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**Technical Note**

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## PSDU CONCEPTUAL DESIGN

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| prepared by/*préparé par* | D. Hagenbeek, L. Chaerle, D. Van Der Straeten |
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# Table Of Contents

## List of Tables



## List of Images

# 1. Introduction

As a general framework, the task to be performed by a PSDU will be to monitor all plants within a higher plant growth chamber (HPC) at regular intervals and with one or more sensors, adopted to detect stress or growth inhibition at an early stage. The conceptual design of the PSDU will be based on the conclusions drawn in the previous TNs. Based on TN89.2 it will be important to operate the PSDU in a fully controlled HPC, in which environmental factors are controlled and logged, preferably a continuous basis and without intervention of an operator. Based on TN89.3 certain environmental parameters will need to be logged and biomass accumulation during and at the end of growth determined, as input for the mathematical model. The major stresses indicated in TN 89.2 each have specific detection requirements. In this conceptual design we will cover the general stress detection techniques and the specific techniques required in addition for the two stresses that will be tested: Pythium infection and ethylene accumulation.

# 2. Higher Plant Chamber

To be able to develop a reliable predictive plant model, including effects of a certain stress on crop growth, it will be important to work in a fully controlled environment. Conditions should be kept constant except for the stress to be tested. However, just controlling the environmental factors and assuming they are constant at all times during a test is not sufficient. Actual environmental conditions will need to be logged with independent sensors to be able to know the amplitude and error margins of set factors. Temperature control for example might be quite constant during the night and during the day, but with on and off switching of lights, temperatures will fluctuate more. Moreover, possible equipment failure will not pass undetected using such setup.

## 2.1. Even temperature, humidity, air quality and air flow

To have a HPC with an as much as possible homogeneous environment in terms of temperature, humidity, air composition, etc, it will be important to have an even airflow through the system and to keep the difference between input and output air temperature minimal. To generate this environment, a large laminar flow will be needed. This means a large volume throughput with a large perforated surface (e.g. one whole wall of the chamber) for air input as well as exit. In this way the volume throughput is high but the air speed in the chamber will be low (adjustable, max. 1m/s) and homogeneous throughout the chamber. This in turn will generate an even climate in the chamber and will avoid excessive air-induced movements of the canopy. The latter prevents mechanical stress to plants and causes less problems monitoring plants, e.g. with imaging techniques. The air can be re-circulated to a large extent, but part of it will need to be refreshed to replenish $CO_2$ and to prevent accumulation of toxic factors, e.g. ethylene. To be able to measure $CO_2$ assimilation, $O_2$ and ethylene production rates it will be important to know the exact amounts of refreshed air.

A climate chamber with the above mentioned requirements, and a temperature accuracy of 0.2 °C for settings in the range of 20 to 30 °C, controllable relative humidity in the range of 60 to 80 % and 'effective' chamber dimensions of 3m length x 1.5m width x 2m height was previously constructed by Witdouck Energietechniek bvba (Kortrijk-Bissegem, Belgium; http://www.witdouck.be; website only available in Dutch and French). The available set-up includes the following.

Equipment:

-Controller RMU730 (http://www.siemens.com/index.jsp)

-Air ventilation; Variable Frequency Drive Hitachi L200 075HFEF (http://www.hitachi-ies.co.jp/pdf/catalog/SM-E242R.pdf).

