

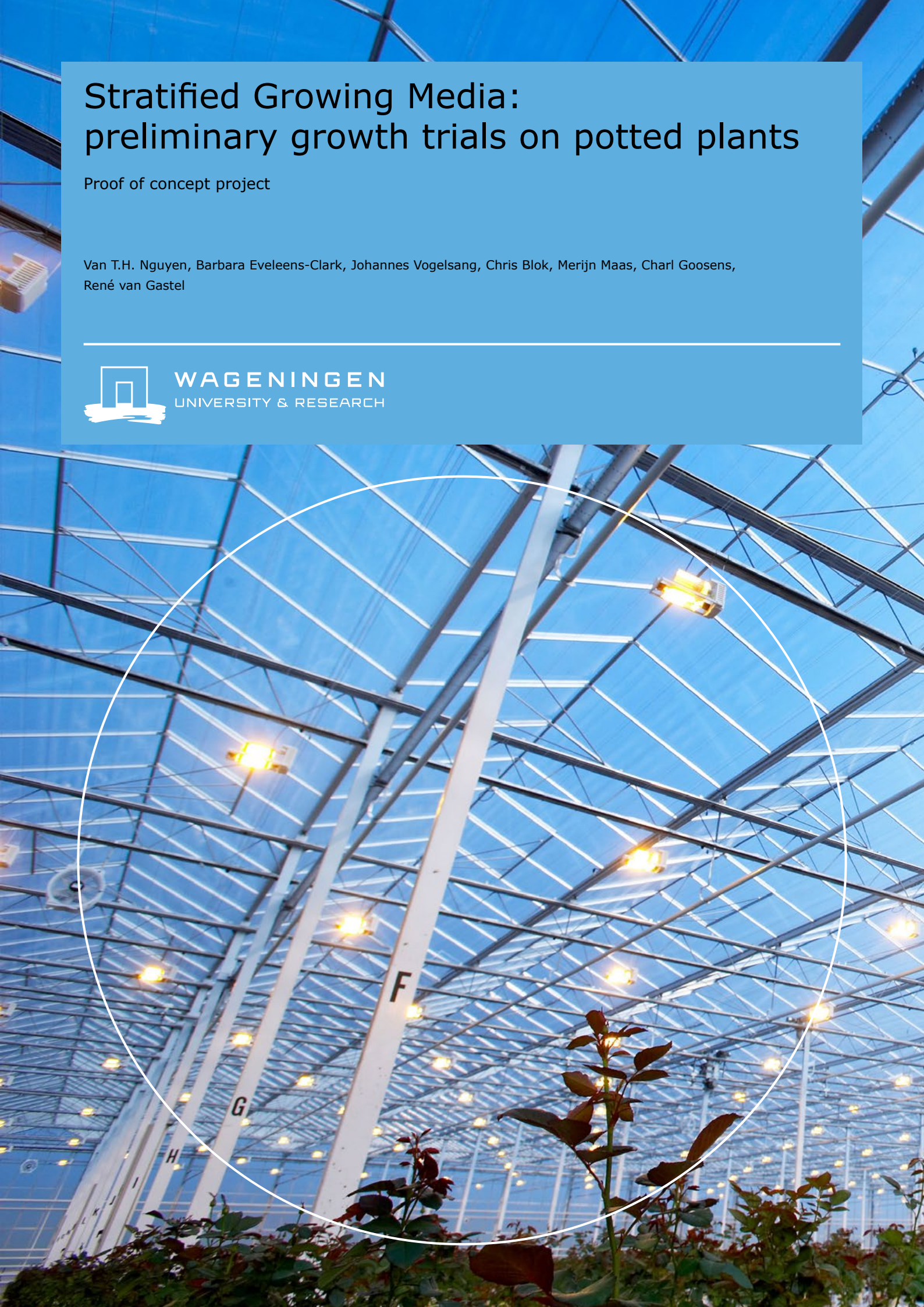
Stratified Growing Media: preliminary growth trials on potted plants

Proof of concept project

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Referaat

Stratificatie is het aanbrengen van lagen in potgrond, oftewel het gebruik van meer dan één enkele laag in de pot. Deze methode is eerder toegepast in boomkwekerijen in de VS, waarbij de onderste helft van de pot werd gevuld met grovere materialen, wat positieve resultaten opleverde. In dit proof-of-concept, gefinancierd door SIGN en het Innovatiefonds Hagelunie, zijn voorlopige groeiproeven uitgevoerd met potplanten. Het doel was om te testen of stratificatie kan worden gebruikt als hulpmiddel om een hoger aandeel veenvrije grondstoffen zoals houtvezels, boomschors, miscanthus, enz. in potten toe te passen. De proeven zijn uitgevoerd op een proeflocatie van Wageningen University & Research (eb- en vloedsysteem) en bij verschillende telers (bovensproeiers). De belangrijkste bevindingen zijn: (1) het vervangen van de onderste laag door veenvrije materialen resulteerde onder beide irrigatiesystemen in een vergelijkbare plantengroei als de referentie (niet-gestratificeerde veenhoudende mengsels), onder normale teeltomstandigheden voor veenmengsels; (2) de berekening van de basisbemesting is essentieel voor succes, vooral bij een hoger aandeel hernieuwbare grondstoffen; en (3) de onderhoudsbemesting moet worden aangepast op basis van de plantengroei, rekening houdend met de nutriëntenbehoefte van de plant en het moment waarop de wortels de onderste laag bereiken. Conclusie: de voorlopige groeiproeven tonen aan dat stratificatie een veelbelovende methode is om het aandeel veenvrije grondstoffen in potten te vergroten.

Abstract

Stratification is the practice of layering growing media in the pots, i.e. more than one single layer. This practice has been used in tree nursery in the US, by replacing the bottom half of the pots with coarse materials, showing positive results. In this proof-of-concept, funded by SIGN and Innovatiefonds Hagelunie, preliminary growth trials were conducted on potted plants. The aim was to test if stratification can be used as a tool to increase higher proportion of non-peat raw materials such as wood fibres, bark, miscanthus, etc. in pots. The trials were conducted at a research station of Wageningen University & Research (ebb and flow) and at several growers (head sprinklers). The main findings are (1) replacing the bottom layer with non-peat materials under both irrigation systems resulted in similar plant growth to the reference non-stratified peat-based mixtures, under normal growing conditions for peat-based mixtures, (2) base fertiliser calculation are essential for success, particularly when a higher proportion of renewable raw materials is used, and (3) the maintenance fertilization should be adjusted based on plant growth, taking into account the plants' nutrient demand and the stage when roots reach the bottom layer. In conclusion, the preliminary growth trials showed that stratification is a promising tool to increase higher proportion of raw materials in the pots).

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Summary

Substrate stratification, the practice of layering different substrate materials or textures in a pot, helps manage water distribution (e.g., preventing excess moisture at the bottom) and facilitates the use of renewable peat-free raw materials. Stratification was tested with peat-based mixtures in the Netherlands in 2013 to enhance water retention in the pots. Stratification also has been tested at the university and at growers in the US from 2015 for tree nursery crops and floriculture crops.

Inspired by promising results in the US, Wageningen University and Research, Business Unit Greenhouse Horticulture (WUR) initiated a pre-test in 2024 to explore stratification for increasing renewable raw materials in potted plants. The project, funded by SIGN and Innovatiefonds Hagelunie, involved WUR, SIGN, Varta, Groeibalans Teelt&Onderzoek, substrate producers (Jiffy and MeeGaa), and four growers (Bestplant, Van der Voort, Van Son en Koot, and Gova). Growth trials were conducted at WUR's research station (ebb-and-flow for *Spathiphyllum*) and at growers' facilities (head sprinklers for *Spathiphyllum*, *Acer*, and *Laurus*).

In the growth trial at WUR, potted *Spathiphyllum* was cultivated in a reference peat-based substrate and five stratified treatments with increasing proportions of less stable raw materials. Three fertigation regimes were tested, a reference regime with a fertigation frequency and nutrient composition optimised for the reference mixture and two modified regimes with either an increased frequency or both, an increased frequency plus an increased nutrient concentration. Replacing the bottom half of the peat-based substrate resulted in comparable plant growth to the reference mixture under normal fertigation. Replacing both layers with renewable raw materials reduced plant growth under normal fertigation, but increasing fertigation frequency and nutrient concentration improved the performance, though not to practically acceptable growth levels. Total pot moisture content in stratified treatments was 10–20% lower than in the reference substrate under normal fertigation, indicating reduced capillary rise in the bottom layer. Increasing fertigation frequency minimized these differences. Nutrient concentration in the bottom layer was lower than in the top layer, likely due to nutrient immobilization by microbial activity, influenced by the proportion of renewable raw materials, and to a lesser extent, nutrient uptake by plant roots, as fibrous thick roots had already reached the bottom layer at the time of sampling. Note the nutrient distribution is highly sensitive to the irrigation type (ebb-and-flow or overhead).

In the growth trials at the growers, potted *Spathiphyllum* in three pot sizes, as well as *Acer* and *Laurus*, were tested. Stratified treatments with various mixture recipes and top-to-bottom layer ratios (50/50 and 75/25, representing layer distribution over pot height) were evaluated under standard grower practices. In general, no significant differences in plant growth were observed between the reference and stratified treatments. This was a positive outcome, partly attributed to the use of overhead sprinklers, to which stratified substrates appear to respond well. In a trial on *Spathiphyllum* in 2.5 L pots at Van der Voort, slight yellowing of older leaves appeared about 12 weeks after transplanting, suggesting that an adjustment in fertigation might be needed. However, from 15 weeks after transplanting onward, this yellowing was not observed anymore. Nutrient levels were generally higher in the top layer than in the bottom layer, likely due to several factors: no base fertilisers used in the bottom layer for all trials (except for the trial at Bestplant), fertigation entering the substrate from the top via overhead sprinklers, higher root activity in the bottom layer at the time of sampling, and differences in raw materials between mixtures. Moisture content, measured for the whole pot, was comparable between the reference and stratified treatments, with some stratified treatments showed slightly higher values (5–10% by volume).

This proof-of-concept project showed that stratified growing media is a promising tool to increase higher proportion of raw materials in the pots, as well as to better manage the gradient of water content over height in pots. In both ebb-and-flow and overhead sprinkler systems, replacing the bottom half of the peat-based substrate maintained plant performance under standard fertigation. Under ebb-and-flow system, fully replacing both layers reduced growth, but increasing fertigation frequency and nutrient levels improved outcomes, depending on the materials used. This leads us to believe adapted fertigation could partly solve the problems with the new raw materials in the top layer. It is however advisable to keep the top soil structure close to the structure of the propagation material. This means that large adaptations of the top soil, might also require similar adaptations to the propagation material media. Proper base fertilization is crucial, with lower initial dressing sufficient if maintenance fertigation meets plant needs. Ebb-and-flow and overhead irrigation require different fertilization strategies. Key knowledge gaps include predicting base fertilization needs based on crop and irrigation parameters and improving moisture gradient predictions.

