

Memorandum of Understanding 19071/05/NL/CP



## **TECHNICAL NOTE: 89.51**

### WORKBENCH DEMONSTRATOR DESIGN

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reference/ <i>référence</i>	Contract number 19787/06/NL/PA
issue/ <i>édition</i>	1
revision/ <i>révision</i>	0
date of issue/ <i>date d'édition</i>	16Oct07
status/ <i>état</i>	Final
Document type/ <i>type dedocument</i>	Technical Note
Distribution/ <i>distribution</i>	

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### APPROVAL

Title <i>titre</i>	issue <i>issue</i>	1	revision <i>revision</i>	0
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author <i>auteur</i>	date <i>date</i>	16Oct 07
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approved by <i>approuvé by</i>	date <i>date</i>
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### CHANGE LOG

reason for change / <i>raison du changement</i>	issue/ <i>issue</i>	revision/ <i>revision</i>	date/ <i>date</i>
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### CHANGE RECORD

Issue: 1 Revision: 0

reason for change/ <i>raison du changement</i>	page(s)/ <i>page(s)</i>	paragraph(s)/ <i>paragraph(s)</i>
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## Walk-in growth chamber

The workbench demonstrator will be positioned in a walk-in chamber with precise environmental control. The chamber measures 3m length x 1.5m width x 2m height. The available set-up includes the following.

### 1.1. Plant growth support

- Six 0.5 m wide shelves which can hold two 1.5 m long gullies, providing a total growing area of 4.5 m<sup>2</sup>.
- A dimmable unit of eight fluorescent lamps per shelf: Master TL-D reflex Super 80 58W / 840 from Philips (<http://www.philips.be>)

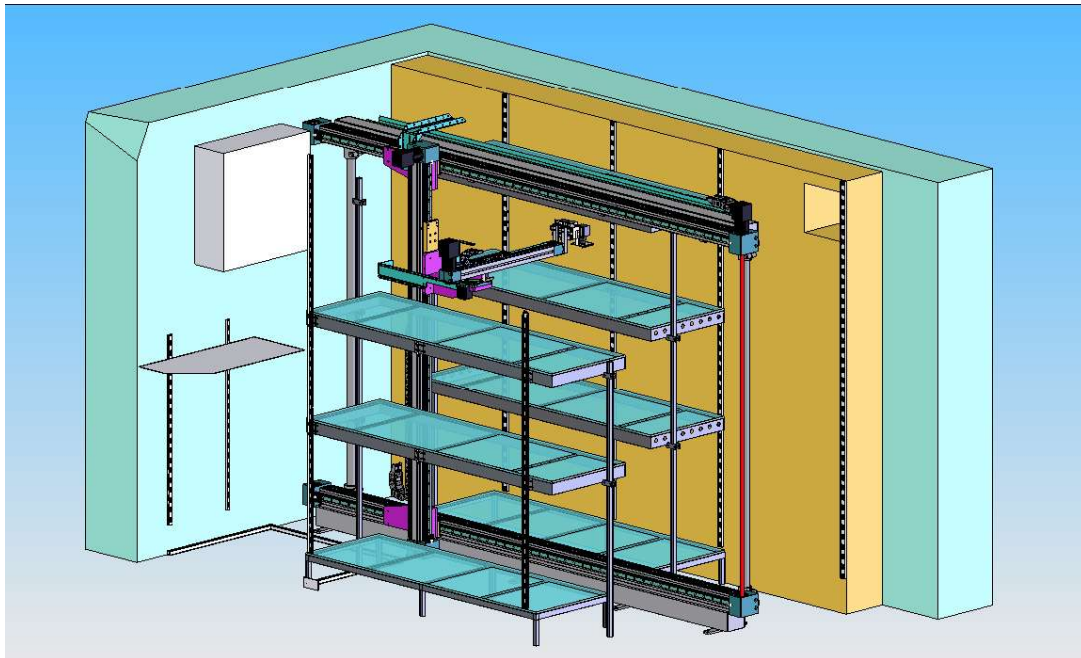


Figure 1: Cut-away view of the growth chamber

### 1.2. Climate control equipment

- PLC Programmable Logic Controller RMU730 (<http://www.siemens.com/index.jsp>) with ACS7 operating/logging software. PI control of cooling, heating and humidification by modulated chilled water valve, steam supply and heating resistances.
- Air ventilation maximum 8000 m<sup>3</sup>/h – adjustable with Variable Frequency Drive Hitachi L200 075HFEF (<http://www.hitachi-ies.co.jp/pdf/catalog/SM-E242R.pdf>).  
Ventilation unit with chilled water coil, electrical resistances and filter section.

