MELiSSA

Technical Note

Memorandum of Understanding 19071/05/NL/CP

UNIVERSITEIT GENT

FACULTEIT WETENSCHAPPEN

TECHNICAL NOTE: 89.2

REVIEW AND TRADE-OFF OF TECHNOLOGIES FOR PLANT STRESS DETECTION

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reference/*réference*	Contract number 19787/06/NL/PA
issue/*édition*	1
revision/*révision*	0
date of issue/*date d'édition*	17Aug07
status/*état*	Final
Document type/*type dedocument*	Technical Note
Distribution/*distribution*	

CONFIDENTIAL DOCUMENT

APPROVAL

Title *titre*	issue 1 *issue*	revision 0 *revision*

author *auteur*	date 17Aug 07 *date*

approved by *approuvé by*	date *date*

CHANGE LOG

reason for change */raison du changement*	issue/*issue*	revision/*revision*	date/*date*

CHANGE RECORD

Issue: 1 Revision: 0

reason for change/*raison du changement*	page(s)/*page(s)*	paragraph(s)/*paragraph(s)*

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AAS	atomic absorption spectroscopy
ICP-AES	inductively coupled plasma-atomic emission spectrophotometry
EC	electrical conductivity
PCR	Polymerase Chain Reaction

1. Detection of environmental parameters

In horticulture, sensors are used for monitoring of many parameters (e.g. light, temperature, CO_2, O_2, EC, humidity), and data are used for process control. Often, sensors are linked to alarm systems. Exceeding specified upper and lower limits, between which stable yields are guaranteed, then results in an alarm, and feed-back actions are carried out to restore the optimal values of the parameter measured. In some cases sensors may be coupled to "expert systems": the parameter measured, together with other related data may be interpreted, leading to an advice for growers (semi-automatic or supervised control actions). The expertise gained in horticulture can be applied to advanced life support systems.
Abiotic or biotic stresses will exert an effect on the growth or development of the plant, which in general can be measured by detection of an altered plant parameter, e.g. reduced growth, enhanced pigment production, enhanced ethylene production, altered gene expression, etc. Methods to detect these changes in plant parameters will be outlined in this technical note, and their specificity as an indicator of critical stresses will be discussed. However, the real-time monitoring of important environmental factors for plant growth and development, and the direct detection of pathogenic microorganisms (and their removal or in-situ destruction) will be the fastest way of avoiding sub-optimal conditions. In case of equipment malfunction this monitoring can give an indication of expected losses, provided that optimal reference conditions are gathered from the literature or where necessary by additional experimentation. Therefore equipment, to measure these environmental factors is listed, before describing monitoring techniques for the critical stresses ethylene production, lack of calcium and the presence of pathogens.

1.1. Light

Quantum meter:
A quantum meter is required for measuring photosynthetic active radiation (PAR) in the wavelength range of 400-700 nm. As an example a quantum meter from Step Systems is given (http://www.stepsystems.de):
Technical data (Art.-No. 32030, Step Systems) Quantum meter:

Measuring range:	0 - 9.999 µmol s^{-1} m^{-2} = PAR
Accuracy:	± 2% µmol s^{-1} m^{-2} = PAR
Operating temp.:	0 - 50 °C
Output voltage:	0.1 mV per 10 µmol s^{-1} m^{-2}

Dimension: 125 x 70 x 25 mm
Weight: 140 g

Other companies delivering comparable quantum meters are: Skye Instruments (PAR Quantum Sensor SKP 215 or PAR Special SKP 210, both can be logged by connection with the DataHog datalogger; http://www.skyeinstruments.com) and Hoogendoorn automatisering (GROWLAB PAR-sensor; http://www.hoogendoorn.nl/).

1.2. Temperature and humidity

Monitoring air temperature and humidity are standard measures in horticulture and in closed environment plant culture. Available devices have sufficient resolution and their output can be easily logged on a PC. The Helios RHT from Skye Instruments Ltd is an example for a data logger for relative humidity and temperature in required range and with required accuracy (http://www.skyeinstruments.com).

