WP T4

D.T4.1.2: Protocol on the practical RDF application for horticultural sector (greenhouse trials)

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Partners:

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1. Introduction

ReNu2Farm aims at increasing recycling of plant nutrients nitrogen (N), phosphorous (P) and potassium (K) from biomass streams like animal manure, sewage sludge and food waste. Despite existing nutrient recovery technologies (and new processes facilitated by the project), usage of recycling-derived fertilisers (RDF) by farmers is limited. The project determined low knowledge and awareness of RDFs in IE, DE, NL, LUX, BE and FR as the main market barrier by farmers. Further, continuously postponed legal enforcement of European Fertiliser Product Regulation (FPR) and end-of-manure status (SafeManure RENURE) prevents a widespread and transregional RDF market uptake. ReNu2Farm results (field trials, policy recommendations on farmer's perspectives) are integrated in the current legal amendment process (FPR). RDF transborder market activities are essential to balance nutrient shortage and surpluses in NWE regions. Upgrading nutrients in surplus regions to mineral fertiliser quality (based on analysed farmer needs) have been created to stipulate demand in nutrient-shortage regions. ReNu2Farm mapped nutrient demand and the use of recycling-derived fertilisers and pinpointed potential RDF demand. Farmer demands of RDFs and willingness to pay were determined through a large-scale survey. Innovative field trials and environmental/biological analyses evaluated RDF efficiency, serving as farmer demonstrations to increase practical knowhow on RDF usage (N- and P-RDFs field trials). Through portraying RDF producer success stories and establishing multiple events, the knowledge gap of farmers and producers was significantly reduced. Preparative activities towards market introduction are successfully processing. Continued active memberships in networks, nutrient platforms, unions, project synergies, research publications and the promotional campaign will ensure long-term project results.

Within the Call for Capitalisation however, ReNu2Farm also started exploring the horticulture as a target group of RDFs, as it is the second biggest market for mineral fertilisers. It is of utmost importance to also understand the agronomical impacts of the use of RDFs. The use of RDFs may be very beneficial, provided that also horticulture crop yields can be maintained and there are no negative effects on the crops. Collected data, as well as results from the field trials on the use of RDFs in agriculture cannot be transposed to soilless cultivation in horticulture. Gathering additional results taking into account the specificities of this cultivation model is necessary. Therefore, ReNu2Farm performed some pot and greenhouse trials with RDFs in different crops.

For these trials, a selection of RDFs was chosen. Important criteria for fertilisers in horticulture is a high nutrient content and a pure product free of organic matter and contamination. Therefore, a selection of fertilisers were tested by Est Horticole, Inagro and University of Limerick in different crops (Table 1).

Table 1: Overview on the RDFs used on what crops by which partner.

Partner	Plant/crop	RDF
Inagro	Lettuce	Ammonium nitrate (from ammonia stripping)
		Ammonium sulphate (scrubber water)
Est Horticole	Chrysanthemum	Ammonium nitrate (from ammonia stripping)
		Ammonium sulphate (scrubber water)
	Petunia	Ammonium nitrate (from ammonia stripping)
		Ammonium sulphate (scrubber water)
	Viola	Ammonium nitrate (from ammonia stripping)
		Ammonium sulphate (scrubber water)
	Basil	Ammonium nitrate (from ammonia stripping)
		Ammonium sulphate (scrubber water)
	Lonicera	Ammonium nitrate (from ammonia stripping)
		Ammonium sulphate (scrubber water)
ULimerick	Lettuce	Struvite (from potato processing wastewater)
		Struvite (from municipal wastewater)
	Tomato	Struvite (from potato processing wastewater)
		Struvite (from municipal wastewater)
	Spinach	Struvite (from potato processing wastewater)
		Struvite (from municipal wastewater)

These RDFs have already been tested in ReNu2Farm in arable farming, yet these fertilisers could also prove to be very valuable in greenhouses in horticulture, which is why they were tested within these greenhouse trials.

2. Greenhouse trials Inagro

In this report, Inagro tested ammonium nitrate and ammonium sulphate in lettuce trials. The objective is to stimulate new markets for increased RDF use and to decrease the use of mineral/synthetic fertilisers. One of those new markets is the horticulture sector which represents the second biggest market for a possible RDF market uptake.

2.1. Material and methods

2.1.1. Experimental set-up

In these trials the effect of RDFs on yield and quality of greenhouse lettuce grown in soil was evaluated. Two promising RDFs were chosen to test: an ammonium sulphate produced by an acid air washer and ammonium nitrate produced by stripping-scrubbing.

The trial was conducted at the following location:
Inagro – Ieperseweg 87, 8800 Rumbeke-Beitem, Greenhouse 7A - lava field

2.1.1.1. Crop and cultivar

- Lettuce (Lactuca sativa var capitata - LACSC)
- Cultivar: Ostria (Rijk Zwaan)

2.1.1.2. Trial design

The lettuce was grown in containers filled with a mixture of greenhouse soil and perlite. Perlite was added to keep the soil light to ensure a good water and air supply to the roots, since greenhouse soil tends to clog together when irrigated. Each container contained 55 L substrate in total with 3.5 L perlite at the bottom and 11 L perlite mixed with the greenhouse soil. The containers were placed on the lava field to catch any drainage of the irrigation water (Figure 1).

Four lettuce plants were planted in a container and irrigated via drip irrigation. Each plant was provided with a separate drip nozzle. The plants were planted with a spacing of 27 cm by 17 cm (Figure 2). Each container (four plants) represented a trial object.

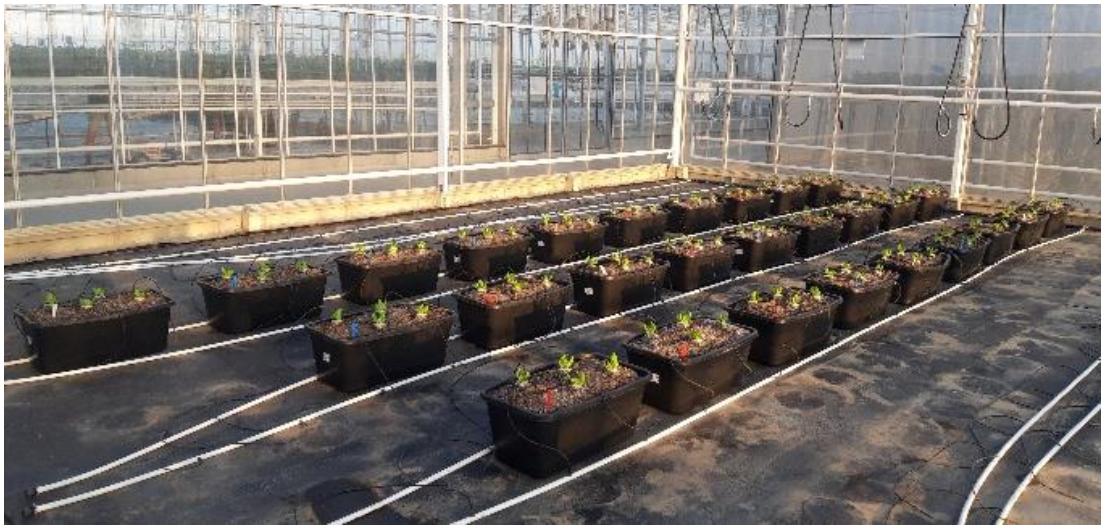

Figure 1: Lava field with containers

Figure 2: (left) Plant distribution in container, (right) irrigation via drip irrigation

2.1.2. Treatments

Two RDF products were chosen for the trial because of their high nitrogen content:

- ammonium sulphate is produced by acid air washer on (primarily) pig farms;
- and ammonium nitrate is produced by a Detricon stripping-scrubbing installation from a manure processing company

In Belgium the soils tend to have a high demand of nitrogen and potassium fertilisation as opposed to a lower demand of phosphate fertilisation.

Before using these products in the trial, they were analysed for their composition and content (analysis was done by the accredited lab at Inagro) (Table 2).

Table 2: Contents RDF products used in lettuce trial

	Ammonium nitrate	Ammonium sulphate	Unit
pH	7.19	3.4	
C	134.12	157.99	kg/1000 kg FM
Electroconductivity (EC)	245.4	191.4	mS/cm 25°C
Total oxidized nitrogen (TON)	46.51	0.01	kg/1000 kg FM
Ammonium-N	33.22	41.48	kg NH_3-N/1000 kg FM
Total N	87.80	45.35	kg/1000 kg FM
Potassium (K)	0.03	0.40	kg/1000 kg K_2O FM
Phosphate (P_2O_5)	0.01	0.13	kg/1000 kg P_2O_5 FM
Dry matter (DM)	246.81	292.45	kg/1000 kg FM
Organic matter (OM)	241.41	284.38	kg/1000 kg FM
Sulphur (S)	0.03	53.41	kg/1000 kg FM

FM = Fresh matter

During this trial the effect of the RDFs in different doses was evaluated. Both products were given at 100%, 70% and 40% of the advised nitrogen fertilisation amount. This meant that seven treatments in total were taken up in this trial (Table 3):

- a control treatment, which is a mineral fertiliser (KAS and chalk nitrate);
- and two RDFs treatments each given in three doses.

Each treatment was evaluated in four repetitions.

Table 3: Overview of treatments.

No.	Name	Fertiliser	% of advised fertilisation	kg N/ha	g product/container
1	Control	Mineral	100	213	9.3 g KAS 15.8 g chalk nitrate
2	N-100	Ammonium nitrate	100	213	62 g ammonium nitrate
3	N-70	Ammonium nitrate	70	149.1	43 g ammonium nitrate
4	N-40	Ammonium nitrate	40	85.2	25 g ammonium nitrate
5	S-100	Ammonium sulphate	100	213	119 g ammonium sulphate
6	S-70	Ammonium sulphate	70	149.1	83 g ammonium sulphate
7	S-40	Ammonium sulphate	40	85.2	48 g ammonium sulphate

The soil used in the container was taken from our greenhouse compartment 11. A KEMA analysis of this soil was performed to specify the fertilisation need. This analysis was done by the Bodemkundige Dienst van België (BDB) and gave a fertilisation need of:

- Potassium (K): 200 kg/ha
- Nitrogen (N): 221 kg/ha

A base potassium fertilisation was mixed with the soil before we filled the containers to fulfil the potassium demand. In total 2 kg/a of potassium chloride and 4 kg/a Vivikali was added. Vivikali also contains some mineral N (2%). If all the potassium would have been given as potassium chloride the salt levels would have risen too much.

So, 8 kg/ha of the 221 kg/ha N demand was already fulfilled by Vivikali. In total to fertilise 100% of the nitrogen demand with RDFs 213 kg/ha is needed, for 70% of the advised dose 149.1 kg/ha and for 40% 85.2 kg/ha. This translates to a different amount of ammonium nitrate and ammonium sulphate for each percentage depending on the N concentration each of the products hold (right column in Table 3).

The RDFs were applied by spraying a mixture of the product and rainwater upon the soil and perlite mix. Everything was mixed thoroughly before filling each container.

2.1.3. Trial conditions

2.1.3.1. Climate conditions

Table 4: Climate conditions in the greenhouse where the trial was carried out.

	Average	Maximum	Minimum
Greenhouse Temperature (°C)	15.7	32.1	5.7
Humidity (%)	73	95	30
Temperature outside (°C)	12.7	24.6	3.2

** Climate data in detail see appendix 1*

2.1.3.2. Overview trial development

Table 5: Overview on the timing of trial activities.

Application time	Activity
30/08/2021	Sowing (5 cm soil blocks)
10/09/2021	Fertilizing + application RDFs
15/09/2021	Planting
28/10/2021	Harvest

Table 6: Overview on the application of plant protection products.

Application time	Plant protection product
15/09/2021	Amistar (0,8 l/ha)
17/09/2021	Previcur Energy (2,5 l/ha) + Decis 15EW (0,833 l/ha) + Serenva (0,6 kg/ha)
24/09/2021	Kenja (1,0 l/ha) + Fubol Gold (1,9 kg/ha)
29/09/2021	Luna Privilege (0,5 l/ha) + Kenja (1,0 l/ha)
06/10/2021	Previcur Energy (2,5 l/ha) + Signum (1,5 kg/ha) + Tracer (0,2 l/ha) + Movento (0,45 l/ha) + Revus (0,6 l/ha)

2.1.4. Measurements

At harvest all four crops of each trial object are harvested and for each crop a couple of plant parameters were evaluated. These parameters can be divided into two categories: **yield and quality**.

Yield:

- Fresh weight: weight of the crop right after harvest, with all leaves still attached to the crop.
- Marketable weight: when delivering crops to the auction for sale, they need to be visually pleasing. This means that often a few leaves are cut off of each crop to present better at the auction. The leaves that are cut off are the lower leaves that often already have turned yellow or began to rot. This results in a lower total weight than the fresh weight depending on how many leaves have been cut off and is an indirect measurement of how healthy the crops are.

Quality:

- Uniformity: A score of how uniform the crops are or look.
- Canopy cover: A score of how much soil surface is covered by the crops. It is best to have a high coverage if possible. It is also an indication of the growth of the crops.
- Crop filling: A score given on how much the crop is filled, if it has formed enough inner leaves. Which is an important quality for lettuce.
- Leaf colour: A score of how green the crops are. If the leaves tend to have a yellowish colour it could be caused by a shortage or a surplus in fertilisation.
- Tip burn: A score of how many tip burn is observed on the crop. Tip burn can be caused by multiple factors but is overall a sign of non-optimal growing circumstances.
- Basal rot: A score on how much rot is observed on the underside of the crop.

After the crops were harvested **soil samples** were taken from each object and sent for analysis to the lab at Inagro. The results were very distorted and made it impossible to interpret them. For this reason, they were not taken up in the processing of the results.

* Raw data of these measurements is included in appendix 2.

2.2. Results and discussion

Remark: When the soil samples were taken it was clear that the soil of container 101 (repetition 1 of treatment 1) (control) was much too wet, maybe caused by a malfunction of one the drip nozzles. This was also reflected in the lower yields of the crops. That is why this object is considered as an outliner and the data will not be included in the statistics.

2.2.1. Yield

Table 7: Results of the yield of the lettuce.

No.	Treatment	Fresh weight (g)		Marketable weight (g)	
1	Control	423	a	382	a
2	N-100	396	abc	359	abc
3	N-70	395	abc	359	abc
4	N-40	368	c	337	c
5	S-100	405	ab	371	ab
6	S-70	385	bc	350	bc
7	S-40	378	bc	344	c
	Average	**393**		**357**	
	L.S.D.	29		27.39	
	C.V. (%)	3.25		3.37	
	p-value	0.001	***	0.002	

The trial showed that it is vital to follow the advised fertiliser amounts. A lower fertilisation led to lower yields. The lower the N-fertiliser, the lower the yield. There was no difference in impact observed in the use of ammonium nitrate or ammonium sulphate. The yield is expressed as the marketable weight shown in Figure 3.

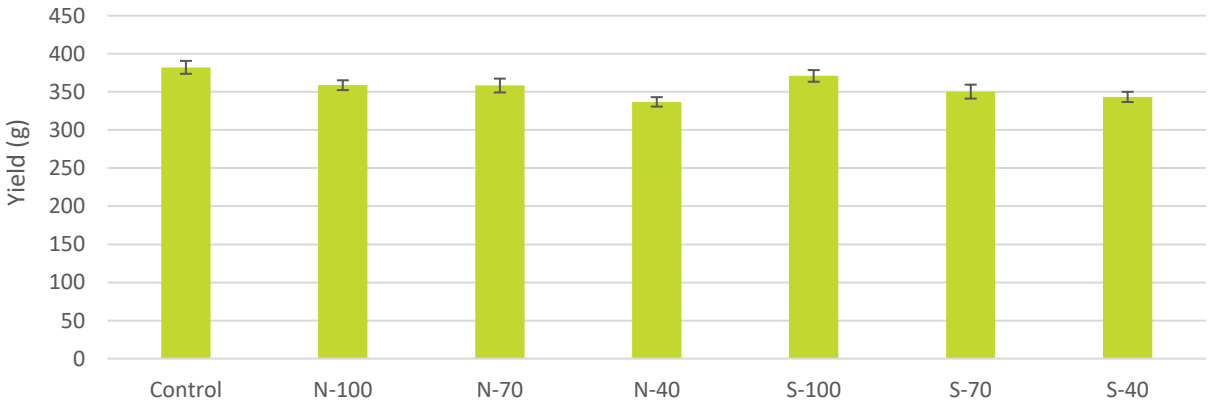

Figure 3: Yield of the lettuce plants.

2.2.2. Quality

Table 8: Quality criteria.

No.	Treatment	Uniformity		Canopy cover		Crop filling		Leaf colour		Tip burn		Basal rot	
1	Control	8.3	a	9.0	a	7.5	a	7.0	a	9.0	a	8.9	a
2	N-100	8.1	a	9.0	a	7.3	a	7.0	a	9.0	a	8.9	a
3	N-70	8.0	a	9.0	a	7.4	a	7.0	a	9.0	a	8.9	a
4	N-40	8.1	a	8.4	b	7.1	a	7.0	a	9.0	a	9.0	a
5	S-100	8.4	a	9.0	a	7.5	a	7.0	a	9.0	a	9.0	a
6	S-70	8.1	a	9.0	a	7.1	a	7.0	a	9.0	a	9.0	a
7	S-40	8.3	a	9.0	a	7.1	a	7.0	a	9.0	a	9.0	a
	Average	**8.2**		**8.9**		**7.3**		**7.0**		**9.0**		**8.9**	
	L.S.D.	0.76				0.55							
	C.V. (%)	4.08		2.07		3.33		0		0		1.83	
	p-value	0.747	N.S.	0.01	*	0.145	N.S.	0.000	***	0.000	***	0.907	N.S.
	9 =	Uniform		Complete soil coverage		Good		Blond		None		None	
	1 =	Heterogeneous		No soil coverage		Bad		Dark green		Very much		Very much	

There were no significant differences between the treatments regarding uniformity, canopy cover, crop filling, leaf colour, tip burn and basal rot (Table 8). However, the crops of S-100 were visually a little bit smaller and more compact (see pictures included in appendix 3). But this compacter growth could not be observed in the other treatments with the same fertiliser S-70 and S-40. Overall, there was no loss in quality between the different treatments.

2.3. Conclusions

The yield and quality of the lettuce grown on the RDFs was good when the fertiliser advice was followed. The crops grown on ammonium sulphate (100%) were a little bit more compact in growth.

It is important to take into consideration that the used fertilisers need to be as pure as possible. Too much ballast elements can cause problems in later crop cycles as they can accumulate in the soil and are not flushed by rainfall or irrigation, in comparison to use in the field.

In greenhouses in Belgium there is a high demand of nitrogen and potassium but a low demand of phosphate. This, as well as legislation, might give some restrictions to the types of RDF that are suited.

3. Greenhouse trials Est Horticole

Est Horticole is responsible for the trials confirming the use of two RDFs for ornamental horticulture in pots, ammonium sulphate and ammonium nitrate. The test pattern implemented by Est Horticole is carried out on 3 crops, *Chrysanthemum*, *Viola* and *Lonicera* according to the cultivation calendar in . The model crops were chosen because of their fast reaction to deficiencies and their representativeness in horticultural farms.

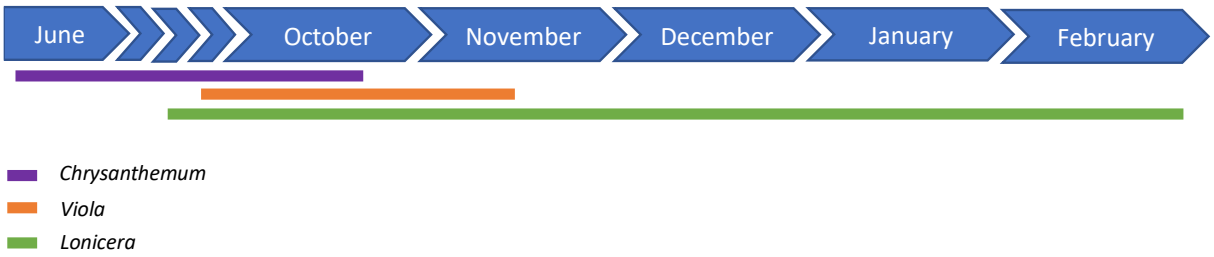

Figure 4: Cultivation calendar of the different crops tested.

3.1. Chrysanthemum

3.1.1. Material and methods

3.1.1.1. Experimental setup

3.1.1.1.1. Crop and cultivar

The three chrysanthemum varieties were evenly distributed between the modalities.

Table 9: Overview on the Chrystanthemum varieties and quantities.

Species	Varities	Quantities	Supplier	Delivery week	Number of plant per modality
Chrysanthemum	Yahou Coco	100	Bernard Jeunes Plants	23	10
Chrysanthemum	Yahou Golden	100		23	10
Chrysanthemum	Yahou Bonbon	100		23	10

3.1.1.1.2. Cultivation conditions

Substrate: the E910 reference of Stender society is used. Its main component is blond peat. Its structure is coarse.

Fertilization at potting time:

- M1: mineral NPK fertiliser, Osmocote (12-7-19) 5-6 months, 4 g/L
- M2: no additional fertilization in the substrate at potting time
- M3 à M8: Patentkali, 2g/L and Superphosphate 45, 1 g/L

Irrigation: plants are watered pot by pot with a watering can. Each plant receives 500 mL water. The watering frequency is defined by the technician, depending on the weather conditions that can cause water stress.

3.1.1.1.3. Trial design and treatments

The trial modalities match the following fertiliser management technique:

- **M1**: Reference mineral controlled-release fertiliser Osmocote
- **M2**: Reference soluble mineral fertiliser Soluplant (16-6-26), 2 g/L
- **M3**: Ammonium nitrate, 100% of ideal concentration, 2 g/L
- **M4**: Ammonium nitrate, 75% of ideal concentration, 1.5 g/L
- **M5**: Ammonium nitrate, 40% of ideal concentration, 0.8 g/L
- **M6**: Ammonium sulphate, 100% of ideal concentration, 6 g/L
- **M7**: Ammonium sulphate, 75% of ideal concentration, 4.5 g/L
- **M8**: Ammonium sulphate, 40% of ideal concentration, 2.4 g/L

Each modality is made up of thirty chrysanthemum arranged in three Fisher blocks counting ten plants each. The three varieties are evenly distributed between each block. The pots stand on single saucers to avoid any contamination from one modality to the other.

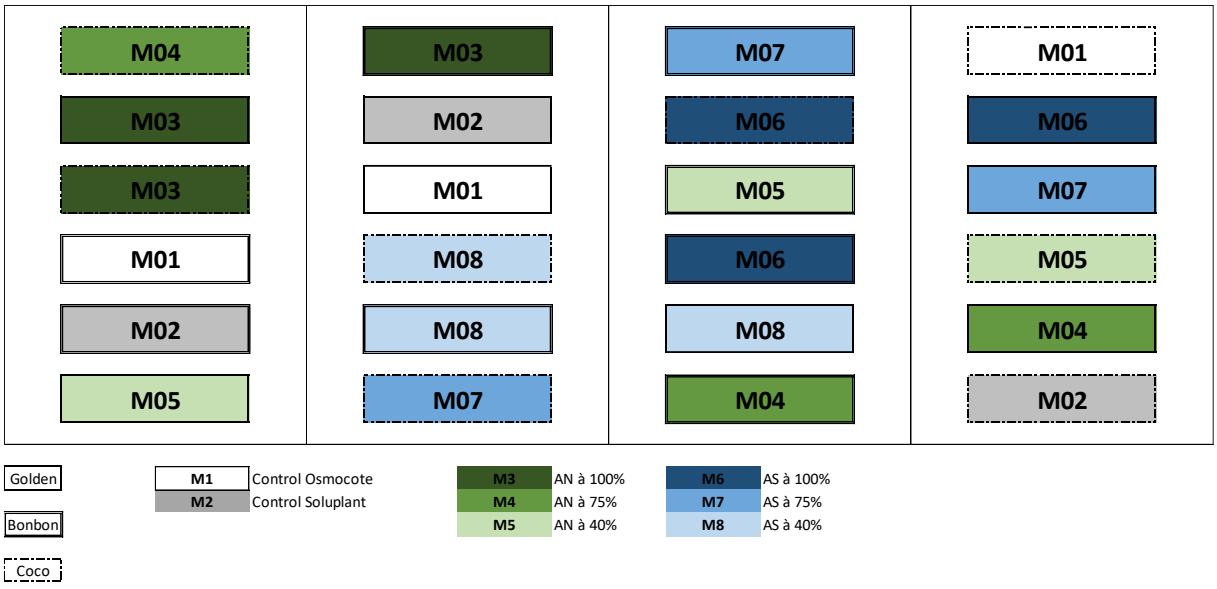

Figure 5: Schematic trial design with the different varieties and objects.

Calculation of the supplied doses:

The calculations of ideal concentrations of ammonium nitrate (AN) and Ammonium sulphate (AS) were based on the Soluplant supplied doses. Indeed, laboratory analyses of RDFs

showed that nitrogen content of AN is almost equal to the Soluplant's one. A three times bigger volume of AS is necessary to get the same nitrogen content.

Supplies frequency:

The control modality M1 gets only one solid mineral controlled-release fertilization supply while potting on week 23. Liquid fertilization modalities (M2 to M8) get one supply per week from week 27 to week 30. Afterwards, there were three supplies per week until the end of the trial on week 42.

Supplies were made through pot by pot fertigation with a 3 L watering can per block. Thus, each plant receives 300mL of the solution. The control modality is watered with the same volume of clear water per plant.

Composition of mineral and recycling-derived fertilisers:

Table 10: NPK composition of mineral and recycling-derived fertilisers.

Fertilisers composition	**N**	**P**	**K**
Osmocote 5-6	12	7	19
Soluplant	16	6	26
Ammonium nitrate (AN)	14.2	0	0
Ammonium sulphate (AS)	17.4 (5.8*3)	0	0

Table 11: Composition of the RDFs.

Product	**Sampling date**	**NO_3-N**	**NH_4-N**	**Kjeldahl N**	**P_2O_5**	**K_2O**	**S**
		g/kg fresh material					
Samples taken from storage before or at the time of trial installation							
Ammonium nitrate	04/15/19	43.4	43.1	43.1	0.0	0.0	0.5
Ammonium sulphate	03/18/19	0.0	33.6	0.0	0.0	0.0	37.9

Table 12: NPK total quantity supplied during the trial.

Fertiliser dose		**N**	**P_20_5**	**K_2O**
		(g) / plant (on the trial's duration)		
Osmocote		1.44	0.84	2.28
Soluplant		3.9	1.4	6.1
AN	**100%**	3.3	3	6
	75%	2.5		
	40%	1.3		
AS	**100%**	3.9		
	75%	3		
	40%	1.64		

3.1.1.2. Trial conditions

3.1.1.2.1. Climate conditions

Greenhouse and cultivation setpoints: the trial was carried out under a cold glasshouse with a high air vent opening on the ridge and a shade system, both controlled by a climate computer. The airing and shading setpoints are set by the technician, depending on the weather conditions.

3.1.1.2.2. Overview trial development

Table 13: Overview on the timing of trial activities.

Date	Activity
08/06/2021	Potting
06/07/2021	Placing on saucers
20/07/2021	Pinching out
20/07/2021 & 04/08/2021	Supply of auxiliaries for integrated crop management
04/08/2021 & 23/08/2021	Distancing
25/10/2021	End of the trial

3.1.1.3. Measurements

Three kinds of follow-up are done:

- Aerial and root development:

Growth measures (height and diameter) were taken every two weeks to follow the plants' development. Moreover, root development measures were taken according to the following scale:

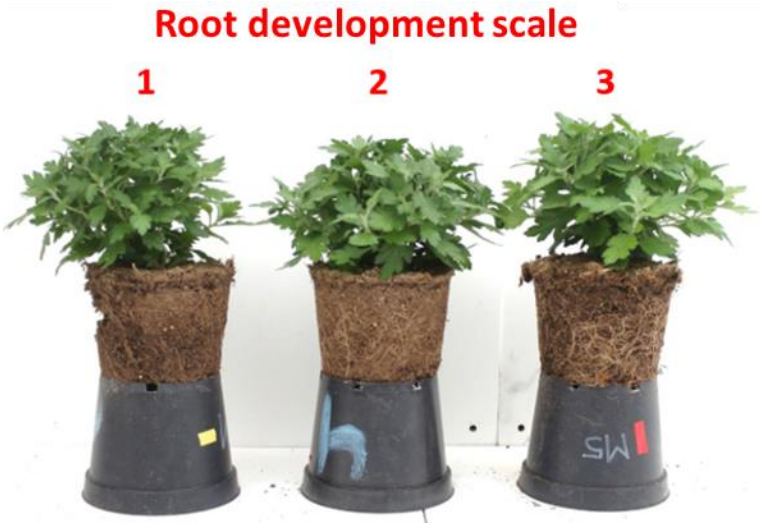

Figure 6: Depicturing of the root development scale.

- Evolution of the soil parameters in the substrate:

Soil parameters analysis are done every three weeks. pH, electroconductivity (EC), nitrate and ammonium contents of the substrate are measured.

An aqueous extract has to be prepared before these analysis. 100 g of substrate was taken in each modality and put in 150 mL of distilled water for 30 minutes. The solution is then filtered to get the aqueous extract on which the soil parameters analysis is done.

The RQflex® device measures the ammonium NH_4^+ content in the substrate, from 5 mg/L to 180 mg/L NH_4^+. The Nitracheck® device measures the nitrates NO_3^- through reflectometry.

Figure 7: The RQflex® (left) and Nitracheck® (right).

- Follow-up of the flowering period:

Weekly evaluations of flowering were made on Yahou Bonbon variety, according to the following scale (Figure 8).

Figure 8: Evaluation scale of the flowering.

3.1.1.4. Statistical data processing

The data of the trial were analysed with R statistical software, version x64 4.1.1. Variance analyses ANOVA were made, or Kruskal-Wallis nonparametric tests when ANOVA conditions were not observed. Newman-Keuls post hoc comparison of means were then used. Eventually, the confidence level of the analysis was of 95%.

3.1.2. Results and discussion

3.1.2.1. Rooting scale

Figure 9: Graphs on the root development and class across the growth season of the different modalities.

The first evaluation, made on the 22nd of July showed early root development on M2 modality with Soluplant supply. The rooting speed is enhanced by phosphorus absorption. It can be assimilated faster through liquid fertilization, which is why there is a difference on M2. Moreover, the supplied phosphorus dose on the M2 modality is slightly higher than on the other liquid fertilization modalities.

On the 10th of August, more than 80% of the chrysanthemum show a class 3 rooting system in most of the modalities. At the end of the trial, root development is even and all modalities show an optimal rooting. RDFs are thus very effective for chrysanthemum rooting. Moreover, the roots are healthy and do not show any burn mark.

Figure 10: Pictures of chrysanthemums root systems on the 26th of October 2021.

3.1.2.2. Height

Height measures were made every three weeks from the 29th of July to the 19th of October.

