Quantifying the spatial and temporal variability of the environmental conditions in a cultivation room for the micropropagation of cannabis.

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Abstract

Controlled environment agriculture (CEA) is an indoor production technique where growth conditions are carefully monitored and controlled to optimize crop production. With the world's population and climate change continuing to rise, CEA has become increasingly important. However, many CEA facilities face challenges in achieving economic viability due to high operating costs. One strategy to enhance efficiency in CEA is gaining a deeper understanding of the variability of environmental conditions within growth enclosures. Through the analysis of data related to temperature, humidity, CO_2 levels, and light intensity in CEA settings, researchers can refine energy models and implement energy-efficient practices. This holds particular relevance in the micropropagation of cannabis, where stringent control of environmental conditions is vital for successful plant growth. Thus, this study aims to quantify the spatial and temporal variability of environmental conditions in a cultivation room used for the micropropagation of cannabis, and to understand the impact of this variability on the growth of stage-two cannabis plantlets. To monitor the environmental conditions in the cultivation room, a low-cost Internet of Things (IoT) sensor system using Arduino technology and InfluxDB software was developed. The system includes sensors that measure temperature, humidity, CO_2, and light levels, and sends the data to a web server. The study tested five different locations within the shelved cultivation room for one-week periods. Basic statistics (i.e., average, mean, standard deviation, skewness, etc.), along with uniformity indexes, were employed to assess spatiotemporal variability of the environmental conditions inside the cultivation room. Notably, an average temperature difference of 1.9°C between locations was detected, which resulted in a relatively low overall uniformity index of 0.52. An analysis of plantlet growth using the Kruskal-Wallis H-test, a non-parametric alternative to ANOVA, revealed a statistically significant difference in plantlet heights at the end of the growth stage across various locations ($H = 12.41$, $p = 0.002$, $p < 0.05$). Furthermore, a strong linear correlation ($R^2 = 0.992$) was observed between temperature variability and plantlet heights. These findings provide valuable insights into assessing microclimate variability in CEA cultivation rooms and underscore the importance of further exploring the impact of these environmental conditions on in-vitro-grown cannabis plants.

Résumé

L'agriculture en environnement contrôlé (AEC) est une technique de production intérieure où les conditions de croissance sont soigneusement surveillées et contrôlées pour optimiser la production de cultures. Avec la population mondiale et les changements climatiques qui continuent de progresser, ce type de culture agricole sera un acteur clé pour assurer une plus grande sécurité alimentaire aux générations futures. Cependant, de nombreux projets en lien avec l'AEC sont confrontées à des défis, tant au niveau de la viabilité économique que de l'impact environnemental. Une stratégie pour améliorer l'efficacité en AEC est d'acquérir une compréhension plus profonde de la variabilité des conditions environnementales à l'intérieur des enceintes de croissance. Grâce à l'analyse des données liées à la température, à l'humidité, aux niveaux de CO_2 et à l'intensité lumineuse dans des enceintes de cultures intérieurs, les chercheurs peuvent affiner les modèles énergétiques et mettre en œuvre des pratiques écoénergétiques. Cela revêt une importance particulière dans le domaine de la micro-propagation du cannabis, où un contrôle strict des conditions environnementales est essentiel pour une croissance réussie des plantes.

Ainsi, cette étude vise à quantifier la variabilité spatiale et temporelle des conditions environnementales dans une salle de culture utilisée pour la micro-propagation du cannabis, et à comprendre l'impact de cette variabilité sur la croissance des plantules de cannabis. Pour surveiller les conditions environnementales dans la salle de culture, un système de capteurs Internet des objets (IoT) utilisant la technologie Arduino et le logiciel InfluxDB a été développé. Le système comprend des capteurs qui mesurent la température, l'humidité, les niveaux de CO_2 et de lumière, et envoie les données à un serveur web. L'étude a testé consécutivement cinq emplacements différents à l'intérieur de la salle de culture pendant des périodes d'une semaine. Des statistiques de base (moyenne, médiane, écart type, asymétrie, etc.), ainsi que des indices d'uniformité, ont été utilisées pour évaluer la variabilité spatiotemporelle des conditions environnementales à l'intérieur de la salle de culture. Notamment, une différence de température moyenne de 1,9 °C entre les différents emplacements testés a été détectée, ce qui a abouti à un indice d'uniformité global relativement faible de 0,52. Une analyse de la croissance des plantules à l'aide du test de Kruskal-Wallis a révélé une différence statistiquement significative de la grandeur des plantules à la fin de la phase de croissance entre les différents emplacements ($H = 12,41$, $p = 0,002$, $p < 0,05$). De plus, une forte corrélation linéaire ($R^2 = 0,992$) a été observée entre la variabilité de la température et la grandeur des plantules. Ces résultats fournissent des informations précieuses pour évaluer la variabilité du microclimat dans les salles de culture AEC et soulignent l'importance d'explorer davantage l'impact de ces conditions environnementales sur les plants de cannabis cultivés in vitro.

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Contributions of Authors

In this thesis, the roles of the authors are delineated as follows: (1) Jérôme Trudel-Brais – responsible for the planning and execution of experiments, data collection, analysis, and presentation of results; (2) Dr. Mark Lefsrud — oversaw the design of the experiments, offered guidance and expertise, and reviewed the thesis. (3) Dr. Valérie Orsat — reviewed the thesis.

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List of Abbreviations and Acronyms

ACU - Air-conditioning units

AI - Artificial intelligence

ANOVA - Analysis of variance

AR - Augmented reality

ARS - Autonomous robotic systems

BDA - Big data and analytics

CC - Cloud computing

CEA - Controlled environment agriculture

CFD - Computational fluid dynamics

CPS - Cyber-physical systems

DT - Digital twins

DWT - Deep-water technique

FLC - Fuzzy logic control

HVAC - Heating, ventilation, and air conditioning

IoT - Internet of things

IRGA - Infrared gas analyzer

KPIs - Key performance indicators

LUI - Local uniformity index

MPC - Model predictive control

NDIR - Non-Dispersive Infrared

NFT - Nutrient film technique

OCGG - Organic, clean, green, and gourmet

PAR - Photosynthetically active radiation

PFEL - Plant factories with electrical lighting

PFSL - Plant factory with solar lighting

PID - Proportional-Integral-Derivative

QS - Quality score

RH - Relative humidity

SI - International system

TI - Temperature integration

TUI - Total uniformity index

VF - Vertical Farm

VPD - Vapour pressure deficit

1. Chapter 1 – General Introduction

1.1 Thesis Motivation

Controlled environment agriculture (CEA) is an innovative method of growing crops indoors, in which the growth conditions such as temperature, humidity, light, and nutrient levels are carefully monitored and controlled to optimize production (Kozai et al., 2020). This includes both commercial greenhouses and plant factories with electrical lighting (PFEL), also known as vertical farms (Otto, 2022; Shamshiri et al., 2018). The key difference between these types of CEA systems is the degree of monitoring and control, as well as the technologies used (Hati & Singh, 2021; Kozai, 2018). PFELs are cultivation rooms with optically opaque, thermally insulated, and airtight walls (Despommier, 2019). These rooms may or may not utilize racks with horizontal multi-tiers to increase crop production (Kozai, 2018). Greenhouses can be defined as plant factory with solar lighting (PFSL). Unlike PFELs, they do not have optically opaque, thermally insulated, and airtight walls.

Controlled environment agriculture is becoming increasingly important as the world's population continues to grow, and climate change becomes more severe. With the global population projected to reach around 10 billion people by 2050, and the likelihood of more frequent and intense droughts, floods, and other extreme weather events due to climate change, there is a need for innovative solutions to sustainably produce food (Benke & Tomkins, 2017; Despommier, 2011). CEA is likely to play a key role in meeting this challenge, as it allows for the controlled production of crops in an indoor environment, regardless of weather conditions or access to fertile land (Krzysztofowicz et al., 2020).

Growing crops indoor, in a controlled environment, offers many benefits, including year-round production, increased crop yields per surface area, reduced dependence on favourable climate conditions and arable lands, and the ability to locate facilities in urban areas, which reduce transportation-related emissions and bring people closer to their food both physically and conceptually (Benke & Tomkins, 2017; Krzysztofowicz et al., 2020; Specht et al., 2013). However, while it is often portrayed as an innovative and sustainable solution, the reality is more nuanced and complex. Important factors such as environmental footprint and economic viability still require thorough investigation. Among these factors, the energy consumption of CEA facilities poses a significant challenge, impacting operational costs and the environment. CEA facilities consume significantly more energy, up to 30 times more than conventional agriculture, leading to a larger environmental footprint (Engler & Krarti, 2021; Van Ginkel et al., 2017). As CEA is a relatively new industry, more innovation and development are needed to increase its sustainability and accessibility to society.

The 4.0 Indoor Farming Industry encompasses the latest innovation avenues in CEA. It is characterized by the adoption and fusion of emerging high-end technologies such as the internet of things (IoT), big data and analytics (BDA), autonomous robotic systems (ARS), augmented reality (AR), artificial intelligence (AI) and much more (Abbasi et al., 2022). One of the core principles of Industry 4.0 is the paramount significance of data. Data monitoring serves as the foundation of Industry 4.0 and is being recognized as a key catalyst for innovation (Abbasi et al., 2022). In the realm of indoor farming, numerous facets can be monitored to optimize processes and production. Examples include soil and irrigation management, plant phenotyping, and, especially, environmental conditions. Multiple environmental factors profoundly impact crop quality and yield. Extensive literature reports highlight temperature, humidity, ambient chemical compounds (e.g., CO2 levels), air movement, and light recipes (i.e., intensity and quality) as the most critical factors (Engler & Krarti, 2021; Iddio et al., 2020; Kozai et al., 2020; Langhans & Tibbitts, 1997a).

Temperature, humidity, ambient CO_2 levels, and air movement stand as crucial environmental factors when cultivating crops in controlled environment agriculture (Ahmed et al., 2020; Engler & Krarti, 2021; Niu et al., 2016). These environmental conditions wield a significant influence over various metabolic processes within plants, including photosynthesis, respiration, transpiration, stomatal conductance, and leaf boundary layer (Downs & Krizek, 1997; Grossiord et al., 2020; Kitaya, 2005; Körner, 2006; Peet & Krizek, 1997; Pritchard et al., 1999; Tibbitts, 1979). Therefore, ensuring uniformity of the microclimate within CEA facilities and cultivation rooms is crucial for achieving consistent, optimized, and predictable crop production (Bhujel et al., 2021; Uyeh et al., 2022). Various factors impact environmental conditions, resulting in spatial and temporal variations in the microclimate within a given space (Kozai et al., 2016). Monitoring environmental data is a fundamental practice to ensure efficient management of microclimates within CEA facilities, as emphasized by recent studies (Bhujel et al., 2021; Uyeh et al., 2022). This practice not only enhances decision-making processes but also plays a pivotal role in designing and controlling the heating, ventilation, and air conditioning (HVAC) systems within these facilities (Chamara et al., 2022; Shamshiri et al., 2018).

1.2 Research Problem

This project explores the realms of microclimates and environmental data monitoring in controlled environmental agriculture.

- Examine the current state of knowledge regarding microclimate variability and environmental data monitoring in CEA. This involves an in-depth investigation of the myriad factors that influence the

microclimate within cultivation rooms and an understanding of their subsequent effects on plant growth.

- Measure the spatial and temporal variations in environmental conditions within a dedicated cultivation room for cannabis micropropagation.
- Evaluate how the variability of the microclimate within the cultivation room affects the growth of stage-2 micropropagated cannabis plantlets.

1.3 Objectives

- Design and construct an IoT-based environmental monitoring system for data collection on temperature, humidity, ambient CO_2 concentration, and light intensity.
- Develop statistical methods for quantifying both the spatial and temporal variations of environmental conditions within a multi-layer cultivation room.
- Conduct environmental data collection in a specialized cultivation room designed for the micropropagation of cannabis plantlets and evaluate the spatiotemporal variability of the microclimate within the facility.
- Cultivate stage-2 cannabis plantlets in various locations within the cultivation room and investigate the effect of the microclimate variability on the plantlet growth.

Connecting Text

The next chapter delved into the environmental factors essential to consider in CEA. It provided insights into fundamental concepts concerning temperature, humidity, carbon dioxide, and air movement, as well as their interplay with plant metabolic processes. Additionally, it examined the key determinants affecting microclimate uniformity and the prerequisites for sensors to monitor variations in these environmental conditions.

2. Chapter 2 – Literature Review

2.1 Controlled Environment Agriculture (CEA)

2.1.1 Definitions and History

2.1.1.1 Definition

Controlled environment agriculture is an innovative method of growing crops indoors, in which the growth conditions such as temperature, humidity, light, and nutrient levels are carefully monitored and controlled to optimize production (Kozai et al., 2020). This includes both commercial greenhouses and plant factories with electrical lighting (Otto, 2022; Shamshiri et al., 2018). The key difference between these types of CEA systems is the degree of monitoring and control, as well as the technologies used (Hati & Singh, 2021; Kozai, 2018).

2.1.1.2 History

The concept of greenhouses dates back to 14 CE in Rome, where the first known protected structure was used to grow Cucumis plants year-round for Emperor Tiberius. From the traditional heating floor known as *Ondol*, used to grow mandarin in Korea during the 1450s, to *Orangeries* in France during the 17th century, which were buildings with large windows used to protect fruit trees from cold temperatures, the concept of greenhouses has evolved and gained popularity over the centuries (Berkers & Geels, 2011; Muijzenberg, 1980; Nemali, 2022). Modern commercial greenhouses made their appearance in the 20th century and are now found worldwide. These greenhouses are designed to accommodate the specific climate conditions of the area in which they are located, including semi-arid, tropical, and Nordic regions. Some of the top greenhouse-producing countries today include the Netherlands, Spain, China, and the United States (Critten & Bailey, 2002; Shamshiri et al., 2018).

Research on vertical farming can be traced back as early as 1949, when the Earhart Plant Research Laboratory at the California Institute of Technology conducted studies on the effect of light and environmental conditions on crop growth. In the 1970s, the first vertical farms were established in the United States and the Netherlands (Otto, 2022). In 2010, Dr. Dickson Despommier's book "The Vertical Farm: Feeding the World in the 21st Century" generated widespread interest in the potential benefits of vertical farms. Today, the vertical farming industry is well established in countries such as Japan, China, Singapore, Israel, the United States, and Canada (Benke & Tomkins, 2017; Kozai et al., 2020).

Controlled environment agriculture is becoming increasingly important as the world's population continues to grow, and climate change effects become more severe. With the global population projected to reach around 10 billion people by 2050, and the likelihood of more frequent and intense droughts,

floods, and other extreme weather events due to climate change, there is a need for innovative solutions to sustainably produce food (Benke & Tomkins, 2017; Despommier, 2011). CEA is likely to play a key role in meeting this challenge, as it allows for the controlled production of crops in an indoor environment, regardless of weather conditions or access to fertile land (Krzysztofowicz et al., 2020).

2.1.1.3 Key CEA Technologies, Concepts, and Terms

2.1.1.3.1 Plant Factory with Electrical Lighting (PFEL)

PFELs are cultivation rooms with optically opaque, thermally insulated, and airtight walls (Despommier, 2019). Of this fact, they thus require a lighting system. These rooms may or may not utilize racks with horizontal multi-tiers to increase crop production (Kozai, 2018). An example of a PFEL without racks is regularly found in indoor cultivation for cannabis plants.

2.1.1.3.2 Greenhouse – Plant Factory with Solar Lighting (PFSL)

Greenhouses can be defined as Plant Factory with Solar Lighting (PFSL). Unlike PFELs, they do not have optically opaque, thermally insulated, and airtight walls. They rely on environmental control units such as heaters, shading screens, thermal screens, insect screens, and roof/fan ventilators or evaporative cooling to regulate temperature and other conditions inside the greenhouse (Critten & Bailey, 2002; Kozai, 2018). A lighting system may be used to supplement low levels of sunlight and extend the illumination period (Nemali, 2022).

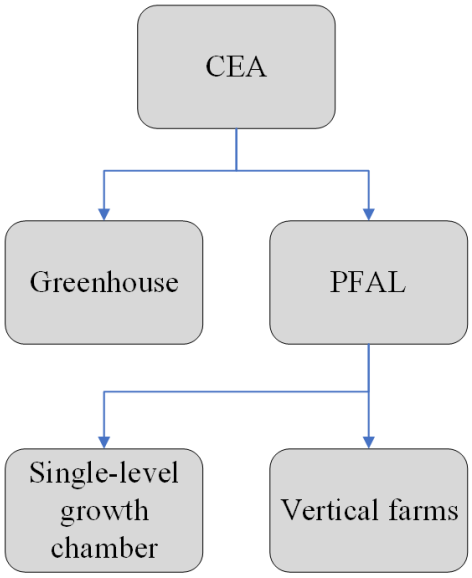

Figure 2.1: Distinction between greenhouses, PFELs and vertical farms

2.1.1.3.3 Vertical farm (VF)

Plant factory with electrical lighting and vertical farms are often used interchangeably, but they are not the same thing. A VF is a type of PFAL that utilizes horizontal multi-tiers to increase crop production. A vertical farm is a PFAL that is specifically designed to grow crops in stacked layers, rather than in a single horizontal plane (Kozai, 2018). The distinction between the different CEA facilities is presented in Figure 2.1.

2.1.1.3.4 Irrigation Systems: Hydroponic, Aeroponics, and Aquaponic

CEA systems require a delivery and circulation unit for the nutrient solution that nourishes the plants (Kozai, 2018). There are various types of irrigation systems currently used in the industry, including hydroponic, aeroponic, and aquaponic systems (Despommier, 2019). Hydroponic systems grow plants in an inert substrate, such as rockwool or clay, and provide them with a mineral nutrient solution on a regular basis. Within the category of hydroponics, there are two main techniques: the deep-water technique (DWT) and the nutrient film technique (NFT) (Son et al., 2020). Aeroponic systems do not use a substrate to support the plant roots. Instead, the roots are suspended in the air and are periodically exposed to a mist or spray of nutrient solution (Niu & Masabni, 2022). Aquaponic systems are similar to hydroponics. However, the source of the nutrients is different: while hydroponics uses chemical nutrients, aquaponics integrates fish into the system and uses their waste as a natural source of nutrients for the plants (Despommier, 2019).

2.1.2 Potential Benefits

Figure 2.2 summarizes the potential benefits and the reality of CEA.

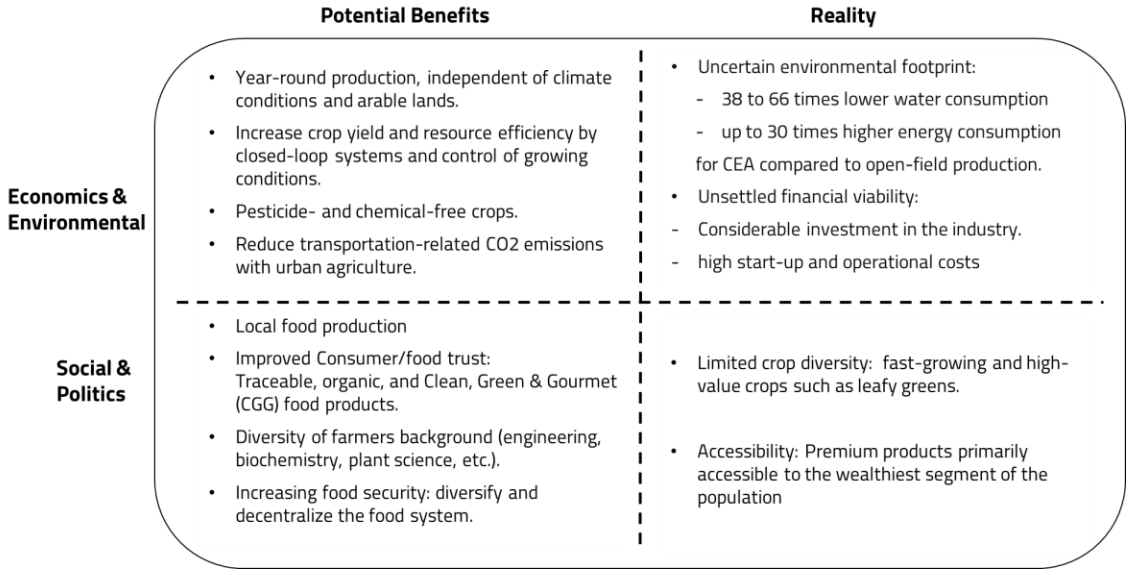

Figure 2.2: Summary of the potential benefits and the reality of CEA

2.1.2.1 Economics and Environmental

Growing crops indoor, in a controlled environment offers many benefits, including year-round production, increased crop yields per surface area, reduced dependence on favourable climate conditions and arable lands, and the ability to locate facilities in urban areas, which reduce transportation-related emissions (Benke & Tomkins, 2017; Krzysztofowicz et al., 2020; Specht et al., 2013). Additionally, closed-loop systems and control over growing conditions can lead to more efficient and sustainable crop production (Van Ginkel et al., 2017). Pesticide- and chemical-free crops can be produced, and lower amounts of fresh water and nutrients are consumed (Benke & Tomkins, 2017). Furthermore, optimal growth conditions can lead to shorter growth periods and higher flavour and nutritional value for the crops (O'Sullivan et al., 2019). Overall, controlled environment agriculture offers a range of economic and environmental advantages (Shamshiri et al., 2018).

2.1.2.2 Social and Politics

CEA facilities bring people closer to their food both physically and conceptually. Physically, by locating facilities in urban and peri-urban areas, people have access to fresh produce grown closer to home. Conceptually, by showcasing the origin of food and providing options for organic, clean, green, and gourmet (OCGG) produce, CEA helps improve people's relationship with their food (Benke & Tomkins, 2017; Specht et al., 2013). Utilizing advanced technologies and innovation, CEA helps create a new diverse group of farmers coming from various fields such as engineering, biochemistry, biotechnology, plant science, construction, finance, etc. (Krzysztofowicz et al., 2020). The establishment of a network of vertical farms helps diversify and decentralize the food system, thus increasing food security in society (Despommier, 2019).

2.1.3 The Reality of CEA

The reality of controlled environment agriculture is more complex and nuanced than how it is typically portrayed in articles advocating for it. The environmental footprint of CEA is not well established yet (Casey et al., 2022; Specht et al., 2013). CEA systems have higher water and nutrient usage efficiency, with water usage being 38 to 66 times lower than open-field production. However, CEA facilities consume significantly more energy, up to 30 times more than conventional agriculture, leading to a larger environmental footprint (Engler & Krarti, 2021; Van Ginkel et al., 2017). Depending on the energy source used, this can result in CEA hydroponically grown lettuce emitting up to 17.8 kg CO_2 eq. per kg of lettuce produced, which is even larger than the 10 kg CO_2 eq. per kg emitted by intercontinental air-freighted lettuce (Casey et al., 2022). Additionally, CEA facilities have high start-up and operational costs, making their financial viability

uncertain (Baumont de Oliveira et al., 2022). The majority of CEA facilities in the US did not achieve a net profit in 2017 (O'Sullivan et al., 2019). As a result, only fast-growing and high-value crops such as leafy greens and herbs are typically grown in CEA facilities, which are then sold at a premium to high-end customers (restaurants, retailers, pharmaceutical industry) (Krzysztofowicz et al., 2020; O'Sullivan et al., 2019). This limits crop diversity and makes CEA produce accessible primarily to the wealthiest segment of the population (Specht et al., 2013). As CEA is a relatively new industry, more innovation and development are needed to increase its sustainability and accessibility.