Measuring range: 0%-100 % RH
-55 - +90 °C
Accuracy: 2% RH
≤ 0.2 °C
Dimension: 11 x 42 x 188 mm
Weight: 450 g

Priva (http://www.agro.priva.nl) sells the E-meetbox, which is commonly used in greenhouses for measurements and data logging of temperature, relative humidity and CO_2.

1.3. CO_2 and O_2

Up to four gases, including CO_2 and O_2 can be measured simultaneously online with multi-gas analysers like the MGA3000 from ADC (http://www.adc.co.uk/). For CO_2 measurements non dispersive infrared absorption with a solid state detector is used, while O_2 measurements are done with a paramagnetic detector cell.

Technical data (MGA3000, ADC) multi-gas analyzer:

Measuring range: 0.001%-100% CO_2
0.1% to 25% O_2
Accuracy: ≤ 1% for CO_2
≤ 0.1% O_2
Operating temp.: 0 - 40 °C
Operating humidity: 0-95%
Dimension: 133 x 483 x 500 mm
Weight: 10-23 kg depending on configuration

Individual or multi-gas analyzers suitable for research purposes are also provided by Qubitsystems (http://www.qubitsystems.com), Phytech (http://www.phytech.co.il), Walz (http://ww.walz.com), PPsystems (http://www.ppsystems.com) and Li-Cor Biosciences (http://www.licor.com).

1.4. Oxygen in solution

Vernier (http://www.vernier.com/) delivers an oxygen probe for measurement in solutions. These devices have sufficient accuracy and option for PC connection.
Technical data Dissolved Oxygen Sensor (order code DO-BTA, Vernier):

Range:	0 to 15 mg/L (or ppm)
Accuracy:	+/-0.2 mg/L
Resolution,	0.007 mg/L
Response Time:	95% of final reading in 30 seconds, 98% in 45 seconds

Temperature Compensation: automatic from 5 to 35 deg C
Pressure Compensation: manual, accounted for during calibration
Salinity Compensation: manual, accounted for during calibration

Qubit Systems (http://www.qubitsystems.com) also delivers oxygen probes for dissolved oxygen such as the S121 oxygen probe. The above mentioned probes work with an oxygen electrode. A new technology uses fiber optic oxygen sensors (http://www.oceanoptics.com). These can measure oxygen in air as well as in solution; they do not consume oxygen themselves, are immune to environmental changes in pH, salinity and ionic strength and have a response time (< 1 sec). An optical fiber carries excitation light produced by a blue LED to a thin-film coating at the probe tip. Fluorescence generated (from an excited ruthenium complex trapped in sol-gel material) at the tip is collected by the probe and carried by the optical fiber to a high-sensitivity spectrometer. When oxygen in the gas or liquid sample diffuses into the thin-film coating, it quenches the fluorescence. The degree of quenching correlates to the level of oxygen.

1.5. pH of nutrient solution

The pH value of the nutrient solution is important for uptake of nutrients. It is rather common and available from different companies, e.g. from Priva (www.priva.nl). The pH meter from Priva consists of two parts, the pH electrode and the measuring instrument. The working part of the pH electrode is formed by a small ball of very thin glass. A voltage proportional to the acidity of the solution is generated across this glass. The meter processes the voltage output by the electrode and uses the saved calibration data and the temperature correction to give a pH value. This pH value is shown on the display. It is important to regularly calibrate the sensor to ensure that readings are reliable.

Technical specification pH meter from Priva:

Range:	0-14 pH
Accuracy:	±0.01 pH
Temperature range:	0 - 80°C
Dimensions:	45 x 90 x 155 mm

Other companies providing pH meters are STEP Systems (http://www.stepsystems.com), Nieuwkoop B.V. (http://www.tuinbouwproducten.nl) and Hanna Instruments (http://www.hannainstruments.com).

