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ABBREVIATIONS

ACC: 1-aminocyclopropoane-1-carboxylic acid

HPC Higher Plant Chamber (Compartment)

PCU Plant characterisation unit

PPU Plant production unit

PSDU Plant Stress Detection unit

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1 Critical assessment of the PSDU design steps

Based on the requirements for monitoring the key as well as the critical plant growth associated parameters, an initial PSDU conceptual design was proposed, identifying the different subsystems and monitoring units to provide the needed measurements (see TN 89.4).
Based on a survey of the available destructive and non-destructive techniques, solutions were implemented to obtain a workbench demonstrator (TN 89.51) in which a preliminary test of feasibility was to be carried out.
During the setup of this system, monitoring of a few parameters identified as critical proved more difficult to implement than presumed based on the available documentation. Automatic gathering/logging of the data was notably a feature of only high end of the price range devices, but the approach remains technically feasible.
This was the case for the dissolved oxygen sensor, which needed repositioning in the PSDU workbench demonstrator to comply with the flow range needed for stable measurements. Measurement at the drain of the gullies, where dissolved oxygen would be minimal (at least before mixing back into the collection tank by gravity) proved impossible.
A single dissolved oxygen probe in the nutrient solution tank (as also proposed in TN 75.3) would be the best solution to monitor this critical parameter with continuous logging.
Calcium concentration measurement could be achieved by using a portable handheld cuvette – based measurement device in which reagents are employed for a colorimetric quantification. However it has little added value (and is less sensitive) compared to the offline chemical analysis using HPLC or ICP-OES. When the latter are available on-site, or analysis results are available within short delay this remains the preferable solution..
The other parameters identified as critical were implemented, but for many if not most of them, integration into a plants stress monitoring strategy requires further development or optimisation. The combination of the monitoring strategy of the MELiSSA HPC system with subunits of the PSDU as described in TN 89.51 has been discussed in TN89.6. For small scale systems emphasis would be on mass energy and space use efficiency. Addition of extra techniques or strategies and further optimisation of already identified techniques and systems are discussed below.

1.1 Plant chamber conditions

The goal of the plant growth chamber air handling system is temperature control and mixing of the chamber air without causing stress to the plants. A temperature difference of 2 degrees between input and output is at the limit of feasibility with regards to the volume taken by the air handling system distribution ducting.

Light distribution is equally difficult to homogenise, and gradients have a clear influence on crop growth and productivity. This is still a critical point that merits continued attention.

The parameters light distribution, and the interconnected temperature, humidity and air flow distribution will exert a clear influence on the plant growth, and will be revealed by individual plant monitoring techniques.
A pro-active approach in which these parameters are mapped along a measuring grid will allow to optimise their homogeneity as far as is feasible within the possibilities of available space and control approach.
By minimising chamber heterogeneity, monitoring techniques will be able to reveal localised suboptimal growth with more confidence. The persisting non-homogeneity can be taken into account by a mathematical model combining both chamber hardware and control characteristics and coupled plant growth kinetics. Obviously the development of such a combined environment-plant first principle model will need considerable development resources.

1.2 Hydroponic growth conditions

The hydroponic gullies and the connection plumbing are characterized by a relatively low flow rate, which is difficult to precisely measure, monitor and control. The relative control of the flows for the different gullies needs to stay stable during the growth period, as it will also influence nutrient and dissolved oxygen availability.

In the case of the PSDU workbench demonstrator this problem was exacerbated by having gullies located at different levels and the need for a distribution box with needle valves. The recirculating system can typically ferry small debris from the plants or died overhanging older leaves. Therefore clogging should be largely prevented by connecting filter pods to the drains. Filter material should be chosen as not to clog by bacterial growth. Autoclavable miracloth rayon-polyester with an acrylic binder with a typical pore size of 22-25 µm proved unsuitable. Crop cover consisting of lightweight polypropylene spunbonded nonwoven fabric performed reasonably well.

1.3 Ethylene measurement

Ethylene monitoring is, given the extensive body of former research on the topic, clearly a requirement for inclusion in a future stress monitoring strategy. In chambers with sealed atmosphere the here tested photo-acoustic system would provide on-line measurement capability.

