The added value of indoor products: the strawberry case

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Report WPR-1322

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Referaat
In Europa vertegenwoordigen aardbeien een markt met een hoge waarde en consumenten wensen continu beschikbare, lokaal geproduceerde aardbeien van hoge kwaliteit. Er is dan ook behoefte aan verder onderzoek om dit te bereiken door middel van indoor productiesystemen.
Deze studie onderzocht de mogelijkheid om aardbeien te produceren met een hoge opbrengst en kwaliteit en het potentieel om het productiepatroon onder gecontroleerde omstandigheden te sturen. In twee daglichtloze cellen met het doordragende aardbeiras *Favori* een stabiel klimaat werd vergeleken met fluctuerende omstandigheden met dezelfde totale licht- en temperatuursom. In elke cel werd het effect van een referentielichtspectrum vergeleken met een hoog blauw spectrum. De resultaten werden vergeleken met een parallel lopende kasteelt. Beide klimaatbehandelingen kenden een grote piekproductie en leverden een 1,8 maal hogere opbrengst op vergeleken met de kasteelt. De hoog blauw behandeling vertoonde geen positieve effecten op de voedingskwaliteit en had een negatief effect op de productie vanwege de compacte architectuur en de lagere lichtonderschepping. Manipulatie van teeltomstandigheden kan worden gebruikt om het gewas in een kort tijdsbestek naar een hoge productie te sturen met nadelen voor de kwaliteit, of om minder te produceren maar voor een langere periode. Bij doordragende aardbeien zijn assimilatieproductie en -beschikbaarheid belangrijke controlefactoren.

Abstract
In Europe, strawberries represent a high-value market and consumers demand continuously available locally produced strawberries of high quality, calling for further investigation to achieve this by means of fully controlled indoor production systems.
This study investigated the possibility of producing strawberries with a high yield and quality and the potential of steering the production pattern under controlled conditions. In two indoor research cells with everbearing strawberry cultivar *Favori* a stable climate treatment was compared to fluctuating conditions with the same final light and temperature sum. In each cell the effect of a reference light spectrum was compared to a high blue spectrum. The results were also compared to a parallel running greenhouse cultivation. Both climate treatments had a large peak production and produced 1.8 times higher yield compared to the greenhouse cultivation. High blue treatment didn't show positive effects on nutritional quality and had a negative effect on production due to compact architecture and lower light interception. By manipulation of cultivation conditions the strawberry crop can be steered towards a high production peak with drawbacks for quality or into producing less but for a longer period. In the case of everbearing strawberries, assimilate production and availability are key control factors.

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1 Introduction

Vertical indoor farming is seen at the latest revolution in agriculture offering a technologically advanced food production system (Van Gerrewey, 2022). In fact, it provides a high-tech and fully controllable, closed environment where it is possible to finetune the growing conditions to achieve a higher yield per unit area and with high quality when compared to field production.

Today, this form of farming is expanding rapidly, with many investors, start-ups, established greenhouse industry companies, and even companies previously unknown to horticulture (e.g., lighting, furniture, and retail industry) entering the vertical farming space. In addition, the interest in vertical farming has amplified research on controlled environment agriculture, which also has a stimulating effect on the greenhouse horticulture industry (Butturini, 2020).

On the other hand, vertical indoor farming still requires high energy input, and therefore cannot compete on costs with other forms of controlled environment such as greenhouses. Therefore, to evolve into a relevant and more economically and environmentally sustainable food production industry, it is necessary to increase the assortment of produced crops with added value, for example fruiting plants, in order to counter high costs and energy input. Initially, these additional crops will have a better opportunity to access a market with an already established large volume demand for high value products (Kozai *et al.*, 2021). Although, vertical farming production started as mainly lettuce and leafy green, the sector is moving towards the production of soft fruits, especially berries, such as strawberries. In fact, strawberries are a potential crop for vertical farming because of their smaller size and lower light requirements compared to other horticultural crops. As a result, the required cultivation space per plant is small, and strawberry plants can be grown using multi-layer, stacked systems (Yoshida, 2016).
From a consumer perspective, the higher product cost will be accepted only when the product has higher quality attributes, namely, an added value (Lubna *et al.*, 2022).

In Europe, the strawberry market is a high-value market and consumers expect quality and freshness. Despite the dominance of local growers, the demand allows for additional import with United Kingdom as the main importer, especially in out-of-season periods of the year[1]. Research into growing conditions to achieve year-round high quality strawberry production would make it possible to develop a new growing model that would fill the production gap between November and February (Read *et al.*, 2021).

Strawberries (Fragara x ananassa) are among the most studied berries given their health-related, economic and commercial importance (Giampieri *et al.*, 2012, Afrin *et al.*, 2016). They are considered as a functional fruit because of their nutritional composition. They are remarkably high in vitamin C and folate as well as in phytochemicals of which anthocyanins and ellagitannins (both polyphenols with high antioxidant capacity) are the most abundant. Di Vittori *et al.* (2018) described the quality of berry fruit as the sum of yield efficiency (yield, harvest speed, resistance to pathogens and fruit size), organoleptic quality (fruit shape, color, firmness, taste, flavor) and nutritional quality (fiber, mineral, vitamin and phenolic content and antioxidant capacity).
Strawberries can be classified into Junebearers, everbearing and day-neutrals cultivars depending on their flowering behavior. Everbearing cultivars are able to produce numerous flower flushes during the growing season. Fruit development and formation of new trusses can take place simultaneously and therefore have the potential to be grown in a 'flat' production pattern with consistently high quality (intended as stable fruit size). On the other hand, this means that plant development must be kept in balance between generative and vegetative growth. Too high production peak and plant load will inhibit vegetative growth which will, in turn, lead to a low production (Elings *et al.*, 2023).

1.1 Research aim and questions

The goal of this project is to investigate the potential added value of strawberries grown under fully controlled environment without sunlight. Because of the increasing demand for out-of-season locally produced strawberries, there is a need for further research into the growing conditions to achieve year-round high quality strawberry production. Strawberries are cultivated in the greenhouse to extend the production season, but there is still a dependency on the outside climate (radiation) which makes the inside climate fluctuating. In fully controlled environment, conditions such as light and climate, can be kept constant throughout the whole cycle. This is unique to this type of cultivation systems and can’t be achieved in other controlled environment systems such as greenhouses. The question arises if stable conditions throughout the cultivation cycles is beneficial for strawberry production and quality compared to fluctuating conditions like in the field or greenhouse. The comparison of a fluctuating climate design, as representation of greenhouse conditions, to a stable one, as representation of vertical farm conditions will help the sector understanding and positioning better both production systems and type of products into the current agrifood system.

Another advantage of indoor cultivation without sunlight is the fully control over the spectral quality which can influence growth, development and quality of the fruits.

Hence, two parameters will be studied:

- Yield consistency throughout the cultivation cycle (peak production versus “flat” production).
- Organoleptic quality (dry matter content, Brix, firmness, and acidity) and nutritional quality (vitamin C and anthocyanins content).

Therefore, the research questions are:

a. What are the effects of climate fluctuations (light and temperature) on yield and quality of strawberries?
b. Can a higher organoleptic and nutritional quality be achieved when strawberries are cultivated under a tailor-made spectrum under fully controlled conditions?

2 Materials & Methods

2.1 Facility

The trial was conducted in the two Multi-Layer Cells (MLCs) of the Vertical Farm facility at the Business Unit Greenhouse Horticulture and Flower bulbs of Wageningen University and Research in Bleiswijk, The Netherlands. Each cell comprised 3 compartments: the lock or pre-chamber (L), the production compartment (P) and the technical installation compartment (Figure 1). The production compartment has an area of 30 m^2 and a volume of 175.8 m^2. It features 2 production layers (ebb and flow table) with each 10.3 m^2 of cultivation area and a free height (distance layer-lamps) of 1.6 m. Each layer is illuminated by an array of LED modules (Philips/Signify GreenPower LED production module 3.0 dynamic) which can controlled (per layer) in intensity (top layer max Par output: 1000 µmol m^{-2} s^{-1}; bottom layer max Par output: 500 µmol m^{-2} s^{-1}) and in spectrum composition (blue, white, red and far red) using Signify's GrowWise system.

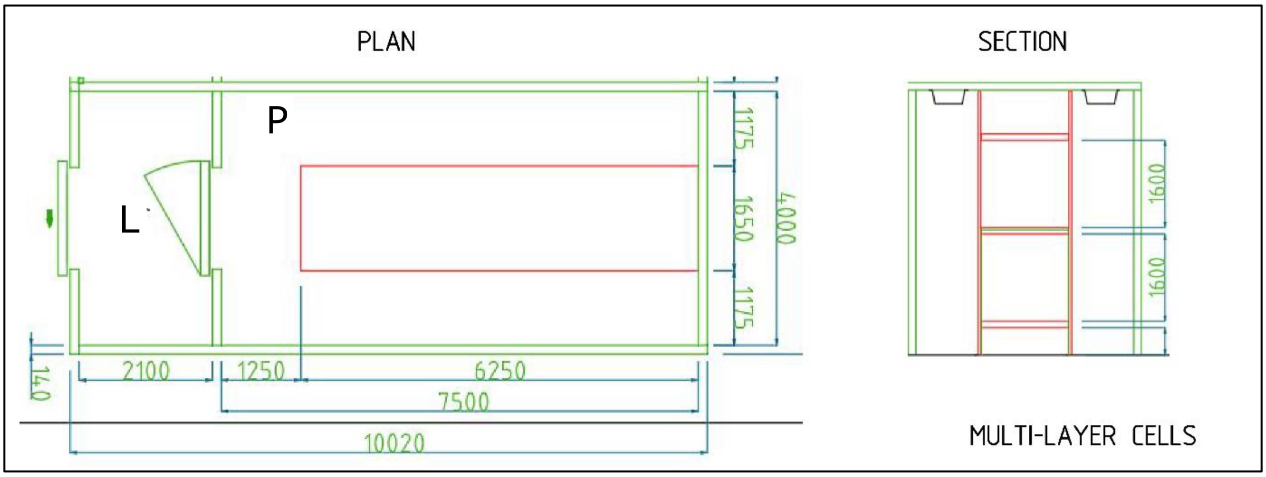

Figure 1 *Dimensions of MLCs (in mm). Top view (left) of lock (L) and production compartment (P) and side view (right) of production compartment.*

Cells are airtight and climate is controlled by the Ridder climate computer running Synopta software. Each cell has its dedicated HVAC unit featuring a ventilator, a heating and a cooling unit. Air is continuously recirculated in the cell. Exhausted air is climatized in the technical installation compartment for temperature humidity and CO_2 content. Climatized air is introduced in the cell via perforated ducts on the side walls (Figure 2 right; blue ducts) and extracted per layer via air ducts above the LED installation. An additional fogging systems with ducts mounted on top of the side ducts enables further humidity control in the cell.

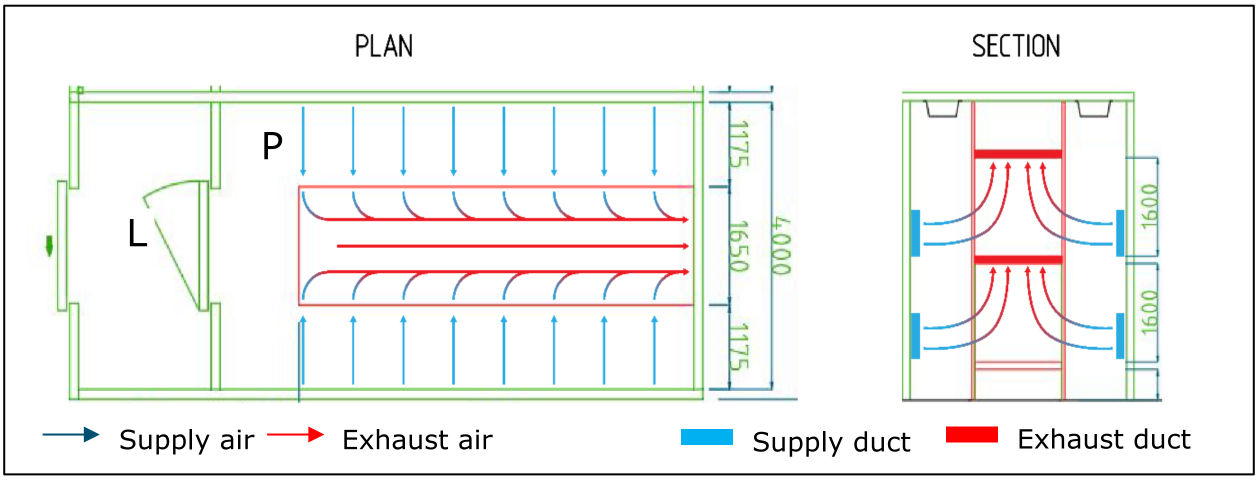

Figure 2 *Climate control in MLCs via air supply and air movement. Air supply is illustrated in blue and air extraction in red. Top view (left) of lock and production compartment and side view (right) of production compartment.*

Each cell has a nutrient solution reservoir of 1000L. Irrigation is controlled per layer either by volume or by time.

2.2 Growth conditions

2.2.1 Plant material and cultivation design

The trial started in week 34 on Tuesday 25th August 2022 and concluded in week 3 on Tuesday 17th January 2023. Seedlings from cuttings of Everbearer strawberries (breeder Flevo Berry) *var. Favori* were rooted in a greenhouse from week 28 to week 34. Young plants with first flowering stem were transplanted in 1 m long potting trays filled with 100% coco (Dutch Plantin) at a plant density of 8 plants m^{-1}. On each Layer, 2 gutters of 6 m each where placed at a distance of 80 cm from each other (smallest distance in greenhouse cultivation – reference to KAS2030 compartment, BU Greenhouse Horticulture and Flower Bulbs, Bleiswijk, The Netherlands) and of 110 cm from the lamps. Calculated plant density is 10 plants m^{-2} (8 plants * 1 m length * 0,8 m width). A total of 6 trays per gutter were placed which makes 48 plants per gutter. On both sides of the gutters, a truss support and a leaf support were installed as standard practice in greenhouse cultivation. During the experiment, only the external truss lines where used as trusses where trained towards the outside of the layer in order to monitor and reach easily the strawberries during their development and harvest.
Part of the plants from the same batch were used in a separate greenhouse trial (Paragraph 3.10).