1.6. EC of nutrient solution

Also the EC meter is a common tool in horticulture and the meter from Priva is given as a good example. The EC meter measures the salt content of fertilizer solutions. EC stands for 'Electrical Conductivity'. The meter measures the total salt concentration. The higher the salt content, the better the solution conducts electrical currents, therefore the higher the EC value. Conductivity also increases as the temperature increases. For this reason the meter also has an automatic temperature correction to ensure that the salt content measurement is correct at any temperature. The accuracy of the meter can be checked using an EC calibration fluid.

Technical specification EC meter Priva:

Range :	0-20 $mS.cm^{-1}$
Accuracy :	± 0.03 $mS.cm^{-1}$ at 20°C
Temperature range :	5 - 35°C
Dimensions :	45 x 90 x 155 mm

Other companies providing EC meters are STEP Systems (http://www.stepsystems.com) and Nieuwkoop B.V. (http://www.tuinbouwproducten.nl).

2. Detection of critical stresses

2.1. Detection of calcium

Destructive calcium (nutrient) determination in tissue or solution:
Elemental analysis in plant tissue is routinely done by reducing plant tissue to ash at high temperature, dissolving the ash and performing ICP-AES (inductively coupled plasma-atomic emission spectroscopy) or atomic absorption spectroscopy (AAS) (Dipietro et al., 1988; Adelantado et al., 1991, Ong, 1992). Calcium determinations in solutions can be performed with the same methods. The approximate optimum calcium concentrations in different parts of a wheat plant are: 1.2, 0.3, 0.1 and 0.2% of total dry weight for leaves, stem, seeds and roots, respectively (http://www.usu.edu/cpl/research_hydroponics3.htm). The limit of detection by AAS for calcium was 100 ng/ ml and the absolute standard deviation was maximal 0.13% (Adelantado, 1991). The typical measurement error associated with the use of ICP-AES for analysis of 1mM calcium concentration in hydroponic solution is 0.2% (which equals an accuracy of 0.002 mM; http://www.usu.edu/cpl/research_hydroponics3.htm).

For future experiments, our samples can be analysed at the Laboratory for Analytical Chemistry and Applied Ecochemistry at Ghent University (http://www.ecochem.ugent.be) and the following equipment is available in this laboratory:

ICP-OES: Inductive coupled plasma optical emission spectrometry – VARIAN Vista MPX with SPS5 Sample Preparation System: Radial ICP for the simultaneous determination of trace elements from levels up to mg/l and down to µg/l
Flame configuration: determination of trace elements down to levels of 0.01 - 0.05 mg/l
Atomic Absorption - VARIAN SpectrAA-10: Atomic absorption with deuterium background correction
Flame configuration: determination of trace elements down to levels of 0.01 - 0.05 mg/l

Calcium can also be measured in solution (from 0 to 2.7 mg L) by using a pocketcolorimeter HI93752 (http://www.hannainstruments.com/), the accuracy is about 5% of the reading. This could be applied to monitor nutrient solutions Ca-levels online.

2.2. Detection of ethylene

Ethylene detection is commonly done by GC measurements after accumulation in a confined space. However, new laser-based photo-acoustic measurements give higher sensitivity and are easier for online measurements. This technique is based on the property of specific gases to absorb certain wavelengths of light in the infrared spectral region, and to transform this energy into kinetic energy resulting in an increase in pressure. When the light source is switched on and off periodically, which is technically obtained with a beam chopper, a pressure wave (sound wave) is generated and detected by a microphone. The most sensitive photo-acoustic detector has a detection limit of around 0.1 ppb, which is about 100 times more sensitive than GC measurement, and a 5-10 sec time response (http://www.sensorsense.nl/).
Ethylene starts to exert growth inhibiting effects in concentrations as little as 10 ppb (Klassen and Bugbee, 2004). Since photo-acoustic measurements have an accuracy of as little as 0.1 ppb they will be adequate. The sufficient accuracy in combination with their short response time also makes them suitable for online measurements.

