reported that increasing stomatal resistance has a negative effect on the transpiration rate [28] and is affected by various environmental factors, including light intensity [26] light quality [15], air velocity [29], plant water status [30] and CO<sub>2</sub> concentration [31]. Light intensity and quality directly affect stomatal opening, and consequently, CO<sub>2</sub> flow rate into the stomatal cavity during photosynthesis [32]. With regard to the effect of air velocity on

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stomata, some studies stated that the stomata are considered to be fully opened at air velocity in the range of 0.5–1 m s<sup>-1</sup> [29]. The same study reported that the vertical air flowing system with an air velocity of 0.7 m s<sup>-1</sup> reduced the resistance of CO<sub>2</sub> and H<sub>2</sub>O diffusion without stomata closing. Lettuce yield was 130% under the vertical air flowing system compared to the horizontal air flowing system [29]. The increase in photosynthetic and transpiration rates was strongly dependent on the decrease in leaf boundary layer resistance to the diffusion of CO<sub>2</sub> and H<sub>2</sub>O. Studies reported that the photosynthetic rate increased with the increase in CO<sub>2</sub> concentration and air velocity. The transpiration rate decreased with the increase in CO<sub>2</sub> concentration and increased with increase in air velocity [33].

Cultivation practices and environmental conditions not only influence growth and development but affect final product quality. Tip burn is the most important symptom of physiological disorder in lettuce. It affects plant quality and causes a significant economic loss. Tip burn occurrence is considered to be caused by poor nutrient availability in the young leaves, especially due to calcium deficiency, despite the presence of calcium in nutrient media. It can occur in mature lettuce crops, before harvesting, due to the presence of a stagnant boundary layer and low transpiration rate [28]. Additionally, its occurrence is highly dependent on lettuce cultivars [36] and affected, among others, by air velocity [37], light intensity [38] and air temperature [35]. Studies showed that sufficient airflow enhances the transpiration rate and thus the uptake of calcium from nutrient media and transport into the inner and newly developed leaves. In some studies, vertical air flowing system was found more effective than the horizontal air flowing system for suppressing the occurrence of tip burn in lettuce plants [36]. Other studies investigated the effect of light intensity in the range between 150 and 300 μmol m<sup>-2</sup> s<sup>-1</sup> on tip burn occurrence in butterhead lettuce grown in a plant factory. They reported that calcium content in the inner leaves decreased with the increase in light intensity, consequently increasing the number of lettuce leaves injured with tip burn. Other studies stated that lettuce quality in a plant factory can be also achieved through the optimal management of temperature since the growth rate is related to tip burn occurrence [36] and also reported the occurrence of tip burn in butterhead lettuce plants in the middle growth stage was observed only at 30 °C and 25 °C during the light and dark periods, respectively, and at 20/25 °C a later stages.

### 1.3 Relation between environmental conditions and plant production in vertical farms

The complex control of indoor production facilities can be attributed to the interaction between physical, chemical and biological processes.

LED Light intensity directly affects CO<sub>2</sub> and H<sub>2</sub>O transport through stomata during the photosynthesis and transpiration processes [29]. The optimum light intensity can enhance the photosynthetic rate, improve dry mass production, and significantly increase the fresh weight, leaf area, and chlorophyll content of lettuce plants [14].

Air velocity directly affects CO<sub>2</sub> and H<sub>2</sub>O diffusion through the boundary layer. Under which influences the photosynthetic and transpiration rates [18][19]. Therefore, an insufficient rate of airflow around plant canopy results in the thickening of the boundary layer [22-24]. Consequently, the photosynthetic and transpiration rates are hampered due to a reduction in the CO<sub>2</sub> uptake from the air and H<sub>2</sub>O diffusion from the stomatal cavity [24]. The reduction in the transpiration rate is more considerable than that of the net photosynthetic rate at low air velocities. Additionally, the high air velocity in the boundary also contributes to the closure of stomata. Lettuce growth tends to decrease with increasing the air velocity under the low concentrations of CO<sub>2</sub>. Conversely, lettuce growth increases with increasing air velocity under the high levels of CO<sub>2</sub> [22]. Temperature affects plant growth in two ways: photosynthesis, transpiration, and organic synthesis. These processes are regulated by enzymes, whose activities are temperature dependent. Enzymes are actively involved in the synthesis of chlorophyll and several other compounds. At high temperatures, enzymes regulating photosynthesis are degraded and lose their function. Changes in temperature can increase the photosynthetic rate up to a plateau after which it drops quickly since photosynthetic reactions have kinetic energy within a particular temperature range [40]. On the other hand, temperature affects the kinetic energy of water molecules, resulting in higher evaporation with an increase in temperature, consequently increasing the transpiration rate and enhancing plant growth [40]. Additionally, temperature affects photosynthetic and transpiration rates through its direct effects on stomatal opening [29]. The rate of CO<sub>2</sub> and H<sub>2</sub>O diffusion through stomata increases with the increase in air temperature [34].

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The effect of relative air humidity on plant growth is mainly due to its direct effect on stomatal behavior. In a VF, relative air humidity can be easily adjusted within the optimal range, but the interaction with the other environmental conditions makes its effect unclear. For instance, increasing the air velocity under high or low relative air humidity increases the potential of stomata closing <sup>[30][29]</sup>. As the air velocity increases, the water potential in the leaves decreases, stomata are sensitive to the leaf water status and tend to close with a decrease in leaf water potential. In addition, leaf conductance linearly decreases with a decrease in leaf water status <sup>[44]</sup>. The effect of relative air humidity on the transpiration rate is also linked to the air velocity. Air velocity removes the water vapor molecules that have passed out through stomata to the leaf surface. Therefore, the air outside the stomata becomes less humid and lower concentration with water vapor. This process maintains the gradient for water to diffuse <sup>[34]</sup>. The increase in relative air humidity increases CO<sub>2</sub> concentration in the leaf, by inducing stomatal aperture. But under low relative air humidity levels, even though with enrichment of CO<sub>2</sub>, slight variation in stomatal aperture occurs <sup>[34]</sup>.



## 2 Fieldlab Vertical Farm project

The Fieldlab Vertical Farming South Holland was launched at the beginning of 2019 with the help of a financial contribution from the Kansen voor West 2 program. Within the Fieldlab, a consortium of SMEs, knowledge and educational institutions is working together on the development of state-of-the-art research and education in the field of vertical farming. The Fieldlab is the place where the previously fragmented knowledge and innovations in the field of vertical farming in the Netherlands are brought together and valorized into concepts that the business community can put on the (international) market. The Fieldlab Vertical Farming thus contributes to the sustainable strengthening of the competitive position of the Dutch horticultural sector and the maintenance of the leading position of the sector in the world. The project has a holistic approach looking at innovating the field from a market and consumer perspective (WP1), innovation and demonstration in indoor plant production (WP2), education and training (WP3) and business accelerator (WP4).

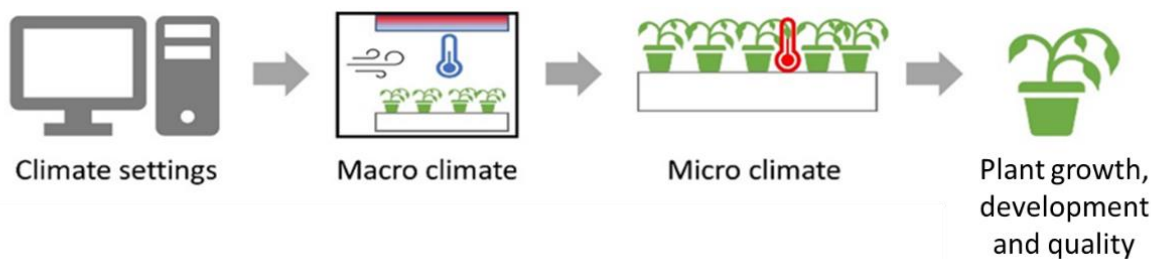
### 2.1 Comparative studies

Within WP2, state-of-the-art indoor facilities of different partners (Delphy Improvement Centre, Logiqs, Philips Horticulture LED Solutions, Vertify and WUR Greenhouse Horticulture) cooperates, among others, to understand how different vertical farming systems work in order to facilitate the standardization of practices. Vertical farms have the premise that, because of the total control of the conditions for plant growing, a standard recipe will lead to plants with standard size, morphology and quality, no matter where the plant is geographically produced. However, some practical experiences show that this level of standardization and reproducibility has not been reached yet. The first and second comparison study with the cultivation of I basil [3,3a] and an exploratory study on lettuce showed that equal inputs and settings could lead to different plants and output.

In this research, we dived deeper into some of the factors that could contribute to creating those differences in the cultivated products.

### 2.2 Research questions and hypothesis

The goal of this trial was to gather further insight into the relationship between setpoints, realized macro- and micro-climate and plant responses at different indoor facilities (Figure 1).



**Figure 1** Schematic overview of the framework.

Research questions are:

1. What is the difference of the climate around plants (=microclimate) among different facilities given the same setpoints (=macroclimate)?
2. How does this difference influence lettuce production and quality?

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The microclimate, the climate just around the leaves of the plants, is determined by the macroclimate and the plant itself. The microclimate is the climate that the plants experience; this will influence growth and development, leading to a plant of certain characteristics.

The hypothesis is that the output (= realized climate condition) and thus plant performance can differ by equal climate settings because of the different facility specifications (design and climate system). Because of the different specifications, the way the climate in the facility is set, monitored, and controlled can be different and therefore the realized climate around the plants can differ between facilities.

# 3 Materials and Methods

## 3.1 Facilities

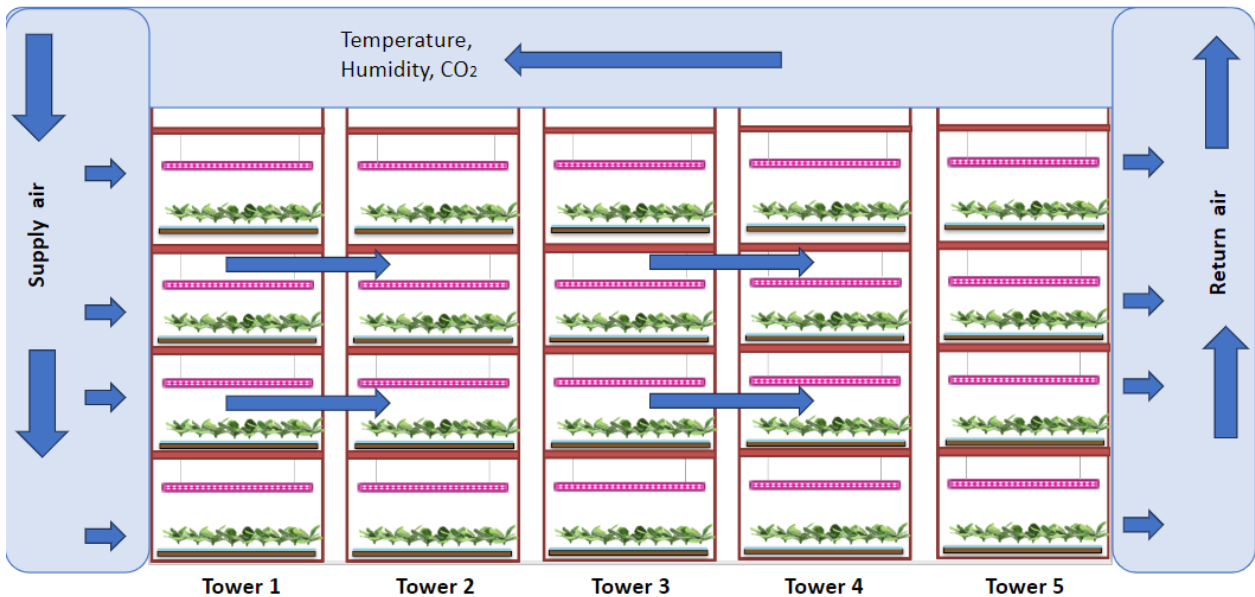
Experiments were carried out in different facilities, from the partners Delphy Improvement Centre (Delphy), Philips Horticulture LED Solutions (Signify), Verify, Logiqs and Business Unit Greenhouse Horticulture and Flower Bulbs of Wageningen University & Research (WUR) with different technical cell specifications shown in Table 1 and described below.