2.2 Environmental Data Monitoring in the 4.0 Indoor Farming Industry

2.2.1 The 4.0 Indoor Farming Industry

The concept of industry 4.0 corresponds to an industrial revolution characterized by the adoption and fusion of emerging high-end technologies such as the Internet of Things (IoT), big data and analytics (BDA), system integration (SI), cloud computing (CC), simulation, autonomous robotic systems (ARS), augmented reality (AR), artificial intelligence (AI), wireless sensor networks (WSN), cyber-physical systems (CPS) and digital twins (DT) (Abbasi et al., 2022). Agriculture, and indoor farming especially, are witnessing this digitalization of processes (Hati & Singh, 2021). The adoption of machine vision systems to help monitor crop health and detect disease development (Ahmad & Nabi, 2021; Siregar et al., 2022), or the implementation of autonomous robotic systems to perform different tasks during the plant development (i.e., seeding/transplanting, pruning, harvesting, etc.), are some examples of this transition (Abbasi et al., 2022). An increase in research and technology adoption related to industry 4.0 has been noticed since 2016 in controlled environment agriculture (Abbasi et al., 2022). In two literature reviews on agriculture 4.0 and Ag-IoT from 2011 to 2021, more than 80% of the selected research studies were published between 2016 and 2022 (Abbasi et al., 2022; Chamara et al., 2022) as illustrated in Figure 2.3. Similarly,

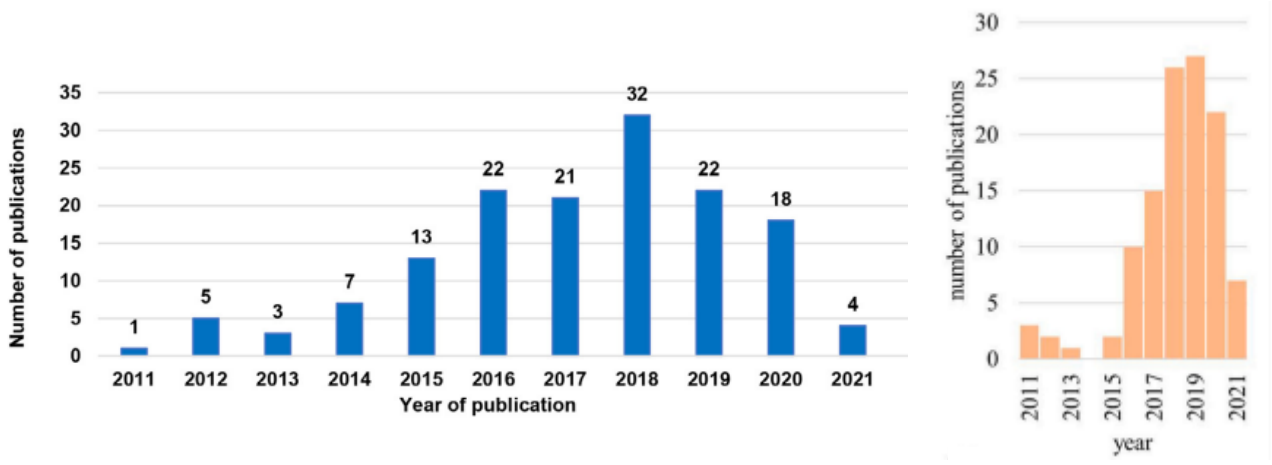

Figure 2.3: Augmentation of the number of publications related to agriculture 4.0 (left) and Ag-IoT (right) from 2001 to 2021 (Abbasi et al., 2022; Chamara et al., 2022).

more than 50% of the selected articles in a 2016-2022 literature review on precision agriculture and artificial intelligence were published in 2021 and 2022 (Cravero et al., 2022).

One of the principles of Industry 4.0 is the paramount significance of data. Data monitoring serves as the foundation of Industry 4.0 and is being recognized as a key catalyst for innovation (Abbasi et al., 2022). The ability to monitor and analyze vast volumes of data empowers organizations to gain valuable insights into the interconnectedness between system inputs and outputs, leading to enhanced decision-making and a profound comprehension of operations (Tsai et al., 2013). In the realm of indoor farming, numerous facets can be monitored to optimize processes and production. Examples include soil and irrigation management, plant phenotyping, and environmental conditions: (1) Monitoring parameters such as pH, electrical conductivity, and water temperature of nutrient solutions has become a standard practice in CEA to uphold optimal irrigation conditions and optimize plant growth and operations (Chamara et al., 2022; Hati & Singh, 2021). (2) Phenotyping entails the observation, measurement, and characterization of observable plant traits. By quantifying plant attributes like morphology and growth using non-destructive techniques, namely imaging technic, valuable insights can be gained into the interaction between the genotype, phenotype, and environment of the plant (Li et al., 2014). (3) Multiple environmental factors profoundly impact crop quality and yield. Extensive literature reports highlight temperature, humidity, ambient chemical compounds (e.g., CO_2 levels), and light recipes (i.e., intensity and quality) as the most critical factors (Engler & Krarti, 2021; Iddio et al., 2020; Kozai et al., 2020; Langhans & Tibbitts, 1997a).

2.2.2 The Importance of Environmental Conditions Data Monitoring

The growth of crops is profoundly influenced by factors such as temperature, humidity, CO_2 levels, and light. Hence, monitoring those environmental conditions within a controlled environment agriculture facility becomes crucial for optimizing yields and overall processes. This practice not only enhances decision-making but also aids in the design and control of the heating, ventilating, and air conditioning systems, as well as efficient resource utilization.

2.2.2.1 Decision-making

Traditional decision-making in CEA facilities and crop management is constrained by several limitations. Typically, it relies on a single set of data, experiences long delays between data generation and decision-making, and tends to focus on addressing one factor at a time, disregarding the fact that crops can be subjected to multiple stresses simultaneously. Monitoring the environmental conditions holds the possibility to revolutionize decision-making at the farm-level by enabling multi-inputs, multi-outputs decision strategies, driven by real-time data processing (Chamara et al., 2022). For example, the ability to

monitor the microclimate within the farm at a high spatiotemporal resolution allows for the precise quantification and management of the variations of the environmental conditions within CEA facilities (Kagan et al., 2022). By uncovering the spatial variability of environmental conditions, it becomes possible to identify yield-limiting factors across different areas of the farm, both horizontally and vertically in multi-level farming setups (Alfred et al., 2021). Similarly, the availability of high temporal resolution data enables the observation of crop responses to environmental cues at finer time intervals. This enriches the understanding of how fundamental plant physiological processes, such as transpiration and photosynthesis, fluctuate in response to short-term changes in the environment (Chamara et al., 2022). Along the same idea, there is a lack of site-specific data to properly parameterize, calibrate, and train crop and CEA facility numerical models/digital twins. The acquired data on environmental conditions provides an opportunity to improve the accuracy of these models for farm-level management assessment (Martini et al., 2021). Environmental condition monitoring helps transform the qualitative, labour-intensive, experience-based decision-making process to a quantitative, automatic, data-driven approach.

2.2.2.2 HVAC System and Resource Efficiency

The HVAC system of a CEA facility plays a crucial role in applying and maintaining uniformed and predefined environmental parameters (e.g., temperature, humidity, CO_2 levels) so that crop yields are optimized and predictable (Iddio et al., 2020). Monitoring the environmental conditions enables a better understanding of the HVAC system's performance and therefore improved design and control (Shamshiri & Ismail, 2013). The performances of the HVAC system are directly dependent of its initial design. Factors such as the placement of air inlet/outlet points, the responsiveness of actuators (e.g., ventilation, heating/cooling elements), and the capacity of the dehumidifier significantly influence the HVAC system's ability to effectively apply and maintain predefined environmental parameters (Kang & van Hooff, 2022). Theoretically, numerical models such as computational fluid dynamics (CFD) simulation can provide insights on thermal fluxes inside a CEA facility (Reichrath & Davies, 2002; Torre-Gea et al., 2011). Multiple studies have worked on modelling temperature distribution and airflow uniformity within growth area in greenhouses and relative error of 3% between simulated and real temperatures was achieved (Norton et al., 2007; Zhou et al., 2020). However, it is much more difficult to model and get accurate results for temperature distribution for large and complex CEA facilities, especially for multi-level farming setups (Baek et al., 2016; Bournet & Rojano 2022; Natarajan et al., 2022). For many variables, interactions and interdependencies need to be considered. Non-uniform distribution of air in the growth area can be caused by several elements; namely uneven air flows from air circulating fans, natural convection due to heat energy generated by lamps, and air flow resistance due to culture shelves or plants (Zhang & Kacira,

2018a). Therefore, it is essential to get empirical data on temperature, humidity, and CO_2 distributions and fluxes to analyze their performances, and to adapt and improve the design of the HVAC systems (Bournet & Rojano 2022).

2.3 Environmental Conditions: General Concepts & Plant Response

Temperature, humidity, CO_2 levels, and air movement stand as crucial environmental factors when cultivating crops in controlled environment agriculture (Ahmed et al., 2020; Engler & Krarti, 2021; Niu et al., 2016). A solid grasp of the fundamental principles governing each of these conditions is vital for comprehending their impact on plant growth. The temperature of both the plants and the surrounding air is regulated by heat transfer mechanisms, encompassing conduction, convection, radiation, and latent heat transfer from evaporation (Hicklenton & Heins, 1997). Temperature wields a significant influence over various metabolic processes within plants, including photosynthesis, respiration, and transpiration (Körner, 2006). Relative humidity (RH) and vapour pressure deficit (VPD) emerge as key psychrometric parameters that directly affect plant transpiration and stomatal resistance (Grossiord et al., 2020; Tibbitts, 1979). The ambient concentration of CO_2, an aspect sometimes under-monitored in CEA, plays a pivotal role in the physiological processes of many plant species. Elevated CO_2 levels impact photosynthesis, stomatal conductance, and overall plant growth (Peet & Krizek, 1997; Pritchard et al., 1999). Air movement, characterized by both its velocity and direction, is closely linked to the leaf boundary layer of plants (Downs & Krizek, 1997; Kitaya, 2005). As for light, while it constitutes a critical environmental factor, this research refrains from delving into it extensively, given the abundance of accessible studies on the subject.

2.3.1 Temperature

2.3.1.1 General concepts

Temperature is a fundamental physical property that quantifies the amount of sensible heat energy present in a substance. It is commonly measured using the International System (SI) unit of Kelvin (K), although it can also be expressed in degrees Celsius (°C) or degrees Fahrenheit (°F) (Hicklenton & Heins, 1997; Pavese, 2014) .

Heat transfer can occur in two main forms: sensible and latent heat transfer (Cho et al., 1998). Sensible heat transfer, which encompasses conduction, convection, and radiation, directly influences the temperature of an object or body (Morris & Langari, 2016a). Latent heat transfer involves energy exchanges that do not directly affect the temperature of a body. Instead, it involves energy fluxes associated with phase transitions, such as evaporation or condensation, occurring at a constant

temperature (Lienhard & Lienhard, 2019; Niu et al., 2016). Temperature reflects the sensible heat energy content of a substance, while heat transfers can occur in the form of both sensible and latent heat.

The plant temperature, which influences many physiological processes, is affected by both sensible and latent heat transfers between the plant and its environment (Gates, 1965; Ye et al., 2013). Equation (2.1) describes the overall energy balance between a plant and its surrounding (Hicklenton & Heins, 1997):

$$E_S = E_R + E_c + E_L + E_M \qquad (2.1)$$

where E_R is the radiative heat transfers (i.e., absorbed and re-radiated), E_C is the conductive and convective heat transfers, E_L is the latent heat transfer (evaporation and condensation of water at the leaf surface), E_M is the balance of heat produced and consumed in the plant's metabolic reaction (negligible) and E_S is the energy stored in the plant, related to plant internal temperature changes.

2.3.1.1.1 Conduction and Convection

Conduction involves the transfer of energy at the molecular level due to a temperature gradient between two separated elements in contact. The heat transfer moves naturally from a region of higher temperature to one with lower temperature (Cho et al., 1998). In the context of plants, energy is conducted between the leaf cells or any part of the plant and the adjacent air molecules (Nobel, 2020b; Rashke, 1960). Conductive heat transfers at the plant/environment interface are limited without convective air movement because of the low thermal conductivity of air (Hicklenton & Heins, 1997; Niu et al., 2016).

Convective heat transfers involves the transfer of energy from one point to another through the movement of a fluid, such as air (Lienhard & Lienhard, 2019). There are two types of convection: free and forced convection. Free convection occurs when air experiences buoyant movement due to variations in air density caused by temperature gradients (Cho et al., 1998; Lienhard & Lienhard, 2019). Under still conditions, a temperature gradient develops between the air in the boundary layer at the surface of the leaf and its surroundings. This results in a corresponding air density gradient, leading the air, and energy, to move from regions of higher density to regions of lower density (Gates, 1965; Nobel, 2020b). Conversely, heat fluxes resulting from air movement generated by fans or wind correspond to forced convection (Cho et al., 1998). Compared to free convection, forced convection is more efficient in promoting heat transfer. In indoor plant production, it is recommended to apply air movement at the leaf canopy in the range of 0.5 to 1.0 $m.s^{-1}$. This controlled airflow enhances the exchange of heat and ensures optimal environmental conditions for plant growth and development (Downs & Krizek, 1997; Hicklenton & Heins, 1997; Niu et al., 2016).

2.3.1.1.2 Radiation

Electromagnetic radiation, characterized by its wavelength (e.g., the visible range is from 400 nm to 700 nm), are a vital source of energy for the development of plants (Gates, 1965; Nobel, 2020b). However, not all wavelengths are equally beneficial. Radiation falling between 400 nm and 700 nm is considered photosynthetically active radiation (PAR) and it is absorbed by the plant to drive photosynthesis (Sager & Mc Farlane, 1997). These wavelengths contribute significantly to the plant's development, but the energy load associated with these wavelengths in the overall energy budget of the plant is relatively low, accounting for only around 3% (Nobel, 2020b). Consequently, their thermal contribution is negligible in consideration to the plant's overall thermal energy balance (Hicklenton & Heins, 1997; Nobel, 2020b). Radiations between 700 nm and 1500 nm (i.e., near-infrared), contribute little to the thermal energy of the plant. These wavelengths are poorly absorbed by the plant, as they are either transmitted through or reflected by the leaves (Niu et al., 2016; Sager & Mc Farlane, 1997). In contrast, absorption in the far-infrared range (1500 nm to 30000 nm) is above 95% and contributes significantly to the thermal energy load of a plant. Managing infrared radiation is essential to obtaining optimal plant temperature (Hicklenton & Heins, 1997; Niu et al., 2016).

2.3.1.1.3 Latent Heat – Transpiration

Evaporation is a phase transition process during which a substance, such as water, absorbs sensible heat from its surroundings and changes from a liquid to a vapour state (Lienhard & Lienhard, 2019). During plant transpiration, water evaporation occurs at the air–liquid interfaces along the pores in the cell walls of mesophyll, epidermal, and guard cells (Gates, 1965; Ye et al., 2013). At 25 °C, 2436 J of energy is transferred from the leaf to the air for every gram of water that evaporates at its surface (Hicklenton & Heins, 1997). This process of transpiration serves as a crucial means for the plant to regulate its temperature through latent heat exchange (Nobel, 2020b). The rate of transpiration of a plant depends on the temperature gradient, as well as the water vapour pressure deficit between the plant and the air; further explanations are provided in section 2.3.2.1.3.

2.3.1.1.4 Air and Plant Temperature

Significant deviations can be observed between the temperature of the air and that of the plant, including its leaves and roots (Gates, 1965). While radiation and latent heat transfers do not directly impact air temperature, they directly influence leaf temperature. Consequently, it becomes essential to consider both the temperature of the plant and the air, as the plant physiology is influenced by both the absolute temperature of the air and the temperature gradient at its interface (Hicklenton & Heins, 1997; Ye et al., 2013).

2.3.1.2 Plant Response

According to relevant thermodynamic principles, all chemical reactions are temperature-dependent due to the concept of activation energy (Atkins & De Paula, 2002). Temperature plays a crucial role in determining whether molecules possess sufficient energy to initiate the reaction. As the temperature increases, the rate of the reaction also increases, up to a certain point where the temperature-dependent energy of the particle (i.e., kinetic energy) is higher than the energy of activation (Ašperger, 2003; Atkins & De Paula, 2002).

Many plant metabolic processes, such as photosynthesis, respiration and transpiration are therefore temperature dependent (Jones, 2014). In those complex biological events, temperature affects the overall process by limiting a single, vital rate-limiting chemical reaction of the process (Penfield & MacGregor, 2014). Two cardinal types of responses to temperature can be observed in plants. The first is associated with photosynthesis, and the second one can be associated with dark respiration, but also stands for the response type of many other metabolic reactions (Körner, 2006).

Photosynthesis response to temperature is known as the bell-shaped response presented in Figure 2.4 (Jones, 2014; Körner, 2006; Kubota, 2016). The function is the net results of two opposite reactions, that being CO_2 fixation and CO_2 release (Körner, 2006). The fixation process is the so-called dark reaction of photosynthesis (i.e., CO_2 fixation by Rubisco) and the two CO_2 release processes are dark- and photo-respirations. The rates increase with temperature up to an optimal temperature and, as the overall process is dominated by CO_2 fixation, photosynthesis is intensified. Above that point, the equilibrium of CO_2 shift from a fixation-dominant process to a release-dominant one and photosynthesis is inhibited (Kaiser et al., 2015; Körner, 2006; Morison & Lawlor, 1999). The temperature-dependent response of photosynthesis is more complex than presented here and is influenced by various elements such as photosynthetic enzymes (e.g., Rubisco, Calvin cycle, electron transport, pi regeneration), thylakoid reactions, or heat shock proteins (HSPs) (Kaiser et al., 2015; Yamori et al., 2014). The temperature dependence of these factors contributes to the spread and horizontal translation of the bell-shaped curve response, making the dependence of photosynthesis on temperature a multifaceted and intricate mechanism (Jones, 2014; Yamori et al., 2014). It's important to mention that photosynthesis is primarily driven by light and not temperature.

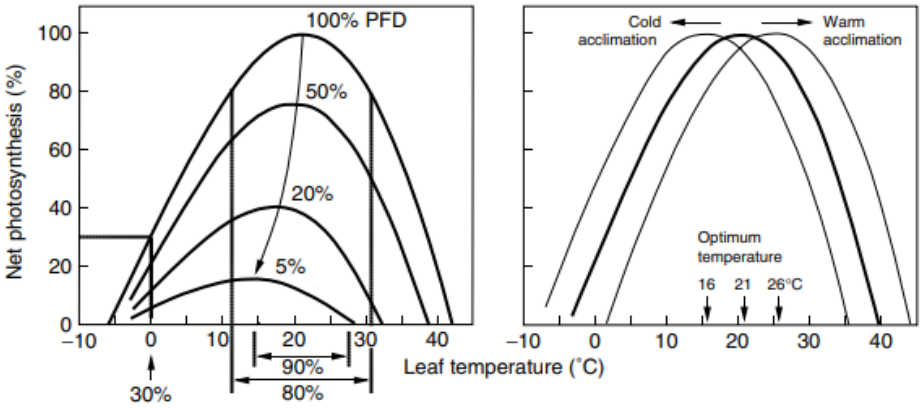

Figure 2.4: Photosynthesis bell-shaped response (Körner, 2006).

Dark respiration (i.e., mitochondrial respiration) serves as a proper example of the temperature response curve exhibited by many reactions in a plant's metabolism (Körner, 2006). The rate of this process exponentially increases with temperature until it reaches the lethal heat limit, leading to a drastic collapse, as illustrated in Figure 2.5 (Jones, 2014). Within biologically relevant temperature ranges, the rate of biological reactions can increase between twofold to threefold with every 10 K temperature rise. At ambient temperature, this ratio, known as Q_{10}, equals 2.3 for dark respiration (Larigauderie & Körner, 1995).

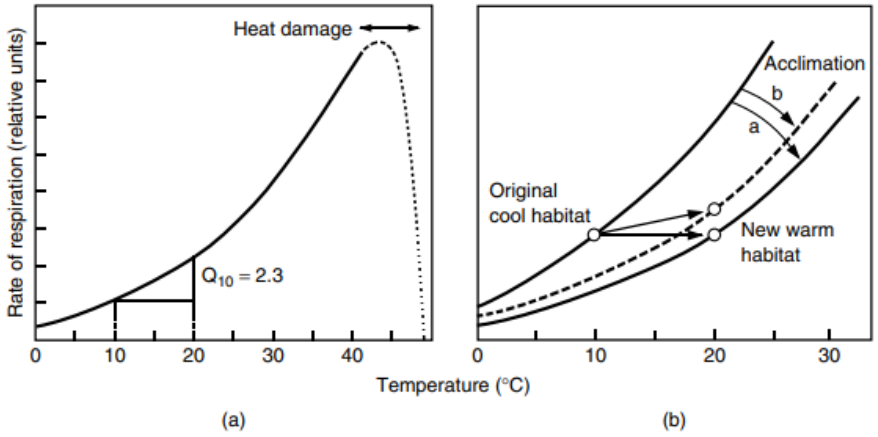

Figure 2.5: Typical Exponential response temperature-dependant metabolic processes (Körner, 2006)

As many plants metabolic reactions are temperature-dependent, the rates of plant growth and development are significantly influenced by temperature. The speed at which plants and their organs progress through developmental phases is directly dependent on temperature conditions (Jones, 2014; Körner, 2006). In controlled environment agriculture, cumulative temperature is a practical tool used to quantify the relationship between production time and the total number of days with optimal temperature

conditions (Kubota, 2016). While temperature generally does not act as a direct signal for developmental phase changes, certain exceptions, like vernalization, occur. Vernalization is the induction of a plant's flowering process by exposure to cold temperatures (Kim & Sung, 2014).

2.3.2 Humidity

2.3.2.1 General concepts

Moisture corresponds to the water content of any solid, liquid or gas, while humidity exclusively refers to the water vapour (i.e., the gaseous phase of water) content of a gas (Spomer & Tibbitts, 1997). Water vapour is produced from diverse sources: evaporation from open water surface (e.g., lakes or ocean), evaporation from wet exposed/contained solid surfaces (e.g., plants), and from chemical reactions (e.g., combustion of organic substance). Conversely, humidity diminishes due to processes such as condensation or chemical reactions (Fehsenfeld & Albritton, 1980). The characterization and quantification of humidity rely on various parameters, all interconnected by the foundational principles of thermodynamics. The psychrometric chart serves as a powerful tool for establishing the relationships between these diverse aspects (ASHRAE, 1990). Concepts such as relative humidity (RH) and vapour pressure deficit (VPD) take centre stage in CEA, serving as essential metrics of humidity levels.

2.3.2.1.1 Psychrometric Chart

Psychrometric employs thermodynamic properties to elucidate processes and conditions involving moist air (ASHRAE, 2013). Specifically, the ideal gas law, presented in Equation (2.2), most accurately characterizes the physical behaviour of mixtures containing dry air and water vapour within the temperature and pressure range relevant to plant growth (Spomer & Tibbitts, 1997).

$$pV = nRT_{db} \qquad (2.2)$$

Where p is the atmospheric pressure (Pa), V is the volume of the gas sample (m^3), n is the gas concentration (mol), R is the ideal gas constant and equals 8.314 $J.K^{-1}.mol^{-1}$, and T_{db} is the dry-bulb temperature of the gas (K).