2.3. Detection of Pathogens

Pathogens can be detected and identified (to a certain extent) by plating on selective media. Molecular techniques however deliver a much faster result and a more accurate identification (Karajeh *et al*., 2006). Detection of *Botrytis* species can be done by PCR based methods after destructive sampling of diseased plant tissue. *Botrytis* species associated with Neck Rot of onion were successfully amplified and separated in five different groups (*B. aclada* types AI and AII; *B. byssoidea*; *B. squamosa* and *B. cinerea*) based a restriction digestion of the PCR product (Nielsen *et al*., 2002).
Fluorescence and confocal microscopy can be applied (after destructive sampling) to visualise plant pathogens and in case of (partial) resistance the plant response (morphological changes or defence compound accumulation) (Scharte *et al*., 2005). Microscopy is useful for localisation and confirmation of pathogen growth but needs destructive sampling and further sample preparation (Caiazzo *et al*. 2006).
Imaging techniques can reveal symptoms due to pathogen-infection both non-destructively and pre-visually; confirmation and identification needs to be based on a follow-up targeted-

sampling and analysis as described above. Efficient stress detection will be highly dependent on software for semi-automated image processing. Early stress-detection can be based on visual assessment of images or image time-series by an experienced operator. Software that can detect heterogeneity in plant canopies can further

3. Detection of plant growth parameters

3.1. Destructive analyses

At set time intervals whole plants can be harvested. Fresh weight can be determined directly after harvesting, from stem, leaves and roots on a precision balance (e.g. PG8001-S, Mettler Toledo, Columbus, OH, accuracy is 0.08g). The leaf area index (LAI = ratio of total leaf area to cultivated surface area) can be determined by making scans of detached leaves and determining the area with an image processing software package (e.g. Image J, http://rsb.info.nih.gov/ij/), or a dedicated apparatus can be used such as the AM300 portable leaf area meter from ADC (http://www.adc.co.uk/index.php) or the LI-3000C portable area meter from Li-Cor (http://www.licor.com).
These leaf area meters can be used by an operator for non-destructive measurements in the growth chamber. The material can then be dried for dry weight determination after a certain drying period; 75 °C for 48 h (Johnstone *et al.*, 2005) or 100-105 °C for 16 h (http://www.biosci.ohio-state.edu/~plantbio/Facilities/abrc/handling.htm). Before weighing the samples need to be cooled down for a short time (30 min) in a vacuum desiccation before weighing to prevent re-adsorption of moisture. Specific leaf area (SLA) is obtained by dividing leaf area by the dry weight of the same leaf area (Garnier et al., 2001).
Concentrations of various compounds in the plant correlate with its health and growth potential. Chlorophyll content determines to a large extent the capacity for photosynthesis, and thus the achievable final yield. Compound quantification after chemical extraction however is laborious; however for several key components alternative sensing methods are available that are labor-saving, and moreover allow repeated measurements without sampling. Importantly, determination by extraction remains valuable and necessary for initial calibration purposes.

3.2. Non-destructive analyses

The approaches described below all depend on image processing and interpretation. Either the software is custom-built using one of the many available programming languages and environments combined with image processing libraries, or specialized image processing applications are used that usually provide macro language support (. For a non-exhaustive overview see http://image.nih.gov/software/ip_packages.html.
Established tools include:
The Mathworks Matlab + Imaging toolbox (http://www.mathworks.com/products/image/)
NIH ImageJ (rsb.info.nih.gov/ij/)

National instruments Labview + IMAQ-Vision (http://www.ni.com/vision/)
Accusoft Visiquest (formerly Khoros http://image.nih.gov/software/ip_packages.html)
Dalsa Coreco WiT http://www.wit-igraph.com/default.htm
The latter three allow visual programming by connecting glyphs that represent IP operations.