**Table 1** Cell technical specifications partners description.

| Partners                                | Delphy                 | WUR                     | Signify                | Verify                 | Logiqs                      |
|---|------------------------|-------------------------|------------------------|------------------------|-----------------------------|
| Cell name                               | MLR                    | MLC4                    | P1                     | WHC                    | POD-1 and 2                 |
| Volume cell                             | 415,06 m <sup>3</sup>  | 175,8 m <sup>3</sup>    | + -98 m <sup>3</sup>   | 68 m <sup>3</sup>      | 2,77 m <sup>3</sup> (1 pod) |
| Net cultivation area                    | 13.2 m <sup>2</sup>    | 20 m <sup>2</sup>       | 14 m <sup>2</sup>      | 11,5 m <sup>2</sup>    | 5,76 m <sup>2</sup> (1 pod) |
| Nr cultivation layer                    | 4                      | 2 – 1 for comparison    | 2 – 1 for comparison   | 4                      | 2                           |
| Distance table-lamp                     | 90 cm                  | 150 cm                  | -<br>85 cm             | 77 cm                  | 48 cm                       |
| Constructor                             | Infinite Acres         | Light 4 Food            | -                      | Priva/infinite Acres   | Logiqs                      |
| Type ventilation                        | 1 Side perforated wall | 2 sides perforated wall | 1 Side perforated wall | 1 Side perforated wall | Air tubes among plants      |
| LED's                                   | Signify Dynamic PM 3.0 | Signify Dynamic PM 3.0  | Signify Dynamic PM 2.1 | HORTILED Multi 4DIM    | Luminaid pixels; max. 220   |
| Sensor box climate control              | Dry-wet bulb           | electronic              | electronic             | electronic             | T/RH at plant level         |
| Realized climate conditions to steer on | Inlet return system    | Measurement box         | Supply system          | Inlet return system    | Supply system               |
| Nr cultivated floaters                  | 28                     | 40                      | 24                     | 24                     | 24                          |

### 3.1.1 Delphy

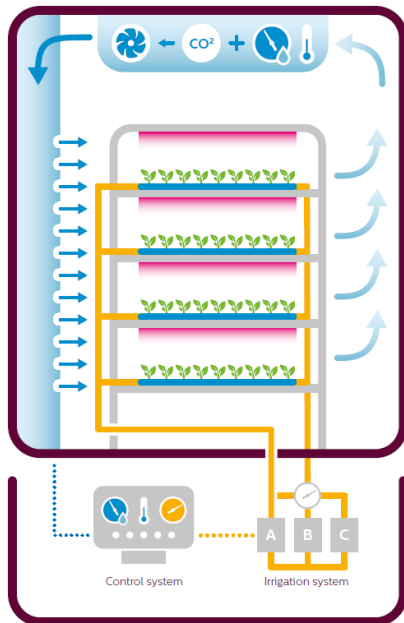
In the vertical farm of Delphy in Bleiswijk, climate (Temperature, Relative Humidity and CO<sub>2</sub>) is controlled by the return air (Figure 2). Climate parameters are measured in the return position and the supply inlet is adapted to reach the setpoint climate. Windspeed is measured as well by Infinite Acres system and can be adjusted if needed for different speeds. The irrigation system is a closed gift-drain loop. The water is measured at the Priva unit and mixed at the right EC and pH. Then it is given by ebb flood, raised drain or dripper to the plant and the drain is reused. The light system in the room consists of Philips dynamic production modules and can reach light levels of approximately 300 μmol m<sup>-2</sup> s<sup>-1</sup> in this specific room, controlled via Signify's GrowWise Control system. Red, Blue, White and Far-red light wavelengths can be adjusted and controlled independently. The light raises the temperature into the cell so the air temperature increases from the intake to the return. The water is heated up by the warmth of the HVAC, fertigation tanks and water management equipment are located outside the farm next to the cultivation room.



**Figure 2** Schematic representation of inner dimensions of the Delphy vertical farming cell which the experiment was performed.

### 3.1.2 Signify

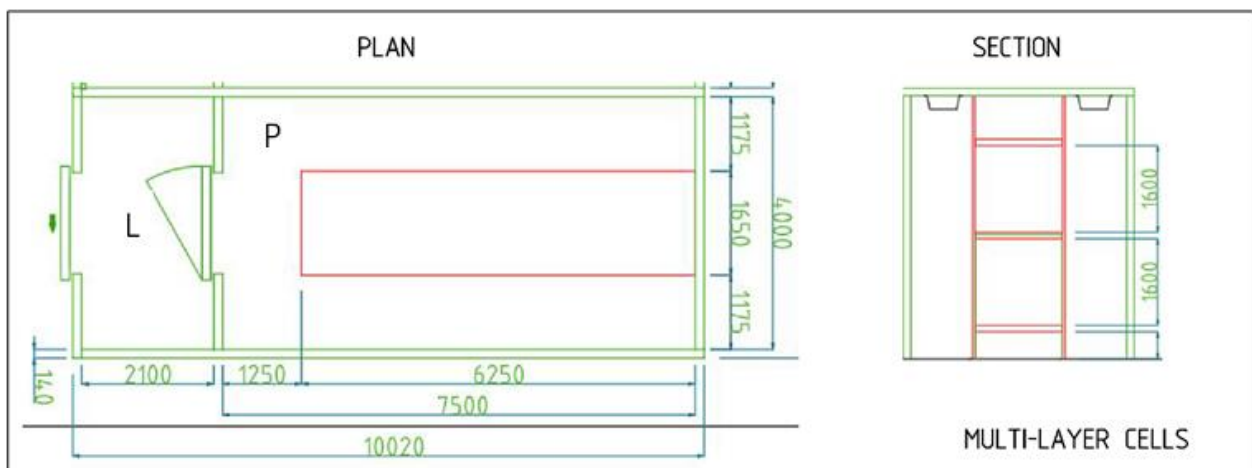
On the High Tech Campus in Eindhoven, The Netherlands, Signify's GrowWise Research Center is located. At the GrowWise center eight climate rooms are built for growth recipe development. One of these eight climate rooms is used for the Fieldlab comparison trial (Figure 3). In the schematic overview below the principals of the cell are visualized. The specific cell for the Fieldlab trials is equipped with 3 growth layers. The first layer has a free height (distance between table and bottom of LED) of approximately 50 cm and the other two layers have a bigger free height of approximately 80 cm. The climate room has a central air handling unit on top of the room. This air handling unit (HVAC) consists of a cold-water cooling system, a heater, humidifier, and dehumidifier to control the air to the right setpoints. Extra Carbon dioxide dosing is also added in the HVAC. The air is continuously flowing inside the room through a perforated stainless steel plenum wall. Inside this wall an overpressure is created to make sure that on all levels the air is moving with similar airspeed over the layers. The layers are 1.2 m wide and 6 m long which results in a growth surface of 7.2 m<sup>2</sup> per layer. The whole climate room is controlled via a Priva blue id control system and irrigated with a Priva minijet irrigation unit. This allows different nutrient recipes automatically to be dosed to each different climate room. The lighting inside the room is equipped with Philips dynamic production modules and can reach light levels of approximately 350  $\mu\text{mol m}^{-2} \text{s}^{-1}$  in this specific room, controlled via Signify's GrowWise Control system. This system allows the grower to tailor the light recipe to each specific crop and trial.



**Figure 3** Schematic representation of the cells at Signify.

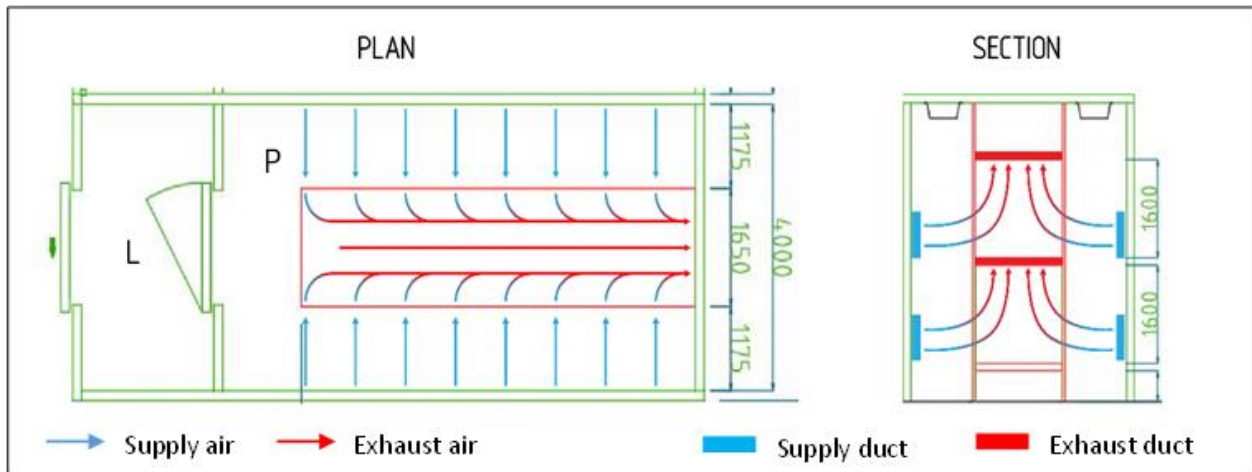
### 3.1.3 WUR

Part of the experiment was carried out in Bleiswijk at the Business Unit Greenhouse Horticulture and Bulbs of Wageningen University & Research. The vertical farm featured two identical airtight multi-layer cells. For the experiment on one cell, MLC4, was used. Each cell comprised 3 compartments: the lock or pre-chamber (L), the production compartment (P) and the technical installation compartment (Figure 4). The production compartment has an area of 30 m<sup>2</sup> and a volume of 175.8 m<sup>3</sup>. It features 2 production layers (ebb and flow table) with each 10.3 m<sup>2</sup> of cultivation area and a free height (distance layer-lamps) of 1.6 m. Each layer is illuminated by an array of LED modules (Philips/Signify GreenPower LED production module 3.0 dynamic) which can be controlled (per layer) in intensity (top layer max Par output: 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ; bottom layer max Par output: 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and in spectrum composition (blue, white, red and far red) using Signify's GrowWise system.



**Figure 4** Dimensions of MLCs (in mm). Top view (left) of lock (L) and production compartment (P) and side view (right) of production compartment.

Cells are airtight and climate is controlled by the Ridder climate computer running Synopta software. Each cell has its dedicated HVAC unit featuring a ventilator, a heating and a cooling unit. Air is continuously recirculated in the cell. Exhausted air is climatized in the technical installation compartment for temperature humidity and CO<sub>2</sub> content. Climatized air is introduced in the cell via perforated ducts on the side walls (Figure 5 right; blue ducts) and extracted per layer via air ducts above the LED installation. An additional fogging system with ducts mounted on top of the side ducts enables further humidity control in the cell.