1 Introduction

1.1 Background

Stratified growing media refers to the practice of layering growing media within pots. Compared to traditional growing media blends, working with two or more layers allows the user to put coarser material in one layer and finer material in the other layer which (1) allows the use of a higher level of peat alternatives, (2) decreases the natural gradient in water content from top to bottom (i.e. the chance to drown the plants), (3) avoids a too wet bottom layer but without going down too much in overall water storage in a container (as this affects transport and shelf life), and (4) increases fertiliser efficiency by different quantities of slow release fertilisers in top and bottom layers.

Substrate stratification other than for potting soil was already a well known concept. In Holland soils with a finer top layer on coarser material were valued as orchard soils. These soils were believed to transport water from the deeper layers upwards “opdrachtige grond” i.e. water lifting soils. In horticulture this was applied since the 90’s when stone wool slabs were produced with a denser top layer (Blok, 1990).

Substrate stratification in container plants was tested in the Netherlands in 2013 for greenhouse container crops (Cyclamen and Monstera) using peat-based substrates (different peat fractions) under ebb-and-flow irrigation conditions (Vennik et al., 2013). The results showed (for this particular system!) improved plant growth when placing coarse peat atop a layer of fine peat. However, the idea was not developed further because at that time there was less pressure on peat reduction and limited enthusiasm of potting machine suppliers. In the US, promising results in practice in the period 2015 till now were presented with substrate stratification in tree nursery and floriculture crops, led by Louisiana State University (Fields, 2023; Fields et al., 2024). Containerized rose grown in stratified bark substrates, with a top layer of aged pine bark and bottom layers of either fine bark or a bark-peat mixture, showed equal or superior growth compared to non-stratified controls (Fields et al., 2021). Similarly, petunia crops grown in stratified substrates, with a peat-based mixture as the top layer and aged pine bark as the bottom layer, produced crops of comparable size and quality to non-stratified controls (Fields & Criscione, 2023).

The initiation of this proof-of-concept project *Stratified Growing Media* was motivated by (1) the promising results of stratified substrates in the US, (2) high pressure on peat reduction, particular for ornamental container crops, and (3) possible techniques in potting machines: probably two machines with a buffer will be needed (one machine for mulch filling and a normal potting machine).

The project consortium consists of Wageningen University and Research, Business Unit Greenhouse Horticulture (WUR), Stichting Innovatie Glastuinbouw Nederland (SIGN), Valorisatielab VARTA (VARTA), Groeibalans Teelt & Onderzoek (Groeibalans), Javo B.V., Jiffy Products International B.V., MeeGaa Substrates B.V., and the growers Bestplant, Van der Voort, Van Son & Koot and Gova. The project is funded by SIGN and Innovatiefonds Hagelunie.

1.2 Goal

The project aims to conduct preliminary growth trials on stratified growing media for selected container crops. The results help to fine-tune technical and research questions for a proposed PPS project focusing on (1) design of stratified growing media to increase the proportion of renewable raw materials, (2) cultivation management (irrigation and fertigation adaptations and possibly changes in propagation), and (3) technical implementation of potting machines.

1.3 Approach

The project encompasses two activities: (1) a growth trial at WUR research station in Bleiswijk on potted *Spathiphyllum* under modified fertigation regimes and (2) five growth trials at the growers on potted *Spathiphyllum*, *Acer* and *Laurus* under normal fertigation at their locations. Moreover, Javo B.V. drafted a machine concept for potting machines. The concept was presented to the project partners in a meeting and is not included in this report.

1.4 Organisation

WUR led the project and organized the growth trial at WUR research station. The growth trials at the growers were organized by VARTA, Groeibalans and the growers Bestplant, Van der Voort, Van Son & Koot and Gova. MeeGaa designed and delivered growing media mixtures for the trial at Bestplant. Jiffy designed and delivered growing media mixtures for the trial at Van der Voort, Van Son & Koot and Gova. Javo prepared a concept for potting machine. SIGN provided the fund and involved in the steering process. Innovatiefonds Hagelunie provided the fund.

Trial visits by project partners were organized on 22nd May 2024 at WUR research station, on 3rd July 2024 at the grower Van der Voort and Van Son & Koot, and on 24th July and 18th September 2024 at the grower Bestplant.

The project was supervised by Van Nguyen of WUR and executed with the help of Barbara Eveleens, Chris Blok and Johannes Vogelsang as well as Joseph Stoener. For the grower experiments Charles Goosens of Varta initiated the work and Merijn Maas supervised and analysed the experiments. René van Gestel of Groeibalans provided advisory services to both, the growers and the project.

2 Growth trial at WUR research station

2.1 Goal

This trial aimed to evaluate the performance of stratified growing media, which consist of increasing proportions of renewable raw materials, under standard ebb-and-flow fertigation conditions for peat-based mixtures, as well as under modified fertigation conditions.

2.2 Methodology

The trial was conducted in one half of a 144 m² glasshouse compartment at Greenhouse Horticulture Business Unit, Wageningen Research (Violierenweg 1, 2665 MV Bleiswijk, The Netherlands). The greenhouse compartment is equipped with 12 tables in 4 blocks with individual fertigation for each table. Each table can contain 63 pots (17-cm pots: height 12.5 cm, diameter 17 cm, volume 1.9 L) (Annex 1, Figure A1).

Spathiphyllum seedlings were supplied by Van der Voort. Two to three seeds were sown in peat-coir plugs (6 mL plugs). Five rooted plugs were transplanted per pot. Fertigation was supplied via ebb-and-flow from individual tank under each table. Cultivation duration was 15 weeks (calendar week 12 to calendar week 27, 2024). Eight weeks after transplanting, gibberellic acid i.e. GA was sprayed (per table 0.46 L GA solution of 182 mg GA/L, Florigib, Royal Brinkman) to stimulate flowering. After week 27, the plants were transported to the grower Van der Voort for further observations.

2.2.1 Growing media

The reference growing medium consists of 70% white peat 0-10 mm and 30% coir pith. The seven mixtures for each layer consist of various proportions of renewable growing media (acrotelm fine, wood fibre coarse, bark coarse 4-8 mm and miscanthus chopped 5-10 mm) (Table 1). Each layer was prepared by hand mixing, then amended with base dressing fertilisers. Mixture recipes and base fertilisers were calculated using the WUR Mixing Model. Five growing media treatments were tested, including one non-stratified reference (T1) and four stratified treatments with increasing proportions of renewable raw materials in each layer (T2, T3, T4 and T5) (Table 2). Pots were filled by hand, ensuring the same height for each layer.

Table 1 Composition of mixtures in each layer.

Layer	Growing media components (% , volume)
M1	70% white peat 0-10 mm/ 30% coir pith
M2	30% wood fibre coarse/ 20% acrotelm fine/ 50% bark coarse
M3	50% coir pith/ 30% wood fibre coarse / 20% acrotelm fine
M4	20% wood fibre coarse / 30% acrotelm fine/ 50% miscanthus
M5	30% coir pith/ 30% wood fibre coarse/ 20% acrotelm fine/ 20% miscanthus
M6	10% coir pith/ 40% wood fibre coarse/ 30% acrotelm fine/ 20% miscanthus
M7	20% wood fibre coarse/ 30% miscanthus/ 50% bark coarse

Table 2 Stratified growing media treatments.

Treatment	Stratified growing media	Top layer	Bottom layer
T1	Non-stratified, reference peat/coir		M1
T2	Stratified: top (reference), bottom (80 LS)	M1	M2
T3	Stratified: top (30 LS), bottom (70 LS)	M3	M4
T4	Stratified: top (50 LS), bottom (70 LS)	M5	M4
T5	Stratified: top (60 LS), bottom (100 LS)	M6	M7

LS: proportion of raw materials in each layer, which are less stable than peat or coir.

2.2.2 Fertigation regimes

After being filled with growing media, the pots were irrigated with a diluted nutrient solution (D1 solution with half of EC) with head sprinkler. After that, three fertigation regimes (F1, F2 and F3) were applied as a combination of two fertigation frequencies and three maintenance dressing recipes (Table 3). Two irrigation frequencies include standard frequency targeted for reference peat/coir mixture and increased frequency by a factor of 1.9 (Note: fertigation frequency was gradually increased according to plant growth) (Annex 1, Table A3). The three maintenance dressing recipes include a standard recipe for reference peat/coir mixture and two adapted recipes with increased nutrient strength (Annex 1, Table A1). Fertigation was done with ebb and flow system.

Table 3 Fertigation regimes.

Treatment	Irrigation frequency	Fertilization recipe
F1	I1 (targeted for reference peat/coir mixture)	D1 (targeted for reference peat/coir mixture)
F2	I2 (increased frequency by a factor of 1.9)*	D2 (NO ₃ increased 1.4x from transplanting to 12 WAT and 1.3x from 12 to 15 WAT)
F3	I2 (increased frequency by a factor of 1.9)*	D3 (NO ₃ increased 1.4x from transplanting to 12 WAT and 1.5x from 12 to 15 WAT)

*Irrigation frequency was gradually increased with a factor of 1.3, 1.7 and 1.9 according to plant growth. WAT: weeks after transplanting.

2.2.3 Measurements

At 5 and 15 weeks after transplanting, above-ground plant biomass was destructively measured and the rooting pattern was qualitatively observed. Moisture content in the pots was monitored by weighing the pots, and calculated from the dry weight of substrates in the pots at the potting day. Substrates were sampled at 5 weeks after transplanting and sent to Eurofins laboratory for the 1:1.5 water extraction analysis.

2.2.4 Data analysis

Differences in means among treatments were analysed using a one-way ANOVA test ($p \leq 0.05$). If a significant difference was detected, Tukey's HSD post-hoc test ($p \leq 0.05$) was performed. Statistical analysis was conducted using R software (version 4.3.1), and graphs were generated using the ggplot2 package in R.

2.3 Results & discussions

2.3.1 Plant growth

For the first week after transplanting no difference in plant growth was observed. From the second to the fourth week, yellowing leaves were first observed in the T4 and T5 treatments, which contained a higher proportion of less stable renewable materials. This may be a combined effect from nitrogen immobilization due to microbial decomposition of renewable components and reduced iron availability caused by high pH in the root zone. To mitigate, measures were implemented from the fourth week, including gradually increase the frequency of fertilization and add extra Fe-EDDHA (15 µmol/L) to the nutrient solution for ensuring iron availability at high pH.