To guarantee long term stability and accuracy of this measurement, a source of pure (hydrocarbon free) calibration air (gas bottle) should be accounted for.

The detector module (30x40x5cm) should be placed in an environment with stable temperature for optimal performance of the laser based detection system.

1.4 pH, EC and water level control logging

Control approaches were tuned as to obtain a fluctuation of 5% around the set value of pH 5.65 and EC of 1500 microS/cm.
In order to have comparable EC and pH readings from the 2 independent circuits in the PSDU workbench demonstrator, mixing and addition by dosing pumps had to be calibrated at the same level.

Specific electrodes for monitoring NO_3^- on NH_4^+ in the nutrient solution will likely be implemented in a next setup, since such sensors are available for process monitoring environments.
A non-invasive microelectrode (MIFE) technique has been used to monitor the dynamics of net fluxes of NH4+, H+,K+ and Ca2+ close to the root surface of canola [1]. Although applied in the context of a specific research topic on the effect of NO_3^- on NH_4^+ uptake, such sensors might be positioned to monitor a few representative 'indicator' plants.
The issue of positioning and data transfer might be eased by the development of wireless sensor arrays that transmit the data and thus overcome the hurdle of cabling or operator interference.

1.5 On-line weight measurement

This approach is in use as an online control technology in horticulture. The Hortimax Prodrain system (www.hortimax.nl) can use up to 4 different weight measurements in order to fine-tune water use in horticultural systems with substrate based hydroponics. Parameters include gully+substrate weight, in case of hanging/supported cultures such as tomatoes or cucumbers crop weight is also amenable to measurement, drain weighing and irrigation weighing. Implementation of load cells al part of a PSDU system depends on geometry of the PPU or PCU as well as the possible redundancy with other monitoring techniques.

1.6 Plant monitoring

The speed of root growth of the developing plants could be considered as equally important as the shoot growth. Accessibility is obviously less, and since shoot growth is finally correlated with the health of the root system. Shoot monitoring might be sufficient for crop growth monitoring. Nonetheless as mentioned above for nutrient uptake, monitoring of a few plants could be a worthwhile addition to the shoot monitoring possibilities. Feasibility and need for root monitoring need to be assessed in the framework of a follow-up study. This aspect can likely be kept as a target for the Plant characterisation unit (PCU) type of chambers, or as a separate approach in a workbench demonstrator setup.

Arrays of fixed camera will likely be limited to visual spectrum CMOS cameras, which are both cheap and relatively insensitive to electromagnetic interference. Since these cameras are also sensitive to near infrared radiation (NIR), presumed to be beyond the window of sensitivity of the plants, they could also be employed for root monitoring. Other types are still too costly for array type deployment.

A mobile camera can be positioned at the desired location, and dependent on the chosen optics automatic focus, zoom can be available, and moreover tilt and pan capability can be added to achieve the needed resolution for trustworthy remote observation. (in the visible spectrum and NIR for dark period monitoring when considered important). The ability to orient the camera focal plane depending on orientation of the target leaf would be a necessity for this type of detailed observation. Tile and pan capability could be considered for the PSDU preliminary design, depending on the added weight and increased height of the monitoring system this would imply. Mass, volume and energy penalties associated with a robotised system can be minimised by using miniaturised technology. However such sensors only provide basic technology.

To monitor plant water usage thermal imaging cameras are a straightforward choice, but as shown also during this study can detect localised stress responses at an early stage. There is an evolution towards higher resolution camera's, which are however not as compact. Compact radiometric thermal cameras (with direct temperature readout) would be ideal. Otherwise extra software has to be developed to implement this functionality based on extra external sensors and references.

The spatial and temporal heterogeneity of temperature conditions within the growth chamber can also affect the readout of the camera. Hence a monitoring and correlation of these factors have to be carried out (see also TN 89.55). Further optimisation will be needed to obtain a software solution that is adaptable to different hardware scenario's.