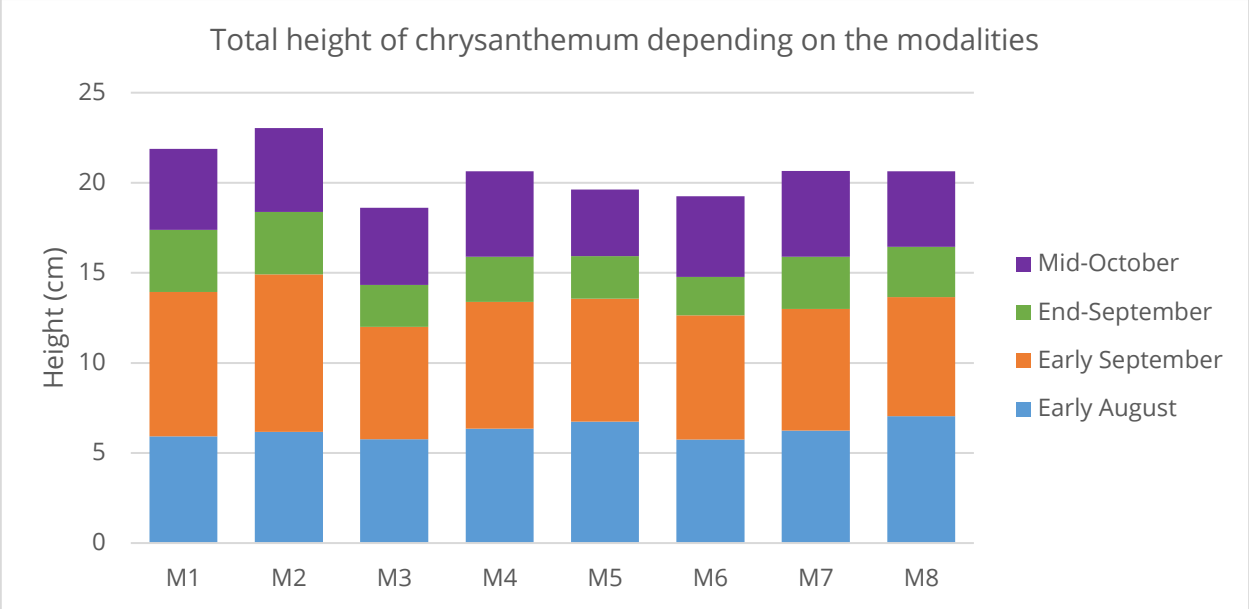

Figure 11: Graph on the total height of Chrysanthemum across the different modalities.

There is no statistical difference between modalities during the whole trial. However, chrysanthemum of control modalities M1 and M2 seem to be higher than the ones fertilized with RDFs. Moreover, M3 and M6 modalities (RDFs supplies at 100% of their ideal concentration) seem to be smaller than the other modalities. Nevertheless, there is no significant difference according to statistical analysis (Kruskal-Wallis, p-value = 0.3572).

Warning: a strong height development does not imply a good commercial quality for chrysanthemum crops. Growers try to get "stocky" plants.

3.1.2.3. Diameter

Diameter measures were made every three weeks from the 29th of July to the 19th of October.

There is no significant difference between modalities for each bi-monthly measure. At the end of the trial, modality M2 (Soluplant) chrysanthemum seems to be larger than the chrysanthemum of the other modalities. However, there is no significant difference (Kruskal-Wallis, p-value = 0.1061).

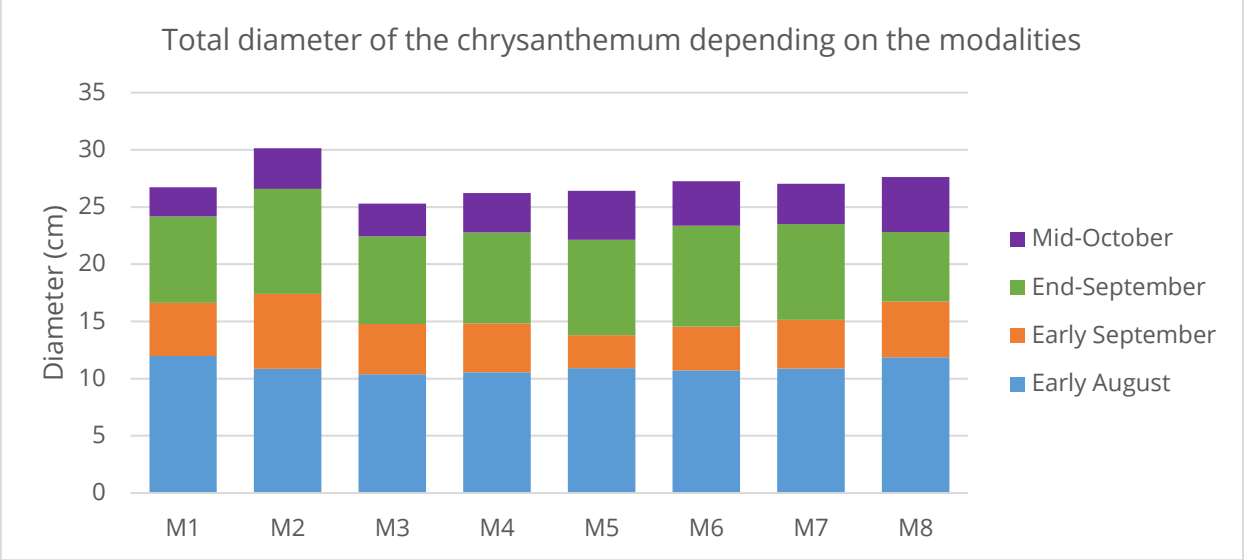

Figure 12: Graph on the total diameter of the Chrysanthemum across the different modalities.

The diameter of chrysanthemum is thus very satisfactory when using RDFs: it is similar to the diameter of chrysanthemum fertilized with a mineral fertiliser.

Figure 13: Picture of the chrysanthemums at the 22nd of September 2021 (M1 to M8).

3.1.2.4. Flowering evolution

Evaluations were made throughout October and two times a week.

Being able to control the flowering period of *Chrysanthemum* is important. Flowers must be open enough to sell them on All Saints' Day on the 1st of November.

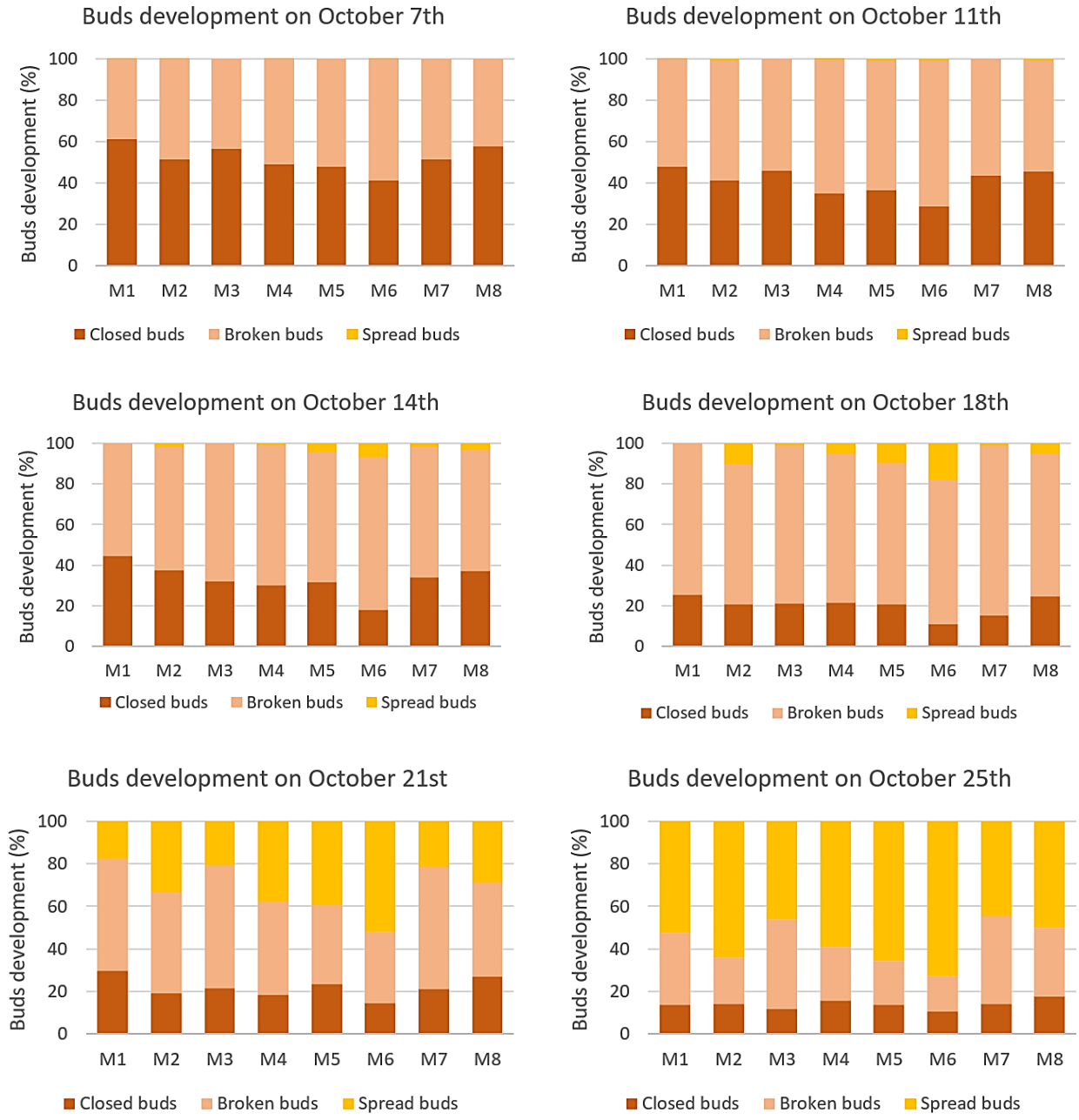

Figure 14: Graphs on the buds development across the growth season of the different modalities.

On the first evaluation, on the 7th of October, half of the buds were still closed. This share reduces when the flowers open up. Differences between modalities appear with time. The control modality M1 (Osmocote) flowers the latest. It can be explained by the slow release of the solid mineral fertiliser.

On the contrary, M6 modality (AS 100%) is flowering the earliest and the spreading of flowers is the fastest.

At the end of the trial, M5 modality (lowest dose of AN) seems to flower faster than M3 and M4 modalities of which AN concentrations were higher. It is the other way around for plants fertilized with AS. Flowers open up faster in the modality with supplies of the AS highest concentration (M6).

Figure 15: Flowering of the Bonbon variety on the 21st of October.

3.1.2.5. Evolution of soil parameters

Soil parameters were measured for the first time after the first supply of liquid fertilization.

Measurement dates are as follows: 22nd of July, 10th of August, 8th of September, 28th of September and 19th of October.

- pH and electric conductivity (EC) evolution

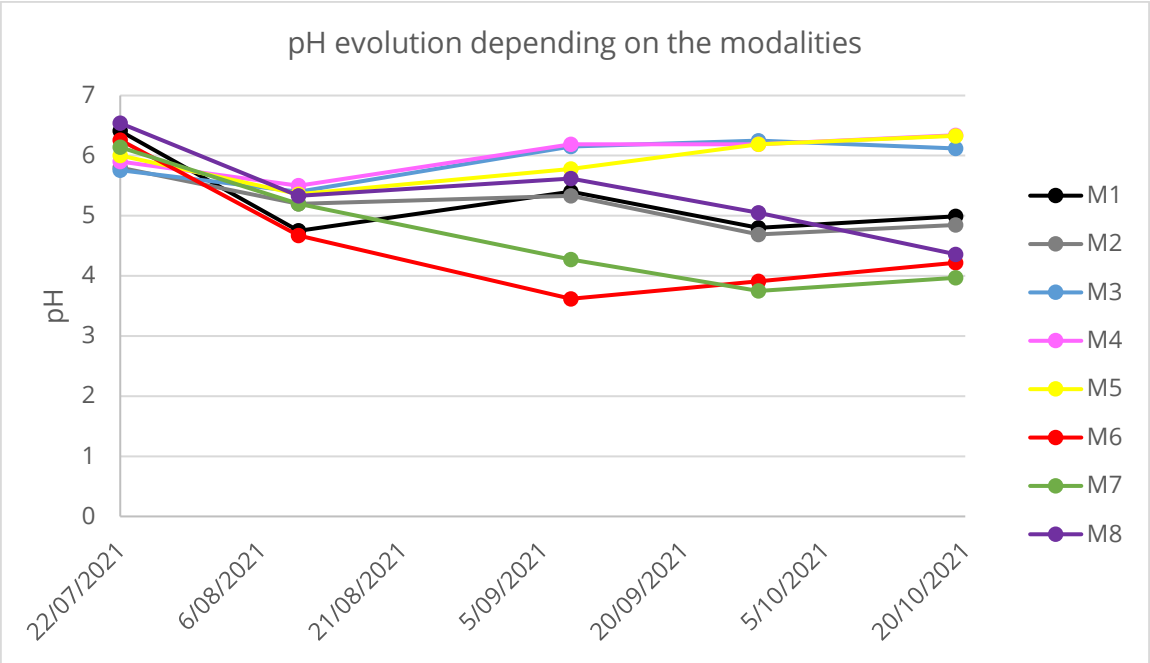

Figure 16: Graph on the pH evolution of the different modalities.

The pH is similar between modalities for the first four weeks after the first supply. Then, modalities with solid or soluble mineral fertiliser have similar pH values, close to 5. The pH values in the substrates of modalities with AN supplies (M3, M4, M5) have the same evolution all along the trial and show that their substrate is more alkaline than the control substrates.

At the end of the trial, the pH measured in the substrates of modalities with AS supplies (M6, M7, M8) is way lower comparing to the other modalities. Culture media get strongly more acidic, the pH reaching 3.6 for the modality with the highest AS concentration (M6). The nitrogen of the AS is mainly in the NH_4^+ form and less in NO_3^- form. The medium term acidifying process of the substrate is thus due to a higher concentration of H^+ ions.

Despite pH value out of the reference optimal range, the acidification of the substrate has no visible influence on the plants quality during the trial.

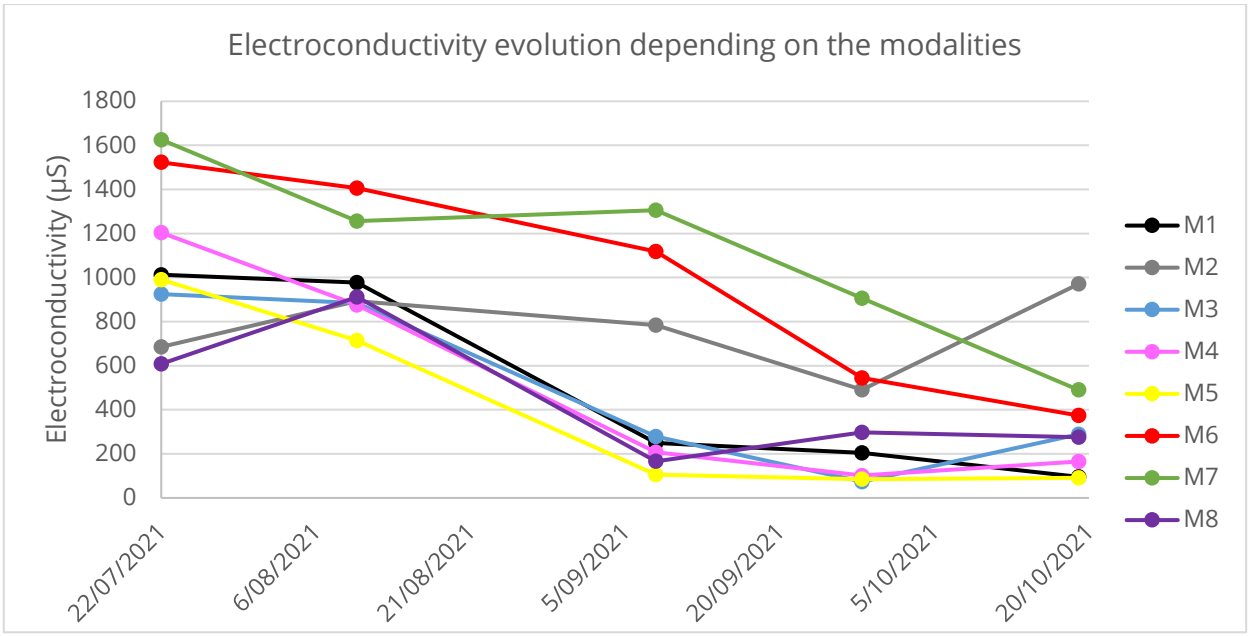

Figure 17: Electroconductivity evolution of the different modalities.

Starting EC values were different between modalities. Modalities with Soluplant (M2) and AS at 40% of its ideal concentration (M8) supplies have low values out of the optimal range.

Modalities with supplies of AS highest concentrations (M6 and M7) have the highest EC values during almost the whole trial.

For the next measurements, starting from August, EC reduces for all the modalities. In this period, plants were in full growth and their roots were already developed. Nutrients supplied were thus quickly used. Only M2 shows an increase during October. There is a peak of nitrate at the same date.

- Nitrate and ammonium contents

On the first measurement, nitrate rate differed between modalities. The Osmocote control (M1) had the highest value with 326 ppm nitrate in the substrate. Nitrate quantity reduces gradually on this modality without additional supply of solid fertiliser. Globally, control modalities with mineral fertilisers have higher nitrate content than modalities fertilized with RDFs.

Chrysanthemum substrates that received AS supplies have lower nitrogen contents than the ones fertilized with AN. 50% of the nitrogen of ammonium nitrate is directly supplied in the form of nitrate. However, the nitrogen supplied with ammonium sulphate is in the form of ammonium. It must be degraded by microorganisms to be transformed into nitrates.

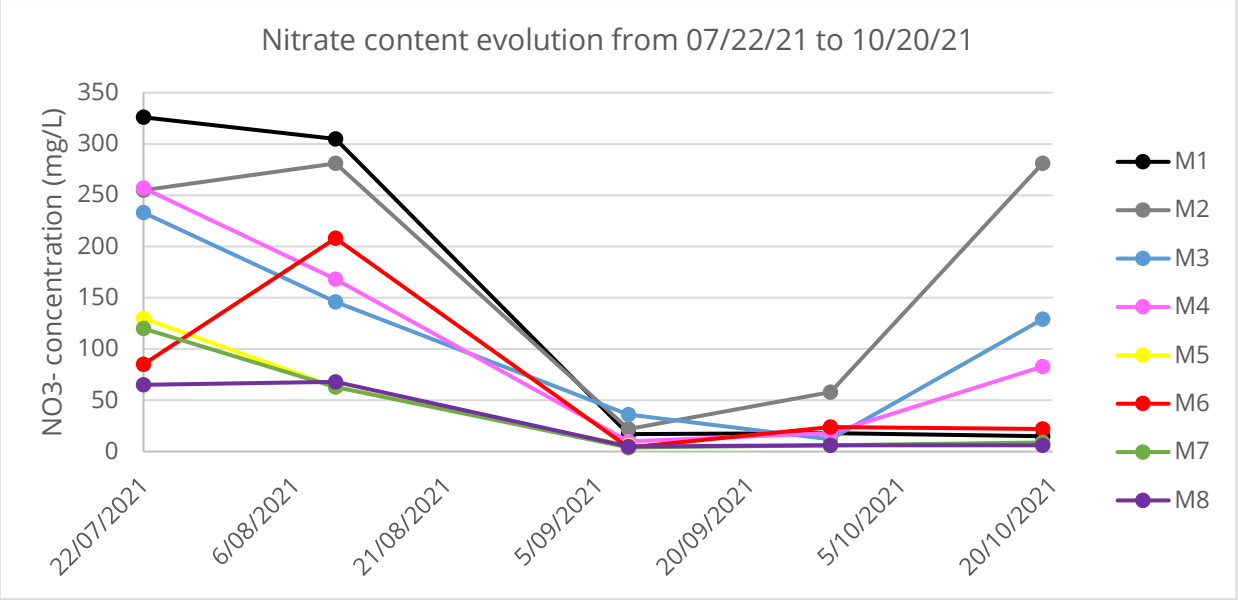

Figure 18: Nitrate content evolution of the different modalities.

There is a strong decrease of the nitrates content in all modalities starting from the 6th of August. Plants are in full growth in this period of time and use a lot nitrogen. Then, nitrates content increases again in M2, M3 and M4 modalities. These supplies should provide slightly too much nitrate comparing to the needs of the plant.

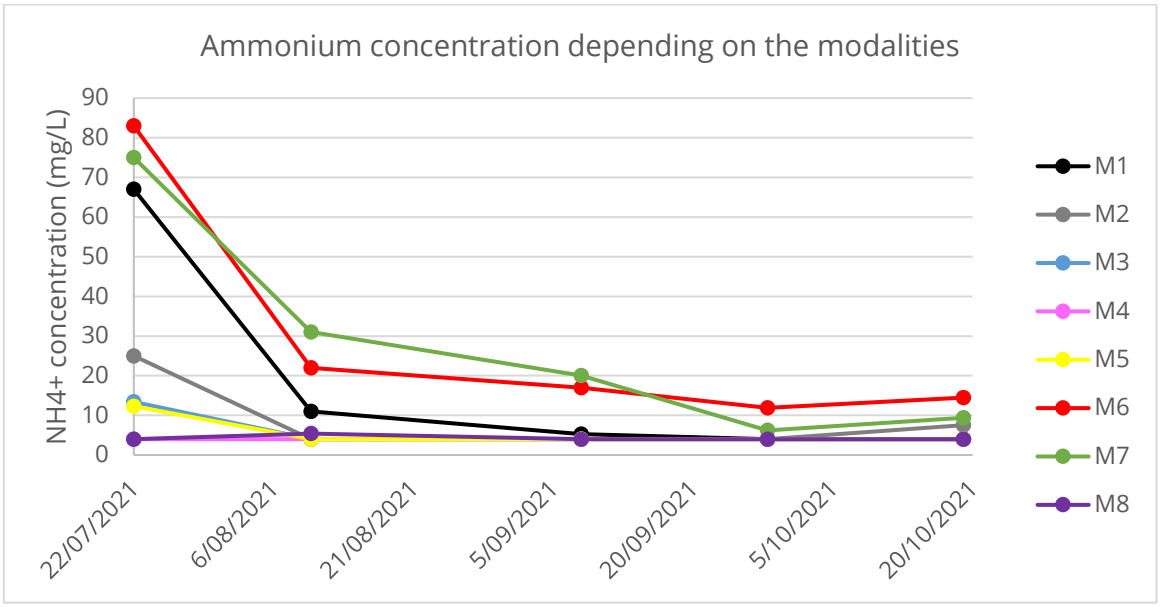

Figure 19: Ammonium concentration of the different modalities.

Two trends can be distinguished at the first date of measurements. The highest values were found for the control with Osmocote (M1), as well as for the modalities M6 and M7 (respectively 100% and 75% AS). The latter received nitrogen inputs containing only

ammonium, which may explain this observation. On the other hand, M8 has the lowest ammonium content. Ammonium is probably rapidly reduced and consumed in the form of NO_3^-.

The amount of ammonium decreases sharply from the 6th of August, despite the application of RDFs and Soluplant for the fertigated modalities. Chrysanthemums have an accelerated aerial and root development, which explains the higher nitrogen consumption during this period.

3.1.3. Conclusions

Chrysanthemum production is very nitrogen consuming and deficiency symptoms can appear quickly if fertilisation is not controlled. However, the use of ammonium nitrate and ammonium sulphate as a liquid recycling-derived fertiliser appears to be as effective as the mineral controls on this crop. At the end of the trial, the plants fertilised with the RDFs have a very good commercial quality and show no signs of nutrient deficiency or excess.

However, the flowering of the chrysanthemums seems to have been influenced by the different concentrations of RDFs. For example, flowering appears earlier with higher concentrations of AS, and the opposite trend is observed for AN.

The use of these products, especially ammonium sulphate, nevertheless requires particular vigilance regarding the acidification of the substrate. It is important to regularly check the pH and EC levels in the media to avoid any growing accidents.

3.2. Petunia

3.2.1. Material and methods

3.2.1.1. Experimental set-up

3.2.1.1.1. Crop and cultivar

Both *Petunia* varieties of the trial were evenly distributed between the modalities.

Table 14: Overview on the Petunia varieties and quantities.

	Species	Varieties	Quantities	Supplier	Delivery week	No. of plants per modality
	***Petunia hybrida***	LTD Sanguna Mega Rose	120	Syngenta	8	15
	***Petunia hybrida***	LTD Shortcake Raspberry	120		8	15

3.2.1.1.2. Cultivation conditions

Substrate: the B400 reference of Stender society is used. Its main component is blond peat.

Fertilisation at potting time:

- **M1**: mineral NPK fertiliser, Osmocote (12-7-19), 5-6 months, 3 g/L
- **M2**: no additional fertilisation in the substrate at potting time
- **M3-M8**: Patentkali, 2 g/L and Superphosphate 45, 1 g/L

Irrigation: plants are watered with a plastic beaker in a saucer containing five pots. Each plant receives 50 mL of water. The watering frequency is defined by the technician, depending on the weather conditions that can cause a water stress.

3.2.1.1.3. Trial design and treatments

The trial modalities match the following fertiliser management technique:

- **M1**: Reference mineral controlled-release fertiliser Osmocote
- **M2**: Reference soluble mineral fertiliser Soluplant (16-6-26), 2g/L
- **M3**: Ammonium nitrate, 100% of ideal concentration, 2g/L
- **M4**: Ammonium nitrate, 75% of ideal concentration, 1.5g/L
- **M5**: Ammonium nitrate, 40% of ideal concentration, 0.8g/L
- **M6**: Ammonium sulphate, 100% of ideal concentration, 6g/L
- **M7**: Ammonium sulphate, 75% of ideal concentration, 4.5g/L
- **M8**: Ammonium sulphate, 40% of ideal concentration, 2.4g/L

Each modality is made of thirty *Petunia* arranged in three Fisher blocks counting ten plants each. Both varieties are evenly distributed between each block. The pots stand on saucers (five pots per saucer) to avoid any contamination from one modality to the other.

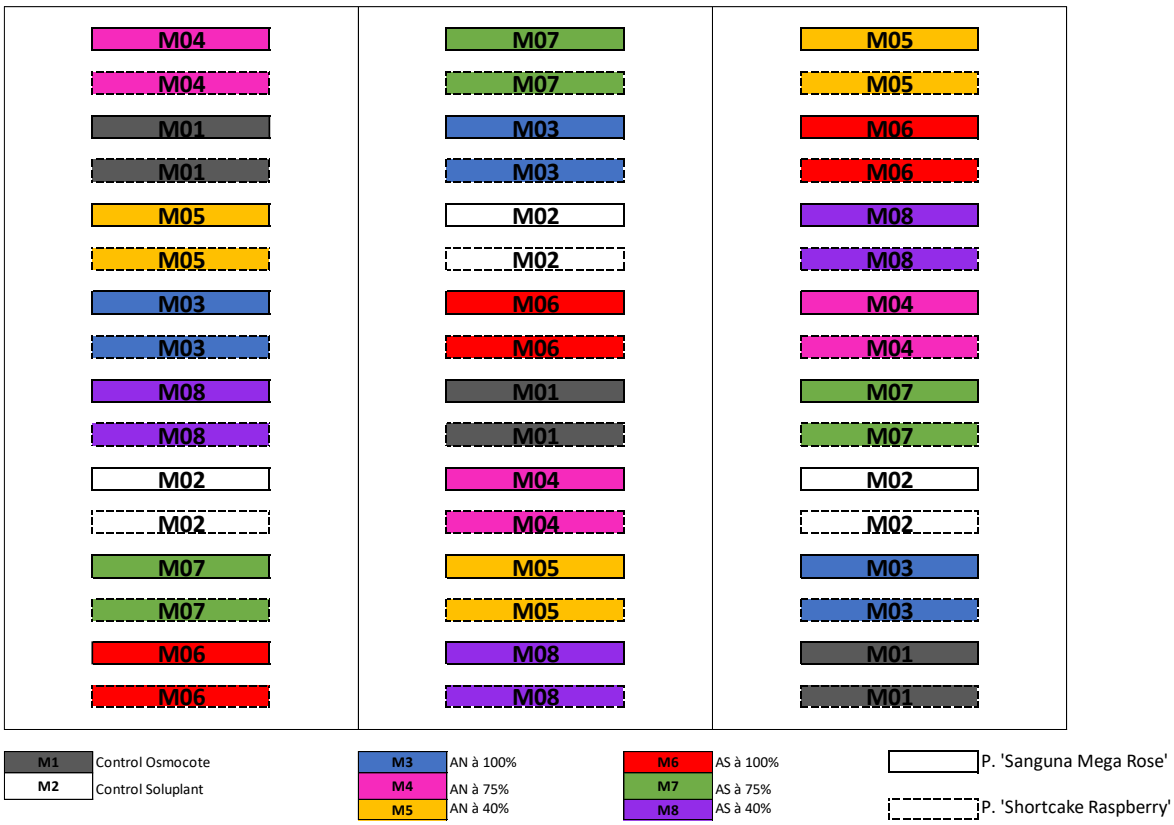

Figure 20: Schematic trial design with the different varieties and objects.

Calculations of the supplied doses:

The calculations of ideal concentrations of ammonium nitrate (AN) and ammonium sulphate (AS) were based on the Soluplant supplied doses. Indeed, laboratory analyses of RDFs showed that nitrogen content of AN is almost equal to the Soluplant's one. A three times bigger volume of AS is necessary to get the same nitrogen content.

Supplies frequency:

The control modality M1 gets only one solid mineral controlled-release fertilisation supply while potting on week 8.

Liquid fertilisation modalities (M2 to M8) get one first supply on week 11, then three supplies per week on week 12 and eventually two supplies per week from week 13 to week 15. There were ten supplies during the whole trial duration.

Supplies were made through pot by pot fertigation with a 1.5 L plastic beaker per block. Thus, each plant receives 50 mL of the solution. The control modality M1 is watered with the same volume of clear water per plant.

Composition of mineral and recycling-derived fertilisers:

Table 15: NPK composition of mineral and recycling-derived fertilisers

Fertilisers composition	N	P	K
Osmocote 5-6	12	7	19
Soluplant	16	6	26
Ammonium nitrate (AN)	14.2	0	0
Ammonium sulphate (AS)	17.4 (5.8*3)	0	0

Table 16: Composition of the RDFs. Samples were taken from the storage before or at the time of trial installation.

Product	Sampling date	NO_3-N	NH_4-N	Kjeldahl N	P_2O_5	K_2O	S
		g/kg Fresh material					
Ammonium nitrate	15/04/19	43.4	43.1	43.1	0.0	0.0	0.5
Ammonium sulphate	18/03/19	0.0	33.6	0.0	0.0	0.0	37.9

Table 17: NPK total quantity supplied during the trial.

Fertiliser dose		N	P	K
		g/plant (on the trial's duration)		
Osmocote		0.18	0.105	0.285
Soluplant		0.16	0.06	0.26
AN	**100%**	0.14	0.5	1
	75%	0.11		
	40%	0.06		
AS	**100%**	0.17		
	75%	0.13		
	40%	0.07		

3.2.1.2. Trial conditions

3.2.1.2.1. Climate conditions

Greenhouse and cultivation setpoints: the trial was carried out under a cold glasshouse with a high air vent opening on the ridge and a shade system, both controlled by a climate computer. The airing and shading setpoints are set by the technician, depending on the weather conditions. The glasshouse is heated to 12°C and the air vents open when the temperature reaches 20°C.