Five weeks after transplanting, plant biomass in the stratified treatment T2 (top layer of reference peat/coir mixture and bottom layer of peat-free mixture M2) was comparable to that in the non-stratified reference (peat/coir treatment T1) across all fertigation regimes (Table 4, Figure 1). However, replacing both layers with peat-free mixtures (treatment T3, T4 and T5) resulted in reduced plant biomass compared to the reference non-stratified peat/coir mixture T1. Under standard fertigation regimes, treatment T3 showed a 20% reduction in biomass, while treatments T4 and T5 experienced reduction of up to 50%. Increasing fertigation frequency (nearly doubling it) and enhancing nutrient strength (by adding 40% more nitrate to the nutrient solution) resulted in a 10% biomass increase in treatment T3 compared to the standard regime. However, these adjustments had no effect on biomass in treatments T4 and T5 at this stage.

By the fifth week, fibrous roots in all treatments had reached the lower layer, while lateral roots were mainly concentrated in the upper layers (Figure 2). Spathiphyllum in treatments T4 and T5 (with higher shares of renewable materials) produced slightly less root biomass and shorter roots compared to those in T1, T2 and T3, based on observation, not measurement (Figure 2). Reduction in root growth could be explained by limited nutrients availability for plant growth, caused by high proportion of renewable materials in each layers.

Table 4 Plant biomass per pot measured at 5 weeks after transplanting.

Treatment		Fresh biomass per pot* (g)			Dry matter percentage (% weight)		
		F1	F2	F3	F1	F2	F3
T1	Non-stratified, reference (M1)	12 ± 0.6 a	na	na	11 ± 0.6 bc	na	na
T2	Stratified: top (M1), bottom (M2)	12 ± 0.8 a	12 ± 0.7 a	11 ± 1.0 a	9 ± 0.4 c	11 ± 0.9 b	10 ± 1.0 c
T3	Stratified: top (M3), bottom (M4)	9 ± 1.2 b	11 ± 0.8 b	10 ± 0.8 a	11 ± 0.8 ab	11 ± 1.0 b	11 ± 0.2 bc
T4	Stratified: top (M5), bottom (M4)	6 ± 0.3 c	6 ± 0.7 c	6 ± 0.8 b	13 ± 1.8 a	13 ± 0.5 a	13 ± 0.6 a
T5	Stratified: top (M6), bottom (M7)	6 ± 1.2 c	6 ± 0.6 c	7 ± 0.7 b	13 ± 1.2 ab	14 ± 0.7 a	12 ± 0.8 ab

* Five plugs per pot; na: non-applicable, not measured; n = 4 pots. F1: standard fertigation treatment for non-stratified peat/coir mixture T1; F2 and F3: modified fertigation treatments with increased fertigation frequency and nutrient strength. Different lower case letters indicate statistically significant differences in means among substrate treatments at each parameter in each column (Tukey's HSD, $p \leq 0.05$).



Figure 1 *Spathiphyllum* plants at 5 weeks after transplanting. Top to bottom: standard fertigation (F1), modified fertigation (F2 and F3). Left to right: reference peat/coir mixture T1, stratified treatments T2, T3, T4, T5.



Figure 2 Rooting of *Spathiphyllum* plants five weeks after transplanting: plant roots reached the bottom layers. Top to bottom: reference peat/coir mixture T1, stratified treatments T2, T3, T4, T5. Left to right (except the first row): standard fertigation (F1), modified fertigation (F2 and F3).

Nine weeks after transplanting, the plants in the stratified treatment T5 performed better than those in treatment T4, although both lagged behind the other treatments (Figure 3). Fifteen weeks after transplanting, plants in stratified treatment T2 were comparable to plants in reference peat/coir mixture under standard fertigation (Table 5, Figure 4). Plants in stratified treatment T3 had only 30% of the biomass of those in reference peat/coir mixture under standard fertigation (F1); however, increasing irrigation frequencies and nitrate concentration in nutrient solution (F2, F3) doubled plant biomass. In the treatments T4 and T5 where higher proportion of less stable raw materials were used, plant fresh biomass reduction was significant; although increased irrigation frequency and adjusted fertiliser recipes improved plant biomass, it remained much below the reference level. Since the maintenance fertiliser was applied at nearly double the frequency and 40% extra nitrate was supplied, the base fertiliser calculation should be improved for mixtures with a high proportion of less stable raw materials (as in treatments T4 and T5). Adding slow-release N fertiliser is an option to enhance nitrogen availability to the plants.

Table 5 Plant biomass per pot measured at 15 weeks after transplanting.

Treatment		Fresh biomass per pot* (g)		
		F1	F2	F3
T1	Non-stratified, reference (M1)	99 ± 9 a	93 ± 12 a	98 ± 8 a
T2	Stratified: top (M1), bottom (M2)	103 ± 10 a	85 ± 8 a	97 ± 13 a
T3	Stratified: top (M3), bottom (M4)	32 ± 9 b	66 ± 13 b	62 ± 9 b
T4	Stratified: top (M5), bottom (M4)	14 ± 4 c	31 ± 8 c	34 ± 5 d
T5	Stratified: top (M6), bottom (M7)	28 ± 5 b	37 ± 7 c	48 ± 6 c

* Five plugs per pot. F1: standard fertigation treatment for non-stratified peat/coir mixture T1; F2 and F3: modified fertigation treatments with increased fertigation frequency and nutrient strength. Different lower case letters indicate statistically significant differences in means among five treatments at each fertigation regimes in each column (Tukey's HSD, $p \leq 0.05$, $n = 8$ pots).



Figure 3 *Spathiphyllum* plants at nine weeks after transplanting. Top to bottom: standard fertigation (F1), modified fertigation (F2 and F3). Left to right: reference peat/coir mixture T1, stratified treatments T2, T3, T4 and T5.

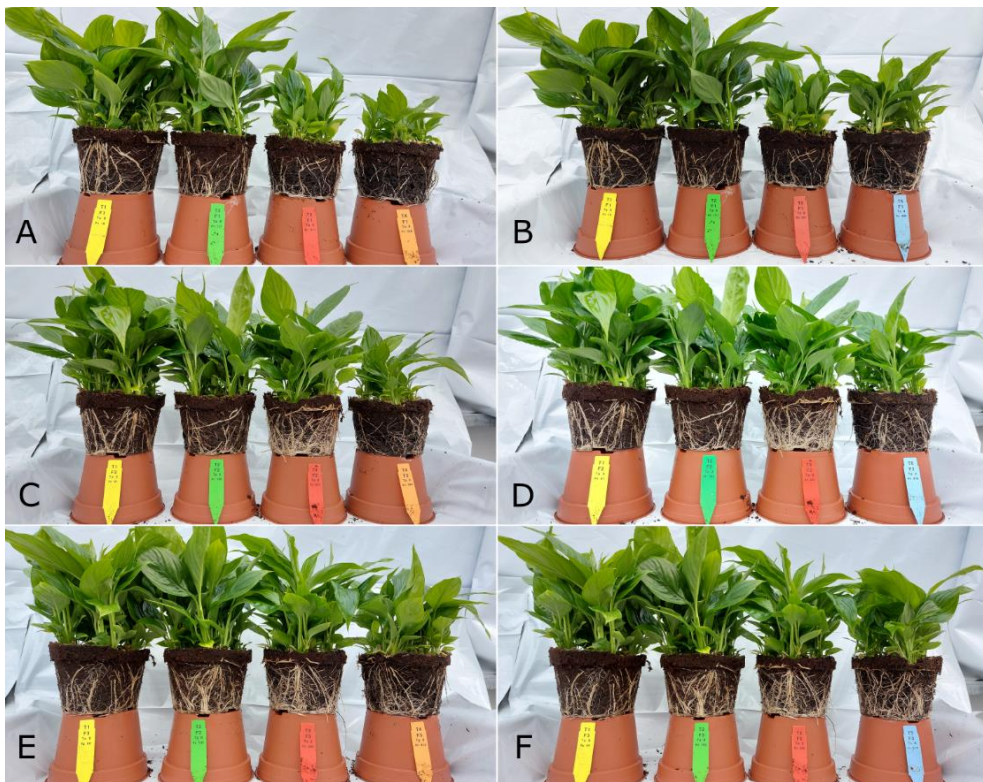


Figure 4 *Spathiphyllum* plants at 15 weeks after transplanting. Top to bottom: standard fertigation F1 (A & B), modified fertigation F2 (C & D) and F3 (E & F). Left to right: T1, T2, T3, and T4 (in A, C and E); T1, T2, T3, and T5 (in B, D, and F).

After the 15th week, the plants were transported to the grower Van der Voort for further observation. All plants eventually flowered. However, the small plants in treatment T3 from F1, T4 and T5 were not marketable, while those in the reference peat/coir mixture, stratified treatment T2, and T3 from F2 and F3 were suitable for sale. Nevertheless, likely due to fertigation via an ebb-and-flow system, the marketable plants reached salable quality later than the typical 15-week growth period for *Spathiphyllum* under overhead irrigation as practiced by the grower.

2.3.2 Moisture content in the pots

Moisture content in the stratified treatments was lower than in the reference treatment, with the decrease ranging from 10% to 20% by volume from treatment T2 to T5 (Figure 5). This reduction was likely due to weaker capillary rise in the bottom layers compared to the reference peat-based mixture. Increasing fertigation frequency resulted in a slight increase in overall moisture content in stratified treatments, with an absolute difference of 5–8% moisture content (Figure 5; compare F2, F3 with F1). Treatments T3 and T4, which shared the same bottom layer M4, exhibited similar moisture retention patterns. Predicted moisture retention in each mixture layer by the WUR Mixing Model showed that the top layer M3 (in stratified treatment T3) holds more water than the top layer M5 (in stratified treatment T4) (Figure 6). This suggests that in an ebb-and-flow system, the bottom layer plays a crucial role in regulating overall pot moisture. However, as moisture content was measured by weighing the entire pot, we do not have data for the changes in moisture content for each layer in between irrigation cycles.

Visual observations showed that the top layers remained moist across all stratified treatments. This suggested that capillary rise of the nutrient solution was occurring to some extent, though not as strongly as in the reference mixture. Comparing treatments T4 and T5, both had similar top-layer textures (20% v/v of chip size as miscanthus), but differences in amount of fibrous materials used in the bottom layers – components like wood fibres or acrotelm, which have a long, thread-like structure. The bottom layer of T5 (20% v/v fibre) had lower moisture content than the bottom layer of T4 (50% v/v fibre) (Figure 6). This suggests that adding fibrous materials in the bottom layers can help improve capillary rise. These findings open up new opportunities to optimize texture and proportion of fibrous materials to improve water distribution in stratified growing media in ebb-and-flow systems. Quantification of this principle justifies dedicated follow-up research.