3.2.1. Plant dimensions

Conventional RGB (visible spectrum) reflectance imaging is a powerful tool for continuous monitoring of growth (Walter and Schurr, 2005). However, the 3rd dimension of the crop canopy will have to be taken into account to enable accurate quantification of canopy growth (Kaminuma *et al.*, 2004). Based on such data, predictive modeling of yield should become possible.
Alterations in optimal environment or stresses cause short term changes in individual plant organ growth rates, which can be measured, e.g. as relative growth rate (increase in area per unit area per time; Schurr *et al.*, 2006). Measurements involve digital image sequence processing to study growth of leaves (Schmundt *et al.*, 1998) and roots (Walter *et al.*, 2002). The respective organs are supported for proper orientation to a CCD camera, making the method only doable for a small set of organs within a canopy, possibly by using a few 'reference' plants.

3.2.2. Agronomic performance parameters

Agronomic performance parameters (APP, Anderson et al., 2005) are amenable to non-destructive measurement (e.g. plant height, fresh weight) although an accurate determination of leaf area index on a plant canopy is technically challenging (see above for contact or destructive determination).

LAI of canopy:
Leaf Area Index (LAI) is the ratio of the foliage area to the cultivated surface area. The measurement of LAI is of fundamental importance in agricultural and ecological research because LAI is a measure of plant growth; it directly affects the interception and absorption of light by the canopy and it influences the heat balance and evaporation from the cultivated substrate. Li-Cor delivers the LAI-2000 Plant Canopy Analyzer (http://www.licor.com) for direct, rapid, non-destructive LAI measurements.
The LAI-2000 provides on-site evaluation of LAI data, and can be used for short or tall canopies (i.e. grass to forest), or row crop canopies, it calculates foliage inclination angle and other relevant parameters. The LAI-2000 calculates Leaf Area Index (LAI) and other canopy structure attributes from radiation measurements made with a "fish-eye" optical sensor (148° field-of-view). Measurements made above and below the canopy are used to determine canopy light interception at 5 angles, from which LAI is computed using a model of radiation transfer in vegetative canopies.

3.2.3. Chlorophyll content measurement

Handheld chlorophyll meters are an easy way to non-destructively estimate chlorophyll content of leaves. Examples are the Opti-Sciences (http://www.optisci.com/) CCM-200

Chlorophyll Meter and the Minolta SPAD (soil plant analysis development) 502 Chlorophyll Meter (http://www.specmeters.com/Chlorophyll_Meters/Minolta_SPAD_502_Meter.html). Both chlorophyll meters measure ratios of radiation transmitted through the leaf at two wavelengths. The SPAD measures the RVI (Ratio Vegetative Index) at 940 nm and 650 nm while the CCM-200 uses 940 nm and 660 nm. The leaf area measured is 0.06 cm^2 by the SPAD 502 and 0.71 cm^2 by the CCM-200. Another alternative is the CL-01 - Chlorophyll Content Meter measuring at 940nm and 620 nm (http://www.hansatech-instruments.com/).