The connection between temperature, water vapour, and air energy, derived from this equation, finds manifestations in the psychometric chart, illustrated in Figure 2.6 (Niu et al., 2016). This chart serves as a tool to extrapolate a comprehensive set of atmospheric parameters from limited information (i.e., two known atmospheric conditions) (Niu et al., 2016; Spomer & Tibbitts, 1997). The psychrometric chart encompasses a range of parameters including dry bulb temperature (T_{db}), wet bulb temperature (T_{wb}), dew point temperature (T_d), humidity ratio (W), relative humidity (RH), specific humidity (γ), absolute humidity (d_v), water vapour pressure (P_v), saturated vapour pressure ($P_{v,sat}$), vapour pressure deficit (VPD), enthalpy

(h), and specific volume (v) (ASHRAE, 2013; Spomer & Tibbitts, 1997). By grasping the interconnections among these moist air properties, one gains the ability to anticipate how diverse environmental control strategies might influence plant behaviour and productivity (Niu et al., 2016).

2.3.2.1.2 Relative Humidity

Relative humidity is a measurement of the water vapour content of the air as a percentage of the air water vapour saturation limit under the same conditions (Anderson, 1936). It is not a direct measurement of water vapour content, but a ratio between the maximum and the actual moisture content under specific conditions. The capacity of air to hold water, thus the saturation limit, is temperature dependent. As temperature increase, the water-holding capacity of air increases rapidly. It approximately doubles for every increase of 10 ^{o}C (Wollaeger & Runkle, 2016). Thus, relative humidity decreases as temperature rises even if there are no changes in water content or vapour density (Spomer & Tibbitts, 1997). RH is one of the most prevalent terms used by growers in CEA to describes humidity. However, RH is not the best measurement to predict humidity effects on plants as it leads to inconsistent conclusions for plant transpiration and water loss (Spomer & Tibbitts, 1997; Wollaeger & Runkle, 2016).

2.3.2.1.3 Vapour Pressure Deficit (VPD)

Another way to express air humidity is vapour pressure deficit (VPD). VPD measures humidity as a difference between the current and the saturation water vapour pressure under specific conditions. The SI unit for VPD is kilopascal (kPa), but it is commonly expressed as kg.m^{-3} (Anderson, 1936; Niu et al., 2016). VPD offers a more accurate means of conveying humidity as it drives evapotranspiration, thus transpiration and water loss (Castellvi et al., 1996). A high VPD indicates the air is relatively dry and can still hold a large amount of water. A low VPD means air water content is near saturation. Optimal VPD for most plants ranges between 0.3 and 1.0 kPa (Niu et al., 2016; Wollaeger & Runkle, 2016). VPD can be calculated from RH and T_{db} using Equation (2.3) (Jin et al., 2019).

$$VPD = (1 - RH) \cdot 610.7 \cdot 10^{\frac{7.5T_{db}}{237.3+T_{db}}} \qquad (2.3)$$

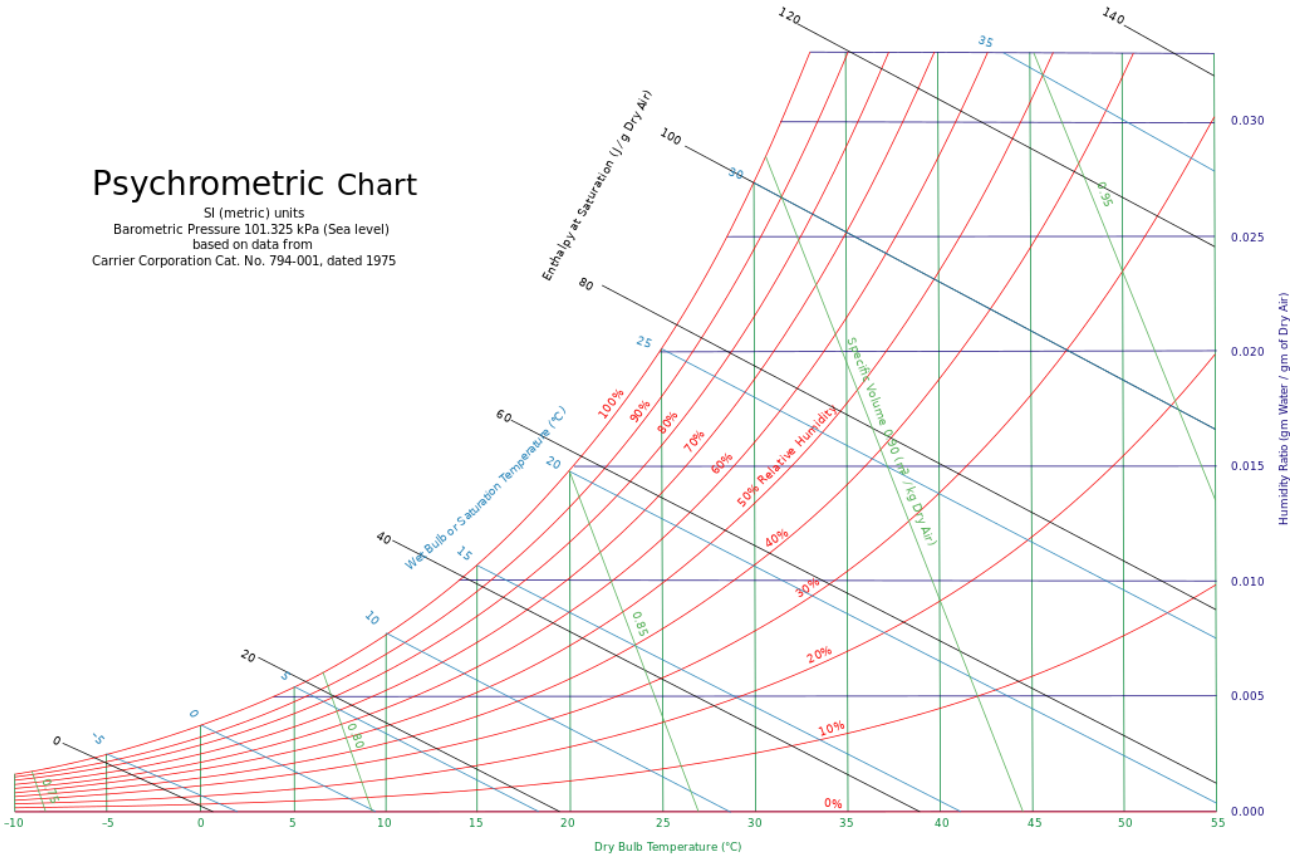

Figure 2.6: Psychrometric Chart (Wikipedia)

2.3.2.2 Plant Response

Humidity directly influences plant transpiration rates and stomatal opening, and thus indirectly affects water potential, photosynthesis, nutriment translocation and plant temperature (Spomer & Tibbitts, 1997; Tibbitts, 1979). Equation (2.4) presents the water diffusion process of transpiration as a function of the vapour pressure deficit, boundary layer and stomatal resistance (Kubota, 2016; Nobel, 2020a).

$$E \propto \frac{VPD}{R_s + R_b} \qquad (2.4)$$

Where E is the leaf transpiration rate, VPD is the vapour pressure deficit at the leaf interface, R_s is the stomatal resistance and R_b is the boundary layer resistance.

Transpiration, which is the evaporation of water from the plant surface, is proportional to VPD at the interface of the leaf. As humidity decreases, the water vapour deficit between the leaf and the environment increases and transpiration is accentuated; and vice versa (Spomer & Tibbitts, 1997). Thus,

water intake from roots increases to prevent wilting. The balance of water loss and gain by transpiration and roots absorption defines plant water status, and influence plant water potential (Kubota, 2016).

Humidity affects transpiration through stomatal opening, which is related to stomatal resistance. When the humidity level is low (i.e., high VPD), stomata close to prevent excessive water loss from transpiration (McAdam & Brodribb, 2015; Tibbitts, 1979). Gas exchange such as CO_2 intake is then limited, and photosynthesis halted. Typically, lower VPD is associated with higher stomatal opening, and thus reduced stomatal resistance (Ahmed et al., 2020). The stomatal response to significant humidity changes happens within 2-25 minutes. The stomatal closing by a sudden increase in VPD is preceded by an opposite transient change, named stomata “pop open” (Grossiord et al., 2020). Stomatal sensitivity, plant stomatal response to VPD change, is highly variable across and within species (Grossiord et al., 2020; Spomer & Tibbitts, 1997).

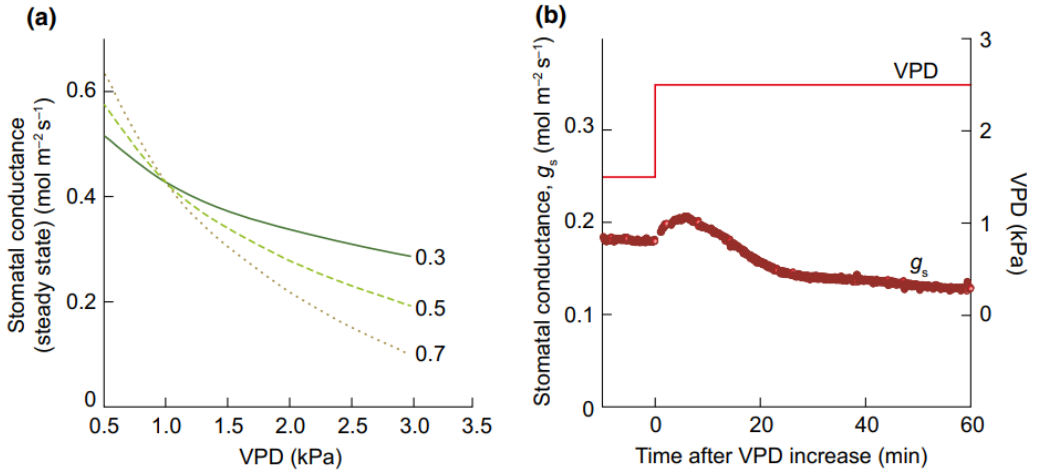

Figure 2.7: Long-term response of stomatal conductance to increased VDP (left). Transient response of stomatal conductance, named stomata "pop open" (right) (Grossiord et al., 2020).

2.3.3 Carbon Dioxide (CO_2)

2.3.3.1 General Concepts

Carbon dioxide is a linear covalent molecule made of two atoms of oxygen and one atom of carbon (Niu et al., 2016). It’s an odourless, colourless, and non-flammable gas at atmospheric pressure and ambient temperature (Peet & Krizek, 1997). It has a molecular mass of 44.009 $g.mol^{-1}$ and density of 1.8714 $kg.m^{-3}$ at 288.15 K (15 °C), which is 53 % heavier than air. At the same temperature, the vapour pressure, and the water solubility equal 5.08 MPa and 1.97 $g.L^{-1}$, respectively (Dean, 1999). The concentration of this naturally occurring compound is commonly express in part per million (ppm) and the SI unit is $\mu mol.mol^{-1}$

(Niu et al., 2016). In May 2023, the atmospheric CO_2 concentration was 421 ppm, which represents a 50% increase since the beginning of the industrial revolution (i.e., 18th century) (NOAA Research, 2023).

The ambient concentration of CO_2 is crucial to consider; however, this measure does not always accurately reflect the CO_2 accessible to plants for their metabolic processes (Leuning, 1983). CO_2 faces a series of impediments as it diffuses from the surrounding air, traversing the boundary layers adjacent to leaf surfaces, through stomata, intercellular air spaces, mesophyll cells, and ultimately reaching the chloroplasts (Nobel, 2020a). The extent of resistance experienced by CO_2 at these distinct stages hinges on various environmental factors such as temperature, humidity, air currents, and light intensity (Buckley, 2017). The cumulative resistance that CO_2 encounters is quantified by Equation (2.5), derived from the general diffusion equation (Nobel, 2020a). This equation finds applicability not only in describing CO_2 diffusion but also in explaining the diffusion of other gases into plants, including water vapour (Leuning, 1983).

$$r_{CO_2}^{total} = r_{CO_2}^{leaf} + r_{CO_2}^{bl} = \frac{1}{D_{CO2}}\left(\delta^{ias} + \frac{\delta^{st} + r^{st}}{na^{st}} + \delta^{bl}\right) \qquad (2.5)$$

Where $r_{CO_2}^{total}$ is the total resistance uncounted by CO_2, $r_{CO_2}^{leaf}$ is the resistance associated with the leaf itself and it includes the stomatal resistance $r_{CO_2}^{st}$ and the intercellular air spaces resistance $r_{CO_2}^{ias}$, $r_{CO_2}^{bl}$ is the resistance of the boundary layer, D_{CO2} is the CO_2 diffusion coefficient ($m^2.s^{-1}$), δ^{ias} is the effective distance associated with intercellular air spaces, and δ^{bl} is the thickness of the boundary layer. δ^{st} is the depth of a stomatal pore, r^{st} is the mean pore radius, n is the number of stomata per unit area of the leaf and a^{st} is the average area per stomatal pore; those variables combined to consider the effective depth of stomatal pores and the fraction of the leaf surface area occupied by stomatal pores.

2.3.3.2 Plant Response

As revealed by Equation (2.6), carbon dioxide stands as a foundational element of photosynthesis (Jones, 2013b). Its significance extends deeply into the intricacies of plant metabolism, exerting direct or indirect influence over a multitude of physiological processes (Peet & Krizek, 1997).

$$6CO_2 + 12H_2O + LightEnergy \rightarrow C_6H_{12}O_6 + 6O_6 + 6H_2O \qquad (2.6)$$

Varied levels of CO_2, whether high or low, intricately mould plant physiology (Terashima et al., 2014). The vitality of plant production hinges significantly on the balance of CO_2 concentrations, especially when they dip below ambient levels (i.e., 400 ppm), resulting in a marked reduction in photosynthetic activity. This occurrence is rooted in the chemical intricacies of photosynthesis that are inherently constrained by the

availability of this compound under such circumstances (Rogers et al., 1997). Research by Heiki and van Uffelen (1984) and Allan et al. (1991) underscores the pronounced sensitivity of dry matter production to sub-ambient CO_2 concentrations for cucumber and soybean, respectively. This sensitivity is vividly demonstrated through empirical data: a mere 50 µmol.mol^{-1} increase in CO_2 concentration precipitates a substantial 26.4% augmentation in cucumber production within the range of 100 to 150 µmol.mol^{-1}, whereas the increase tapers to a mere 3.6 % within the span of 350 to 400 µmol.mol^{-1} (Heiji & van Uffelen, 1984).

The influence of heightened CO_2 concentrations on photosynthesis, stomatal conductance, and plant growth has undergone meticulous examination within the realm of CEA (Niu et al., 2016). It entails a multifaceted interplay of physiological processes, intricately linked to species diversity, growth stages, and prevailing environmental conditions (Xu et al., 2016). Elevated CO_2 levels surpassing ambient levels distinctly invigorate photosynthesis, thereby fostering escalated growth and augmented biomass production (Nowak et al., 2004). A comprehensive assessment by Ainsworth and Rogers (2007) reveals that elevated CO_2 concentrations provoke a noteworthy 31% upsurge in light-saturated photosynthesis within C3 plants, as elegantly illustrated in Figure 2.8. However, the magnified photosynthetic activity's impact on plant growth turns out to be less profound than initial expectations. This could be elucidated by several factors, including the constrained leaf area vis-à-vis unit biomass, accumulation of non-structural carbohydrates, and the influence of various environmental elements (Ainsworth & Rogers, 2007; Poorter & Perez-Soba, 2002). Notably, this phenomenon is contingent on the species under scrutiny.

A consistent decline in stomatal conductance is observed in plants cultivated under elevated CO_2 conditions (Kubota, 2016). This phenomenon is posited to stem from the substantial depolarization of guard cells caused by elevated CO_2 levels, consequently inducing stomatal closure (Xu et al., 2016). An

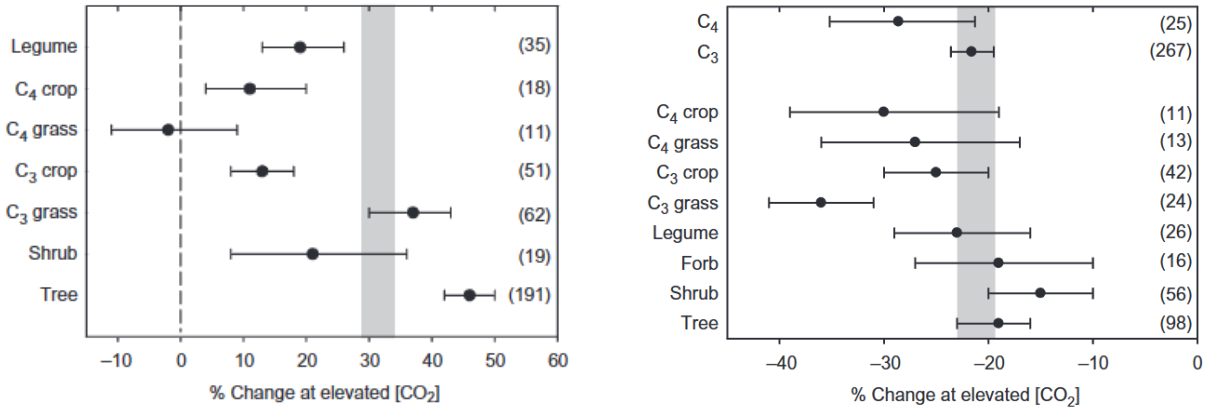

Figure 2.8 : Left - Photosynthesis response of multiple C3 plants to increased ambient CO_2 concentrations. Right - Response of the stomatal conductance of C3 plants to increased ambient CO_2 concentrations (Ainsworth & Rogers, 2007).

insightful meta-analysis, as illustrated in Figure 2.8, aggregates data from a multitude of free air CO_2 enrichment experiments, revealing an average 22 % reduction in stomatal conductance across diverse plant species (n ≈ 580) (Ainsworth & Rogers, 2007). While the influence of enriched CO_2 concentration on stomatal density remains a topic of ongoing debate, whether it ultimately diminishes the density remains uncertain (Ainsworth & Rogers, 2007; Estiarte et al., 1994; Xu et al., 2016). Thus, it is plausible that the modulation of stomatal aperture, rather than stomatal density, serves as the pivotal determinant governing the response of stomatal conductance to elevated CO_2 concentrations (Ainsworth & Rogers, 2007; Tricker et al., 2005).

Most of the consequences of CO_2 depletion or saturation are hard to detect as no obvious visual effects occur. This emphasizes the need for continuous monitoring of CO_2 levels inside CEA facilities (Peet & Krizek, 1997).

2.3.4 Air movement

2.3.4.1 General Concepts

Air movement is a critical environmental condition to consider in CEA facilities (Baptista et al., 1999). Diverse nomenclature is employed to assess air dynamics in CEA settings. Air velocity is defined as the distance travelled by a volume of air over a period of time in a specific direction and the units are expressed in $m.s^{-1}$ (Downs & Krizek, 1997). Commonly, air velocity varies between 0.01 $m.s^{-1}$ and several $m.s^{-1}$, encompassing a range of conditions (Kitaya et al., 2000). Air movement mirrors air velocity but diverges as it defines the distance travelled by a volume of air over a period of time in $m.s^{-1}$, omitting directional specifics. Air flow and air speed serve as interchangeable terms for air movement (Niu et al., 2016). The dynamic of the air can either be laminar of turbulent. Laminar airflow refers to the controlled, smooth, and uniform movement of air in a parallel or layered manner, with minimal turbulence. Turbulent airflow characterizes the chaotic and irregular movement of air, involving intricate swirling and mixing patterns that disrupt its smooth and organized flow (Jones, 2013a).

2.3.4.2 Plant Response

The foremost influence of air movement on plant physiology stems from its interaction with the boundary layer (Zhang & Kacira, 2018b). In botanical contexts, the leaf boundary layer refers to the thin and often turbulent layer of air that envelopes the surface of a leaf surface due to the friction induced by the passage of air across it (Jones, 2013a). The boundary layer's dimension is governed by a convergence of factors, including the leaf's attributes (dimensions, configuration, and texture) as well as the characteristics of the air movement itself (velocity, trajectory, and turbulence) (Jones, 2013a). The equivalent boundary layer

thickness (δ_{bl}) is defined by the following approximated equation (Downs & Krizek, 1997; Zhang & Kacira, 2018b).

$$\delta^{bl} = 4.0\sqrt{\frac{l}{v}} \qquad (2.7)$$

Where l is the mean length of leaf in the downwind direction in m, v is the ambient wind speed in m.s^{-1}. The coefficient 4.0 (m.s$^{-1/2}$) and the exponent 0.5 vary with different leaf shapes and sizes. The order of magnitude of the boundary layer is typically 10^{-3} m. The equivalent boundary layer thickness is 1.33 mm for an air velocity of 0.45 m.s^{-1} over leaf surface of 5 cm. This value decreases to 0.89 mm if the air velocity is 1 m.s^{-1}. The complete development of the equations related to the boundary layer are presented by Jones (2013a).

The boundary layer functions as a resistance barrier for the exchange of heat and gases between the plant and its environment (Zhang & Kacira, 2018b). Molecular diffusion mediates the transfer of heat and gases. Thus, the characteristic of the boundary layer, the thickness as well as the turbulence, influence the extent of those diffusions (Jones, 2013a). The boundary layer thereby influences: 1. Sensible heat transfer at the plant interface; 2. Transpiration, evaporation, and latent heat transfer between the plant and the surrounding; and 3. CO_2 uptake by leaves (Downs & Krizek, 1997). Generally, optimal air velocity between 0.3 and 1 m.s^{-1} helps reduce the boundary layer thickness and the resistance associated, which leads to enhanced photosynthesis and transpiration (Ahmed et al., 2020; Downs & Krizek, 1997). Numerous studies delved into the realm of optimal air movement and velocity within CEA. In a study by Kitaya et al. (2000), it was revealed that increasing air velocity from 0.01 to 0.3 m.s^{-1} resulted in a doubling of transpiration and photosynthesis rates in sweet potato leaves. In the same study, the transpiration and photosynthesis of rice plant canopies exhibited 2- and 2.5-fold increases as the air current speed was elevated from 0.01 to 0.8 m.s^{-1}. Shibuya et al. (2006) observed that the implementation of forceful upward and downward ventilation within tomato seedling canopies led to a 1.4-1.5 times enhancement in the CO_2 exchange rate of the canopy and 1.2-1.3 times increase in the dry masses of the seedlings, in contrast to conventional horizontal airflow strategies. Similarly, Nishikawa et al. (2013) demonstrated a remarkable 17.3% increase in the fresh mass of lettuce plants when subjected to an air velocity of 0.9 m.s^{-1}, as opposed to 0.1 m.s^{-1}. The incorporation of a rotational mechanical fan led to an additional 8.7 % gain. Optimal air movement also proves effective in preventing tip burn occurrences, as illustrated by Goto and Takakura (1992), who showcased the efficacy of vertical airflow through lettuce crop canopies in mitigating tip burn incidents.

Above 1 $m.s^{-1}$ air velocity must be avoided as it may cause mechanical stress and damage to the plants. The mechanical stress caused by air movement-induced shaking has been reported to lead to a reduction in the length of internodes and the size of leaves in plants (Downs & Krizek, 1997).

2.4 Microclimate variability in CEA cultivation room.

Various factors impact environmental conditions, resulting in spatial and temporal variations in the microclimate within a given space (Kozai et al., 2016). Several elements contribute to the non-uniform distribution of air and temperature in the growth area, including uneven air flows from circulating fans or ventilation inlets/outlets, natural convection from heat energy emitted by lamps, and air flow resistance due to culture shelves or plants (Zhang & Kacira, 2018a). Air movement can be attributed to either natural or forced ventilation mechanisms (Niu et al., 2016). The genesis of natural ventilation arises from pressure disparities instigated by two primary forces: the force of wind and the buoyancy effect (Baptista et al., 1999). Wind-induced natural ventilation is applicable exclusively to greenhouses equipped with window openings. However, the buoyancy effect manifests in both greenhouses and enclosed growth chambers. Temperature gradients inside CEA facilities lead to pressure gradient, and subsequently natural buoyancy air movement (Jones, 2013a). As buoyancy effect remains relatively modest inside growth enclosure, forced ventilation through mechanical fans or conditioned air inlet/outlet is needed (Zhang & Kacira, 2018b). This approach not only enables precise regulation of air movement around the plants but also ensures a consistent supply of fresh air, contributing to the overall health and growth of the vegetation (Downs & Krizek, 1997).