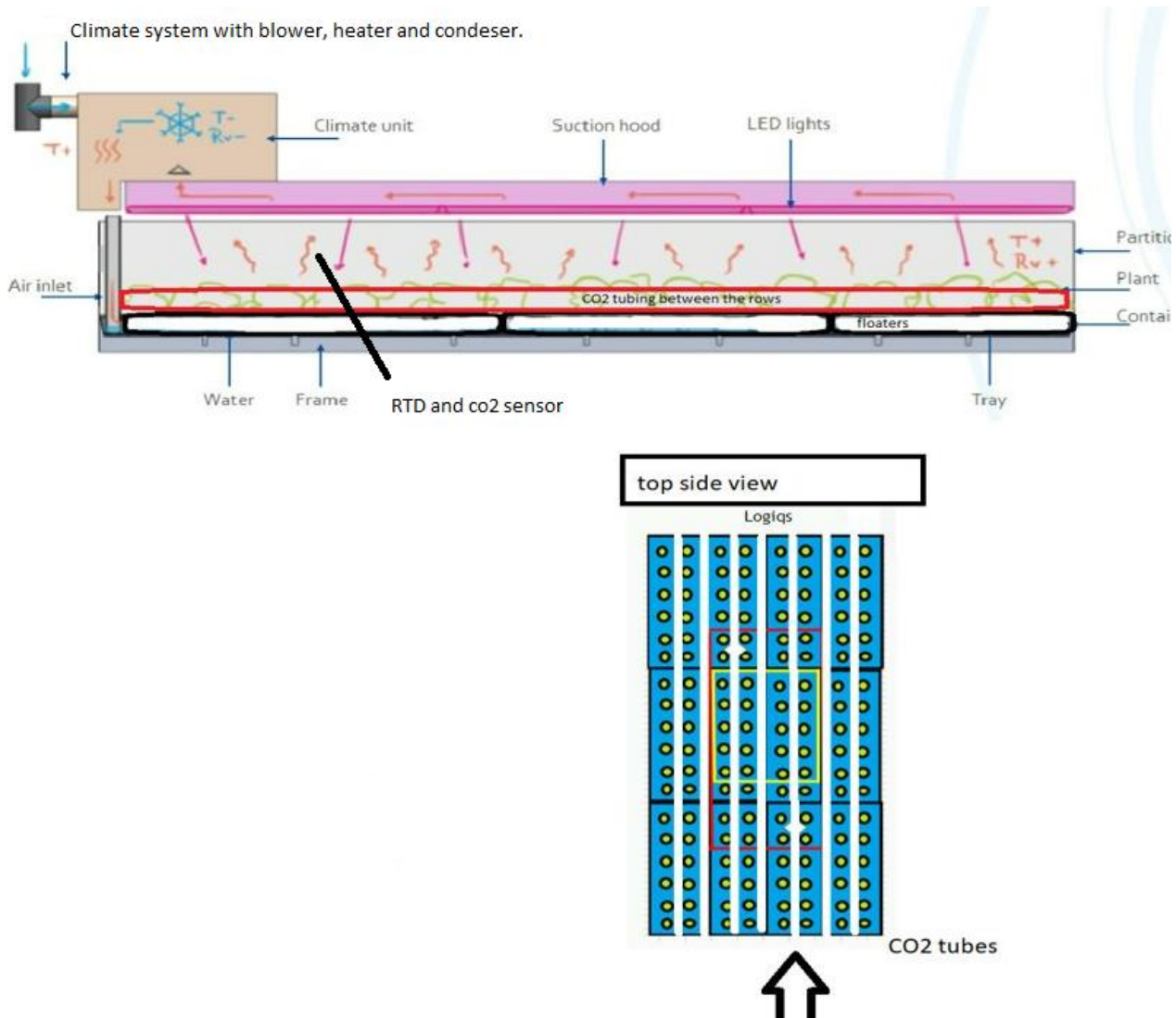
**Figure 5** Climate control in MLCs via air supply and air movement. Air supply is illustrated in blue and air extraction in red. Top view (left) of lock and production compartment and side view (right) of production compartment.

Each cell has a nutrient solution reservoir of 1000L. Irrigation is controlled per layer either by volume or by time and nutrient solution is mixed by a FertiMix Unit (Ridder).

### 3.1.4 Logiqs

The location of Logiqs B.V. is at Maasdijk, The Netherlands. Situated on the 2<sup>nd</sup> floor is an insulated grow chamber with six sections. Each section is a "Pod." (Figure 6). The Pods are stacked 3 high and 2 wide and for the Fieldlab Lettuce test there were 2 reserved at the base and middle. These pods are fitted with an ebb and flood system and each pod has its own climate system. The table has a cultivation area of 6 m<sup>2</sup>. The climate (temperature, relative humidity, and CO<sub>2</sub> concentration) within each compartment was monitored separately via a Raspberry pi computer running custom software developed by Logiqs. It can also alter the spectrum of the LEDs in the pod. These are mounted at a height of 0.5 m with a maximum output of 330  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Normally air movement is archived via Logiqs Airflow trays, with this system air is pressed from underneath the trays, passing the plants and sucked back through the ceiling and passing the climate system again. Because of the choice for floaters the system has changed out the Airflow trays for floaters with air tubes attached to them. See Figure X. Temperature, humidity and CO<sub>2</sub> are measured inside the growing area. The air that reaches the climate box will be cooled or heated and CO<sub>2</sub> or humid air could be added before blowing the air back underneath the Logiqs Airflow trays or, in this specific case, in tubes among the crop.





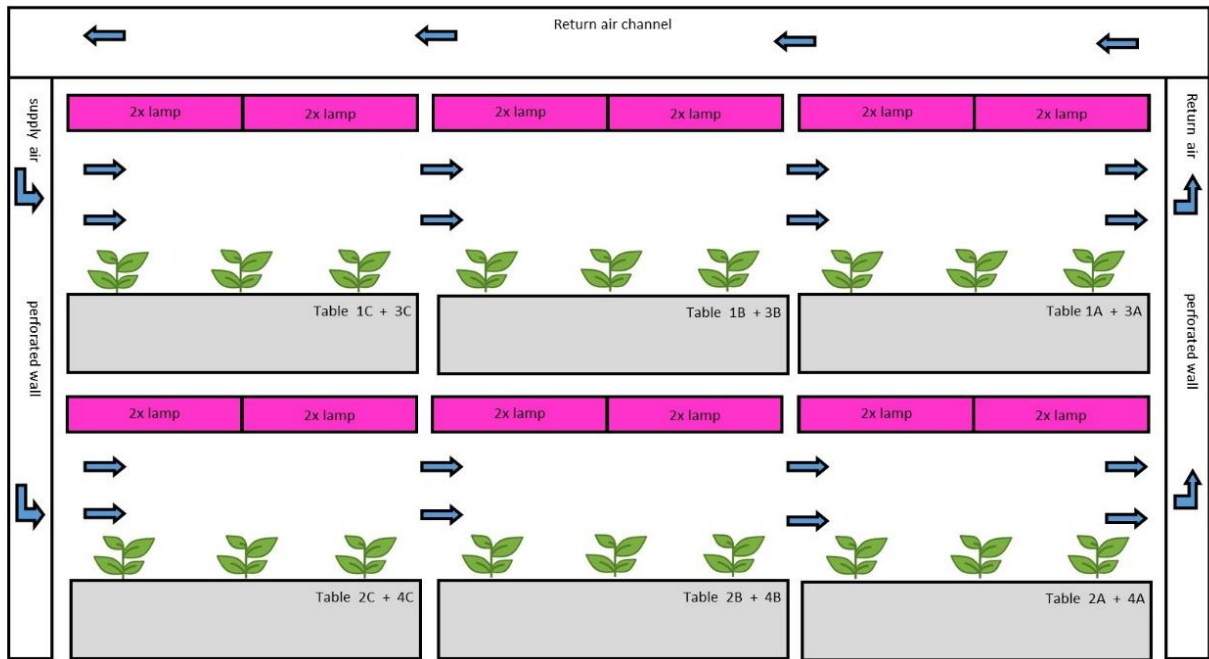
**Figure 6** Schematic representation of inner dimensions of the multi-layered Logiqs Pod in which the experiment was performed. The side view can be found on the top, while the top view with the floaters can be found on the bottom.

### 3.1.5 Verify

Verify has different locations; this trial was performed at the World Horti Center in Naaldwijk, The Netherlands.

In the container indoor facility (Figure 7), temperature, relative humidity, and CO<sub>2</sub> are controlled by the return air (Figure x). The temperature of the B section is measured with 30 MHz sensors and the return is adapted to reach the right climate. Windspeed is measured by hot wire of 30MHz. This is a check if the ventilator is working properly. There is a closed gift drain loop. The water is measured at the Priva unit and mixed at the right EC and pH. Then it is given by ebb flood, raised drain or dripper to the plant and the drain is reused. Hortilux LED are used to give separately Red, Blue, White or Far-red light. This is divided into 4 light recipes.

These factors interact with each other. The light raises the temperature into the cell so the air temperature increases from the intake to the return. The water is heated up by the warmth of the light. This is the one of the interactions that are known to be there but how to cope with this variation is the key to success.

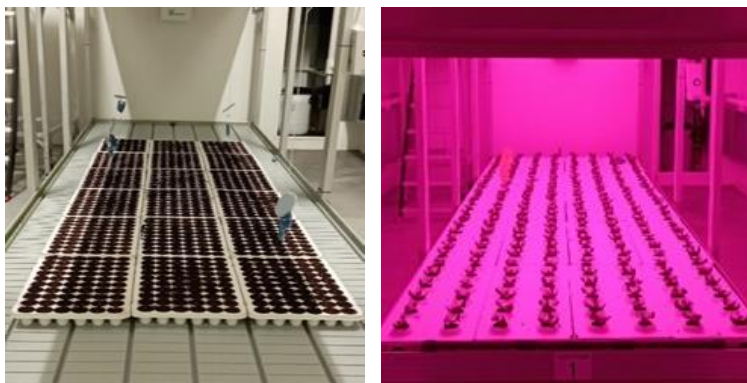


**Figure 7** Schematic representation of the cells at Verify.

## 3.2 Cultivation conditions

For this trial, Batavia lettuce cv. *Caipira* (Enza Zaden) was selected. This variety is known for his low sensitivity to tip burn.

Germination and propagation took place at one common location for all partners, in MLC4 of WUR. Caipira seeds were sown in Light4Food propagation trays (60cm x 40 cm) filled with Jiffy pots and pot soil mix at a density of 250pl/m<sup>2</sup>. Before sowing, soil was moisture with irrigation water. Trays were placed in in the cell and covered with plastic for 2 days in which seeds germinated in darkness under 22 °C, 85% RH and 1000ppm CO<sub>2</sub> level (Figure 8). After two days, plastic was removed, light was turned on for 16 hours per day at 220 μmol m<sup>-2</sup> s<sup>-1</sup> (90% Red, 10% Blue). After 15 days of propagation in MLC4 (WUR, Bleiswijk), propagation trays were randomized and distributed among the partners who came collecting them. On 28<sup>th</sup> of March cultivation started at all facilities and lasted for 21 days until 18<sup>th</sup> of April. Plants were transplanted in Light4Food floaters with a plant density of 24 pl/m<sup>2</sup> at the different partners location (Figure 8). To prevent the substrate from drying out during the first days after transplanting, propagation trays were dipped in 2 cm shallow water for 30 minutes before transplanting. Plants were grown at a temperature of 24-20°C (day-night) for three weeks, with 70-80% (day-night) relative humidity and a Par light intensity of 220 μmol m<sup>-2</sup> s<sup>-1</sup> (90%Red - 10%Blue with extra 5%Farred) for 16 hours per day resulting in a DLI of 12.7 mol m<sup>-2</sup> d<sup>-1</sup> (Table 2). The CO<sub>2</sub> level was set to 1000 ppm when the lights were on. The climate was bridged between day and night conditions over two hours (Table 4).