2.2.2 Watering system and strategy

Irrigation system was modified on each layer in order to provide water by drip irrigation. Nutrient solution was applied with 4 drippers per box. Irrigation was managed in order to achieve a drain of 30%. The supply volumes (S) were recorded daily from one dripper per layer into a 500 ml beaker. The drain water (D) was collected through a gutter system and collected into a 25 L bucket per layer. The volume of drain water was recorded daily and the bucket was then empty into a 60 L container. The drain fraction was calculated as D/S and it was calculated per layer. At the end of each week, a sample of the cumulative drain water collected in the 60 L container was sent to the laboratory Eurofins to determine nutrients concentration (C_D). The container was then empty in the sewage. The same day also a sample of supply water was collected (one per cell) and sent to the laboratory Eurofins to determine nutrients concentration (C_S). For the first 2 weeks of cultivation a nutrient solution for vegetative growth was supplied (Annex 1), after this a nutrient solution for generative growth was applied (Annex 1) and the composition was managed with the rule of thumb: EC irrigation + EC drain = 3. From these standard recipes, specific adjustments have been carried out throughout the cultivation according to the figures in the drain water and following the guidelines of the Bemestingadviesbasis (Kreij *et al.*, 1999).

The nutrients uptake concentration (U_c) was calculated per week per layer as:

- $U_c = U/U_w$
- U = input – (drain + buffer)
- U_w = water uptake = S – D
- input = S * C_s
- drain = D * C_d

2.2.3 Pest and disease control

After rooting in the greenhouse compartment, plant were found affected by leaf wasps, aphids and powdery mildew. A plant protection product based on fatty acids and potassium salts (Flipper) was sprayed twice to reduce the aphid infestation. The sawflies were controlled with one correction with *Bacillus thuringiensis* subsp. aizawai (Xentari). After that, plants were scouted weekly and biological control was applied throughout the entire cultivation. Aphidophagous hoeverfly *Eupeodes corollae*, predatory gall midge *Aphidoletes aphidimyza* and brown lacewing *Micromus variegatus* were introduced to prevent aphid outbreak. Predatory mite *Transeius montdorensis* was introduced preventively to control thrips and whiteflies. In order to contain the powdery mildew infection, first a mechanical action was employed: affected fruits were removed twice per week. This action was not sufficient and when too many fruits had to be removed the choice was made about spraying the crop with Karma or Abir (2 times at the beginning of cultivation) and Sonata or Taegro as preventive action (once per week from week 9 of cultivation). For detailed schedule see Annex 2.

2.2.4 Pollination

To facilitate crop pollination, the hoverfly specie *Eristalis tenax* (Polyfly) was employed. First time adult hoverflies were introduced to each layer of cells four days after transplanting the plants. The introductions were repeated weekly in the first 4 weeks of the experiment (week 36 to 39). One more introduction took place in week 45. To sustain the hoverflies, feeders containing pollen and nectar (Biogluc, Biobest) were positioned at the center of each table. Additionally, black spot bulbs (BUV27 UV lamps, Tronios BV, Almelo, The Netherlands) were positioned in the middle of each layer, just below the climate measurement box. The UV light plays a vital role for many foraging insects, aiding them in locating flowers and maintaining their orientation during flight.

2.2.5 Climate treatment

Based on a literature study and greenhouse experiences (trials in greenhouse compartment 6.04, season 2021-2022, At Business Unite Greenhouse Horticulture and Flower Bulbs. Personal communication.) two different climate treatments were designed (one per cell):

- “Stable” climate (MLC3) has a constant climate during the whole cultivation The climate recipe was: temperature 20-14 °C (day-night), relative humidity 80-85% (day-night), vapour-pressure deficit (vpd) 4.68-2.40 mbar (day-night), CO_2 concentration 700 ppm, photoperiod 16 h, and average of Daily light integral 11.5 mol m^{-2}.
- “Fluctuating” climate (MLC4) has fluctuating climate conditions in terms of temperature, light, and relative humidity with same vpd. Therefore, 4 typical days (Table 2, Figure 3) were designed based at realized climate fluctuations of greenhouse trials; a typical day was repeated for 1 week creating typical weeks. The 4 weeks were randomized to make sure that at the end of the trial the two cells were comparable in terms of total light and temperature sum.

Table 1 *Climate recipe of the 4 typical days (a, b, c, and d).*

Climate	Description	Average DLI (mol/m^2)	Average DLI (mol/m^2)	Average T (°C)	Average T (°C)
			bright+dull		bright+dull
a	bright days	17.3	11.5	19.00	17.97
b	dull days	5.8		16.93	
c	variation	11.5		18.04	
d	big fluctuations	11.5		18.00	

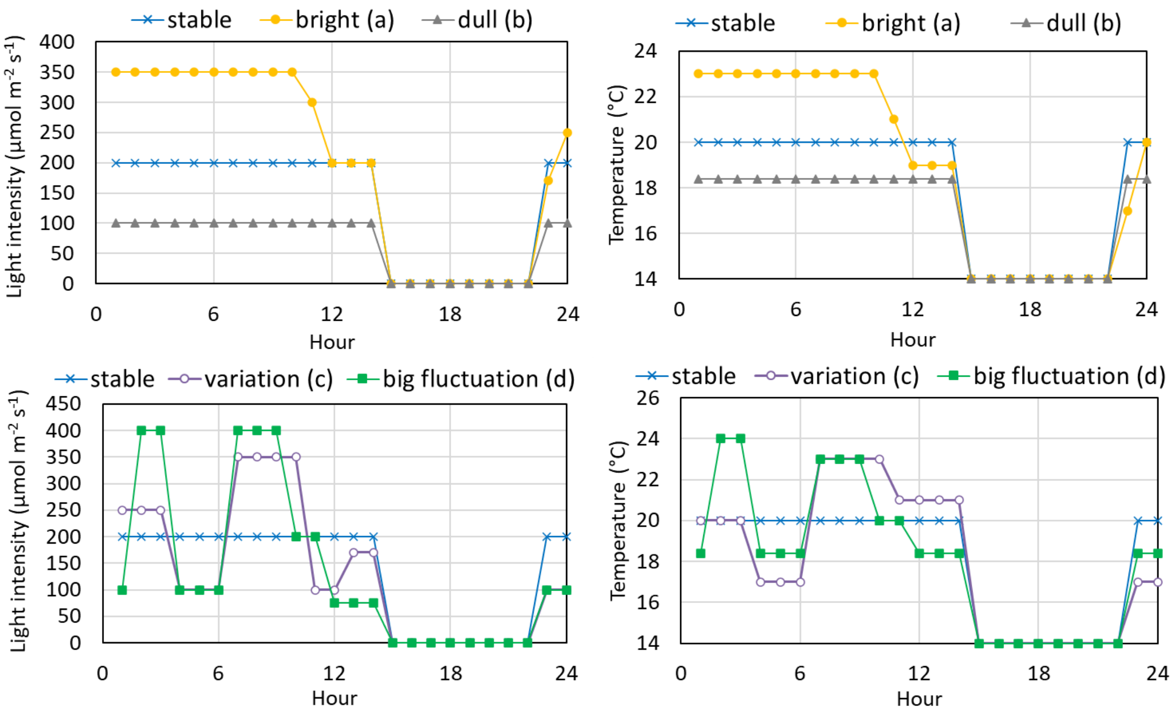

Figure 3 *Patterns of light intensity and temperature during the day for the 4 typical climates and the stable climate.*

The trial started in both cells with 1 week of stable climate in order to adapt the plants to the new facility. After that, a mix of the 4 typical weeks (repetition of a typical climate for 7 days) was applied in MLC4 as "Fluctuating" climate as shown in Annex 3.

2.2.6 Light treatment

Plants were grown under two different light treatments applied in each cell (one per layer). In both cells, the bottom layer was used for reference light treatment and the top layer was used for high blue light treatment (Figure 4). According to the literature (Meinen et al, 2018) and greenhouse experience (trials in greenhouse 6.04, season 2021-2022), reference light treatment was designed as broad spectrum with continuous far-red in order to promote truss elongation; therefore, the reference composition was: 15% blue, 25% green, 60% red, and 20% far-red. High blue light treatment was designed to improve nutritional quality aspects of the strawberry (Kadomura-Ishikawa *et al.*, 2013), especially the anthocyanins content; the high blue spectrum composition was: 30% blue, 10% green, 60% red, and 20% far-red. The extra blue in this treatment was at the expense of green, so the red and far red amounts were comparable in both light treatments. Reference Par photo flux density (PPFD) was 200 µmol m^{-2} s^{-1}. The trial started in both cells with 2 weeks of reference light treatment in order to ensure a good crop (vegetative growth), than it was applied the high blue treatment on the top layer.

Figure 4 *Bottom layer with reference treatment (left) and top layer with high blue treatment (right).*

2.3 Set-up

For 21 weeks, plants were cultivated under stable climate conditions (Figure 5 left) or fluctuating (light and temperature). This was possible by growing the plants in two different cells. In each cell plants were exposed to a “reference” light spectrum treatment (on the bottom layer) or to a “high blue” light spectrum treatment (top layer). This generated 4 different treatment combinations:

Climate treatment	Light treatment
Stable	Reference
	High Blue
Fluctuation	Reference
	High Blue

On each gutter, 12 plants located centrally at the gutter were used as experimental plants.

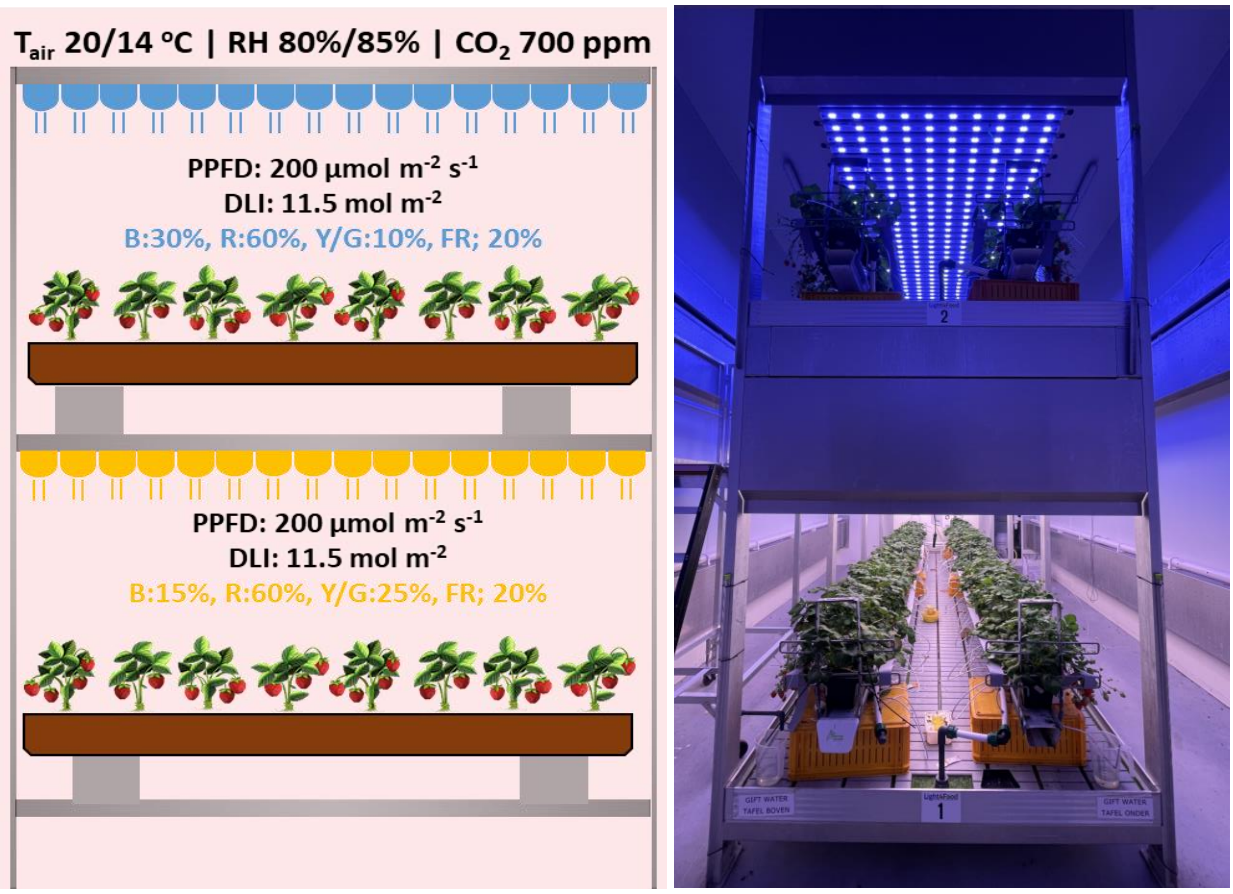

Figure 5 *Schematic overview (left) and picture (right) of the experimental set-up of the Stable treatment.*

2.4 Measurements

2.4.1 Plant growth and development

Once every two weeks, morphological measurements were performed on 3 of the 12 experimental plants per gutter for both cells (n=6). In order to monitor crop development during the cultivation, plant height and width and trusses length, number of leaves, leaflet length and petiole length of the youngest fully developed leaf, were recorded at week of treatment (WOT) 5, 7, 9, 11,13, 15, 19, 20 (Figure 6). With leaflet length, the area of the young leaf was calculated as: ((leaflet length / 2)^2)*3.