3.2.4. Chlorophyll fluorescence emission

A plant leaf only partially absorbs the incident visible light energy, which is partially reflected and transmitted. The light energy absorbed by chlorophyll is either used for photosynthetic assimilation, or is dissipated as thermal energy and for a small fraction (max 2%) as light (chlorophyll fluorescence, Krause and Weis 1991).
Chlorophyll fluorescence can be measured non-destructively using leaf-clip devices, with either stand-alone devices (Hansatech plant-efficiency analyzer PEA (http://www.hansatech-instruments.com), and Walz pulse amplitude modulated PAM fluorescence meter (http://ww.walz.com)), or in combination with gas-exchange equipment (see above paragraph 1.3, e.g. PPSystems CIRAS, Licor Li-6400). The measuring areas need to be dark-adapted before carrying out the chlorophyll fluorescence measurement. The PAM technique can be used under growing conditions since the short periodic pulses of measuring light can be separated from the resulting fluorescence light (Lenk et al. 2007), and are measured as an increase compared to the ambient light. These PEA and PAM measuring devices are only suitable for manual point measurements of an area of approximately 0.5cm^2, and preclude any automatisation. Measurments of chlorophyll fluorescence using a spectrometer can also be carried out under growth chamber light conditions (Norikane et al., 2003)
Imaging of chlorophyll fluorescence has been applied to detect bacterial, viral, fungal, and oömycete infections before visual symptoms became apparent (Berger et al., 2004, Chaerle *et al*., 2004 and 2006, Oerke et al., 2006, Scharte et al., 2005). In addition this technique could also reveal abiotic stresses at the early pre-visual stages (Buschmann *et al*., 2000). Pixel resolution is at least 640x480, imaging rates are determined by the used cameras. Fluorescence imaging has a sub-cellular resolution, and can be used at the microscopic scale (Oxborough, 2004).

3.2.5. Leaf temperature measurement

Plants leaves have a hydrophobic surface (cuticula) which has a very low transpirational water loss. To further avoid excessive water loss plants have evolved turgor-controllable microscopical valves that close upon early signals of drought stress and in the absence of light, when no CO_2 uptake is needed. In addition, cuticula impermeability and active stomatal control can avoid pathogen ingress (Melotto et al. 2006). The regulation of stomatal conductance by CO_2 availability can also be revealed efficiently by thermography (Hashimoto *et al.*, 2006). Stomatal closure causes transpiration to cease and hence leaf temperature increases. Leaf temperature can be measured by thermocouples and radiometers, but thermal cameras have the important benefit of visualising plant heterogeneity (Jones, 1994). The level

of stomatal opening can be derived from the thermal measurements, if the necessary calibrations are performed (Leinonen et al., 2006). From the captured images, plants can be discerned selectively from the background, using complementary video imaging (Leinonen and Jones, 2004). There are radiometric and non-radiometric IR cameras. Radiometric IR cameras provide a temperature reading via software, have a detector which is well insulated from the environment (bulky isolating casing) and include internal calibration sources. The pixel resolution is 320x240 and the temperature resolution is better than 0.1°C, which is sufficient for plant-physiological applications. Examples are the JadeUC from CEDIP (http://www.cedip-infrared.com/instrumentation/produit_detail.php?id=C0302.J300), the TH7800 from NEC (http://www.necsan-ei.co.jp/osd/thermography/thermography_f.htm) and the A20 and A40 from the A-series, and the E-series from FLIR (http://www.flirthermography.com). Regarding measuring conditions, growth rooms with limited T-fluctuations and constant airflow/mixing are needed for stable measurements.
Non-radiometric cameras need an external calibration source and dedicated software, but are miniature and weight and space-efficient. An example is the A10 from FLIR (http://www.flirthermography.com/cameras/camera/1043).

3.2.6. Multispectral and hyperspectral imaging

Ultraviolet light excited fluorescence detection has the potential for non-destructive quantification (and possibly identification) of individual substances (Lenk et al. 2007). Fluorescence after UV-excitation can be measured in at least 4 different wavelength bands: blue, green, red and far–red, the last two corresponding to chlorophyll fluorescence (Lichtenthaler at al., 2005). New types of diodes for illumination with higher intensity and in a wider spectral range (from UV-C to infrared) will help to improve the signal detection (e.g. Khan, 2006). Combination of UV-induced fluorescence and hyperspectral imaging has already shown promise for in-field detection of plant diseases (Moshou *et al.*, 2005).