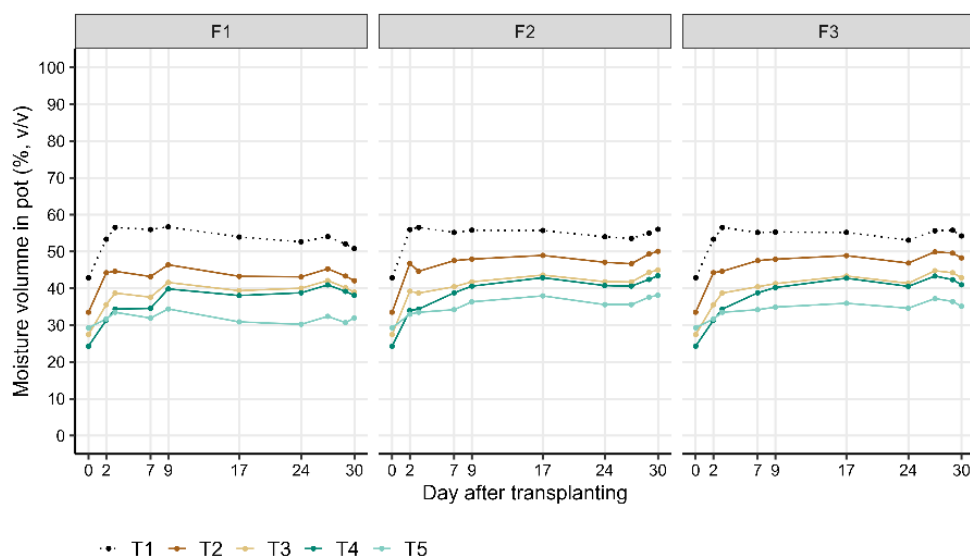


Figure 5 Moisture content of stratified treatments T1, T2, T3, T4 and T5 measured by weighing the pots under standard fertigation treatment for non-stratified peat/coir mixture (F1) and modified fertigation treatments with increased fertigation frequency and nutrient strength (F2, F3).

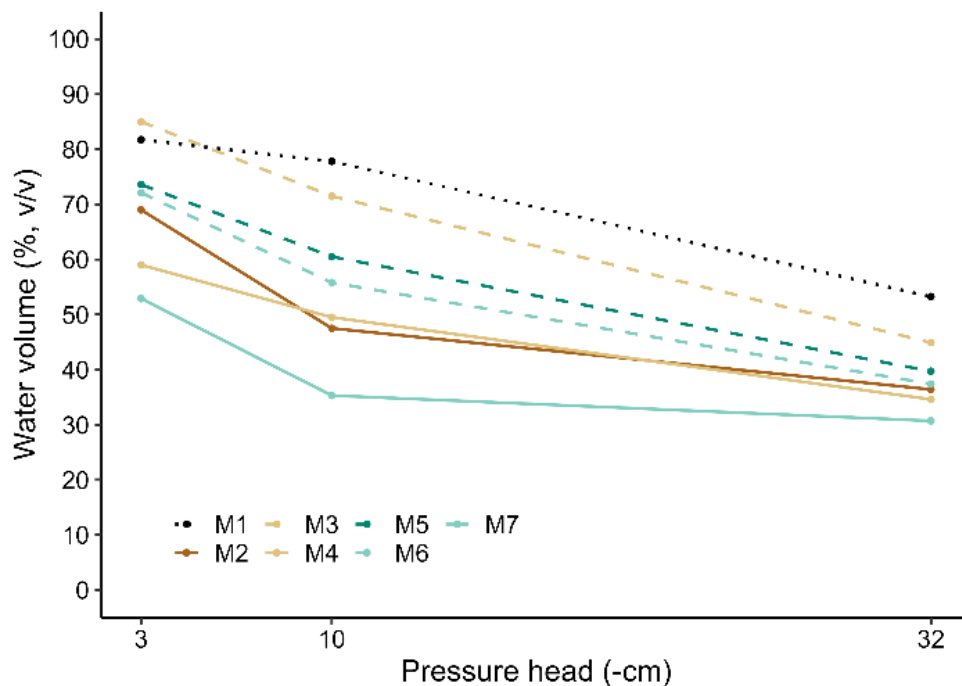


Figure 6 Predicted water retention curves of 7 mixtures (M1, M2, M3, M4, M5, M6, and M7) from WUR Mixing Model.

2.3.3 pH, EC and nutrients in the substrate extraction

In stratified treatment T2, where the top layer is the reference peat-based substrate and the bottom layer is peat-free with a high proportion of less stable raw materials, plant growth was comparable to the reference treatment T1 (Table 4). Nutrient concentration in the top layers were similar between non-stratified peat-based treatment T1 and stratified T2, while nutrient concentration in the bottom layer showed lower values than the top layer (Ca, Mg, NO₃, SO₄, P, Si, Fe, Mn, and B) (Table 6). Nutrient depletion in the bottom layer could be explained by (1) nutrient immobilization due to microbial activity, likely influenced by the proportion of renewable raw materials in the mixtures, and (2) to a lesser extent, nutrient uptake by plant roots, as the fibrous thick roots had already reached the bottom layer at this point. At 5 weeks after transplanting, lateral roots were observed only in the top layers. This suggested that nutrients supplied from both base fertilisers in the top layer and fertigation were sufficient for plant growth at this stage.

Later, as the plant roots developed further into the bottom layers, they exploit the nutrients available in this zone. Increasing fertigation frequency and nutrient dosage would likely be highly effective at this stage. The similar plant growth observed up to the 15th week supports the idea of higher root activity in the lower layer in stratified treatment T2. Although root biomass was not measured, visual observations indicated that the bottom layer in T2 had more roots compared to the bottom layer in non-stratified T1 (Figure 7).

In stratified treatment T3, where the top layer was peat-free with 50% coir, plant growth was reduced by 25% (Table 5). Substrate extraction showed lower nutrient levels in the top layers compared to the reference peat-based (Ca, Mg, NO₃, P, Fe, Mn, B) (Table 6). In the bottom layer, compared to T2, the most significant difference was in nitrate level, while Ca and Mg were not different. When fertigation frequency and nutrient dose were increased in F2 and F3, nitrate levels doubled, leading to an increase in biomass in F2 and F3. Thus nitrate availability is a key factor to explain and in future to avoid growth reductions.

In stratified treatments T4 and T5, where the top layers were peat free with a low percentage of coir pith, the addition of base fertilisers and extra fertigation was not sufficient to significantly improve plant growth. This means the model used must be adapted to become more valid for the new materials.

Table 6 pH, EC and nutrients in the substrate 1:1.5 water extraction analysis, samples collected at 36 days after transplanting.

Fertigation	Treatment	Layer	EC	pH	NH ₄	K	Na	Ca	Mg	NO ₃	Cl	SO ₄	HCO ₃	P	Si	Fe	Mn	Zn	B	Cu	Mo
			mS/cm	mmol/L extract										µmol/L extract							
F1	T1	whole	1.3	5.8	<0.1	2.9	0.4	2.6	1.2	6.9	0.3	1.5	0.1	0.71	0.12	4.8	1.4	1.9	3.9	0.1	0.1
	T2	top	1.4	5.6	<0.1	3.4	0.4	2.4	1.2	6.7	0.2	1.8	<0.1	0.77	0.11	7.3	2.2	1.5	6.6	<0.1	<0.1
	T2	bottom	0.6	6.4	<0.1	2.2	0.1	0.5	0.3	2.2	<0.1	0.6	<0.1	0.29	<0.02	2.1	0.6	1.6	2.2	<0.1	<0.1
	T3	top	0.5	7	<0.1	2.4	0.3	0.3	0.1	<0.2	0.5	1.2	<0.1	0.23	0.4	3.4	<0.4	2.7	2.6	0.1	<0.1
	T3	bottom	0.5	7.5	<0.1	2.5	0.1	0.4	0.3	0.4	0.1	0.6	1.8	0.22	0.24	2.6	<0.4	1.2	2.1	<0.1	<0.1
	T4	top	0.4	7	<0.1	2.3	0.3	0.2	<0.1	0.2	0.5	0.9	0.1	0.15	0.34	4.4	<0.4	1.4	3	0.2	0.2
	T4	bottom	0.5	7.4	<0.1	2.5	0.2	0.4	0.2	0.3	0.1	0.7	1.6	0.23	0.27	3	<0.4	1.2	2.2	0.1	<0.1
	T5	top	0.4	6.9	<0.1	2.2	0.3	0.3	0.1	<0.2	0.4	1.1	<0.1	0.27	0.23	5.4	<0.4	2.1	2.5	0.2	<0.1
	T5	bottom	0.3	7.3	<0.1	1.6	0.1	<0.1	<0.1	0.5	0.1	0.5	0.2	0.13	0.12	2.2	<0.4	1.3	1.8	0.1	<0.1
	F2	T2	top	1	5.8	<0.1	2.7	0.3	1.7	0.8	4.8	0.2	1.3	<0.1	0.53	0.1	7.2	1	1.4	3.9	<0.1
T2		bottom	0.7	6.2	<0.1	2.6	<0.2	0.7	0.4	3.1	0.1	0.8	<0.1	0.37	0.03	2.8	0.9	2.1	2.8	0.1	<0.1
T3		top	0.6	7.1	<0.1	3	0.4	0.3	0.2	0.2	0.5	1.4	<0.1	0.31	0.49	4.1	0.4	3.5	3	0.2	<0.1
T3		bottom	0.6	7.4	<0.1	2.5	0.1	0.5	0.3	1	0.2	0.7	1.4	0.26	0.26	2.8	0.4	0.9	2.6	<0.1	<0.1
T4		top	0.5	7	<0.1	2.8	0.3	0.3	0.1	<0.2	0.7	1.2	<0.1	0.2	0.35	4.1	<0.4	1.6	2	0.2	<0.1
T4		bottom	0.5	7.3	<0.1	2.1	0.2	0.4	0.2	0.5	0.2	0.5	1.4	0.2	0.23	2.6	<0.4	0.6	2	<0.1	<0.1
T5		top	0.5	7	<0.1	2.4	0.3	0.3	0.2	<0.2	0.3	1.3	<0.1	0.26	0.32	5.8	<0.4	2.1	2.6	0.2	<0.1
T5		bottom	0.4	7	<0.1	1.7	<0.1	0.2	0.1	1	<0.1	0.4	0.2	0.17	0.12	1.4	<0.4	0.6	1.3	<0.1	<0.1
F3	T2	top	1.5	5.5	<0.1	3.5	0.5	3	1.5	8.8	0.3	1.6	<0.1	0.85	0.15	8	2.3	1.9	5.2	<0.1	<0.1
	T2	bottom	0.6	6.1	<0.1	1.9	0.1	0.6	0.3	2.8	<0.1	0.4	<0.1	0.25	0.02	1.7	0.6	1.2	1.7	<0.1	<0.1
	T3	top	0.5	7	<0.1	2.3	0.3	0.2	0.1	0.2	0.4	0.9	0.2	0.2	0.4	3.6	<0.4	2.7	2	0.2	<0.1
	T3	bottom	0.7	7.3	0.1	2.7	0.1	0.7	0.5	1.7	0.2	0.5	2.4	0.3	0.31	3.3	<0.4	1	2.8	<0.1	<0.1
	T4	top	0.6	7.1	<0.1	2.9	0.4	0.5	0.2	0.2	1.2	1	0.3	0.19	0.32	9.8	1.1	2	2.4	0.3	<0.1
	T4	bottom	0.6	7.3	<0.1	2.3	0.1	0.6	0.4	0.9	0.1	0.4	2.3	0.26	0.24	2.4	0.5	0.7	1.7	<0.1	<0.1
	T5	top	0.4	6.9	<0.1	1.8	0.2	0.2	<0.1	0.2	0.3	0.7	<0.1	0.18	0.25	5.3	<0.4	2	1.9	0.3	<0.1
	T5	bottom	0.4	7	<0.1	1.7	0.1	0.2	0.2	1.4	0.1	0.3	0.2	0.17	0.11	1.3	<0.4	0.4	1.3	<0.1	<0.1



Figure 7 *Spathiphyllum* roots of the best plants in each treatment at the 15 week after transplanting. Left to right: reference non-stratified peat-based mixture T1 at reference fertigation F1, stratified mixture T2 with top layer as reference mixture at reference fertigation F1, stratified peat-free mixture T3 at modified fertigation F2, stratified peat-free mixture T4 and T5 at modified fertigation F3.