**Figure 8** Propagation (left) and cultivation (right) at the cell MCL4 of WUR, Bleiswijk.

**Table 2** Lettuce cultivation recipe executed in the cells of the partners.

| Cultivation recipe partners  |                                      |                     |           |       |                     |                  |
|--|--------------------------------------|---------------------|-----------|-------|---------------------|------------------|
|  | Temp (°C)                            | RH (%)              | VPD (kPa) | Slope | [CO2] (ppm)         | Air speed (m/s)  |
| Day  | 24                                   | 70%                 | 0.9       | 2h    | 1000                | Default setpoint |
| Night  | 20                                   | 80%                 | 0.47      | 2h    | climate (no supply) | Default setpoint |
|  | Intensiteit (µmol/m <sup>2</sup> /s) | Spectrum (ratio)    |           |       | Photoperiod         | Dim              |
| Day  | 220 at table height                  | R=90% B=10% + 5% FR |           |       | 16h (00:00 -16:00)  | 0h               |
| Night  | 0                                    |                     |           |       | 8h                  | 0h               |
| Irrigation   |                                      |                     |           |       |                     |                  |
| Shallow Flow irrigation EC=1,8 – pH=6.0 Irrigation Recipe: (Table 4)                     |                                      |                     |           |       |                     |                  |
| Water level (height): 2cm from table surface Irrigation time (fill-drain): +- 10 minutes |                                      |                     |           |       |                     |                  |
| Frequency: 6 times per day at 00:00; 4:00; 8:00; 12:00; 16:00; 20:00                     |                                      |                     |           |       |                     |                  |

**Table 3** 24-h Schedule of the cultivation recipe executed by the partners.

| Time (h) | Temperature (°C) | RH (%) | Par (µmol/m <sup>2</sup> /s) | CO2  |
|----------|------------------|--------|------------------------------|------|
| 0        | 24               | 70     | 220                          | 1000 |
| 1        | 24               | 70     | 220                          | 1000 |
| 2        | 24               | 70     | 220                          | 1000 |
| 3        | 24               | 70     | 220                          | 1000 |
| 4        | 24               | 70     | 220                          | 1000 |
| 5        | 24               | 70     | 220                          | 1000 |
| 6        | 24               | 70     | 220                          | 1000 |
| 7        | 24               | 70     | 220                          | 1000 |
| 8        | 24               | 70     | 220                          | 1000 |
| 9        | 24               | 70     | 220                          | 1000 |
| 10       | 24               | 70     | 220                          | 1000 |
| 11       | 24               | 70     | 220                          | 1000 |
| 12       | 24               | 70     | 220                          | 1000 |
| 13       | 24               | 70     | 220                          | 1000 |
| 14       | 22               | 75     | 0                            | -    |
| 15       | 20               | 80     | 0                            | -    |
| 16       | 20               | 80     | 0                            | -    |
| 17       | 20               | 80     | 0                            | -    |
| 18       | 20               | 80     | 0                            | -    |
| 19       | 20               | 80     | 0                            | -    |
| 20       | 20               | 80     | 0                            | -    |
| 21       | 20               | 80     | 0                            | -    |
| 22       | 22               | 75     | 220                          | 1000 |
| 23       | 24               | 70     | 220                          | 1000 |

Throughout the growth cycle, plants were irrigated using the standard nutrient solution for lettuce on NFT (Yara Teras) shown in Table 4. The water was supplied as shallow flow with a water level (height) of approximately 3 cm from table surface. Water was refreshed in 10 minutes for 50% 6 times per day at 00:00; 4:00; 8:00; 12:00; 16:00; 20:00.

**Table 4** Nutrient solution composition.

| pH | EC  | NH4      | K    | Ca   | Mg  | NO3  | Cr - SO4 2 | H2PC | Si | H | Fe | Mn       | Zn | B  | Cu | Mo |
|----|-----|----------|------|------|-----|------|------------|------|----|---|----|----------|----|----|----|----|
| dS | m-1 | mmol l-1 |      |      |     |      |            |      |    |   |    | µmol l-1 |    |    |    |    |
| 6  | 1.5 | 1        | 9.61 | 4.63 | 1.1 | 16.2 | 2.1        | 1.55 |    |   | 40 | 7        | 7  | 40 | 1  | 1  |

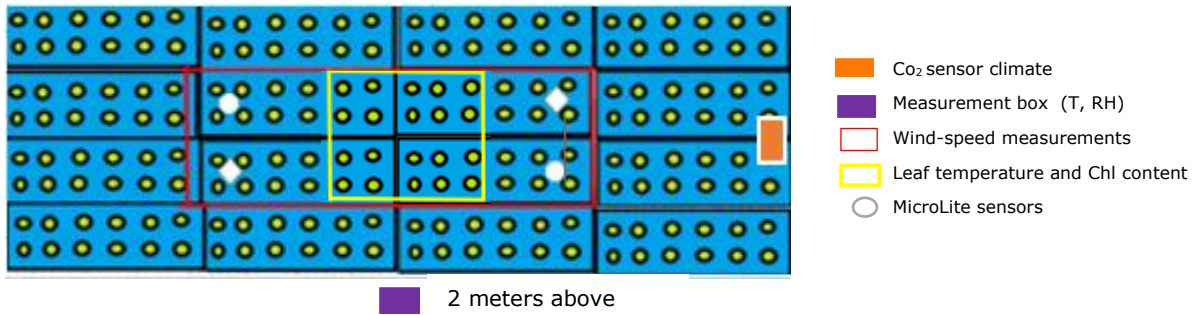
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### 3.3 Measurements

On weekly basis, non-destructive measurements were taken at crop level (synthesized in Table 5 and described in Appendix 1), and a crop description was made regarding the development of lettuce in the different cells. Two destructive measurements were performed during the experiment: one at the end of propagation phase (DAS 15), once the plants were transplanted at different locations, and one at the end of the trial (DAS 35). Non-destructive measurement set-up is described in Table 5. Measurements were taken at each location on plants grown in predefined measurement areas, as shown in the experimental design (Appendix 2 and example WUR, Figure 9). At each destructive harvest, fresh weight, leaf area, length longest leaf, root length and tip burn score were measured for 10 plants per cultivar per treatment (at transplant) and on 20 measurement plants per layer per facility (at final harvest).

**Table 5** Measurement scheme and devices.

| Parameter                       | Crop level                                      | Chlorophyll content   | Wind-Speed              | Macroclimate level       | O2 content                                  | T(°C) & RH (%) micro&macro climate                           | T(°C) , RH (%)                    | CO2 (ppm)                               |
|---------------------------------|---|---|-------------------------|--------------------------|---|--|-----------------------------------|---|
| Sensor                          | INFRARED THERMOMETER (IR) sensor (Testo) 830-T2 | MPM-100 (Opti-Science).   | TESTO 440 climate meter | LI-600 compact porometer | PHORP Meter                                 | MicroLite USB Temperature and Humidity data Logger (Fourtec) | Rotronic HC2A-S Ridder Hoogendrom | Vaisala CARBOC AP® Carbon Dioxide Probe |
| Accuracy                        | 150%  |   | ±(0.03 m/s + 4 % of mv) | ±10%                     | Ph ( 0 to 14 pH) and T ( 0 to 80°C)         | 2%   |                                   | ±40 ppm                                 |
| Range                           | (-30°C to +400°C)                               | fluorescence (from 720 nm to 900) transmission ( from 720 nm to 850 nm) | 0 to 5 m/s              |                          | 0 to 100 ppm dCO2                           | T(-40 to 80 °C) , RH (5 to 95 %)                             | ( 0 to 100%) &(-50 to 100 °C)     | 0-3000 ppm                              |
| Pattern                         | *Yellow square=20 samples                       |   | *Red square= 9 points   | Random along the layer   | No pattern, submerge each layer in stagnant | *White points scheme   | *Purple square                    | *Orange square                          |
| Number of repetition (x sample) | 3   | 3   | 1                       | 10                       | 3 x layer                                   |  |                                   |   |
| Moment of photoperiod           | Daynight 2h slope                               | Day   | Daynight 2h slope       | Daynight 2h slope        | Day   | All period   |                                   |   |
| Frequency                       | 1 x week x partner                              |   |                         |                          |   | continuously, interval 15                                    | continuously,                     | interval 5 minutes                      |



**Figure 9** Location non-destructive measurements taken at MCL4 cell Bleiswijk bottom layer.

### 3.3.1 Statistical analysis

Statistical analysis was performed using GenStat 22nd edition, thereby considering a single plant as an independent repetition. To determine the main effect of all parameters, a one-way analysis of variance (ANOVA) was performed. In addition, a post-hoc protected Tukey test was performed to determine whether the means differed significantly between the partners ( $p < 0.05$ ).



## 4 Results

Abbreviations used are listed in Appendix 1.

### 4.1 Propagation climate and harvest

After 2 days of germination, seedlings were propagated for 13 days. Realized climate is shown in Table 6.

**Table 6** Realized climate during propagation.

| Partner | Day               |        |            |                       |  | Night             |        |            |                       |
|---------|-------------------|--------|------------|-----------------------|--|-------------------|--------|------------|-----------------------|
|         | Temperature (C °) | RH (%) | VPD* (kPa) | CO <sub>2</sub> (ppm) | LI (μmol m <sup>-2</sup> s <sup>-1</sup> ) | Temperature (C °) | RH (%) | VPD* (kPa) | CO <sub>2</sub> (ppm) |
| WUR     | 23.4              | 85.6   | 0.39       | 1087.8                | 227.2                                      | 21.1              | 84     | 0.40       | 1065                  |

\*VPD, Vapour Pressure Deficit.

After 15 days of propagation in MLC4 (WUR, Bleiswijk), propagation trays were randomized among the partners who came collecting them. On 28<sup>th</sup> of March cultivation started at all facilities and lasted for 21 days until 18<sup>th</sup> of April.

All partners set their cells with the reference cultivation recipe (see materials and methods).

### 4.2 Realized environmental conditions during cultivation

From this section onwards, partners names will be hid in order to avoid competition between the results of the farms.

Different facilities controlled the climate differently to make sure that the setpoints were reached, this was intrinsic to the cell design and set up. The average realized climate at each partner during cultivation is shown in Table 7 and Table 8 and followed by the percentage error (Table 9) to show how close the realized climate was to the setpoints. Percentage error was calculated as ((average realized parameter – setpoint)/ setpoint) \* 100).