3.2.1.2.2. Overview trial development

Table 18: Overview on the timing of trial activities.

Date	Activity
22/02/2022	Potting
28/02/2022	Placing on saucers
16/03/2022	Supply of auxiliaries for integrated crop management: predatory mites THRIPEX (*Neoseiulus cucumeris*)
15/04/2022	End of the trial

3.2.1.3. Measurements

There are three kinds of follow-up:

- Aerial and root development:

Growth measures (diameter) were taken every two weeks to follow the plants' development. Moreover, root development measures were taken according to the root development scale (Figure 21).

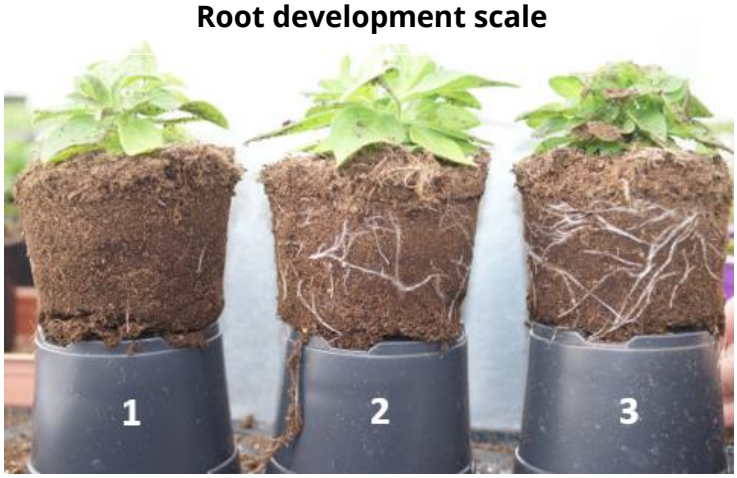

Figure 21: Depicturing of the root development scale.

- Evolution of the soil parameters in the substrate:

Soil parameters analysis are done every three weeks. pH, EC, nitrate and ammonium contents of the substrate are measured.

An aqueous extract has to be prepared before these analysis. 50 mL of substrate was taken in each modality and put in 75 mL of distilled water for thirty minutes. The solution is then filtered to get the aqueous extract on which the soil parameters analysis is done.

The RQflex® device measures the ammonium NH_4^+ content in the substrate, from 5 mg/L to 180 mg/L NH_4^+. The Nitracheck® device measures the nitrates NO_3^- through reflectometry.

Figure 22: The RQflex® (left) and Nitracheck® (right).

- Follow-up of the flowering period:

Weekly evaluation of the phenological stage were made (vegetative, presence of buds, presence of flowers) and the number of flowers per plant was counted.

3.2.1.4. Statistical data processing

The data of the trial were analysed with R Studio statistical software. Variance analysis ANOVA were made, or Kruskal-Wallis nonparametric tests when ANOVA conditions were not observed. ANOVA conditions were checked with the Shapiro-Wilk normality test and the Bartlett equality of variance test. Newman-Keuls or Duncan post hoc comparison of means were then used. Eventually, the confidence level of the analysis was of 95%.

3.2.2. Results and discussion

3.2.2.1. Rooting scale

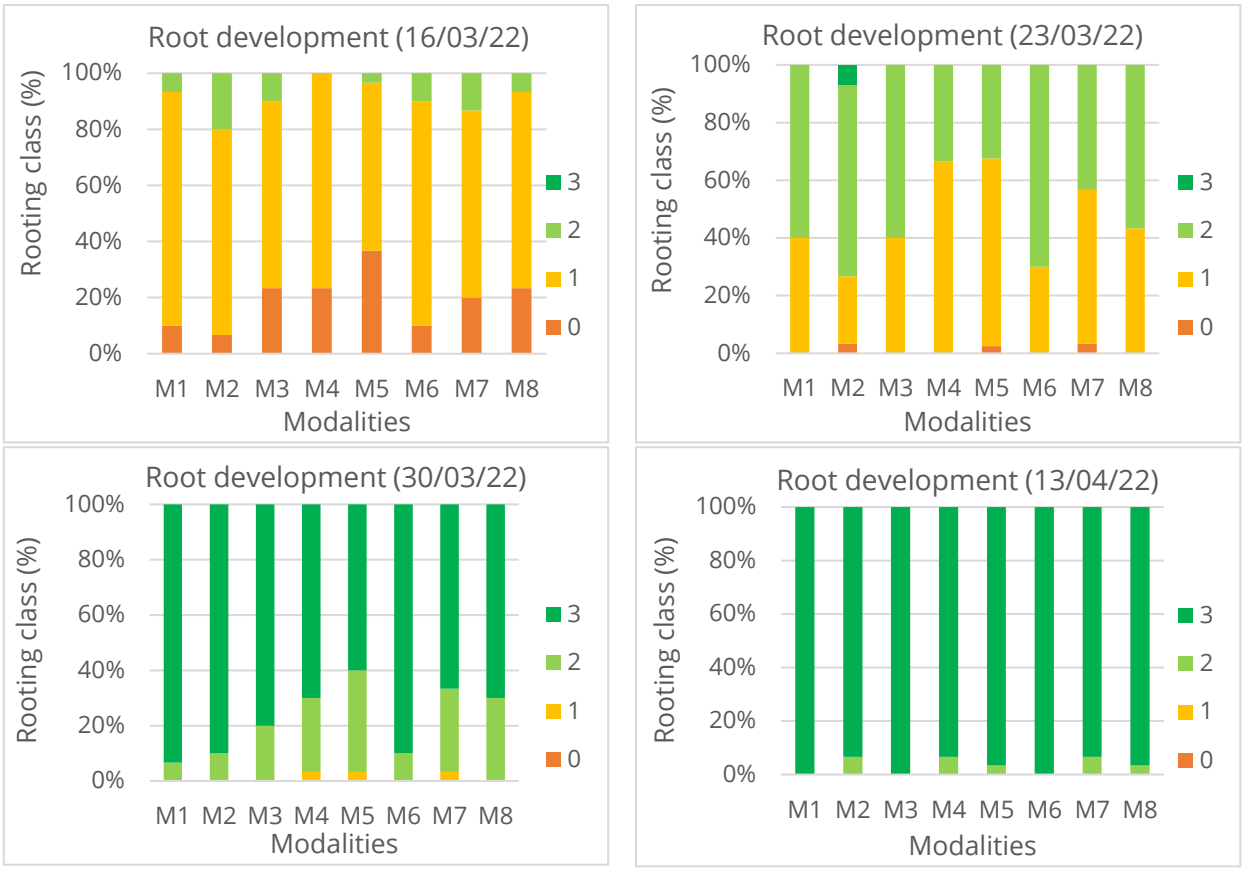

Figure 23: Graphs on the root development and class across the growth season of the different modalities.

On the first evaluation, the rooting is similar between modalities, but slightly stronger with the Soluplant supply. On the intermediate evaluation, with the RDFs supplied at 100% of the ideal concentration, the plants seem to root faster than with the RDFs supplied at 75% and 40%. This rooting speed is similar to the speed in the modalities with mineral fertilisation.

At the end of the trial, the root development is homogeneous and it is optimal in all modalities. The use of RDFs enables a good rooting of Petunia. Moreover, the roots are healthy and there is no burn.

Figure 24: the roots of the Petunia 'Sanguna Mega Rose' variety across the different modalities (from left to right: M1 – M8).

Figure 25: the roots of the Petunia 'Shortcake Raspberry' variety across the different modalities (from left to right: M1 – M8).

3.2.2.2. Diameter

3.2.2.2.1. Diameter of combined varieties

Diameter measures were made from 23/03/22 to 13/04/22.
There is no significant diameter difference between modalities on the first three measures (*diameter 1: Kruskal-Wallis: p-value = 0.3579, D2: Kruskal-Wallis: p-value = 0.6631, D3: Kruskal-Wallis: p-value = 0.3218*). There are significant differences on the final diameter of Petunia. When there was RDFs supplies, there is no significant difference with the control Osmocote. Plants with RDFs supplies at 40% of ideal concentration are smaller than the ones fertilized

with Soluplant. They are larger with RDFs supplies at 100% of the ideal concentration than with 40% of the ideal concentration.

The use of RDFs enables to get a satisfactory Petunia size, similar to the ones that got mineral fertilisation.

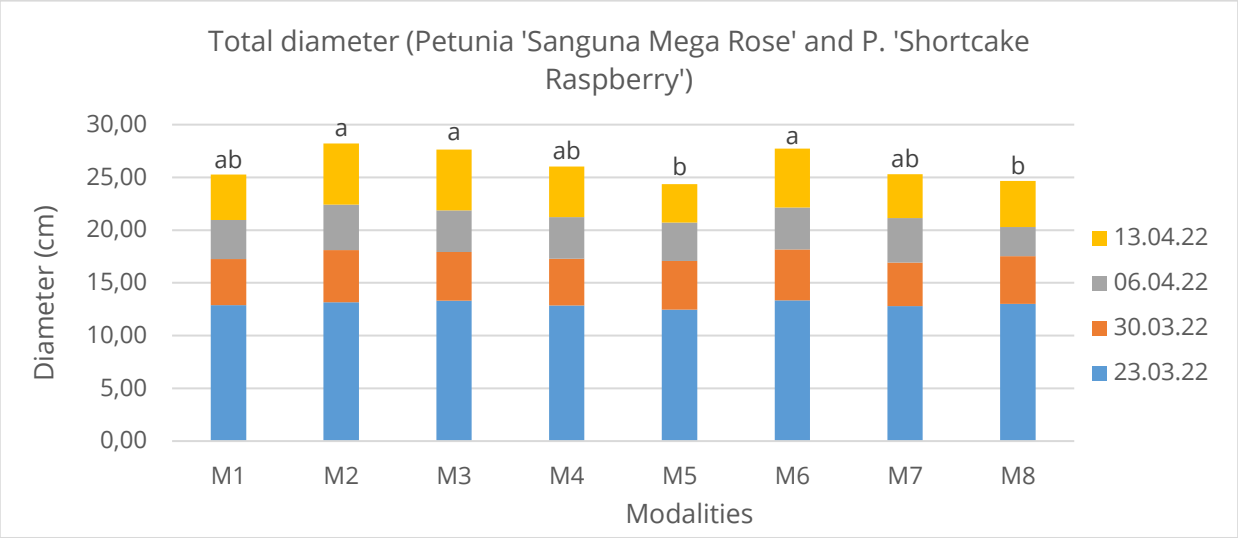

Figure 26: Total diameter of the different modalities across the growing period. Kruskal-Wallis, p-value = 0.008162, Duncan post hoc test.

Table 19: Significant diameter differences between the RDFs and the two mineral fertiliser controls. Kruskal-Wallis, p-value = 0.008162, Duncan post hoc test.

	Diameter in relation to	
Modality	**M1**	**M2**
M3 (AN 100%)	=	=
M4 (AN 75%)	=	=
M5 (AN 40%)	=	-
M6 (AS 100%)	=	=
M7 (AS 75%)	=	=
M8 (AS 40%)	=	-

3.2.2.2.2. Diameter of Petunia 'Sanguna Mega Rose'

There is no significant diameter difference between plants on the first two measures (*diameter 1: Kruskal-Wallis: p-value = 0.3629, D2: Kruskal-Wallis: p-value = 0.141*). There start to be differences at the third measure (06.04.22) (*D3: Kruskal-Wallis: p-value = 0.03213*).

At the end of the trial, the growth of plants with AN and AS is as substantial as the one of the plants with mineral fertilisation (Osmocote M1 or Soluplant M2). The only ones with a

significantly smaller diameter than the ones which got Soluplant supplies were fertilised with AN at 40% (*final diameter: Kruskal-Wallis: p-value = 0.00363*).

Taking into account only the 'Sanguna Mega Rose' variety, there is no diameter difference depending on the AN or AS concentration. The average size of this variety is 30 cm in diameter.

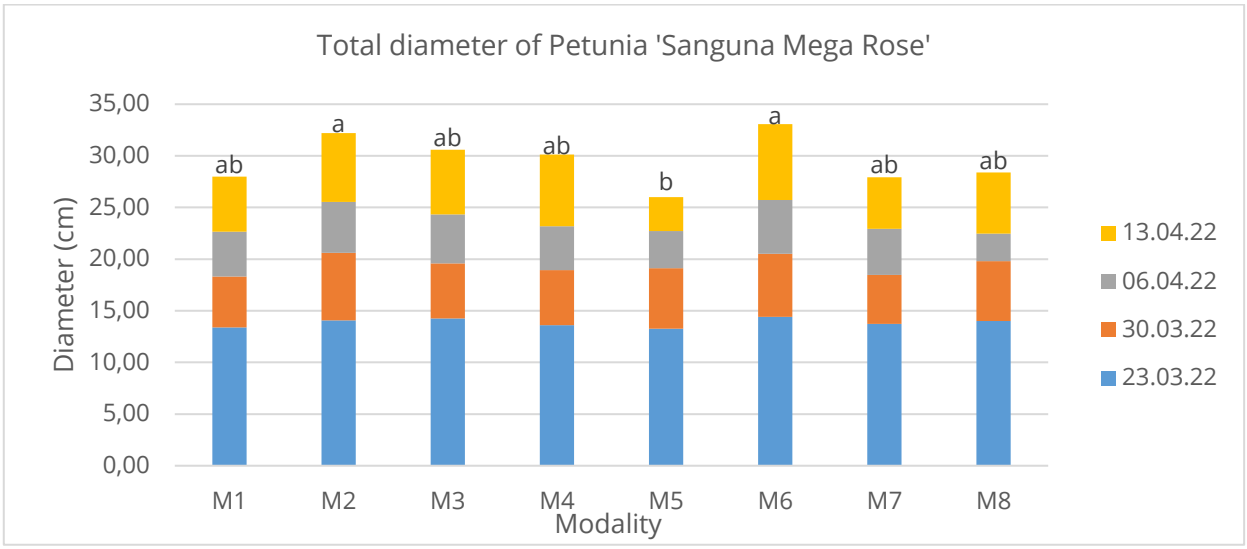

Figure 27: The total diameter of the Petunia 'Sanguna Mega Rose' variety across the different modalities. Kruskal-Wallis, p-value = 0.00363, Student-Newman-Keuls post hoc test.

Figure 28: Picture of the Petunias taken at the same moment across the different modalities.

3.2.2.2.3. Diameter of Petunia 'Shortcake raspberry'

There is no significant diameter difference between Petunia of the 'Shortcake Raspberry' variety on the first three measures (*D1: Kruskal-Wallis: p-value = 0.5342, D2: Kruskal-Wallis: p-value = 0.1332, D3: Kruskal-Wallis: p-value = 0.2105*).

At the end of the trial, there are some significant differences (D4: Kruskal-Wallis: p-value = 0.0005874). The diameters are similar with RDFs supplies and Osmocote supply. Only the AN at 100% of the ideal concentration enables to get a larger diameter. The average size of this variety is 23 cm in diameter. The use of RDFs enables to get a satisfactory *Petunia* size, similar to the ones that received a mineral fertilisation.

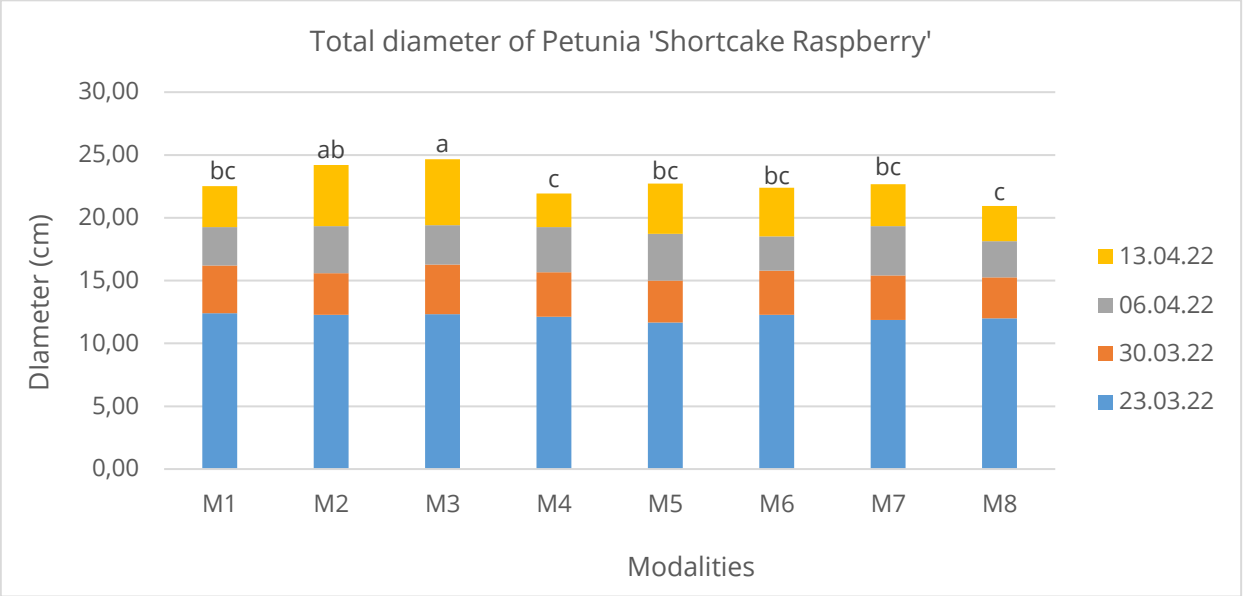

Figure 29: The total diameter of the Petunia 'Shortcake Raspberry' variety across the different modalities. Kruskal-Wallis, p-value = 0.0005874, Student-Newman-Keuls post hoc test.

Figure 30: Picture of the Petunias taken at the same moment across the different modalities.

3.2.2.3. Phenological stage evolution

Most *Petunia* are in the vegetative stage at the first rating. Plants are homogeneous between modalities.

At the second rating (06/04/22), it seems that more *Petunia* have buds or flowers when they received an AN supply, especially with 40% of the ideal concentration. Most *Petunia* are still in the vegetative stage.

At the last rating (13/04/22), most *Petunia* have buds. More flowers are blooming in the modalities with AS supplies at 100% and 75% of the ideal concentration. Blooming *Petunia* on 13/04 have one to two open flowers in average in all modalities.

Petunia's development is similar with both RDFs and mineral fertilisers.

Figure 31: The percentage of plants per class depending on the phenological stage on three different data across the different modalities.

3.2.2.4. Evolution of the soil parameters

Soil parameters were measured for the first time after the fifth supply of fertilisers and a second time after all ten supplies of fertilisers. Measure dates were as follows: 31st of March and 14th of April. Soil parameters were measured on *Petunia* of the 'Sanguna Mega Rose' variety.

- Evolution of pH and electroconductivity

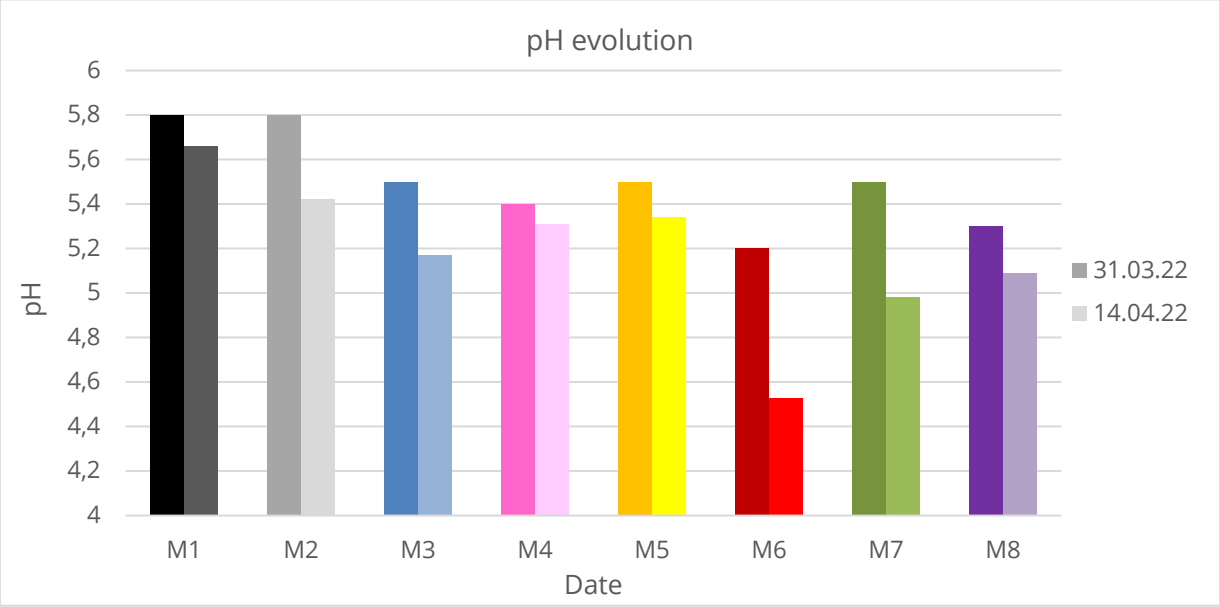

Figure 32: The evolution of the soil pH across the different modalities measured on two different dates.

At the first measure, pH ranges between 5.2 and 5.8. It is slightly higher in the modalities with mineral fertilisation than with the RDFs supplies.

At the second measure, the substrate is becoming more or less acid depending on the modality. The pH in the modalities with mineral fertilisers remains higher than in the other modalities. Moreover, the pH in the modalities with AS supplies is the most acidic, especially with the highest concentration of AS (pH = 4.5 at the end of the trial). The nitrogen of the ammonium sulphate is mostly in the shape of NH_4^+ and less in the shape of NO_3^-. Since there are more H^+ ions in the substrate, these should be the reason for the acidification of the substrate over the medium term.

The substrate acidification did not impact the quality of the plants during the trial.

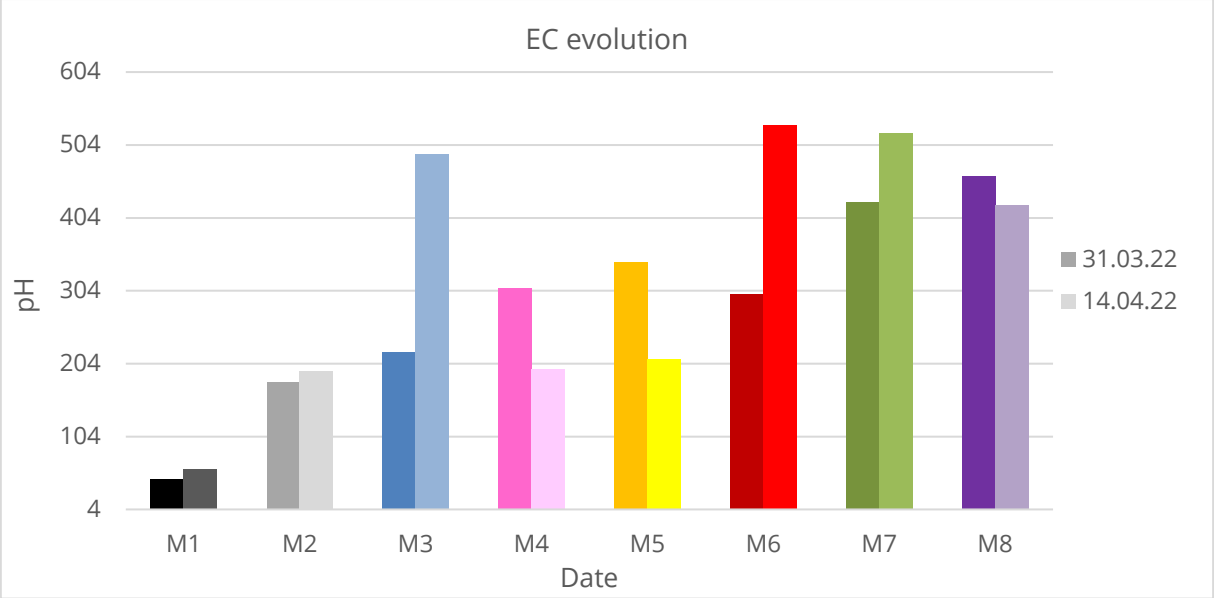

Figure 33: The evolution of the electroconductivity across the different modalities measured on two different dates.

On 31/03, after five fertilisation supplies, there were EC differences between modalities. Mineral fertilisation (Osmocote and Soluplant) induced lower EC values than RDFs.

At the end of the trial (14/04), the EC decreased in the modalities with AN supplies at 75% and 40% and AS supplies at 40%. The EC increased in the modalities with AN supplies at 100% and AS supplies at 100% and 75%. Thus, the nutrients provided by the fertilisers had been quickly used in the modalities with the lowest concentrations.

- Evolution of nitrate and ammonium concentration

Ammonium concentration

Halfway through the trial and at the end of the trial, ammonium concentration was lower than 5 mg/L in all modalities. Microbial activity in the substrate enabled to transform ammonium into nitrate.

Nitrate concentration

Halfway through the trial, there is a higher and satisfactory nitrate concentration (90 mg/L) with the Soluplant supplies than with Osmocote and RDFs supplies (10 to 30 mg/L). At the end of the trial, nitrate concentration decreases gradually in all modalities. Plants use a lot of nitrogen to grow and root at this time. The final nitrate concentration is close to 0, except in the modality with Soluplant supplies.

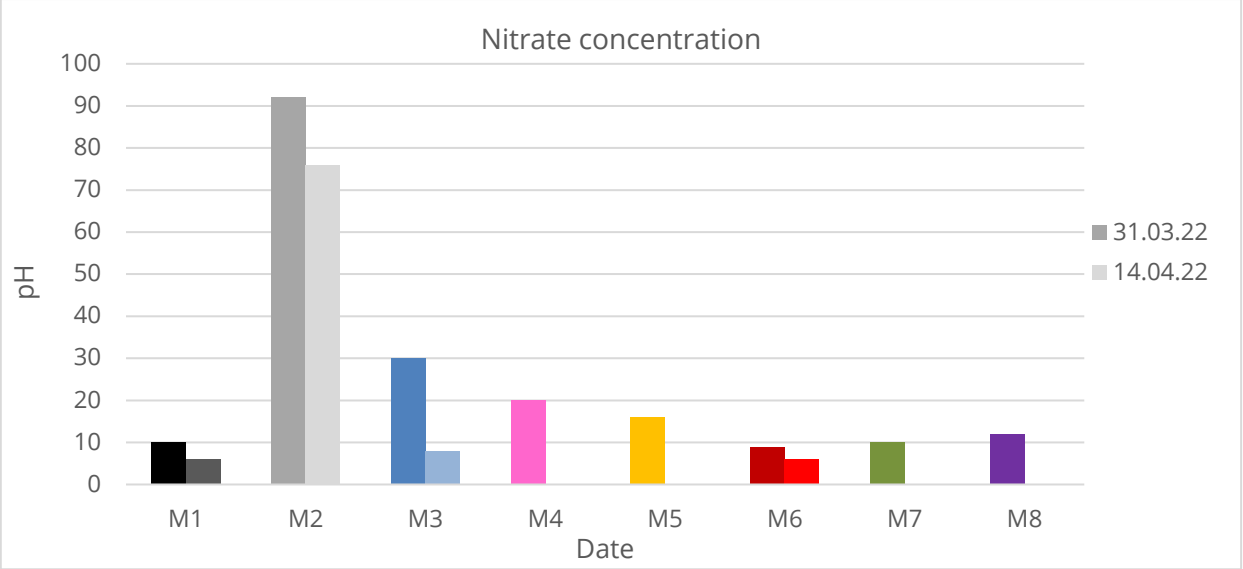

Figure 34: The evolution of the nitrate concentration across the different modalities measured on two different dates.

3.2.3. Conclusions

Ammonium nitrate and ammonium sulphate seem to be as efficient as mineral control fertilisers when used as liquid recycling-derived fertilisers on *Petunia*. At the end of the trial, the quality of plants fertilized with RDFs is very good. They do not have any deficiency or nutrient excess.

The use of these products, especially the ammonium sulphate, requires to be particularly careful in the follow-up of the substrate acidification. It is important to regularly check pH and EC in the substrate. Moreover, ammonium nitrate and sulphate are liquid fertilisers and the supplies are done through fertigation. They require regular supplies. On the contrary, the solid fertiliser Osmocote is brought only once, at the beginning of the trial.

3.3. Viola

3.3.1. Material and methods

3.3.1.1. Experimental setup

3.3.1.1.1. Crop and cultivar

The pansy species used for this Viola experiment was *Viola cornuta* 'Rocky Plum Antique' from the Syngenta Group.

3.3.1.1.2. Cultivation conditions

Substrate: the B400 reference of Stender society is used. Its main component is blond peat. Its structure is medium.

Fertilization at potting time:

- **M1**: mineral NPK fertiliser, Osmocote (12-7-19) 5-6 months, 3g/L
- **M2**: no additional fertilization in the substrate at potting time
- **M3 - M8**: Patentkali, 2g/L and Superphosphate 45, 1g/L

Irrigation: Plants are watered pot by pot with a beaker. Each plant receives 50mL water. The watering frequency is defined by the technician, depending on the weather conditions that can cause a water stress.

Plants were potted on week 36 in Ø10,5 cm pots and the trial is implemented on week 38.

3.3.1.1.3. Trial design and treatments

The trial modalities match the following fertiliser management technique:

- **M1**: Reference mineral controlled-release fertiliser Osmocote
- **M2**: Reference soluble mineral fertiliser Soluplant (16-6-26), 2g/L
- **M3**: Ammonium nitrate, 100% of ideal concentration, 2g/L
- **M4**: Ammonium nitrate, 75% of ideal concentration, 1.5g/L
- **M5**: Ammonium nitrate, 40% of ideal concentration, 0.8g/L
- **M6**: Ammonium sulphate, 100% of ideal concentration, 6g/L
- **M7**: Ammonium sulphate, 75% of ideal concentration, 4.5g/L
- **M8**: Ammonium sulphate, 40% of ideal concentration, 2.4g/L

Each modality is made of thirty pansies arranged in three Fisher blocks counting ten plants each. The pots stand on saucers (five pots per saucer) to avoid any contamination from one modality to the other.