In large CEA facilities with high crop density, the air experienced by the different part of the plant (e.g., upper canopy and lower canopy) can be drastically different (Kitaya et al., 1998). The design of the ventilation system is thereby critical to obtain optimal air movement. The key parameters to scrutinize encompass air velocity and direction for both the overall and localized ventilation system (Zhang & Kacira, 2018b). Air flow can either be vertical (i.e., bottom to top, or top to bottom) or horizontal. Downward vertical airflow is preferred because it mitigates temperature and humidity stratification, thus enhancing plant growth uniformity and production (Shibuya et al., 2006). Following intensive studies at Cornell University, it was observed that vertical temperature gradient was half with downward airflow compared to upward airflow (Downs & Krizek, 1997). Research on humidity distribution concluded that downward flow leads to more consistent air movement (Matsui et al., 1980). Shibuya et al. (2006) confirmed that downward air movement results in enhanced CO_2 exchange and consequently improved plant production and plant growth uniformity. Both overall and localized control system may be implemented in CEA facilities to obtain optimal air movement (Zhang & Kacira, 2018b). Usually, the overall system supplies

ventilation air at a high velocity to mix and dilute the air in the entire room. Localized control is an air distribution strategy to enhance air circulation and uniformity. It includes equipment such as mechanical fans or perforated air tubes (Zhang & Kacira, 2018a).

In a standard cultivation room, the primary factor influencing humidity levels is plant transpiration, which refers to the process of water evaporation from the plant's surface (Anderson, 1936; Niu et al., 2016). Plant transpiration can result in rapid and significant increases of humidity in a closed room.

Carbon dioxide is one of the least controlled environmental conditions (Peet & Krizek, 1997). Yet, it plays a major role in many plants physiological processes. Human activity in growth enclosure has major effects on ambient CO_2 concentration (Niu et al., 2016). The air exhaled by a human is typically composed of 78 % nitrogen, 13 to 16 % oxygen, 4 to 5 % CO_2, and 1 % argon and other trace gases (Ejaimi & Saeed, 2016). When several people are working in the cultivation rooms, the CO_2 concentration can increase far above ambient level of 350-400 ppm within a few minutes for poorly ventilated rooms (Peet & Krizek, 1997). Plant physiological processes are also decisive for the fluctuations of the CO_2 concentration inside growth chambers. Plant respiration during the night is a CO_2-releasing process and photosynthesis assimilates ambient CO_2 during the day. Those processes need to be considered to avoid critical CO_2 depletion or accumulation in the cultivation room (Wheeler, 1992).

2.5 Environmental Sensors in CEA: Measurements and Specifications

2.5.1 Introduction to IoT System

The Internet of Things refers to a network of interconnected physical objects or devices that communicate and share data with each other over the internet (Chamara et al., 2022). As shown in Figure 2.9, the IoT framework can be defined as a five-layer architecture that comprises: (1) Perception layer, (2) Connectivity layer, (3) Intermediate layer, (4) Service layer, and (5) Application Layer (Abbasi et al., 2022; Hati & Singh, 2021). The perception layer contains the hardware devices such as sensors, actuators, microcontrollers, tags, etc. The connectivity layer corresponds to all the methodologies, technologies, and tools that allow the connectivity of the IoT devices: routers, bridges, wireless network devices (Wi-Fi, Bluetooth, etc.), etc. The intermediate layer handles the data collected at the two previous layers. It includes the data transmission protocol (e.g., message queuing telemetry transport (MQTT)), cleaning, and aggregation. The service layer corresponds to the data analysis. Different technologies such as big data, artificial intelligence, machine learning, cloud management, etc. are used in this layer to improve management and decision-making. The application layer combines the end-user and the business part of the IoT process (Abbasi et al., 2022; Chamara et al., 2022; Kamilaris et al., 2016). Several variations of the IoT architecture

exist; the number of layers and the exact definition of each layer slightly changes from one variation to the other. Nonetheless, the sensors remain a fundamental component of any IoT structure, serving as the crucial link between the physical and digital realms (Hati & Singh, 2021).

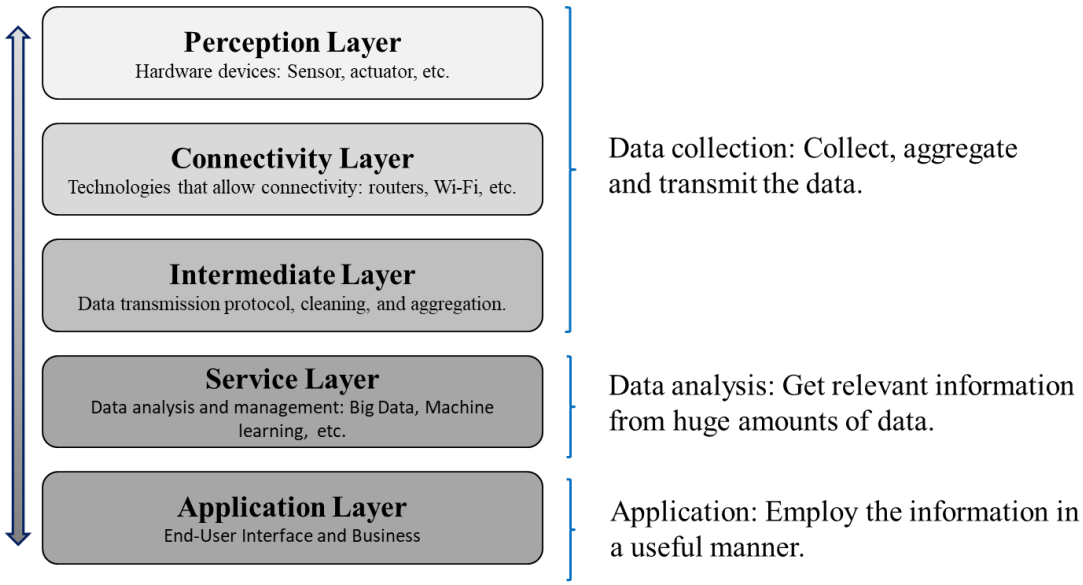

Figure 2.9: Five-layer architecture of the internet of things.

2.5.2 Perception layers : Environmental Sensors

2.5.2.1 General Concepts about Sensors

A sensor is a device that converts a stimulus into an electrical signal. A stimulus corresponds to a physical quantity, property, or condition that is sensed and converted to an electrical signal. Examples of stimulus are light intensity, temperature, motion, or chemical composition (Kenny, 2005). The electrical signal is the sensor's output and may be in the form of voltage, current, or charge (Morris & Langari, 2016a). A transducer is a converter of any one type of energy or property into another type of energy or property. Thus, a sensor is a transducer that converts any type of energy into electrical energy. Dependant on the stimulus, different types of transducers need to be used (Fraden, 2016a).

Sensors may be passive or active (Morris & Langari, 2016b). Passive sensors do not need any additional energy source as they generate the electrical signal in response to the stimulus; examples are thermocouples, photodiodes, or piezoelectric. Active sensors need an external power supply to operate, most commonly called the excitation signal. This signal is modulated by the sensor to generate the output signal. Temperature-sensitive resistors (i.e., thermistor) and resistive strain gauges are examples of active sensors (Fraden, 2016a). A sensor is either absolute if it detects a stimulus in reference to an absolute

physical scale, independent of the measurement conditions, or relative if the output signal is dependent on a reference/baseline (Fraden, 2016a). Sensors are defined by performance specifications. Those characteristics, defined in the datasheet of the sensor, change with the measurement conditions. The specifications of a temperature sensor would not be the same to monitor the temperature inside a CEA facility or a cooking oven. Table 2.1 presents the most common sensor characteristics (Eren, 2014; Kenny, 2005).

Table 2.1: Description of Sensor Specifications (Fraden, 2016c; Kenny, 2005; Morris & Langari, 2016b)

Characteristic	Definition
Transfer function	The transfer function represents the functional relationship between the physical input and the electrical output signal. Mathematically, it's defined as the ration between the output signal and the input signal.
Sensitivity	It defines as the magnitude of change of the output electrical signal for a change in the input physical signal. Mathematically, it's the derivative of the transfer function with respect to the physical signal.
Accuracy	Also named uncertainty, it is defined as the largest expected error between the quantity's actual value and the sensor output signal.
Resolution	The resolution is defined as the minimum detectable input signal fluctuation.
Precision	The precision corresponds to the degree to which a sensor produces consistent and closely clustered measurements when exposed to the same input or conditions.
Dynamic Range	The range of input physical signals that may be converted to electrical signals by the sensor. Input values outside of this range leas to faulty values from the sensor.
Time response	The time response of a sensor refers to the speed at which the sensor can accurately detect and reflect changes in the input signal over time.
Stability	The stability of a sensor refers to its ability to maintain consistent and reliable performance over an extended period of time and varying conditions.
Other	Hysteresis, Linearity, Cost, size, weight, Operating life.

The calibration of a sensor is essential to confirm the accuracy of the output value (Morris & Langari, 2016c). The definition of the International Bureau of Weights and Measures (BIPM) states: "Operation that, under specified conditions, in a first step, establishes a relation between the quantity values with

measurement uncertainties provided by measurement standards and corresponding indications with associated measurement uncertainties (of the calibrated instrument or secondary standard) and, in a second step, uses this information to establish a relation for obtaining a measurement result from an indication." In other words, calibration is defined as the comparison between the measurements values made by a sensor under test and those of a calibration standard of known accuracy (Eren, 2014). The theoretical model of the transfer function of the sensor is fitted to the experimental data to compensate systematic errors such as improper zero reference, drift over time, or mechanical damage to the sensor (Fraden, 2016e). Calibration processes should be performed periodically (Both et al., 2015).

2.5.2.2 Measurement Device and Specifications

Every environmental condition can be monitored by several sensor technologies, i.e., type of transducer. Each technology has its own trade-off: reliability, accuracy, price, etc. An appropriate choice of transducer technology is case specific and depends on the characteristic of the physical phenomenon to monitor (Fraden, 2016c). It is possible to make an enlightened choice by knowing the different technologies available and the sensor specification needed (Fontes, 2005b).

Sensor specifications are different for each environmental condition. Characteristics such as the range, the accuracy, the resolution, or the time response depend on the plant response to the environmental conditions (Both et al., 2015). Logically, the higher the sensitivity of the plant to a specific environmental condition, the higher the resolution needs to be to grasp the intricacies of the phenomenon in action. The resolution of the sensor is dictated by the control system needs (e.g., HVAC system) (Fontes, 2005b). Likewise, the sensor's range must intersect with the physiologically relevant range of the environmental condition for the plant (Niu et al., 2016). Some sensor specifications are shared across environmental conditions due to the reality of CEA facilities. The sensor needs to be compact in order to be inserted among the plants without causing any disruption to them or the operations of the facility. Minimizing overtime drift (i.e., maximize stability) helps lower maintenance operation for calibration and enhance data reliability for management and decision-making (Shamshiri et al., 2018).

2.5.2.2.1 Temperature

An air temperature sensor range spanning from 0 °C to 60 °C is a reasonable baseline interval, effectively encompassing critical aspects of plant physiology temperature requirements while accounting for potential extremes caused by HVAC system malfunctions or excessive heat generated by LED lighting (Hicklenton & Heins, 1997; Yu et al., 2023; Zhang et al., 2016). The resolution and sensitivity of the sensor should be maximal from 10 °C to 30 °C, the temperature ranges relevant to physiological processes of most

plants (Langhans & Tibbitts, 1997b). For best practice, the resolution and the accuracy must be as low as 0.1 °C and 0.2 °C respectively (Both et al., 2015). Practically, those specifications are often selected to be below 0.5 °C and dictated by the HVAC control system (Bhujel et al., 2021). Temperature sensors should be shielded again radiant heat from the lighting system. Additionally, a fan can be integrated to the sensor to expose the sensing element to airflow above 3 $m.s^{-1}$, maximize convective heat transfer and obtain accurate readings (Both et al., 2015; Niu et al., 2016).

Temperature sensors can be separated in two general categories: contact and non-contact sensors. Contact temperature sensors have to be in direct physical contact with the media. This type of sensor can monitor both air and plant temperature. Non-contact sensors monitor the infrared radiant energy heat emitted to quantify the temperature (Fontes, 2005b). This type of sensor is useful for determining the plant temperature; however, it is not effective for gases (Hicklenton & Heins, 1997). Thermocouple, thermistor, and infrared temperature sensor technologies are the most common in CEA (Niu et al., 2016). Thermistors are components that alter their electrical resistance as a response to temperature fluctuations. Typically, they comprise a blend of 2 or 3 metal oxides, fused within a ceramic substrate (Morris & Langari, 2016a). As it is relatively inexpensive, and responds quickly to temperature changes, it makes them ideal for CEA (Fontes, 2005b). For similar reasons, thermocouple sensors are frequently used in greenhouses and vertical farms. A thermocouple is formed by joining two distinct metals, generating an electrical voltage proportional to the temperature difference at their junction (Fraden, 2016d). Multiple variations of thermocouples exist, encompassing diverse metal compositions, wire diameters, consequently leading to varying price and levels of precision. The prevalent choice in CEA is the type T (copper) thermocouple, which is secured through welding (Niu et al., 2016). Infrared sensors use lenses and thermopile to monitor the radiant energy emitted by a body, thus correlated to temperature. This type of sensor is employed to measure the temperature of the plant body and its leaves (Hicklenton & Heins, 1997).

2.5.2.2.2 Humidity

The vapour pressure deficit is the best measurement to quantify ambient water vapour content as it is directly relevant to plant physiological processes. However, relative humidity remains the preferred measurement of humidity in CEA (Bhujel et al., 2021; Spomer & Tibbitts, 1997). VPD can easily be calculated from RH and T_{db} (Section 2.3.2.1.3). Typical humidity values for the growth of plants are between 50 % and 80 % (Both et al., 2015). Sensor range should exceed those values and should include the full RH spectrum (i.e., 0 % to 100 %) to ensure complete humidity control. RH can rapidly change inside a growth enclosure; an 8 °C temperature increase from 24 °C to 32 °C leads to a RH variation from 41% to

71% as the VPD remains constant at 1.38 kPa (Wollaeger & Runkle, 2016). The accuracy of the sensor should be equal or exceed that resolvable by the plant's response, although the precision of plant response is generally unknown (Spomer & Tibbitts, 1997). A resolution of 2 % and accuracy of 5 % are recommended, but it should be as high as possible under sensor constraints (Both et al., 2015). Calibration is primordial for relative humidity sensors as they are often exposed to high drift and hysteresis. It should be performed at least once a year against vapour standard or with accurate psychrometer (Niu et al., 2016). Even with frequent calibration, RH sensors have low accuracy for extreme values; below 10 % and above 95 % (Fontes, 2005a).

The most common relative humidity sensor technologies in CEA are psychrometer, capacitance, dewpoint, and infrared gas analyzer (IRGA) (Both et al., 2015). Wet-dry bulb psychrometer is the traditional method to measure relative humidity. Both dry and wet bulb temperatures are measured using a standard thermometer, either covered with a wetted fabric wick or not (Spomer & Tibbitts, 1997). This method is not suitable for continuous monitoring as the wet bulb needs to be wet all the time which presents practical constraints (Niu et al., 2016). Capacitive RH sensors are prevalent in CEA because of their simplicity, low maintenance requirements (i.e., minimal long-term drift and hysteresis), and reliability. Based on the change of capacitance of a material when exposed to variation in the surrounding humidity levels, it allows for RH measurements with typical accuracy of 2 % over most of the range (Fontes, 2005a). This type of sensor can also be produced with incorporated dewpoint temperature measurement units to increase accuracy in small RH values (Fontes, 2005a; Fraden, 2016b). An infrared gas analyzer is an apparatus that quantifies the concentration of specific gases in a sample by measuring the absorption of infrared light. It can be used for both water vapour and ambient CO_2. This type of device is usually expensive (Spomer & Tibbitts, 1997).

2.5.2.2.3 Carbon Dioxide (CO_2)

Detecting CO_2 depletion or saturation in plants can be challenging due to the absence of readily apparent visual effects (Peet & Krizek, 1997). Therefore, it is crucial to continuously monitor a broad spectrum of ambient CO_2 concentrations. Monitoring CO_2 levels ranging from 0 ppm to 1500 ppm and beyond is of paramount importance. Complete depletion of ambient CO_2 is possible due to the daytime drawdown by plant photosynthesis (Wheeler, 1992). Depending on the specific crop, maintaining concentrations around 1000 ppm can prove beneficial (Bhujel et al., 2021; Pritchard et al., 1999). Additionally, it is important to be aware that CO_2 levels can exceed these thresholds due to the accumulation caused by the presence of workers in the growth room (Niu et al., 2016; Peet & Krizek, 1997). A resolution of 50 ppm is adequate to consider plant sensitivity to the environmental condition (Heiji & van Uffelen, 1984), but lower resolution

might be needed if a CO_2 control system is implemented (Peet & Krizek, 1997). For CO_2 sensors, a two-point calibration process is the essential baseline. Zero calibration involves exposing the sensor exclusively to pure nitrogen gas. Subsequently, additional calibration points can be carried out by introducing a calibration gas with a precisely known CO_2 concentration, typically at the upper end of the measurement range (Both et al., 2015).

Non-dispersive infrared sensors are the most common technology for CO_2 monitoring is CEA (Bhujel et al., 2021). A Non-Dispersive Infrared (NDIR) sensor is a type of gas sensor that detects the presence and concentration of specific gases in the surrounding environment based on the principle of infrared absorption spectroscopy. The crucial components include an infrared emitter, an optical path, a wavelength filter interface, and an infrared detector. The gas sample is either pumped or allowed to diffuse into the optical path, and the sensor's electronics gauge the extent of infrared light absorption by the gas within the optical path (Dinha et al., 2016; Jha, 2022). This type of sensor offers a high accuracy and short-term reliability (Both et al., 2015). Typical cost for NDIR sensor range between US $100 and $1000, depending on the specification (Niu et al., 2016). Since CO_2 measurements can be expensive, a single sensor can be supplied with air samples from different greenhouse section (Peet & Krizek, 1997). Conductometric semiconducting metal oxide (MOX) gas sensors are another option to monitor CO_2 concentration (Bhujel et al., 2021). The working mechanism is based on the change of electrical conductivity of the sensing element due to the absorption/desorption of the target gas. This type of sensor is less expensive and provides fast response-recovery rates, high sensitivity, and low limit of detection. However, the performance of the sensor shows high dependence on environmental humidity and temperature, and the presence of other gases, including CO, NO_2, and VOCs (Wang et al., 2010).

2.5.2.2.4 Air Movement

An anemometer is a device designed to measure both air velocity and direction. When considering an anemometer for CEA, it is essential to prioritize high sensitivity at low velocities (Kubota, 2016; Morris & Langari, 2016b). The recommended velocity range falls between 0.1 $m.s^{-1}$ and 15 $m.s^{-1}$ (Both et al., 2015), with optimal sensitivity occurring between 0.1 $m.s^{-1}$ and 1 $m.s^{-1}$, aligning with the ideal conditions for plant growth (Alveringh et al., 2022). Targeted resolution and accuracy should fall within the range of 2 % and 5 % of the reading, respectively (Both et al., 2015). Anemometers can be categorized as either directional or omnidirectional. Directional sensors exhibit variations in measuring air velocity across different directions, whereas omnidirectional sensors do not have this limitation (Hennessy, 2005). In the context of vertical farming, it is crucial to select an anemometer with compact dimensions due to the limited space between growing shelves (Niu et al., 2016). Continuous monitoring of air movement within the growth enclosure

may not be necessary. Instead, periodic punctual measurements at a minimum of five uniformly distributed locations throughout the room can effectively map the air movement inside the growth room (Downs & Krizek, 1997). This approach can be repeated at different stages of the growth cycle to ensure that optimal air movement is consistently maintained and to assess the impact of plants on air circulation.

Hot-wire anemometers are the most popular instrument used to quantify air movement in CEA (Alveringh et al., 2022). The electrical resistance of the sensing element, consisting of two wires heated above the ambient temperature, undergoes proportional changes as it cools due to the flow of air (Morris & Langari, 2016b). Their rapid response time and ability to be manufactured in a compact size make them ideal for taking measurements in close proximity to the plant. As a trade-off, this device typically exhibits directional characteristics (Alveringh et al., 2022; Downs & Krizek, 1997). Ultrasonic anemometers are another option to measure air movement in confined spaces (Norton et al., 2007). Precise measurement of the time of flight of sonic pulses between pair of transducers allows to quantify air velocity. It is possible to combine up to 3 pairs of transducers to obtain 3-dimensional flow measurements. These expensive devices provide highly accurate measurement of turbulent airflow (Hennessy, 2005; Norton et al., 2007).

2.5.2.3 Sensor Location and Spatiotemporal Resolution

Optimal sensor placement and spatiotemporal resolution are essential to obtain an adequate representation of the microclimate inside greenhouses and vertical farms (Bhujel et al., 2021). As limited measurement locations are often monitored due to economic implication, it is crucial to carefully choose the optimal sensor placement (Uyeh et al., 2022). Simple strategies involve positioning the sensors near the plant or at locations with minimal variation of the environmental conditions. Ryu et al. (2012) suggested that measurements related to light and carbon dioxide should be done at the canopy height to portray the environmental conditions experienced by the plants. For temperature and relative humidity measurements, the most suitable locations were identified as being near the ground floor and at mid-height within the crop canopy (Both et al., 2015; Ryu et al., 2012). Using CFD simulations, Lee et al. (2019) determined that the most advantageous positions for sensors were those where environmental parameters, such as air temperature and wind speed, exhibited minimal frequent fluctuations.

The problem of optimal sensor position and spatiotemporal resolution have been intensively studied for greenhouses (Ajani et al., 2023; Bhujel et al., 2021; Reza et al., 2023). Uyeh et al. (2022) used K-Means++ algorithms, a data-driven learning approach based on clustering, to ascertain both the ideal quantity and precise placement of temperature and humidity sensors within a greenhouse, using a total of 56 sensors as their data source. For temperature, the 56 sensors were clustered into 3 groups, each associated with

an optimal sensor location. The analysis led to an optimal number of sensors of 4 for relative humidity monitoring. Similarly, Balendonck et al. (2010) performed trials in 4 commercial greenhouses in the Netherlands with 100 sensors to evaluate microclimate variability and determine the amount of sensors needed to assess this spatiotemporal distribution. 2D kriging interpolation and a uniformity index algorithm were used to quantify static climate variation and analyze sensor density. Long-term significant spatial variations in temperature and humidity were observed within the greenhouse, ranging from 1.0 °C to 3.4 °C for temperature and 10 % to 40 % for humidity. The study showed that a density of 9 sensors per hectare (±33 m) was needed to detect cold/hot and dry/wet spots. Other algorithms such as principal component analysis (Lee et al., 2019), or descriptive statistics (mean, standard deviation, outliers, and z-index) (Arnesano et al., 2016) can be used to assess best sensor location.