Noise level of the chosen (imaging) sensors has to be assessed in the targeted measuring environment. Dependent on the type, noise can be filtered out by dedicated software routines.

Low weight, power consumption are important requirements for a PSDU compatible with the space, weight and power use constraints of HPC, PPU or PCU plant growing hardware.

Camera's with embedded DSP (digital signal processing) capability enable to carry out pre-processing of the images, possibly including filtering of the essential information. This functionality should be user-programmable to be able to customize according to changing needs as research progresses.

With the gradual increase in light output per unit power supplied, miniature LED illumination units for fluorescence imaging will allow to supply the necessary intensity to excite chlorophyll (a measure of photosynthetic efficiency) and blue-green (linked to the contents of secondary metabolites possibly indicative of stress) fluorescence. This will be the preferable way to add extra sensing capability without increasing size or weight of the monitoring system.

1.7 Plant performance characterisation

Observation of plant performance will consist of expert assessment (human plus multi-sensor imaging coupled to a stress-classification expert-system).

The **expert system** will be based on gathered info on imaging of plant pathogen interactions. In addition to nutrient deficiency and toxicity catalogues, a symptom catalogue can be provided for the operator. Baseline datasets obtained from 'type' experiments would function as reference guidelines, and be employed to build a predictive first principle model of plant growth.

From the overview of available literature on **mathematical modelling** (TN 89.3) a lack of predictive (mechanistic, first principle, based on metabolic reaction kinetics) plant growth models that go beyond the simulation of the photosynthetic process emerged. Although it is not deemed feasible to immediately target a model describing all aspects of the very versatile plant metabolism, a prioritization will be needed in order to simulate the metabolic (and physicochemical) processes that have the largest influence on plant growth yield. In terms of closed loop bioregenerative life support, this could be summarised as the influence of possible stressors on the water, carbon and nitrogen cycles. Key processes involved would be nutrient and water uptake from the liquid phase and CO_2 from the air phase; water loss to the air phase as under leaf stomatal control; biomass (food) production consisting of carbohydrates, lipids and proteins.

The expert system will need to gradually evolve with the accumulating knowledge and remediation scenarios. Predictive modelling will offer the possibility to issue warnings when crop growth or performance is lower than expected.

After observation and first deduction (based on stress catalogue expert system) of the level of emergency and control needed, characterisation is needed for prompt selection of a corrective action.

In order to proceed with **stress factor characterisation**, sampling is needed. This can be accomplished by manual action as long as the tray is within reach of the glove boxes of the airlocks at the ends of the growing chamber. This allows to proceed without breaching the closed atmosphere of the system. The access door on the side of the chamber can be used to manually sample, but this will temporary interfere with gas phase assessment and associated mass balance calculations.

A robotised system that can **sample individual leaves**, based on vision system outputs, could avoid such interference. This would imply deposition of the sample in a holder and placement within reach of a glovebox for further manual .handling through one of the 2 airlocks.

Whole plant removal would be hard to achieve with a single robotic-arm gripper. Given the possible intertwining of plants and roots; the need to temporary remove part of the gully cover; the impossibility lo severe the shoot from the root and to leave the latter in place, such an operation is likely to be handled by an operator in case such a drastic intervention would be needed.

Robotisation in current horticultural settings is not hampered by confined spaces or small overall growing volumes. The existing (prototype) systems prove the feasibility of **robotised harvest** (cucumber [2]/ strawberry [3]) and **robotised plant maintenance** (leaf removal – cucumber [4]/ tomato). The latter issue raises also the question of handling of maturing crops, in which the drying leaves would detach. Monitoring and timing of harvest would need to be consolidated into a fixed schedule after a few tests.

When adhering to **proper sanitation** (in a space-context, sterilisation etc) and correct environmental control concerning temperature and humidity, pathogens are supposed to pose little threat. As a preventive measure air as well as nutrient solution can be sterilised while circulating. For air handling an ethylene removal strategy can also lead to potential pathogen spore suppression (oxidative approach). Avoiding plant debris spread could be another point of attention.