-Steam humidifier Carel UR010HL001 (http://www.carel.com/carelcom/web/eng/catalogo/prodotto_dett.jsp?id_gamma=34&id_tipologia=9&id_prodotto=67)

-Chilled water primary circuit Chiller Daikin EUWA5KZW1 (http://global.daikin.com/global/our_product)

-Perforated walls from RMIG (http://www.rmig.com/):

| | |
|---|---|
| Material: | stainless steel |
| Diameter perforation: | 5 mm |
| Distance between perforations: | 8 mm (centre to centre) |
| Distance to real walls: | 0.24 m |
| Dimensions perforated wall input: | 3 x 2 m (effective surface) |
| Dimensions perforated wall output: | 2 x 2 m (effective surface) |

Settings and errors:

| | |
|---|---|
| Temperature range: | 20 to 30 °C |
| Error temperature: | ± 0.2 °C |
| Humidity range: | 60 to 80 % RH |
| Error humidity: | ± 5 % RH |
| Fresh air supply: | 0 to 2 $m^3 h^{-1}$ |
| Air circulation: | 3000 to 8000 $m^3 h^{-1}$ |

## 2.2.Light

The artificial lighting systems should provide stable light output with spectral properties suitable for plants (sufficient red and blue light), with sufficient intensity and preferably adjustable. For this purpose fluorescent lamps are the best choice. They are limiting in intensity output compared to metal halide lamps and therefore not suitable for maximum growth of all crops but intensity is easily adjustable and this characteristic might be more important for a test facility than maximum light output. The mounting of the lamps will be as close as possible to maximize PAR, which is not straightforward for metal halide lamps. The Master TL-D reflex Super 80 58W / 840 from Philips (http://www.philips.be) is an energy efficient lamp with proper spectral properties for plant growth and long lifetime (20000 h with 10% reduced output over that period). The following light mounting frame with dimmer was used: cat. No. EURO-FFL158 (Fluolite, Tettnang, Germany), IP 65 (dust-tight and protected against water jets).

### 2.3.Hydroponics

For advanced life support systems hydroponic systems are generally considered as the standard choice. It makes it easier to optimise supply of water and minerals since soil properties are not a concern anymore. Absence of soil also reduces the risk for soil-bound pathogen contamination. For a test facility, hydroponics is also a good choice because it allows reliable testing of (individual) nutrient composition. NFT (nutrient film techniques) systems are used in the research facilities of Guelph (Controlled Environment Systems Research Facility, Ontario Agricultural College, University of Guelph, Guelph, Ontario, Canada) as well as in commercial lettuce hydroponic systems (personal communication Belgian Research Centres). Hortiplan (http://www.hortiplan.com) delivers mobile gully systems for optimized lettuce production with NFT. Besides the gullies in which the plants grow with their roots in a thin (few millimetre) film of nutrient solution, a reservoir for the nutrient solution and a pump for recirculation is required.

## 3. Sensors

Based on the conclusions in technical note 89.3 the following parameters will need to be logged on regular time intervals to be able to test the mathematical model: $O_2$, $CO_2$, light quantity, temperature and humidity. For additional possible parameters for the mathematical model and for future reference it will also be advisable to monitor and preferably log the temperature of the nutrient solution, the oxygen in solution, the individual nutrient levels, the EC and pH of the nutrient solution and the levels of ethylene in the surrounding air. Except for the nutrients these parameters can be logged online. For calcium an individual probe that can be logged should be considered. Remaining nutrients are best checked by weekly sampling and consecutive analyses in a dedicated laboratory by ICP-AES (inductively coupled plasma-atomic emission spectroscopy) or atomic absorption spectroscopy (AAS). At the same time plant samples can be taken for analysis on nutrient element composition, by the same laboratory.
The following sensors are suggested for online measurement of the respective parameters (Table 1).

**Table 1: Sensors of choice for online measurement of required environmental parameters**

| Parameter | Sensor |
|---|---|
| **In air:** | |
| $O_2$ | MGA3000 from ADC |
| $CO_2$ | MGA3000 from ADC |
| Light | Quantum Sensor SKP 215 and DataHog data logger from Skye Instruments |
| Temperature | Vaisala Humicap® |
| Humidity | Vaisala Humicap® |

| | |
|---|---|
| Ethylene | ETD-300 from Sensor Sense |
| **In solution:** | |
| Temperature | HI-9141 dissolved oxygen meter (Hanna Instruments) |
| $O_2$ | HI-9141 dissolved oxygen meter (Hanna Instruments) |
| Calcium* | HI-93720 hardness (Calcium) meter (Hanna Instruments) |
| EC | S125 Conductivity Sensor (Qubit Systems) |
| pH | S165 pH sensor (Qubit Systems) |

* manual measurement and data transfer

## 3.1. $O_2$ and $CO_2$ in air

For online measurements of $O_2$ and $CO_2$ the MGA3000 multi-gas analyser from ADC is suggested. 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.