**Table 7** Average realized climate conditions at room level during day period (lights on).

| Partner   | Average Temperature (C °) | StdDev | Average RH (%) | StdDev | VPD (kPa) | Average CO <sub>2</sub> (ppm) | StdDev |
|-----------|---------------------------|--------|----------------|--------|-----------|-------------------------------|--------|
| Company A | 23,7                      | 0,7    | 71             | 1,6    | 0,85      | 1139                          | 163    |
| Company B | 23,8                      | 1,8    | 62             | 6,8    | 1,12      | 1159                          |        |
| Company C | -                         | -      | -              | -      | -         | -                             | -      |
| Company D | 24,1                      | 0,4    | 81             | 0,9    | 0,57      | 1106                          | 163    |
| Company E | 23,9                      | 0,3    | 70             | 1,6    | 0,89      | 1030                          | 36     |

| Partner   | Average LI (μmol m <sup>-2</sup> s <sup>-1</sup> ) | Photoperiod (h) | DLI (mol m <sup>-2</sup> d <sup>-1</sup> ) |
|-----------|--|-----------------|--|
| Company A | 220  | 16              | 12,7                                       |
| Company B | 139  | 16              | 8,0  |
| Company C | 220  | 16              | 12,7                                       |
| Company D | 225  | 15              | 12,2                                       |
| Company E | 228  | 16              | 13,1                                       |

**Table 8** Average realized climate conditions at room level during night period (lights off).

| Partner   | Temperature (C °) | StdDev | RH (%) | StdDev | VPD (kPa) | CO <sub>2</sub> (ppm) | StdDev |
|-----------|-------------------|--------|--------|--------|-----------|-----------------------|--------|
| Company A | 20,4              | 1,0    | 79     | 2,1    | 0,50      | 831                   | 165    |
| Company B | 21,7              | 1,5    | 66     | 6,2    | 0,88      | 1146                  |        |
| Company C | -                 | -      | -      | -      | -         | -                     | -      |
| Company D | 20,4              | 0,8    | 88     | 2,4    | 0,29      | 612                   | 134    |
| Company E | 20,2              | 0,5    | 79     | 2,0    | 0,50      | 1083                  | 101    |

**Table 9** Ratio of deviation of realized room climate from setpoints during day and night period for each partner shown as percentage error.

| Partner   | Day      |           |                     |         | Night    |           |           |
|-----------|----------|-----------|---------------------|---------|----------|-----------|-----------|
|           | Tair (%) | RHair (%) | CO <sub>2</sub> (%) | VPD (%) | Tair (%) | RHair (%) | VPD (kPa) |
| Company A | -0.8     | 5.8       | 13.9                | -5,5    | 0.9      | -15.1     | 6,4       |
| Company B | -1.5     | -20.4     | 15.9                | 0.18    | 10.3     | -18.3     | 87,2      |
| Company D | -1.0     | 3.8       | -10.1               | -36,6   | 5.5      | -4.4      | 38,3      |
| Company E | -0.39    | 0.5       | 3                   | -1,1    | 0.8      | -1.0      | 6,4       |

Temperature realization during the day was close to setpoint at all partners, as shown by the percentage error which was at maximum 10.3% at Company B. During the night, the same results were found except for Company B, where the temperature was 2 degrees higher than setpoint, showing proportionally percentage error of 18.3%. In terms of relative humidity, Company D and Company E were the closest to setpoint with percentage error values of 4.4 and 1.0 during the day respectively, and 3.8 and 0.5 during the night. Given that relative humidity reflects the current state of absolute humidity relative to a maximum humidity at the same temperature, percentage values cannot be compared when they are associated with different temperatures. Therefore, we used vapour pressure deficit (VPD) for comparison purposes since it indicates the humidity deficit present in the air. VPD, is the difference (deficit) between the amount of moisture in the air and how much moisture the air can hold when it is saturated. Contrary to relative humidity, VPD has a more straightforward relationship with transpiration activity of the crop. In general, average VPD values achieved by all partners deviated from the recipe, except for the case of Company E and Company D during the dark period for which average VPD was around 0.5 as programmed. During the day period, Company A, Company D and Company E recorded average VPD conditions (0.77, 0.84, 0.89 kPa respectively) below the setpoints (0.9 kPa) which indicates their climate humidity stayed higher than the programmed recipe. In the case of Company B, the average VPD (1.08 kPa) was higher than the setpoints (0.9 kPa) indicating the environment executed tended to be drier than programmed. LI and DLI values showed the same results as day temperature for all partners, except for Company B, with LI and DLI values 1.6 and 0.6 times respectively lower due to wrong filled in setpoint in the climate system. CO<sub>2</sub> levels during day had a high percentage error value compared to the other climate parameters at Company A (13.9%) and Company B (15.9%), and lower at Company D (10.2%) respect the setpoint. At night, there was no CO<sub>2</sub> supplementation. At Company E the cell is airtight so CO<sub>2</sub> level during night was higher due to crop respiration. On the other hand, cells that are less tight, such as Company D, had lower night CO<sub>2</sub> values. Climate cell data from Company C was not available due to technical problems (data loss) during the experiment.

### 4.3 Realized environmental conditions during cultivation

Wind-speed, temperature and relative humidity measurements were taken at microclimate level (on floater) and macroclimate level (25 cm on top of floater) in all facilities (Table 12, 13, 14). Comparison between climate cell data and MicroLite data during cultivation weeks is shown in Appendix 3.

**Table 10** Average wind-speed measurement.

| Partners  | Wind speed (m/s) |        |        |       |                         |       |         |      |
|-----------|------------------|--------|--------|-------|-------------------------|-------|---------|------|
|           | on floater       |        |        |       | 25 cm on top of floater |       |         |      |
|           | day              | SEM    | night  | SEM   | day                     | SEM   | night   | SEM  |
| Company A | 0,04 b           | 0.0045 | 0,04 b | 0.042 | 0,17 c                  | 0.019 | 0,18 bc | 0.18 |
| Company B | 0,06 a           | 0.07   | 0,11 a | 0.1   | 0,27 b                  | 0.03  | 0,22 b  | 0,22 |
| Company C | 0,05 ab          | 0.0045 | 0,03 b | 0.26  | 0,35 a                  | 0.04  | 0,34 a  | 0,02 |
| Company E | 0,04 b           | 0.0045 | 0,05 b | 0.045 | 0,15 c                  | 0.016 | 0,15 c  | 0.02 |

Wind speed measured between the crop (on floater) was highest at Company B, on average 0.06 m/s during the day, 1.3 times higher than Company A and Company C and 1.5 than Company E. At night the same trend was found (Table 12). This can be explained by the fact that air at Company B was supplied by tubes placed within the crop.

However at 25 cm from the floater, Company C showed the highest speed during day, on average 0,35 m/s, which was 2.2 times higher than Company A and Company E and 1.4 than Company B. ( $p < 0.001$ ). At night, the same trend was found.

**Table 11** Average MicroLite sensors: microclimate measurement within the crop.

| Partners  | On floater |      |        |     |           |      |           |      |        |      |           |      |
|-----------|------------|------|--------|-----|-----------|------|-----------|------|--------|------|-----------|------|
|           | Day        |      |        |     |           |      | Night     |      |        |      |           |      |
|           | Temp (°C)  | SEM  | RH (%) | SEM | VPD (kPa) | SEM  | Temp (°C) | SEM  | RH (%) | SEM  | VPD (kPa) | SEM  |
| Company A | 25.4 b     | 0.03 | 77.3 c | 0.2 | 0.81 b    | 0.01 | 20.6 b    | 0.04 | 89.6 b | 0.19 | 0.28 c    | 0.01 |
| Company B | 23.9 d     | 0.03 | 67.1 e | 0.2 | 1.02 a    | 0.01 | 20.5 b    | 0.03 | 75.3 d | 0.21 | 0.61 a    | 0.01 |
| Company C | 25.5 ab    | 0.06 | 84.1 a | 0.4 | 0.56 d    | 0.01 | 20.5 b    | 0.06 | 90.8 a | 0.35 | 0.23 d    | 0.01 |
| Company D | 25.6 a     | 0.05 | 82.6 b | 0.2 | 0.65 c    | 0.01 | 22.3 a    | 0.07 | 89.5 b | 0.25 | 0.33 b    | 0.01 |
| Company E | 24.2 c     | 0.03 | 73.9 d | 0.1 | 0.82 b    | 0.00 | 19.7 c    | 0.03 | 86.4 c | 0.13 | 0.32 b    | 0.00 |

In terms of microclimate measured on top of the floater and therefore below the crop (Table 13), partners achieved significantly different conditions with the greatest differences found during the day period. During the day, average temperature at Company C was similar to Company A and Company D and above the setpoints (24 °C). Humidity was variable, both above and below the setpoints resulting as well in variable VPD (70% and 0.9 kPa). During the night period, air temperature at floater level was close to the programmed value (20 °C) for most of the partners except for Company D where the average temperature was 2 degrees higher. In terms of humidity, there was more variation and each partner had significantly different levels of humidity with a tendency towards higher humidity levels than the setpoints (80%, 0.47 kPa). This is expected, especially after canopy closure.

**Table 12** Average MicroLite sensors microclimate measurement facilities.

| Partners  | At 25 cm on top of floater |      |        |      |           |      |           |      |        |      |           |      |
|-----------|----------------------------|------|--------|------|-----------|------|-----------|------|--------|------|-----------|------|
|           | Day                        |      |        |      |           |      | Night     |      |        |      |           |      |
|           | Temp (°C)                  | SEM  | RH (%) | SEM  | VPD (kPa) | SEM  | Temp (°C) | SEM  | RH (%) | SEM  | VPD (kPa) | SEM  |
| Company A | 25.4 a                     | 0.02 | 75.8 c | 0.1  | 0.79 c    | 0.00 | 20.9 b    | 0.03 | 83.0 b | 0.15 | 0.43 b    | 0.00 |
| Company B | 24.3 e                     | 0.03 | 64.8 e | 0.19 | 1.1 a     | 0.01 | 20.6 c    | 0.03 | 73.1 c | 0.22 | 0.67 a    | 0.01 |
| Company C | 24.7 c                     | 0.03 | 83.1 a | 0.15 | 0.53 e    | 0.01 | 20.4 c    | 0.05 | 85.9 a | 0.15 | 0.34 d    | 0.00 |
| Company D | 25.3 b                     | 0.02 | 77.0 b | 0.08 | 0.77 d    | 0.00 | 21.7 a    | 0.04 | 85.1 a | 0.14 | 0.42 b    | 0.00 |
| Company E | 24.5 d                     | 0.02 | 71.1 d | 0.06 | 0.89 b    | 0.00 | 20.1 d    | 0.03 | 83.6 b | 0.07 | 0.39 c    | 0.00 |

MicroLite sensors also monitored climate at 25 cm on top of the floater which was in close range with the top canopy of the crop during the cultivation. In general, we observed the same trend as with the microclimate on the floater surface: plants experienced significantly different canopy climate conditions among the partners during the cultivation. Average temperature registered during the day period deviated slightly from the setpoints and tended to be warmer than the setpoints (24 °C). Humidity levels were slightly higher than the setpoints (70%, 0.9 kPa) for most of the partners except for Company B whose climate tended to be drier than the desired level. During the night period, a similar trend to the climate measured on the floater was observed. Average temperatures deviated little from the setpoints for most of the partners. Humidity levels tended to be above setpoint. VPD level were closer to setpoint for Company D and Company A, lower for Company C and Company E (more humid), and higher in the case of Company B (drier).

## 4.4 Destructive harvest

After 35 days of production (seed to harvest), lettuce heads were harvested at all partners. Average values of fresh and dry weight (kg m<sup>-2</sup>), dry matter content of lettuce (g), tip burn score, plant height (cm), longest length leaf (cm) and roots length (cm) for each partner are shown in Table 13 and Table 14.

**Table 13** Average fresh weight and tip burn scores measured at final harvest of the lettuce plants for each partner.

| Partner   | FW crop (g) | SEM  | DW crop (g) | SEM  | DMC (%) | Tip burn score | SEM  |
|-----------|-------------|------|-------------|------|---------|----------------|------|
| Company A | 226,1 c     | 0.87 | 5,6 b       | 0.03 | 2,5 a   | 1,6 a          | 0.01 |
| Company B | 68,3 a      | 0.56 | 2,5 a       | 0.01 | 3,7 c   | 1,2 a          | 0.01 |
| Company C | 252,1 c     | 0.57 | 8,2 c       | 0.02 | 3,3 bc  | 1,2 a          | 0.01 |
| Company D | 220,9 c     | 0.66 | 6,2 b       | 0.03 | 2,8 ab  | 1,2 a          | 0.01 |
| Company E | 173,6 b     | 0.62 | 7,6 c       | 0.02 | 4,4 d   | 1,6 a          | 0.02 |

The results (Table 13) showed that Company C, Company A and Company D produced the largest fresh lettuce head, on average 233 g/head, which were 1.3 times heavier than those produced at Company E and 3.4 times heavier than those produced at Company B ( $p < 0.001$ ). Different trend was found with dry weight (DW), were Company C and Company E produced the highest average, 7.9 g/head, which was 1.3 higher respect Company A and Company D, and 3 times respect Company B ( $p < 0.001$ ). The lower dry weight and, in general, smaller heads produced at Company B was due to the 1.6 times lower DLI. In terms of dry matter content, Company E had the highest value, on average 4.4%, which was 1.3 times higher than the rest of the partners. Average tip burn score showed no differences between the partners, a tendency with slightly higher values was found at Company A and Company E. In all facilities plants showed thus quality loss due to tip burn, moreover, heterogeneity among the heads was found with an average standard deviation of 27.5 g compared to the average crop fresh weight.