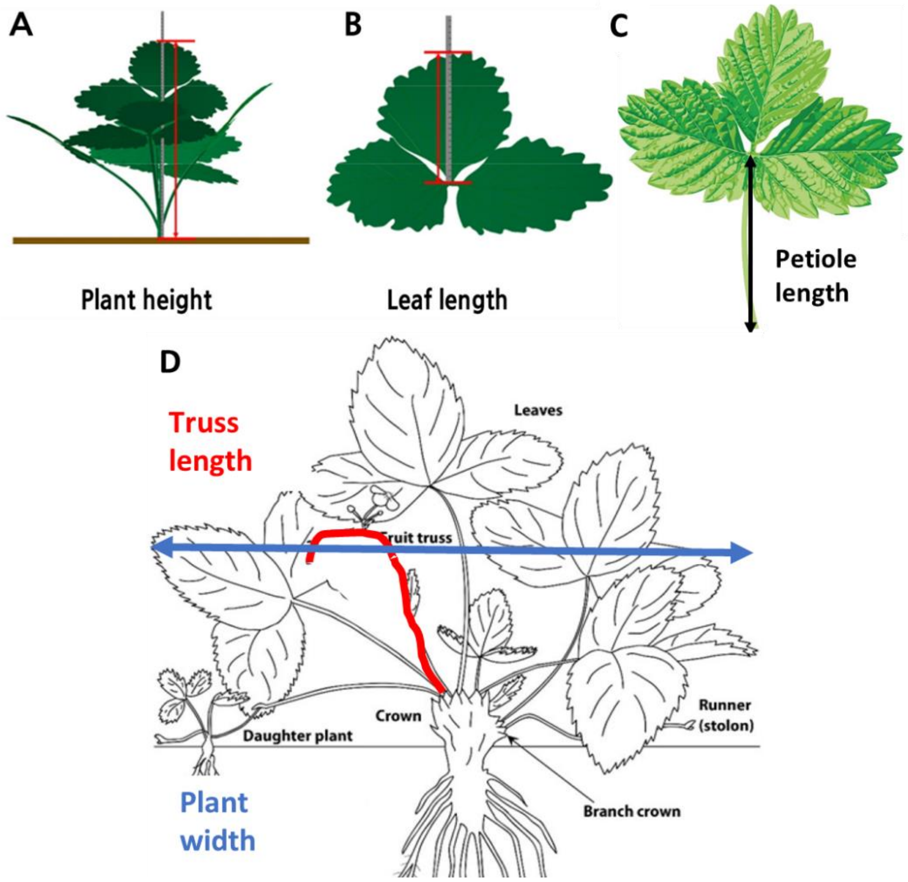

Figure 6 *Plant development measurements.*

Twenty-one weeks after transplanting in the vertical farm, a final destructive harvest was performed on the experimental plant of each layer (n=12). Plant height and width, number of leaves, total leaf aera, number of trusses, number of branch crowns, number of flowers, number of fruits, fresh and dry weight of aboveground organs were measured. Total light interception measurements were carried out in order to analyze differences in crop architecture between different treatments. Measurements were performed at 3 timepoints during the whole cultivation cycle (cultivation week 7, 11, 15). Measurements were taken on 4 spots per gutter within the measurement area using a spectral line meter (LIDIS LED light Interception and Distribution Spectrometer, Jeti Gmbh, Jena, Germany). 2 measurements above and below the canopy respectively were taken; the light stick was positioned in the length of the plant row (Figure 7). The following equation has been used to describe the total light interception: (1-(PAR*calibration factor)/Total*correction factor))*100.

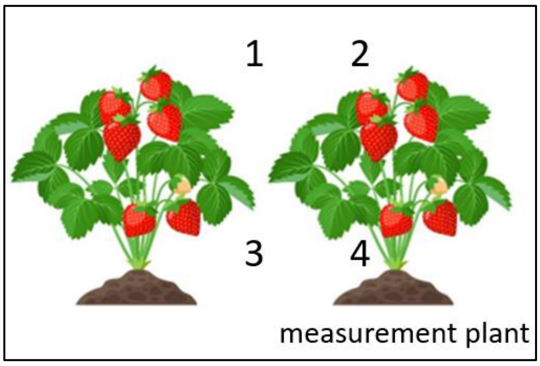

Figure 7 *Measurement points of light interception: point 1 on the top between two plants; point 2 on top close to the experimental plant; point 3 on the bottom between two plants; point 4 on the bottom close to the experimental plant.*

2.4.2 Fruit yield and quality

Twice a week strawberries at full red stage were harvested from the measurement plants and pulled together (n=1) per layer for both cells in order to monitoring the total production (kg m^{-1}). Therefore, number of fruits per (diameter) class and total fruit fresh weight per (diameter) class were measured collectively on the 12 experimental plants. The classes were, in accordance to the UNECE standard, 2021: class 1 (Ø > 30 mm), class 1 (30 < Ø < 27 mm), class 1 (Ø < 27 mm); class 2 (slightly deformed fruits, fruits with some spots of damaged tissue, fruits with weird looking seeds); fruits with powdery mildew; waste fruits (fruits that could not be market). In order to determine the percentage of dry matter, the total dry weight of class 1 fruits was also measured once per week. On non-measurement plants, the fruits were harvested, and total yield (kg) were noted.

Detailed quality measurements were carried out per treatment for a total of 4 timepoints during the whole cultivation (cultivation weeks 7, 9, 12, 15). For the quality assessment, 40 strawberries were weighted and the average fruit weight has been determined. To determine the bite and juiciness, 12 samples of 1 cm^3 were punched out of the fruit flesh of 12 different strawberries. The samples were pressed down using a texture analyser (Instron Universal Testing Machine). The breaking force was determined as an indicator of the perceived firmness during chewing and the percentage of the juice pressed from the fruit wall of the strawberries is determined. The remaining strawberries were de-crowned and mixed together using a blender. The total soluble solids of these mix-samples were determined using a handheld refractometer (Refracto 30PX, Mettler Toledo). The titratable acid and vitamin C level were determined using titration-methods with a Mettler Toledo T50 titrator set. Based on the flavour related instrumental data, the flavour score was calculated using the Strawberry Flavour Model version 1.0 (2017) (Labrie and Verkerke, 2017). For the anthocyanins quantification, 0.2 g of fruit material was extracted with 1.2 mL of 80% methanol with 1% formic acid. The extracts were analyzed using LC-PDA-MS and anthocyanins were identified using mass spectra and quantified using PDA at 515 nm wavelength. This resulted in semi-quantitative analysis which provided the absorption value of pelargonidin glucoside and pelargonidin acetylglucoside (peak area, AU).

2.4.3 Pollinators behavior

On a weekly basis, the behavior of the hoverflies was monitored. The measurements consisted of one-minute observations during which the quantities of insects engaged in flight, at rest (either within the crop or cell structure), and foraging on flowers or feeders was recorded. During three-minute observations, the average foraging duration and the number of visits to flowers was calculated. These observations were repeated a total of 15 times. In the fifth week of data collection, observations were divided into two distinct segments: one with the UV light activated and another with the UV light turned off. This division allowed to investigate the extent to which hoverflies are sensitive to UV light.

2.5 Statistical analysis

Data were subjected to analysis of variance using the Genstat data analysis software (22^{nd} Edition, VSN International, England UK) using the measurement plants as repetition, where possible. To determine the main effect of all parameters, a two-way analysis of variance (ANOVA) was performed. In addition, a post-hoc protected Tukey test was performed with a one-way ANOVA to determine whether the means differed significantly between treatments ($p < 0.05$).

3 Results

3.1 Growth conditions

The average realized conditions after 145 days of cultivation under the two climate treatment are shown in Table 2. Larger standard deviation during lights on in Fluctuating treatment reflects the temperature and humidity fluctuations of the typical days while same deviation is found during lights off as it was kept equal in both treatments.
On average the temperature sum expressed in degree days (using a base temperature of 10 °C) and light sum (Figure 8) were comparable between the 2 climate treatments and the 2 treatments can be compared (average of 1678 mol/m^2 and 1181 d°C).

Table 2 *Realized climate conditions of the 2 climate treatments during the experiment with standard deviation.*

	Lights on				Lights off			
Climate treatment	**Average Temperature (°C)**	**Average RH (%)**	**Average Vapor Pressure (kPa)**	**Average CO_2 (ppm)**	**Average Temperature (°C)**	**Average RH (%)**	**Average Vapor Pressure (kPa)**	**Average CO_2 (ppm)**
Stable	19,9 ± 0,5	76,2 ± 2,7	1,8 ± 0,1	743 ± 51	14,1 ± 0,6	79,4 ± 2,4	1,3 ± 0,1	801 ± 71
Fluctuating	20,0 ± 2,2	76,1 ± 4,2	1,8 ± 0,3	748 ± 69	14,1 ± 0,5	79,6 ± 2,5	1,3 ± 0,1	799 ± 72

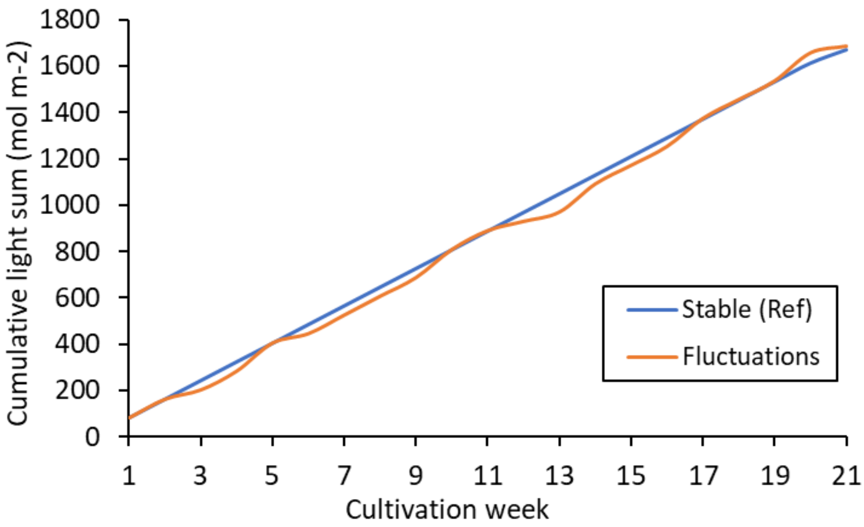

Figure 8 *Cumulative light sum under Stable and Fluctuating conditions.*

The supply EC was higher at the start of the cultivation to support the vegetative growth and lower from cultivation week 6. The two cells were irrigated with similar recipe and therefore also the EC of the supply solution was similar. The supply pH was mostly around 5.5. The trend of the drain EC was similar between treatments and it was lower (average 2.0 dS/m) at the start and higher later (average 3.0 dS/m) with a peak in December (cultivation week 15 to 18). Trends were in general similar among treatments but in week 14 an increase in drain EC was noticed under Fluctuating climate, during this week, plants received a higher temperature and light sum due to the “bright week”. The drain pH was quite stable around 6.0 throughout the cultivation (Figure 9). The total water use and drain rate was similar between layers with a tendency for higher amount of water under Reference treatment and under Fluctuating climate.

The drain rate was on average slightly lower than aimed (around 20%). A summary table (Table 3) is shown below. Based on the drain analysis all nutrients were found back in the drain at acceptable range. A summary table of the average concentration per each element per each treatment is provided in Annex 1.

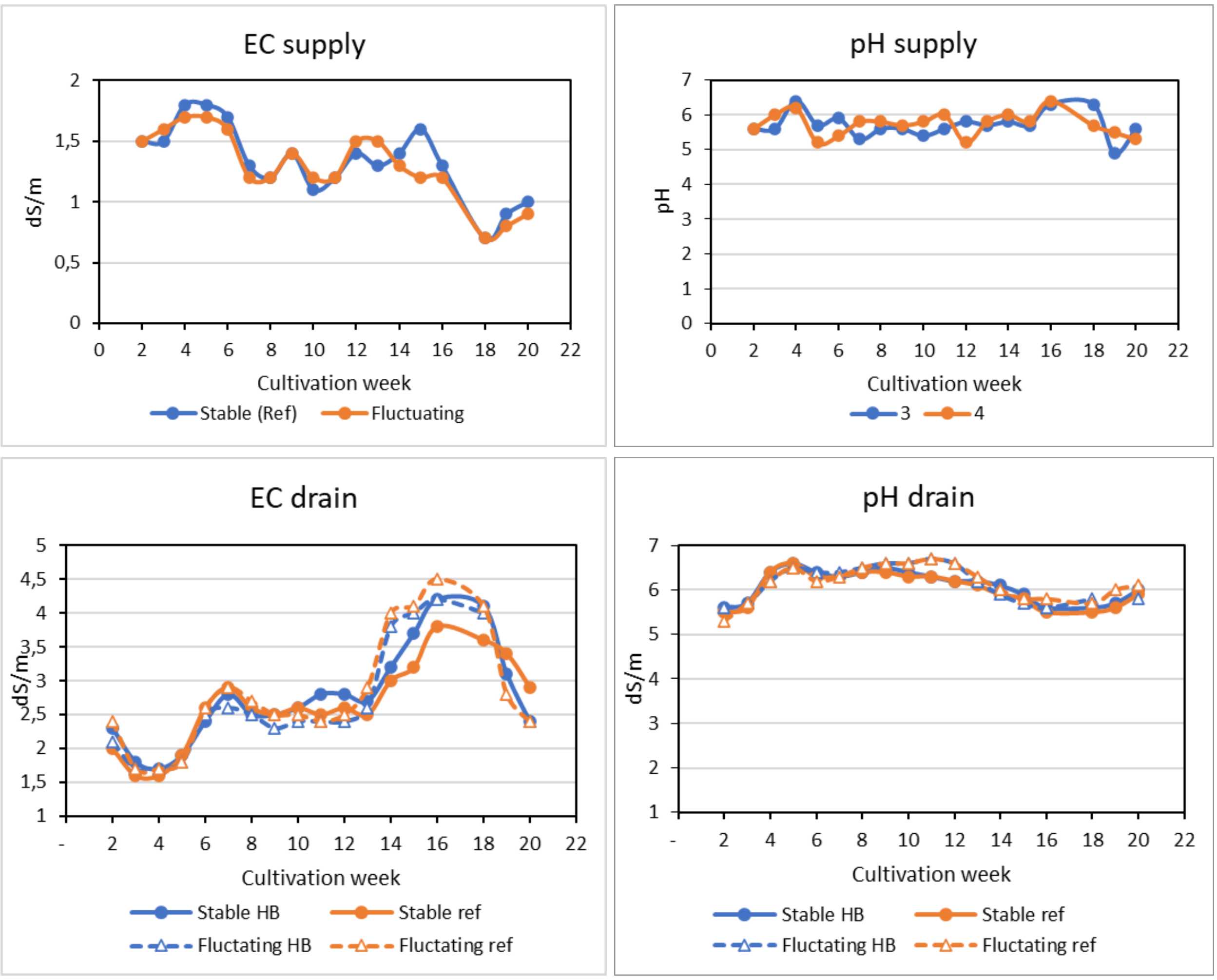

Figure 9 *EC and pH composition of supplied and drain water during the experiment.*

Table 3 *Realized water use and average drain percentage during the whole cultivation.*

Climate treatment	Light treatment	Total water use (L)	Average drain (%)	Average drain EC	Average drain Ph
Stable	High Blue	2194	23	2.8	6.1
	Reference	2226	23	2.7	6.0
Fluctuating	High Blue	2253	21	2.7	6.2
	Reference	2307	20	2.8	6.2

3.2 Cultivation description

After 6 weeks of rooting in greenhouse compartment 9.06 (BU Greenhouse Horticulture and Flower Bulbs, Bleiswijk), young everbearing strawberry plants (*cv. Favori*) where selected and transplanted in the two multi-layer cells. All plants had 1 crown with on average 6 leaves and 1 flowering truss with a length on 16 cm (Figure 10).