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%
Data signal output: RS-232C

## 3.2. Light quantity

For online measurement of light quantity, the Quantum Sensor SKP 215 and DataHog2 data logger (SDS5050, 1 channel) from Skye Instruments are a good choice (http://www.skyeinstruments.com/products.htm).

Measurement range: 0 to $5x10^4$ µmol $m^{-2}$ $s^{-1}$
Accuracy:
Linearity error < 0.2 %
Absolute calibration error: typically < 3 %, 5 % max
Temperature coefficient: ± 0.1 %/ °C
Long term stability: max 2 %/ year
Operating environment:
Temperature: -35 to +75 °C
Humidity: 0-100%
Data signal output: RS-232

Calibration check recommended at least every two years.

## 3.3. Air temperature and relative humidity

For online measurement of air temperature and relative humidity, the Vaisala Humicap® temperature and humidity transmitter (HMT333; http://www.vaisala.com/businessareas/instruments/products/humidity/fixed/hmt330) is suggested.

Technical specifications for relative humidity:

| | |
|---|---|
| Measurement range: | 0-100 % RH |
| Accuracy at +20 °C: | ± 1.0 % RH (0-90 % RH) |
| | ± 1.7 % RH (90-100 % RH) |
| Sensor: | Vaisala Humicap® 180 |

Technical specifications for temperature:

| | |
|---|---|
| Measurement range: | -40 to+80 °C |
| Accuracy at +20 °C: | ± 0.2 °C |
| Sensor: | PT100 RTD 1/3 Class B IEC 751 |

Operating environment:

| | |
|---|---|
| Temp. for probe: | Measurement range |
| Temp. for transmitter body: | -40 to +60 °C |
| Pressure: | Vapor tight |

| | |
|---|---|
| Data signal output: | RS-232 |
| Controllable from: | LabView |

## 3.4. Ethylene

For online ethylene measurements the photo-acoustic laser PTD-300 from Sensor Sense (http://www.sensor-sense.nl) is suggested. The specifications are given below.

| | |
|---|---|
| Detection range: | 0.3 to 5000 ppbv |
| Accuracy: | < 1% of measured value or 0.1 ppbv, whichever is larger. |
| Measuring time: | 4 seconds |
| Gas flow rate: | 1 - 5 liter per hour (through instrument) |
| Operating temperature: | 10 to 28 °C |
| Data signal output: | RS232 and USB |
| Controllable from: | LabView |
| Software: | MS Windows compatible. |

Calibration can be performed using a calibrated gas mixture, this is recommended annually by the company.

## 3.5. Dissolved $O_2$

For online measurement of dissolved oxygen and temperature the HI-9141 portable dissolved oxygen meter from Hanna Instruments (http://www.hannainst.co.uk/acatalog/HI91410.pdf) appears adequate.

| | |
|---|---|
| Range: | 0.00- 19.99 mg $L^{-1}$ $O_2$ |
| | 0.0 to 50.0 °C |
| Resolution: | 0.01 mg $L^{-1}$ $O_2$ |
| | 0.1 °C |
| Accuracy (at 20°C): | ± 1.5% |
| | ± 0.5 °C |
| Data signal output: | RS232 |

Automatic temperature compensation (in the range of 0 to 30 °C)

Salinity compensation (in the range of 0 to 40 g $L^{-1}$)