2.4 Conclusions for the trial at WUR

For *Spathiphyllum* in ebb-and-flow fertigation systems, some considerations for designing stratified growing media:

- **Top layer:** When using a reference substrate as the top layer and a peat-free mixture as the bottom layer (in this trial: 20% acrotelm fine/30% wood fibre coarse/50% bark coarse), plant performance was comparable to that in a non-stratified reference peat-based mixture (70% white peat/30% coir pith) under standard fertigation conditions.
- **Importance of the top layer at the beginning:** base fertilization is crucial in ebb-and-flow systems to support early plant development. Addition of slow-release nitrogen fertiliser can further enhance nutrient availability.
- **Bottom layer:** to facilitate capillary rise to the top layer, the bottom layer should contain about 50% fibrous materials (by volume).

This proof of concept has just scratched the surface of the possibilities with stratification. Some practically relevant ideas for follow-up research include:

- **Moisture and oxygen dynamics in different layers:** How do moisture content and oxygen content in each layer change between irrigation cycles? Understanding these dynamics is key to optimizing the moisture profile throughout the growing medium. The goal is to find the ideal moisture distribution over the height of the system that supports both plant growth and efficient transport to customers.
- **Nutrient dynamics in different layers:** It remains unclear whether microbial activity was initially higher in the bottom layer and decreased over time. Identifying the optimal distribution of directly available nutrients versus slow-release nutrients in each layer is critical for improving plant health and maximizing nutrient efficiency.
- **Base fertilization in the bottom layers:** in ebb-and-flow systems, is it necessary to add base fertilisers to the bottom layer, or is fertigation alone sufficient? Finding the right balance between base fertilization and fertigation is crucial to ensure adequate nutrient supply throughout the system. This research should aim to optimize these processes for various irrigation and fertilization systems, or alternatively, provide guidance on adapting current systems for better performance.

3 Growth trials at the growers

3.1 Goal

This activity aimed to evaluate the performance of stratified growing media under normal practise at the growers.

3.2 Methodology

Five growth trials were conducted at growers' facilities for Spathiphyllum, Acer, and Laurus (Table 7). The reference and stratified substrates were managed using the same standard cultivation practices. The substrate mixtures were formulated by the substrate producers and remain confidential. However, despite this confidentiality, some insights into the stratified design are provided in this section to aid in result interpretation.

Plant growth was monitored using non-destructive measurements, including shoot length, crown diameter, and SPAD values (measured with a SPAD meter). At the final crop visit, plant biomass (including both shoot and root, without separation) was assessed. Moisture content in the pots was determined by weighing them, and substrate samples were collected for 1:1.5 water extraction performed by Normec Groen Agro Control.

Differences in mean values among treatments were analyzed using a one-way ANOVA test ($p \leq 0.05$). If a significant difference was found, Tukey's HSD post-hoc test ($p \leq 0.05$) was performed. Statistical analyses were conducted using R software (version 4.3.1).

Table 7 Overview of five growth trials at the growers in 2024.

	Crop	Grower	Pot diameter (cm)	Pot volume (L)	Duration (week)	Calendar week	Irrigation	Number of plants per treatment
1	Spathiphyllum	Bestplant	24	7.5	24	Wk 9 – wk 33	Head sprinkler	20
2	Spathiphyllum	Van der Voort	12	0.9	15	Wk 15 – Wk 30	Head sprinkler	40
3	Spathiphyllum	Van der Voort	17	2.5	15+	Wk 15 – Wk 30	Head sprinkler	40
4	Acer	Van Son & Koot		3.2	18	Wk 15 – Wk 33	Head sprinkler	20
5	Laurus nobilis	Gova	14	1	17	Wk 16 – Wk 33	Head sprinkler	40

3.2.1 Spathiphyllum in 7.5 L pots at the grower Bestplant

Two reference mixtures (one peat-based and one coir-based) and four stratified treatments were tested in this trial (Table 8). Two mixtures were designed for the top layer and two for the bottom layer. By combining these four mixtures in equal proportions (each occupying half of the pot volume), four stratified mixtures were created. All four stratified mixtures were peat-free. Base fertilisers were added to all mixtures to achieve a target pH of 5.2 and an EC of 1.0 mS/cm.

Table 8 *Treatments used at the grower Bestplant.*

Treatment	Color code	Top layer	Bottom layer	Proportion top/layer
Ref 1	Blue	Non-stratified coir-based (Mix B) (45 LS)		
Ref 2	Yellow	Non-stratified peat-based		
Proef 1	Purple	Mix_A (50 LS)	Mix_B (45 LS)	50/50
Proef 2	Orange	Mix_A (50 LS)	Mix_D (20 LS)	50/50
Proef 3	Green	Mix_C (30 LS)	Mix_B (45 LS)	50/50
Proef 4	white	Mix_C (30 LS)	Mix_D (20 LS)	50/50

LS: proportion of raw materials in each layer, which are less stable than peat or coir.

3.2.2 Spathiphyllum in 1 L pots at the grower Van der Voort

One reference mixture (peat-based) and two stratified treatments were tested in this trial (Table 9). The top layer consisted of the reference mixture, while the bottom layer was a peat-free mixture. Both layers occupied equal proportions of the container. Reference mixture B is the same as reference mixture A, but contains double the amount of base fertiliser.

Table 9 *Treatments used at the grower Van der Voort location 1.*

Treatment	Color code	Top layer	Bottom layer	Proportion top/layer
Ref	Blue	Non-stratified peat-based (Ref A) (31 LS)		
Proef 1	Purple	Ref_A (31 LS)	Mix_C (100 LS)	50/50
Proef 2	Orange	Ref_B (31 LS)	Mix_C (100 LS)	50/50

LS: proportion of raw materials in each layer, which are less stable than peat or coir.

3.2.3 Spathiphyllum in 2.5 L pots at the grower Van der Voort

One reference mixture (peat-based) and two stratified treatments were tested in this trial (Table 10). In both stratified treatments, the top layer consisted of the reference mixture, while the bottom layer was a peat-free mixture. One treatment had equal proportions of both layers (50/50), while the other had a greater proportion of the top layer (75/25).

Table 10 *Treatments used at the grower Van der Voort location 2.*

Treatment	Color code	Top layer	Bottom layer	Proportion top/layer
Ref	Yellow	Non-stratified peat-based (Ref A) (31 LS)		
Proef 1	Green	Ref_A (31 LS)	Mix_B (100 LS)	50/50
Proef 2	White	Ref_A (31 LS)	Mix_B (100 LS)	75/25

LS: proportion of raw materials in each layer, which are less stable than peat or coir.

3.2.4 Acer in 3.2 L pots at the grower Van Son en Koot

One reference mixture (peat-based) and four stratified treatments were tested in this trial (Table 11). The top layers consisted of peat-based mixtures, while the bottom layers were peat-free mixtures. Two treatments had equal proportions of both layers (50/50), while the other two had a greater proportion of the top layer (75/25). Five different strains of Acer were used in all treatments: Going Green, Little Princess, Katsura, Orange Dream, and Beni-Maiko.

Table 11 Treatments used at the grower Van Son en Koot.

	Color code	Top layer	Bottom layer	Proportion top/layer
Ref	Blue	Non-stratified peat-based (Ref A)		
Proef 1	Purple	Ref_A (0 LS)	Mix_B (100 LS)	50/50
Proef 2	Orange	Ref_A (0 LS)	Mix_B (100 LS)	75/25
Proef 3	Green	Ref_B (35 LS)	Mix_B (100 LS)	50/50
Proef 4	White	Ref_B (35 LS)	Mix_B (100 LS)	75/25

LS: proportion of raw materials in each layer, which are less stable than peat or coir.

3.2.5 Laurus nobilis in 1 L pots at the grower Gova

One non-stratified coir-based reference mixture and four stratified treatments were tested (Table 12). The stratified treatments consisted of two different top layers combined with a single bottom layer in two proportions (50/50 and 75/25).

Table 12 Treatments used at the grower Gova.

	Color code	Top layer	Bottom layer	Proportion top/layer
Ref	Blue	Non-stratified coir-based (Ref A) (35 LS)		
Proef 1	Purple	Ref_A (35 LS)	Mix_B (100 LS)	50/50
Proef 2	Orange	Ref_A (35 LS)	Mix_B (100 LS)	75/25
Proef 3	Green	Ref_B (35 LS)	Mix_B (100 LS)	50/50
Proef 4	White	Ref_B (35 LS)	Mix_B (100 LS)	75/25

LS: proportion of raw materials in each layer, which are less stable than peat or coir.

3.3 Results & discussions

3.3.1 Spathiphyllum in 7.5 L pots at the grower Bestplant

Final biomass (including both shoot and root mass, without separation) in stratified treatments Proef 1, Proef 3, and Proef 4 showed no significant differences compared to the two reference mixtures. However, stratified treatment Proef 2 had significantly lower fresh biomass, approximately 75% of the biomass of the reference mixtures. This could be explained by the fact that Proef 2 contained a higher proportion of less stable raw materials (wood and bark) compared to the reference mixtures (Table 8).

Figures 8–10 clearly show that the peat-based Reference mixture 2 resulted in more compact plants compared to the peat-free Reference mixture 1 (Table 13). The plants in the peat-based Reference mixture produced fewer roots compared to the peat-free reference mixture and all peat-free stratified mixtures. These differences can be explained by the fact that peat-based mixtures retain water and nutrients more effectively for a longer period than peat-free mixtures. As a result, plants in peat-free mixtures develop more roots to actively search for water and nutrients.