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### Robotized imaging system

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-Steam humidifier Carel UR010HL001

([http://www.carel.com/carelcom/web/eng/catalogo/prodotto\\_det.jsp?id\\_gamma=34&id\\_tipologia=9&id\\_prodotto=67](http://www.carel.com/carelcom/web/eng/catalogo/prodotto_det.jsp?id_gamma=34&id_tipologia=9&id_prodotto=67))

-Chilled water primary circuit Chiller Daikin EUWA5KZW1

([http://global.daikin.com/global/our\\_product](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)
Open area due to perforation:	35% (see TN89.52 for air speed calculation)
Distance to real walls:	0.24 m
Distance to backside of air-duct - an inclined plate ensures an even distribution of air flow along the vertical:	0.24 m at air entry from ceiling of room 0.04 m at bottom of room
Dimensions perforated wall input:	3 x 2 m (effective surface)
Dimensions perforated wall output:	2 x 2 m (effective surface)

### 1.3. Settings and error limits

Light range:	0-400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR
Temperature range:	20 to 30 °C
Error temperature:	$\pm 0.2$ °C
Humidity range:	60 to 80 % RH
Error humidity:	$\pm 5$ % RH
Fresh air supply:	0 to 2 $\text{m}^3 \text{h}^{-1}$
Air circulation:	3000 to 8000 $\text{m}^3 \text{h}^{-1}$

Video, chlorophyll fluorescence and thermal time-lapse imaging of plants gives invaluable data regarding the growth, developmental and health status of those plants. To be able to position the required camera's at set time intervals above the crop, a gantry robot was designed within the walk-in chamber. The robot needed to be able to access all the plants on the six shelves (at 3 different heights) in the room. The robot arm carrying the camera's had to be small, as had to be the camera's, to maximize growing space.

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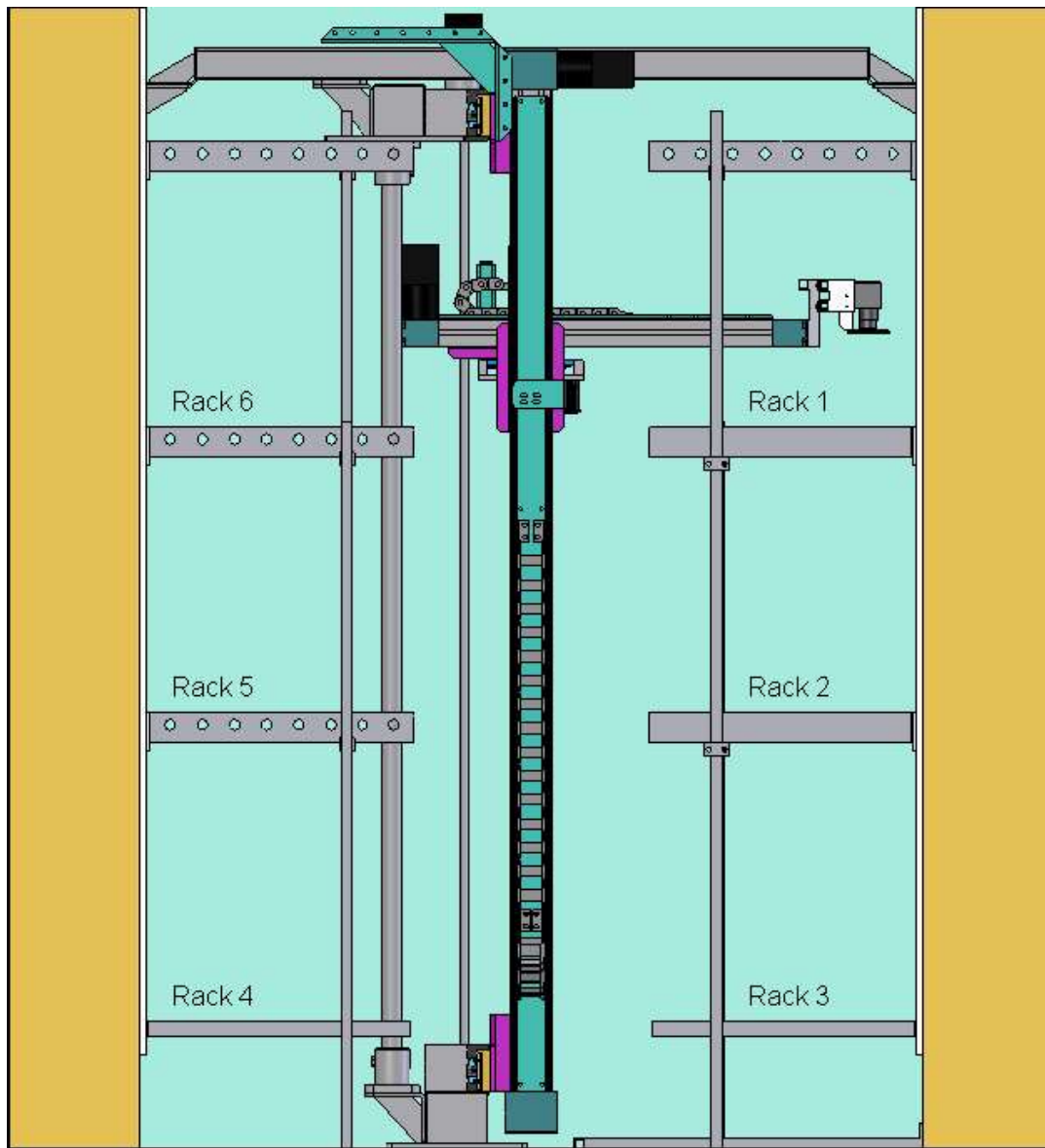