## 3.6.Calcium in solution

For manual measurements of Calcium levels in nutrient solution the pocket colorimeter HI93752 from Hanna Instruments (http://www.hannainstruments.com/) seems an appropriate choice.

| | |
|---|---|
| Range: | 0.00 to 2.70 mg $L^{-1}$ |
| Resolution: | 0.01 mg $L^{-1}$ |
| Accuracy (at 20°C): | ± 0.11 mg $L^{-1}$, ± 5 % of reading |
| Method: | Calmagite method. The reaction between Calcium and the reagents causes a red tint in the sample |

## 3.7.EC

For online measurements of the EC of the nutrient solution the S125 Conductivity Sensor from Qubit Systems (http://www.qubitsystems.com) is suggested.
The Conductivity Sensor comes with a Sodium Chloride Calibration Standard (equivalent to 1000 uS/cm, 491 mg/L as NaCl, or 500 mg/L TDS) and the Conductivity Sensor Manual.

| | | |
|---|---|---|
| Range: | Low Range: | 0 to 200 µS/cm |
| | Mid Range: | 0 to 2000 µS/cm |
| | High Range: | 0 to 20,000 µS/cm |
| Resolution: | Low Range: | 0.1 µS/cm |
| | Mid Range: | 1 µS/cm |
| | High Range: | 10 µS/cm |
| Accuracy: | +/- 1% of full-scale reading for each range | |
| Response time: | 98% of full-scale reading in 5 sec., 100% of full-scale in 15 sec | |
| Operation range: | 0 to 80 °C | |
| Compensation: | automatic from 5 to 35 °C | |
| Data transfer: | C901 Logger Pro Software | |
| Data analysis: | C410 LabPro Interface | |

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## 3.8.pH

For online measurements of the pH of the nutrient solution the S165 pH sensor from Qubit Systems (http://www.qubitsystems.com) is suggested. Specifications for the pH Electrode include:

| | |
|---|---|
| Range: | 5 to 80 °C |
| Resolution: | 12 bit resolution, 0.005 pH units |
| Response time: | about 1sec |
| Operation range pH: | 0 to 14 |
| Data transfer: | C901 Logger Pro Software |
| Data analysis: | C410 LabPro Interface |

The pH sensor is factory-calibrated. If re-calibration is required, it is a simple two point procedure using buffer solutions.

# 4. Plant and canopy analysis

## 4.1.Destructive analyses

All the stresses indicated in TN89.12 have repercussions on biomass accumulation and therefore techniques measuring changes in biomass should certainly be included in a PSDU.
For a final PSDU, the purpose is to develop non evasive techniques that can measure predictive plant parameters online. However to develop and test these it will be necessary to take destructive plant samples at set time intervals to reliably check crop growth curves (especially dry weight) and nutrient content. For destructive sampling, the required crop area for testing will increase quickly with decreasing time intervals. If sampling is done within one chamber, crop reduction during the growth periods also needs to be taken into account for the modelling.
Depending on the crop and stress to be tested the interval times for destructive sampling will need to be determined. At every harvest the following parameters need to be determined:

-Whole plant fresh weight
-Fresh weight of roots, stem and leaves
-LAI
-Dry weight of roots, stem and leaves

Instrumentation needed is a precise balance, an oven, which is standard laboratory equipment, and a leaf area meter.
**LAI meter** LI-3100C area meter from Li-Cor (http://www.licor.com)

- Measures Area, Length, Maximum Width and Average Width
- Fast and continuous operation for large quantities of samples with individual or cumulative area recorded
- Scanning area: 25 cm wide and 2.5 cm thick
- Resolution 1 $mm^2$
- Accuracy ± 2.0 %
- Quiet belt system with adjustable press roller to flatten curled leaves
- LED display
- Data output via RS-232 or USB
- MSWindows® software compatible

When the plant pathogen *Pythium* is applied as stress, both the increase in plant biomass and the progression and accumulation of the pathogen in the plant needs to be determined at set time intervals. The progression of *Pythium* growth in the plant can be assessed by (fluorescent) light microscopy. This will require a high quality (fluorescent) light microscope, as for example the Axiovert 200 from Zeiss (http://www.zeiss.com/micro). The fluorescent light microscope will be useful in general for analyses and confirmation of stress symptoms at the tissue and cellular level, e.g. collapse of cells and production of fluorescent secondary compounds.