**Table 14** Average plant height, leave length and root length measured on the final harvest of the lettuce plants for each partner.

| Partner   | Plant height (cm) | SEM  | Length longest leaf (cm) | SEM  | Root length (cm) | SEM  |
|-----------|-------------------|------|--------------------------|------|------------------|------|
| Company A | 14,3 b            | 0.04 | 17,5 b                   | 0.04 | 25,5 a           | 0.13 |
| Company B | 8,9 a             | 0.03 | 13,2 a                   | 0.05 | 36,0 b           | 0.21 |
| Company C | 16,9 bd           | 0.09 | 17,7 b                   | 0.02 | 27,3 a           | 0.16 |
| Company D | 14,1 b            | 0.01 | 14,2 a                   | 0.02 | -                | -    |
| Company E | 16,5 bd           | 0.08 | 12,6 a                   | 0.07 | 34,1 b           | 0.10 |

The plant morphology results (Table 14) showed that Company C and Company E produced the most elongated plants, on average 16.7 cm/head, which were 1.7 times taller than Company A and Company D and 1.8 than Company B ( $p < 0.001$ ). Length of the longest leaf values were higher at Company A and Company C, with an average of 17.6 cm/leaf, 1.3 times longer than Company B ( $p < 0.001$ ). Root length was higher at Company B and Company E, with an average of 35,1 cm/head, 1.3 times longer than Company A and Company C ( $p < 0.001$ ). During the trial, plants at Company E suffered water stress during the first days after transplant due to the distance between substrate and water surface. This can be one of the reasons for root development promotion as well as for the high DMC.

**Table 15** Estimated average fresh weight yield and annual production level for each partner.

| Partner   | Yield FW (kg m <sup>-2</sup> ) | Yearly production (kg m <sup>-2</sup> y <sup>-1</sup> ) | LUE FW (g/mol) | LUE DW (g/mol) |
|-----------|--------------------------------|---|----------------|----------------|
| Company A | 5,6                            | 91  | 18,1           | 0,45           |
| Company B | 1,6                            | 26  | 5,5            | 0,20           |
| Company C | 6,0                            | 98  | 20,2           | 0,66           |
| Company D | 5,3                            | 86  | 17,7           | 0,50           |
| Company E | 4,1                            | 67  | 13,9           | 0,62           |

Yield (kg m<sup>-2</sup>) and yearly production (kg m<sup>-2</sup> y<sup>-1</sup>) were calculated for all partners to indicate the production that could be achieved under the set conditions and reflected towards a commercial application (Table 17). The highest yields were calculated at Company C, Company A and Company D, reflecting the highest average head weight which led to a calculated yearly production of 98, 88, 86 kg m<sup>-2</sup> y<sup>-1</sup> respectively. Light use efficiency (LUE) was calculated from seed to harvest with the total light sum received and the weighted average plant density over the total growth cycle from seed until harvest. The highest LUE was achieved at Company C with 20,2 g FW/mol and 0.66g DW/mol.

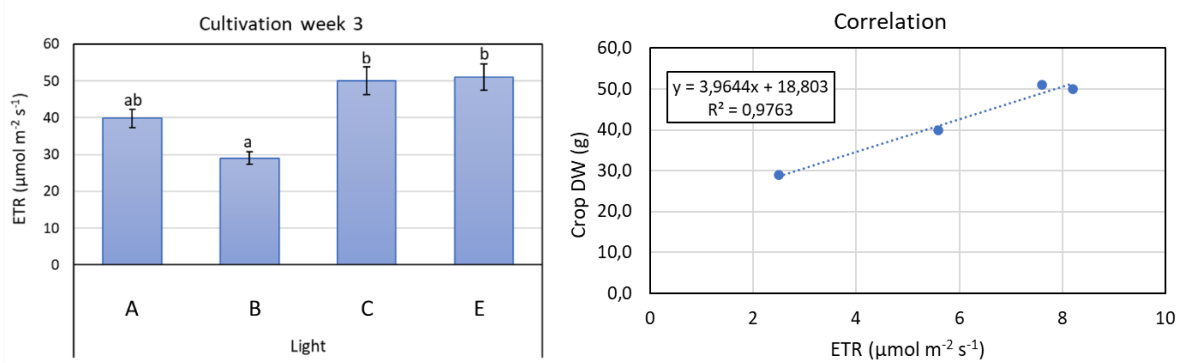
## 4.5 Crop responses during cultivation

Day and night plant temperature (°C) and pigment content (Chlorophyll and anthocyanins) were measured during two cultivation weeks in the different facilities (Table 16), relative electron transport rate (ETR), stomatal conductance (gsw) and fluorescence (Fv/Fm) were measured during the third cultivation week (except at Company D), when the growth stage of the plant (leaf size) could allow it.

**Table 16** Photochemical average measures.

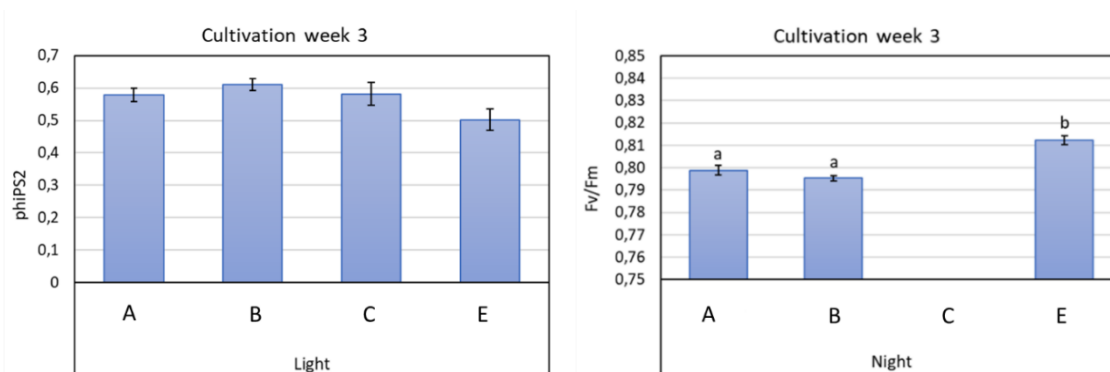
| Partners  | Chlorophyll | SEM  | Anthocyanins | SEM   | Plant temp. Day (°C) | SEM  | Plant temp. Night (°C) | SEM  |
|-----------|-------------|------|--------------|-------|----------------------|------|------------------------|------|
| Company A | 0,43b       | 0.04 | 0,05b        | 0.001 | 21,2b                | 0.01 | 16,7b                  | 0.02 |
| Company B | 0,19a       | 0.19 | 0,03a        | 0.001 | 19,9a                | 0.02 | 18,0d                  | 0.01 |
| Company C | 0,40b       | 0.40 | 0,05b        | 0.001 | 22,5c                | 0.03 | 17,1c                  | 0.03 |
| Company D | 0,39b       | 0.40 | 0,06b        | 0.001 | 21,7b                | 0.01 | 17,4c                  | 0.01 |
| Company E | 0,47b       | 0.44 | 0,07b        | 0.016 | 20,2a                | 0.02 | 16,1a                  | 0.01 |

Chlorophyll and anthocyanins content did not significantly differ among plants cultivated at Company A, Company C, Company D and Company E. Plants from these partners had an average of 0.42 chlorophyll content and 0.05 anthocyanins content. Plants at Company B had significantly lower chlorophyll content (0.19) and anthocyanins content (0.3) than the rest of the partners ( $p < 0.001$ ). This can be explained by the lower DLI the plants at Company B were exposed to. In terms of plant day temperature, measured with the IR sensor, Company C showed the highest leaf temperature, on average 22.5°C, 1 degree higher than Company D and Company A and 2.5 degrees than Company E and Company B. However, at night, Company B showed the highest results, on average 18°C, 0.7 degrees higher than Company D and Company C, 1.3 than Company A and 1.9 than Company E partners. The higher night leaf temperature at Company B is also due to the higher temperature realized in the Pods.



**Figure 10** Graph left) electron transport rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) in the 3<sup>rd</sup> cultivation week. Graph right) correlation electron transport rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) and biomass production.

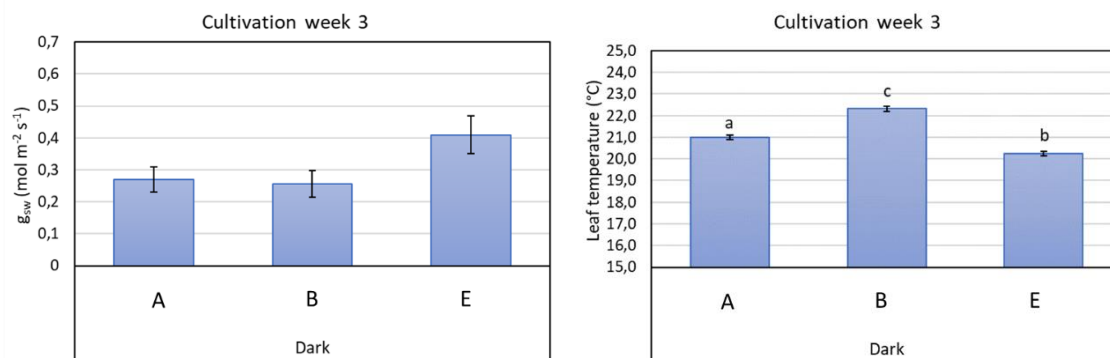
Company C, Company E and Company A had the highest ETR values measured during the 3<sup>rd</sup> cultivation week, on average  $46.7 \mu\text{mol m}^{-2} \text{s}^{-1}$  (Figure 10), 1.6 times higher than Company B ( $p < 0.001$ ). The electron transport rate is a function of the absorbed light thus it is expected that plant a Company B had a lower ETR due to the 37% lower light received during cultivation. In the second graph, the correlation between biomass production and ETR, with an  $R^2$  of 0.98, is shown to underline the relationship between electron transport rate, photosynthesis, sugar production and thus biomass accumulation.



**Figure 11** Graph Left) photosystem II efficiency in the 3<sup>rd</sup> cultivation week with light period (day) between different facilities. Graph Right) Dark-adapted correlation fluorescence in the 3<sup>rd</sup> cultivation week with night period between different facilities.

Results showed a trend in efficiency of the photosystem II at Company C, Company B and Company A on average 0.59, 1.2 times more than Company E. Fluorescence during the night period showed no stress at any partner with values higher or very close to 0.8 (Figure 11).





**Figure 12** Graph Left) Stomatal conductance ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) in the 3rd cultivation week during dark (night) period between different facilities. Graph Right) Leaf temperature ( $^{\circ}\text{C}$ ) in the 3rd cultivation week with during dark (night) period between different facilities.