Figure 10 *Plants in the multi-layer cell at transplant (left; before LED lamps were switched on)) and after 4 days (DAT 4) (right).*

After 4 days of cultivation, the first introduction of hoverflies with one feeder per layer took place (Figure 11).

Figure 11 *Hoverflies on the feeder (left) and foraging a flower (right) on DAT 5.*

Under indoor conditions, trusses where stretched and some of the developing strawberries where found malformed. This was due to pollination of the first flower truss during rooting in the greenhouse. Overall, it takes on average 5 to 6 weeks from the moment the flower stem is visible in the crop (under development) to fruit harvest of which on average 4 weeks from open flower to fruit harvest. This means that at least the fruits harvested during the first 6 weeks have been developed during rooting in the greenhouse.

On DAT 19, pests and diseases infection were found on the plants in both cells (Figure 12). They came with the young rooted material. In order to reduce to the minimum the necessity to spray which would, among others, negatively affect pollinators, mechanical pest control (pinching the caterpillars and harvesting fruits with mildew even if not fully ripe) and biological control was applied. This was not sufficient to bring the infestation under control so use of green agents was started as well (paragraph 2.2.3).

Figure 12 *Caterpillar of leaf wasp (left) mildew on fruit (central) aphids on flower truss (right) on DAT 19.*

During the third week of cultivation (Figure 13), the crop under Fluctuating conditions looked more elongated towards the lamps while crop under Stable conditions looked more wide. A size different in crop under Reference and High Blue treatment started appearing. The first harvest took place on DAT 20 and on few new young leaves showed tip burn. This is typical of this strawberry variety.

Figure 13 *Crop under Stable climate and Reference light (top) and Fluctuating climate and Reference light (bottom) during the 3rd week of cultivation.*

Between week 5 and week 7, the first small production peak was harvested. These fruits were already present in the trusses in the young plant and were developed under 4 different temperature and light regimes. At the same time, plant canopy was fully grown, looking a bit vegetative (high leaf biomass). Old yellowing leaves are removed from the plants under all treatments in week 10. Plants were producing many trusses with flowers thus a second large production flush was expected around cultivation week 12 (Figure 14).

Figure 14 *Crop under Stable climate and Reference light (left) and Stable climate and High Bue light (right) during the 10th week of cultivation.*

During the following 5-7 weeks, the large peak of production was harvested. These fruits were initiated and developed under the 4 different climate and light treatments. Plants under both cells and both treatment looked exhausted with very few flowers and strawberries and with old leaf material especially under Stable climate (Figure 15).

Figure 15 *Crop under Stable climate and Reference light (top) and Fluctuating climate and Reference light (bottom) during the 19th week of cultivation.*

After 21 weeks of cultivation in the multi-layer cells, plants were destructively measured. To capture the difference between treatments in older material and in new fresh material, organs at the two different developmental stages where harvested separately (Figure 16).

Figure 16 *Example of plant from Fluctuating climate and Reference light at destructive harvest. Picture on the left shows plant directly out of the cell with both old and new organs. Picture on the right shows same plant after "old material" (yellowing or burnt leaves; harvested flowering stems) are harvested and measured leaving the "new material".*

3.3 Plant morphology

Morphological analysis showed that light treatment had a significant effect on the process of elongation of plants, thus on their architecture (Table 4). Under Stable climate conditions, High Blue treatment created significantly more compact plants compared to Reference treatment. Petiole length was 19% shorter ($p=0.003$), crop height was 21% lower ($p<0.001$) and crop width 18% lower ($p<0.001$), young fully developed leaf was on average 17% less expanded ($p=0.011$) and trusses were 13% less elongated ($p=0.041$). Under fluctuating climate, similar tendency was found but difference between the two light treatments was not significant. An interaction between climate and light treatment was indeed found for crop height ($p<0.001$), crop width ($p<0.001$) and petiole length ($p=0.007$).

Table 4 *Plants morphology results reported as mean values of measurements taken on WOT 5, 7, 9, 11,13, 15, 19, 20 ± SEM and significance letters with Tukey test (n=6).*

Climate treatment	Light treatment	#leaves	Petiole length (cm)	Calculated leaf area (cm^2)	Crop height (cm)	Crop width (cm)	Truss length (cm)
Stable	Reference	20,9±2,1 a	14,4±0,4 a	42,6±1,2 a	25,4±0,3 a	56,6±1,2 a	24,3±0,8 a
	High Blue	18,0±1,3 a	11,7±0,4 b	35,2±1,9 c	20,2±0,6 c	46,9±1,3 c	21,1±1,1 a
Fluctuation	Reference	16,3±1,2 a	12,9±0,4 ab	40,6±1,4 ab	22,8±0,5 b	51,3±1,0 bc	23,5±0,9 a
	High Blue	18,1±1,5 a	12,7±0,3 ab	38,1±1,2 ab	22,1±0,8 bc	52,0±1,2 ab	20,9±0,8 a

In Figure 17, the time trends of crop height and truss length are showed. For both plant characteristics, a depression is shown between week 13 and 17 which correspond to the production peak (Figure 16).

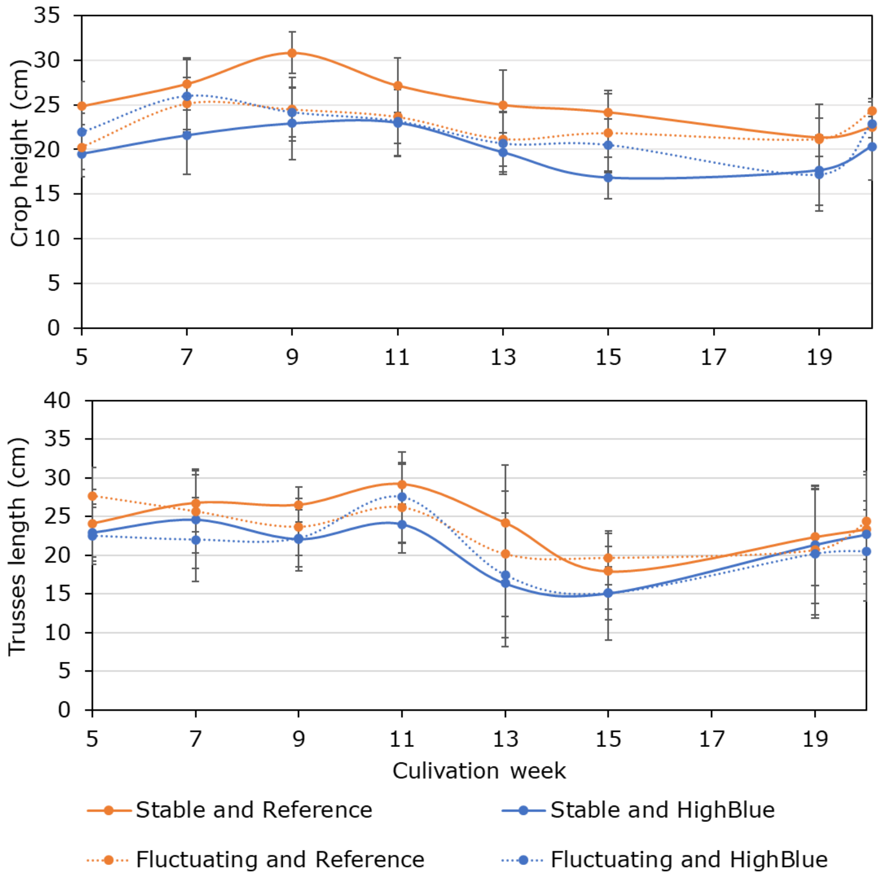

Figure 17 *Plant height and truss length (cm)of plants grown under the different light and climate treatments from cultivation week 5 to 20. Mean values are reported with standard deviation (n=6).*

Sufficient elongation of petioles is important for a good light interception. No significant differences were found between the different climate and light treatments in terms of total light interception (Figure 18). A trend was observed for High Blue treatment under Stable climate where light interception was 9% lower compared to Reference light treatment in accordance with the more compact architecture and shorter petioles (Table 3; p=0.055). Similarly to the plant development results, same average light interception was found under Fluctuating climate.

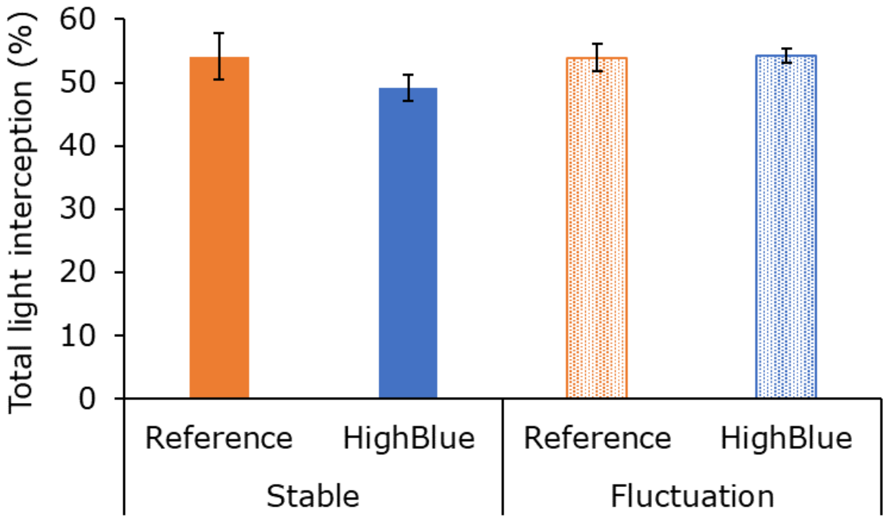

Figure 18 *Total LED light interception expressed as average of measurements taken in cultivation week 7, 11, and 15 respectively. Mean values and standard deviation are reported (n=8).*

3.4 Plant growth

At the end of the experiment, all the produced biomass was calculated and expressed in average grams dry weight per plant (Table 5): fruit harvests during cultivation (n=1) and destructive harvest measurement (n=12). Under Fluctuating climate a total higher biomass was produced compared to Stable climate. This difference was due only to the difference found in vegetative organs (+35% under Fluctuating climate) as no differences are shown in biomass in fruits. Climate treatment seems to have an effect on the biomass partitioning towards vegetative and generative organs. Under Fluctuating climate, more biomass was partitioned towards the vegetative organs. In week 11 of cultivation, after the first production flush, old leaf material was removed once for all treatments. This biomass was not measured. From the crop observations, no difference was described in presence and/or quantity of old material under the different treatments but the possibility of a difference created by this action cannot be ignored. Light treatment had an effect on biomass production. Reference light treatment produced on average 12% higher biomass both in vegetative and in generative organs. This is in line with the higher light interception results.

Table 5 *Dry biomass produced during the entire experiment (except pruned old material during week 10) and its partitioning towards vegetative and generative (fruits) organs.*

Climate treatment	Light treatment	Total biomass (g/plant)	Vegetative biomass (g/plant)	Fruit biomass (g/plant)	Biomass partitioning to vegetative organs	Biomass partitioning to fruits
Stable	Reference	91,0	28,3	62,6	31%	69%
	High Blue	76,0	21,1	54,9	28%	72%
Fluctuation	Reference	96,6	34,2	62,4	35%	65%
	High Blue	86,9	32,4	54,5	37%	63%

After 21 weeks of cultivation, the trial ended with a destructive harvest of 12 plants per layer. All aboveground organs where measured separately for new fresh organs and old organs (see Figure 15). Total aboveground biomass measured per plant at final destructive harvest was 37% higher in plants grown under Fluctuating climate compared to Stable climate (p=0.018) (Figure 19).

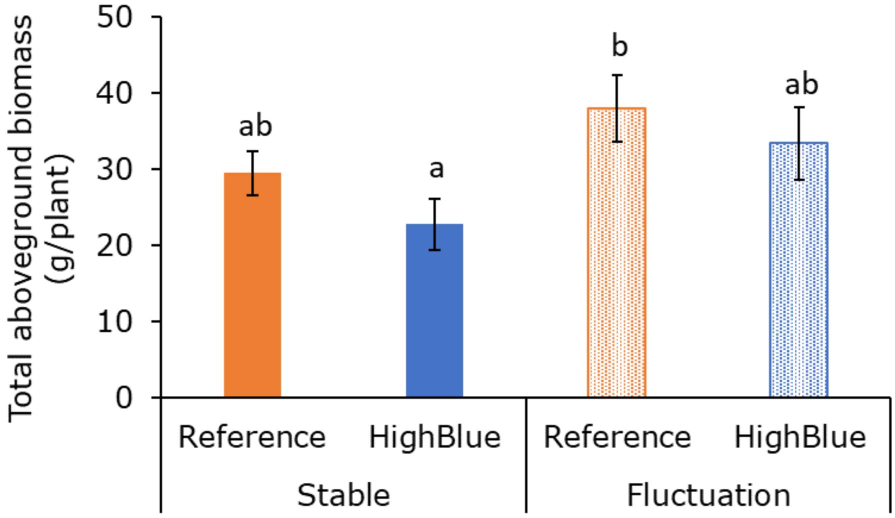

Figure 19 *Total aboveground biomass at the final destructive harvest. Graph shows the sum of leaves, trusses, branch crowns, and young-unripe fruits expressed as grams of dry weight per plant; (n=12 with SEM).*

Looking at the total number of leaves and leaf area (new + old) and at the size of the crop (height and width), the average plant morphology per treatment was described at final harvest. No significant differences were found for crop size and total number of leaves although a tendency was found for more compact plants under High Blue treatment compared to Reference treatment under both climates. On the other hand, climate treatment had a significant effect on total leaf area and number of producing trusses. Plants grown under Fluctuating climate had 32% higher total leaf area at the end of the experiment (p=0.021) and produced 30% more flower trusses (p= 0.025) (Table 6).
The differences were mainly significant due to the new vegetative organs (Table 7) rather than the old organs (Table 8). New leaves were 44% more (p =0.013) under Fluctuating climate compared to Stable climate and had 60% higher biomass (p=0.004). New leaf area was positively affected both by Fluctuating climate (+46%; p=0.006) and Reference light treatment (+36%; p=0.022).