Quantization of *Pythium* levels is commonly done by polymerase chain reaction (Wang *et al*., 2003: Kong *et al*., 2004: Bala *et al*., 2005). The required specific primers can be found in these references.

## 4.2.Non-destructive analyses

Video imaging will be a corner stone of a plant stress detection system not only because it can serve multiple purposes, but also given its resolution at the single leaf level. Images gathered as a function of time can be accessed from a central storage place, but remote from the actual growth chamber, which will prevent disturbance of the chamber environment. The images can be used for direct visualization and assessment of the health status of a crop. With image analyses routines automatic stress detection can be developed. The imaging can also be used as a reference for directing equipment, e.g. other sensors or harvest robots. With time series they also can be used to follow developmental stages of crops and to follow quantitative traits of plant growth, e.g. projected leaf area. In the presence of stress, projected leaf area will change and can serve as an indicator. Video image analyses could also serve as input for crop volume calculations and for visualization of development of crop volume in time, but the third dimension (height) will need to be incorporated. The third dimension can be incorporated by an ultrasonic sensor, which measures the top of the canopy from a set point. For basic imaging, a video camera and an ultrasonic sensor will be needed. Both camera and sensor will need to monitor different plants or parts of the canopy. Therefore the plants will need to be moved to a fixed measurement set-up or the sensors will need to be robot-guided over the plants. The latter option will not disturb the canopy and therefore be preferable. Thus, a robotic frame will be needed. Other sensors and imaging equipment mentioned further in this TN can also be positioned by such a robot.

**Ultrasonic sensor** 18GM Shorty (Pepperl and Fuchs GmbH, Mannheim, Germany; www.pepperl-fuchs.com).

Range: 30 to 300 mm
Output: analogue 0 to 10 V
Compensation for temperature

Another interesting application to use this sensor for (besides measuring the height of the canopy) is as a flooding-protection switch for fluid levels in nutrient reservoirs and plant growing gullies.

**Video camera** BCi5 CMOS camera (http://www.vector-international.be). This camera uses a CMOS (complementary metal oxide semiconductor) pixel sensor for detection instead of a CCD (charge coupled device) and is therefore preferable. Two important characteristics of CMOS devices are high noise immunity and low static power supply drain. Specifications:

- 1280 x 1024 pixels (H x V)
- Synchronous shutter
- Color
- 12 bit digital output
- Serial LVDS, USB 2.0, IEEE-1394 or Camera Link interface

A relatively cheap and continuous way to monitor crop growth and transpiration might be achievable by placing plots of plants on a balance. Unexpected changes in daily growth or transpiration will indicate stress and require action. For tomatoes this method has advanced till a practically applicable stage (Helmer *et al*., 2005; http://res2.agr.ca/parc-crapac/fs-ft/fsft002_e.htm). In this system a lower load cell (on which the substrate is resting) and an upper load cell (to which the plant shoot is suspended) are used to monitor water use and shoot biomass accumulation, respectively. In case of determinate crops, which will be the choice for ALS systems (because they are more compact and require less manual labor), only a lower load cell can be used. Granier *et al*. (2006) used one balance which was transported by a robot to measure individual *Arabidopsis* plants automatically at set time intervals; transpiration rates could be determined from the gathered data. For larger crop plants a fixed set-up for the balances might be more practical and in combination with controlled NFT this should be sufficient to monitor crop growth (fresh weight increase) and transpiration rate. Besides weighing a gully holding several plants the difference between nutrient solution supply and re-circulated nutrient solution should be determined to know the transpiration rate of the plants. This could be done by weighing the recirculation tank separately. Determining the flow in and out of the gully might help to improve the accuracy of the measurements. Since the humidity in the climate chamber is strictly controlled, the transpiration of the plants can also be determined at the place where it condenses, by volume (in a measurement cylinder) or weight (balance). Weighing a gully system in the absence of plants can be used as control for non-plant related evaporation. The gullies will be maximally 1.5 m long. With lettuce as test crop, such gully can hold 7 plants. The balance will need to hold about 5 kg max (the weight of the lettuce plants plus the gully and nutrient solution) and will need to be programmable for measurements at set time intervals. Since the gullies are rather long two balances might be needed per gully for accurate measurements and for stability of the whole set up. The accuracy needed will be about 0.1g. Sartorius AG (http://www.sartorius.com) delivers suitable balances. For the workbench demonstrator design, a final choice will be made for the number and type of balances.