On-line assessment of air composition can also highlight a stress-situation by increased accumulation of ethylene or other compounds. Spot-wise initiation of a potential disease will however be hard to timely monitor given the dilution.

For nutrient solution handling, care has to be taken not to degrade chelating components performance when applying treatments, otherwise **essential element deficiencies** will occur. Iron availability is one of the more critical nutrient-linked parameters. Iron deficiency severely stunts root growth and inhibits further crop development. Long term recycling of the same nutrient solution has to be monitored for iron availability. Leaf colour pattern and root morphology changes are first signs that however would need to be monitored remotely or through the window in the middle compartment of the HPC. Off-line nutrient solution analysis provides an indication; as a precautionary measure, extra chelated iron could be dosed to the nutrient solution at fixed intervals during the growth period.

Sampling of the nutrient solution will allow to assess abnormal nutrient levels or depletion profiles, **organic root-zone compound accumulation** (allelochemicals as secreted by the roots), as well as pathogenic or protective/beneficial **micro-organism development**. The latter can be characterised by a Q-PCR (quantitative real time polymerase chain reaction) based approach in which multiple strains can be detected in parallel on one sample [5].

This QPCR approach likely can also be adapted to the characterisation of genes involved in **stress-responses known to limit productivity**. Within the context of a plant characterisation unit (PCU) setup such a tool can aid in defining an optimal growing environment based on parameter settings with associated safety margins..

Automatic sampling of plant fluids could be used as an indication of actual transport and thus immediate availability of the different elements in the plant [6]. This is seen as more relevant than the composition of the whole plant, which reflects already fixed elements not necessary involved in biomass production. Such an approach could indicate at a very early stage possible developing nutrient deficiencies. However this seems not compatible with the sealed atmosphere setup of the HPC, and would also rather be a research target for PCU type of chambers.

1.8 Data acquisition, processing and management

The effectiveness of the PSDU in detecting plant stress and damage is still subject to extensive optimisation. As described in TN89.53 54 and 55, the existing software needs to be updated to allow online analysis and comparison during the crop growth test.

Other non-imaging sensors could be added to the robotised system such as T, RH and PAR. Their positioning should make the measurement independent of the robotised system structure.

An effective HPC monitoring strategy, based on the monitoring infrastructure of the MELiSSA pilot plant architecture (Human Machine Interface/SCADA supervising system) (see TN 89.6) would enable to realise the goals of the PSDU design.

In comparison with the Workbench demonstrator system, an ideal monitoring system should integrate all parameters from

1. air handling system control. The workbench demonstrator relied on a dedicated computer with Siemens logging software
2. logging system gathering weight, light level, CO_2, O_2, VPD, pH, EC, water level, nutrient, pH, water addition volumes. The workbench demonstrator functionality was assured by a DeltaT logger interface that needed manual download and graphing via macro commands.
3. Image acquisition from multiple sensors, associated data visualisation, processing and output to supervising system. The workbench demonstrator system custom-built NI labview software captured the images on a dedicated PC. A remote server preprocessed the images and synchronised the data to a backup location. Processing of the individual images to overview image sequences was provided by the same server for the video images. Such overview generation permits an operator to assess both the correct functioning of the system while monitoring individual plant growth.

In the workbench demonstrator, **thermal image interpretation** and plant detection was implemented in a preliminary way in Matlab, and needs to be further developed to obtain an automated functionality. From the obtained images during the workbench tests, necrotic damage could be detected at an early stage due to the evaporation of cellular liquid emanating from damaged cells at the leaf surface, thus proving the concept of early high-contrast visualisation.

Distance measurement from sensor to plant could be envisaged. However this would seem mostly suited for closed canopies, for which the average distance would be similar. Open canopies would need a moiré elaborate system in which the distance measurement would need to be controlled by a vision system as to target individual leaves within the field of view. 3D plant visualisation by using LIDAR (light detection and ranging) based systems is feasible at the lab and growth chamber scale, but needs multi-angle measurements for whole plant 3D representation [7]. With a system able to work at close range, a distance mapping to the developing plant canopy should be feasible and is open to further research.