In everbearing strawberries there is an antagonistic relationship between vegetative and generative growth. Conditions under which flowering is stimulated generally have a negative effect on leaf area, elongation of leaf and flower stems and the formation of runners (Heide *et al.*, 2013). In turns, the amount of leaves, which translates into assimilate availability, seems to play a role in inducing flowering (Elings *et al.*, 2023). In the experiment, a positive linear correlation was found between the total number of leaves and the total number of producing trusses (R^2 = 0.87).
With everbearing varieties, the size of flowering stems depend on day length, temperature and plant load (Elings *et al.*, 2023). Under Stable climate, truss size (expressed as #fruits/truss) (Table 6) was higher and total leaf area and leaf count was lower compared to Fluctuating climate showing a large disbalance between vegetative and generative growth. This also support that, at the end of the experiment, plants looked more exhausted under Stable climate compared to Fluctuating climate (see Figure 14).

Table 6 *Plant morphology at final destructive harvest. Mean value are reported per plant with SEM (n=12).*

Climate treatment	Light treatment	Total nr. leaves	Total nr. producing trusses	Nr. fruits/truss	Total leaf area (cm^2)	Crop height (cm)	Crop width (cm)	Nr. branch crowns
Stable	Reference	33.6±3.3	11,3±1,2	9,1	1795±148 ab	23.6±1.3	41.6±2.8	6.8±0.7
	High Blue	27.5±3.6	9,8±1,0	10,3	1364±188 a	21.2±1.1	39.8±1.9	6.2±0.7
Fluctuation	Reference	39.9±4.1	14,8±1,5	7,0	2205±233 b	24.3±1.4	47.5±2.8	7.4±0.6
	High Blue	37.8±5.4	12,6±1,6	7,5	1960±257 ab	23.8±1.1	41.8±1.8	7.6±0.8

Table 7 *Results new organs final destructive harvest. Mean value and SEM are reported (n=12).*

Climate treatment	Light treatment	Nr. New leaves	Nr. new flowering trusses	New leaf area (cm²)	New leaves DW (g)	Branch crowns DW (g)
Stable	Reference	20,5±2,9 ab	3,5± 2,1	898±120 ab	5,4±0,9 ab	6,9±2,8
	High Blue	15,6±2,3 a	3,4± 3,1	603±85 a	4,1±1,0 a	4,7±3,3
Fluctuation	Reference	27,4±3,2 b	5,8± 4,6	1232±138 b	8,6±1,0 b	7,9±3,4
	High Blue	24,7±3,8 ab	4,6± 3,8	959±130 ab	6,6±0,9 ab	7,6±4,0

Table 8 *Results old organs at final destructive harvest. Mean value and SEM are reported (n=12).*

Climate treatment	Light treatment	Nr. old leaves	Old leaf area (cm²)	Nr. Old trusses	Old leaves DW (g)	Old trusses DW (g)	Old crown DW (g)
Stable	Reference	13,1±1,5	897±115	7,8±1,3	6,7±0,9	4,3±0,8	1,9±0,2
	High Blue	11,9±1,6	762±122	6,4±0,9	5,2±0,7	3,2±0,6	1,5±0,2
Fluctuation	Reference	12,5±1,4	973±128	9,1±1,3	7,2±1,1	4,7±0,8	1,8±0.2
	High Blue	13,1±2,0	1001±149	8,0±1,2	7,7±1,4	4,3±0,7	1,6±0,3

3.5 Yield

From week 3 to week 21 after transplanting into the cells, strawberries were harvested twice per week until the final destructive harvest (week 21) where the whole plant was harvested. Highest yield was found under Stable climate and Reference light treatment (11,6 kg/m²; Table 9). Yield was 10% higher for Reference treatment compared to High Blue treatment in Stable climate and 8% higher in Fluctuating climate. The lower harvest of both High Blue treatments had, on the other hand, a positive effect on the proportion of Class 1 strawberries harvested as percentage of total production (69% under Stable climate and 67% under Fluctuating climate). High Blue treatment had as well a positive effect on lowering the amount of strawberries harvested with mildew which was 10% lower under Stable climate and 24% lower under Fluctuating climate.
Stable climate cell produced on average 4% higher yield compared to Fluctuating cell which was on average composed by 7% more Class 1, 5% more Class 2 and less mildew (-9%) and not sellable strawberries (-9%). This was not caused by different fruit weight as the same pattern was found for the number of fruits harvested (Table 10).

Table 9 *Total fresh yield per class.*

Climate treatment	Light treatment	Class 1 (Kg/m²)	Class 2 (Kg/m²)	Mildew (Kg/m²)	Total production (Kg/m²)	Not sellable (Kg/m²)
Stable	Reference	7,7	1,9	2,0	11,6	0,7
	High Blue	7,3	1,4	1,8	10,5	0,5
Fluctuation	Reference	7,1	1,6	2,4	11,1	0,7
	High Blue	6,9	1,6	1,8	10,3	0,6

Table 10 *Total number of harvested fruits per class.*

Climate treatment	Light treatment	Class 1 (#fruits/m²)	Class 2 (#fruits/m²)	Mildew (#fruits/m²)	Total production (#fruits/m²)	Not sellable (#fruits/m²)
Stable	Reference	591	104	190	885	122
	High Blue	590	81	190	861	113
Fluctuation	Reference	542	90	225	857	125
	High Blue	527	80	189	797	126

Harvested fruits were dried during the experiment and dry matter content was calculated. Using weeks as repetition (week 3 to week 21; n= 19) DMC varied between 8.1 and 8.5% and no statistical difference was found for the average fruit DMC during the cultivation (Table 11) A tendency is shown with 4% higher DMC under Reference light treatment compared to High Blue treatment. An average dry matter content of 8.6% was found in the year-round greenhouse cultivation of strawberry in greenhouse (KAS2030, Source: Elings *et al.*, 2023).
With the dry biomass of all harvested fruits during the experiment (class 1, Class 2, mildew and non-sellable) the light use efficiency was calculated. Highest LUE was found for strawberries grown under Stable climate and Reference light (0,62 g/mol) and lower LUE was found for the High Blue treatment, independently from the climate (0.54 g/mol).

Table 11 *Average fruit dry matter content during the experiment and light use efficiency.*

Climate treatment	Light treatment	DMC (%)	LUE (g/mol)
Stable	Reference	8,5	0,62
	High Blue	8,1	0,54
Fluctuation	Reference	8,5	0,59
	High Blue	8,3	0,54

Plants had a clear high production peak during the experiment followed by a very low production (average of 0,2 kg/week/m²). When describing the size of the production peak as number of weeks in which the production was higher than 0,60 kg/m², plants under Reference light treatment and Stable climate had a peak of 7 weeks in which they produced in total 7,97 kg/m² while plants under Reference light treatment and Fluctuating climate had a peak of 9 weeks in which they produced 8,21 kg/m². This difference in flush size started from week 12 (Figure 20).

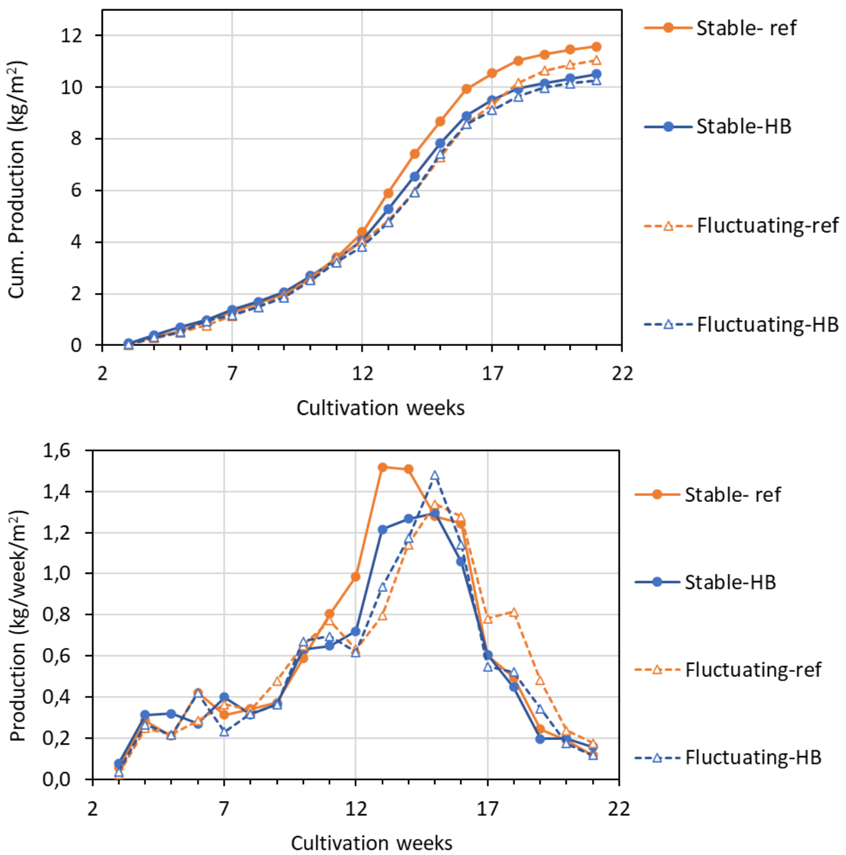

Figure 20 *Total production of strawberry fruits per different climate and light treatments combination and in the separate greenhouse experiment. Graphs show the cumulative production per cultivation week (top) and the weekly production (bottom) of all harvested fruits (class 1, 2 and mildew).*

At the final destructive harvest, number of flowering trusses, of flowers and of developing fruits were counted per plant as an indication of the size of the next production flush. Given the large variation per plant, no statistical difference was found although a tendency is observed with a higher number of trusses (+26%), flowers (+61%) and fruits (+33%) in the Fluctuating climate compared to the Stable climate. A significant interaction between climate and light treatments was found for the amount of fruits per plant (p=0.034) (Table 12).

Table 12 *New generative organs counted per plant at final destructive harvest. Values are averages of experimental plants ± SEM and significance letters with Tukey test (n=12).*

Climate treatment	Light treatment	Nr. trusses	Nr. flowers	Nr. fruits
Stable	Reference	6,7±1,0	10,7±3,1	2,0±0,9
Stable	High Blue	6,3±1,1	6,8±1,7	4,3±2,0
Av. Stable		6,5	8,8	3,2
Fluctuating	Reference	8,2±1,6	14,5±3,8	6,2±1,6
Fluctuating	High Blue	8,1±1,6	13,8±3,2	2,3±0,9
Av. Fluctuating		8,1	14,1	4,2

To try to explain the more "flat" production obtained with Fluctuating-Reference treatment, weekly production was compared to the radiation temperature ratio (RTR) calculated with average 24h temperature and DLI per week (Figure 21). While Stable climate cell had a constant radiation temperature ratio (RTR) of 0.6, Fluctuating climate cell had both higher and lower RTR depending on the week climate. RTR value was either higher or lower compared to Stable climate due to both higher or both lower DLI and temperature with a different ratio. Looking at 6 weeks before the difference started (cultivation week 6), thus when trusses are initiated, a lower RTR under Fluctuating climate was applied. Lower RTR means lower light compared to temperature which was also lower (18,4 °C during day in Fluctuating and 20 °C in Stable climate) which slows down production creating a lower plant load. The plant will probably initiate less fruits which will appear 6 weeks later with a lower weekly production (WOT 12). Because a lower maximum weekly production is achieved, the plant has more assimilates to invest in new trusses and fruits so in week 18 a new small peak is observed (only under Fluctuating-Reference) and at the end of the trial a higher number of trusses are counted at destructive harvest (week 21).

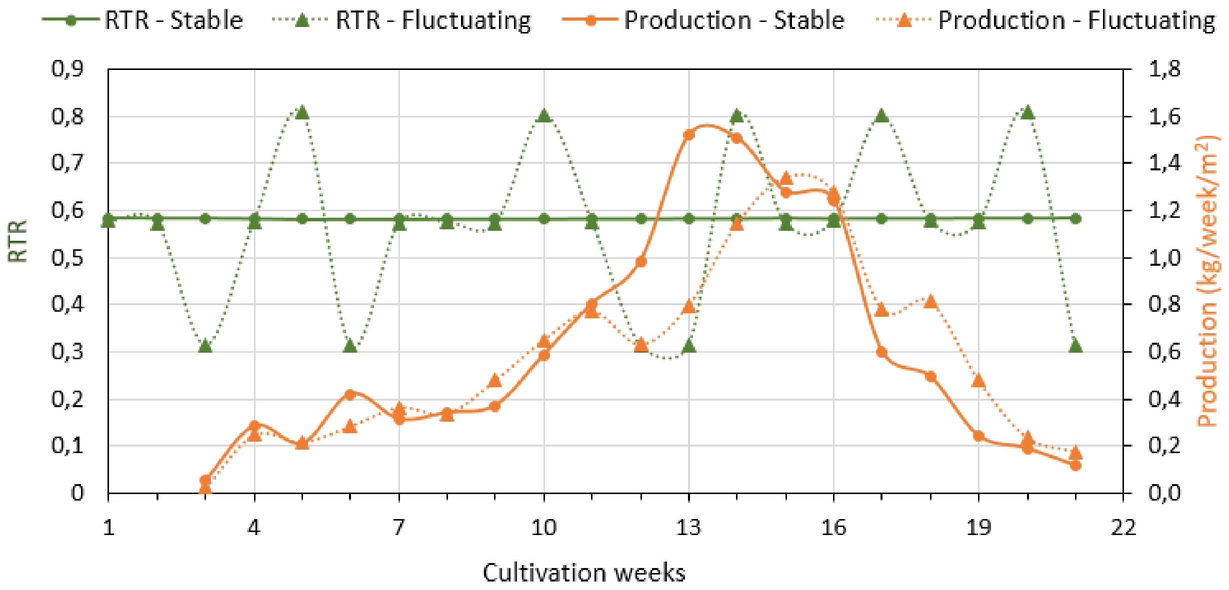

Figure 21 *Weekly RTR value and strawberry production.*

3.6 Quality

Important quality characteristics for strawberries are fruit weight, size, shape, color, taste, firmness and shelf life. During each harvest, fruits were classified depending on their size and presence of defects (Class 1, Class 2, Mildew and Not-sellable) and, within Class 1, also by diameter size (> 30 mm; 27-30 mm; <27 mm). All these quality attributes determine the price category of the final product. Strawberries harvested as Class 1 >30 are those sold for the highest Kg price while mildew-infected and not-sellable strawberries never reach the consumers.
On average, 37% of the yield was represented by Class 1 >30 strawberries, 13% by Class 1 27-30, 12% by Class 1 <27 and 14% by Class 2 (Figure 22) and no big effects were found between treatments. A slightly higher percentage of mildew was found in the Fluctuating climate in the Reference treatment compared to High Blue treatment. Overall, quite a high percentage of mildew fruits was harvested. This was due to the fact that no UV treatment was possible in the cells and spraying with green fungicides was limited in order to avoid any interference with the pollination.