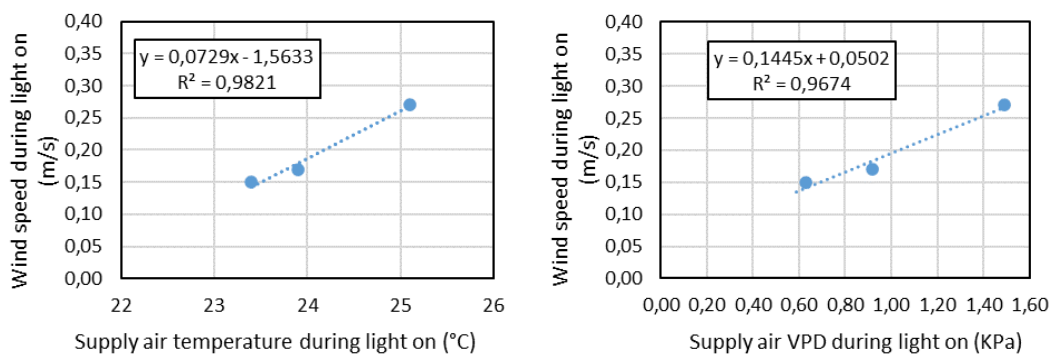
Company E plants showed highest stomatal conductance values during the night, on average  $0.4 \mu\text{mol m}^{-2} \text{s}^{-1}$ , which was 1.5 times higher than Company A, and 1.6 than Company B ( $p=0.03$ ) (Figure 12). In the right graph, night leaf temperature measured via the poro-fluorometer is reported. At Company B leaf temperature was founded 1.07 times higher than Company A, and 1.2 than Company E ( $p<0.001$ ) higher during the night, on average  $22.5^{\circ}\text{C}$ , which was close to the realized temperature in the Pods.

# 5 Discussion

## 5.1 Crop-climate interaction

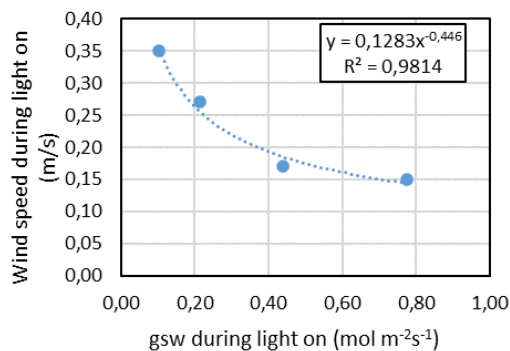
Young lettuce plants were cultivated for 3 weeks in 5 different indoor farming facilities. Fresh head weight varied significantly between facilities at the end of the trial and heterogeneity in the final harvested crop was found at all companies. Only in one case (Company B), the low final fresh weight could be explained by the wrong setpoints (37% lower DLI, 10% higher night temperature). For the other 4 facilities, correct climate settings were applied and realized.

Looking closer to the realized conditions around the plants it is interesting to highlight that wind speed within each facility didn't vary between day and night. This means that the climate is realized only (or mainly) by changing the characteristics of the air but the airflow stays constant. For example, there is a correlation between wind speed and supplied air temperature (Appendix 3): the facilities with lower wind speed blow in cooler air compared to the cells with higher wind speed (Figure 13). At the same time, lower VPDs at cell level are measured when lower wind speeds are found. For this correlation, when multiple growing areas were used, the wind speed measurements taken at 25 cm from floater at all areas were averaged as a representation of the whole cell.



**Figure 13** Correlation of wind speed during light on and average supplied air (temperature of the blown in air) (left) and supply air VPD (right) at different companies.

At 25 cm from the floater, low wind speeds were found at Company A and Company E (on average 0.16 m/s) while almost double speed was measured at Company C and Company B being closer to the optimum wind speed of 0.3-0.7 m/s reported by Kitaya et al. (2000). Wind speed has an effect on stomatal conductance: when higher wind speeds were measured, lower stomatal conductance was found showing that the plant is responding to it by closing the stomata in order to prevent too high transpiration (Figure 14). At Company E, lower wind speeds were measured at 25 cm from the floater in combination with higher VPD during the day which pushed the plant to keep the stomata more open and thus had an effect on transpiration. In addition, at night at Company E the largest values for conductance were measured. This implies that the plant is transpiring significantly during the night (and during the day), which leads to insufficient nutrient transport towards the young leaves, causing the higher tipburn appearance.



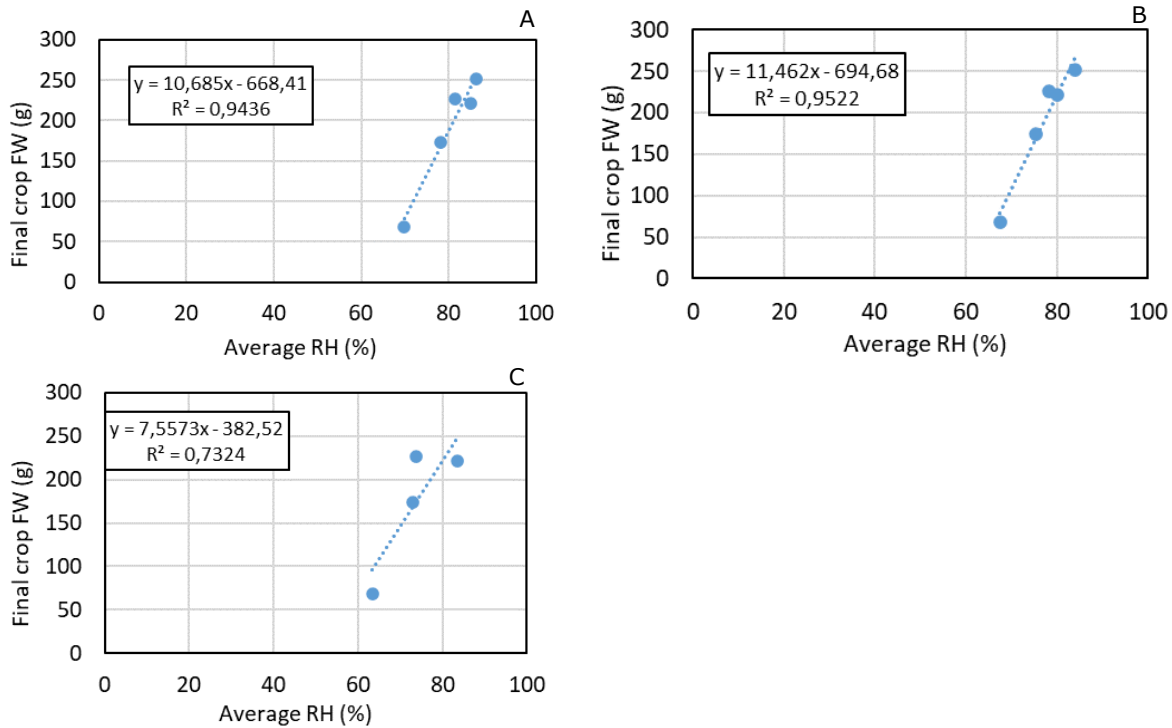
**Figure 14** Correlation of wind speed during light on and stomatal conductance (gs) at different companies.

At crop level (floater, Table 11) Company E had a lower temperature both during the day and during the night compared to the other 3 companies. Temperature influences plant development so a lower temperature can lead to lower yields. Indeed, the final head fresh weight at company E was 32% lower compared to the average of 233 g achieved at Company C, Company D and Company A (pooled together as no statistical difference was found). With a lower developmental rate but same growth rate (same DLI as other 3 partners) it is also explained why a higher DMC was found at Company E (Table 13).

## 5.2 Towards standardization

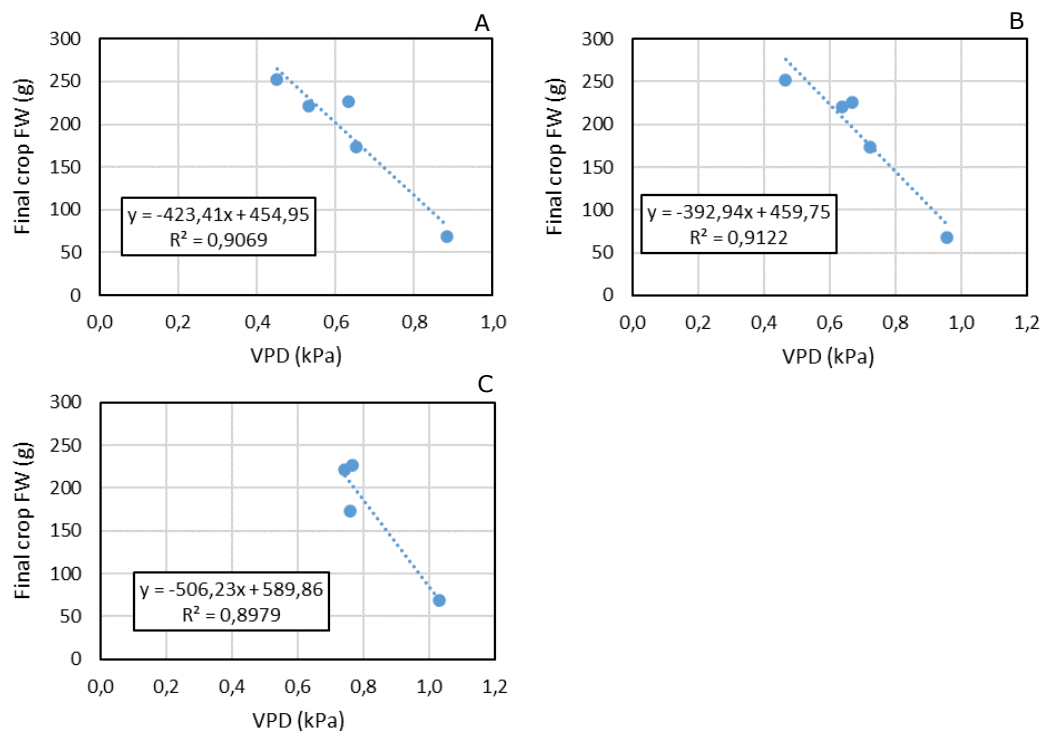
At the end of the trial it was clear that the same climate recipe can lead to different realized climates around the plant. This means that a cultivation recipe expressed at cell level can't be used as a standard way to discuss setpoints among Vertical Farms to expect the same crop growth and development patterns. When we want to assess and/or predict crop performance, which parameters do we need to monitor and where? As shown in the previous paragraphs, during the third week of cultivation, ETR was measured at all patterns and a good correlation ( $R^2 = 0.98$ ) was found with the final crop dry weight (Figure 10). During the same week, wind speed and stomatal conductance were measured and a strong correlation was found between the two ( $R^2 = 0.98$ ; Figure 14).

Final fresh weight was also correlated with the average realized climate of the cultivation weeks. A strong correlation was found with the average RH and the average VPD. For RH, the position where this was measured played a role in how strong the correlation was with final fresh weight: the strongest correlation was found in close proximity to the crop with  $R^2 = 0.94$  when measured on the floater and  $R^2 = 0.95$  when measured at 25 cm on top of the floater. Poorest correlation was found with the cell measurement (point where the cells are steering climate) with  $R^2 = 0.73$  (Figure 15).



**Figure 15** Correlation of average final crop weight (FW, grams) and average relative humidity during 3 weeks of cultivation at floater level (A) at 25cm from floater (B) and cell level (C).

Interestingly, when the correlation is made with average VPD, the correlation at cell level, 25cm on top of the floater and on the floater is similar with an  $R^2=0.90$  (Figure 16). This is possibly due to the fact that differences in humidities at different locations in the farm are usually coupled to different temperatures. VPD depends on these two climate parameters and it is thus less sensitive to the location where it is measured.



**Figure 16** Correlation of average final crop weight (FW, grams) and average VPD during 3 weeks of cultivation at floater level (A) at 25cm from floater (B) and cell level (C).