The tilted PSDU Cartesian monitoring system proposed for the PSDU preliminary design to be implemented in the HPC could be a space and actuator-complexity saving approach, depending on the handling capability expected from such a system. Given the relatively small size and particular geometry (optimally adapted to continuous production of plant food)) of the HPC and its interfaces for manual labour for planting and harvest, plant manipulation will likely for this level/scale of production not be feasible, or would need a redesign of the HPC.

2 Plant stress detection test approach

Although the comparative detection test carried out in the workbench demonstrator PSDU confirmed an effect of the ethylene-precursor ACC added to the nutrient solution, the result did not comply with all the known effects of ethylene on plant growth, and presented some puzzling outcomes. The setup of as comparative study on the effect of a gaseous compound in a measuring environment with 2 independent liquid circuits but common atmosphere is not ideal, although no increases in ethylene level of the growth chamber air could be measured. More likely underlying the unexpected results is the fate of the ACC added to the nutrient solution, where it can be metabolised by the resident bacteria and possibly generate yet uncharacterised or toxic compounds.

A nutrient deficiency (Fe or Ca) or salt stress (overall high EC or nutrient imbalance) would be a better alternative for follow up experiments when a common gaseous atmosphere is part of the setup characteristics.

Ethylene effect studies are by preference targeted with hardware incorporating separated liquid and gaseous phases.

Monitoring of microbial presence at the root level (rhizosphere) would ideally be targeted by an available commercial Q-PCR monitoring approach targeted at horticultural production [5]. A similar approach for leaf level (phyllosphere) microbial characterisation would be target for additional research.

The presence of non-microbial but mm to sub-mm size organisms (booklice, mould mites, collembola) is in itself not immediately harmful to plants, but can become so by spreading fungi and bacteria, especially in the seeding or propagation steps of plant growth, and can become critical when in vitro propagation facility would be used for certain crops. Microscopical monitoring of any suspect symptoms therefore should be carried out to identify the possible presence of animalia and to characterize histology of root and shoot.

3 Consolidated requirements assessment

As a conclusion of the TN 89.55, the following combination of techniques was proposed to constitute a per formant early stress monitoring system.

The combination of monitoring

1. atmosphere monitoring, CO_2 assimilation – NCER (net carbon exchange rate), Ethylene
2. nutrient solution pH EC H_2O compensation volume input rates
3. nutrient solution composition
4. shoot and root observation
5. weight

and characterisation

6. expert system with database based on baseline and stress test runs
7. Q-PCR for pathogen and stress factors
8. Microscopy for localisation or structural info

will lead to a setup and protocol for growth under optimal safeguard and adaptive control.

Subsystem or function 3) is currently limited to off-line analysis. Hence it is used as a confirmation tool that nutrient availability is within the needed limits for optimal plant growth. In case of clear depletion, manual intervention is needed to alleviate the problem. This represents a rather coarse control strategy, and could thus be improved by the use of element specific sensors for a few key elements (the macronutrients N P K as well as Ca and Mg).

Function 4) needs to be further developed to permit automated deployment. Root imaging is to be considered a new development task.

Function 5) is a possible new system, depending on the level of added complexity its implementation would require.

Function 6) will be based on the output of the image processing in 4), consequently this functionality needs to be developed in follow up research to this project. It will also take advantage of the accumulated knowledge of predictive modelling.

Function 7) will likely take advantage of existing horticultural applications to be deployed on site using a dedicated PCR machine.

4 Conclusion – future outlook

Considerable progress is needed to obtain a monitoring system as described in the above text that can quasi-autonomously follow up on plant growth, and provide trends and warnings (based on comparison with baseline data and derived scenario's based on predictive mathematical models) to a supervision system (of the MELiSSA Pilot Plant).

The combination of optimised plant growth hardware with a performant logging and monitoring system that keeps track of all parameters should allow swift straightforward diagnosis of occurring problems (hardware or plant linked), timely remediation of the problem, and hence assurance of continuous plant food production that could be integrated into an autonomous bioregenerative life support system, proposed for remote deployment during future long term human space missions..

5 References

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