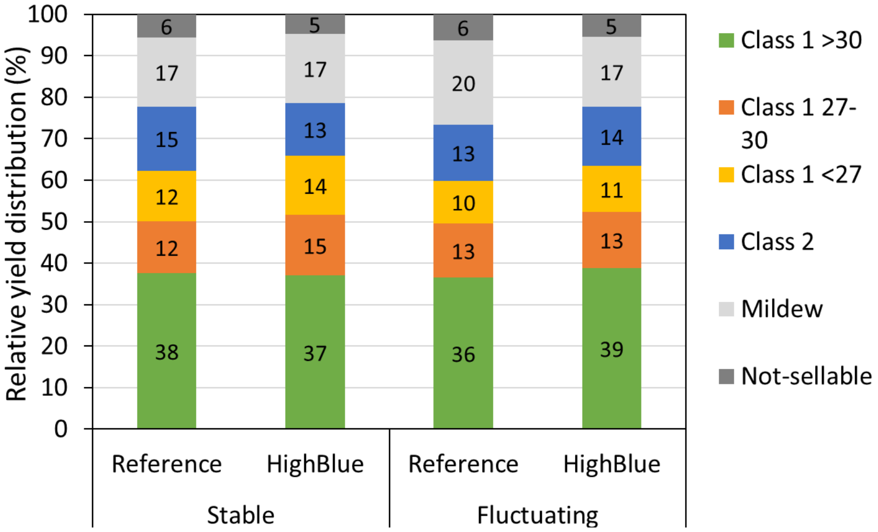

Figure 22 *Relative yield distribution in different appearance and size classes.*

Organoleptic and nutritional quality attributes have been measured as well during the experiment (Table 13). Highest Brix was found under Stable climate and Reference light conditions. In general, under Reference light, strawberries had a higher Brix, acidity and DMC and a lower %juice. A lower Brix value was found under High Blue treatment compared to Reference treatment. This is in line with the results of Avendano-Abarca *et al.* (2023) where treatment with high blue (20%) had a lower content of total soluble solids compared to the treatment with low blue (6%). The liking score was slightly higher under Fluctuating climate (55,5) compared to Stable climate (54) but overall the light treatment played a larger effect on quality attributes compared to climate treatment. Vitamin C content was not affected by the treatments.

Table 13 *Quality results of vertical farming cultivation expressed as average of 4 measurement date (n=4). *DMC is the average of the whole cultivation (n=21; weeks).*

Climate treatment	Light treatment	Taste liking number	Refraction (°Brix)	Acid (mmol H_3O+/100g)	%Juice	Bite (N)	Vit C (mg/100g)	DMC* (%)
Stable	Reference	55	8,6	12,8	56,0	7,3	48	8,5
	High Blue	53	7,8	12,6	57,7	7,6	49	8,1
Fluctuation	Reference	56	8,1	12,7	57,0	7,8	50	8,5
	High Blue	55	7,8	12,3	57,2	7,8	49	8,3

For two strawberry harvest, at DAT 85 and 109, a sample was prepared to measure semi-quantitatively the concentration of anthocyanins (Table 14). More than 25 anthocyanin pigments have been described for strawberries and pelagonidin-3-glucoside is the principal one (Giampieri *et al.*, 2012). In general, a higher quantity (expressed as peak area unit) was found at DAT 109 compared to DAT 85. No positive effect was found under the High Blue treatment as a higher amount was found in general under Reference light treatment.

Table 14 *Anthocyanins peak absorption area of vertical farming cultivation at DAT 85 and DAT 109 (n=1).*

Climate treatment	Light treatment	DAT 85 Pelargonidin glucoside (AU)	DAT 85 Pelargonidin acetylglucoside (AU)	DAT 109 Pelargonidin glucoside (AU)	DAT 109 Pelargonidin acetylglucoside (AU)
Stable	Reference	183839	84884	178133	76093
	High Blue	184535	82049	171760	80754
Fluctuation	Reference	182410	78000	168486	71786
	High Blue	177930	73537	161945	74514

3.7 Nutrient/EC uptake

The water uptake was very stable throughout the cultivation and no difference was observed between treatments (Figure 22). The nutrient uptake concentration calculation did not show specific differences between treatments. The main difference was observed between a first phase (week 1 to 5), which corresponds to the vegetative growth of the plant in combination with truss development, and a second phase (week 6 to 15) which corresponds with generative growth. The calculation for the uptake concentration from week 15 to the end (week 21) was negative and therefore omitted from the graphs. The first phase was characterized by a higher uptake concentration. In this period the calculated EC uptake concentration was about 1.3 dS/m. From week 6 to 15 the nutrients uptake was much lower (0.6 dS/m). Since no large differences have been seen between elements, the EC uptake concentration is showed as summary example of the trend (Figure 23). The K:Ca ratio between the uptake concentration of K and Ca showed eventually a remarkable results. In the cell with Stable climate the ratio decreased quite proportionally from start to end, whereas in the Fluctuating climate it seemed to be opposite. This is probably explained by the different balance between vegetative and generative growth which was found under the two climates.

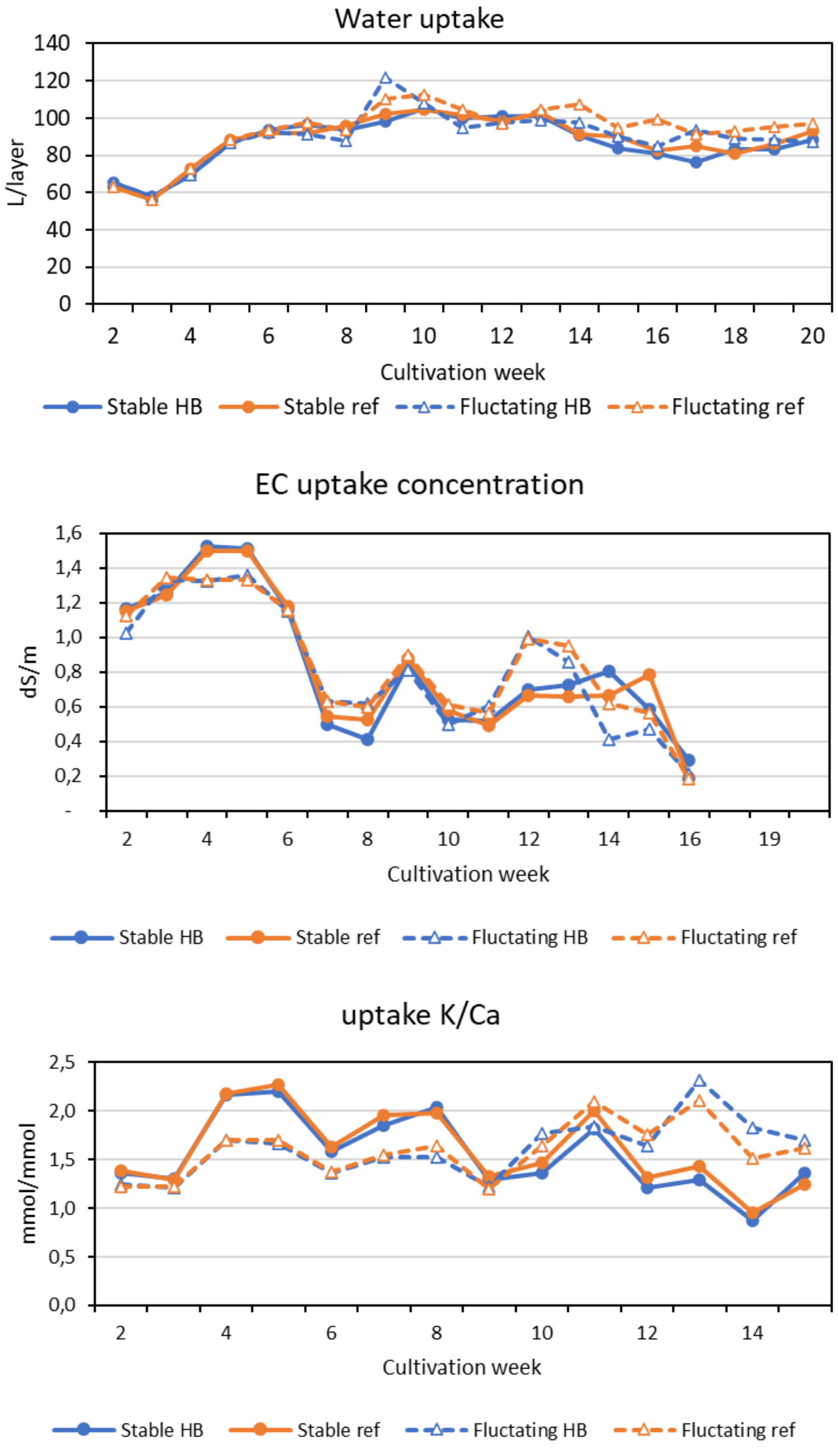

Figure 23 *Water uptake, nutrient uptake (EC) and K/Ca uptake during the experiment.*

3.8 Pollinators behavior

The mobile behavior of hoverflies was dependent on the presence of the UVA lamp. Under both climate conditions and under both light spectra, the hoverflies were affected in their behavior when the black spot lamp emitting UVA light was off (Figure 24). The number of hoverflies that flew in the crop and actively foraged was lower or even not observed during the observations when the UV lamp was off. The number of flowers visits was also depending on the presence of the UV light and was higher when the UV lamp was on but only under the stable climatic conditions (Figure 25). This leads to the conclusion that hoverflies, in the same way as bumblebees and honeybees, orient themselves better when UVA light is present and use the contrasting colors (including UV) for flower recognition (target versus background) (Peitsch *et al.*, 1992; Kevan *et al.*, 2001), however, they can still provide pollination services without UVA light in the spectrum.

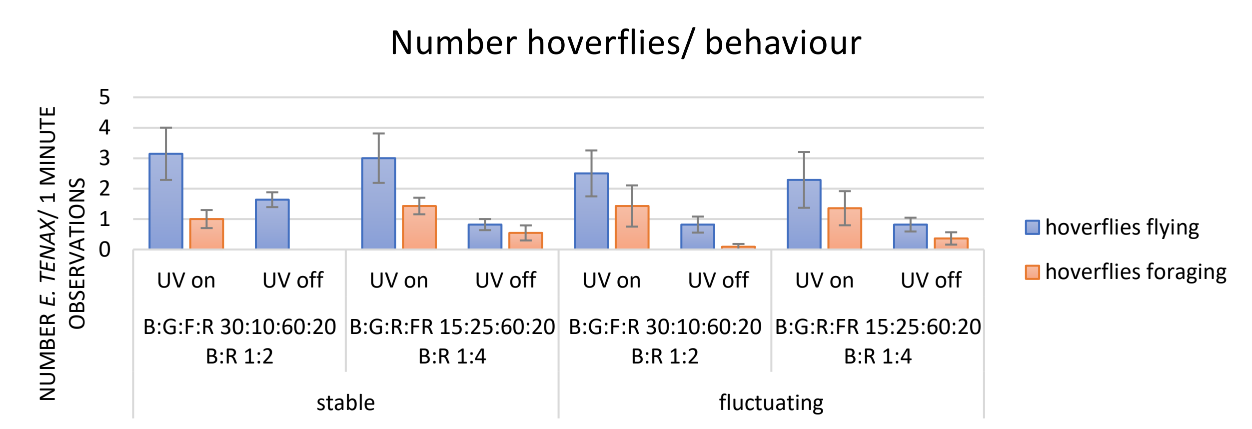

Figure 24 *Number hoverflies active in the crop under stable and fluctuating climatic conditions and two light spectra, with and without additional UV light.*

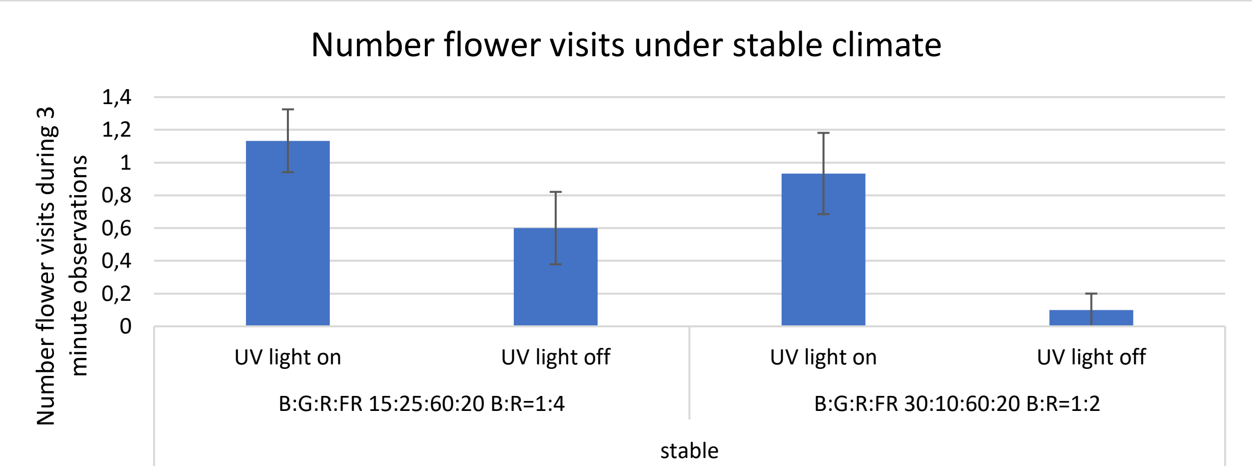

Figure 25 *Number flower visits under stable climatic conditions and two light spectra, with and without additional UV light.*

3.9 Resource use

At the end of the experiment, resource use of the four treatments was calculated for the whole cultivation area (9.6 m^2). Water Use (WU) was calculated as liter of water taken up by the plant per unit fresh yield. Electricity Use from lamps (EU_{lamps}) was calculated as electricity used by lamps per unit fresh yield. WU was on average 10% higher under Reference light treatment compared to High Blue treatment. Energy use from the HVAC system was not measured, Kozai at al 2013 presented as a rule of thumb an additional 25% in energy use for the environmental control. Avendano-Abarca *et al.* (2023) found as well that an increase in green light, associated with the decrease in blue light, caused an increase in water use efficiency due to a decrease in transpiration and stomatic conductance. Moreover, Stable climate conditions had a positive effect on water use efficiency (-5%). EU was lowest under Stable climate and Reference light treatment. Compared to the same spectrum under Fluctuating conditions the energy use of the lamps was 15% lower under Stable conditions. Under Fluctuating climate the higher EU is possibly due to the lower efficacy at higher light intensities.