Another observation is that Proef 2 and Proef 4 also produced more compact plants. This appears to be due to the use of coarser materials in the bottom layers (Mix D), which led to shorter and more compact plants compared to Proef 1 and Proef 3, which used a less coarse bottom layer (Mix B).

Table 13 Shoot length, crown diameter and SPAD values of *Spathiphyllum* at the grower Bestplant.

Treatment	Shoot length (cm)				Crown diameter (cm)				SPAD value		Shoot & root biomass (g)
	start	5 WAT	14 WAT	24 WAT	start	5 WAT	14 WAT	24 WAT	14 WAT	24 WAT	24 WAT
Ref 1	23.4	23.8	40.8	73.2	22.6	38.6	64.4	76.4	52.0	57.0	1267 ± 58 a
Ref 2	22.0	26.0	45.6	68.0	22.8	35.2	57.2	72.8	53.9	58.1	1233 ± 153 a
Proef 1	22.6	25.6	45.2	77.0	24.4	32.4	59.4	79.4	55.1	58.6	1200 ± 100 ab
Proef 2	21.4	24.2	45.4	71.0	19.8	37.8	56.0	73.2	54.1	60.3	900 ± 173 b
Proef 3	22.0	23.6	40.6	74.4	19.8	36.2	63.4	76.0	51.8	58.9	1133 ± 58 ab
Proef 4	21.4	25.2	47.4	69.4	23.2	35.6	62.2	79.6	53.9	59.3	1100 ± 100 ab

Mean ± standard deviation, n = 5 pots. WAT: weeks after transplanting. Different lower case letters indicate statistically significant differences in means among treatments (Tukey's HSD, $p \leq 0.05$, n = 5 pots).



Figure 8 *Spathiphyllum* plants at the grower Bestplant at 24 weeks after transplanting. Left to right: ref 1, ref 2, proef 1, proef 2, proef 3 and proef 4, with 2 plants each.



Figure 9 *Spathiphyllum* plants at the grower Bestplant at 24 weeks after transplanting. Left to right: ref 1, ref 2, proef 1, proef 2, proef 3 and proef 4.



Figure 10 *Spathiphyllum* roots of the trial at Bestplant at the 24 week after transplanting. Top left to bottom right: ref 1, ref 2, proef 1, proef 2, proef 3 and proef 4.

Table 14 pH, EC and nutrients in the substrate 1:1.5 water extraction analysis of substrates sampled from the trials.

Treatment	Layer	pH	EC	NH ₄	K	Na	Ca	Mg	NO ₃	Cl	SO ₄	HCO ₃	P	Si	Fe	Mn	Zn	B	Cu	Mo	
			mS/cm																		mmol/L extract
Spathiphyllum at the grower Bestplant (24 weeks after transplanting)																					
Ref 1	Whole	5.9	0.87	< 0.1	1.9	1	1.5	1.2	0.2	4.2	< 0.1	1.4	0.3	0.89	2.9	0.2	1.3	6.2	0.5	< 0.1	
Ref 2	Whole	5.6	1.2	< 0.1	2.5	1.1	2.1	1.8	0.1	5.5	< 0.1	2.1	0.3	1.3	10.6	0.3	1.6	4.8	0.1	< 0.1	
Proef 1	Top	5.3	1.9	< 0.1	3	1.5	4.9	3.2	0.3	7.3	< 0.1	5.5	< 0.1	2	11.8	3.5	4	14	0.8	< 0.1	
Proef 1	Bottom	6.1	0.86	< 0.1	1.8	0.8	1.5	1.1	0.2	4.5	< 0.1	1.2	0.6	0.83	2	< 0.1	1.3	5.8	0.5	< 0.1	
Proef 2	Top	5.3	2.1	< 0.1	3.4	1.7	5.3	3.4	0.3	8.6	< 0.1	5.8	< 0.1	2	8.4	2.5	4	13.8	0.7	< 0.1	
Proef 2	Bottom	6.4	0.76	< 0.1	1.6	0.8	1.3	0.9	0.2	3.4	< 0.1	1.2	0.7	0.73	1.1	< 0.1	1.4	4.3	0.5	< 0.1	
Proef 3	Top	6.2	2	< 0.1	3.8	1.8	5.3	3.1	0.3	7.8	< 0.1	6.3	0.5	1.4	2.1	0.1	5.4	8.7	2	< 0.1	
Proef 3	Bottom	6.3	0.78	< 0.1	1.7	0.8	1.4	1	0.2	4.4	< 0.1	1.1	0.6	0.74	1.6	< 0.1	1.5	5.3	0.6	< 0.1	
Proef 4	Top	6.1	2.1	< 0.1	4.3	2	5.4	3.2	0.4	8	< 0.1	6.5	0.6	1.4	2.6	0.2	4.3	9.6	1.8	< 0.1	
Proef 4	Bottom	6.4	0.76	< 0.1	1.7	0.8	1.3	1	0.2	3.5	< 0.1	1.2	0.6	0.73	1.2	< 0.1	1.8	< 4	0.7	< 0.1	
Spathiphyllum in 1 L pots at the grower Van der Voort, location 1 (15 weeks after transplanting)																					
Ref	Whole	6.6	0.88	< 0.1	0.7	0.8	2.6	1.2	0.2	2	1.3	2.2	0.6	0.58	2.7	< 0.1	2.3	< 4	0.3	< 0.1	
Proef 1	Whole	6.4	0.79	< 0.1	0.4	0.9	2.4	1.1	0.1	1.4	1.2	2.2	0.6	0.55	3.2	0.2	1.8	< 4	0.2	< 0.1	
Proef 2	Whole	6.2	0.72	< 0.1	0.6	0.7	2	0.9	0.1	1.3	1.2	1.9	0.3	0.49	3.1	0.1	1.4	< 4	0.3	0.1	
Spathiphyllum in 2.5 L pots at the grower Van der Voort, location 2 (15 weeks after transplanting)																					
Ref	Whole	6.7	0.88	< 0.1	1.7	0.9	1.8	1.2	0.2	2.5	0.9	1.9	0.6	0.67	5.3	< 0.1	4.4	< 4	1	0.2	
Proef 1	Whole	6.3	0.8	< 0.1	1.4	1	1.6	1.2	0.2	1.9	0.9	1.8	0.6	0.72	6.6	0.2	3.5	< 4	0.7	< 0.1	
Proef 2	Whole	6.6	0.84	< 0.1	1.3	1.1	1.8	1.3	0.2	1.7	0.9	2.3	0.6	0.67	6.4	0.1	4.3	< 4	0.9	0.1	
Acer at the grower Van Son & Koot (18 weeks after transplanting)																					
Ref	Whole	4.9	0.30	< 0.1	0.5	0.6	0.5	0.3	< 0.1	0.1	0.1	1.0	< 0.1	0.07	7.2	1.0	0.5	6.2	0.2	< 0.1	
Proef 1	Top	5.0	0.24	< 0.1	0.5	0.4	0.4	0.3	< 0.1	0.4	0.1	0.7	< 0.1	0.09	6.9	0.6	0.5	< 4	0.2	< 0.1	
Proef 1	Bottom	4.9	0.20	< 0.1	0.6	0.4	0.2	0.1	< 0.1	< 0.1	0.1	0.7	< 0.1	< 0.05	4.6	2.4	0.4	4.8	< 0.1	< 0.1	
Proef 2	Top	4.9	0.25	< 0.1	0.5	0.4	0.4	0.2	< 0.1	0.2	0.1	0.7	< 0.1	0.08	7.4	0.8	0.5	< 4	0.2	< 0.1	
Proef 2	Bottom	4.9	0.21	< 0.1	0.6	0.4	0.2	0.1	< 0.1	< 0.1	0.1	0.6	< 0.1	< 0.05	2.8	2.0	0.3	5.0	< 0.1	< 0.1	
Proef 3	Top	5.1	0.27	< 0.1	0.5	0.5	0.4	0.2	< 0.1	0.2	0.1	0.8	0.2	0.08	7.9	0.6	0.6	4.7	0.2	< 0.1	
Proef 3	Bottom	5.1	0.18	< 0.1	0.5	0.4	0.2	0.1	< 0.1	< 0.1	0.1	0.6	0.3	< 0.05	5.2	1.4	0.4	5.0	< 0.1	< 0.1	
Proef 4	Top	5.0	0.31	< 0.1	0.6	0.5	0.7	0.4	< 0.1	0.2	0.2	0.9	< 0.1	0.08	10.3	1.6	0.8	4.8	0.4	< 0.1	
Proef 4	Bottom	5.2	0.15	< 0.1	0.5	0.3	< 0.1	< 0.1	< 0.1	< 0.1	0.1	0.4	< 0.1	< 0.05	3.7	0.7	0.2	4.8	< 0.1	< 0.1	
Laurus at the grower Gova (17 weeks after transplanting)																					
Ref	Whole	5.4	0.63	< 0.1	1.2	0.4	1.4	0.7	0.3	3.2	< 0.1	0.9	0.1	0.61	4.4	0.8	1.3	12.5	0.2	< 0.1	
Proef 1	Whole	5.2	0.67	< 0.1	1.2	0.3	1.6	0.7	0.2	3.9	< 0.1	0.7	< 0.1	0.63	4.9	1.1	1.5	10.1	0.2	< 0.1	
Proef 2	Whole	5.3	0.56	< 0.1	1.1	0.2	1.3	0.5	0.2	3.3	< 0.1	0.6	0.2	0.56	4.2	0.6	1.7	6.4	0.1	< 0.1	
Proef 3	Whole	5.4	0.56	< 0.1	1.1	0.3	1.2	0.6	0.2	3.1	< 0.1	0.7	0.3	0.51	3.6	0.4	0.9	8.7	0.1	< 0.1	
Proef 4	Whole	5.2	0.66	< 0.1	1.2	0.3	1.6	0.7	0.3	3.8	0.1	0.7	< 0.1	0.63	4.3	0.7	2.0	7.8	0.1	< 0.1	

Substrate analysis showed that mixtures at the same position (either top or bottom) had similar values, regardless of the composition of the other layer (Table 14). Also, nutrient levels were higher in the top layers than in the bottom layers. This could be attributed to several factors, including (1) nutrient application via overhead sprinklers, (2) potentially stronger root activity in the bottom layer, and (3) differences in the biostability of raw materials used in each mixture.

Moisture content was slightly higher in the stratified treatments compared to the peat-free reference mixture (Ref 1), with an increase of 5% to 10% (v) (Table 15). However, moisture levels in the stratified treatments were comparable to those in the peat-based reference mixture (Ref 2).

Table 15 Moisture content in the whole pots (% , volume) at the grower Bestplant.