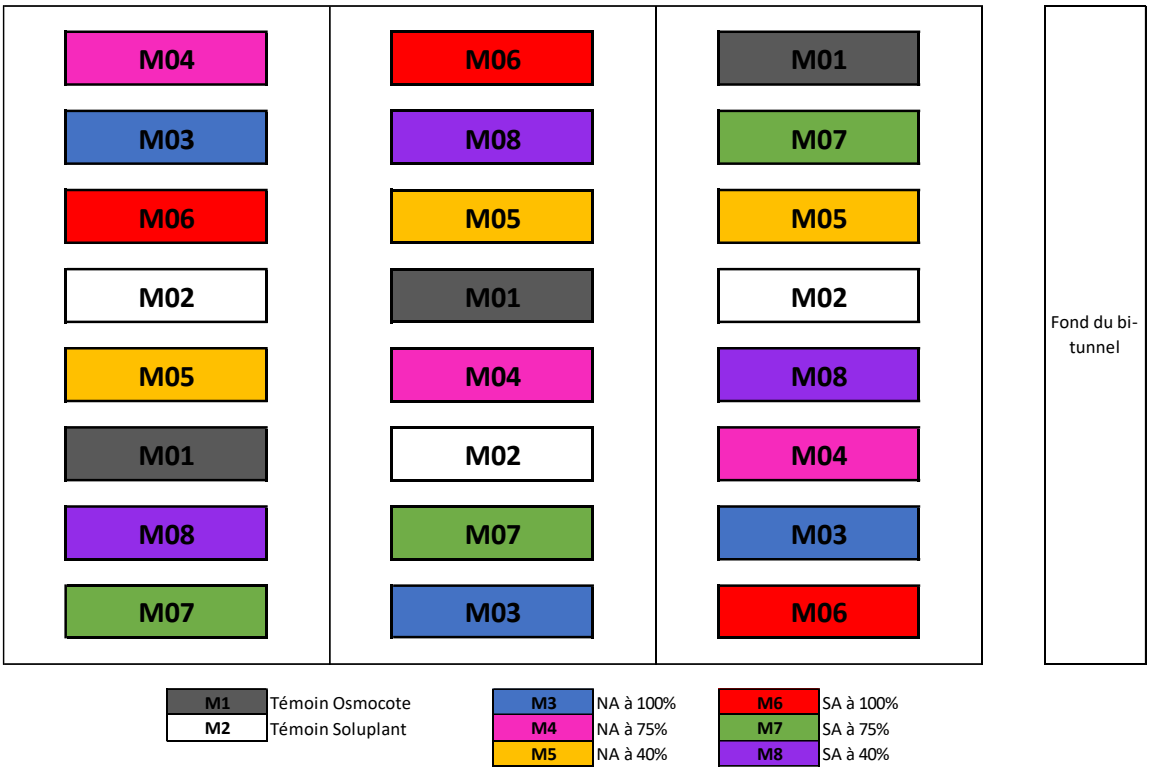

Figure 35: Schematic trial design with the different varieties and objects.

Calculation of the supplied doses:

The calculations of ideal concentrations of ammonium nitrate (AN) and ammonium sulphate (AS) were based on the Soluplant supplied doses. Laboratory analyses of RDFs showed that nitrogen content of AN is almost equal to the Soluplant's one. A three times bigger volume of AS is necessary to get the same nitrogen content.

Supplies frequency:

The control modality M1 gets only one solid mineral controlled-release fertilisation supply while potting on week 36.

Liquid fertilization modalities (M2 to M8) get one supply on week 39, two supplies on week 40, three supplies per week on week 41 and 42 and one supply on week 43. Starting from week 44, almost all pots are soaked with water. There were only water supplies on the plants which needed it (week 44 : water supply of 500mL on M1 Block 3; week 45 : water supply on M2). The trial ended on week 46.

Supplies were made through pot by pot fertigation with a 1,5 L beaker per block. Thus, each plant receives 50 mL of the solution. The control modality is watered with the same volume of clear water per plant.

Composition of mineral and recycling-derived fertilisers:

Table 20: NPK composition of mineral and recycling-derived fertilisers.

Fertilisers composition	N	P	K
Osmocote 5-6	12	7	19
Soluplant	16	6	26
Ammonium nitrate	14.2	0	0
Ammonium sulphate	17.4 (5.8*3)	0	0

Table 21: Overview on the RDF composition. Samples are taken from storage beforehand or at the time of trial installation.

Product	Sampling Date	NO_3-N	NH_4-N	Kjeldahl N	P_2O_5	K_2O	S
		g/kg Fresh material					
Ammonium nitrate	04/15/19	43.4	43.1	43.1	0.0	0.0	0.5
Ammonium sulphate	03/18/19	0.0	33.6	0.0	0.0	0.0	37.9

Table 22: NPK total quantity supplied during the trial.

Fertiliser dose		N	P_2O_5	K_2O
		g/plant (on the trial's duration)		
Osmocote		0.18	0.105	0.285
Soluplant		0.16	0.06	0.26
AN	**100%**	0.14	0.5	1
	75%	0.11		
	40%	0.06		
AS	**100%**	0.17		
	75%	0.13		
	40%	0.07		

Osmocote 5-6 months: the speed of release of the fertilising elements depends on the temperature. The advised period of use is given for an average temperature of 21°C.

3.3.1.2. Trial conditions

3.3.1.2.1. Climate conditions

Greenhouse and cultivation setpoints: the trial was carried out under a cold greenhouse.

3.3.1.2.2. Overview trial development

See Figure 36.

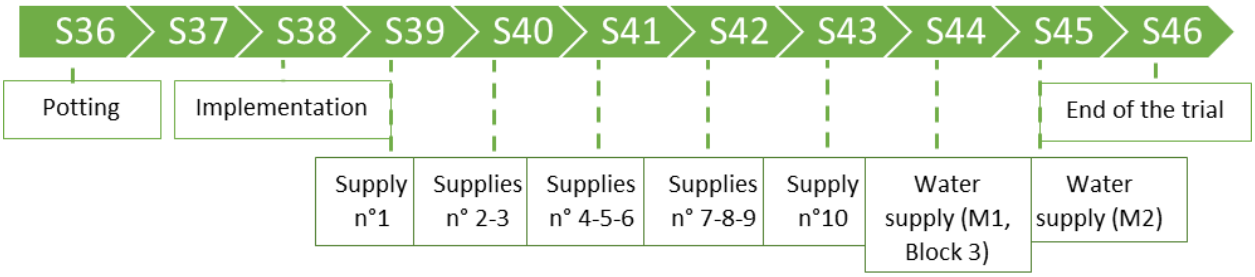

Figure 36: Trial overview from potting until end of the trial.

3.3.1.3. Measurements

Several kinds of follow-up are done :

- Aerial and root development:

Growth measures (height and diameter) were taken once per week every week (from week 38 to week 46) to follow the plants' development. Moreover, root development observations were done according to the scale in Figure 37.

Figure 37: Depicturing of the root development scale in pansies.

- Evolution of the soil parameters in the substrate:

Soil parameters analysis are done every two weeks (on weeks 40, 42 and 44). pH, electroconductivity, nitrates and ammonium contents of the substrate are measured.

An aqueous extract has to be prepared before these analysis. 100mL of substrate were taken in each modality and put in 150mL of distilled water for 30 minutes. The solution is then filtered to get the aqueous extract on which the soil parameters analysis is done.

The RQflex® device measures the ammonium NH_4^+ content in the substrate, from 5 mg/L to 180 mg/L NH_4^+. The Nitracheck® device measures the nitrates NO_3^- through reflectometry.

Figure 38: The RQflex® (left) and Nitracheck® (right).

- Follow-up of the flowering period:

Weekly evaluations of flowering were made from week 41 to week 46. The number of flowers was counted. Each week, counted flowers were removed from the plants.

- Commercial grade of the plants:

Commercial grades were given to the plants at the end of the trial, on week 46. The different grades are as follows: 1, the plant is not marketable; 2, the plant is marketable, but is second-class; 3, extra quality plants. The observations were done according to the following scale:

Figure 39: Depicturing of the commercial grade of the pansies.

3.3.1.4. Statistical data processing

The data of the trial was analyzed with R Studio statistical software. Variance analyses ANOVA were made, or Kruskal-Wallis nonparametric tests when ANOVA conditions were not observed. ANOVA conditions were checked with the Shapiro-Wilk normality test and the Bartlett equality of variances test. Newman-Keuls post hoc comparison of means were then used. Eventually, the confidence level of the analysis was of 95%.

3.3.2. Results and discussion

3.3.2.1. Rooting scale

Every week the roots are given a grade. The scale is from 1 to 3 (according to the scale in Figure 37). «1» means that the rooting is weak, «3» means that the rooting is strong.

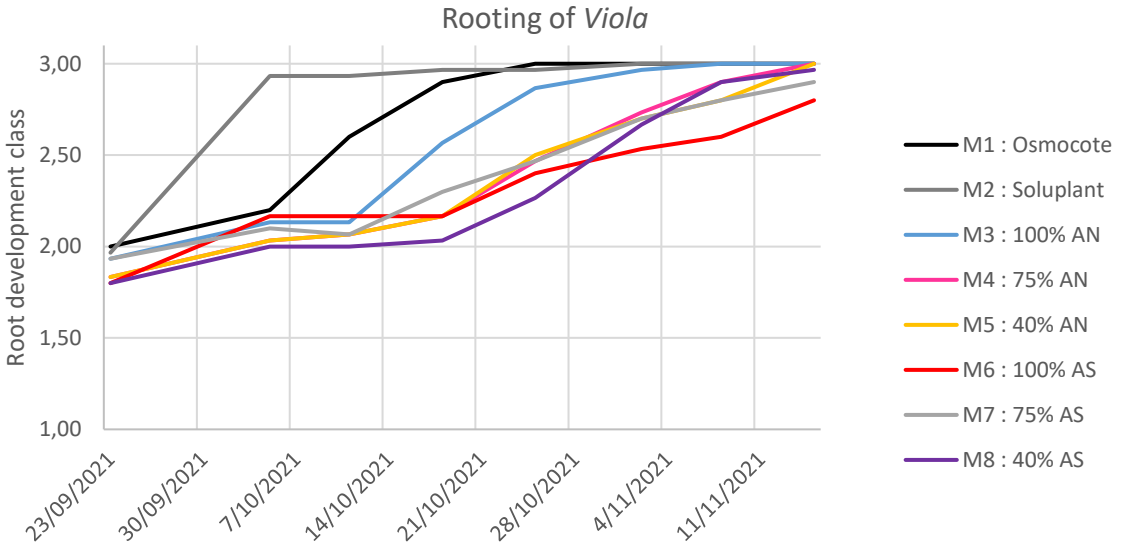

Figure 40: The rooting development over time of the different modalities in Viola.

Plants with Soluplant supply (M2) took root faster. Then come the ones with Osmocote supply (M1) and later the ones with 100% AN (M3). The plants of the other modalities took root with the same speed, slower than the latter.

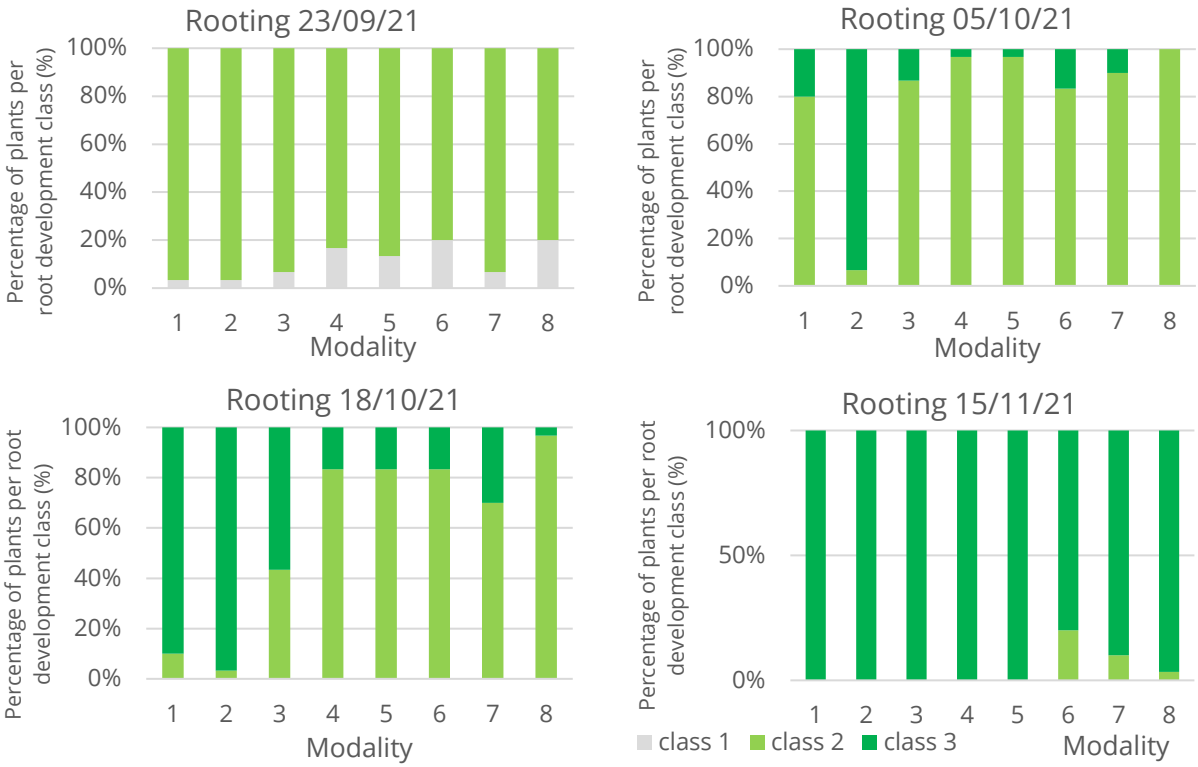

Figure 41: The rooting class of the different modalities on different times during the growth.

At the beginning of the trial (23/09), most plants belong to the root development class «2».

On the 5th of October, there is a difference in rooting. Nearly all plants of the M2 modality belong to the root development class «3», while the others remain in the root development class «2».

On the 18th of October, namely after six supplies, the plants of the M1 and M2 modality and 50% of the plants of M3 modality are strongly rooted (class «3»).

At the end of the trial, on 15th November, all plants of the M1 to M5 modalities belong to the root development class 3. A few plants of the M6 to M8 modalities remain in the root development class « 2 ».

3.3.2.2. Diameter

The average diameter of pansies and its evolution on every period (growth 1 to 7) were measured.

Table 23: Statistical analysis on the average diameter and its evolution each growth period.

Growth	No. of supplies	Statistical test	p-value	Difference
Total	10	ANOVA	< 2.2e-16	significant
1	2	ANOVA	< 2.2e-16	significant
2	3	Kruskal-Wallis	= 1.336e-10	significant
3	6	Kruskal-Wallis	= 5.065e-12	significant
4	9	Kruskal-Wallis	= 1.449e-07	significant
5	10	Kruskal-Wallis	= 1.266e-05	significant
6	10	Kruskal-Wallis	= 0.2096	not significant
7	10	Kruskal-Wallis	= 0.05576	not significant

In the first period, from the 23rd of September 2021 to the 5th of October 2021, namely after two supplies of fertilization, plants with Osmocote supply (M1) grew the most. There is no difference between the other modalities. On the second period, the Soluplant supply (M2) lead to a greater development.

From the 2nd of November (week 44), there is no more difference in growth between the pansies of all the modalities. There is no more supply of fertilization on this date.

Figure 42: Overview on the growth of Viola throughout the growth season.

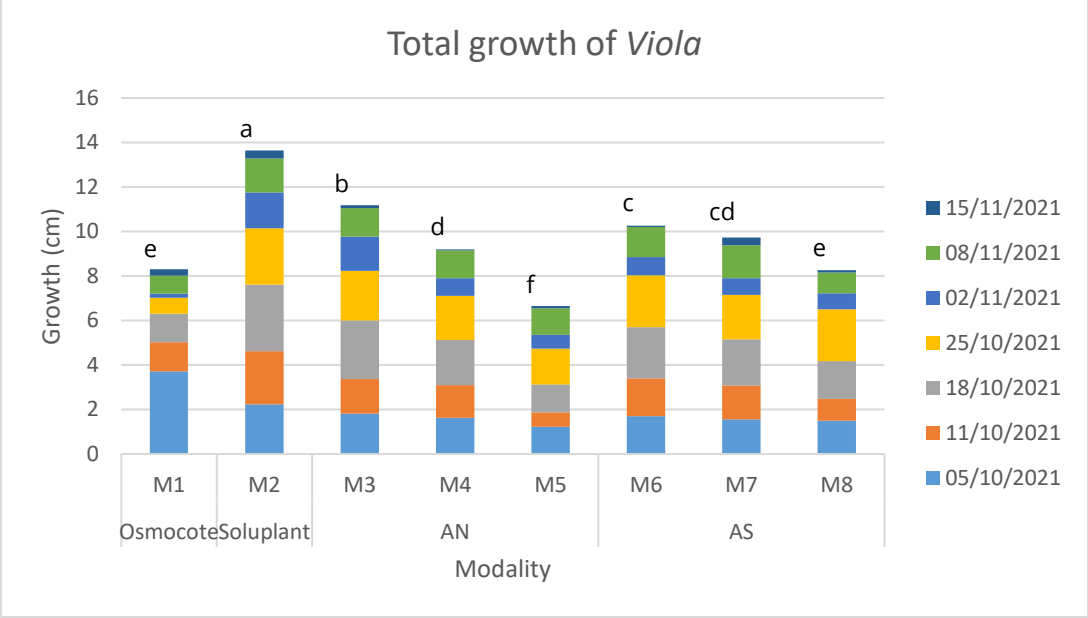

Figure 43: Global overview of the growth of Viola across the different modalities.

The average diameter of pansies after ten fertilization supplies, from the 23rd of September until the 15th of November, was measured.

The pansies of the M2 modality, with Soluplant supplied, have the greatest growth across the whole length of the trial.

There are significant development differences depending on the supplied dose between the plants which got AN supplies. 100% of AN (M3) enables the greatest growth, 75% (M4) the second greatest and then 40% (M5). 100% and 75% of AS enables to get plants with a greater diameter than Osmocote (M1) and the plants which got 40% of AS supply grew less than the control plants.

There is no growth difference between the modalities with 100% and 75% of AS (M6/7). These plants grew more than the ones of the modality with 40% of AS (M8) which have a similar growth as the control plants with Osmocote (M1).

There is a similar development with 75% of AS and 75% of AN.

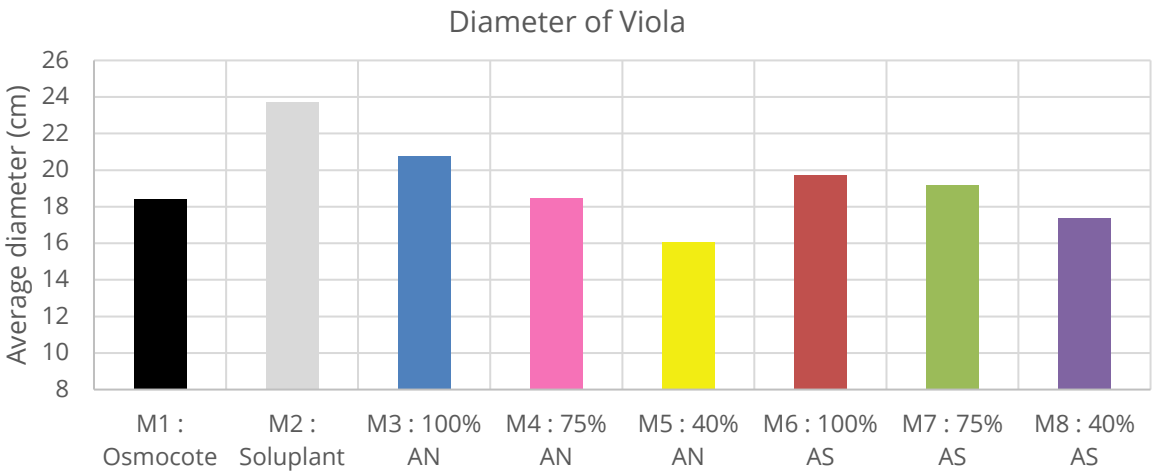

Figure 44: The average Viola diameter across the different modalities.

The average diameter of plants from the control modality M1 (Osmocote) is approximately 18.5 cm. With Soluplant supplies, the average diameter if 23.5 cm.

3.3.2.3. Flowering evolution

Figure 45: The flowering evolution on the 8th of November across the different modalities.

Every week, the flowers are counted then removed.

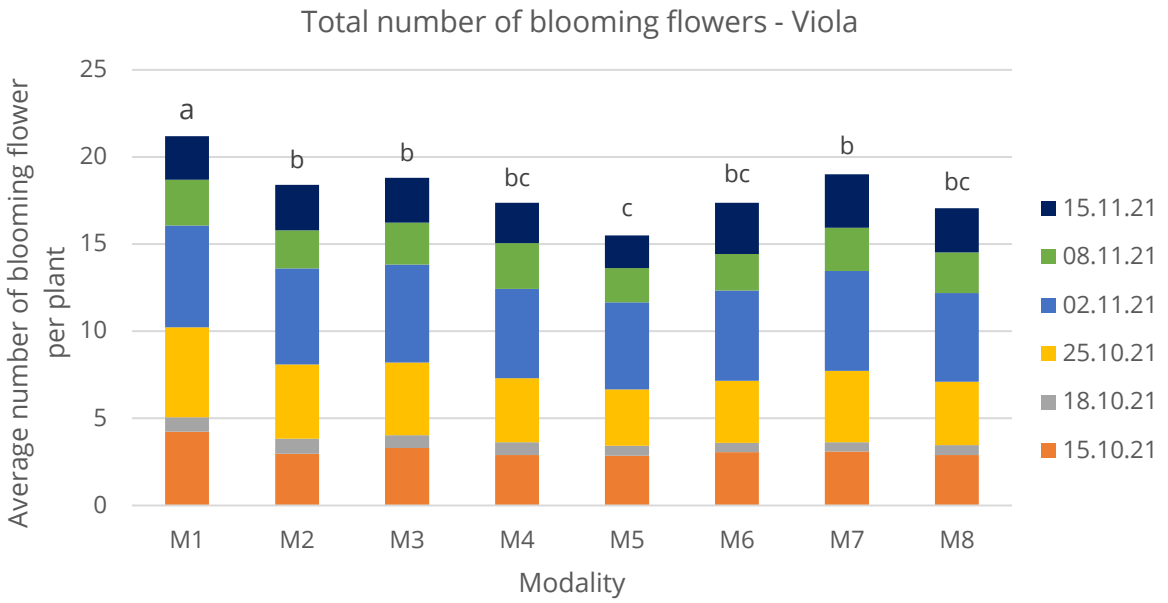

Figure 46: The total number of blooming flowers across the different modalities. Kruskal-Wallis: p-value = 2.2e-10.

There is between 21.2 (M1) and 15.5 (M5) flowers per plant on average on the total length of the trial.

The control M1 have significantly more flowers than the plants of the other modalities. Indeed, at the beginning of the trial, these plants grew more than the others and produce more flowers during the first observations. Then, the number of flowers is similar to the other modalities.

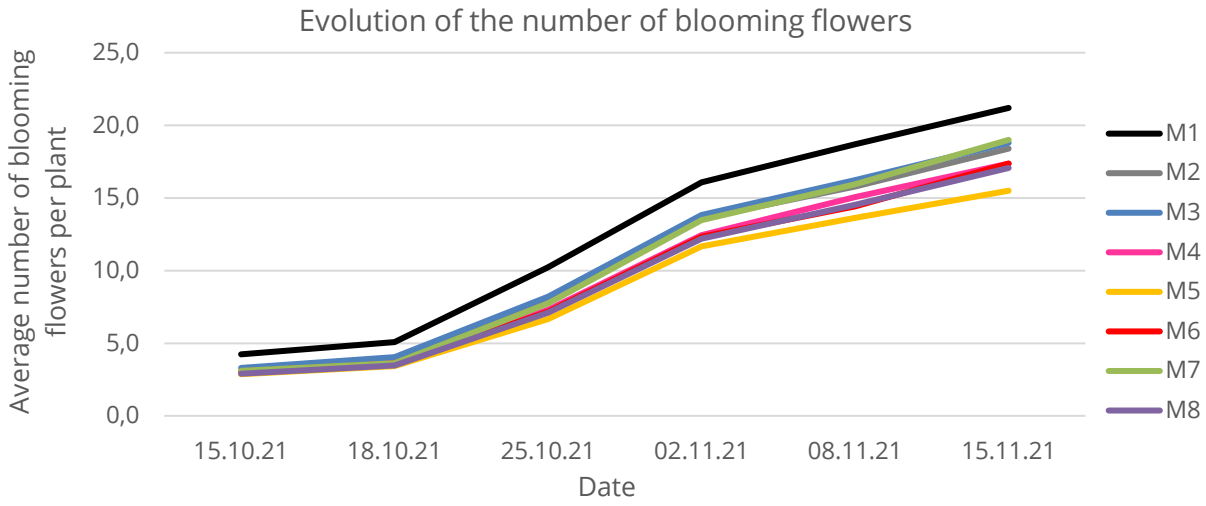

Figure 47: Evolution of the number of blooming flowers over the growth period across the different modalities.

In the first periods, the number of flowers increases a lot until the 2nd of November. Then the increase of the number of flowers slows down as does the growth at the end of the trial.

*Figure 48: Flowers on 02/11, total of eight modalities * 3 blocks – namely 1293 flowers.*

- Contorted leaves

Contorted leaves were mainly noticed on M1 modality (Osmocote), but also less considerably on a few plants of the other modalities.

The leaf distortion can be caused by several things: a calcium deficiency, a problem with the Osmocote release or even the water excess.

Figure 49: Example of leaf distortion. The same plant on 11/10/2021 (left) and 25/10/2021 (right).

3.3.2.4. Commercial grade

At the end of the trial, a commercial grade depending on the leaves colour was given to each pansy. The scale is as follows:

- **3**: the leaves are perfectly green
- **2**: a few leaves have purple edges
- **1**: a lot of leaves have purple edges

Figure 50: Depicturing of the commercial grade scale.

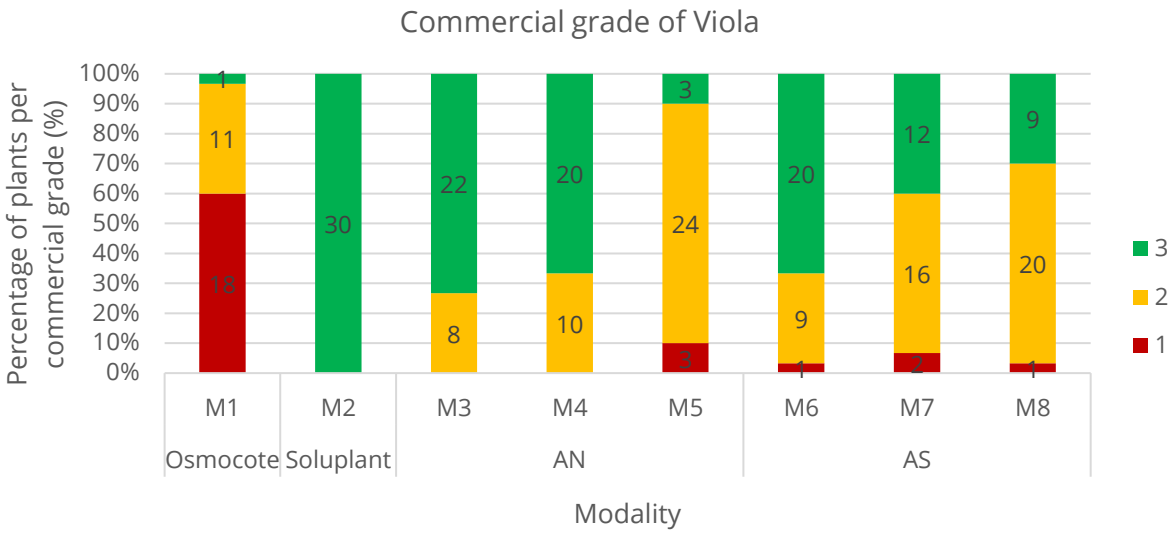

Figure 51: The commercial grade of the pansies across the different modalities.

The purple colouring appeared as soon as the 18th of October on the edges of old leaves. First, all the plants of M1 modality had leaves with purple edges. Then, the symptoms were spotted on modalities with 40% of AN (M5) and with 40% of AS (M8) and only afterwards on the ones with 75% and 100% of AN and AS.

These symptoms are on the leaves because of a water stress. Too much water was added when the supplies were made three times per week. All plants were quickly soaked with water.

Plants of modality M2 had the greatest growth. Their rooting was also faster. Maybe it can explain why they did not suffer from the water stress. Moreover, on week 45, only the plants of modality M2 were watered because they were the only one which were not soaked with water.

The plants of modality M1 had a greater growth at the beginning of the trial, but then it slowed down and became weaker, probably because of the water stress or a problem with the release of Osmocote which slowed down the growth, making the plants more sensitive to water stress.

The modalities with 40% of RDFs (M5 and M8) have a weaker growth than the others, that is why the water stress had a bigger impact on these plants and there were more symptoms.

3.3.2.5. Evolution of soil parameters

pH, EC, NO_3^- content (using Nitracheck®) and NH_4^+ content (using RQflex®) were measured. It is worth noting that plants were under water stress since the 18th of October.

The pH decreases a bit during the trial. It could play a role in nutrients absorption problems and cause the deficiencies seen on the pansies' leaves. It was stepped up by the increase of EC. Supplies were probably too much for the uptake capacity of the plants. The water stress during the last supplies also obstructed the nutrients absorption.

NO_3^- content decreased as well, except on Soluplant and 100% AN modalities. The values are overall very low.

Table 24: The different parameters across the different modalities at three different times during the growth.