The complexity of the problem of sensor placement and spatiotemporal resolution increases for vertical farms compared to greenhouses (Kozai et al., 2016). Vertical stratification of environmental condition is critical as crops are grown on multi-layer shelves, which generate a higher resistance to airflow (Zhang & Kacira, 2018b). Best location and spatiotemporal resolution of sensors are closely related to the microclimate uniformity of the environment (Tamimi et al., 2013), and it has so far mainly been studied through CFD simulation (Zhang & Kacira, 2022). Those studies usually test different ventilation and HVAC designs on the uniformity of the microclimate. Naranjani et al. (2022) developed a 3-dimensional numerical model to optimize air flow and heat transfer inside a closed production system. They used an objective uniformity parameter to assess the degree of uniformity; the most efficient design provided a score of 91.7 %. The simulated results were not compared to measured data points. (Zhang et al., 2016) performed computational fluid dynamics analysis to improve environmental and airflow uniformity inside a small-scale plant factory. The best design studied led to an average air velocity of 0.42 $m.s^{-1}$ with a coefficient of variation of 44 %. The validation process was done on a single shelf, and the simulated data for air temperature and velocity showed 8.9 % and 7.5 % accuracy compared to the measured data. Those different studies provided relevant insights on environmental uniformity as well as the optimal ventilation and HVAC designs, but the findings remain limited (Norton et al., 2007). They are performed on small-scale growth enclosures, and the validation process is either non existent or executed on constricted portion on the growing area (Bournet & Rojano 2022). These constraints suggest that more intensive environmental data collection should be done in large-scale plant factories to properly assess microclimate uniformity and thus the best location and spatiotemporal resolution for sensors (Engler & Krarti, 2021; Norton et al., 2007).

The design of the HVAC system and the placement of the air inlet/outlet can influence the best sensor location and spatiotemporal resolution. Baek et al. (2015) performed an experiment to improve the uniformity of a cultivation environment, and thus the grow rate of the plants. They implemented hybrid control of air flow devices to decrease temperature deviations between upper and lower beds of a cultivation shelf. The system utilized three sets of integrated sensors (i.e., environmental sensor (EMS) that monitor temperature, humidity, and CO_2) fixed at a height of 70 mm, 1400 mm, and 2370 mm above the ground to control the air conditioning (AC) and air-circulation fan (ACF) units. In a similar experiment, (Jiang et al., 2018) developed a wireless sensor network-based temperature monitoring and fan-circulating system to eliminate uneven temperature distributions (UTDs) within each cultivation shelf in a plant factory. They used a highly dense temperature sensor network (i.e., between 8.93 and 16.63 sensors per m^2 of growing area) for the detection of the UTDs.

Connecting Text

The following chapter explores the core of this project, which centers on the measurement of spatial and temporal variations in environmental conditions within a cultivation room dedicated to cannabis micropropagation.

3. Chapter 3 – Quantifying the spatial and temporal variability of the environmental conditions in a cultivation room for the micropropagation of cannabis.

Abstract

One strategy, to enhance the efficiency in CEA, is gaining a deeper understanding of the variability of environmental conditions within growth enclosures. The monitoring and analysis of data related to temperature, humidity, CO_2 levels, and light intensity in CEA settings not only enhances decision-making processes but also plays a pivotal role in designing and controlling the heating, ventilation, and air conditioning (HVAC) systems within these facilities. This has particular relevance in the micropropagation of cannabis, where stringent control of environmental conditions is vital for successful plant growth. Thus, this study aims to quantify the spatial and temporal variability of environmental conditions in a cultivation room used for the micropropagation of cannabis, and to understand the impact of this variability on the growth of stage-two cannabis plantlets. To monitor the environmental conditions in the cultivation room, a low-cost Internet of Things (IoT) sensor system using Arduino technology and InfluxDB software was developed. The system includes sensors that measure temperature, humidity, CO_2, and light levels, and sends the data to a web server. The study tested five different locations within the shelved cultivation room for one-week periods. Basic statistics (i.e., average, mean, standard deviation, skewness, etc.), along with uniformity indexes, were employed to assess spatiotemporal variability of the environmental conditions inside the cultivation room. Notably, an average temperature difference of 1.9 °C between locations was detected, which resulted in a relatively low overall uniformity index of 0.52. An analysis of plantlet growth using the Kruskal-Wallis H-test, a non-parametric alternative to ANOVA, revealed a statistically significant difference in plantlet heights at the end of the growth stage across various locations ($H = 12.41$, $p = 0.002$, $p < 0.05$). Furthermore, a strong linear correlation ($R^2 = 0.992$) was observed between temperature variability and plantlet heights. These findings provide valuable insights into assessing microclimate variability in CEA cultivation rooms and underscore the importance of further exploring the impact of these environmental conditions on in-vitro-grown cannabis plants.

Keywords

Controlled Environmental Agriculture, Microclimate, Environmental Conditions, Temperature, Vapour Pressure Deficit, Monitoring System, IoT, Cannabis Micropropagation.

3.1 Introduction

Temperature, humidity, ambient CO_2 levels, and air movement stand as crucial environmental factors when cultivating crops in controlled environment agriculture (CEA) (Ahmed et al., 2020; Engler & Krarti, 2021; Niu et al., 2016). These environmental conditions wield a significant influence over various metabolic processes within plants, including photosynthesis, respiration, transpiration, stomatal conductance, and leaf boundary layer (Downs & Krizek, 1997; Grossiord et al., 2020; Kitaya, 2005; Körner, 2006; Peet & Krizek, 1997; Pritchard et al., 1999; Tibbitts, 1979). Therefore, ensuring uniformity of the microclimate within CEA facilities and cultivation rooms is crucial for achieving consistent, optimized, and predictable crop production (Bhujel et al., 2021; Uyeh et al., 2022).

Various factors impact environmental conditions, resulting in spatial and temporal variations in the microclimate within a given space (Kozai et al., 2016). Several elements contribute to the non-uniform distribution of air and temperature in the growth area, including uneven air flows from circulating fans or ventilation inlets/outlets, natural convection from heat energy emitted by lamps, and air flow resistance due to the culture shelves or plants (Zhang & Kacira, 2018a). In a standard cultivation room, the primary factor influencing humidity levels is plant transpiration, which refers to the process of water evaporation from the plant's surface (Anderson, 1936; Niu et al., 2016). Plant transpiration can result in rapid and significant increases of humidity in a closed room. Moreover, human activities within the growth enclosure exert a substantial impact on ambient CO_2 concentration (Niu et al., 2016). When several people are working in a cultivation room, the CO_2 concentration can increase far above ambient level of 350-400 ppm within a few minutes with poorly ventilated rooms (Peet & Krizek, 1997). Plant physiological processes are decisive for the fluctuations of the CO_2 concentration inside growth chambers (Wheeler, 1992).

Monitoring environmental data is a fundamental practice to ensure efficient management of microclimates within CEA facilities, as emphasized by recent studies (Bhujel et al., 2021; Uyeh et al., 2022). This practice not only enhances decision-making processes but also plays a pivotal role in designing and controlling the heating, ventilation, and air conditioning (HVAC) systems within these facilities (Chamara et al., 2022; Shamshiri et al., 2018). Traditional decision-making methods in CEA rely on limited datasets, often leading to significant delays between data generation and informed decisions. Moreover, these traditional methods tend to address individual factors one at a time, overlooking the complex interplay of multiple environmental stresses affecting crops simultaneously (Chamara et al., 2022). By actively monitoring environmental conditions, it becomes possible to revolutionize decision-making at the farm-level through the incorporation of multi-input and multi-output strategies driven by real-time data analysis (Chamara et al., 2022; Chaterji et al., 2021). Furthermore, environmental monitoring provides valuable

insights into the performance of HVAC systems, ultimately contributing to their improved design and control (Shamshiri & Ismail, 2013).

Theoretically, numerical models such as computational fluid dynamics (CFD) simulation can provide valuable insights on thermal fluxes and microclimate uniformity inside a CEA facility (Reichrath & Davies, 2002; Torre-Gea et al., 2011). Several studies have focused on modeling the temperature distribution and airflow uniformity within greenhouse growth areas, successfully achieving a relative error of 3 % between simulated and empirical temperature data (Norton et al., 2007; Zhou et al., 2020). However, it is more challenging to model and get accurate results for the temperature distribution for large and complex CEA facilities, especially those with multi-level farming configurations (Baek et al., 2016; Bournet & Rojano 2022; Natarajan et al., 2022). Naranjani et al. (2022) developed a 3-dimensional numerical model to optimize air flow and heat transfer within a closed production system. They used an objective uniformity parameter to assess the degree of uniformity. The most efficient design provided a score of 91.7%, though the simulated results were not compared to measured data points. Zhang et al. (2016) performed computational fluid dynamics analysis to improve environmental and airflow uniformity inside a small-scale plant factory. The best design studied leads to an average air velocity of 0.42 $m.s^{-1}$ with a coefficient of variation of 44 %. The validation process, however, was limited to a single shelf, with the simulated data for air temperature and velocity displaying accuracies of 8.9 % and 7.5 % when compared to measured data. Those different studies provided relevant insights on environmental uniformity as well as the optimal ventilation and HVAC designs, but the transfer of these findings to industry remains limited (Norton et al., 2007). The studies are performed on small-scale growth enclosures, and the validation process is either non existent or executed on constricted portion on the growing area (Bournet & Rojano 2022). Therefore, it is essential to obtain empirical data on temperature, humidity, and CO_2 distributions and fluxes to further assess the uniformity of the microclimate inside a plant factory with electrical lighting (PFEL) and within vertical farming (VF) (Bournet & Rojano 2022).

Sensor placement and spatiotemporal resolution are key parameters to obtain an adequate representation of the microclimate inside greenhouses and vertical farms (Bhujel et al., 2021). As limited measurement locations are often monitored due to economic implication, it is crucial to carefully choose the optimal sensor placement (Uyeh et al., 2022). This issue has been intensively investigated, particularly in the context of greenhouses (Ajani et al., 2023; Bhujel et al., 2021; Reza et al., 2023; Uyeh et al., 2022). Using CFD simulations, Lee et al. (2019) determined that the most advantageous positions for sensors were those where environmental parameters, such as air temperature and wind speed, exhibited minimal

fluctuations. Uyeh et al. (2022) used K-Means++ algorithms, a data-driven learning approach based on clustering, to ascertain both the ideal quantity and precise placement of temperature and humidity sensors within a greenhouse, using a total of 56 sensors as their data source. Other research used algorithms such as principal component analysis, or descriptive statistics (mean, standard deviation, outliers, and z-index) to assess best sensor location in greenhouses (Arnesano et al., 2016; Lee et al., 2019). The complexity of the problem of sensor placement and spatiotemporal resolution increases for vertical farms compared to greenhouses (Kozai et al., 2016). Vertical stratification of environmental condition is a critical phenomenon to consider and efficient sensor placement and spatiotemporal resolution allow identifying yield-limiting factors across different areas of the farm, both horizontally and vertically in multi-level farming setups (Alfred et al., 2021). More intensive environmental data collection should be done in large-scale plant factories to properly assess microclimate uniformity and thus the best location and spatiotemporal resolution for sensors (Bournet & Rojano 2022; Engler & Krarti, 2021; Norton et al., 2007).

This research project is dedicated to highlight the significance of environmental data monitoring within the context of controlled environment agriculture. The core objective is the meticulous monitoring of environmental conditions within a multi-layer shelves growth enclosure to precisely quantify both the spatial and temporal variability of critical environmental parameters, specifically focusing on temperature, relative humidity, and ambient CO_2 concentration. A low-cost sensor system was designed and utilized to monitor the microclimate at several locations inside a cultivation room.

Subsequently, another goal of this study is to assess how the spatial and temporal fluctuations in environmental conditions impact plant growth. It is worth noting that a non-uniform microclimate within the growth enclosure may not necessarily result in statistically significant differences in key plant parameters. This outcome hinges on the sensitivity of the plant to these specific environmental conditions. In a productivity context, it prompts a decision-making process regarding whether it is worthwhile, in terms of both time and resources, to mitigate this non-uniformity or not.

3.2 Materials and Methods

3.2.1 Problem formulation

The primary goal of the study is to quantify the spatial and temporal variability of the microclimate inside a multi-layer cultivation room. As limited quantity of sensors are used in the industry due to economic implications (Uyeh et al., 2022), a low-cost, and easy-to-implement approach is proposed. Instead of an extensive array of sensors, a restrained quantity of devices is used to subsequently monitor the environmental conditions at several locations inside the growth enclosure. Within a specific temporal frame marked by various factors influencing environmental conditions—such as HVAC response time, plant growth phases, and activities within the growth enclosure—the fluctuation patterns of these conditions are expected to cyclically reoccur. By identifying the temporal limiting factor, it is possible to determine how long a device should stay at each location. In the specific case of the study, operations made by the workers in the cultivation room are the main factors influencing environmental conditions, and they cyclically reoccur every week.

The cultivation room is dedicated to the micropropagation of cannabis plantlets. Cannabis micropropagation is a cutting-edge horticultural technique that involves the cultivation of cannabis plants from tiny, sterilized plant tissue samples, such as meristematic cells or nodal segments, in a controlled laboratory environment (Monthony et al., 2021). This method offers numerous advantages for cannabis cultivation, including rapid and efficient clonal propagation, reduced risk of pests and diseases, and the potential to maintain genetic consistency in plant strains (Jin et al., 2019). The micropropagation process can be divided into 5 stages: 0. Stock plant management and selection of explant; 1. Establishment of aseptic culture; 2. Multiplication; 3. In vitro rooting; and 4. Transplanting and acclimatization (Kyte et al., 2013). During the whole process, the plantlets are typically grown within sterile containers to provide a controlled and contamination-free environment for their development (Kyte et al., 2013; Monthony et al., 2021). This study focuses on the second stage of the process, whose objective is to rapidly increase the number of plantlets. Cannabis micropropagation research to date has been limited due to historical prohibitions and restrictions on cannabis. In particular, little is known about the effects of environmental and physical conditions, such as temperature, humidity, CO_2, and light, on *in vitro*-grown cannabis plants (Monthony et al., 2021).

3.2.2 Site Description

The study site is a multi-layer growth chamber exclusively used for the micropropagation of cannabis plantlets at EXKA Inc., situated in Mirabel, Quebec. The chamber is a fully insulated and aseptic room with dimensions of 11 metres in length, 3.5 metres in width, and 3.8 metres in height. Within this space, there

are 15 sliding cultivation shelves aligned adjacent to the back wall. Each individual shelf measures 0.6 metres in length, 2.5 metres in width, and 4.8 metres in height, further subdivided into a 2-by-4-array configuration, resulting in 8 cultivation areas, each measuring 0.6 metres in length, 1.25 metres in width, and 1.2 metres in height. To regulate the environmental conditions, the HVAC system consists of two air-conditioning units (ACU) positioned on the front wall of the chamber, on either side of the entry door, approximately 3 metres above the ground. The HVAC system is programmed to maintain a temperature range of 22±2 °C. The temperature sensor, used to control the air-conditioning units, is located next to the entry door. Each of the cultivation areas is equipped with an LED light source featuring a wide spectrum spanning from 400 to 700 nanometres. These LEDs deliver a light intensity of 125 $\mu mol.m^{-2}.s^{-1}$, following a photoperiod of 16 hours of illumination followed by 8 hours of darkness. It is noteworthy that the relative humidity and CO_2 concentration are not actively controlled within this chamber, and there are no supplementary heating units in place. The growth room is presented in Figure 3.1.

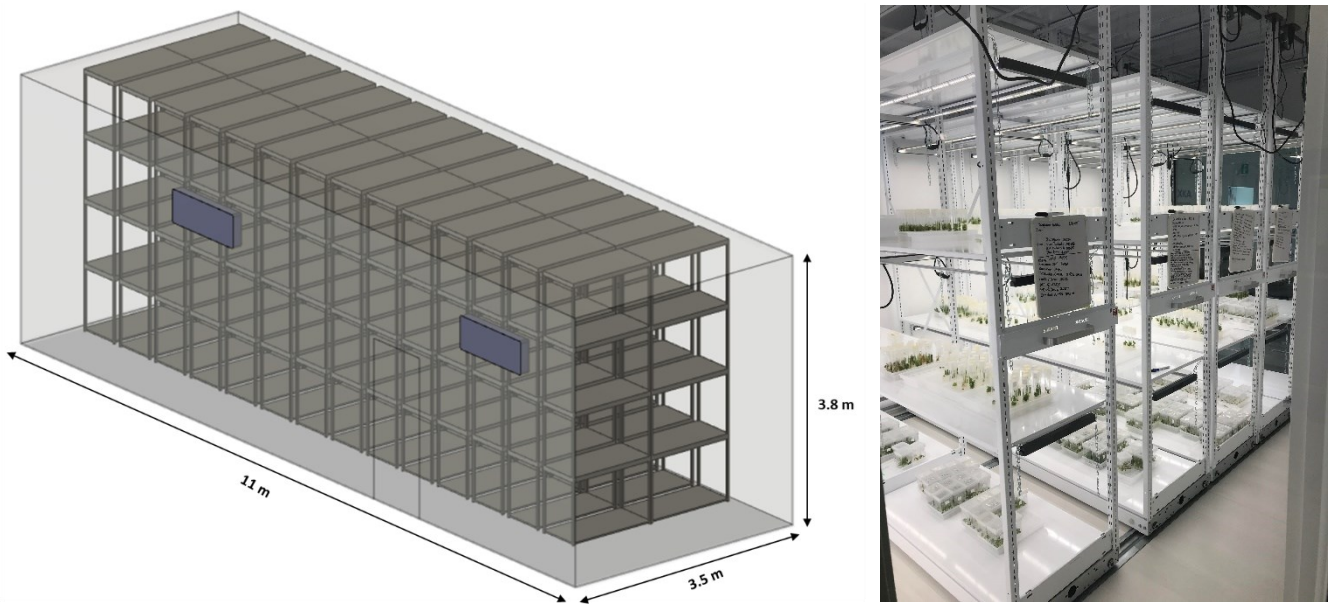

Figure 3.1: (Left) Schematic of the study multi-layer growth enclosure for the micropropagation of cannabis plantlets. (Right) Picture of the growth room at EXKA Inc.

3.2.3 IoT-based Environmental Monitoring System

3.2.3.1 Introduction to the Internet of Thing (IoT)

IoT monitoring systems are increasingly used in CEA facilities to automate environmental data collections, aggregation, cleaning and analysis (Chamara et al., 2022; Kamilaris et al., 2016). The IoT framework can be divided into a 5-layer architecture comprising: 1. Perception layer, 2. Connectivity layer, 3. Intermediate layer, 4. Service layer and 5. Application layer (Abbasi et al., 2022; Kagan et al., 2022). The system

implemented for this experiment covers layers 1 to 3, which include all the hardware (i.e., sensors, microcontroller, wireless network device, etc.) and the software (i.e., data transmission protocol, and aggregation) needed. The analysis of the environmental data collected during the experiment is equivalent to the service layer, and the application layer was out of the scope of the study.

3.2.3.2 Environmental Sensors

Four distinct types of sensors were selected to oversee the environmental conditions both within the growth room and inside the Magenta™ plant growth vessels (GA-7 model), which are essential for the micropropagation of cannabis plantlets. Since the plantlets are cultivated in sealed containers, they are not directly exposed to the environmental conditions within the room. Therefore, monitoring the temperature, relative humidity, and CO_2 levels inside these containers allows elucidating the connection between the internal and external environmental conditions and their impact on the growth of cannabis plantlets.

BME680 sensors (Bosh Sensortec, Reutlingen, Germany) were used to monitor both ambient temperature and relative humidity inside the growth room and the containers. These sensors are capable of measuring temperatures from -40 °C to 85 °C, offering an absolute accuracy of ±0.5 °C over the majority of this range, with an output resolution of 0.01 °C. The operating range for relative humidity is 10 % to 90 % to obtain accurate measurements of ±3 %, including typical hysteresis of ±1.5 %. The sensor provides readings exceeding these limits (i.e., below 10 %, and above 90 %), but the accuracy drops drastically. The sensor response time for RH is 8 seconds (i.e., $\tau_{63\%}$). Two different types of sensors were used to quantify ambient CO_2 concentration. The first ones, K30 CO_2 sensors (Senseair AB, Delsbo, Sweden) are the most accurate of the two. Based on Non-Dispersive Infrared (NDIR) measurements, these sensors provide readings between 0-5000 ppm with an accuracy of ±30 ppm ±3 % of the value. The dimensions of the sensors are 51 x 57 x 14 mm. The CSS811 sensor (ams-OSRAM AG, Premstaetten, Austria) is the second CO_2 sensor integrated into the system. Smaller than the K30 sensor with dimensions of 2.7 x 3 x 1.1 mm, this metal oxide (MOX) gas sensor was deployed to monitor CO_2 levels within the micropropagation containers. It is important to note that this type of sensor exhibits some limitations, as its performance is significantly influenced by environmental humidity, temperature, and the presence of other gases, including CO, NO_2, and VOCs (Wang et al., 2010). The CSS811 sensor provides measurements of equivalent carbon dioxide (eCO_2) within the range of 400 ppm to 32768 ppm. Its primary utility lies in offering insights into CO_2 trends rather than providing absolute values. A VEML7700 light sensor was integrated to the system. This LUX sensor played a crucial role in distinguishing light on/off periods, to subsequently correlate patterns in environmental conditions with changes in lighting. Table 3.1 summarizes the specification of the sensors.

Table 3.1: Summary of the sensors employed for one monitoring unit.

Sensor	# of device	Environmental Condition	Units	Monitoring location	Operation range	Accuracy	Resolution
BME680	3	Temperature	°C	Both	-40 to 85	±0.5	0.01
		Humidity	%	Both	10 to 90	±3	1
K30	1	CO_2 levels	ppm	Room	0-5000	±(30 +3 %)	20
CSS811	2	CO_2 levels	ppm	Container	400 to 32768	-	-
VEML7700	1	Light intensity	Lux	Room	-	-	-

3.2.3.3 Circuit Diagram

A total of 7 sensors were used for the environmental monitoring system. Temperature, relative humidity, and CO_2 levels were monitored within two Magenta™ vessels, with one BME680 and one CSS811 sensor allocated to each container. Additionally, the conditions inside the growth room were monitored using BME680, K30, and VEML7700 sensors. The connection of all sensors was established via I2C communication protocol to an ESP8266 NodeMCU V3 (Espressif Systems, Shanghai, China), which is a microcontroller unit with an integrated Wi-Fi module. Given that multiple sensors sharing the same I2C address were integrated into the system, an I2C multiplexer (TCA9548A, Texas Instrument, Dallas, US) was employed to expand the microcontroller's communication capabilities. To simplify component connections, printed circuit boards (PCBs) were designed using EasyEDA software and then manufactured by JLCPCB in China. Figure 3.2 presents the circuit diagram of the environmental monitoring system.

3.2.3.4 3D-Printed Enclosure

To consolidate all the components, a 3D-printed enclosure was designed. This enclosure consists of two distinct sections: one section accommodates the microcontroller, the multiplexer, and all associated connections, while the other section is designated for the sensors responsible for monitoring the environmental conditions within the growth chamber. The enclosure was purposefully designed to ensure that the heat generated by the hardware does not interfere with sensor measurements. Typically, this is achieved by incorporating an aspiration fan into the design (Hicklenton & Heins, 1997). Additionally, the sensors employed to monitor the environmental conditions inside the Magenta™ vessels are directly integrated into the containers. Figure 3.3 presents the 3D model of the monitoring system as well a picture of the actual system.