Leaf pigment content and their relative concentrations change when plants are exposed to a range of environmental stresses. Such changes can be detected by reflectance measurements, using spectrometers and imaging techniques.
Hyperspectral reflectance imaging provides information from multiple narrow wavelength zones [typically of a few (tens of) nanometers], the combination of which can be indicative of specific stresses (e.g. revealing the accumulation of particular compounds; Muhammed, 2005; Moshou *et al.*, 2005). Hyperspectral imaging systems are based on fast responding liquid crystal (Saito et al. 2005) or acousto-optic filters (Vila-Frances et al., 2006), and might in the future enable real-time imaging of fluorescence spectra for each pixel covering a leaf area. At least one system is commercially available (http://www.cri-inc.com - Nuance system).

Multispectral camera's image in 3 broader wavelength bands: the green red and near-infrared channels (Noh et al. 2006) Systems are available from http://www.terraverdetech.com/ and http://www.specim.fi.

3.2.7. Other imaging and spectral techniques

Autoluminescence imaging or biophoton imaging, which captures the spontaneous ultra weak emission of photons typically associated with oxidative stress reactions (Havaux et al., 2006) has been shown to discriminate the hypersensitive disease reaction (indicating plant resistance) from a lesions caused by attack of a virulent pathogen (Bennett *et al.*, 2005). A drawback is that this technique needs to be carried out in a dark cabinet.
Nuclear magnetic resonance imaging is not considered since it needs a bulky infrastructure (Windt et al., 2006). The same constraint applies to polarography (Savenkov et al., 2004).

4. Trade off

The suitability of using the abovementioned techniques in a semiautomatic monitoring system was evaluated using a trade off with 10 parameters (see Table 1)

4.1. Accuracy

For the considered techniques accuracy is independent of the developmental stage of the crop.
After canopy closure, intra-canopy (automated) air sampling for ethylene measurement can be considered.
Quantification and processing of imaging data will need to take into account the growth of the crop, by using distance ranging (infrared or ultrasonic) to determine crop height and adjust imaging distance accordingly.

4.2. The time of detection

The environmental factors can be measured on-line in nearly real time, and immediate corrective actions can be taken to avoid a stressing environment.
The critical stress ethylene can be detected on-line and this info can be used to adjust ethylene removal efficiency.
The critical stress pathogen presence can be monitored by imaging techniques at an early stage. Although the exact cause of the observed local deviations cannot be derived from these measurements, the early warning info can be used to remove the affected plant part and to sample it for determination of the causal agent by e.g. PCR.
The critical stress lack of calcium can be minimized by monitoring calcium levels in the feeding solution. Preventive sampling would be needed to assess tissue level calcium. Imaging will likely detect changes at the leaf surface, but only after verification of the existence of a specific signature will allow to discriminate it from other stresses.
Plant biomass and plant morphology monitoring will allow detection of deviations from optimal growth when baseline data on growth in optimized conditions are available.

4.3. The duration of analysis

From the above point it follows that detection and analysis of the environmental factors, ethylene and calcium in solution are real-time.

The efficiency of detection of local plant stress responses depends on the development and optimization of an analyzing software module that detects such alterations autonomously and issues a warning, thereby saving operator time. This approach does not require operator presence in the growing room, remote assessment of the image sequences suffices.
Destructive analysis needs operator presence for sampling and further operator time for performing the actual analysis.

4.4. The procedures for data collection, handling and processing

The real-time monitoring sensors have a low complexity and time usage level.
Ethylene detection at the growth chamber level (continuous air sampling from a ventilation duct) has the same characteristics. Setting up a dual sampling capability intra-canopy at several locations will increase complexity but has the potential of being fully automatic.
Destructive sampling and weighing is simple, needs operator and extensive drying time, hence its medium classification.
The procedures for imaging are termed highly complex since they depend on robotization and automated image processing, and a final operator decision step. However, this can be automated with exception of the last step.
Destructive determination of calcium, PCR and microscopy depend on multiple operator-performed steps and will be difficult or impossible to automate.

4.5. The possibility of automation

For destructive analysis automation could be achieved by robotizing the sampling procedures, which implies imaging capability.
Whether the sample preparation and the analysis can be automated without a high mass and energy penalty should be issue to a trade-off.
Stress detection by imaging over the whole plant growth area can be automated by using robot technology. However customized pattern recognition software routines and an expert system that issues warnings need to be implemented for autonomous functioning.