Leaf temperatures will be monitored by a thermal camera. Based on leaf temperature, stomatal conductance can be derived (Chaerle *et al*., 2005; Omasa and Takayama, 2003) and daily transpiration estimated. Heterogeneous patterns of transpiration can be detected. The optimal distance from the crop will be determined based on image resolution. Since the thermal camera will need to be transported by a robot, it will be optimal to select a compact camera compliant with the required resolution. The thermoVision® A10 Infrared Camera (http://www.flir.com) is the smallest available infrared camera (though other types can be used as well) with the following specifications:

-Ultra-compact, lightweight
-Solid state uncooled microbolometer detector, 7.5 to 13 micrometer sensitivity
-Thermal resolution (<0.04 ºC when normalized to f/1.0)
-Both analog RS-170A (CCIR) and 14-bit digital outputs (Firewire) for direct support of machine vision and imaging applications, and camera control via RS-232 interface.
-Operating temperature range: 0 to 40ºC

The last imaging technique to be incorporated is chlorophyll fluorescence imaging. In adverse conditions, e.g. pathogen infection, photosynthetic activity is reduced, and this is accompanied by either increased or decreased chlorophyll fluorescence (Chaerle *et al*., 1999 and 2004). In the HSB unit of UGent, a chlorophyll imaging unit mounted on a gantry robot is in use since 2000. Tobacco mosaic virus infection, Cercospora and Botrytis (fungal) infection, Mg nutrient deficiency and ROS-induced cell death (Chaerle *et al*., 2004), were successfully pre-visually detected with this imaging technique. However, to be implemented the new plant culture room this chlorophyll fluorescing unit requires miniaturization. This miniaturization will be achieved by replacing the 6 halogen excitation lamps, fitted with a blue filter, by blue LEDs.

## 5. Data acquisition system and management

Most sensors that are chosen can be automatically logged. As many loggers as possible (multiple entry) will be shared among the sensors. Software should be included for sensors of choice and preferably be compatible with the NI LabView visual programming environment or at least data capture software should be available for central data management. Data needs to be displayed in real time, and be organized in automatically generated daily, weekly and monthly time graphs for incoming data of all sensors. Routines need to be written for this or purchased as standard software packages. Automatic graphical display can be achieved using Labview, MS Excel macro's, and proprietary software from e.g. environmental control equipment suppliers.
The gully weighing application needs to be equipped with automatic interval weighing, which is available from the mentioned suppliers.
The image data needs to be organized automatically according to plant position and treatment, which can be done by generating overview images combining the different modalities per position (thermal, fluorescence, video etc. images), converting the overview images to time series and indexing this information for the different experimental trials under a standard web-browsable interface (unix scripting combined with Imagemagick image compositing software and a javascript based indexer). Such an approach could be further implemented or complemented by image database software (http://sidb.sourceforge.net/, http://www.mediacy.com/ IQbase), or more generally data management software.
Analysis of the organized data can be done offline using ImageJ, Matlab or Labview. Depending on the level of automatization achievable, Labview could be used to analyze the data captured in a labview-written acquisition program.