Date	Parameter	M1	M2	M3	M4	M5	M6	M7	M8
06/10/2021 ***After 2 supplies***	pH	6	6	5.38	5.66	5.98	5.87	5.7	5.69
	Ec	75.8	165.5	346	697	232	245	425	561
	RQflex®	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0
	Nitracheck®	9	14	27	11	8	7	7	8
18/10/2021 ***After 6 supplies***	pH	5.54	5.87	5.84	5.78	5.86	5.47	5.55	5.51
	Ec	134.5	204	429	575	443	748	527	749
	RQflex®	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0
	Nitracheck®	8	9	0	7	8	9	6	6
02/11/2021 ***After 10 supplies***	pH	5.47	5.68	5.53	5.66	5.67	4.9	5.09	5.27
	Ec	182.9	280	655	552	510	947	823	827
	RQflex®	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0
	Nitracheck®	6	22	5	5	7	7	7	8

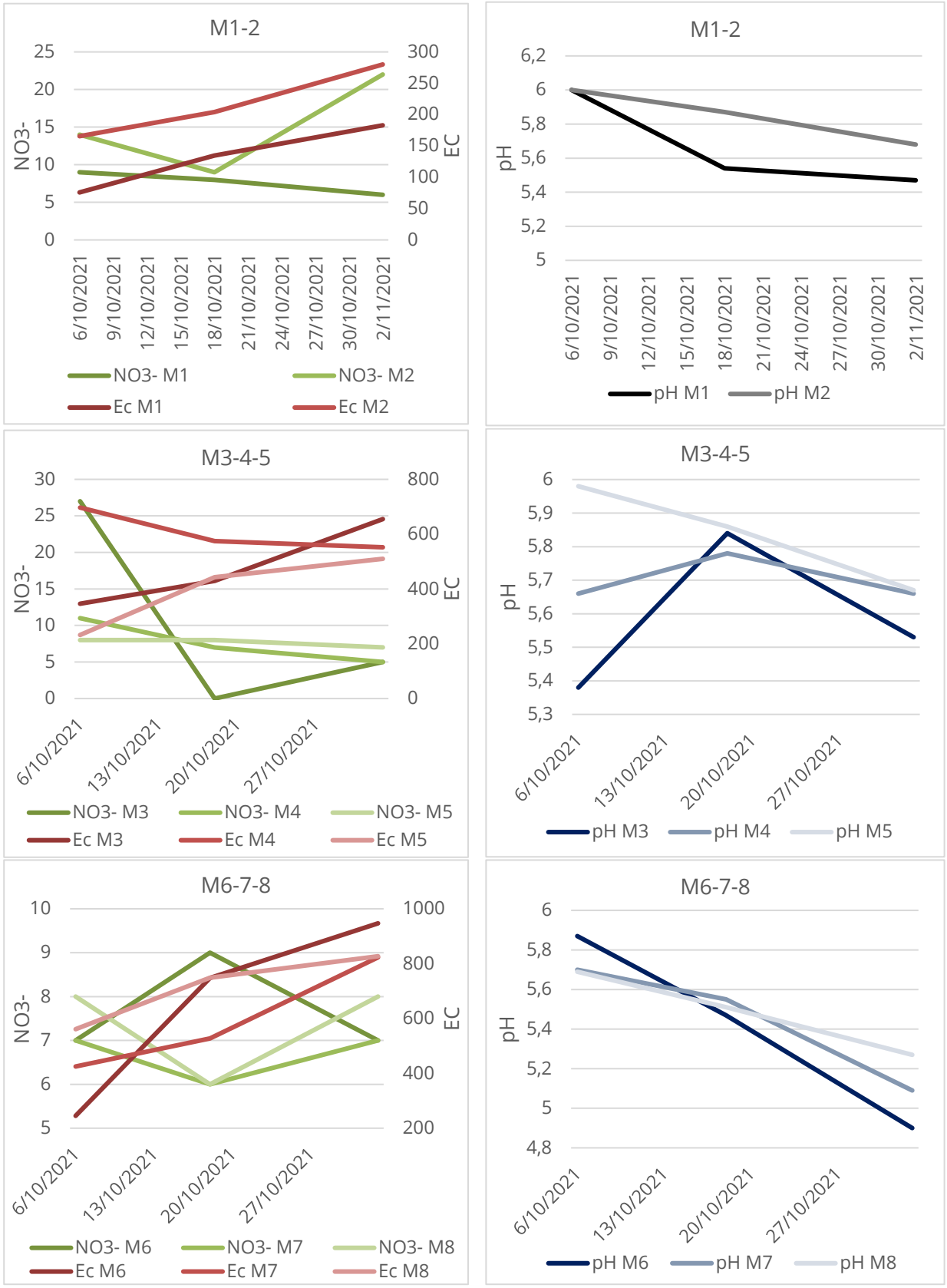

Figure 52: Different graphs on the evolution of the different parameters across different modalities. NO3- and EC values on the left, and pH on the right for modalities 1 & 2 at the top, 3, 4 & 5 in the middle and 6, 7 & 8 at the bottom.

3.3.3. Conclusions

In general, blooming and rooting were satisfactory with RDFs. Nevertheless, the plants took root faster with Soluplant. The growth was also greater with Soluplant, 75% and 100% of AN and AS than with Osmocote.

Table 25: Synoptic table on the comparison of RDFs to Osmocote in Viola.

Modality		**Growth compared to the control Osmocote**
Soluplant		+++
AN	**100%**	++
	75%	+
	40%	-
AS	**100%**	+
	75%	+
	40%	=

3.4. Basil

3.4.1. Material and methods

3.4.1.1. Experimental setup

3.4.1.1.1. Crop and cultivar

The basil sowing was made in honeycomb plates in a non-fertilized substrate. At potting time, four to five basil seedlings were put by pot to create homogeneous batches.

Table 26: Overview on the Basil variety and quantities.

Species	Varieties	Pot size	Number of plants/modality
Ocimum basilicum	Loki	10.5L	30

3.4.1.1.2. Cultivation conditions

Substrate: the B400 reference of Stender society is a substrate mainly made of blond peat. Its structure is medium.

Fertilization at potting time: fertilizing programs were implemented at potting time on 21st of July 2022:

- **M1**: NPK mineral fertiliser, Osmocote Bloom (12-7-18) 2-3 months, 3g/L
- **M2** : no additional fertilization in the substrate at potting time
- **M3 - M8**: Patentkali, 2g/L and Superphosphate 45, 1g/L

Irrigation: plants were watered pot by pot with a 1.5 L graduated beaker. Each plant receives 50mL water. The watering frequency is defined depending on the weather conditions to avoid any water stress.

3.4.1.1.3. Trial design and treatments

The trial modalities match the following fertiliser management technique:

- **M1**: Reference mineral controlled-release fertiliser Osmocote
- **M2**: Reference soluble mineral fertiliser Soluplant (16-6-26), 3g/1.5L
- **M3**: Ammonium nitrate at 100% of the ideal concentration, 3g/1.5L
- **M4**: Ammonium nitrate at 75% of the ideal concentration, 2.3g/1.5L
- **M5**: Ammonium nitrate at 40% of the ideal concentration, 1.2g/1.5L
- **M6**: Ammonium sulphate at 100% of the ideal concenration, 9g/1.5L
- **M7**: Ammonium sulphate at 75% of the ideal concentration, 6.8g/1.5L
- **M8**: Ammonium sulphate at 40% of the ideal concentration, 3.6g/1.5L

Each modality is made of thirty basils dispatched in three Fisher blocks composed of ten plants each. The pots were placed on saucers (five pots per saucer) to avoid any contamination from one modality to the other.

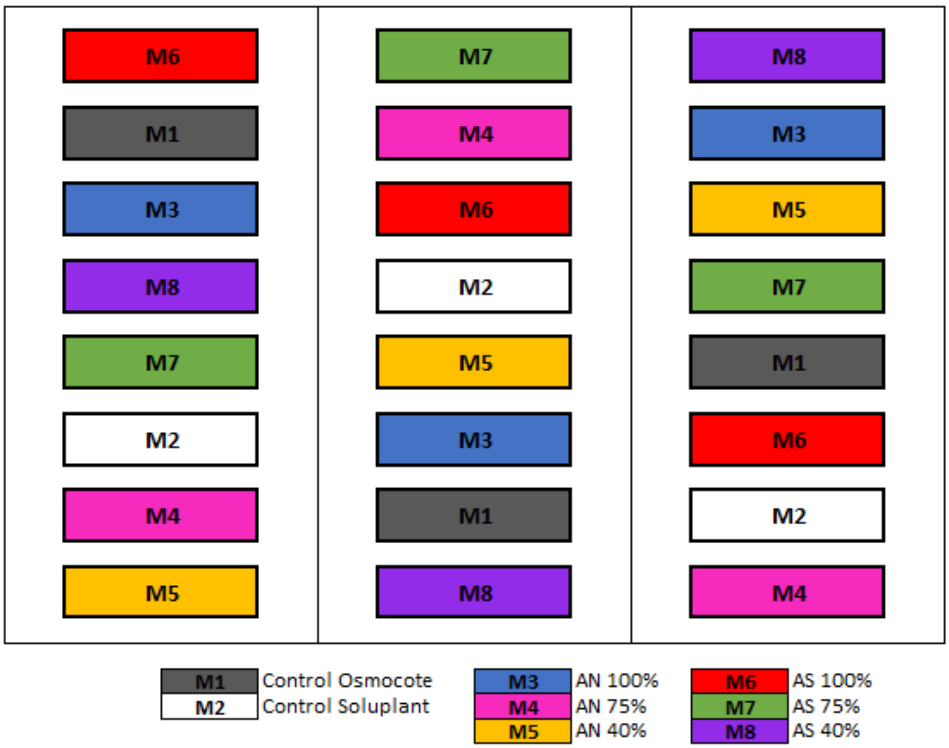

Figure 53: Schematic trial design with the different modalities.

Calculation of the supplied doses:

The calculations of the ideal concentrations of ammonium nitrate (AN) and ammonium sulphate (AS) were based on Soluplant supplied doses. The RDFs laboratory analysis showed that the nitrogen content of AN is almost the same as in Soluplant. A three times bigger volume of AS is necessary to get the same nitrogen content.

Supplies frequency:

The plants of the control modality M1 had only one supply of solid mineral controlled-release fertilization at potting time on week 29. In the other modalities, there were three supplies per week from week 31 to week 35.

The supplies were made through pot by pot fertigation with a 1.5 L graduated beaker for thirty plants. The control modality is watered with the same volume of clear water.

Composition of mineral and recycling-derived fertilisers:

Table 27: NPK composition of mineral and recycling-derived fertilisers.

Fertilisers composition	N	P	K
Osmocote	12	7	18
Soluplant	16	6	26
Ammonium nitrate (AN)	14.2	0	0
Ammonium sulphate (AS)	17.4 (5.8*3)	0	0

Table 28: Composition of the RDFs. Samples taken from storage before or at the time of trial installation.

Product	Sampling date	NO_3-N	NH_4-N	Kjeldahl N	P_2O_5	K_2O	S
		g/kg fresh material					
Ammonium nitrate	04/15/19	43.4	43.1	43.1	0.0	0.0	0.5
Ammonium sulphate	03/18/19	0.0	33.6	0.0	0.0	0.0	37.9

Table 29: NPK total quantity supplied during the trial.

Fertiliser dose		N	P_2O_5	K_2O
		g/plant (on the trial's duration)		
Osmocote		0.18	0.11	0.27
Soluplant		0.21	0.08	0.34
AN	**100%**	0.18	3	6
	75%	0.14		
	40%	0.07		
AS	**100%**	0.23		
	75%	0.17		
	40%	0.09		

3.4.1.2. Trial conditions

3.4.1.2.1. Climate conditions

Greenhouse and cultivation setpoints: the trial was carried out in a cold glasshouse with a high air vent opening on the ridge and a shade system, both controlled by a climate computer. The airing and shade setpoints are set depending on the weather conditions.

3.4.1.2.2. Overview trial development

Table 30: Overview on the timing of trial activities.

Date	Activity
21/07/2022	Repotting
21/07/2022	Setting of the experimental design

01/08/2022	First fertiliser supply
31/08/2022	Last fertiliser supply
13/09/2022	End of the trial

3.4.1.3. Measurements

Several kinds of follow-up are done:

- Aerial and root development

Growth measures (height and diameter) were taken every two weeks to follow the plants' development. Moreover, root development measures were taken according to the following scale:

Figure 54: Depicturing of the root development scale.

- Fresh mass

At the end of the trial, the fresh aerial mass of each plant was weighed.

- Chlorophyll content

The chlorophyll content is measured with the N-Tester. It indicates the nitrogen nutrition status of the plant.

Figure 55: N-Tester.

- Evolution of the soil parameters in the substrate

Soil parameters analyses are done every three weeks. pH, EC, nitrate and ammonium contents of the substrate are measured.

An aqueous extract has to be prepared before this analysis. 100g of substrate were taken in each modality and put in 150 mL of distilled water for thirty minutes. The solution is then filtered to get the aqueous extract on which the soil parameters analysis is done.

The RQflex® device measures the ammonium NH_4^+ content in the substrate, from 5 mg/L to 180 mg/L NH_4^+. The Nitracheck® device measures the nitrates NO_3^- through reflectometry.

Figure 56: The RQflex® (left) and Nitracheck® (right).

3.4.1.4. Statistical data processing

The data of the trial were analysed with R statistical software, version x64 4.1.1. Variance analysis ANOVA were made, or Kruskal-Wallis nonparametric tests when ANOVA conditions were not observed. Newman-Keuls post hoc comparison of means were then used. Eventually, the confidence level of the analysis was of 95%.

3.4.2. Results and discussion

Growth measures were done every week since the first supply and until the end of August.

3.4.2.1. Rooting scale

The rooting is faster in Soluplant (M2), 75% AS (M7) and 100% AN (M3) modalities. Since mid-August, there is no difference anymore and all modalities are well rooted on the 22nd of August.

Figure 57: Rooting percentage over time and according to modalities.

3.4.2.2. Height

Figure 58: Representative photograph of the basil's development on the 22nd of August.

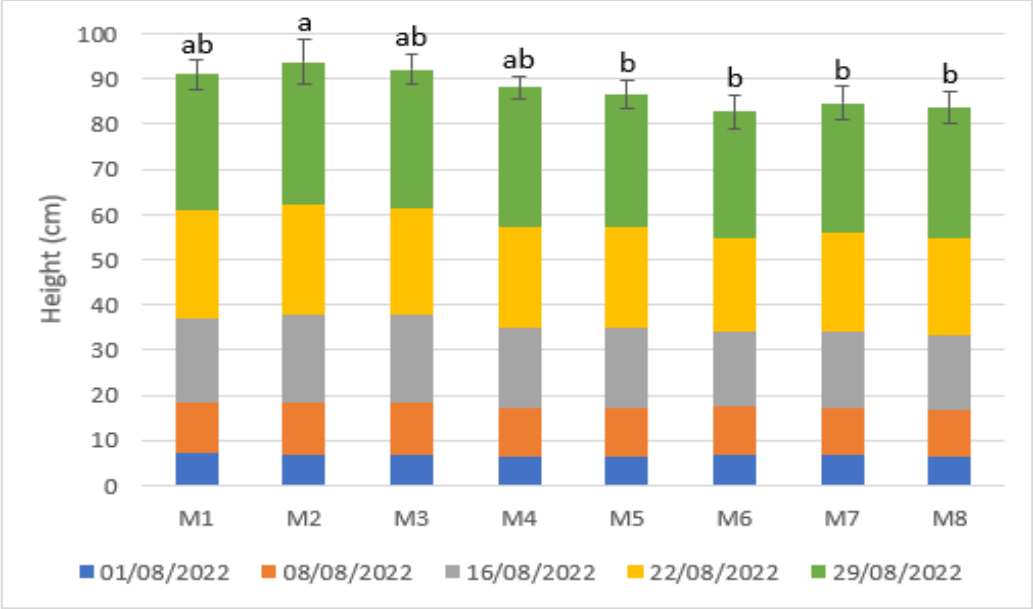

Figure 59: Height evolution over time and according to the different modalities.

Since the 16th of August, there were significant differences: plants fertilized with AS (ammonium sulphate M6, M7 and M8) grew less. This difference remains for all the trial's duration. The highest plants are in the Soluplant modality (M2).

3.4.2.3. Fresh mass

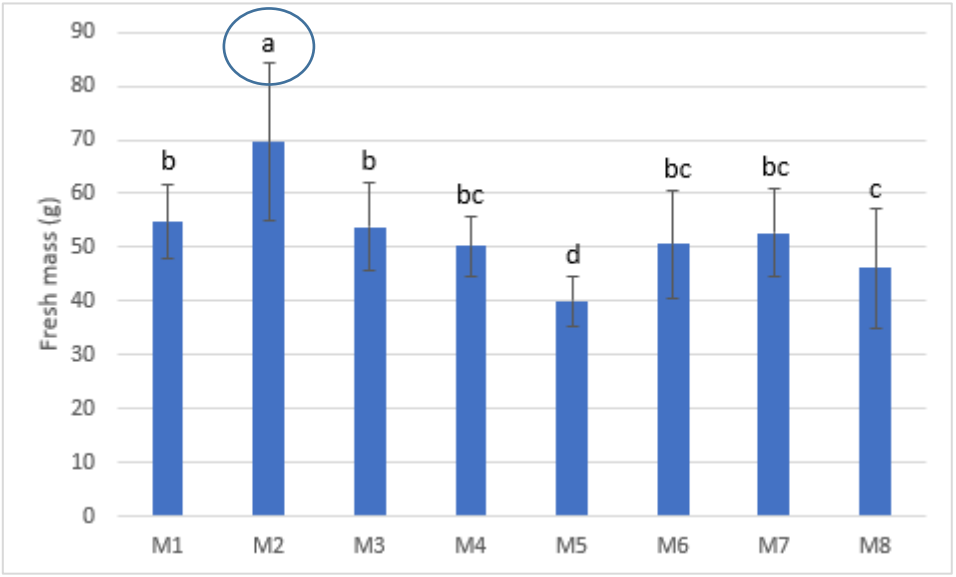

Figure 60: Aerial fresh mass on the 13th of September according to the different modalities.

The aerial fresh mass was weighed at the end of the trial. Differences between modalities were highlighted. The heaviest fresh mass was observed in the Soluplant modality (M2). It correlates the height measures.

The lightest fresh mass was observed in 40% RDFs modalities (M5 ammonium nitrate and M8 ammonium sulphate). In the other modalities, the fresh mass is similar as the one in the Osmocote control (M1).

3.4.2.4. Nitrogen content

The nitrogen content was estimated through the leaves chlorophyll content.

The statistical analysis showed a significantly lower nitrogen content in the plants fertilized with 40% AN (M5). On the contrary, the plants fertilized with 75 and 100% of AS have the most nitrogen.

The low chlorophyll content in modality M5 was visible on the basil leaves at the end of the trial: they turned yellow. However, plants were marketable by the end of August and producers would not have kept the crop for a longer time. As a consequence, even if there are some deficiencies, they are not that important because they appear after the marketable time.

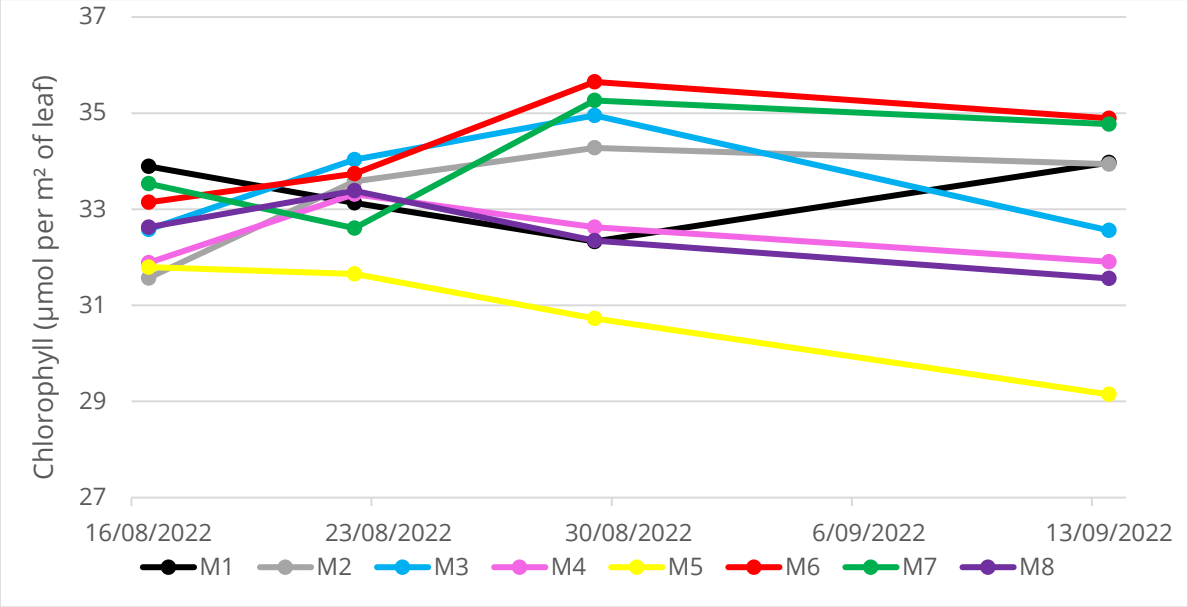

Figure 61: Chlorophyll content of the leaves over time and according to the different modalities.

3.4.2.5. Evolution of soil parameters

- pH evolution

The pH was rather low in all modalities. Ammonium sulphate solutions really acidified the substrate because values between 4.7 and 5 were measured. It impacted the aerial development which was weaker in these modalities.

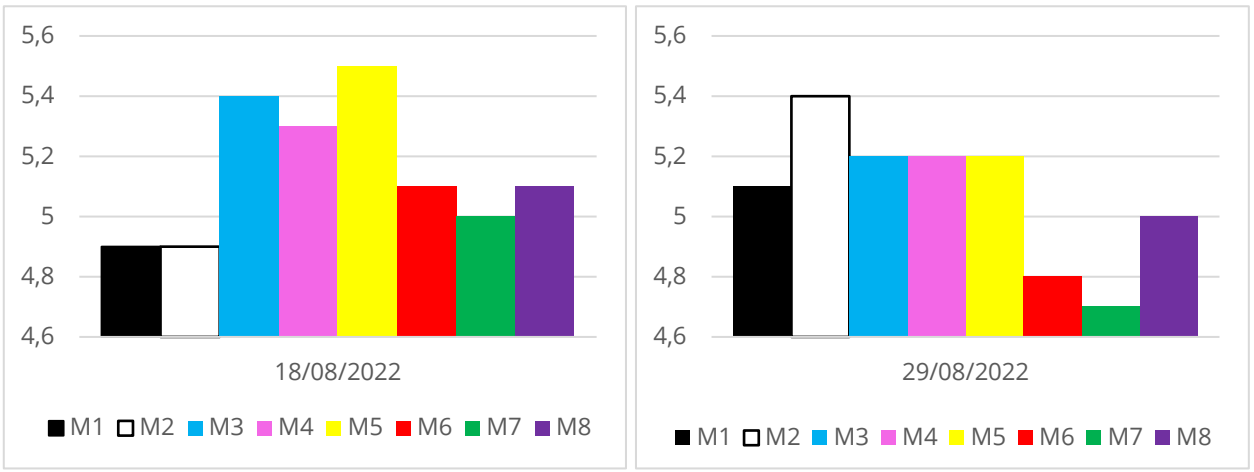

Figure 62: pH evolution over time and across the different modalities.

- Electroconductivity (EC) and ammonium and nitrate content

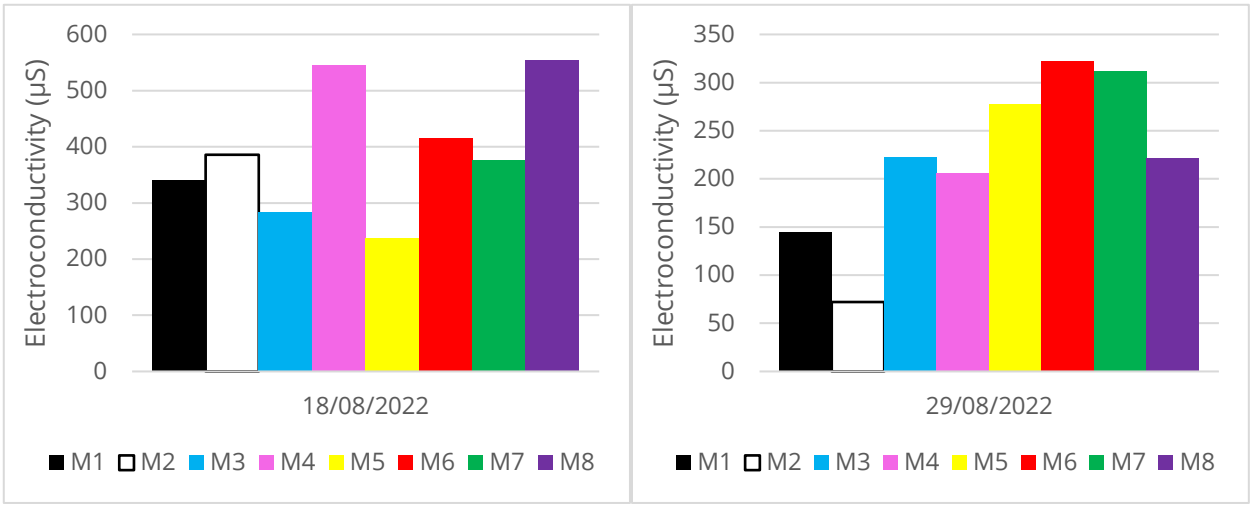

Figure 63: Evolution of EC over time and across the different modalities.

The EC of the substrate globally decreases except in the 40% AN modality (M5): the nutrients brought to the crop are absorbed by plants. The decrease of the ammonium quantity shows that it is transformed in nitrate by microorganisms.

The nitrate is then absorbed by plants because the data does not show any increase of the NO_3^- content in the substrate.

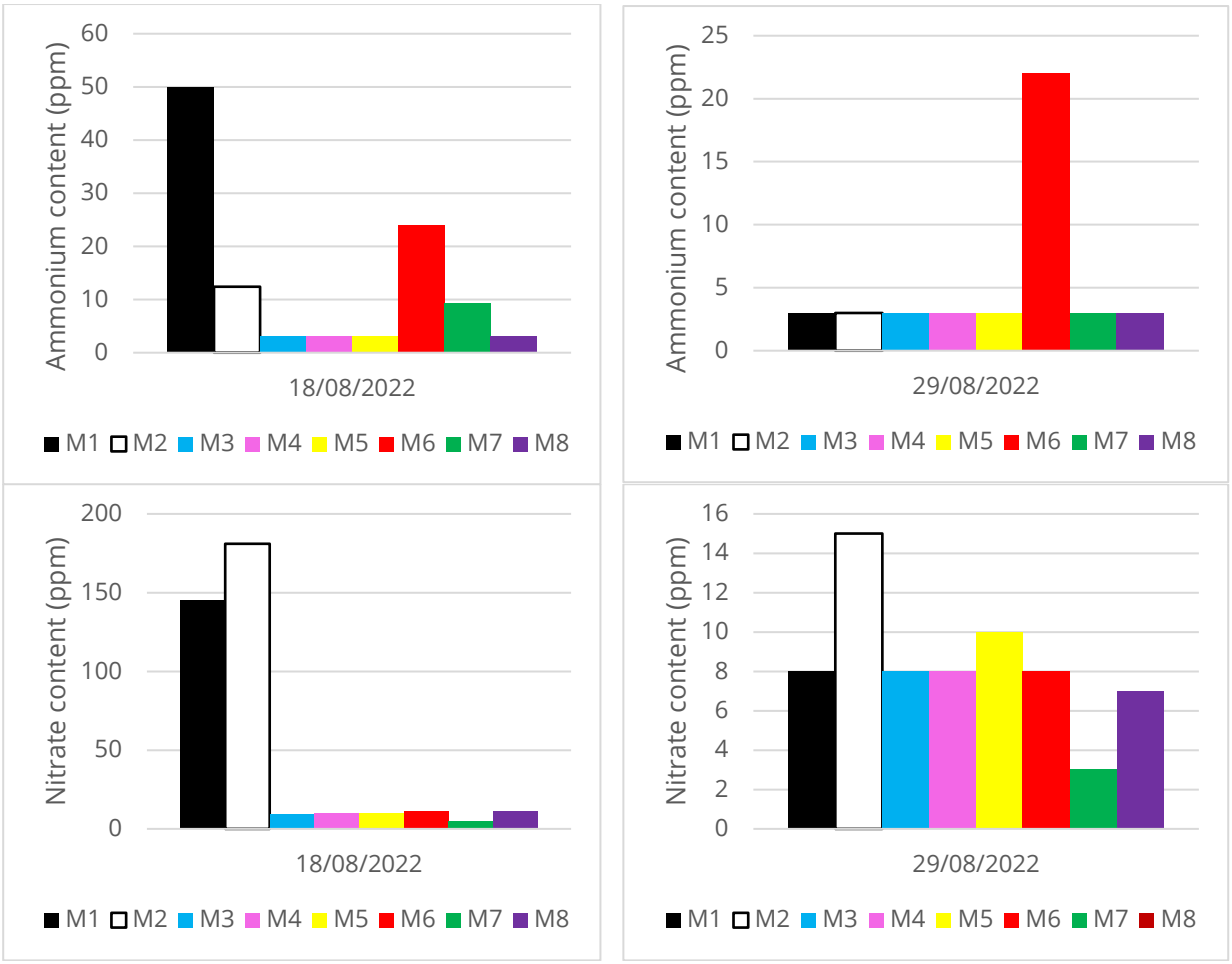

Figure 64: Evolution of ammonium (on the left) and nitrate (on the right) content over time and across the different modalities.

3.4.3. Conclusions

The basil is a short crop which requires a good fertilization. It uses a lot of nitrogen and can show deficiency issues. RDFs impacted the plants according to the different concentrations. The plants fertilized with AS and 40% AN were smaller than the others. For all that, ammonium and nitrate rates measures showed a good absorption of these nutrients.

It is worth noting that the ammonium sulphate solutions strongly acidify the substrate. However, it did not immediately lead to any stress on the plants. At the end of the trial, plants were marketable and did not show any deficiency symptom on the leaves.

3.5. Lonicera

3.5.1. Material and methods

3.5.1.1. Experimental setup

3.5.1.1.1. Crop and cultivar

The plants of this trial are taken from cuttings of *Lonicera nitida* 'Maigrun' grown in aeroponics. The cuttings were taken at Est Horticole's experimental site.

Table 31: Overview on the Lonicera variety and quantities.

Species	Variety	Pot size	Number of plants per modality
Lonicera nitida	Maigrun	3L	30

3.5.1.1.2. Cultivation conditions

Potting: the *Lonicera* were repotted in 3L pots with the Klasmann substrate 233. It is commonly used at Est Horticole to pot nursery plants. It is made of 80% blond peat and 20% wood fiber.

Fertilisation at potting time:

- **M1**: Osmocote (15-9-11) 8-9 months, 4g/L
- **M2**: no fertiliser at potting time
- **M3 - M8**: Patentkali, 2g/L and Superphosphate, 1g/L

Irrigation: plants were watered pot by pot with a watering can. Each plant received 300 mL of water. Supplies frequency depends on weather conditions to avoid any stress on the plants.

3.5.1.1.3. Trial design and treatments

The trial modalities match the following fertiliser management technique :

- **M1**: Reference mineral controlled-release fertiliser Osmocote
- **M2**: Reference soluble mineral fertiliser Soluplant (16-6-26), 6g/3L
- **M3**: Ammonium nitrate, 100% of the ideal concentration, 6g/3L
- **M4**: Ammonium nitrate, 75% of the ideal concentration, 4.5g/3L
- **M5**: Ammonium nitrate, 40% of the ideal concentration, 2.4g/3L
- **M6**: Ammonium sulphate, 100% of the ideal concentration, 18g/3L
- **M7**: Ammonium sulphate, 75% of the ideal concentration, 13.5g/3L
- **M8**: Ammonium sulphate, 40% of the ideal concentration, 7.2g/3L

Each modality is made of thirty *Lonicera* dispatched in three Fisher blocks made of ten plants each. Pots were placed on single saucers to avoid any contamination from one modality to the other.