4.6. The mass, volume and energy consumption of the detection system

The sensors for real-time measurements will on average weigh less than 500g each (low category).
Gas analyzers for CO_2 and ethylene are in the medium range (specifications in above section).
Destructive calcium determination and (confocal) microscopy need extensive hardware and are thus qualified as ‘high’. Imaging sensors get more and more miniaturized (medium category), thus also the supporting robotized equipment can be dimensioned smaller.

4.7. The required maintenance time and procedures of the detection system

Equipment with moving parts such as gas detectors are placed in the medium category, as are imaging and microscopy with more extensive electronics than the real time sensors (low).

4.8. Integration in the HPC alarm system

- The real-time sensors are the first level of alarm signals at chamber to plant level.
- Imaging based stress detection is proposed for alarms at the plant or leaf level.
- PCR and microscopy are needed for stress characterization.
- Destructive calcium determination can indicate a deficiency which has to be correlated with imaging results to realize the possibility an on-line calcium lack alarm.
- Destructive weighing is needed as a calibration for imaging based plant biomass estimation.
- This estimation as function of time can be applied as a third level of alarm for detection of growth retardation.

4.9. The technical readiness level

Real time environmental sensors are in extensive use in the horticultural sector. Gas analyzers for CO_2 and O_2 are widely used in research. Photo acoustic ethylene detection equipment was recently commercialized and proved reliable during a test experiments.
Imaging sensors are reliable, as are the robot systems that can implement sensor mobility in a growth chamber. However the stress detection and decision support software systems need to be further developed.

4.10. The space adaptability

For the on-line sensors no implementation problems are foreseen. The sensors that involve diffusion (e.g. dissolved oxygen) would need further research, but this is outside the scope of this project. Imaging sensors likely only need minor modifications, but this is not the subject of this research.

Table 1: detection technique trade off

Measure	Technique	Accuracy (error)	Detection time	Duration of analyses	Complexity of procedures	Possibility of Automation	Mass, volume, energy	Main-tenance	Technical readiness
Light	Quantum meter	± 2% µmol/s.m^2 sufficient	sec	sec	low	yes	low	low	ready
Temperature	Thermocouple	≤ 0.2 °C sufficient	sec	sec	low	yes	low	low	ready
CO_2	Infra-red gas analyser	≤ 1% sufficient	sec	sec	low	yes	medium	medium	ready
Humidity	Hygrometer	2% RH sufficient	sec	sec	low	yes	low	low	ready
Dissolved oxygen	Oxygen electrode	0.2 mg/L sufficient	< min	< min	low	yes	low	medium	ready
	Fiberoptic oxygen probe	sufficient	< min	< min	low	yes	low		Needs testing
Calcium	ICP-AES/ AAS	0.2% sufficient	min	min (in solution) h (in tissue)	complex sampling	complex operator	high	high	ready
	Colorimeter	5% sufficient	min	min (in solution)	low	possible	low	low	ready
	Imaging	Accurate but not specific	min	days	high	complex	medium	medium	Analysis to be further developed
Ethylene	Acoustic laser	1ppb sufficient	< min	< min	Low on chamber level	yes	medium	medium	ready
Pathogen	PCR	accurate	h	h	high sampling	Complex operator	medium	medium	ready
	microscopy	accurate	h	h	High sampling	No operator	high		ready
	Imaging	Accurate but not specific	min	days	high	complex	medium	medium	Analysis to be further developed
Plant biomass	Destructive weighing	accurate	min	Days (drying)	Medium sampling	No operator	medium	low	ready
	Imaging	Estimation	min	days	high	complex	medium	medium	Analysis to be further developed
Plant morphology	Imaging	Estimation	min	days	high	complex	medium	medium	Analysis to be further developed

5. References

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