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## 6 Conclusions

When the same climate recipe was correctly applied in 5 different vertical farms, all systems were able to realize the setpoints and maintain them for 3 weeks of cultivation. Although at cell level realized climate matched with setpoints and was thus similar among the farms, closer to the plants the realized climate differed. Depending on how the farm works (ex. wind speed) and where the measurements are of the realized conditions (ex. in the cell or in the return air duct), the climate achieved at crop level is different. The climate around the plant (air flow, temperature and VPD) directly determines crop development and physiological processes such as transpiration which in the end affect the yield and quality of the final product.

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# Appendix 1 List of abbreviations

DMC = Dry Matter Content

DLI = Daily Light Integral

DW = Dry Weight

FW = Fresh Weight

LI = light intensity

RHair = Air relative humidity

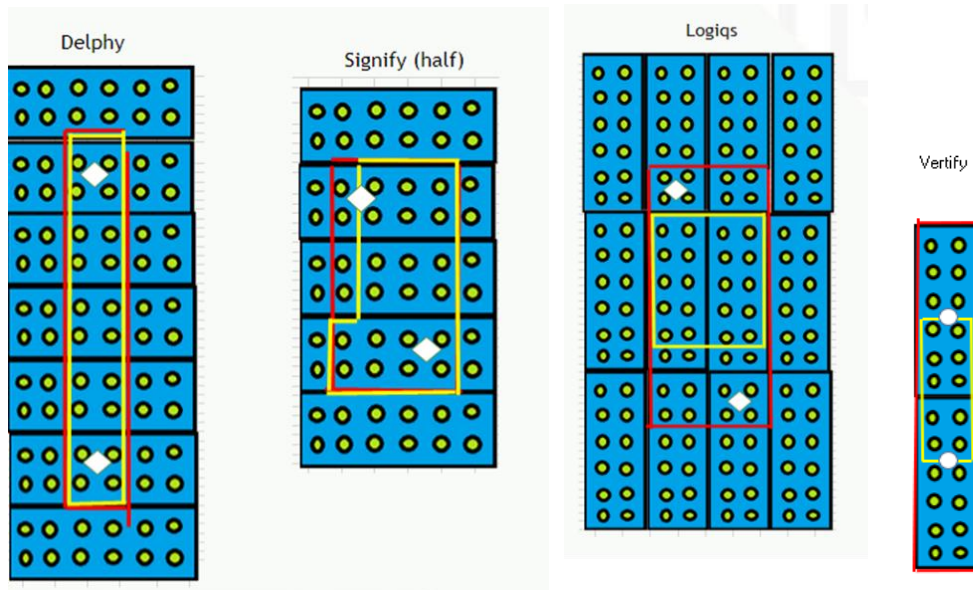
SEM = Standard Error of Mean

StdDev = standard deviation

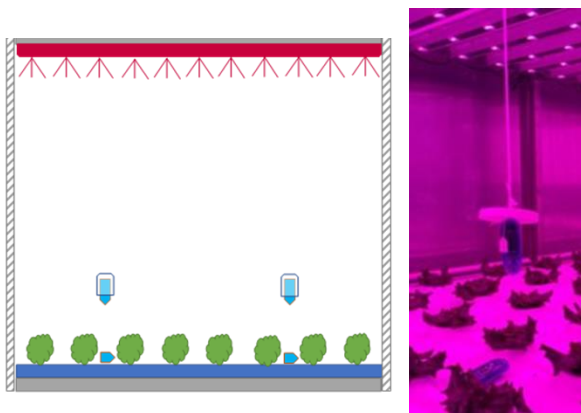
Tair = Air temperature

VPD = Vapor Pressure Deficit

## Appendix 2 Set-up at partners



Measurement area of the rest of facilities were non-destructive and destructive measurements were taken during the trial.



Scheme and picture of the position of MicroLite dataloggers at WUR, Bleiswijk. Microclimate T-RH sensor placed laying on the floaters; 4 MicroLite/layer. Macroclimate sensor placed 25 cm above floaters protected by direct light with a plastic lit; 4 MicroLite/layer.

# Appendix 3 Climate per cultivation week

## Company A

| Temp<br>Cultivation week | Light           |            |                        |            |            | Dark            |            |                        |            |            |
|--------------------------|-----------------|------------|------------------------|------------|------------|-----------------|------------|------------------------|------------|------------|
|                          | Measurement box | Supply air | 25cm on top of floater | Below crop | Return air | Measurement box | Supply air | 25cm on top of floater | Below crop | Return air |
| 1                        | -               | 23,8       | 26,0                   | 27,0       | 23,7       | -               | 20,3       | 20,9                   | 21,0       | 20,4       |
| 2                        | -               | 23,9       | 25,4                   | 26,1       | 23,7       | -               | 19,9       | 20,9                   | 20,9       | 20,4       |
| 3                        | -               | 23,9       | 24,4                   | 23,7       | 23,7       | -               | 19,7       | 20,6                   | 20,4       | 20,4       |

| RH<br>Cultivation week | Light      |            |                        |            |            | Dark       |            |                        |            |            |
|------------------------|------------|------------|------------------------|------------|------------|------------|------------|------------------------|------------|------------|
|                        | Calculated | Supply air | 25cm on top of floater | Below crop | Return air | Calculated | Supply air | 25cm on top of floater | Below crop | Return air |
| 1                      | 70,6       | 67,3       | 71,0                   | 65,5       | 68,0       | 79,0       | 75,6       | 80,0                   | 82,8       | 75,2       |
| 2                      | 70,5       | 69,1       | 75,9                   | 73,4       | 69,7       | 79,0       | 80,2       | 83,3                   | 87,0       | 77,6       |
| 3                      | 70,4       | 70,8       | 81,2                   | 83,0       | 71,8       | 79,0       | 82,3       | 84,4                   | 90,0       | 78,7       |

## Company B

| Temp<br>Cultivation week | Light           |            |                        |            |            | Dark            |            |                        |            |            |
|--------------------------|-----------------|------------|------------------------|------------|------------|-----------------|------------|------------------------|------------|------------|
|                          | Measurement box | Supply air | 25cm on top of floater | Below crop | Return air | Measurement box | Supply air | 25cm on top of floater | Below crop | Return air |
| 1                        | 22,1            | 19,8       | 25,6                   | 25,5       | 22,9       | 21,0            | 20,2       | 21,9                   | 22,1       | 21,5       |
| 2                        | 23,6            | 24,4       | 24,6                   | 24,2       | 23,2       | 21,0            | 21,7       | 21,0                   | 21,0       | 20,9       |
| 3                        | 25,2            | 29,0       | 22,9                   | 22,4       | 23,0       | 22,7            | 25,8       | 20,3                   | 20,1       | 21,0       |

| RH<br>Cultivation week | Light           |            |                        |            |            | Dark            |            |                        |            |            |
|------------------------|-----------------|------------|------------------------|------------|------------|-----------------|------------|------------------------|------------|------------|
|                        | Measurement box | Supply air | 25cm on top of floater | Below crop | Return air | Measurement box | Supply air | 25cm on top of floater | Below crop | Return air |
| 1                      | 69,7            | 71,4       | 56,7                   | 58,8       | 68,4       | 71,9            | 70,3       | 68,0                   | 69,8       | 72,5       |
| 2                      | 61,9            | 55,1       | 60,9                   | 63,1       | 67,7       | 67,6            | 61,0       | 70,4                   | 71,5       | 72,7       |
| 3                      | 57,9            | 40,4       | 76,4                   | 76,4       | 75,7       | 60,6            | 44,7       | 80,0                   | 78,7       | 76,6       |

## Company C

| Temp<br>Cultivation week | Light                  |            | Dark                   |            |
|--------------------------|------------------------|------------|------------------------|------------|
|                          | 25cm on top of floater | Below crop | 25cm on top of floater | Below crop |
| 1                        | 25,2                   | 27,2       | 20,9                   | 21,4       |
| 2                        | 24,8                   | 26,4       | 20,6                   | 20,8       |
| 3                        | 24,4                   | 23,1       | 20,2                   | 19,8       |

| RH<br>Cultivation week | Light                  |            | Dark                   |            |
|------------------------|------------------------|------------|------------------------|------------|
|                        | 25cm on top of floater | Below crop | 25cm on top of floater | Below crop |
| 1                      | 75,1                   | 68,6       | 80,0                   | 79,4       |
| 2                      | 79,9                   | 80,6       | 83,5                   | 89,2       |
| 3                      | 85,3                   | 95,1       | 86,2                   | 97,1       |

## Company D

| Temp | Light            |                 |            |                        |            | Dark       |                 |            |                        |            |
|------|------------------|-----------------|------------|------------------------|------------|------------|-----------------|------------|------------------------|------------|
|      | Cultivation week | Measurement box | Supply air | 25cm on top of floater | Below crop | Return air | Measurement box | Supply air | 25cm on top of floater | Below crop |
| 1    | 24,0             | 23,5            | 26,9       | 28,6                   | 24,8       | 20,5       | 19,8            | 20,9       | 21,1                   | 20,9       |
| 2    | 24,2             | 23,9            | 26,2       | 27,3                   | 24,8       | 20,6       | 20,1            | 20,8       | 20,8                   | 20,9       |
| 3    | 24,0             | 23,9            | 24,9       | 24,2                   | 24,4       | 20,3       | 20,0            | 20,3       | 20,2                   | 20,5       |

| RH | Light            |                 |            |                        |            | Dark       |                 |            |                        |            |
|----|------------------|-----------------|------------|------------------------|------------|------------|-----------------|------------|------------------------|------------|
|    | Cultivation week | Measurement box | Supply air | 25cm on top of floater | Below crop | Return air | Measurement box | Supply air | 25cm on top of floater | Below crop |
| 1  | 81,3             | 81,9            | 69,4       | 64,6                   | 74,2       | 87,3       | 91,4            | 83,3       | 85,9                   | 83,2       |
| 2  | 80,8             | 80,0            | 74,2       | 75,2                   | 74,4       | 87,7       | 90,6            | 87,1       | 91,9                   | 84,1       |
| 3  | 80,5             | 78,2            | 80,9       | 92,4                   | 74,9       | 88,6       | 90,5            | 90,3       | 95,9                   | 85,5       |

## Company E

| Temp | Light            |                 |            |                        |            | Dark       |                 |            |                        |            |
|------|------------------|-----------------|------------|------------------------|------------|------------|-----------------|------------|------------------------|------------|
|      | Cultivation week | Measurement box | Supply air | 25cm on top of floater | Below crop | Return air | Measurement box | Supply air | 25cm on top of floater | Below crop |
| 1    | 23,9             | 22,4            | 25,1       | 25,5                   | 25,4       | 20,1       | 19,5            | 20,4       | 20,1                   | 20,4       |
| 2    | 23,9             | 23,1            | 24,6       | 25,1                   | 25,2       | 20,2       | 19,7            | 20,2       | 20,0                   | 20,3       |
| 3    | 23,9             | 24,6            | 23,8       | 22,5                   | 25,0       | 20,2       | 20,7            | 19,9       | 19,2                   | 20,3       |

| RH | Light            |                 |            |                        |            | Dark            |            |                        |            |            |
|----|------------------|-----------------|------------|------------------------|------------|-----------------|------------|------------------------|------------|------------|
|    | Cultivation week | Measurement box | Supply air | 25cm on top of floater | Below crop | Measurement box | Supply air | 25cm on top of floater | Below crop | Return air |
| 1  | 70,4             | 90,7            | 67,6       | 65,8                   | 79,3       | 90,0            | 81,6       | 82,2                   |            |            |
| 2  | 70,3             | 79,9            | 70,1       | 69,0                   | 79,2       | 87,3            | 82,8       | 83,5                   |            |            |
| 3  | 70,3             | 66,3            | 74,7       | 83,8                   | 79,2       | 78,7            | 85,6       | 91,4                   |            |            |

\* Supply air doesn't account for extra humidifier.



To explore  
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quality of life



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