Treatment	Moisture content at potting before irrigation (% , v)	Moisture content at the 24 WAT (% , v)
Ref 1	30%	29%
Ref 2	11%	36%
Proef 1	29%	34%
Proef 2	29%	39%
Proef 3	31%	36%
Proef 4	30%	36%

WAT: weeks after transplanting.

3.3.2 Spathiphyllum in 1 L pots at the grower Van der Voort

No major differences in plant growth were observed between the stratified treatments and the reference mixture, based on visual assessments (shoot length, crown diameter, and SPAD values) as well as total fresh biomass (shoot and roots). The only differences observed were that the reference and Proef 2 were slightly taller and produced flowers one week earlier than those in Proef 1. Additionally, more root formation was observed in Proef 1 and these roots were bigger with fewer root hairs (Table 16, Figure 11-13). Nutrient data was collected for the entire pot, so there is no conclusion about the nutrient dynamic among the layers (Table 14). The moisture content in the whole pot was slightly lower in the stratified treatment of Proef 1, with an absolute difference of 4% compared to the reference and 6% compared to the stratified treatment of Proef 2 (Table 17). No clear explanation can be drawn here, as both Proef 1 and Proef 2 shared the same mixture recipes for both layers, with the only difference being the base fertilisers in the top layer. One possible explanation is that the difference in plant biomass (shoot and root) contributed more to the calculation of moisture content, especially in the small pot size (1L). The average total biomass of Proef 1 was slightly higher than that in the reference and Proef 2, which may have contributed to the observed differences.

Table 16 Shoot length, crown diameter and SPAD values of *Spathiphyllum* in 1 L pots at the grower Van der Voort.

Treatment	Shoot length (cm)				Crown diameter (cm)				SPAD value			Shoot & root biomass (g)
	start	3 WAT	9 WAT	15 WAT	start	3 WAT	9 WAT	15 WAT	3 WAT	9WAT	15 WAT	15 WAT
Ref	4.3	5	12.6	26.2	5.0	7.8	22.6	37.4	23.9	34.8	44.3	96.3 ± 12 ns
Proef 1	4.5	4.8	13.0	22.2	5.0	7.4	21.8	38.8	23.6	36.5	45.5	112.7 ± 3
Proef 2	4.1	5	12.0	24.4	4.8	7.8	21.8	41.6	24.1	37.4	47.8	97.4 ± 15 a

WAT: weeks after transplanting. Shoot & root biomass: mean ± standard deviation, ns = non-significant difference (ANOVA, p ≤ 0.05, n = 3 pots).



Figure 11 *Spathiphyllum* plants in 0.9 L pots at the grower Van der Voort at 15 weeks after transplanting. Left to right: ref, proef 1 and proef 2, three plants each.



Figure 12 *Spathiphyllum* plants in 0.9 L pots at the grower Van der Voort at 15 weeks after transplanting. Left to right: ref, proef 1 and proef 2.



Figure 13 *Spathiphyllum* roots of the trials at Van der Voort in 0.9 L at 15 weeks after transplanting. Left to right: ref, proef 1 and proef 2.

Table 17 Moisture content in the whole pots (% , volume) at the grower Van der Voort (1 L pot).

Treatment	Moisture content at potting before irrigation (% , v)	Moisture content at 15 WAT (% , v)
Ref 1	14%	20%
Proef 1	13%	16%
Proef 2	13%	22%

WAT: weeks after transplanting.

3.3.3 Spathiphyllum in 2.5 L pots at the grower Van der Voort

No significant differences in plant growth were detected (Table 18). However, around 12 weeks after transplanting, older leaves began to show slight yellowing, suggesting that a change in fertigation may be needed in this trial. Unexplainably the discoloration in the leaves was no longer observed during the final visit to the grower (Figure 14). Moisture content in the whole pot was similar between the reference and stratified treatments (Table 19). The only notable difference was that the green-labeled plants (Proef 1) exhibited significantly more root development (Figure 14-15). This can be explained by the absence of base fertilisers in the bottom layer (in both Proef 1 and Proef 2) and by Proef 1 receiving only half the base fertiliser dose in its top layer compared to Proef 2.

It is important to note that the bottom layer in this experiment (Proef 1 and Proef 2) did not contain any base fertilisers. As a result, the total fertiliser content in the substrate was only half that of Reference 1. Nevertheless, the plants still grew well.

Table 18 Shoot length, crown diameter and SPAD values of *Spathiphyllum* in 2.5 L pot at the grower Van der Voort.

Treatment	Shoot length (cm)				Crown diameter (cm)				SPAD value			Shoot & root biomass (g)
	start	3 WAT	9 WAT	15 WAT	start	3 WAT	9 WAT	15 WAT	3 WAT	9WAT	15 WAT	
Ref 1	15.4	20.2	33.2	58.2	21.8	29.2	50.4	65.6	36.1	43.0	44.7	405 ± 81 ns
Proef 1	16.8	22.4	37.4	58.0	21.0	30.0	47.0	62.4	34.6	42.3	46.2	412 ± 55
Proef 2	17.0	21.4	35.4	56.8	21.6	30.8	46.8	66.6	35.7	40.7	45.5	434 ± 34

WAT: weeks after transplanting. Shoot & root biomass: mean ± standard deviation, ns = non-significant difference (ANOVA, $p \leq 0.05$, $n = 3$ pots).



Figure 14 *Spathiphyllum* plants in 2.5 L pots at the grower Van der Voort at 15 weeks after transplanting. Left photo: ref, proef 1 and proef 2 in the order from left to right. No yellowing leaves observed anymore.



Figure 15 Roots of *Spathiphyllum* plants in 2.5 L pots at the grower Van der Voort at 15 weeks after transplanting. Left photo: ref, proef 1 and proef 2 in the order from left to right.

Table 19 Moisture content in the whole pots (% , volume) at the grower Van der Voort (2.5 L pot).

Treatment	Moisture content at potting before irrigation (% , v)	Moisture content at 15 WAT (% , v)
Ref 1	25%	38%
Proef 1	22%	38%
Proef 2	24%	38%

WAT: weeks after transplanting.

3.3.4 Acer in 3.2 L pots at the grower Van Son en Koot

No significant differences in plant growth were observed (Table 20). The only difference observed was that plants in the reference treatment produced fewer roots, and those roots were less dispersed throughout the whole pot (Figure 16-17). The differences in shoot length and crown diameter between sampling times were due to pruning for propagation purposes. Substrate extraction showed that nutrient levels were higher in the top layers than in the bottom layer (Table 14). Moisture content was comparable across all treatments, although stratified Proef 1 retained slightly more moisture (approximately 5% v/v) than the reference mixture (Table 21). All treatments in this trial received repeatedly 10 g/m² of calcium nitrate due to nitrogen deficiency. This was likely caused by a later potting date: these plants were transplanted 2–3 weeks after the other plants.

Table 20 Shoot length, crown diameter, SPAD values and biomass of *Acer* plants at the grower Van Son en Koot.

Treatment	Shoot length (cm)				Crown diameter (cm)				SPAD value			Shoot biomass (g)
	start	5 WAT	12 WAT	18 WAT	start	5 WAT	12 WAT	18 WAT	5 WAT	12 WAT	18 WAT	18 WAT
Ref	26.4	35.8	27.4	49.4	22.4	40.4	26.2	49.8	31.1	35.8	37.4	77.1 ± 17 ns
Proef 1	26.8	39.2	27.0	54.2	24.8	34.8	27.6	47.6	30.3	34.5	37.6	65.0 ± 16
Proef 2	24.6	30.6	27.4	61.4	25.8	31.6	28.8	55.2	28.8	34.0	36.7	69.4 ± 6
Proef 3	26.6	32.0	29.2	47.2	20.4	41.0	28.6	58.4	29.0	35.4	37.2	67.4 ± 0.5
Proef 4	27.4	34.6	26.8	49.8	19.2	37.6	26.8	56.0	30.5	36.0	36.7	74.1 ± 12

WAT: weeks after transplanting. Shoot & root biomass: mean ± standard deviation, ns = non-significant difference (ANOVA, $p \leq 0.05$, $n = 3$ pots).



Figure 16 Acer plants at the grower Van Son & Koot 18 weeks after transplanting. Left to right: Ref, proef 1, proef 2, proef 3, and proef 4. Three plants per variable of 3 different strains, with 1. Going Green, 2. Little Princess, 3. Katsura.



Figure 17 Roots of Acer plants at the grower Van Son & Koot at 18 weeks after transplanting. Left to right: Ref, proef 1, proef 2, proef 3, and proef 4. Top: 3 plants per variable of 3 different strains, with 1. Going Green, 2. Little Princess, 3. Katsura. Bottom: Going Green (Proef 1 (Purple) is unrepresentable for the whole batch).

Table 21 Moisture content in the whole pots (% , volume) at the grower Van Son en Koot.

Treatment	Moisture content at potting before irrigation (% , v)	Moisture content at 15 WAT (% , v)
Ref 1	13%	39%
Proef 1	15%	44%
Proef 2	15%	42%
Proef 3	13%	41%
Proef 4	13%	41%

WAT: weeks after transplanting.

3.3.5 Laurus nobilis in 1 L pots at the grower Gova

No significant differences in plant growth were observed among the treatments (Table 22). Since nutrient levels were measured for the whole pot, no conclusions could be drawn about nutrient dynamics between layers (Table 14). Moisture content in the whole pot appeared slightly higher in the stratified treatments compared to the reference non-stratified mixture, though the difference was small (2–5% by volume) (Table 23). Plants in reference treatment had the least root development. Proef 1 and Proef 2 had average root development, whereas Proef 3 and Proef 4 developed more roots (Figures 18–20). This difference in rooting may be attributable to the different top-layer composition used in Proef 3 and Proef 4 compared to that of Proef 1 and Proef 2.

Table 22 Shoot length, crown diameter, SPAD values and biomass of Laurus plants at the grower Gova.

Treatment	Shoot length (cm)				Crown diameter (cm)				SPAD value			Shoot biomass (g)
	start	5 WAT	11 WAT	17 WAT	start	5 WAT	11 WAT	17 WAT	5 WAT	11 WAT	17 WAT	17 WAT
Ref	18.2	18.1	24.8	37.0	19.4	19.58	22.2	26.2	45.6	45.5	47.7	54 ± 7 ns.
Proef 1	18.8	18.5	24.4	36.0	17.0	16.58	22.4	24.0	48.1	47.6	48.3	51 ± 14
Proef 2	17.4	20.4	26.6	41.0	15.8	20.4	23.2	28.4	44.6	45.7	47.6	68 ± 13
Proef 3	18.4	19.8	25.2	36.0	19.2	19.7	24.2	24.8	49.3	46.2	50.4	63 ± 20
Proef 4	16.6	21.4	28.4	47.6	15.8	25.42	27.4	31.8	38.0	46.4	46.2	75 ± 16

WAT: weeks after transplanting. Shoot & root biomass: mean ± standard deviation, ns = non-significant difference (ANOVA, $p \leq 0.05$, $n = 3$ pots).