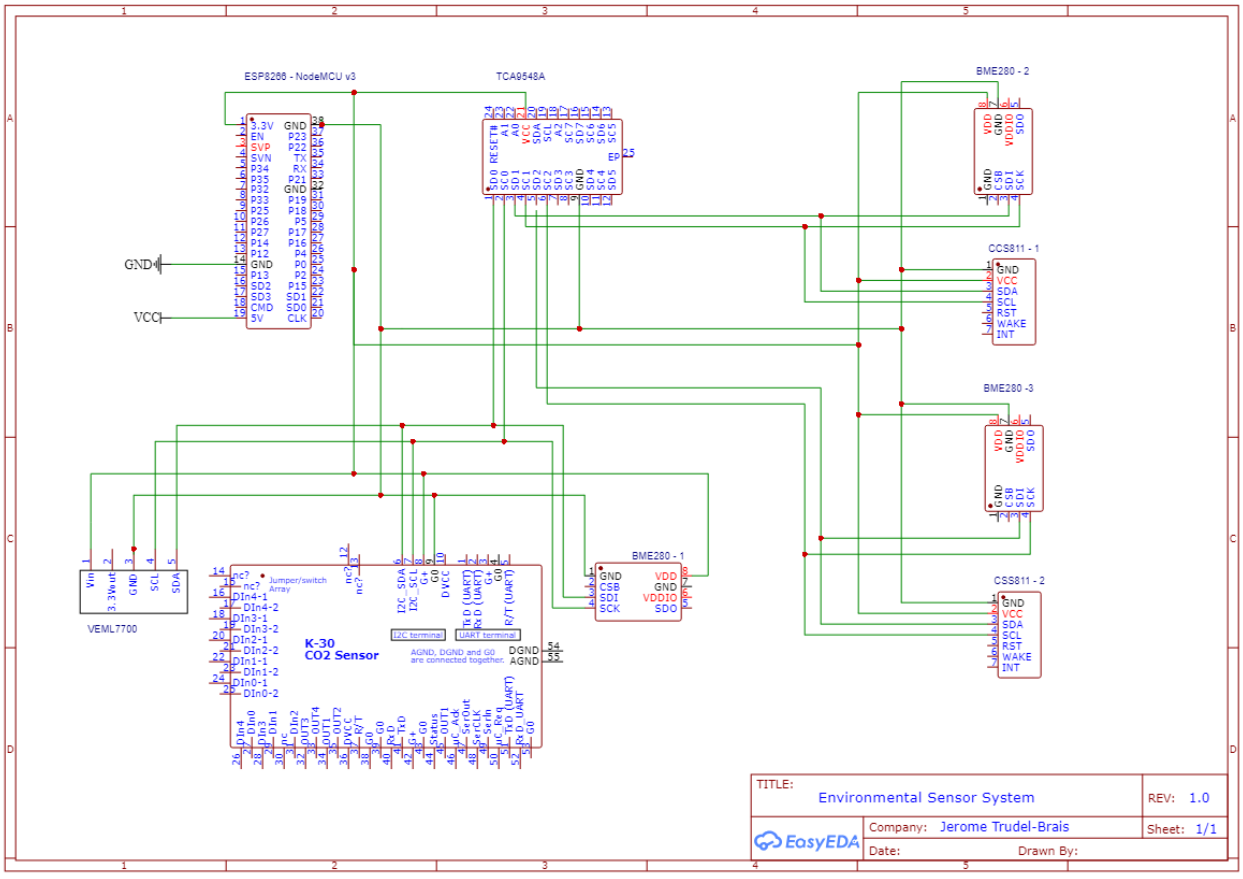

Figure 3.2: Circuit Diagram of the environmental monitoring system.

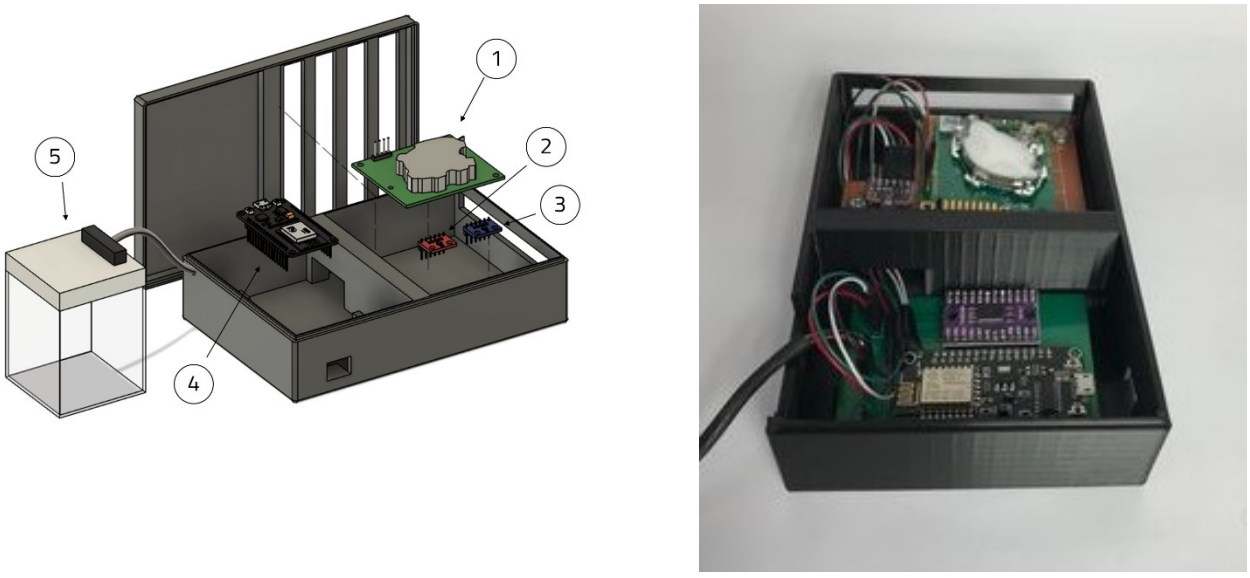

Figure 3.3: (Left) - 3D-model of the monitoring system. (Right) - Picture of the monitoring system.

3.2.3.5 Software

The operating software of the monitoring system was programmed in C++. A class/object-oriented architecture was utilized to ease the interaction between the necessary libraries and to ensure flexibility and scalability of the program. One class, name *Sensor*, handles the libraries related to the sensors: Adafruit_BME680, Adafruit_VEML7700, Adafruit_CSS811, and K30. The class includes a function to initiate the sensors (e.g., connect, and configure), a function to check the status of the sensors (i.e., active, or non-active), and a function to update the value of the sensor measurement. The class *Device* handles all the data associated with the sensors: configuration parameters, status, and measurement values. The class *Connectivity* handles the connectivity between the microcontroller, the Wi-Fi, and the web server InfluxDB. InfluxDB is an open-source time series database software made by InfluxData which can be used for the storage, visualization, and retrieval of time series data. The program's overall workflow is illustrated in Figure 3.4, delineating the functions and classes in action for each step. The C++ program can be found on the *GitHub repository* associated with the project.

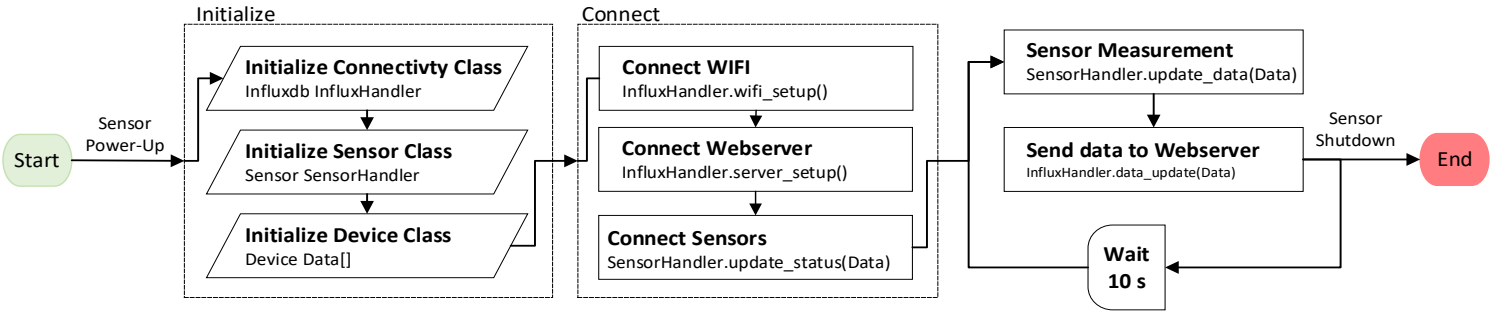

Figure 3.4: Flowchart of the C++ program.

3.2.3.6 Calibration

Most of the sensors used in the system do not allow for an easy calibration process after the initial manufacturing calibration. To overcome this problematic, two individual monitoring units were used per location during the data collection. The increased number of sensors enables to cross-validate the data collected by the two set of sensors, and thus increase confidence in the measurements.

3.2.4 Experimental design

3.2.4.1 Microclimate Uniformity

To quantify the spatiotemporal variability of the microclimate inside the growth room, the environmental conditions were monitored subsequently at 5 different growth areas. Two monitoring systems were used simultaneously per location for periods of one week; the overall experiment lasted 5 weeks. Operations made by the workers in the room are the main factors influencing environmental conditions, and they cyclically reoccur every week. The locations were selected semi-randomly to effectively evaluate the

horizontal and vertical component of the spatial variability of the microclimate. The 5 locations tested are presented on Figure 3.5. Each monitored location is represented by an orange rectangle labelled from 1 to 5. Additionally, the letters "f" and "b" are used to indicate whether the monitoring pertained to the front (f) or the back (b) cultivation area. Temperature, relative humidity, CO_2 concentration and light on/off statues were the monitored environmental conditions.

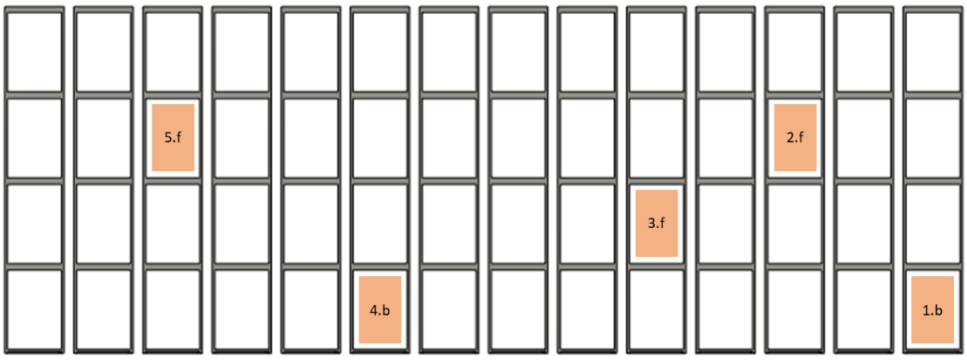

Figure 3.5: The 5 locations tested within the cultivation room. Each monitored location is represented by an orange rectangle labelled from 1 to 5. The letters "f" and "b" indicate whether the monitoring pertained to the front (f) or the back (b) cultivation area.

3.2.4.2 Plant Growth

Three different locations in the shelved cultivation room were selected following the evaluation of the uniformity of the microclimate in the growth room. Location A is situated near the entry door and air conditioner unit, B is located in a lower corner of the growth room with suboptimal ventilation, and C was selected randomly. Those locations were partially selected as they are subject to the highest variability in environmental conditions. They correspond to location 3, 1, and 4, respectively. 90 stage-two *C. sativa* plantlets ('Black Mountain Side' accession) were grown at each location, using Magenta™ GA-7 vessels (Magenta LLC, Lockport, IL) with 50 mL of D2470 medium supplemented with 2.5% $w.v^{-1}$ sucrose, pH 5.7, 7 $g.L^{-1}$ agar, and 1 $\mu mol.L^{-1}$ of Meta-topolin. Three plantlets were grown in each vessel, using either apical or nodal explants.

Plantlet height, multiplication rate (i.e., number of nodes), and total mass at the end of the 4-week growth cycle were measured to quantify plant growth. To assess total mass, each plantlet was separately weighted with an 0.01 g precise balance, and the plantlet's height was measured with a millimetric ruler. The number of nodes was counted visually. Given the challenge of determining the precise node count, intervals were employed to derive an ordinal dataset score ranging from 0 to 5, mirroring Murphy and Adelberg (2021) method for assessing ex-vitro cannabis plantlet rooting quality. The specific interval delimitation for node evaluation is case-specific, contingent on variables like accession or growth medium. This delimitation

relies on empirical data obtained during preliminary tests preceding the experiment. The following table summarizes the intervals used.

Table 3.2: Ordinal dataset score for the number of nodes the cannabis plantlets

Number of Nodes	Ordinal Score
[0,1]	1
[2,3]	2
[4,5]	3
[6,7]	4
> 8	5

The two most crucial key performance indicators (KPIs) for the growth of stage-two cannabis plantlets are height and the multiplication rate. In the second stage of the micropropagation process, which involves multiplying the number of individual plants, a plantlet with a high number of nodes and long internodal distance is considered superior. The plant is easier to manipulate and expedites the multiplication process. Conversely, a compact plantlet with a high number of nodes is difficult to handle, and a plant with a low number of nodes hinders multiplication. Thus, a quality score (QS) was developed from those two metrics to quantify the quality of plantlets from a multiplication process perspective. The quality score corresponds to the sum of the score of the multiplication rate and a weighted height score (Equation *(3.1)*). The QS has a maximum value of 10, with each factor ranging from 0 to 5.

$$Quality\ Score = \text{Multiplication Rate} + \frac{Plantlet\ Height}{Maximum\ Plantlet\ Height} \cdot 5 \qquad (3.1)$$

3.2.5 Data Analysis

3.2.5.1 Spatiotemporal variability of the environmental conditions

3.2.5.1.1 Sensor Data Preprocessing

Preprocessing of the sensor data was performed to clean the dataset and ease the subsequent data analysis processes. The sensors utilized in the monitoring system occasionally generated faulty values (e.g., NaN, negative value, predefined error code) or outliers due to defective measurement by the sensor. Those invalid data points were identified and replaced using simple two-point interpolation between the previous and subsequent data point of the time series. The interquartile method was used to detect outlier data points by calculating the minimum and maximum boundary and identifying out of range values. The preprocessing was done using Python, especially the library *Panda* to manage the time series, as well as the libraries *SciPy* and *NumPy* to perform the simple arithmetic operations. Moreover, specific subsets of

data, corresponding to particular scenarios (e.g., light on/off periods or workers in/out periods), were extracted using the Panda library.

3.2.5.1.2 Simple Statistics

Simple descriptive statistics were extracted from the environmental sensor data to get a first glimpse into the microclimate variability in the growth room. The mean, standard deviation, median, skewness, kurtosis, and Lyapunov exponential are the features extracted from the data set (Uyeh et al., 2022). The mean equation served as a point estimator of the data. The standard deviation provided insights into the data's dispersion, the median represented the central point between the highest and lowest values in the dataset, skewness and kurtosis gauged the distribution's symmetry, and the Lyapunov exponential served as an indicator of how data points changed within the dataset, signifying the system's chaotic nature. These initial metrics enable to compare various subsets of the data, which may be associated with different scenarios such as light being on or off, different locations, and the presence or absence of workers. This helps shed light on the potential factors that contribute to the variability of the microclimate within the growth room. Additionally, it aids in comparing the variability of the data with the accuracy of the sensors, helping determine whether the fluctuations are significant.

Table 3.3 summarizes the equations for each statistical calculation. N corresponds to the number of points in the time series, x_i is one point of the time series, δZ_0 is the initial separation vector divergence, and $\delta Z(t)$ is the separation vector divergence at a time t. The python libraries *Panda*, *SciPy* and *Nolds* were used to calculate the mean, the median, the standard deviation, skewness, kurtosis, and Lyapunov exponential.

Table 3.3: Equations for the simple statistics.

Statistic	Equation	Reference Number
Mean	$\mu = \frac{1}{N}\sum_{i=1}^{N} x_i$	*(3.2)*
Standard Deviation	$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}$	*(3.3)*
Skewness	$skew = \frac{1}{N\sigma^3}\sum_{i=1}^{N}(x_i - \mu)^3$	*(3.4)*
Kurtosis	$skew = \frac{1}{N\sigma^3}\sum_{i=1}^{N}(x_i - \mu)^3$	*(3.5)*
Lyapunov Exponential	$\lambda = lim_{t\to\infty}\frac{1}{t} ln \frac{\delta Z(t)}{\delta Z_0}$	(3.6)

3.2.5.1.3 Uniformity Indexes

A local uniformity index (LUI) was utilized to further assess the temporal uniformity of the environmental conditions at one location inside the room and, thus the performance of the HVAC system. The LUI, presented in Equation *(3.7)*, is a dimensionless parameter defined as the fraction of the data points of one environmental condition within a suitable growth interval. This interval is either defined by the sensitivity of the plant to that specific environmental parameter, or in a similar vein by the specification of the control system (e.g., HVAC system) (Balendonck et al., 2010).

$$LUI_{j,k} = \frac{|[x_i | x_i \in T, C(x)]|}{|T|} \qquad (3.7)$$

Where $LUI_{j,k}$ is the local uniformity index for one specific environmental condition (i.e., *j* = temp, RH, or CO_2) and location (i.e., *k* = 1, 2, 3, 4, or 5), x_i is one data point of the time series T, |T| corresponds to the number of data points within this time series (i.e., or any list), and C(x) corresponds to the condition x must satisfy to be within the suitable growth interval. For the temperature condition inside the growth room, the suitable growth zone is defined based on the HVAC system. The temperature is considered optimal if it remains between the specification of the HVAC system, which is 22±2 °C.

$$C(x) \equiv |22 - x_i| \leq 2 \qquad (3.8)$$

The uniformity index was exclusively computed for temperature since the other environmental conditions either remain uncontrolled within the study room or their optimal ranges for the micropropagation of cannabis are not well established in the literature (Monthony et al., 2021).

A total uniformity index (TUI) was employed to quantify the overall microclimate uniformity of the room for one environmental condition:

$$TUI_j = 1 - \left(\frac{\sigma_{LUI_J}}{\mu_{LUI_j}}\right) \qquad (3.9)$$

TUI_j is the total uniformity index for one environmental condition (i.e., *j* = temp, RH, or CO_2). σ_{LUI_J} and μ_{LUI_j}, defined by Equations *(3.2)* and *(3.3)*, are the standard deviation and the mean of the LUI_j of the 5 locations tested in the room. Equation *(3.9)* considers an optimal growth interval when calculating the uniformity index. Alternatively, it is possible to define an equivalent equation without this consideration, replacing σ and μ with the values calculated for each location. The calculation related to the uniformity indexes were performed using Python. Equations *(3.7*), *(3.8)*, and *(3.9)* were manually coded.

3.2.5.2 Plant Growth

The data on the metrics height, multiplication rate, and total mass of the plantlets were analyzed using one way analysis of variance (ANOVA). The location of growth inside the room corresponds to the treatment applied to the plantlets, each location is a different factor. The null hypothesis (H_0), which states that the different microclimates at each location do not generate significant difference in the plantlet growth (i.e., height, multiplication rate, etc.), was tested with a confidence interval of $P < 0.05$. The ANOVA assumptions (i.e., normally distributed, and homogeneity of variance) were tested using Shapiro-Wilk and Levene's tests. If they were not respected, the Kruskal-Wallis H-test, which is a non-parametric version of ANOVA, was used as an alternative. The analysis was performed using the Python library S*tats* from *SciPy* and *Statsmodels*.

3.3 Results and Discussion

3.3.1 Spatiotemporal variability of the environmental conditions

3.3.1.1 Temperature

Figure 3.6 presents the evolution of the temperature over a period of 168 hours (i.e., one week) at the 5 different locations monitored. Two distinct trends are visible in the data: long plateaus with periodic variations and slowly decreasing slopes. These visually distinct trends in temperature data are consistent with lights on/off periods. Locations 1 and 2 show higher absolute temperature values than locations 1, 4 and 5.

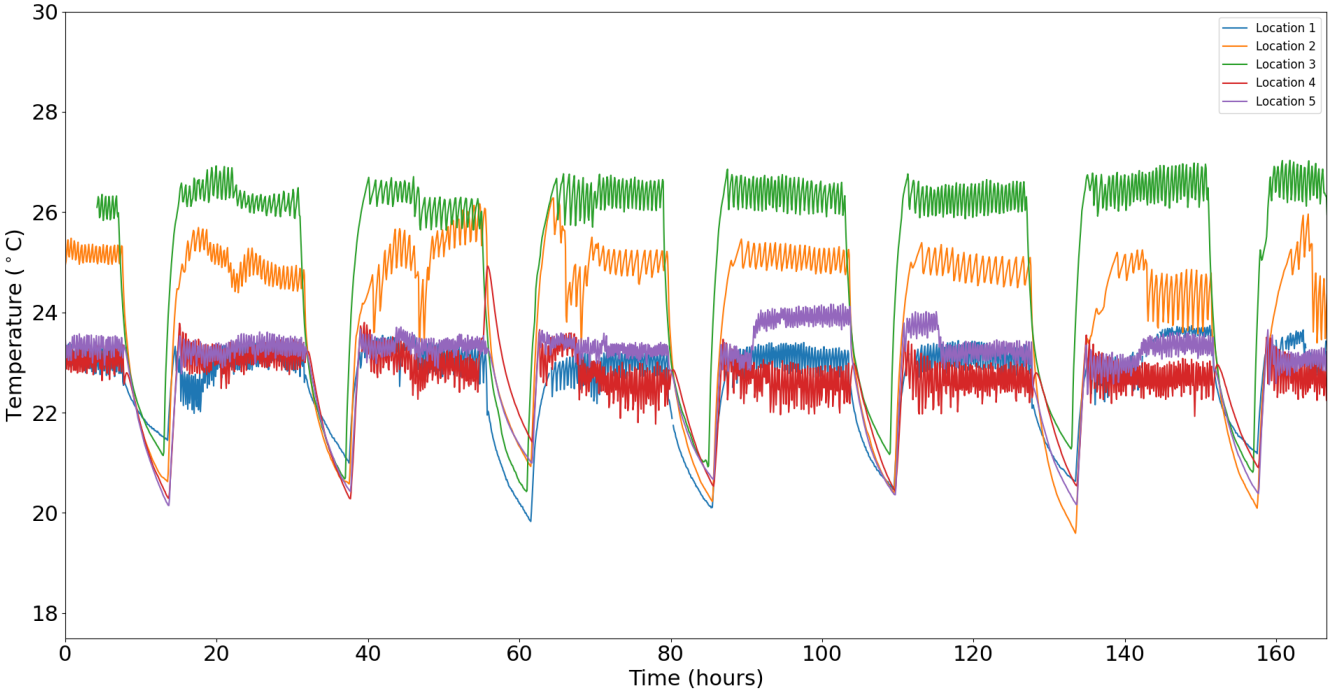

Figure 3.6: Evolution of the temperature at 5 different locations in cultivation room over a period of 168 hours.

The basic statistics presented in Table 3.4 provide a more comprehensive insight into temperature variability across these distinct scenarios. In terms of the temporal variability, a notable disparity is observed in temperature distributions between light-on and light-off periods (Figure 3.7). During light-on periods, the convective and radiative heat generated by the LED system increase the air temperature in the growth room, which is subsequently counterbalanced by the ACUs. In this situation, the average temperature across the 5 locations monitored is 24.0 °C, with standard deviations between 0.4-0.6 °C. The increased average room's temperature during light-on periods, observed at 24.0 °C instead of the programmed 22 °C, may be attributed to the placement of the temperature sensor used by the control system. Positioning the sensor near the wall, adjacent to the entry door, does not provide an accurate single-point estimation of the room's temperature as it exhibited frequent fluctuations (Lee et al., 2019). Thus, the HVAC system does not adjust the temperature adequately. The periodical variations of the

temperature during light-on periods are associated with the HVAC control system's responsiveness. It proves to be satisfactory, considering a standard deviation around 0.5 °C for the five locations.