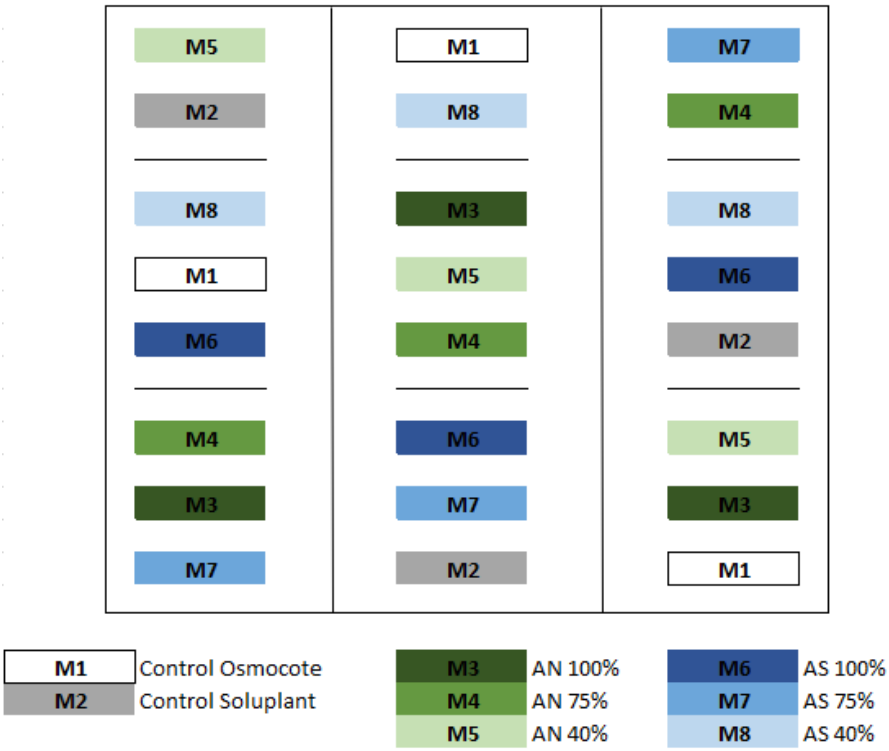

Figure 65: Schematic trial design with the different modalities.

Calculation of the supplied doses:

The ideal concentrations of ammonium nitrate (AN) and ammonium sulphate (AS) were based on the Soluplant supplied doses. Laboratory analyses of RDFs showed that the nitrogen content of AN is approximately the same as in Soluplant. However, a three times bigger volume of AS is necessary to get the same nitrogen content.

Supplies frequency:

The control modality M1 had only one supply of solid mineral controlled-release fertilization while potting on week 43, year 2021.

Liquid fertilization started in 2022. There was one supply per week from week 10 to week 14, then two supplies per week from week 14 to week 17. Eventually, there were three supplies per week until the end of the trial on week 35.

Supplies were made through pot by pot fertigation with a 3L watering can for ten plants. The control modality was watered with the same volume of clear water.

Composition of mineral and recycling-derived fertilisers:

Table 32: NPK composition of mineral and recycling-derived fertilisers.

Fertiliser composition	N	P	K
Osmocote 8-9	15	9	11
Soluplant	16	6	26
Ammonium nitrate (AN)	14.2	0	0
Ammonium sulphate (AS)	17.4 (5.8*3)	0	0

Table 33: Composition of the recycling-derived fertilisers. Samples were taken from storage before or at the time of trial installation.

Product	Sampling date	NO_3-N	NH_4-N	Kjeldahl N	P_2O_5	K_2O	S
		g/kg Fresh material					
Ammonium nitrate	04/15/19	43.4	43.1	43.1	0.0	0.0	0.5
Ammonium sulphate	03/18/19	0.0	33.6	0.0	0.0	0.0	37.9

Table 34: NPK total quantity supplied during the trial.

Fertiliser dose		N	P_2O_5	K_2O
		g/plant (on the trial's duration)		
Osmocote		1.8	1.1	1.3
Soluplant		5.2	1.9	8.4
AN	**100%**	4.6	3	6
	75%	3.5		
	40%	1.8		
AS	**100%**	5.6		
	75%	4.2		
	40%	2.3		

3.5.1.2. Trial conditions

3.5.1.2.1. Climate conditions

The trial was carried out under a cold glasshouse.

3.5.1.2.2. Overview trial development

Table 35: Overview on the timing of trial activities.

Date	Activity
28/10/2021	Repotting of small pots into 3L pots
04/03/2022	Setting of the experimental design inside the greenhouse
09/03/2022	First supply of fertiliser

27/04/2022	Setting of the experimental design outside
31/08/2022	Last supply of fertiliser
13/09/2022	End of the trial

3.5.1.3. Measurements

Several kinds of follow-up are done:

- Aerial and root development

Growth measures (height and diameter) were taken every two weeks to follow the plants' development. Moreover, root development measures were taken according to the following scale:

Figure 66: Depicturing of the root development scale.

- Commercial grade

A commercial grade is given at the end of the trial based on plant uniformity, size and coloration. The scoring scale used is as follows:

Figure 67: Depicturing of the commercial grade scale.

- Chlorophyll content

Figure 68: N-Tester.

The chlorophyll content is measured with the N-Tester. It indicates the nitrogen nutrition status of the plant.

- Evolution of the soil parameters in the substrate

Soil parameters analysis are done every three weeks. pH, EC, nitrate and ammonium contents of the substrate are measured.

An aqueous extract has to be prepared before this analysis. 100g of substrate were taken in each modality and put in 150 mL of distilled water for thirty minutes. The solution is then filtered to get the aqueous extract on which the soil parameters analysis is done.

The RQflex® device measures the ammonium NH_4^+ content in the substrate, from 5 mg/L to 180 mg/L NH_4^+. The Nitracheck® device measures the nitrates NO_3^- through reflectometry.

Figure 69: The RQflex® (left) and Nitracheck® (right).

3.5.1.4. Statistical data processing

The data of the trial were analysed with R statistical software, version x64 4.1.1. Variance analysis ANOVA were made, or Kruskal-Wallis nonparametric tests when ANOVA conditions were not observed. Newman-Keuls post hoc comparison of means were then used. Eventually, the confidence level of the analysis was of 95%.

3.5.2. Results and discussion

The results showed here do not take into account the measures of the 9th of March and the 27th of July. Data of August are not shown because plants stopped growing.

3.5.2.1. Rooting scale

The first observation was done on the day when there was the first supply of RDFs.

The observations in March show an earlier rooting of the plants from Soluplant and Osmocote modalities. Over time and supplies, the plants of the other modalities are rooting well. On 21st April, the plants of the control modalities are less rooted with only 70 to 75% of them in class 3, while more than 80% of the plants from the modalities with ammonium nitrate and ammonium sulphate belong to this class. However, there are no differences anymore between the modalities since May.

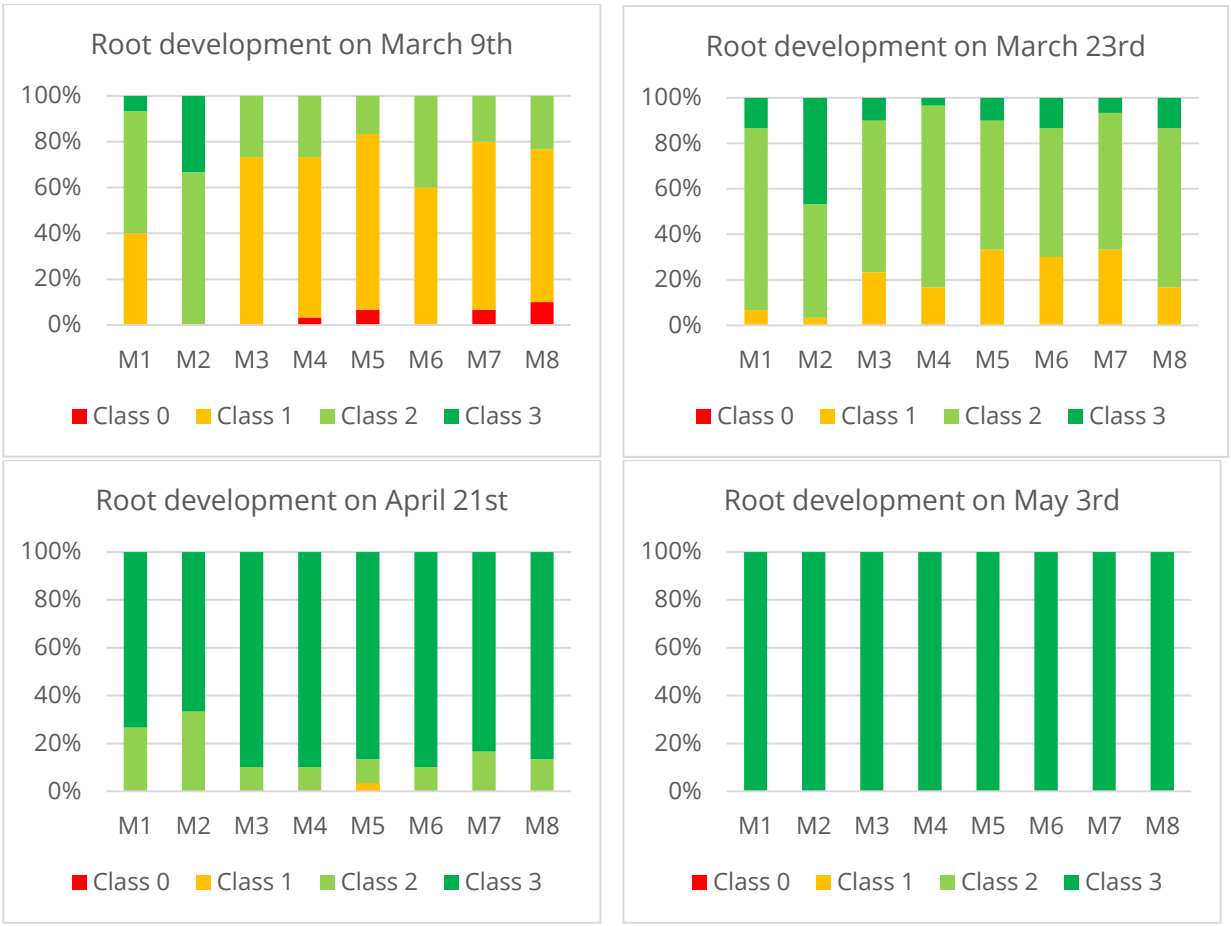

Figure 70: Percentage of rooting over time and across the different modalities.

3.5.2.2. Diameter

Statistical differences were noticed. The plants from the modalities with 100% AN (M3) and 100% AS (M6) have larger diameters, similar as in Soluplant modality (M2) (see Annex).

Plants fertilized with a RDFs concentration of 40% (M5 and M8) grew less. Additionally, plants of the modality with 75% of AN and AS have a final development similar to the control plants fertilized with Osmocote.

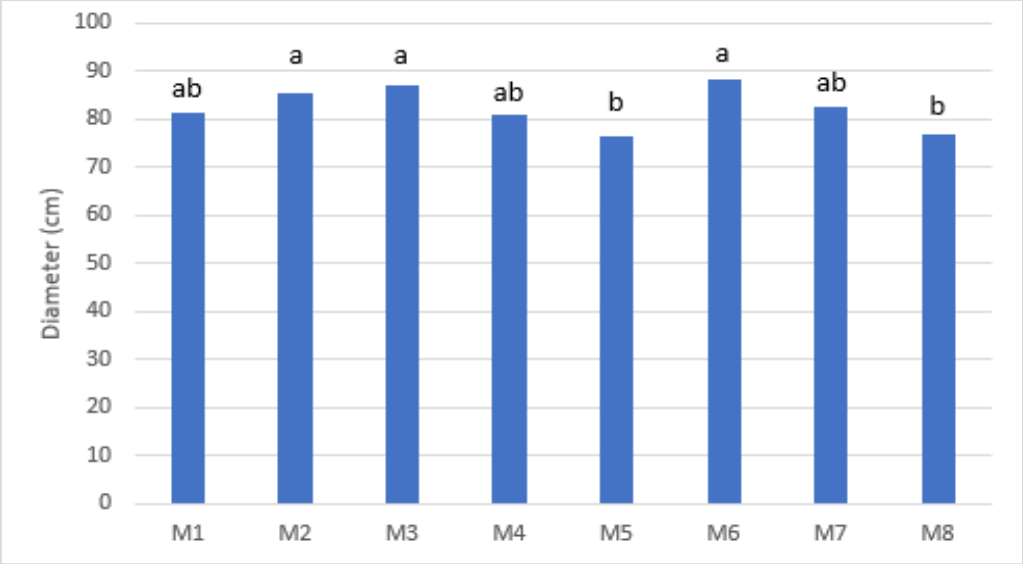

Figure 71: Total diameter on the 27th of July.

3.5.2.3. Longest branch

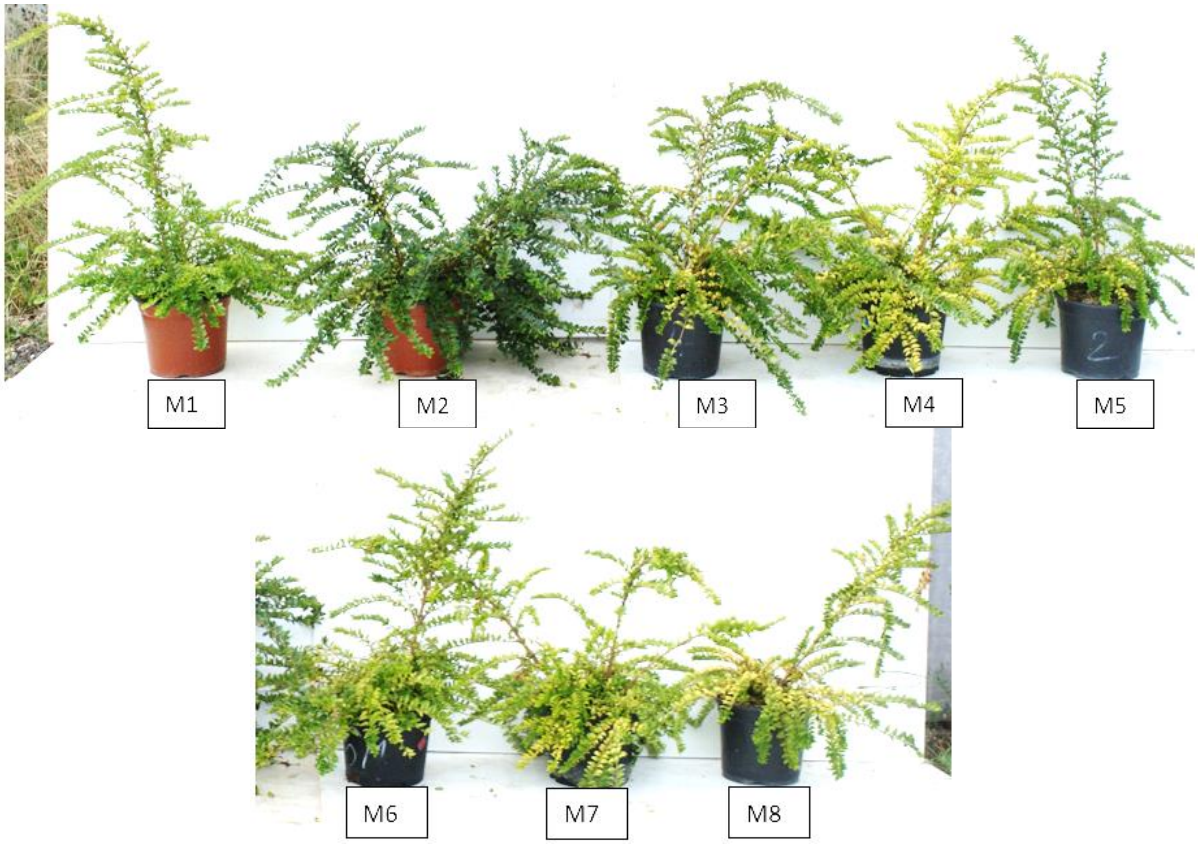

Figure 72: Representative photograph of the Lonicera's development on the 17th of August.

The branches' growth was great between April and May. The statistical analysis show that the Soluplant modality (M2) is significantly different from the others (see Annex). Between the 9th and 23rd of March, the length of the longest branch was clearly shorter than in the other modalities. The assessment is the same at the beginning of July.

The last two measurements showed a weaker growth on plants fertilized with 40% AN. On the contrary, the plants of the modalities with 100% RDFs (M3 and M6) have significantly longer branches.

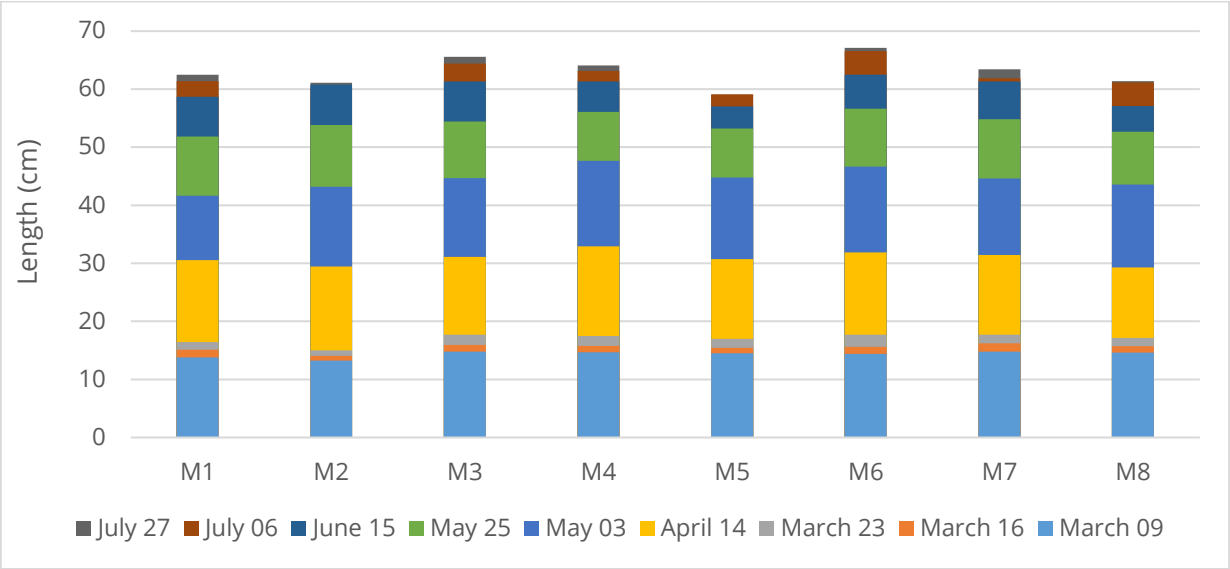

Figure 73: Length of the longest branch over time and across the different modalities.

3.5.2.4. Nitrogen content

The measured chlorophyll content reflects the nitrogen content of the leaves. The modality where the highest nitrogen content was measured is Soluplant (M2). The average values are about 66µmol. The values in the other modalities are rather low.

Ammonium sulphate solutions exclusively bring the nitrogen in the form of ammonium. The microorganisms need to break down ammonium into nitrate for it to be absorbed by plants. On the contrary, ammonium nitrate solutions is already made of 50% of nitrogen in the form on NO_3^-. It explains the significantly higher nitrogen content in the plants from 100% AN modality (M3) compared to the other RDFs concentrations.

Thus, the use of RDFs caused nitrogen deficiencies in *Lonicera*. It strongly affected their quality.

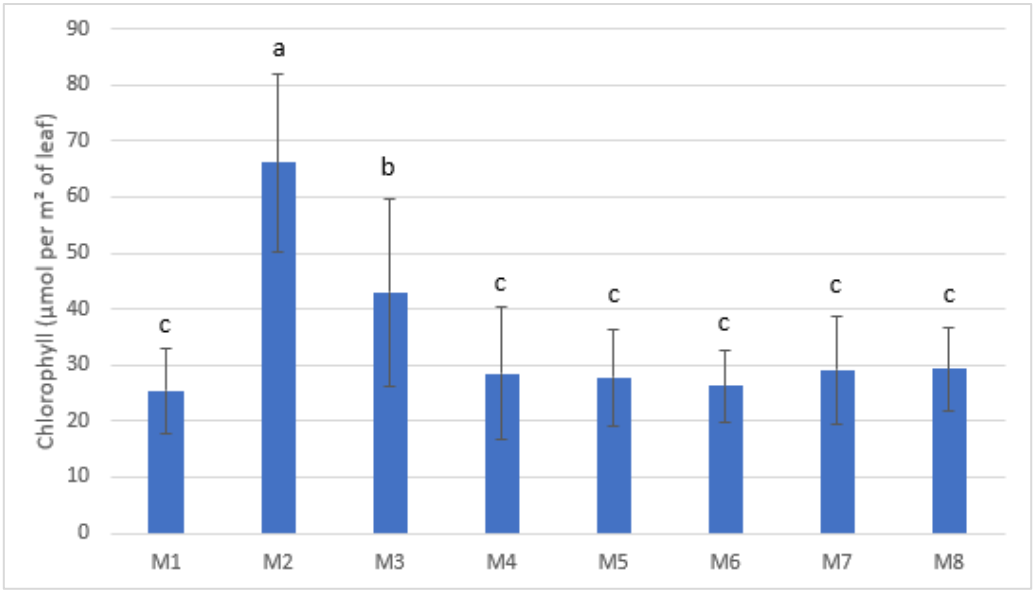

Figure 74: Chlorophyll content in the leaves in September according to the different modalities.

3.5.2.5. Commercial grade

The plants habits were overall balanced but the quality was low. Indeed, as soon as the end of May, the leaves started to turn yellow in several modalities. The first yellowing observations were on the plants of modality M1: all the Osmocote was released and used. Then, the yellowing appeared on the plants of the modalities with 40% and 75% of RDFs.

Figure 75: The yellowing of the leaves, as clearly seen in modality M1, compared to M2.

At the end of the trial, only the plants fertilized with Soluplant remained perfectly green, except on a few old leaves at the base of the branches.

The graph hereunder shows that the plants fertilized with AS (M6, M7 and M8) show a higher rate of deficiencies than the ones fertilized with AN (M3, M4 and M5). These observations correlate the low nitrogen content of the leaves.

Half of the plants fertilized with 100% AS (M6) loss their leaves at the base of the branches. The combination of nutrient and temperature stress (see Annex) may be the cause.

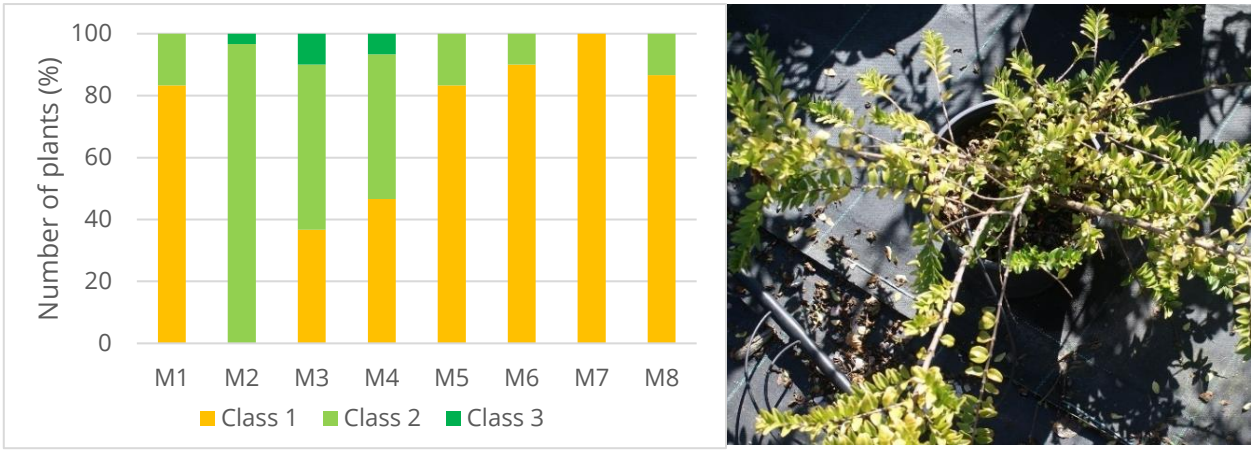

Figure 76: Commercial grade according to modalities on the 13th of September. Right: leafless branches from modality M6.

3.5.2.6. Evolution of soil parameters

- pH evolution

The measurements of January, before the supplies, showed two groups in the values. The first one is made of the controls Osmocote and Soluplant with a pH close to 6. The second group gathers the other modalities with a pH around 5. The pH fluctuates over time until reaching acidic values in the AS modalities.

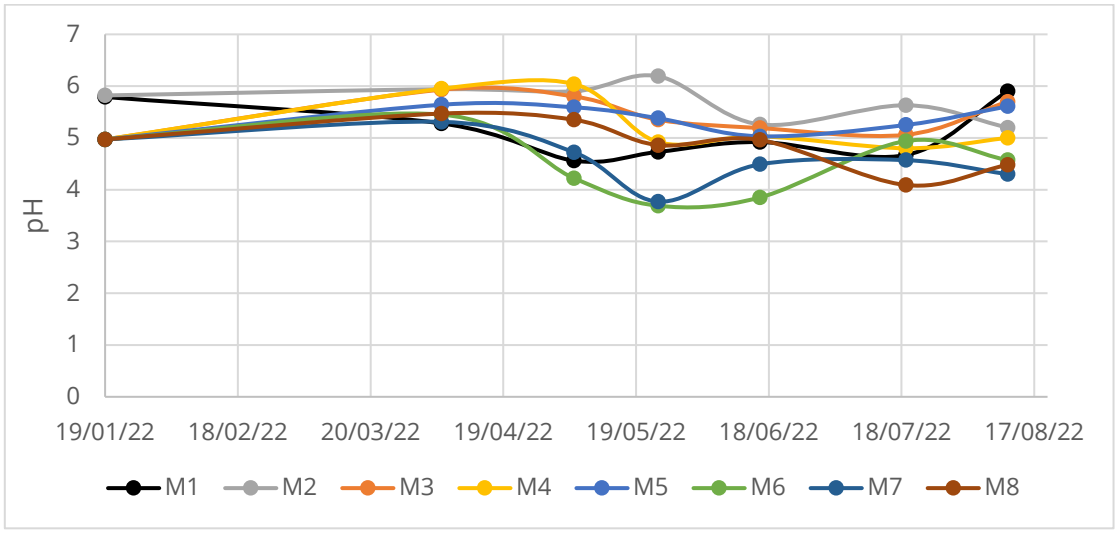

Figure 77: pH evolution over time across the different modalities.

- EC evolution

There was a peak EC between May and June in the 100% AS (M6) modality. The sulphate probably accumulated in the pot. The same assessment is made in the M3 modality with 100% AN on the 16th of June. At the same date, there was a peak EC as high as 1200 µS in the

Osmocote (M1) modality. The cause may be the increase of temperatures in June (see Annex) speeding up the release of Osmocote.

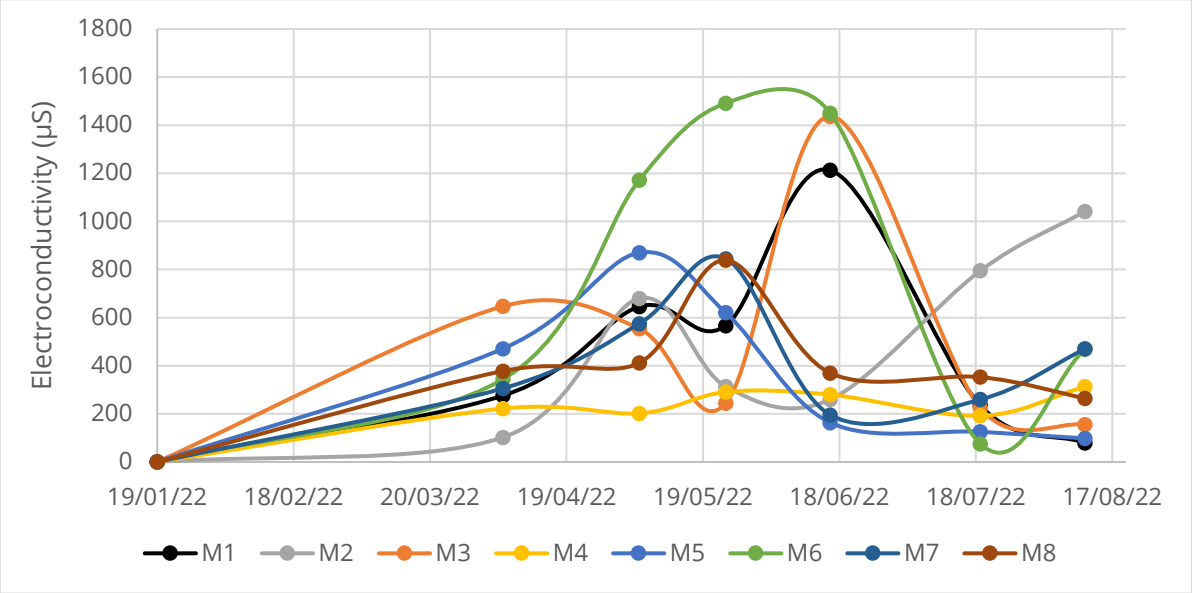

Figure 78: EC evolution over time and across the different modalities.

- Nitrate and ammonium content

Between April and the end of May, the nitrate content was high in the Osmocote modality with values between 250 and 150ppm. The nitrate quantity gradually decreased, absorbed by plants.

In RDFs modalities, nitrate content was under 50 ppm and ammonium content was under 5 ppm. From April to June, the explanation is the consumption of nitrogen for the plants' growth. Then, the high summer heat may have affected nitrification by microorganisms. This may explain the low nitrate values and a slight increase of the ammonium values (maximum 22 ppm) in the substrate in July and August.

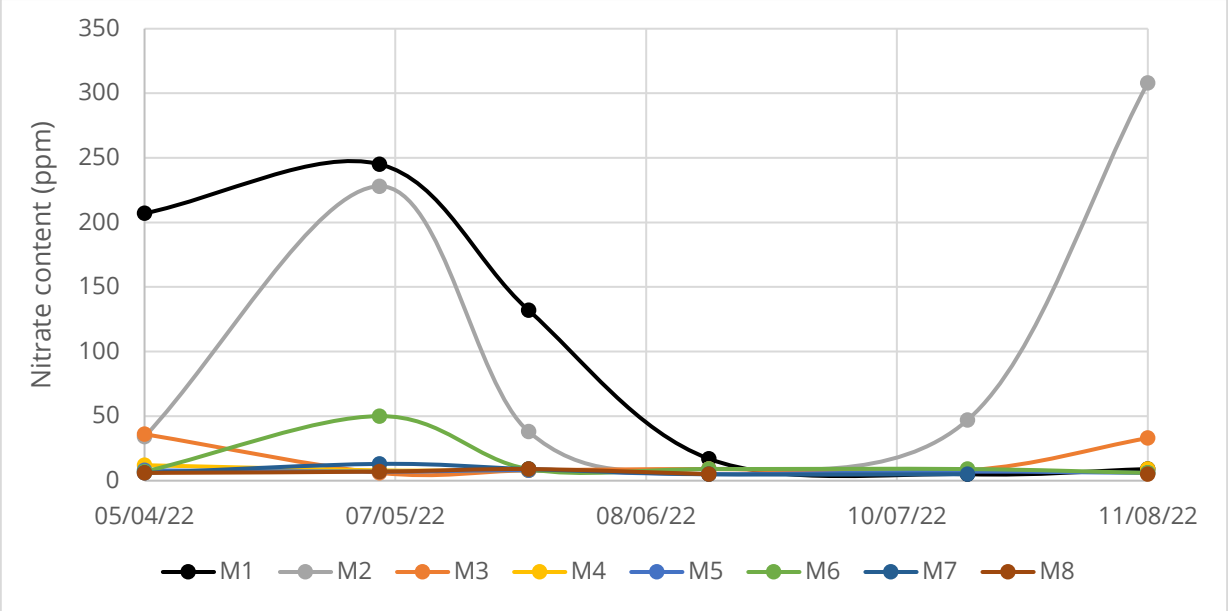

Figure 79: Nitrate content evolution over time and across the different modalities.