## 6. Actuators

For all monitored parameters upper and lower limits need to be entered as threshold values. Incoming data need to be checked in the data management routines against these thresholds. If surpassed at lower or higher limit, an alarm signal needs to be issued.

For imaging purposes, a positioning repeatability of 0.1 mm will be needed to have time-lapse image series of the same positions (and thus plants) over time. Controllers and motors have to be adapted to these requirements. The standard 6 degree of freedom robots used in industry (6DOF with articulated arm and spherical working area) are not able to reach all positions in a growth chamber as defined above, therefore a gantry (Cartesian 3 axis XYZ) system is chosen as optimal.

For control of the nutrient solution, a continuous flow of nutrient will be guaranteed; compensation of evaporated water, used nutrient and adjustment of pH will be accomplished by dosing pumps.

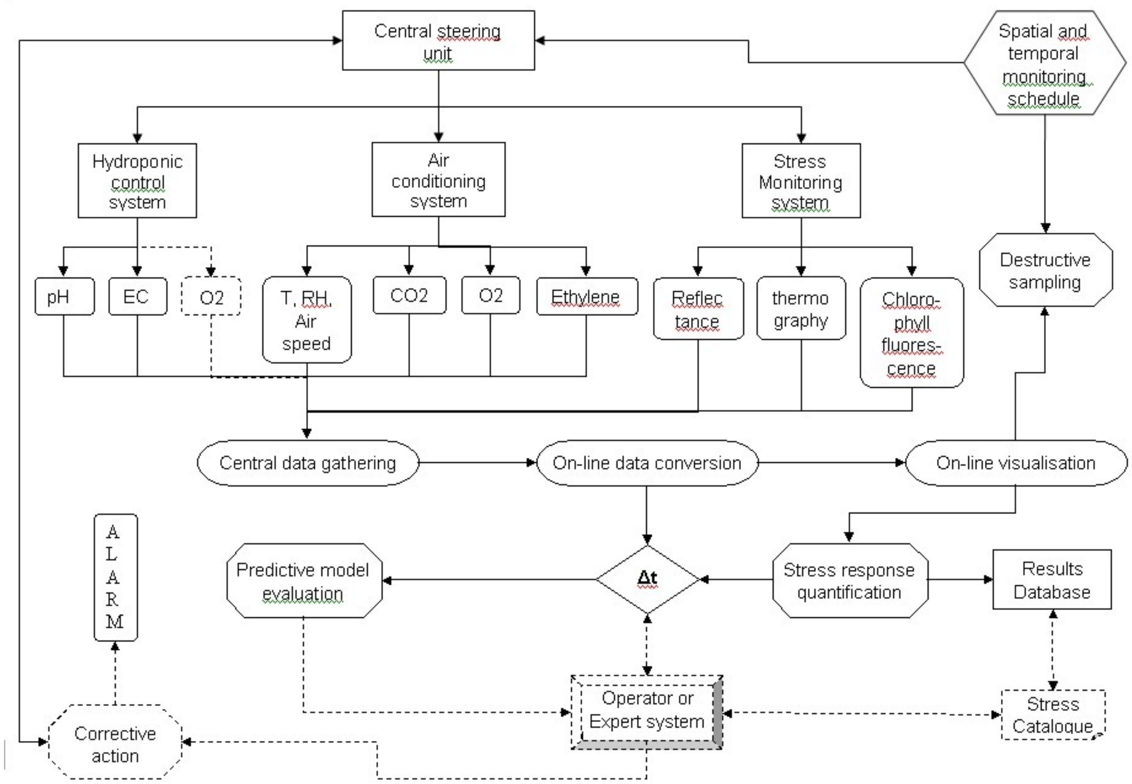


**Figure 1 Proposed data mangement scheme**

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