Table 15 *Water use efficiency and Energy use efficiency.*

Climate treatment	Light treatment	WU (L/Kg)	EU_{lamps} (KWh/Kg)
Stable	Reference	15,8	26,1
	High Blue	17,2	29,2
Fluctuation	Reference	17,5	30,4
	High Blue	18,1	30,0

3.10 Comparison with greenhouse trial

The same propagated plant material that was transplanted in the multi-layer cells was also used for a greenhouse trial in the greenhouse compartment 6.04 at the BU Greenhouse Horticulture and Flower Bulbs, Bleiswijk, The Netherlands. Plants grew in the same potting trays with the same substrate composition and irrigation system (drip irrigation) as in the vertical farm experiment. A total of 8 plant per tray (8 plant m^{-1}) were transplanted but, given the distance between gutters of 1 m, plant density in the greenhouse was 7,3 plant m^{-2} (vertical farm was 10 plants m^{-2}). In the greenhouse, sunlight was supplemented by HPS lights which extended the photoperiod to 16,5 hours from DAT (days after transplanting) 81 to DAT 94 and to 18 hour from that 95 to DAT 165 in order to keep the measured Par in the greenhouse (point measurement on top of the crop) on average around 11,5 mol/m^2/day. Greenhouse trial ended with a destructive harvest on 16th February 2023 (175 DAT).
This greenhouse trial is here used to compare the realized yield and quality of the vertical farming experiment with a greenhouse cultivation experiment with the advantage that same starting material was used.
In order to compare production and quality, an overview of the realized growing conditions in the two experiments is presented in Table 16.

Table 16 *Average realized climate between 26 August 2022 and 17 January 2023 (145 DAT).*

Factor	Vertical Farm	Greenhouse
DLI calculated (mol m^{-2} d^{-1})	11.5	13.2
• DLI lamps	11.5	6.5
• DLI sun	0	6.7
Temperature (24 h) (°C)	18	18.8
• Temp light	20.0	20.4
• Temp dark	4.1	15.4
CO_2 (light) (ppm)	745	503
RH (24 h) (%)	77	84
• RH light	76	81
• RH dark	80	89

In both experiments, fruit harvest started after 3 weeks of cultivation (Figure 26). During the whole production period, greenhouse production had a lower peak production compared to vertical farm and managed to achieve a more constant "flat" production (Figure 26). Between week 8 and week 18 of cultivation, weekly production in the vertical farm went from a minimum of 0,32 Kg/m^2 to a maximum peak of 1,35 kg/m^2 (4,2 times higher) in week 15. In the greenhouse, weekly production was 0,14 Kg/m^2 in week 8, it achieved the highest peak of 0,50 (3,6 times higher) in week 16 and kept above 0,24 Kg/m^2 until week 26. In the vertical farm weekly production went down to 0,14 Kg/m^2 at 21 weeks.
At 200 micromoles, and leaf temperature of 20 degrees, CO_2 levels below 600 ppm have been found limiting for photosynthesis rate (Elings at al. 2023). This can explain the lower fruit production achieved in the greenhouse compared to the vertical farm were CO_2 was not limiting. The more stable production could be attributed to the lower CO_2 availability which limits assimilate production thus keeps the plant in a better balance between vegetative and generative.

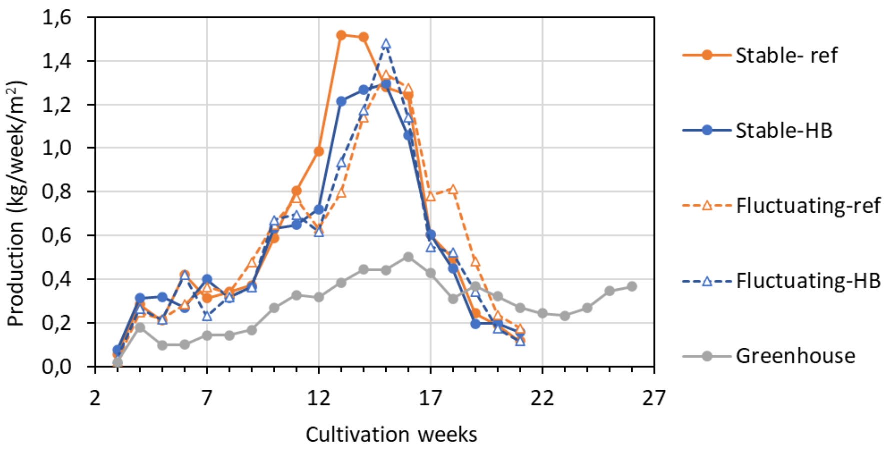

Figure 26 *Total production of strawberry fruits per different climate and light treatments combination and in the separate greenhouse experiment. Graph shows weekly production of all harvested fruits (class 1, 2 and mildew).*

To compare total yield after 21 weeks of cultivation, calculations are made both per unit area and per linear meter since plant density was different in the two facilities but number of plants per linear meter was the same (Table 17). When the best performing vertical farm treatment (Stable – Reference) is compared to the greenhouse, yield was 1,5 times higher when expressed per linear meter and 2,2 times higher when expressed per unit area in the vertical farm. Cultivation in the greenhouse continued for 5 more weeks which were not sufficient to achieve the same total yield as in the best performing vertical farm treatment: 8,1 Kg per linear meter and 6,7 Kg per square meter were achieved.

Table 17 *Yield results (Class 1, Class 2 and mildew) of vertical farming and greenhouse cultivation expressed per square meter and per linear meter during 21 weeks.*

Climate treatment	Light treatment	Yield (km/m^2)	Yield (Kg/m)
Stable	Reference	11,6	9,3
	High Blue	10,5	8,4
Fluctuation	Reference	11,1	8,8
	High Blue	10,3	8,2
Greenhouse		5,2	6,3

To try to explain the "flat" production obtained in the greenhouse and compare it with the vertical farm, weekly production was compared to the radiation temperature ratio (RTR) calculated with average 24h temperature and DLI per week up to cultivation week 25 (Figure 27). In the greenhouse, the average RTR after 21 weeks of cultivation was 0,7 while in the vertical farm it was 0,6.

In the greenhouse both average DLI and temperature where compared to the vertical farm and a higher RTR means that more light was provided compared to the temperature. In the greenhouse and average of 65 fruits/plant (Class 1+ Class 2 + mildew) are harvested after 21 weeks while in the vertical farm 85 fruits/plant. In everbearing varieties, a higher 24-h temperature is correlated with a higher number of fruits per plant (Sønsteby & Heide, 2007). This is in contrast with what found in the two experiment where higher number of fruits was achieved in the conditions with lower 24-h temperature. Similar controversial results are found when the two cultivations are compared for the average DLI. When more light is supplied, more assimilates will be produced leading to a higher fruit set and development (Hidaka *et al.*, 2013). Greenhouse cultivation had a light sum of 1914 mol/m^2 which was 14% higher than the average light sum in the vertical farm yet less fruits were produced. This leads back to the hypothesis that another factor, namely the CO_2 concentration, was the limiting factor in the greenhouse cultivation for assimilate production. Close to this, the higher average night temperature would have an effect on the respiration rate during the night which would lead to a higher consumption of assimilates during the night, thus less assimilates are available during the day, compared to the vertical farm.

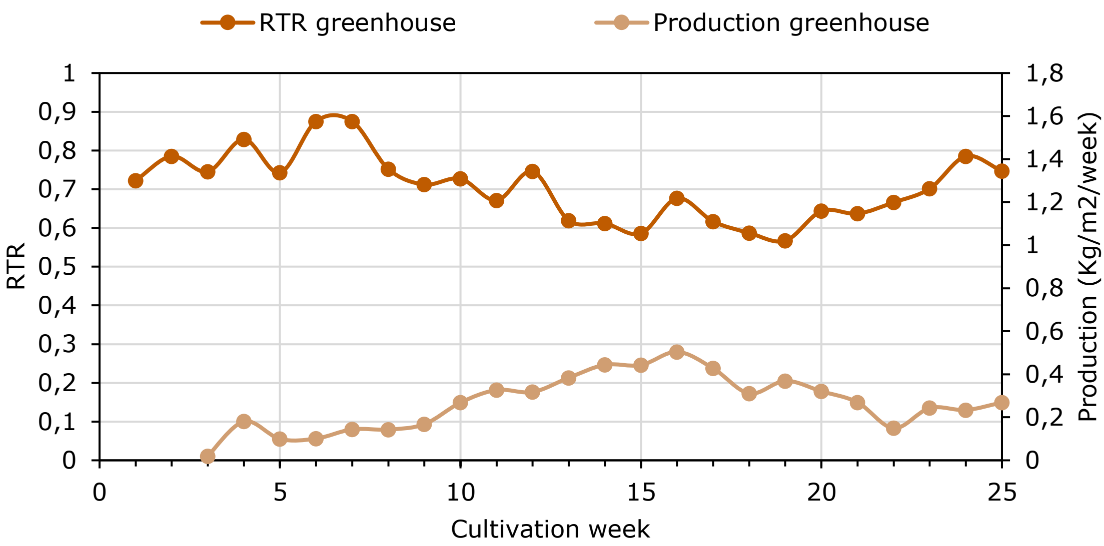

Figure 27 *Weekly RTR value and strawberry production in the greenhouse experiment.*

One of the attributes of strawberry quality is measured by the size of the fruits. Class 1 fruits are the highest category, with class 1 > 30mm as "extra quality". When only Class 1 fruit yield is observed production per square meter is higher in the vertical farming experiment while it is similar to the production per linear meter obtained in the Stable climate. A higher percentage of larger strawberries was harvested in the vertical farm compared to the greenhouse.

Table 18 *Yield results for Class 1 fruits of vertical farming and greenhouse cultivation expressed per square meter and per linear meter and yield distribution in different size classes.*

Climate treatment	**Light treatment**	**Yield Class 1 (km/m²)**	**Yield Class 1 (Kg/m)**	**% Class 1 >30**	**% Class 1 27-30**	**% Class 1 <27**
Stable	Reference	7,7	6,1	60	20	20
	High Blue	7,3	5,8	56	22	22
Fluctuation	Reference	7,1	5,7	61	22	17
	High Blue	6,9	5,5	61	21	17
Greenhouse		4,9	5,9	56	18	26

Organoleptic measurements (Brix and acidity) and nutritional measurements (vitamin C content) were performed in both cultivation experiment (Table 19). Brix value was on average higher in the greenhouse compared to the vertical farm where it was found similar to the best performing treatment (Stable – Reference). In general Brix is affected by plant load (Correia *et al.*, 2011). Values were indeed more stable in the greenhouse over the whole cultivation while they went below a Brix value of 8 between week 11 and 17 (Stable-Reference) which corresponded to the high production peak in the vertical farm (Figure 28).

Vitamin C was measured once in the greenhouse trial during cultivation week 9 and it is compared to same measurement date taken in the vertical farm. A slightly positive effect of High Blue treatment was observed in the vertical farm with values which are similar to the greenhouse. The slightly higher value in the greenhouse could be attributed to the higher exposer of the fruits to light (in the vertical farms, fruits were hanging at the border of the light installation) or to the exposure of the UV treatment against mildew.

Table 19 *Quality results of vertical farming cultivation expressed as average of 4 measurement date (n=4) and of greenhouse trial (n=4) for Brix and acidity.*
**Vitamin C is expressed with measurement on WOT 9 (n=1).*
***DMC for vertical farm is the average of the whole cultivation (n=21; weeks) while for greenhouse is the average of WOT 12 and 21 (n=2).*

Climate treatment	Light treatment	Refraction (°Brix)	Acid (mmol H_3O^+/100g)	Vit C (mg/100g)	DMC* (%)
Stable	Ref	8,6	12,8	52	8,5
	High Blue	7,8	12,6	56	8,1
Fluctuation	Ref	8,1	12,7	52	8,5
	High Blue	7,8	12,3	54	8,3
Greenhouse		8,8	14,0	57	7,6

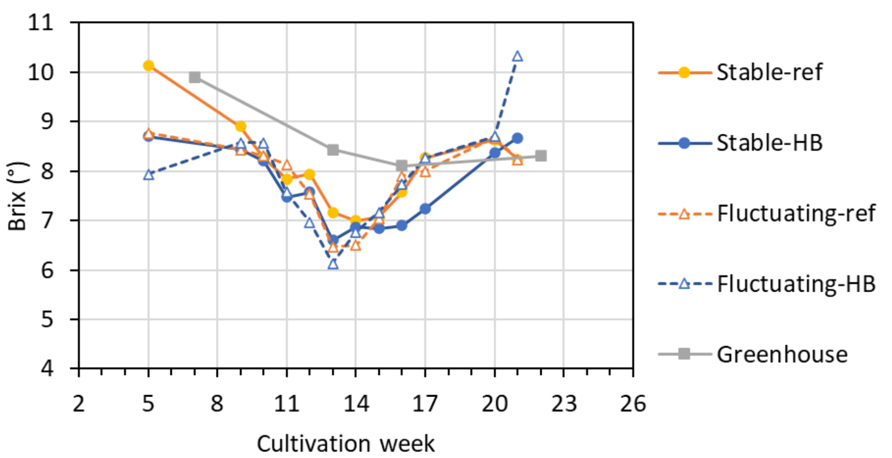

Figure 28 *Brix value during different cultivation weeks in the vertical farm and in the greenhouse.*

4 Discussion

4.1 Plant growth and production

At the end of the trial, a positive effect of Fluctuating climate treatment was found on aboveground biomass production ($p=0.018$) which was mainly due to new organs growing on the plants. Fruit biomass was not affected by the climate treatments after 21 weeks of cultivation. Showing a higher partitioning of biomass towards fruits under Stable climate compared to Fluctuating climate. Due to their simultaneous vegetative and generative growth behavior, everbearing strawberries need to allocate their sugars into both developing leaves and fruits. Fruits are strong sinks (Jia *et al.*, 2013) and when the fruit load is too high, plants will not have enough assimilates to destine to vegetative growth which will in turn reach a stand still bringing the plant out of balance (Elings *et al.*, 2023). This phenomenon was noticed, in this experiment, under both climate treatments and it was more accentuated under Stable treatment. With Stable conditions, at the end of the experiment, plants had a lower leaf area and a higher number of flowers per truss, showing a more generative growth pattern (Heide *et al.*, 2013). This was also clear at destructive harvest with a crop which looked more exhausted and had 1.5 times more new leaf compared to old leaves while Fluctuating had 2 times more. This disbalance was also shown in the harvest pattern. Under Stable and Reference climate, a shorter production peak of 7 weeks was achieved while under Fluctuating and Reference climate a longer but not yet flat production was achieved during 9 weeks.