3.5.3. Conclusions

The nursery plants have a longer growth that requires regular nutrient supplies over time. *Lonicera nitida* was chosen because of its responsiveness to deficiencies.

At the end of the trial, the plants fertilized with RDFs had nitrogen deficiency symptoms, like in the Osmocote modality. Only the plants fertilized with Soluplant remained perfectly green. Thus, the three supplies per week were not enough. The number of supplies should be increased, or the concentration of the solutions should be adjusted.

Nevertheless, the use of ammonium nitrate and ammonium sulphate as liquid fertilisers seems to be efficient on *Lonicera*'s growth. The 75% and 100% concentrations enabled to get similar or higher values than the controls. However, pH and electroconductivity levels in the substrate must be checked regularly because the ammonium sulphate acidifies it.

4. Pot trials ULimerick

At the University of Limerick, greenhouse experiments were set up to find answers to two important hypotheses: "does the slow-release nature of struvite fertilisers synchronise well with short term horticultural crops for good agronomic effectiveness?" and "what effect, if any, does struvite fertiliser application have on soil dwelling micro-organisms?". These hypotheses were tested in three different crops, namely lettuce, tomato and spinach.

While the publication of some scientific articles are pending, the following summaries can be provided.

4.1. Lettuce

4.1.1. Material and methods

4.1.1.1. Seed germination

Seeds were cultivated in the propagation tray with the same soil as sued for potting and left to grow for six days before transplantation into pots in the first experiment. Seeds were placed in the propagation tray as for the first experiment, but left to grow for fourteen days before transplantation into pots in the second experiment. Transplantation was done by removing plants and soil from the propagation tray and inserting the seedlings into the pots.

4.1.1.2. Potting soil and experimental setup

The soil that was used for growing was sieved through a 5.6 mm mesh size sieve to remove stones, twigs, and gravel, and kept moist (approximately 10% water w/w) before being used as potting soil. This soil was a sandy loam soil (54% sand, and 8.6% clay) that had a bulk density of 1037 kg/m³ and a water holding capacity (WHC) of 22.4%. The soil was chosen because it had a low available P content (Table 31), which is ideal for robust P application agronomic comparisons. Other chemical attributes of the soil are given in Table 36. Briefly, the soil was slightly alkaline and had low exchangeable Na, Mn, and Zn.

Table 36: Properties of soil used as growing media.

pH	OM (%)	N_{tot} (%)	Available P (mg P/L)	CEC (meq/100g)	Exchangeable cations (ppm)					
					K	Mg	Ca	Zn	Mn	B
7.5	7.5	0.43	4.6	15.0	154	217	4252	4.9	43	1.38

A sheet of filter paper was placed at the bottom of the pots, large enough to cover the holes at the bottom of the pot and prevent soil loss. Pots were filled with 650 g of 3.35 mm sieved potting soil (Table 36). Potato wastewater struvite (PWS) and municipal wastewater struvite (MWS) was ground into a fine powder and added to the top two centimetres of six of the pots. All pots were kept in a growth chamber set for 12-hour days at 23°C and 12-hour nights at 18°C. They were equally watered an average of three times a week. One week after

transplantation KH_2PO_4/K_2HPO_4 was added to the phosphate control (replicate of six pots) as positive control in liquid form. Another six pots received no phosphorus fertiliser (negative control), while six pots each received PWS or MWS respectively. Within the course of the next week, three liquid fertilisers were added to all 24 pots in equal amounts in the following order: NH_4NO_3, KNO_3, then $MgSO_4$. Plants were grown in the growth chamber for 44 days for the first experimental set and 39 days for the second set.

Table 37: Nutrient concentrations applied to pots in potting experiments 1 and 2.

	Concentration applied (kg/ha)	Mass per pot (mg)
Sulfur	10	10.9
Magnesium	7.6	8.26
Nitrogen	200	218
Potassium	200	219.07
Phosphorus	40	-

KNO_3, NH_4NO_3, $MgSO_4$ were used to provide K, N, Mg and S in the desired amounts in the first and second experiment, while granular superphosphate (Westland Superphosphate Salad & Vegetables Fertiliser Granules, NPK value of 0-17.5-0) was used exclusively in the second experiment.

4.1.1.3. Harvest and soil sampling

Lettuce plants were harvested by cutting the plant just above the soil surface. After measuring the wet weight of all shoots, they were labelled and dried in a fan oven set for 50°C for 144 hours or until no moisture remained. Dry weights were then recorded. The bulk soil was removed from the roots by shaking it free. The rhizosphere soil was then collected by placing the root in a plastic bag and gently shake the soil free, without causing the roots to break off into the bag. The soil samples from the rhizosphere of each plant was labelled and stored in 4°C fridge for later use in creating soil suspensions for the colony-forming unit (CFU) analysis.

Soil was collected from the rhizosphere of each plant. This involved shaking loose the bulk soil, then placing the root in a plastic bag to shake of the remaining rhizosphere soil. Two grams of this soil was added to 50 ml centrifuge tubes and labelled with the pot number the soil was collected from. 20 mL of sterile saline solution was added and tubes were placed in an ELMI Intellimixer RS-2M rotating at 75 rpm for 30 minutes at 4°C to create a suspension for serial dilution in saline.

Tri-calcium agar plates (TCP) were prepared as described in Fox et al 2014 to identify growing bacteria capable of dissolving calcium-bonded phosphorus from the serial dilution. 100 µL was applied per TCP plate. TCP plates were incubated for eight days at 25°C. Plates were

examined with back lighting to find colonies with zones of clearance, where the microbes had used the tri-calcium phosphate.

4.1.2. Results and discussion

In the first pot trial, mean dry weight for the shoots was 0.36 g for the negative control and 1.06 g for the chemical P fertiliser plants. The mean for PWS was 1.34 g which was significantly higher than the no P control. MWS plants had the highest mean dry weight, of 1.56 g which was also significantly higher than the no P control but significance over SSP was not reached (Figure 80).

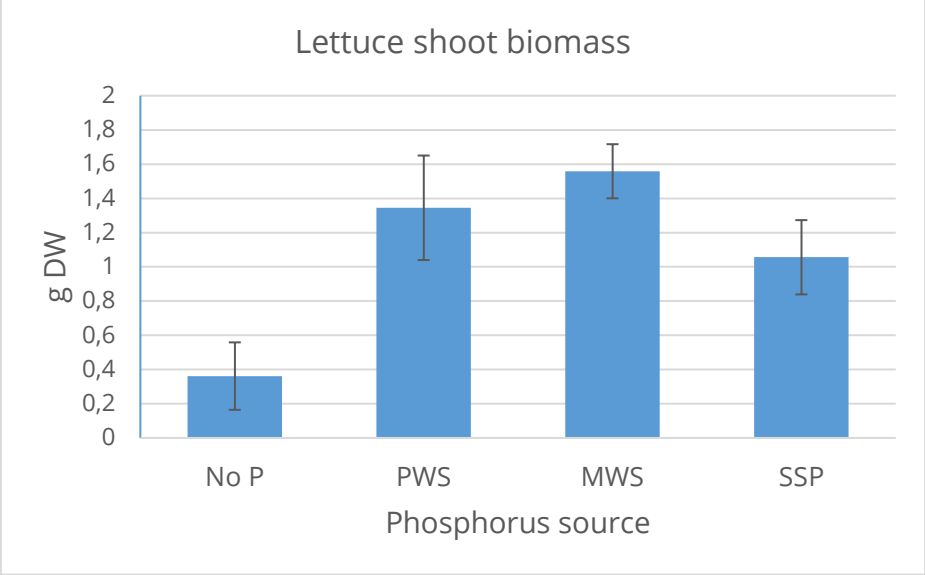

Figure 80: Lettuce shoot biomass from first pot trial with P free fertiliser (No P), potassium phosphate fertiliser (SSP) or struvite fertiliser from potato or municipal waste water (PWS, MWS).

While in the first experiments, several plants grew poorly or even died, this was prevented in the second trial as seedlings were kept in the propagators for longer before transplanting.

The second trial with superphosphate as particulate fertiliser, applied at the same time as the struvite fertiliser resulted in a different outcome where the struvites no longer provided on average the highest yields of lettuce (Figure 81). Nevertheless, statistical analysis showed that significant differences were neither reached in the first or the second trial when it comes to differentiating ortho or superphosphate with the secondary mineral struvite.

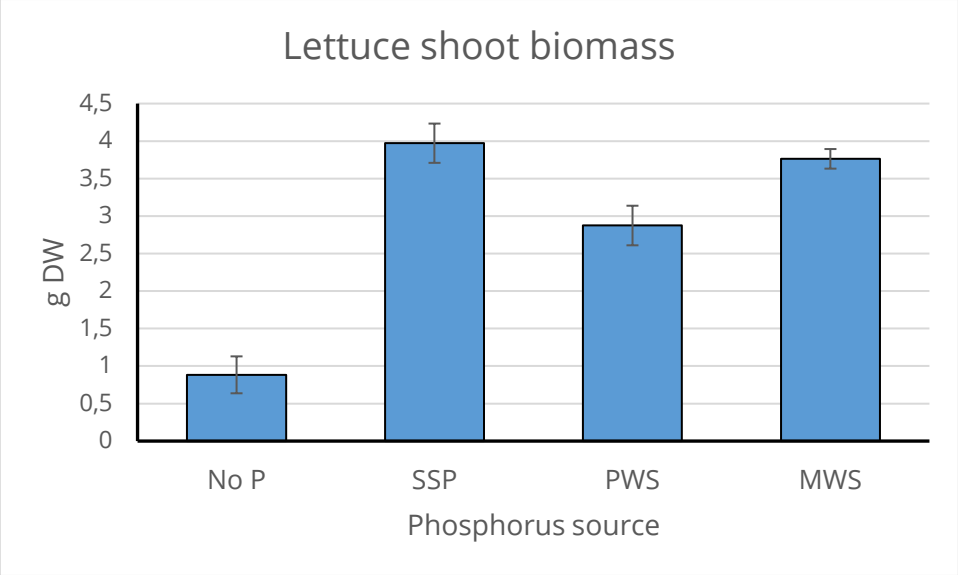

Figure 81: Lettuce shoot biomass from second pot trial with P free fertiliser (No P), super phosphate fertiliser (SSP) or struvite fertiliser from potato or municipal waste water (PWS, MWS).

Available P in the potting soils differed greatly at the time of harvest. Expectedly, No P soil had the lowest amount of Morgan's P that was below the amount at the start of the experiment. Morgan's P was highest in the SSP treatment. Values of SSP and MWS overlapped, while the value of PWS were the lowest between all three fertilized treatments (Figure 82).

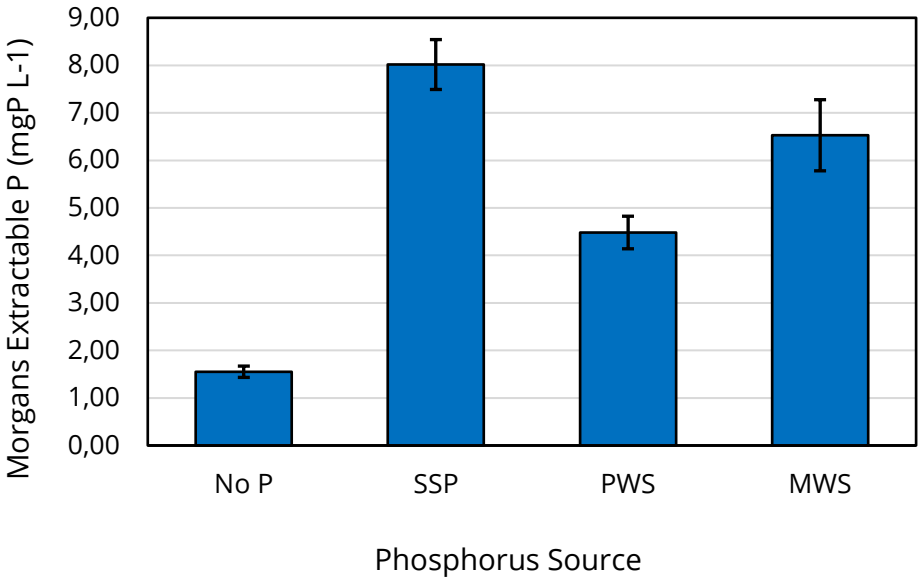

Figure 82: Soil Morgan's at harvest of lettuce from 2nd pot trial with P free fertiliser (No P), super phosphate fertiliser (SSP) or struvite fertiliser from potato or municipal waste water (PWS, MWS).

Soil enzymatic analysis of alkaline and acid phosphatase potential revealed that there were no substantial changes in the enzymatic activity in the second pot experiment (Figure 83).

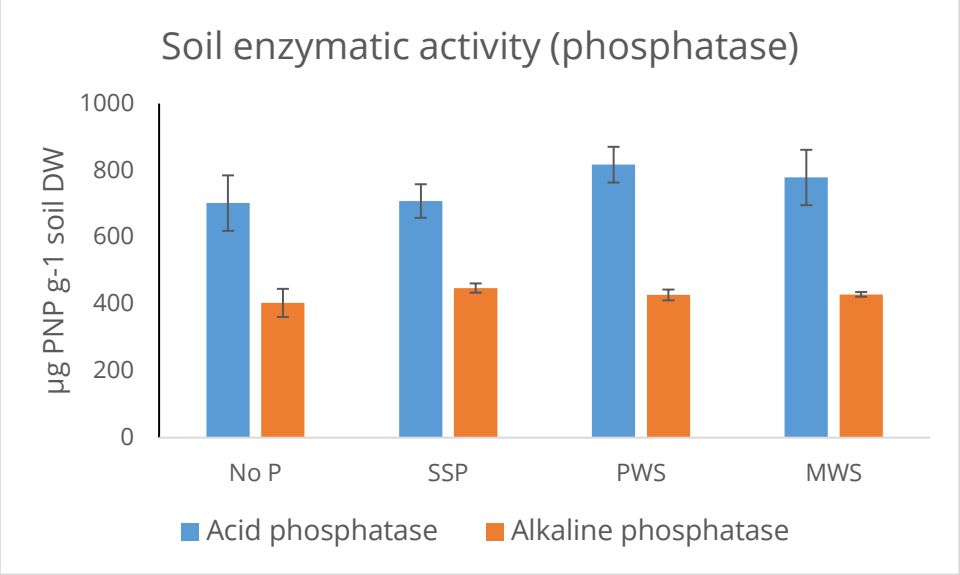

Figure 83: Alkaline and acid phosphatase soil activity at harvest of lettuce from second pot trial with P free fertiliser (No P), super phosphate fertiliser (SSP) or struvite fertiliser from potato or municipal waste water (PWS, MWS).

Quantification of tricalcium-phosphate mobilizing bacterial colonies was attempted. However, the percentage of colonies that displayed a halo around their colonies was low and thus it was not possible to determine an accurate CFU g-1 soil. However, CFU g^{-1} of calcium-phosphate mobilizing bacteria ranged from 10^4 to 10^5 throughout the experiment with no clear differences between the treatments.

Overall, growth conditions were better adjusted in the second pot trial for consistent growth to minimize random poor performance of individual plants (Fig. 84)

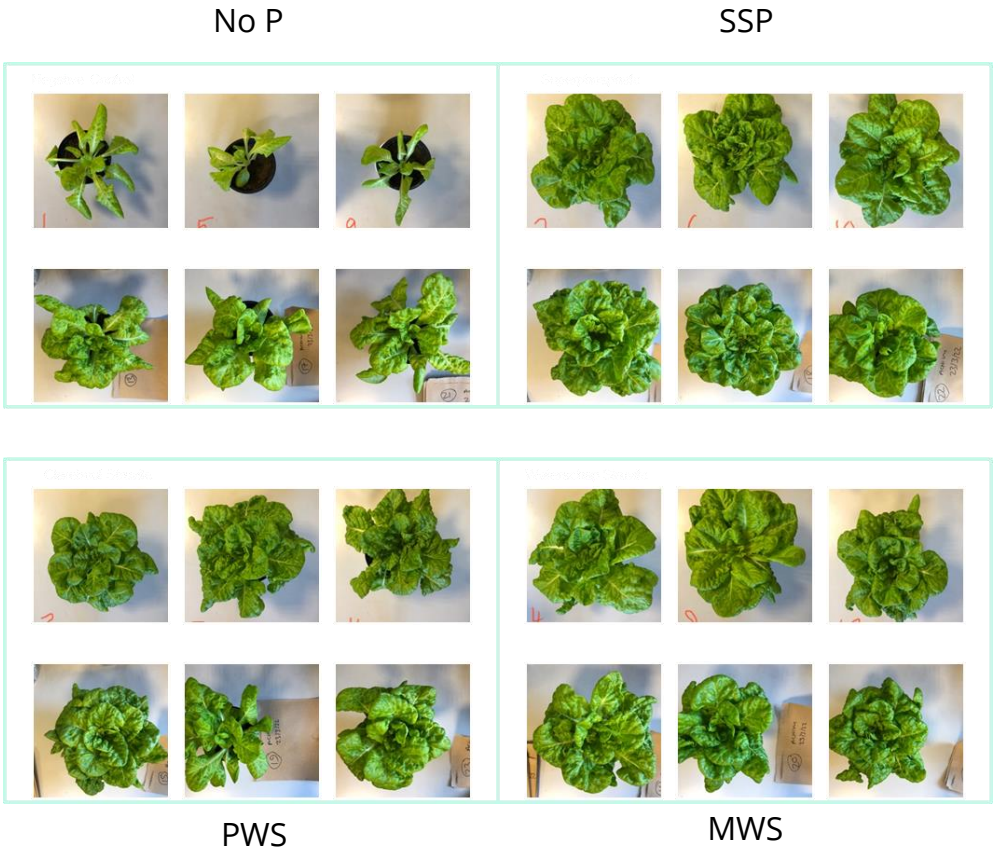

Figure 84: Lettuce growth at day of harvest from second pot trial with P free fertiliser (No P), super phosphate fertiliser (SSP) or struvite fertiliser from potato or municipal waste water (PWS, MWS).

4.1.3. Conclusions

P application resulted in improved biomass production and higher soil available P at the time of harvest for lettuce. However, lettuce biomass differences between the potato and municipal wastewater-based struvite, potassium-phosphate and superphosphate did not reach significance in the short term. First results indicate that the applied struvite may be a suitable substitute for superphosphate to cultivate lettuce, especially if cultivation will be conducted for a longer period of time with repeat harvests. Future long-term studies are needed to confirm this hypothesis.

4.2. Tomato

A glasshouse trial was initiated at University of Limerick to evaluate the agronomic efficiency of two struvite fertilisers as P sources for tomato and their impact on soil microbial activity. There is limited agronomic and soil genomic research of struvite fertilisers using short term horticultural crops.

4.2.1. Material and methods

4.2.1.1. Potting soil and experimental setup

The soil that was used for growing was sieved through a 5.6 mm mesh size sieve to remove stones, twigs, and gravel, and kept moist (approximately 10% water w/w) before being used as potting soil. This soil was a sandy loam soil (54% sand, and 8.6% clay) that had a bulk density of 1037 kg/m^3 and a water holding capacity (WHC) of 22.4%. The soil had a pH of 7.5 and a relatively low available P content (Irish P index of 2), which is ideal for robust P application agronomic comparisons.

The experiment consisted of four treatments (three P fertiliser types and a no P control) replicated six times. Phosphorus treatments consisted of single super phosphate (SSP) (7.6% P, 0% N, 0% K), struvite from municipal wastewater (MWS) (14.8% P, 5.5% N, 0.06% K), and struvite from potato processing wastewater (PWS) (15.3% P, 5.1% N, 1.9% K). About 1.75 kg of soil was used per pot and fertilized with 80mg P/ kg soil while N and K were applied at 182mg N/ kg soil and 142mg K/ kg soil respectively. Micronutrients were applied routinely once every week to each pot as a micronutrient solution.

Cherry tomato (var. *Mr Fothergill's Garden Pearl*) seedlings at the three-leaf stage were transplanted into pots (two seedlings per pot) and watered to approximately 75% WHC. Average day temperatures in the glasshouse ranged between 19 – 34^{o}C and night temperatures ranged between 14 – 25^{o}C during the experiment.

4.2.1.2. Soil sampling

Rhizosphere soil sampling was done for microbiological analysis and molecular analysis, while for chemical analysis bulk soil was collected and air-dried. Soil available P was measured calorimetrically after extraction from soil in Morgan's extractant (10% NaOAc pH4.8). Soil pH was measured using a pH meter in a 1:5 (w/v) soil: water mixture. Dried tomato aboveground biomass was ground and digested for P, Ca, K, S, Mg, Fe and Na quantification in an inductively coupled plasma spectrophotometer (ICP). Soil alkaline and acidic phosphatase activity of soil was analysed as described previously (Tabatabai, 1994).

Soil DNA extraction from 0.25g of rhizosphere soil was performed using a DNeasy PowerSoil Pro Kit (Qiagen, Hilden Germany) according to the manufacturer's guidelines. DNA was used

for next generation sequencing of 16S rRNA gene fragments and qPCR of the alkaline phosphatase gene *ssuD*.

Figure 85: Tomato plants during early fruit development and just before harvest.

4.2.2. Results

At the time of harvest, PWS and MWS applied pots produced about 20% less aboveground biomass than when SSP was applied. This trend was visually observable during the vegetative growth stages of the tomato plants up to flowering. Nevertheless, addition of PWS and MWS fertilisers led to an increase of about 30% biomass over the control that received no P fertiliser. The same trend for aboveground biomass was observed for fresh fruit yield where PWS, MWS and SSP produced about twice as much fruit than the no P control.

Phosphorus uptake was similar across all treatments, while Na and S uptake was higher for SSP. Calcium uptake was lowest in the no P control and highest in SSP, while Mg uptake was similar among the P fertiliser treatments but lower in the no P control. In contrast, K uptake was highest in the no P treatment and lowest in the SSP treatment.

Soil available P was lowest in the no P control and increased by a factor of around two when P fertilisers were applied, which was at a similar level to the P availability before the tomato cultivation started. Compared to the no P control, addition of MWS and SSP reduced soil pH to around 7.1, while the pH reduction in the no P and PWS treatment was smaller and the values were closer to the pH of 7.5 at the start of the experiment.

4.2.3. Discussion and conclusions

The presented study suggest that struvite fertilisers applied for tomato cultivation to replace superphosphate may not release available P as quickly as that is the case with superphosphate. Therefore, early tomato growth leads to less vegetative vigour initially and

delayed fruiting compared to super phosphate. However, struvite application performed more similarly to conventional mineral P application than the no P control. Further long-term experiments are needed to identify whether the initial delay in P supply to the plants would have a sustained effect in fruit production. In a related field trial with grasslands, the authors observed that within a year, the struvite applications performed equally well when compared to superphosphate and outperformed superphosphate fertilization after a two year period. For short-term gains in tomato cultivation, the present authors recommend a combinational approach of superphosphate and struvite application, where the superphosphate is serving short-term needs of the plant, while struvite P will be beneficial for plant growth in the medium to long term. Future experiments shall identify the optimal combination of superphosphate and struvites for optimal yields.

4.3. Spinach

A glasshouse trial was initiated at University of Limerick to evaluate the agronomic efficiency of two struvite fertilisers as P sources for spinach and their impact on soil microbial activity. There is limited agronomic and soil genomic research of struvite fertilisers using short term horticultural crops such as baby spinach.

4.3.1. Material and methods

4.3.1.1. Potting soil and experimental setup

The soil that was used for growing was sieved through a 5.6 mm mesh size sieve to remove stones, twigs, and gravel, and kept moist (approximately 10% water w/w) before being used as potting soil. This soil was a sandy loam soil (54% sand, and 8.6% clay) that had a bulk density of 1037 kg/m^3 and a water holding capacity (WHC) of 22.4%. The soil had a pH of 7.5 and a relatively low available P content (Irish P index of 2), which is ideal for robust P application agronomic comparisons.

The same treatment structure as previously described for tomato was employed. Briefly, there were four treatments (three P fertiliser types and a no P control) replicated six times and arranged in a randomised complete block design (RCBD). Phosphorus was applied at 51 mg P/kg soil as SSP or MWS or PWS. Nitrogen and K were applied at 160mg N/ kg soil and 143mg K/ kg soil respectively.

Baby spinach seeds (var. *Trumpet F1*) seedlings were germinated by putting them on moistened filter paper in petri dishes that were then placed in a cold room for three days and afterwards placed in a plant growth chamber for another three days and the healthiest seedlings were then transferred to the pots (ten plants per pot). Watering was done routinely to approximately 75% WHC.

Figure 86: The second baby spinach trail after the first harvest.

4.3.1.2. Harvest and soil sampling

Two harvests of spinach biomass were collected. The first harvest was performed by pinching off outer leaves to leave the 3 most inner leaves at 40 days after transplanting (DAT). The second and final harvest was done by cutting the stems at soil level at 63 DAT (see Figure 86). The aboveground biomass was weighed after drying to a constant mass. For soil sampling and analysis, please refer to the methods section of the tomato experiment described above.

4.3.2. Results

Aboveground biomass production of spinach was highly similar for all four treatments. However, when biomass production values were cumulated, then the PWS treatment achieved the highest yields, while the lowest yields were achieved by the no P control. Nevertheless, increases in yield with PWS over no P were around 10% and increases in yield over the superphosphate treatment was around 5% (i.e. 3.4 – 3.8 g DW). Soil pH was generally lower after spinach growth compared to initial pH in all treatments with the no P treatment resulting in the smallest pH drop of around 0.5, while P fertiliser use resulted in a pH drop of around 0.5. P fertilization treatments had a Morgan's P that was above the original soil level by around 10%. While available P levels in the no P control dropped by around 20%. Acid phosphatase measurements were highest in the struvite treatments, while no P and SSP had similar levels of acid phosphatase, which was around 10-20% below that of the struvite treatment. Alkaline phosphatase activity was highest in SSP and lowest in no P (about 20% difference) with the struvite treatments in between the two.

4.3.3. Discussion and conclusions

In comparison to the tomato cultivation, P application and source for spinach cultivation may not translate to a strong biomass agronomic response for baby spinach in the short term. This is mostly due to the lower dry biomass generated over the same timeframe as the tomato cultivation. The reduced biomass production is resulting in lower nutrient demands in the short-term. Nevertheless, the current pot trials indicate that struvites can be a suitable substitute for superphosphate during spinach cultivation. When repeated harvests are taking into consideration over the entire growing season, then the observed slower release of available P from struvites when compared to superphosphate may result in a clear advantage. Long-term studies are needed in the future in order to assess the potential benefit of struvites as P fertiliser when cultivation of spinach is conducted over a full cultivation period. Application of struvite fertilisers appeared to result in greater soil acidic phosphatase activity compared to SSP under spinach growth. Unlike alkaline phosphatase activity, acid phosphatase activity is the combinational effect of microbial and plant enzymatic activity, hence further research is needed to investigate whether spinach roots are actively contributing to P availability when struvites are used as P fertiliser. In conclusion, the use of struvites appears to have clear potential to replace conventional mineral P

fertilisers in baby spinach cultivations, albeit further studies over long term in the field is needed to confirm the initial pot trial results.

5. General conclusions

Overall, relatively positive results were achieved in horticulture, depending on the fertiliser and with some points of attention.

Struvites, as slow release fertilisers, might not provide sufficient phosphorous in sync with the phosphorous need of some crops just as well as single super phosphate does. However, mineral phosphorous could still be positively replaced by struvites for long-term cultivation with repeated harvests.

Both ammonia salts, ammonium nitrate and ammonium sulphate, showed similar results as mineral fertiliser in the majority of plants tested. It became clear that following fertilisation advice remains important. Even though some of the plants initially showed slower rooting, this delay had disappeared by the time they were marketed. Ammonium sulphate showed earlier flowering in some plants, with no impact on the floridity. This is mainly a major area of attention with chrysanthemums, as the marketing time should be aligned with All Saints' Day. For long-cropped plants, the low N-content is also not ideal. Either the plants need to receive fertilisation more often, or the concentration should be higher, although the nutrient recovery process makes this difficult. One more point of attention is the pH of ammonium sulphate. As it had a pH of 4, the ammonium sulphate acidified the substrate. This should be monitored closely to be able to correct the pH value when necessary.