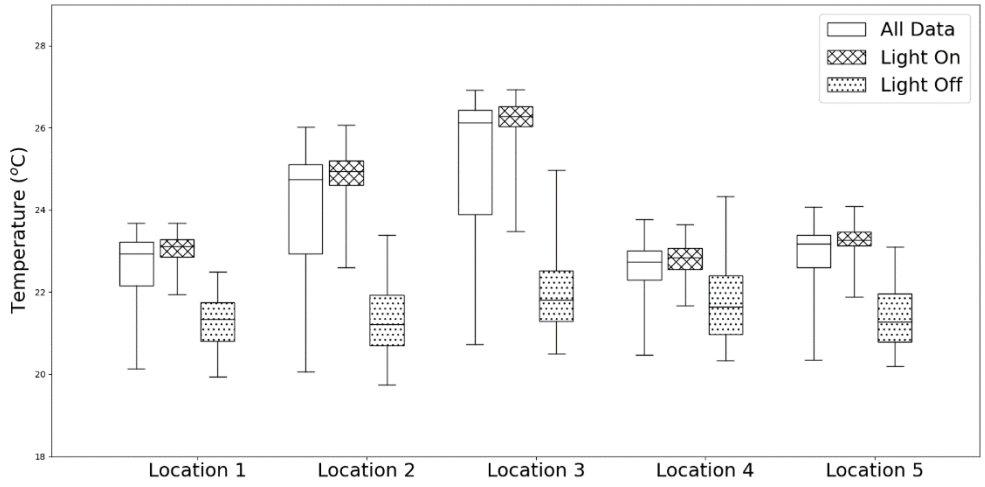

Figure 3.7: Boxplot representation of the temperature distribution of the 5 different locations for different scenarios.

During the light-off periods, there are no actuators (i.e., heating system) in place to compensate heat loss in the room; thus, the presence of the decreasing temperature slopes. However, temperature distribution remains within the HVAC specifications. The average temperature and standard deviation for the 5 locations are respectively 21.6 °C and 0.8 °C, which leads to an average difference of 2.4 °C between light-on/off periods. The two temperature trends also exhibit distinctive characteristics in terms of skewness and kurtosis statistics. The absolute value of both statistics is higher for light-on periods, compared to light off periods. As the skewness values are negative, an asymmetrical temperature distribution towards the right side is detected. The positive values of kurtosis, exceeding the expected value of 3 for normal and symmetric distributions, suggest a narrower and lighter-tailed distribution (Blanca et al., 2013; Richard A. Groeneveld, 1984).

Spatial variability of the temperature within the growth room has been observed. Locations 2 and 3 exhibit higher average temperatures compared to locations 1, 4, and 5. Specifically, the former group registers an average temperature of 24.5 °C, while the latter records 22.6 °C, resulting in a temperature difference of 1.9 °C. Several factors, including the positioning of ACUs, the arrangement of culture shelves, and the heat generated by lamps, influence air circulation and heat dispersion within the room. These factors could potentially account for the significant temperature variation observed. The local uniformity index further accentuates this distinction. LUIs for locations 2 and 3 are 0.32 and 0.25, signifying that only 32 % and 25 % of the data points fall within the optimal growth range. In contrast, LUIs average at 0.99 for locations 1,

4, and 5. This leads to a relatively low total uniformity index (TUI_{Temp}) of 0.52. Values above 0.8 are considered satisfactory (Balendonck et al., 2010). Therefore, even if the average room temperature remains within the optimal growth range during both light-on and light-off periods, significant spatial discrepancies are evident within the growth room. The integration of strategies such as supplemental mechanical fan could prove beneficial to help mitigate those spatial fluctuations (Zhang & Kacira, 2018b). No vertical temperature stratification, a prevalent phenomenon in CEA facilities, was detected in the cultivation room (Downs & Krizek, 1997; Shibuya et al., 2006).

Table 3.4: Statistic and uniformity indexes on the temperature distributions of the 5 different locations.

Scenario	Statistic	Loc 1	Loc 2	Loc 3	Loc 4	Loc 5
All Data	Mean	22.6	23.9	25.1	22.5	22.8
	STD	0.9	1.7	1.9	0.7	1.0
	Median	22.9	24.7	26.1	22.7	23.2
	Skewness	-1.21	-1.04	-1.14	-0.99	-1.25
	Kurtosis	0.46	-0.32	-0.33	1.08	0.41
	Lyapunov Exp.	0.012	0.006	0.011	0.012	0.014
	LUI	1.00	0.32	0.25	0.99	0.98
Light On	Mean	23.1	24.8	26.2	22.8	23.3
	STD	0.4	0.6	0.6	0.4	0.4
	Median	23.1	24.9	26.3	22.8	23.3
	Skewness	-1.014	-1.348	-2.559	-0.164	-1.110
	Kurtosis	2.082	3.224	8.227	2.509	5.398
	Lyapunov Exp.	0.012	0.001	0.026	-0.005	0.008
	LUI	1.00	0.11	0.02	1.00	0.97
Light Off	Mean	21.3	21.4	22.0	21.7	21.4
	STD	0.6	0.9	1.0	0.9	0.8
	Median	21.3	21.2	21.8	21.6	21.3
	Skewness	-0.17	0.46	1.08	0.60	0.47
	Kurtosis	-0.71	-0.40	1.01	0.11	-0.73
	Lyapunov Exp.	0.011	0.049	0.006	0.018	0.009
	LUI	0.98	0.96	0.94	0.98	1.00

3.3.1.2 Carbon Dioxide

Figure 3.8 presents the evolution of CO_2 levels at the five locations in the cultivation room over one week.

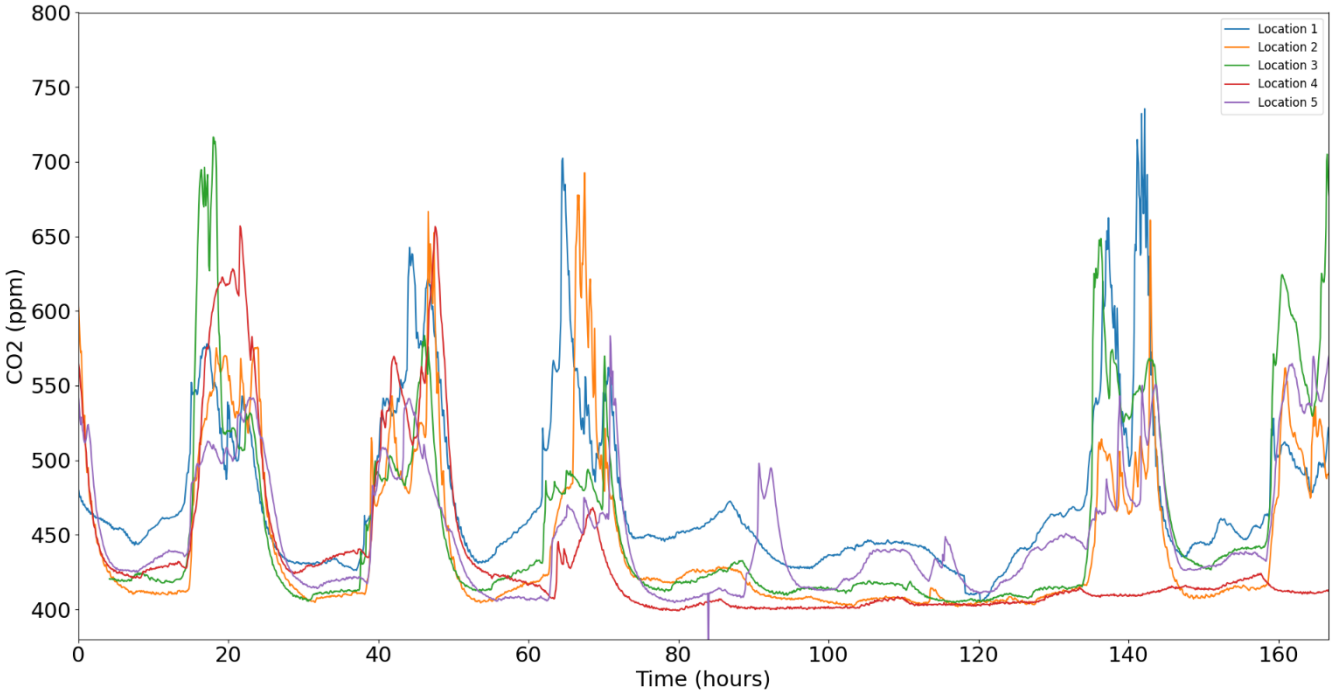

Figure 3.8: Evolution of the ambient CO_2 concentration at 5 different locations in cultivation room over a period of 168 hours.

Clear temporal variations of ambient carbon dioxide concentrations are apparent. Over a one-week production cycle inside the growth room, the CO_2 peaks in the data correlate with working hours. As presented in Figure 3.9 and Table 3.5, the presence of the workers in the cultivation room leads to higher CO_2 levels. The room average CO_2 levels are 505 ppm and 435 ppm for workers-in and workers-out periods, respectively. The average difference between those two scenarios is 70 ppm for the 5 locations monitored. Ambient CO_2 increases due to human activity inside the cultivation room is a known phenomenon to consider in CEA facilities (Niu et al., 2016). Likewise, the CO_2 concentration remains stable without any human activity inside the cultivation room. This translates into a small standard deviation and high kurtosis values. The standard deviation average at 39 ppm during worker-out periods. As the K30 sensor accuracy is specified at 30 ppm + 3% of the measurement value, those fluctuations can be considered negligible. The averaged kurtosis values of the five locations are much higher during workers-out periods than workers-in periods: $kur_{on,avg}$=10.08 and $kur_{off,avg}$=0.28. High kurtosis values imply narrower peaks of the data distribution, which is equivalent to smaller data fluctuations in this situation (Blanca et al., 2013). The CO_2 fluctuations spawned by plant metabolic processes are not readily apparent in the cultivation room due to the restricted air exchange with the Magenta™ vessel (Huang & Chen, 2005).

The ambient CO_2 concentration is not actively regulated in the cultivation room. Thus, the adjusted total uniformity index presented in Equation *(3.9)* had to be used. The TUI_{CO2} value, which stands at 0.96, indicates that fluctuations in CO_2 concentration remain relatively modest when compared to the overall room average. Namely, the metric indicates that, even if human activity leads to CO_2 increases during working hours, spatiotemporal deviations from the room average are limited. Uniformity value above 0.8 is associated with satisfactory long-term homogeneity of the room microclimate (Balendonck et al., 2010).

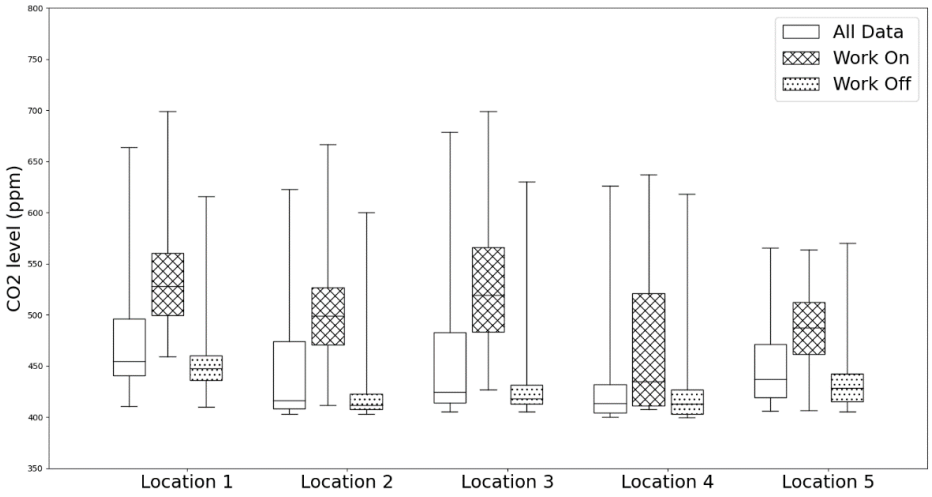

Figure 3.9: Boxplot representation of the CO_2 distribution of the 5 different locations for different scenarios.

Table 3.5: Statistic on the temperature distributions of the 5 different locations.

Scenario	Statistic	Loc 1	Loc 2	Loc 3	Loc 4	Loc 5
All Data	Mean	474	444	456	435	451
	STD	53	53	64	53	43
	Median	455	416	424	414	437
	Skewness	1.73	1.65	1.72	2.38	1.22
	Kurtosis	3.08	2.32	2.41	4.88	0.81
	Lyapunov Exp.	0.0159	0.0106	0.0105	0.0070	0.0141
Workers On	Mean	537	501	531	467	489
	STD	53	52	64	72	38
	Median	528	499	519	435	488
	Skewness	1.09	0.66	0.82	1.06	0.07
	Kurtosis	1.10	1.10	0.13	-0.25	-0.67
	Lyapunov Exp.	-0.0143	-0.0017	-0.0075	-0.0044	-0.0002
Workers Off	Mean	455	426	433	424	439
	STD	36	40	43	41	37
	Median	447	412	418	413	428
	Skewness	3.08	3.03	3.21	3.39	2.11
	Kurtosis	12.61	9.65	11.33	12.04	4.76
	Lyapunov Exp.	-0.0015	0.0255	0.0043	0.0053	0.0237

3.3.1.3 Humidity

The first metric used to quantify the ambient humidity inside the cultivation room is the relative humidity, which is the most common parameter utilized in CEA to express humidity (Wollaeger & Runkle, 2016). As seen in Figure 3.10, and further detailed in Table 3.6, the evolution of RH at location 1 differs drastically from all the other locations (i.e., 2, 3, 4 and 5).

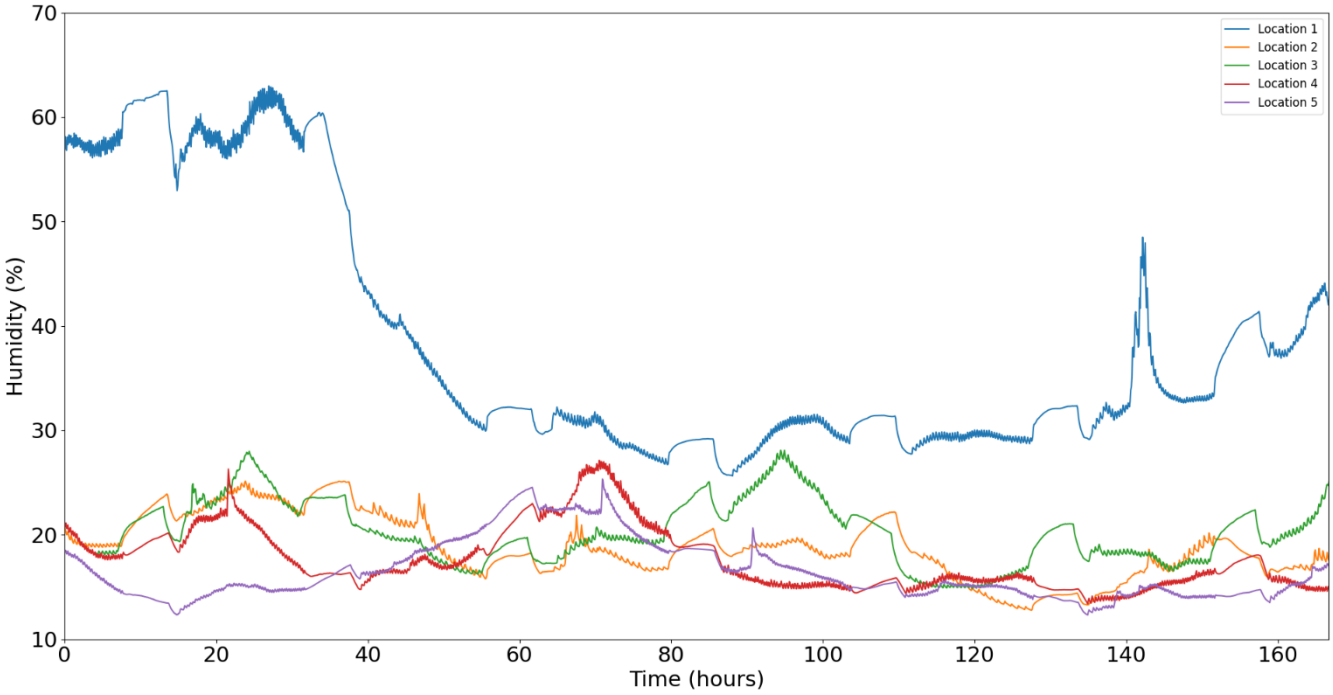

Figure 3.10: Evolution of the relative humidity at 5 different locations in cultivation room over a period of 168 hours.

The average relative humidity for location 1 is 38.5 % with a standard deviation of 11.7 %, while for locations 2 to 5, the values are μ_{RH} = 18.3 % and σ_{RH} = 3.0 %. This potentially suggests a significant spatial variability of RH inside the cultivation room, especially evident with the notably high relative humidity at location 1 compared to the others. However, this highlights one limitation of the experimental methodology employed. Since the different locations are tested sequentially, temporary divergent trends in an environmental condition caused by an external factor may be erroneously attributed to spatial variability instead of temporal. This scenario was verified with the assistance of the environmental sensor already integrated into the room for the control of the HVAC system. During the location 1 test period, the RH measurements from the HVAC sensor closely matched those collected by the experimental system. This confirms that the discrepancy in the data collected at location 1 was a result of a temporary malfunction in the ACUs rather than a consequence of spatial variability.

Table 3.6: Statistic on the relative humidity distributions of the 5 different locations.

Scenario	Statistic	Loc 1	Loc 2	Loc 3	Loc 4	Loc 5
All Data	Mean	38.5	18.8	20.4	17.6	16.5
	STD	11.7	2.9	3.1	3.0	2.8
	Median	32.2	18.5	20.0	16.4	15.3
	Skewness	0.99	0.19	0.27	1.10	1.10
	Kurtosis	-0.62	-0.54	-0.66	0.57	0.34
	Lyapunov Exp.	-0.0010	0.0051	0.0070	0.0030	0.0010

Excluding location 1 from the analysis, the relative humidity distribution inside the room is considered uniform both spatially and temporally. The TUI_{RH} value and the average standard deviation stands at 0.84 and 3 %, respectively. TUI values above 0.80 are considered acceptable and the RH variations are considered minimal as the standard deviation value remains within the accuracy specification of the RH sensor.

In controlled environment agriculture, the primary factor affecting humidity levels within a standard cultivation room is plant transpiration (Spomer & Tibbitts, 1997). Plant transpiration can result in rapid and significant increases of humidity in a closed room (Niu et al., 2016). However, this phenomenon is not observed in a micropropagation-dedicated cultivation room. Since the plantlets grow inside enclosed containers, the water vapour they produce remains contained. Additionally, there is limited air exchanges between the interior and exterior of the containers (Huang & Chen, 2005).

An alternative method to express air humidity is through vapour pressure deficit. VPD measures the humidity as a difference between the current and the saturation water vapour pressure under specific conditions (Anderson, 1936). VPD can be calculated from RH and T_{db} using Equation (3.8) (Jin et al., 2019).

$$VPD = (1 - RH) \cdot 610.7 \cdot 10^{\frac{7.5T_{db}}{237.3+T_{db}}} \qquad (3.8)$$

Figure 3.11 illustrates the variations in both VPD and RH over the span of one week at location 2 within the cultivation room. Distinct disparities are observable in the patterns of these metrics. The relationship between VPD and RH is not linear, as it is influenced by the interconnected factors of temperature, water vapour, and air energy (Wollaeger & Runkle, 2016). Figure 3.11 aids in providing a more comprehensive understanding of the divergence between fluctuations in RH and VPD within a cultivation room. If relative humidity remains within an optimal growth range, it does not necessarily imply the same for VPD. Greater emphasis should be placed on monitoring VPD, as it drives plant evapotranspiration, transpiration and water loss (Castellvi et al., 1996).

Summarizing the results, temporal variations were observed for both temperature and CO_2 concentration. These fluctuations, influenced by either human activities or control systems (i.e., LEDs or HVAC), remained

relatively limited and within the required specifications. Spatial variations in temperature were identified, with locations 2 and 3 exhibiting higher average temperatures and unsatisfactory LUIs compared to locations 1, 4, and 5. Under a properly functioning HVAC system, no spatiotemporal fluctuations were discerned in relative humidity. Most of the basic statistics and uniformity indexes provided valuable insights into the variability within the cultivation room. However, the Lyapunov exponential, as initially proposed by Uyeh et al. (2022), did not contribute significantly to the analysis.

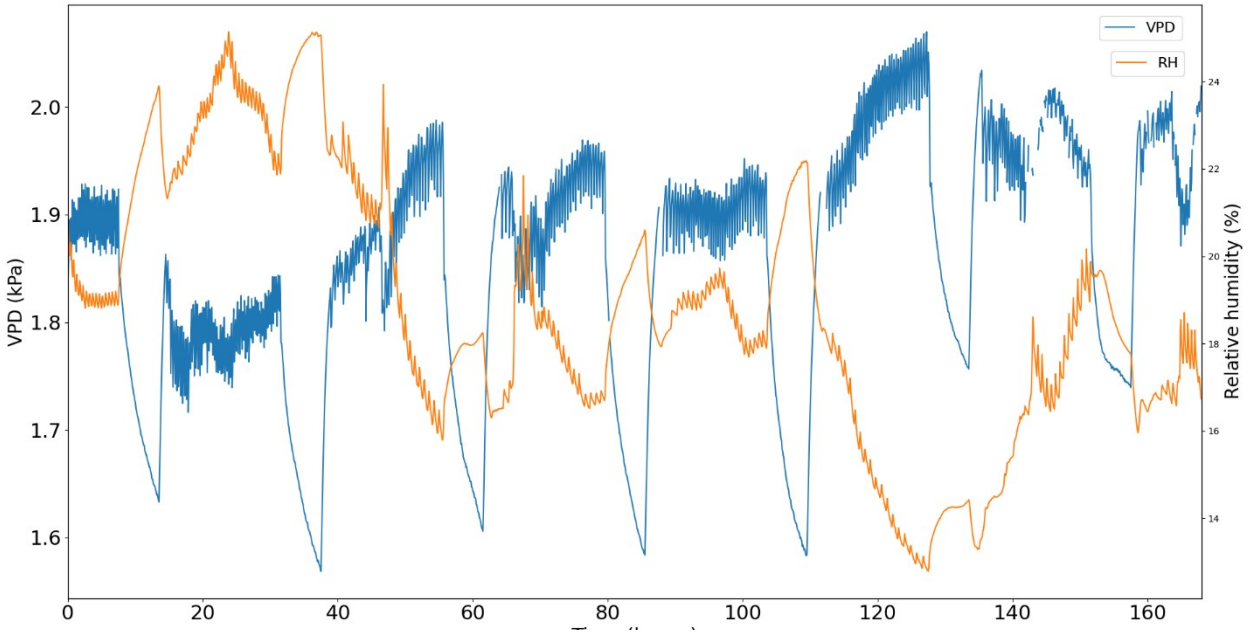

Figure 3.11: Evolution of the vapour pressure deficit and relative humidity at location 2 in the cultivation room over 168 hours.

3.3.2 Plant Growth

The ANOVA assumptions were tested to determine which analysis (i.e., ANOVA or Kruskal-Wallis H-test) is appropriate for each dataset. Table 3.7 summarized the results from the Shapiro-Wilk and Levene's tests.