Under Fluctuating condition, the steep increase in weekly harvest, which started in week 9 – 10 under Stable climate, was delayed with a couple of weeks. Under Fluctuating conditions, light and temperature were, depending on the weeks, either lower or higher compared to Stable climate. Wang & Camp (2000) found a temperature effect on dry matter distribution: at higher temperatures assimilates moved more to leaves and stems (day/night temperatures 25/12°C), and at lower temperatures (18/12°C) more to the roots and possibly fruits (the latter cannot be determined with certainty because the numbers of fruits per plant are not give). It is hypothesized that the lower light and temperature during week 6 influenced truss size and brought the plant to a lower plant load after 6 weeks compared to Stable climate. Twitchen at al., (2021) found that the combination of low temperature and lower light slowed down strawberry ripening. Weekly production was thus lower after 6 weeks (at cultivation week 12) and kept the vegetative and generative growth more in balance which sustained the production for a longer period and resulted in a higher vegetative biomass at the end of the trial.

Light treatment had as well an effect on plant growth and yield. Under High Blue treatment, plants produced 12% less biomass through the whole cultivation. It is know that increasing blue light fraction decreases growth mainly due to its effects on plant morphology and light interception (Kalaitzoglou *et al.*, 2021). In this experiment strawberry under High Blue treatment had 18% lower crop width ($p<0.001$) which caused, under Stable conditions, a 9% lower light interception and explains the lower biomass and yield. Interestingly, the effect of more “flat” production was not observed und Fluctuating climate and High Blue light treatment. Under both climates, High Blue treatment was delayed with 2 weeks as the Fluctuating- Reference crop but dropped the production already at week 17 as the Stable-Reference crop. An interaction was as well found for plant morphology between climate treatment and light treatment. It is thus hypostasized that the delay at the beginning of the production peak was due to low assimilates availability due to either low light interception (High blue treatment) or lower light and temperature (Fluctuating treatment). Yoshida *et al.* (2012) found that blue light promoted flowering of everbearing strawberries compared to white and to red light. The missing small peak at week 17-18 could be due to the disbalance between assimilates produced and flower initiation in the Fluctuating – High Blue treatment.

4.2 Quality

Overall, no effect of the climate treatment was found on strawberry size, organoleptic quality and nutritional quality.

Reference light treatment had a positive effect on sugar accumulation in the fruits with higher dry matter content and higher Brix compared to High Blue treatment. A good Brix value for commercial strawberry is 8 (minimum is 7) as reported by the industry standard for import in Europe (MFA, 2019). High fruit loads are known to have a negative effect on fruit sugar content. During the high production peak, between week 12 and 17, Brix values were therefore below the industry standard. Light intensity has a positive effect on Vitamin C levels. Hanenberg *et al.* (2016) found that strawberries Vitamin C content depend on the amount of light the crop receives and it is increased when light is directly supplemented towards the fruits. As no difference was applied in total light sum, this can explain why no difference in vitamin C content was found among the treatment.

Blue light positively influenced anthocyanins accumulation in many fruiting crops (Xiao 2022, Choi 2015) In the present experiment, no effect of blue was found on anthocyanin concentration. Similarly, Piovene *et al.* (2015) didn't find any effect of different blue:red light treatments on strawberry antioxidant compounds.

4.3 Water and nutrients

The similar water uptake and nutrients uptake concentration between treatments would suggest that the transpiration and nutrients requirement was not much different between treatments. The 10% difference in yield might be not high enough to be seen from this data. The negative nutrient uptake concentration calculation for the last part of the cycle (week 15 -21) was probably due to the flushing of nutrients from the growing media. This is often the case when nutrients uptake is reduced but still high volumes of nutrient solution are supplied. In fact, also the drain rate was a bit higher (30%) in that period compared to the rest of cultivation (20%). The two different observed phases (week 1-5 and 6-15) in nutrients uptake concentration could coincide with respectively the vegetative and generative phase. Because of the high nutrients uptake concentration (1.3 dS/m) in the vegetative phase, this period must be supported with high enough drip EC (1.5-2.0 dS/m). From week 6 to 15 the nutrients uptake was much lower (0.6 dS/m). The drip EC can therefore be reduced to about 1.0 dS/m. The difference in the uptake K:Ca ratio between the cell with stable light and the fluctuating one seemed to reflect well the higher number of new flower trusses, flowers and fruits registered in this treatment.

4.4 Comparison with a greenhouse trial

The comparison between the greenhouse and the vertical farming experiments is presented in order to gain insight on how a more flat production can possibly be achieved. In 21 weeks of cultivation, Stable-Reference treatment in the vertical farm produced 2.2 times yield per square meter compared to the greenhouse. Light conditions in the greenhouse pointed towards a high assimilate production given the 14% higher light sum. The availability of assimilates is a trigger for flowering in strawberry (Elings *et al.*, 2023) so a higher assimilate production combined with similar average day temperature in the greenhouse compared to vertical farm should have led to higher productions in the greenhouse. It is thus hypothesized that other factors limited the availability of assimilates which reduced flowering namely the 33% lower average CO_2 concentration in the air, which limited photosynthesis, and the higher night temperature which increased crop respiration.

The large fruit yield in the vertical farm brought, on the other hand, to a steep peak production pattern while in the greenhouse a flat production was achieved. By modulating the "energy state" of the plant or, in other words, with the assimilate availability, different type of production curves can be achieved with everbearing strawberries.

5 Conclusions

Strawberries are suitable for cultivation in fully controlled environment. It is fundamental to be able to start with disease-free plants, especially mildew-free plants, when no UV treatment can be applied. If plants are not pest-free, a standing army of biological control agents is able to keep the infestation under control. Pollinators can also orient and forage flowers under LED-only lighting conditions. Depending on the specie, the addition of UVA light may be necessary or anyways beneficial.

With everbearing varieties, both a large peak production or a more “flat” production can be achieved in indoor cultivation because climate can be fully controlled. This depends on how the environmental conditions are controlled and whether they are favorable for keeping the vegetative and generative growth in balance. During this experiment, both the Fluctuating and the Stable climate were not able to achieve a “flat” production. Stable conditions had a more generative growth type and produced higher yield while Fluctuating and Reference light had a more balanced growth. After 21 weeks of cultivation, Stable climate and Reference treatment produced the highest yield with 11,6 Kg/m^2 which was 1.8 times higher than the yield achieved in the greenhouse (6,3 Kg/m^2).

Conditions which modulate truss initiation and assimilate production can lead to year-round production of strawberries. In the greenhouse a more flat production was realized which can provide insights in critical environmental conditions during cultivation which led to this flat production. It is hypothesized that a lower availability of assimilates was generated by limiting CO_2 levels during the day and possibly by the higher night respiration during the night caused by higher night temperatures. Depending on the business model, a large peak production could also be favorable to fill the market gaps. Pushing the crop towards high production can, on the other hand, have a negative influence on the organoleptic quality of the fruits.

With the application of tailor-made spectra, plant growth, production and quality can be steered as well. With a blue rich treatment, a good balance between the positive effects on quality attributes and the possible reduction on plant growth and consequently yield has to be found. In the current experiment, no beneficial effect of High Blue treatment (B:R = 1:2) were found on anthocyanins content (AU) compared to a reference treatment (B:R =1:4).

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Annex 1 Nutrients

Table A1.1 *Vegetative and generative recipe.*

		Vegetative	Generative
dS/m	EC	1,2	1,2
mmol/l	NH4	0,7	0,4
mmol/l	K	4,1	5,5
mmol/l	Ca	3,1	2,4
mmol/l	Mg	0,9	0,9
mmol/l	NO3	9,8	9,6
mmol/l	Cl	0,0	0,0
mmol/l	SO4	1,1	1,1
mmol/l	H2PO4	0,9	0,7
umol/l	Fe	30,0	20,0
umol/l	Mn	15,0	10,0
umol/l	Zn	7,0	7,0
umol/l	B	12,0	12,0
umol/l	Cu	0,8	0,8
umol/l	Mo	0,5	0,5

Table A1.2 *Average element concentration throughout the cultivation per treatment. Data are corrected to an EC of 1.7 dS/m.*

	cell	layer		
	3	3	4	4
Values	**bot**	**top**	**bot**	**top**
Average of EC(mS/cm)	2,8	2,7	2,7	2,8
Average of pH	6,1	6	6,2	6,2
Average of NH4(mmol)	0,2	0,2	0,2	0,2
Average of K(mmol)	7,0	7,1	6,3	6,8
Average of Na(mmol)	0,9	0,8	0,9	0,9
Average of Ca(mmol)	6,8	6,5	6,7	6,9
Average of Mg(mmol)	2,0	1,9	1,9	2
Average of NO3(mmol)	17,2	17	16	18
Average of Cl(mmol)	0,3	0,2	0,3	0,3
Average of SO4(mmol)	2,8	2,6	2,8	2,8
Average of P(mmol)	1,7	1,7	1,4	1,5
Average of Fe(µmol)	23,7	24	24	26
Average of Mn(µmol)	4,9	5,5	5,9	6,3
Average of Zn(µmol)	10,9	11	10	11
Average of B(µmol)	18,8	19	20	22
Average of Cu(µmol)	0,9	0,7	0,8	0,8
Average of Mo(µmol)	0,1	0,1	0,1	0,1

Annex 2 Sprayed agents

Table A2.1 *List of sprayed agents against pests and diseases.*

Date	Product name	Active ingredient	Pest/disease
1-09	Serenade/ Silwet (adjuvant)	*Bacillus amyloliquefaciens*	Powdery mildew
14-09	Flipper/ Karma	Fatty acids and potassium salts/ potassium hydrogen carbonate	Aphids/powdery mildew
14-09	Xentari	*Bacillus thuringiensis*	Sawflies
22-09	Karma/ Taegro	Potassium hydrogen carbonate/ *Bacillus amyloliquefaciens*	Powdery mildew
12-10	Abir	Bupirimate	Powdery mildew
14-10	Flipper	Fatty acids and potassium salts	Aphids
19-10	Serenade	*Bacillus amyloliquefaciens*	Powdery mildew
31-10	Taegro	*Bacillus amyloliquefaciens*	Powdery mildew
9-11	Serenade	*Bacillus amyloliquefaciens*	Powdery mildew
17-11	Serenade	*Bacillus amyloliquefaciens*	Powdery mildew
23-11	Serenade	*Bacillus amyloliquefaciens*	Powdery mildew
1-12	Serenade	*Bacillus amyloliquefaciens*	Powdery mildew
7-12	Abir	Bupirimate	Powdery mildew
21-12	Abir	Bupirimate	Powdery mildew

Annex 3 Climate set-up

Table A3.1 *Daily climate composition of stable climate and fluctuating climates (a=bright day; b=dull day; c=variation; d=big fluctuations).*

	µmol/m2/s	T (°C)		µmol/m2/s	T (°C)		µmol/m2/s	T (°C)		µmol/m2/s	T (°C)		µmol/m2/s	T (°C)	
Average	133.3	18.00		200.0	19.0		66.7	16.9		132.9	18.04		133.3	18.00	
hour	**stable**	**stable**	**stable**	**a**	**a**	**a**	**b**	**b**	**b**	**c**	**c**	**c**	**d**	**d**	**d**
	µmol/m2/s	T (°C)	RH (%)	µmol/m2/s	T (°C)	RH (%)	µmol/m2/s	T (°C)	RH (%)	µmol/m2/s	T (°C)	RH (%)	µmol/m2/s	T (°C)	RH (%)
1	200	20	80	150	17	76	100	18.4	79	100	17	76	100	17	76
2	200	20	80	200	19	79	100	18.4	79	100	17	76	100	17	76
3	200	20	80	300	21	82	100	18.4	79	250	20	80	100	17	76
4	200	20	80	350	23	83	100	18.4	79	250	20	80	400	24	84
5	200	20	80	350	23	83	100	18.4	79	250	20	80	400	24	84
6	200	20	80	350	23	83	100	18.4	79	100	17	76	50	19	79
7	200	20	80	350	23	83	100	18.4	79	100	17	76	50	19	79
8	200	20	80	350	23	83	100	18.4	79	100	17	76	50	19	79
9	200	20	80	350	23	83	100	18.4	79	350	23	83	500	24	84
10	200	20	80	350	23	83	100	18.4	79	350	23	83	500	24	84
11	200	20	80	350	23	83	100	18.4	79	350	23	83	100	19	79
12	200	20	80	350	23	83	100	18.4	79	350	23	83	100	19	79
13	200	20	80	350	23	83	100	18.4	79	100	21	82	300	22	83
14	200	20	80	300	21	82	100	18.4	79	100	21	82	300	22	83
15	200	20	80	200	19	79	100	18.4	79	170	21	82	75	17	76
16	200	20	80	150	17	76	100	18.4	79	170	21	82	75	17	76
17	0	14	85	0	14	85	0	14	85	0	14	85	0	14	85
18	0	14	85	0	14	85	0	14	85	0	14	85	0	14	85
19	0	14	85	0	14	85	0	14	85	0	14	85	0	14	85
20	0	14	85	0	14	85	0	14	85	0	14	85	0	14	85
21	0	14	85	0	14	85	0	14	85	0	14	85	0	14	85
22	0	14	85	0	14	85	0	14	85	0	14	85	0	14	85
23	0	14	85	0	14	85	0	14	85	0	14	85	0	14	85
24	0	14	85	0	14	85	0	14	85	0	14	85	0	14	85

Table A3.2 *Climate recipe distribution in Fluctuating climate treatment.*

Week	Climate type
1	stable
2	variation
3	dull
4	big fluctuations
5	bright
6	dull
7	variation
8	big fluctuations
9	variation
10	bright
11	big fluctuations
12	dull
13	dull
14	bright
15	variation
16	big fluctuations
17	bright
18	big fluctuations
19	variation
20	bright
21	dull

To explore the potential of nature to improve the quality of life

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The mission of Wageningen University & Research is "To explore the potential of nature to improve the quality of life". Under the banner Wageningen University & Research, Wageningen University and the specialised research institutes of the Wageningen Research Foundation have joined forces in contributing to finding solutions to important questions in the domain of healthy food and living environment. With its roughly 30 branches, 7,600 employees (6,700 fte) and 13,100 students and over 150,000 participants to WUR's Life Long Learning, Wageningen University & Research is one of the leading organisations in its domain. The unique Wageningen approach lies in its integrated approach to issues and the collaboration between different disciplines.