Figure 18 Laurus plants at the grower Gova at 17 weeks after transplanting. Left to right: Ref, proef 1, proef 2, proef 3, and proef 4, with two plants each.



Figure 19 Roots of *Laurus* plants at the grower Gova at 17 weeks after transplanting. Left to right: Ref, proef 1, proef 2, proef 3, and proef 4.



Figure 20 Roots of *Laurus* plants at the grower Gova at 18 weeks after transplanting. Left to right: Ref, proef 1, proef 2, proef 3, and proef 4.

Table 23 Moisture content in the whole pots (% , volume) at the grower Gova.

Treatment	Moisture content at potting before irrigation (% , v)	Moisture content at 17 WAT (% , v)
Ref 1	17%	18%
Proef 1	12%	23%
Proef 2	13%	20%
Proef 3	11%	23%
Proef 4	11%	21%

WAT: weeks after transplanting.

3.4 Conclusions from the trials at the growers

Under head sprinkler irrigation, trials on *Spathiphyllum*, *Acer*, and *Laurus* led to the following conclusions:

- Replacing the bottom half of the substrate in the pot with a peat-free alternative is effective, as it resulted in similar biomass to the reference under normal practices.
- Replacing the top half of the substrate in the pot with a peat-free alternative is also effective, as it resulted in similar plant growth and similar biomass to the reference under commercially used peat-based practices.
- Initial plant growth, before the roots reached the bottom layer, was comparable between the stratified substrate experiments and the references, indicating that the fertiliser in the substrates combined with overhead sprinkler irrigation supplied sufficient water and nutrients to the top layer.
- To test extremes in all stratified substrate experiments—except for the one at Bestplant—no fertilisers were added to the bottom layer.
- In the *Spathiphyllum* trial at Bestplant, using coarser materials in the bottom layer produced more compact plants, whereas increasing the proportion of wood fibre in the bottom layer resulted in taller plants.
- In the trials at Gova, Van Son & Koot, and Van der Voort, the bottom layers were made extremely coarse to validate the stratified substrate concept. However, this approach overshot the intended effect: instead of achieving a more evenly distributed moisture profile and nutrient gradient, the bottom layer became drier than the top layer (visual observation, no measurements for each layer). Additionally, the absence of fertiliser in the bottom layer may have contributed to reduced plant growth during the later growth phase.

This proof of concept has just scratched the surface of the possibilities with stratification. Some practically relevant ideas for follow-up research include:

- For crops with a longer cultivation period, the timing of root exploration in the bottom layers should be considered, and the fertigation solution should be adjusted accordingly. When plant roots reach the bottom layer, which contains a higher proportion of renewable components, nutrient availability may decrease due to nitrogen immobilization in this layer. Increasing nutrient strength in the fertiliser solution and/or increasing fertigation frequency is necessary to ensure sufficient nutrients to the plants. One possible solution is to add slow-release fertiliser into the bottom layer.
- For future study, it would be valuable to adjust the coarseness of the bottom layer relative to the top layer so that moisture content remains uniform throughout the pot. This could involve testing several bottom-layer mixes with increasing proportions of coarse material, combined with the addition of fertiliser to the bottom layer. Such an approach may enhance plant growth compared to non-stratified substrates, especially given that—even under unadjusted conditions in these pre-trials—plants in the stratified treatments performed as well as those in the reference.
- Additionally, moisture and oxygen dynamics in each layer should be monitored throughout irrigation cycles. Understanding how moisture and oxygen levels fluctuate between cycles is crucial for optimizing the moisture profile in the growing medium. The goal is to establish the ideal vertical moisture distribution that maximizes plant growth and supports efficient post-harvest handling.
- Investigating nutrient dynamics across substrate layers is important to determine whether microbial activity was initially higher in the bottom layer and then declined over time. Additionally, identifying the optimal balance of readily available versus slow-release nutrients in each layer is critical for improving plant health and maximizing nutrient-use efficiency.
- The selection of test plants should include crops that are sensitive to root rot caused by high moisture content at the bottom of the pots. These plants could particularly benefit from stratified substrate layers.
- Finally, in this project, the pots were hand-filled to ensure accurate ratios between the top and bottom layers. In the future, tests should be conducted to automate this process—using two separate potting machines, a combined potting machine capable of adding two layers, or a bark spreader.

4 Conclusions

In this proof-of-concept project, stratified growing media demonstrated potential as a tool for incorporating renewable raw materials into substrate mixtures.

Several conclusions can be drawn from the two activities: the growth trial at the WUR research station and the growth trials at the growers' facilities.

- In both fertigation systems (ebb-and-flow and head sprinkler), stratification—by replacing the bottom half of the peat-based reference substrate—performed similarly to the original peat-based substrate under standard fertigation practices.
- Under standard fertigation practices for the reference mixture, monitoring plant growth (both shoot and root development) is essential to adjusting fertigation regimes in a timely manner.
- For head sprinkler fertigation systems, replacing the top half of the potting substrate with a peat-free alternative is also effective, as it resulted in plant growth and biomass comparable to those achieved with commercially used peat-based practices.
- In ebb-and-flow fertigation, replacing both the top and bottom layers of the peat-based reference substrate with renewable materials resulted in lower performance compared to peat. Several solutions are to be considered:
 - Not using the tested renewable raw materials in the top layer may not be innovative but at least temporarily a safe way forward while additional research is done.
 - Adjusting fertilization improved performance, but the extent of improvement varied depending on the types and proportions of renewable growing media used. The base dressing based on calculations needs to be increased while better taking the materials used into account.
 - In the base dressing, to avoid high EC's, it is needed to increase the fraction of slow release nitrogen fertiliser.
 - The propagation material must be adapted to resemble the cultivation medium enough to allow proper and speedy root growth. For the same reason nutrient levels should be similar.
- Pre-calculating the required base fertilization is essential for success. The base dressing of the lower layer does not need to be very high, as long as the maintenance dressing (via irrigation water) is sufficient when the roots begin to explore the lower layer.
- Ebb-and-flow and overhead irrigation differ significantly in how they supply fertilisers to the top and bottom layers, necessitating distinct base fertilization strategies for each layer.

The key knowledge gaps identified in this study are:

- Predicting base fertilization requirements, taking into account crop type, crop stage, and irrigation system parameters.
- Improving the accuracy of moisture gradient predictions by optimizing the coarseness of the bottom layer for each specific top layer.
- Automating the potting in stratified layers in a commercially viable manner.

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Annex 1 Growth trial at WUR research station

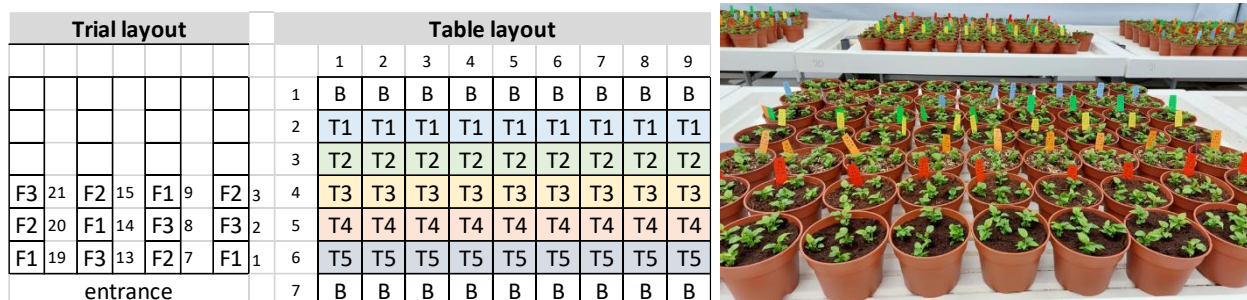


Figure A1 Trial layout showing the distribution of tables with fertigation treatments (F1, F2 and F3). Five growing media treatments (T1-T5) were placed on each table.

Table A1 Fertiliser recipes of maintenance nutrient solutions (D1, D2 and D3).

Time		Transplanting - 12 WAT		12 WAT - 15 WAT		
Parameter	Unit	D1	D2 & D3	D1	D2	D3
EC	mS/cm	2.5	3.0	2.5	3.0	3.5
pH		5.5	5.5	5.5	5.5	5.5
NH ₄	mmol/L	1.0	1.0	1.0	3.0	3.0
K	mmol/L	10.5	10.8	10.5	9.9	8.8
Na	mmol/L	0.0	0.0	0.0	0	0.0
Ca	mmol/L	4.4	6.5	4.4	4.1	7.2
Mg	mmol/L	2.3	3.0	2.3	4.4	4.3
NO ₃	mmol/L	16.3	23.5	16.3	21.3	24.9
Cl	mmol/L	0.0	0.0	0.0	0.0	0.0
SO ₄	mmol/L	3.6	2.4	3.6	3.3	3.8
P	mmol/L	1.4	1.6	1.4	2.1	2.5
HCO ₃	mmol/L	0.0	0.0	0.0	0.0	0.0
Fe	µmol/L	15.0	15.0	15.0	19.5	19.5
Mn	µmol/L	4.5	2.5	4.5	6.2	6.2
Zn	µmol/L	2.8	3.7	2.8	3.7	3.7
B	µmol/L	14.9	14.4	14.9	14.4	14.4
Cu	µmol/L	0.7	0.7	0.7	0.7	0.7
Mo	µmol/L	0.0	0.0	0.0	0.0	0.0

WAT: weeks after transplanting. Note: Extra Fe-EDDHA (15 µmol/L) was added to the prepared nutrient solution in each tank from 4 WAT. This measure was taken to correct the symptom of yellowing leaves.

Table A2 Base fertilisers for the mixtures used in each layer.

Layer	Lime (kg/m ³ GM)	Ca(NO ₃) ₂ (kg/m ³ GM)	PG mix 14-16-18 (21) (kg/m ³ GM)	Fe-EDDHA (kg/m ³ GM)
M1	2.940	0.374	0.758	0.0133
M2	1.090	0.539	0.521	0.0135
M3	0.840	0.429	0.679	0.0127
M4	1.260	0.674	0.269	0.0135
M5	0.840	0.527	0.514	0.0130
M6	1.260	0.550	0.482	0.0134
M7	0.250	0.618	0.372	0.0135

Table A3 Fertigation frequencies.

	Number of fertigation cycle per week	
	F1	F2 & F3
Week 12	3	3
Week 13	1	3
Week 14	1	2
Week 15	2	3
Week 16	1	4
Week 17	2	3
Week 18	2	4
Week 19	1	3
Week 20	2	4
Week 21	2	3
Week 22	2	4
Week 23	1	3
Week 24	2	4
Week 25	2	3
Week 26	3	4
Week 27	3	3

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