Table 3.7: Results of the Shapiro-Wilk and Levene's tests for ANOVA assumptions

Metric	Shapiro-Wilk Test		Levene Test	
	Statistic Value	p-Value	Statistic Value	p-Value
Height	0.97	2.56E-04	1.84	0.16
Mass	0.928	2.90E-08	0.909	0.405
Number of Nodes	0.868	4.29E-12	0.155	0.856
Quality Score	0.982	0.013	0.580	0.561

The p-value for the Shapiro-Wilk test falls below the statistical significance threshold of 0.05, thus the normality assumption is not met for the four tested metrics. The ANOVA assumption regarding

homogeneity of variance is satisfied for the dataset examined. The Kruskal-Wallis H-test, a more robust and nonparametric variant of ANOVA, was employed to determine statistical difference between samples distribution. Table 3.8 present the results for this analysis. No statistically significant effect associated with the treatment Location was identified for the plant growth metrics of Mass ($H = 3.63$, $p = 0.163 > 0.05$), Multiplication Rate ($H = 0.32$, $p = 0.854 > 0.05$), and Quality Score ($H = 2.20$, $p = 0.333 > 0.05$). For the Height metric, there is a significant difference between the treatment groups as the p-value is lower than the significance level 0.05; $H = 12.41$, $p = 0.002 < 0.05$.

Table 3.8: Results of the Kruskal-Wallis H-test.

Metric	Kruskal-Wallis H-test	
	Statistic	p-Value
Mass	3.63	0.163
Height	12.41	0.002
Multiplication Rate	0.32	0.854
Quality Score	2.20	0.333

A multiple pairwise-comparison, using Wilcoxon Signed-Rank Test, was performed to further assess the influence of the Location treatment and confirm which pair of groups are different. Table 3.9 presents the results of this analysis. The height of the plantlets cultivated at location B exhibits a notable difference compared to those grown at locations A and C. The post hoc test uncovers a significant distinction between location A and B ($p\text{-value}_{AB} = 0.0008 < 0.5$), and also between location A and C ($p\text{-value}_{AB} = 0.0006 < 0.5$). No significant difference was detected between locations A and C as the p-value is above the significant threshold; $p\text{-value}_{AB}=0.49 > 0.05$. As illustrated in Figure 3.12, there are distinct differences between the average height of the plantlets at 3 locations, as well as for the median values. Location B presents higher average and median plantlets height values ($\mu_{locB} = 3.56$ g, $median_{locB} = 3.5$ g) compared to locations 1 ($\mu_{locA} = 2.89$ g, $median_{locA} = 2.55$ g) and 3 ($\mu_{locC} = 2.93$ g, $median_{locC} = 2.8$ g).

Table 3.9: Wilcoxon Signed-Rank Test for pair comparison between location 1, 2 and 3 considering the Height metric.

Location #	Location #	Statistic	p-Value	Reject
A	B	541	0.0008	TRUE
A	C	968	0.4926	FALSE
B	C	572	0.0006	TRUE

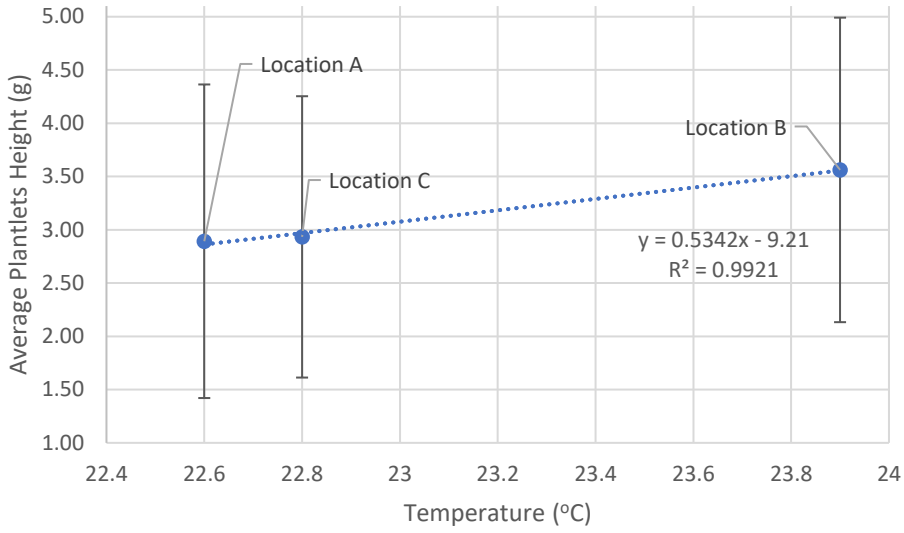

Figure 3.12: Average plantlets height (g) for the 3 locations compared to the average temperature (°C) at each of those locations.

The environmental conditions of incubation, particularly temperature, significantly impact the growth of cannabis plantlets (Monthony et al., 2021). Thus, the findings obtained for the height metric of the plantlet growth may potentially be elucidated by the temperature difference at each location. Locations A, B and C present average temperatures of 22.6 °C, 25.1 °C and 22.8 °C, respectively. A correlation is present between average temperature and plantlets height at each location. A strong linear correlation (R^2=0.992) is obtained from the data collected. As mentioned in literature, this finding strengthens the need to further assess the effect this environmental condition has on in-vitro-grown *Cannabis*.

The spatiotemporal variability of humidity and ambient CO_2 concentration have a limited effect on the growth of cannabis plantlets. The plantlets are not directly exposed to those environmental conditions inside the cultivation room. As shown in Figure 3.13, the evolution of the relative humidity and CO_2 levels inside the Magenta™ containers is considerably different from those inside the cultivation room. The agar- and water-based substrate leads to highly humid environment inside the container (Kyte et al., 2013). The relative humidity fluctuates between 90 % and 100 % inside the vessel, compared to 30-40 % inside the cultivation room. A similar situation is observable for the CO_2 concentration. The average CO_2 concentration is higher inside the container and the temporal variations diverge slightly between inside and outside.

Consequently, the spatial and temporal variability in the cultivation room's environmental conditions only marginally impacts the growth of cannabis plantlets. While temperature variation significantly influences the Height growth metric, other metrics, especially QS, remain unaffected. Furthermore, there is no observable impact on plantlet growth in relation to humidity and CO_2 levels.

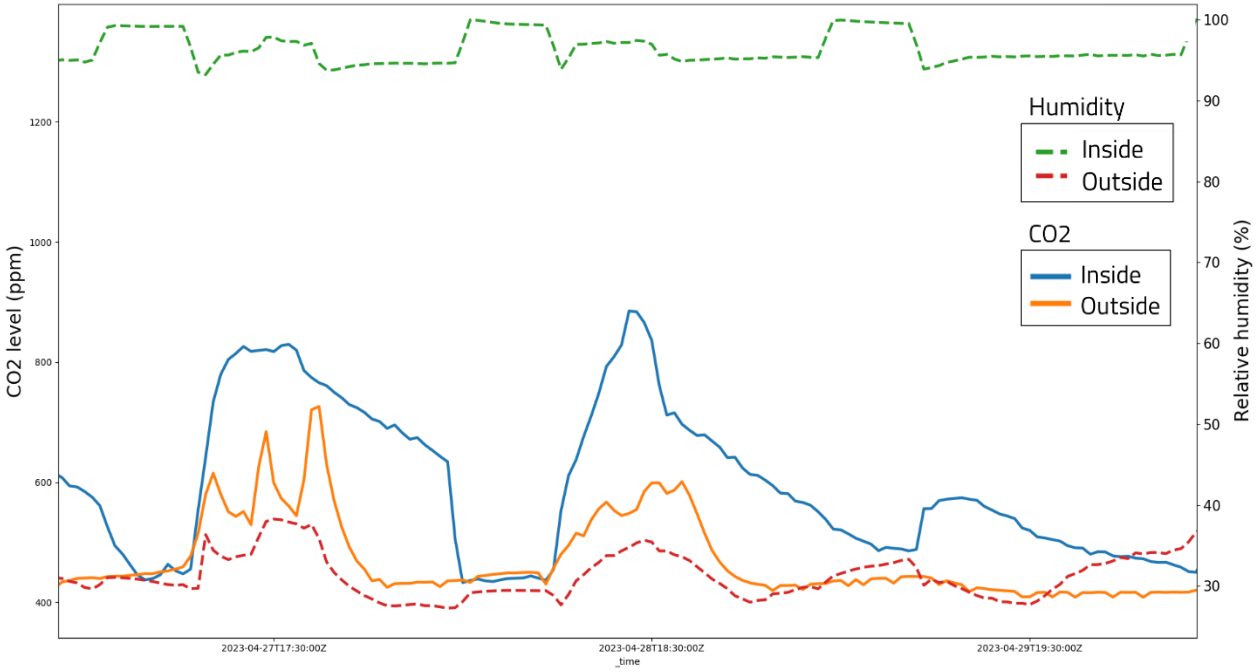

Figure 3.13: Comparison of the environmental conditions inside and outside the Magenta™ vessel at one location for a period of 48 hours.

3.4 Conclusion

Enhanced environmental data collection is essential in large-scale plant factories to comprehensively evaluate microclimate uniformity and determining the most optimal sensor placement and spatiotemporal resolution. Hence, a methodology is proposed to quantify spatial and temporal variations in environmental conditions within a cultivation room dedicated to cannabis micropropagation. An IoT sensor system, utilizing Arduino technology and InfluxDB software, was designed to monitor temperature, humidity, CO_2, and light levels, with data transmitted to a web server. Five different locations in the shelved cultivation room were tested subsequently for periods of one week. Assessing spatiotemporal environmental condition variability relied on basic statistics and uniformity indexes. Notably, the study identified a 1.9 °C average temperature difference between locations, resulting in a relatively low total uniformity index for temperature of 0.52. The plantlet growth analysis, employing the Kruskal-Wallis H-test as a nonparametric ANOVA alternative, unveiled a significant difference in plantlet heights among various locations ($H = 12.41$, $p = 0.002$, $p < 0.05$). Additionally, a strong linear correlation ($R^2 = 0.992$) was noted between temperature

variability and plantlet heights. These findings offer valuable insights into assessing microclimate variability within CEA cultivation rooms and emphasize the need for further exploration into the impact of these environmental conditions on in-vitro-grown cannabis plants.

The proposed methodology has limitations when it comes to distinguishing temporal variability from spatial variability. To address this issue, it is necessary to monitor at least two locations simultaneously. This approach enables the cross-validation of data trends and a more comprehensive assessment of spatial variability. Similarly, the procedure developed in the study is effective for adequately mapping the microclimate inside a cultivation room. However, it is not suitable for continuous real-time monitoring of environmental condition variability within the room. To achieve this, an array of sensors covering the entire growing area would be required. Time series analysis would serve as a valuable tool for detecting such variations. In an industrial context, a discussion is essential to determine whether mitigating this non-uniformity is worthwhile, considering both time and resource constraints.

Connecting Text

In the subsequent section, a thorough discussion of all four project objectives is provided, along with potential directions for future research.

4. Chapter 4 - General Discussion

4.1 IoT-based Environmental Monitoring System

The first project objective was to design and construct an IoT-based environmental monitoring system, which has demonstrated satisfactory performance. The system's performance has been evaluated based on various factors, including specifications, uniqueness, low cost, and reliability. The sensors used in the system adhere to the specifications outlined in the relevant literature. For instance, the temperature sensor has a range from -40 °C to 85 °C, surpassing the minimal range of 0 °C to 60 °C. The thermocouple exhibits excellent precision (0.01 °C) and slightly higher accuracy (0.3 °C) than the recommended values. The accuracy of the humidity sensor is slightly below the suggested threshold of 5 %. The primary carbon dioxide sensor (K30) meets the required specifications, with an accuracy of less than 50 ppm, a range spanning from 0 to 1500 ppm, and immunity to interference from other chemicals like CO, NO_2, and VOCs.

A commercially available sensor system suitable for monitoring environmental conditions within the Magenta™ vessel was not identified in the market. The developed system successfully completed this task. The requirement for the sensor to maintain an aseptic environment was a critical consideration during the development of this component of the system. The established procedure proved to be successful, as the introduction of the sensor did not result in contamination of the plantlets, and no statistical differences in plantlet growth were observed. The total cost of the sensor amounts to $284 per unit, and a breakdown of the component prices is provided in Table 4.1.

Table 4.1: Price (CAD$) estimate for one monitoring unit.

Component	# Unit	Price/unit	Price (CAD$)
BME280 – Temperature	3	20	60
K30 – CO_2	1	128	128
CSS811 – CO_2	2	25	50
VEML7700 – Light	1	8	8
I2C multiplexer TCA9548A	1	9	9
ESP8266 NodeMCU V3	1	9	9
PCBs	1	1	1
Box	1	4	4
Wires	-	-	15
		Total	284

The system demonstrates reliability as it successfully operates across the first three layers of the IoT architecture without any interruptions during the entire experiment. The system flawlessly collected sensor data (i.e., perception layer), and transmitted this data via Wi-Fi to a web server (i.e., connectivity

layer), where it was efficiently aggregated and visualized (i.e., intermediate layer). Moreover, the system's dependability is evident in its performance, with fewer than 5 % of the transmitted data points exhibiting faults or errors.

While the system has performed well, there is room for improvement in a couple of key areas. One of the significant challenges encountered was the necessity for extensive wiring to power the sensor system. Due to the centralized location of the power outlets in a confined section of the cultivation room, long wires had to be laid out, which proved to be a logistical hurdle. To address this issue, a battery-powered system could serve as a promising alternative, effectively eliminating the need for extensive wiring. Additionally, a crucial aspect of any sensor system is the calibration process. A substantial improvement could be achieved by implementing sensor units that offer manual calibration capabilities.

An affordable IoT environmental monitoring system was successfully designed, built, and operated, raising questions about the relevance of existing commercial environmental sensor systems. Commercial sensors tend to be expensive, have a larger physical footprint, and frequently incorporate aspiration fans. Future research could involve a comprehensive performance comparison between these two sensor categories, exploring the benefits of commercially available sensor systems in comparison to more budget-friendly, minimalist alternatives.

4.2 Statistical analysis for the spatiotemporal variability of the environmental conditions

Basics statistics and uniformity indexes were used to assess the spatiotemporal variability of the environmental conditions inside the cultivation room. The basic statistics, encompassing average, median, standard deviation, skewness, kurtosis and Lyapunov exponent, allowed for analysis of the dataset based on different features, such as a one-point estimation, variability, or symmetry distribution. Those descriptive metrics helped better understand the causes of the temporal or spatial fluctuation of the microclimate and to compare different subsets of data associated with different locations or scenarios (e.g., light-on/off periods). Two different types of uniformity index were used. The local uniformity index evaluates if the environmental conditions are constricted within a specified interval. The total uniformity index addressed the spatial uniformity of the local uniformity index across the cultivation room.

Time series analysis is an additional statistical technique that proves useful in quantifying the spatiotemporal variability of environmental conditions. This approach extends the analysis by providing a method for examining and modeling the patterns, trends, and interrelationships within the collected data points. A time series is essentially a sequence of data points or observations, usually recorded at consistent time intervals, with each data point denoting a value or measurement at a specific moment in time.

Consequently, the data pertaining to temperature, humidity, and CO_2 concentration are all considered time series.

Table 4.2: Euclidean distance similarity matrix for the temperature time series of the 5 locations.

Loc	1	2	3	4	5
1	0	75	127	32	25
2	75	0	68	83	64
3	127	68	0	137	119
4	32	83	137	0	29
5	25	64	119	29	0

Table 4.3: Euclidean distance similarity matrix for the CO2 time series of the 5 locations.

Loc	1	2	3	4	5
1	0	2262	2156	3078	2218
2	2262	0	1942	2036	1652
3	2156	1942	0	3001	1812
4	3078	2036	3001	0	2380
5	2218	1652	1812	2380	0

Table 4.4: Euclidean distance similarity matrix for the RH time series of the 5 locations.

Loc	1	2	3	4	5
1	0	988	941	1069	1144
2	988	0	145	167	214
3	941	145	0	219	261
4	1069	167	219	0	123
5	1144	214	261	123	0

As an introduction to time series analysis, similarity metrics were derived from the dataset collected to assess the spatial uniformity of each environmental condition. Common similarity metrics, which are also known as distance measures, used in time series analysis include Euclidean distance, dynamic time warping (DTW), Pearson correlation, or cosine similarity. Each of these metrics evaluates time series based on various characteristics like value offset, scale, noise, time warping, and more. In this particular case, the Euclidean distance metric was employed. Table 4.2, Table 4.3, and 4.4 display the results of the analysis, reaffirming the conclusions drawn from the previous examination employing basic statistics and uniformity indexes. The dissimilarity between two time series increases as the Euclidean distance value rises. In the case of temperature, it is evident that locations 2 and 3 exhibit significant differences compared to locations 1, 4, and 5. The average value for intergroup comparisons (i.e., 2 and 3 vs. 1, 4, and 5) is 100, while the average for intragroup comparisons (i.e., 2 vs. 3 and 1 vs. 4 vs. 5) is 38. Regarding carbon dioxide,

location 4 shows the greatest difference, although the absolute disparity between the time series remains relatively low compared to temperature. In the case of relative humidity, the most notable difference is observed between location 1 and the others.

A wide array of techniques can be harnessed within the scope of time series analysis, including descriptive analysis, forecasting, intervention analysis, cross-correlation analysis, clustering, and anomaly detection. Forecasting and intervention analysis, for example, can be invaluable for predicting and assessing the impact of external perturbations (e.g., operational changes or power shortages) on the temperature's evolution within the cultivation room. Cross-correlation analysis offers a means to evaluate the interrelationship between different locations inside the room or the relationship between environmental conditions (e.g., temperature and humidity). Clustering is apt for grouping sensors that share similar features and behaviors, effectively aiding in the assessment of the spatial distribution of environmental conditions within the room. Last, anomaly detection plays a pivotal role in identifying malfunctioning sensors.

4.3 Environmental data collection inside a multi-layer cultivation room

The project introduced an economical and straightforward method for monitoring the environmental conditions within the cultivation room. Rather than employing an extensive array of sensors, a restrained number of devices were strategically placed to subsequently monitor conditions at various locations inside the growth enclosure. Within a specific temporal frame marked by various factors influencing environmental conditions—such as HVAC response time, plant growth phases, and activities within the growth enclosure—the fluctuation patterns of these conditions are expected to cyclically reoccur. By pinpointing the temporal limiting factor, it becomes possible to ascertain how long each device should remain at a particular location.

This methodology has proven to be effective, enabling the quantification of microclimate variability and the identification of disruptive factors in action. Spatial temperature variations were observed in the room, with an unexpectedly higher average temperature during light-on periods. Several factors could explain these fluctuations, such as the placement of ACUs, the arrangement of culture shelves, or the positioning of the temperature sensor used by the control system. Additionally, temporal CO_2 variability was linked to the presence of workers inside the cultivation room. However, the data collected on relative humidity revealed a limitation in the proposed methodology. The results indicate significant spatial variability of RH inside the cultivation room, especially notable with the remarkably high relative humidity at location 1 compared to the others. Yet, because different locations are tested sequentially, temporary divergent

trends in environmental conditions caused by external factors may be mistakenly attributed to spatial variability rather than temporal. To address this concern, it is necessary to monitor more than one location simultaneously.

Future research should prioritize investigations involving extensive array of sensors to monitor environmental conditions at various locations simultaneously. Such an approach has the potential to offer more precise insights into spatial variability within multi-layer cultivation rooms and the underlying factors at play. Furthermore, it would allow for experiments related to optimal sensor placement, akin to what has been conducted in greenhouse scenarios. Data on air movement should be included in future research to provide a more comprehensive representation of the environmental conditions experienced by the plants.

4.4 Growth of stage-2 cannabis plantlets

Assessing the impact of the variability of the environmental factors on plant growth is essential for optimizing crop production and resource allocation. Therefore, 90 stage-two *C. sativa* plantlets ('Black Mountain Side' accession) were grown at three different locations in the shelved cultivation room. The location of growth inside the room corresponds to the treatment applied to the plantlets, each location is a different factor. Plantlet height, multiplication rate (i.e., number of nodes), and total mass at the end of the 4-week growth cycle were measured to quantify plant growth. The plantlet growth analysis, employing the Kruskal-Wallis H-test as a nonparametric ANOVA alternative, unveiled a significant difference in plantlet heights among various locations ($H = 12.41$, $p = 0.002$, $p < 0.05$). Additionally, a strong linear correlation ($R^2 = 0.992$) was noted between temperature variability and plantlet heights.

However, when evaluating the general quality of the plantlets, considering both height and multiplication rate metrics, we did not observe any significant differences among the various locations. This finding suggests that while microclimate variability affects individual plantlet height, it may not impact overall plantlet quality. These results accentuate the need for a broader discussion on the level of control exerted over the microclimate in plantlet growth environments. Increasing control over the microclimate comes with associated costs and resource requirements, which may not necessarily translate into higher crop yields or improved plant growth metrics. This presents an optimization problem where the balance between microclimate control, resource allocation, costs, and plant growth needs to be carefully considered. Decisions regarding the extent of control over the microclimate must be weighed against potential benefits and economic feasibility to make informed choices in agricultural practices.

5. Chapter 5 – General Conclusion

Extensive environmental data collection is indispensable for large-scale plant factories, offering a comprehensive assessment of microclimate uniformity. This practice not only enhances decision-making but also aids in designing and controlling heating, ventilating, and air conditioning systems, optimizing resource utilization. This project delved into the realms of microclimates and environmental data monitoring in controlled environmental agriculture.

The literature review explored the environmental conditions to be considered in CEA, elaborating on general concepts related to temperature, humidity, carbon dioxide, and air movement, along with their interactions with plant metabolic processes. Furthermore, the principal factors that influence the uniformity of the microclimate and the sensor requirements for monitoring those environmental condition variations were investigated.

The central focus of the project was quantifying the spatial and temporal variations in environmental conditions within a cultivation room designed for cannabis micropropagation. To accomplish this, an affordable Internet of Things sensor system was developed using Arduino technology and InfluxDB software to monitor key parameters, including temperature, humidity, CO_2 levels, and light intensity. The study involved the examination of five distinct locations within the shelved cultivation room for periods of one week. To gauge the spatiotemporal variability in environmental conditions, basic statistical metrics such as average, mean, standard deviation, and skewness, alongside uniformity indices, were employed. Notably, the findings revealed a noteworthy temperature disparity of 1.9 °C among these locations, culminating in an overall uniformity index of 0.52. Additionally, employing the Kruskal-Wallis H-test, a non-parametric alternative to ANOVA, a statistically significant difference in plantlet heights at the conclusion of the growth stage across these varied locations was identified ($H = 12.41$, $p = 0.002$, $p < 0.05$). Further analysis unveiled a robust linear correlation ($R^2 = 0.992$) between temperature fluctuations and plantlet heights.

Furthermore, a comprehensive discussion was conducted, covering all four project objectives, and exploring potential areas for future research. The development of the IoT-based environmental monitoring system was deemed satisfactory, prompting a critical examination of existing commercial environmental sensor systems. The addition of time series analysis as a complementary statistical tool to the basic statistics and uniformity indexes used during the project was suggested. The proposed methodology, which employs a cost-effective approach involving a limited number of strategically placed devices to monitor conditions at various locations within the growth enclosure, has proven to be effective

in quantifying microclimate variability and identifying disruptive factors. Lastly, the results regarding the impact of microclimate variability on plantlet growth underscore the need for a broader discourse on the level of control exerted over microclimates in plantlet growth environments. Enhancing microclimate control entails additional expenses and resource commitments, which may not always result in increased crop yields or improved plant growth metrics.

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