6. Annex

6.1. References

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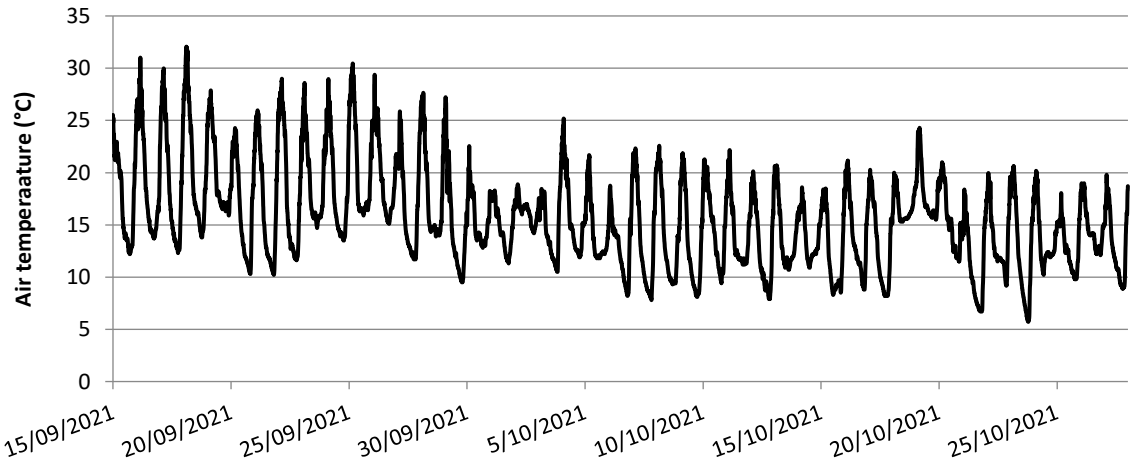
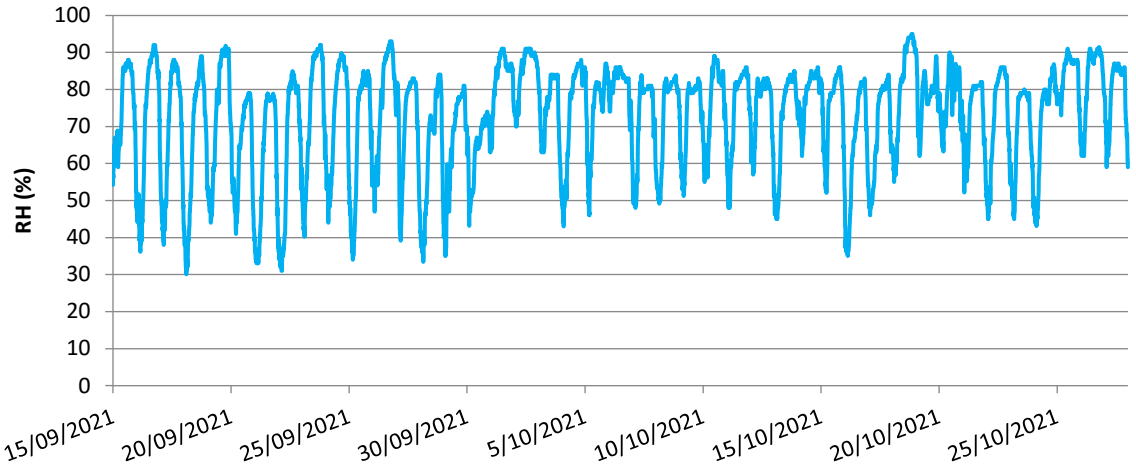
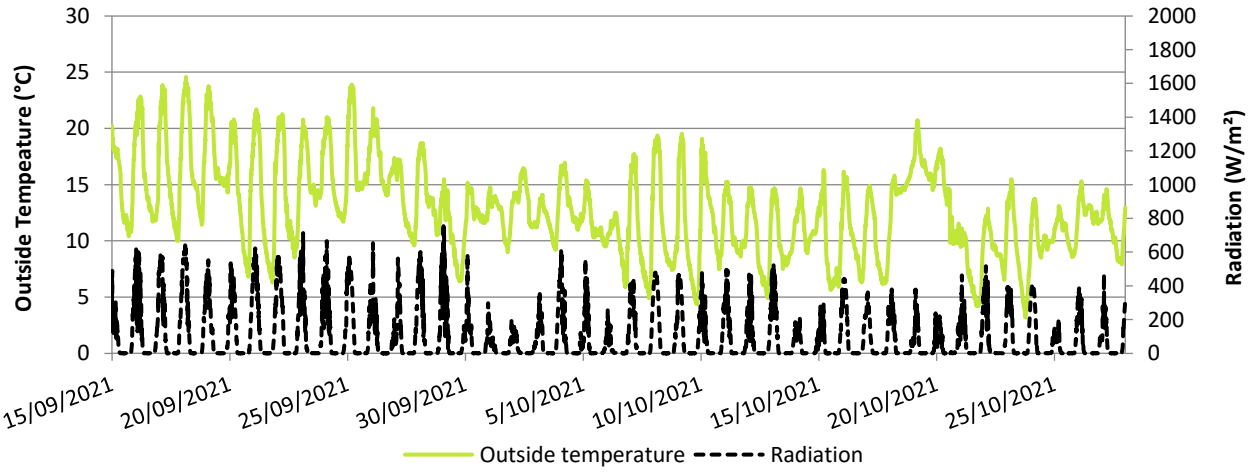
6.2. Lettuce

6.2.1. Annex 1 - Climate conditions







6.2.2. Annex 2 – Raw data

	Canopy Cover	Uniformity	Crop filling	Leaf colour
~~101~~	~~9.0~~	~~7.0~~	~~7.0~~	~~7.0~~
102	9.0	7.5	7.5	7.0
103	9.0	8.0	7.5	7.0
104	9.0	8.0	7.0	7.0
105	9.0	8.5	7.5	7.0
106	9.0	8.5	7.0	7.0
107	9.0	8.0	7.0	7.0
201	9.0	8.5	7.5	7.0
202	9.0	8.5	7.5	7.0
203	9.0	8.0	7.5	7.0
204	8.0	8.5	7.5	7.0
205	9.0	8.0	7.5	7.0
206	9.0	8.0	7.0	7.0
207	9.0	8.5	7.0	7.0
301	9.0	8.0	7.5	7.0
302	9.0	8.5	7.5	7.0
303	9.0	8.0	7.5	7.0

304	8.5	8.0	7.0	7.0
305	9.0	8.5	7.5	7.0
306	9.0	8.5	7.5	7.0
307	9.0	8.5	7.5	7.0
401	9.0	8.5	7.5	7.0
402	9.0	8.0	6.5	7.0
403	9.0	8.0	7.0	7.0
404	8.0	8.0	7.0	7.0
405	9.0	8.5	7.5	7.0
406	9.0	7.5	7.0	7.0
407	9.0	8.0	7.0	7.0

		Fresh weight (g)	Marketable weight (g)	Tip burn	Basal rot
101	Crop 1	~~346~~	~~310~~	~~9.0~~	~~9.0~~
	Crop 2	~~292~~	~~266~~	~~9.0~~	~~8.5~~
	Crop 3	~~327~~	~~299~~	~~9.0~~	~~9.0~~
	Crop 4	~~324~~	~~285~~	~~9.0~~	~~9.0~~
102	Crop 1	416	366	9.0	9.0
	Crop 2	398	362	9.0	9.0
	Crop 3	417	370	9.0	7.5
	Crop 4	367	336	9.0	8.5
103	Crop 1	422	387	9.0	9.0
	Crop 2	403	375	9.0	9.0
	Crop 3	347	313	9.0	9.0
	Crop 4	397	359	9.0	9.0
104	Crop 1	360	323	9.0	9.0
	Crop 2	331	313	9.0	9.0
	Crop 3	410	375	9.0	9.0
	Crop 4	367	341	9.0	9.0
105	Crop 1	420	377	9.0	9.0
	Crop 2	372	344	9.0	9.0
	Crop 3	448	418	9.0	9.0
	Crop 4	422	392	9.0	9.0
106	Crop 1	385	359	9.0	9.0
	Crop 2	322	291	9.0	9.0
	Crop 3	403	370	9.0	9.0
	Crop 4	398	360	9.0	9.0
107	Crop 1	395	365	9.0	9.0
	Crop 2	358	327	9.0	9.0
	Crop 3	378	341	9.0	9.0
	Crop 4	357	323	9.0	9.0
201	Crop 1	408	372	9.0	8.0
	Crop 2	390	347	9.0	9.0
	Crop 3	467	407	9.0	9.0
	Crop 4	400	361	9.0	9.0
202	Crop 1	414	380	9.0	9.0
	Crop 2	407	377	9.0	9.0
	Crop 3	448	402	9.0	9.0
	Crop 4	391	357	9.0	9.0
203	Crop 1	410	373	9.0	9.0
	Crop 2	440	393	9.0	9.0
	Crop 3	368	325	9.0	9.0
	Crop 4	419	388	9.0	9.0
204	Crop 1	333	314	9.0	9.0
	Crop 2	387	348	9.0	9.0
	Crop 3	401	365	9.0	9.0
	Crop 4	402	365	9.0	9.0

		Fresh weight (g)	Marketable weight (g)	Tip burn	Basal rot
205	Crop 1	405	370	9.0	8.5
	Crop 2	451	418	9.0	8.5
	Crop 3	344	324	9.0	9.0
	Crop 4	397	368	9.0	9.0
206	Crop 1	459	421	9.0	9.0
	Crop 2	437	397	9.0	9.0
	Crop 3	350	305	9.0	9.0
	Crop 4	398	357	9.0	9.0
207	Crop 1	373	347	9.0	9.0
	Crop 2	349	308	9.0	9.0
	Crop 3	427	386	9.0	9.0
	Crop 4	386	340	9.0	9.0
301	Crop 1	497	435	9.0	9.0
	Crop 2	405	356	9.0	9.0
	Crop 3	419	394	9.0	9.0
	Crop 4	425	383	9.0	9.0
302	Crop 1	400	360	9.0	9.0
	Crop 2	379	337	9.0	9.0
	Crop 3	420	379	9.0	9.0
	Crop 4	431	393	9.0	9.0
303	Crop 1	408	373	9.0	9.0
	Crop 2	367	325	9.0	9.0
	Crop 3	449	403	9.0	9.0
	Crop 4	449	410	9.0	9.0
304	Crop 1	342	321	9.0	8.0
	Crop 2	355	316	9.0	9.0
	Crop 3	332	308	9.0	9.0
	Crop 4	414	367	9.0	9.0
305	Crop 1	461	416	9.0	9.0
	Crop 2	426	394	9.0	9.0
	Crop 3	370	337	9.0	9.0
	Crop 4	435	378	9.0	9.0
306	Crop 1	344	321	9.0	9.0
	Crop 2	428	404	9.0	9.0
	Crop 3	384	361	9.0	9.0
	Crop 4	383	351	9.0	9.0
307	Crop 1	329	297	9.0	9.0
	Crop 2	447	407	9.0	9.0
	Crop 3	390	348	9.0	9.0
	Crop 4	373	339	9.0	9.0
401	Crop 1	387	355	9.0	9.0
	Crop 2	417	399	9.0	9.0
	Crop 3	470	429	9.0	9.0
	Crop 4	394	349	9.0	9.0

		Fresh weight (g)	Marketable weight (g)	Tip burn	Basal rot
402	Crop 1	338	297	9.0	9.0
	Crop 2	362	334	9.0	9.0
	Crop 3	357	331	9.0	9.0
	Crop 4	390	360	9.0	9.0
403	Crop 1	390	363	9.0	9.0
	Crop 2	392	343	9.0	7.0
	Crop 3	302	271	9.0	9.0
	Crop 4	359	333	9.0	9.0
404	Crop 1	379	349	9.0	9.0
	Crop 2	322	292	9.0	9.0
	Crop 3	358	330	9.0	9.0
	Crop 4	391	364	9.0	9.0
405	Crop 1	385	355	9.0	9.0
	Crop 2	361	324	9.0	9.0
	Crop 3	404	378	9.0	9.0
	Crop 4	373	343	9.0	9.0
406	Crop 1	322	294	9.0	9.0
	Crop 2	374	345	9.0	9.0
	Crop 3	350	319	9.0	9.0
	Crop 4	417	351	9.0	8.5
407	Crop 1	380	352	9.0	9.0
	Crop 2	344	319	9.0	9.0
	Crop 3	387	359	9.0	9.0
	Crop 4	370	337	9.0	9.0

6.2.3. Annex 3 – pictures

N-100

N-70

N-40

Control

S-100

S-70

S-40

6.3. Viola

6.3.1. Annex 1 – Pictures throughout the growth period

23/09/2021

05/10/2021

11/10/2021

18/10/2021

25/10/2021

02/11/2021

08/11/2021

15/11/2021

23/09/2021

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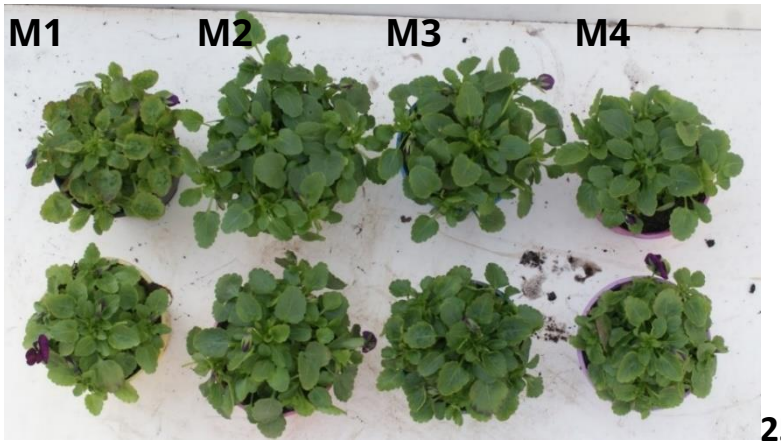
18/10/2021

25/10/2021

02/11/2021

08/11/2021

15/11/2021



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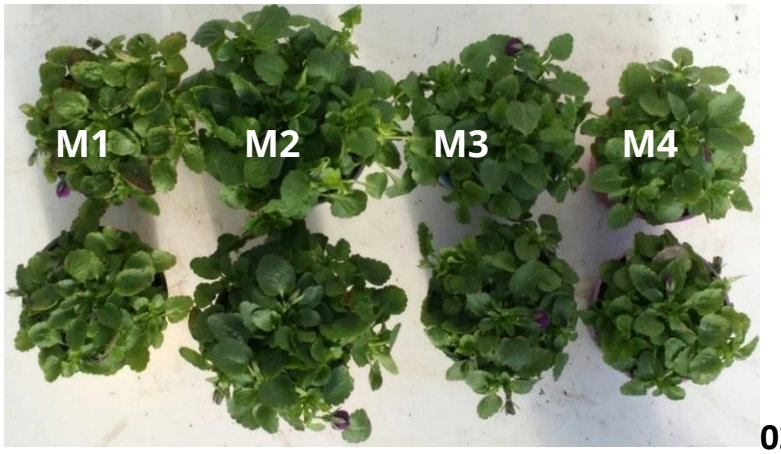

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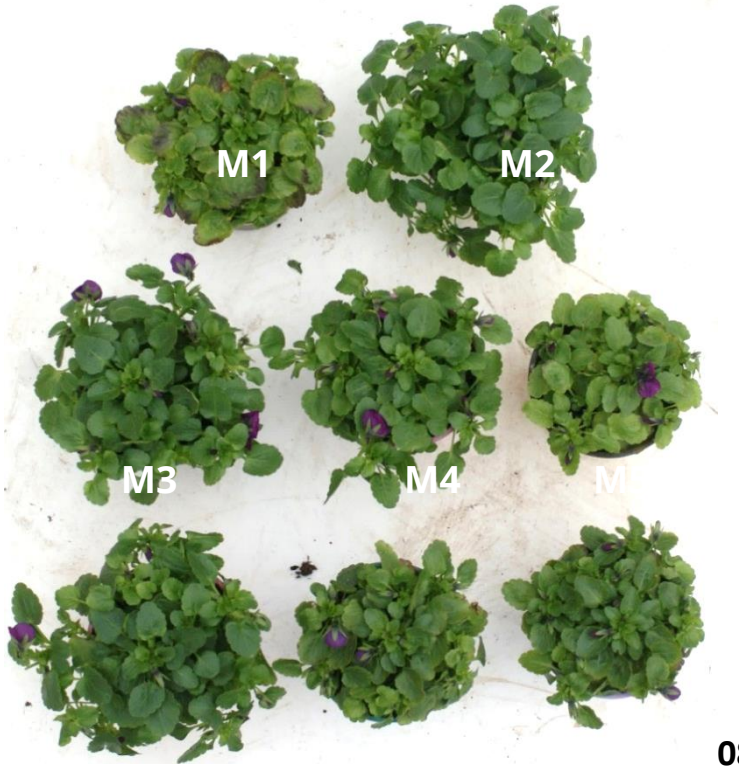

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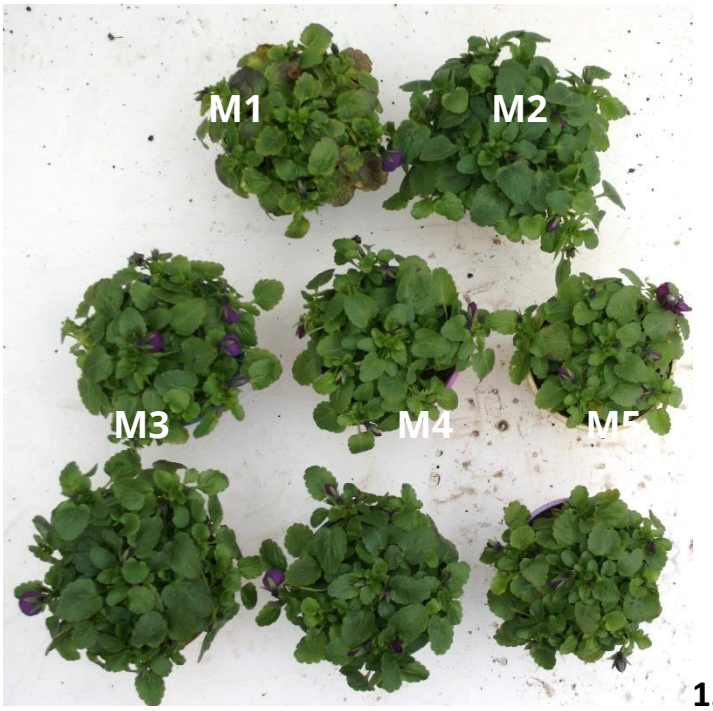
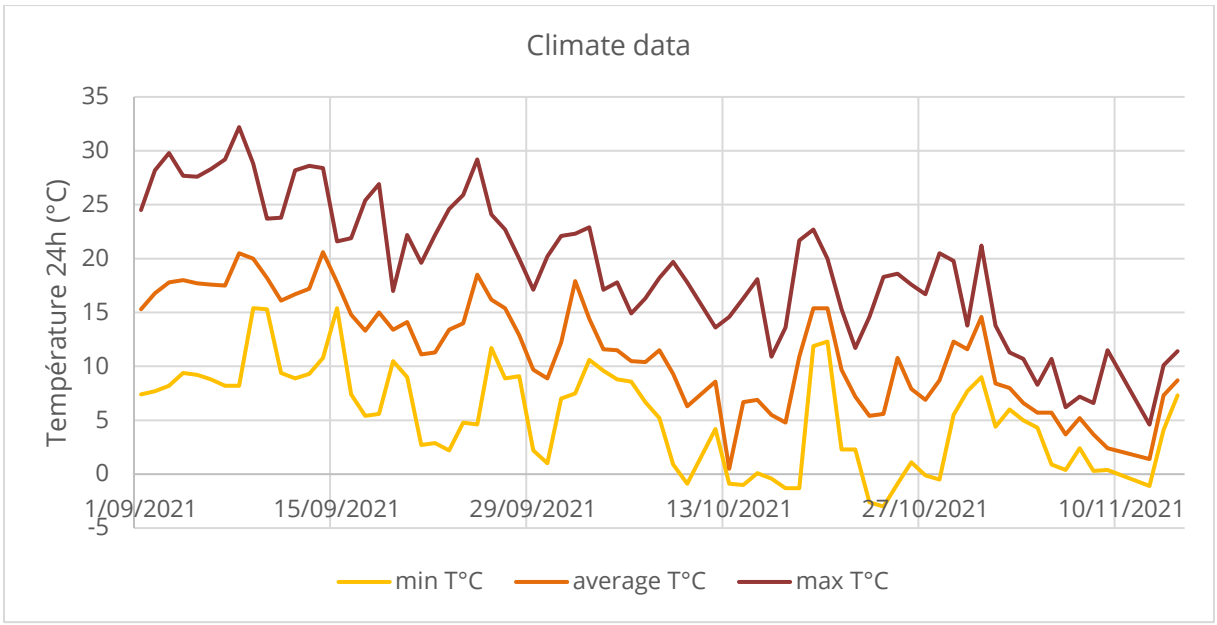
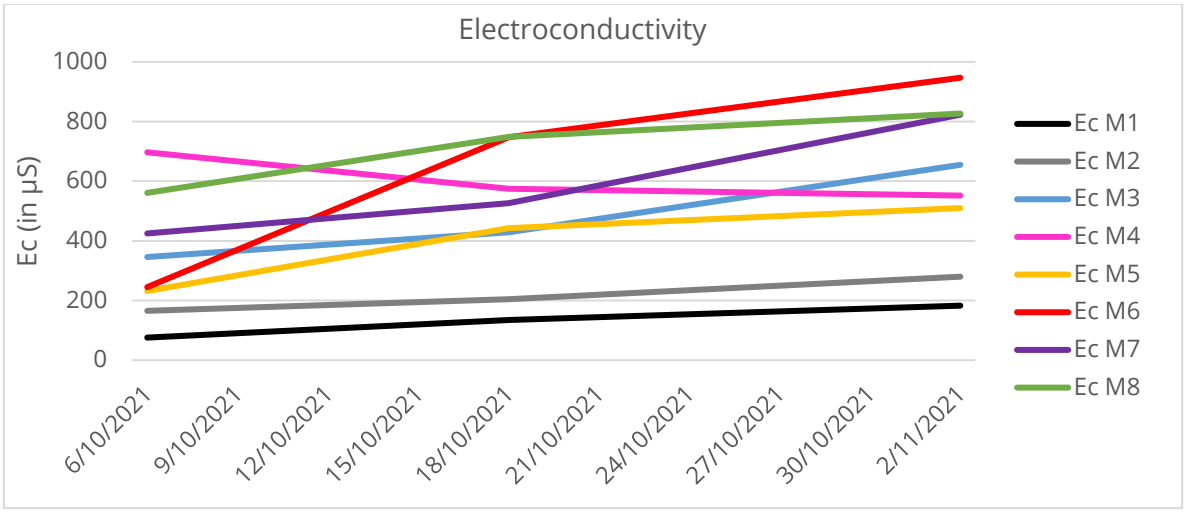


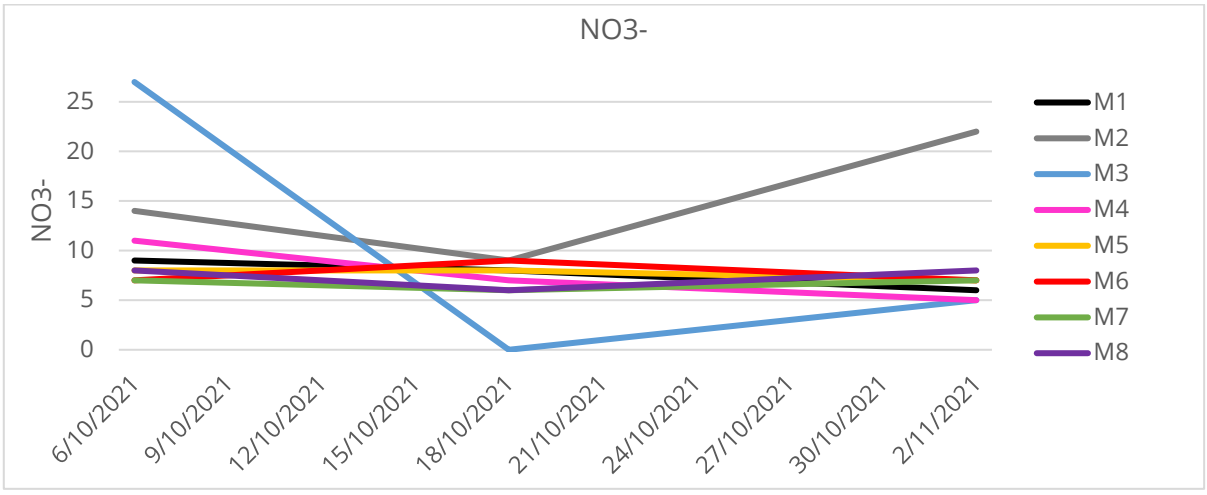
15/11/21

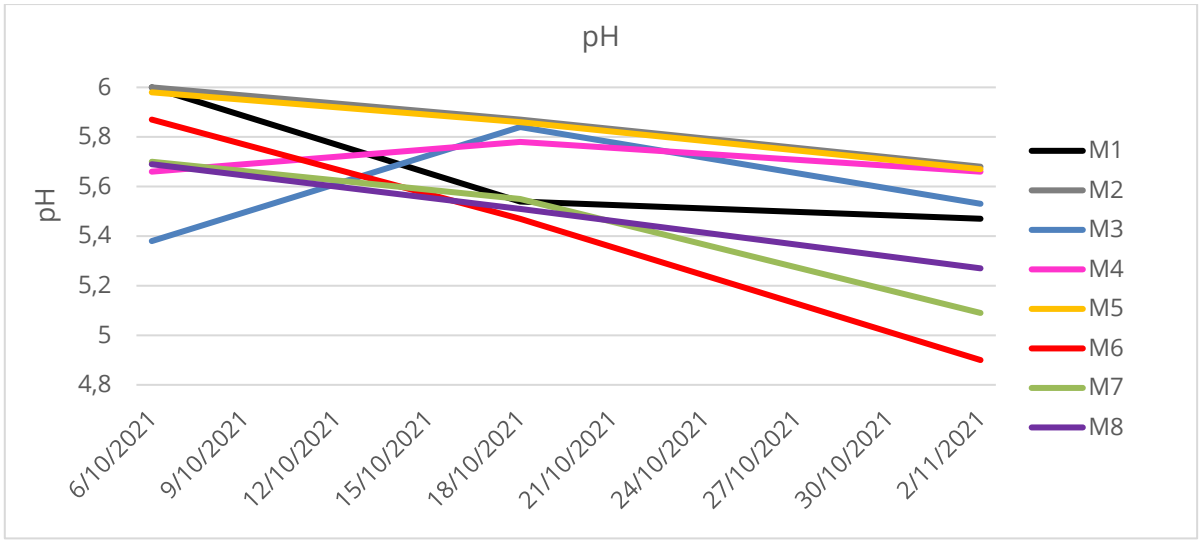
6.3.2. Annex 2 – Climate data



6.3.3. Annex 3 – Other graphs

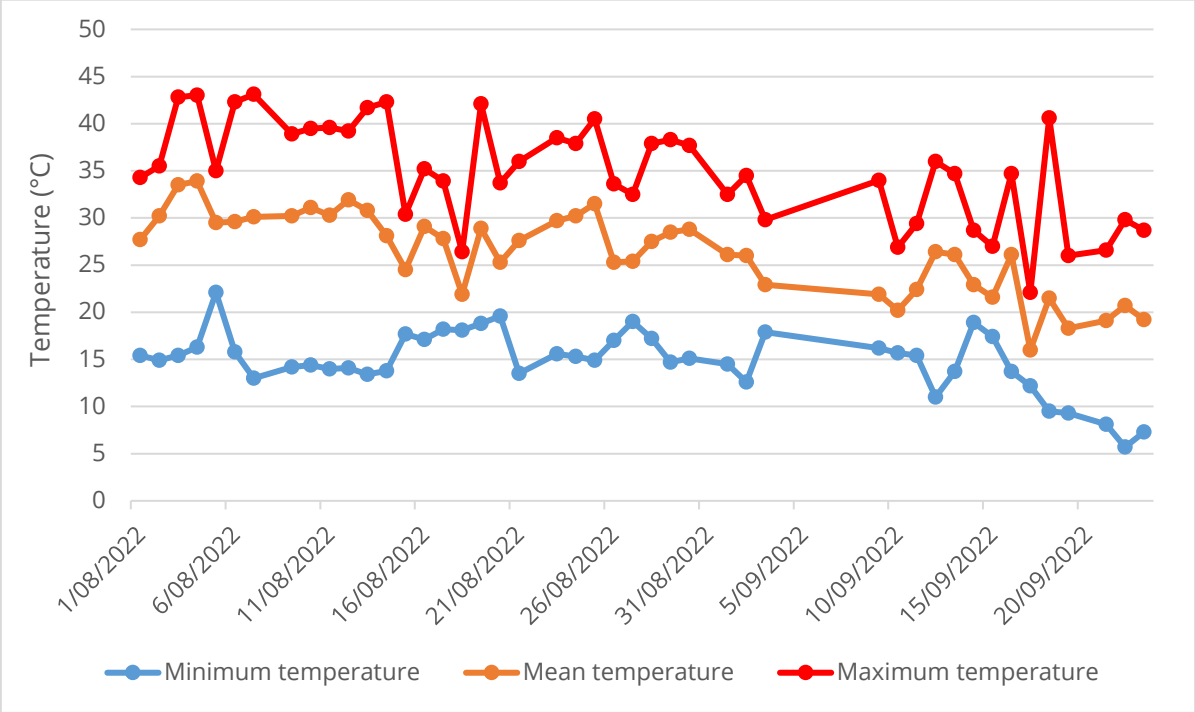






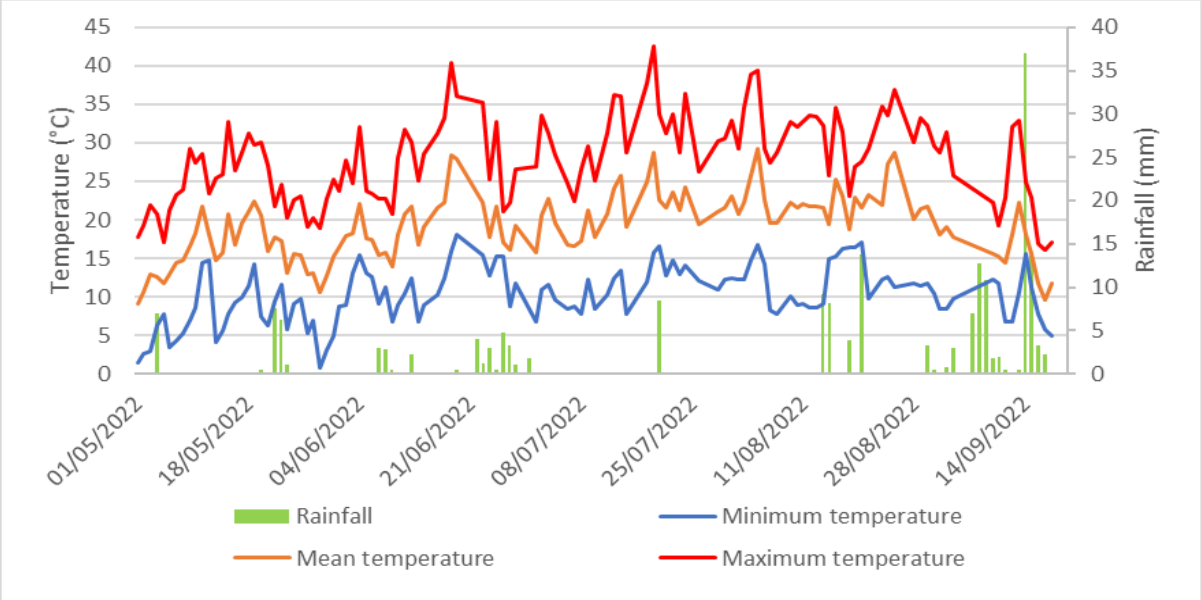
6.4. Basil

6.4.1. Annex 1 – Weather records of the greenhouse between August and September



6.5. Lonicera

6.5.1. Annex 1 – Outdoor weather records from May to September 2022



6.5.2. Annex 2 – Statistical analysis

Table 38: Recap of the statistical analysis (in green the significantly high values and in red the significantly low values).

Date	Kruskal-wallis p-value	Differences	M1	M2	M3	M4	M5	M6	M7	M8
3rd May	$0.14x10^{-3}$	Significant	c	ab	abc	a	bc	abc	ab	bc
15th June	$1.9x10^{-7}$	Significant	bc	a	ab	b	c	ab	b	c
6th July	$0.35x10^{-3}$	Significant	abc	a	a	abc	c	a	ab	bc
27th July	$0.88x10^{-3}$	Significant	ab	a	a	ab	b	a	ab	b

Table 39: Recap of the statistical analysis on the length of the longer branch (in green the significantly high values and in red the significantly low values).

Date	Kruskal-wallis p-value	Differences	M1	M2	M3	M4	M5	M6	M7	M8
9th March	$0.3x10^{-3}$	Significant	ab	b	a	a	a	a	a	a
16th March	$0.2x10^{-3}$	Significant	a	b	a	a	a	a	a	a
23rd March	$0.9x10^{-6}$	Significant	a	b	a	a	a	a	a	a
14th April	0.1823	Non significant	a	a	a	a	a	a	a	a
3rd May	0.0153	Significant	b	ab	ab	a	ab	ab	ab	ab
25th May	0.0765	Non significant	a	a	a	a	a	a	a	a
15th June	0.0222	Significant	a	a	a	a	a	a	a	a
6th July	0.0038	Significant	ab	b	ab	ab	b	a	ab	ab
27th July	0.0029	Significant	ab	ab	a	ab	b	a	ab	ab