Figure 2: Side view of plant racks

Calculations for the basic requirements of the gantry robot and the delivery of the hardware were performed by ROTERO Belgium bvba (<http://www.rotero.com>). Final design, testing and installation were performed by Atlantic Engineering bvba (<http://www.atlantic-engineering.be>). The gantry robot is carried by a frame that is mounted vertically in the middle of the room. User access is through the same corridor. For user safety it is not possible to reach

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### Hydroponic system:

into the working area of the robot while an experiment is running – see safety zone in Figure 3. The robot can move in the x, y and z direction and furthermore the arm carrying the camera's can rotate (toothed belt linear axis: <http://www.indunorm.eu>). All these movements are very precise (repeatability 0.1 mm) due to the use of closed-loop stepping motors ([www.orientalmotor.de](http://www.orientalmotor.de)) and planetary gears (<http://www.alphaetriebe.de>). An application written with national instruments Labview ([www.ni.com](http://www.ni.com)) by CIT ([www.citengineering.com](http://www.citengineering.com)) controls both robot and image acquisition.

#### 1.4.Vision components

The Thermal infrared camera ThermoVision® A10 (<http://www.flir.com>) was chosen based on compactness. The camera is not radiometric and therefore does not provide a direct temperature reading. A Matlab application was written that converts the heat intensity data to temperatures, based on the chamber temperature settings and measurements on a reference surface placed next to the plants. The camera detector is an array of microbolometers which needs periodic recalibration by performing a NUC (non-uniformity correction). Since the imaging room has stable temperature conditions, this calibration has to be performed only at the start of the experiment by using the ThermoVision Micron software.

A BCi5 CMOS greyscale camera (<http://www.vector-international.be>), capturing intensity images of 10bit depth will be fitted with a LED-illuminating source.

A BCi5 CMOS colour camera (<http://www.vector-international.be>) was selected to capture high-resolution colour images functioning as references for the thermal and chlorophyll fluorescence images.

These 3 cameras are fitted on a purpose-built camera head at the end of the horizontally moving linear positioning module (see Figure 2). Based on a maximal total weight of 2kg of the 3 camera's plus the measuring head the system could be built to achieve 0.1mm repeatability by using 80x80mm axis for the vertical and inter-rack movement and a 40x40mm axis for the horizontal intra-axis positioning axis. A smaller axis helps to keep the space needed for imaging lower and minimizes blocking of the overhead light.

The firewire (IEEE1394) output of the A10 and the USB2 output of the 2 BCi5 cameras are captured by a Labview application upon positioning of each of the cameras above the target plant. The software provides a module to compensate for the difference in alignment and field of view of the camera's, to obtain a perfect match between the different images sequences when analyzing the data.

A custom-built hydroponics system was implemented by the company Ecotechnic ([www.ecotechnic.be](http://www.ecotechnic.be))

Two types of gullies are employed:

- 1) 8 closed PVC gullies 1.5 m long, 100 mm width and 50 mm height (from the Mobile Gully System, [www.hortiplan.be](http://www.hortiplan.be))
- 2) 4 Polyethylene gullies 1.75m long 105mm width and 50mm height, with removable upper lids (adapted DLP system, [www.legrand.be](http://www.legrand.be)).

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### Sensors

Two separate recirculation systems (“Control / C” and “Treatment / T”) are provided (6 gullies per system, one T and one C gully per shelf, connections switchable to vary spatial layout of treatments among repeat experiments). For each system the main supply is split into 6, and adjustable needle valves ensure even supply to the 6 gullies. Solution outlet of the six gullies is combined. A pump fills the gullies with nutrient solution at a speed of 200ml/ gully<sup>-1</sup> h<sup>-1</sup>. Gullies are positioned with a downward angle of 1% and empty by gravity.

The recycled solution is automatically readjusted for volume (from a distilled water reservoir with pump controlled by a liquid level detector: Nivofix, [www.honsberg.com](http://www.honsberg.com)), pH (with nitric acid) and EC (with 10 x nutrient stock solution) under control of a pH and an EC

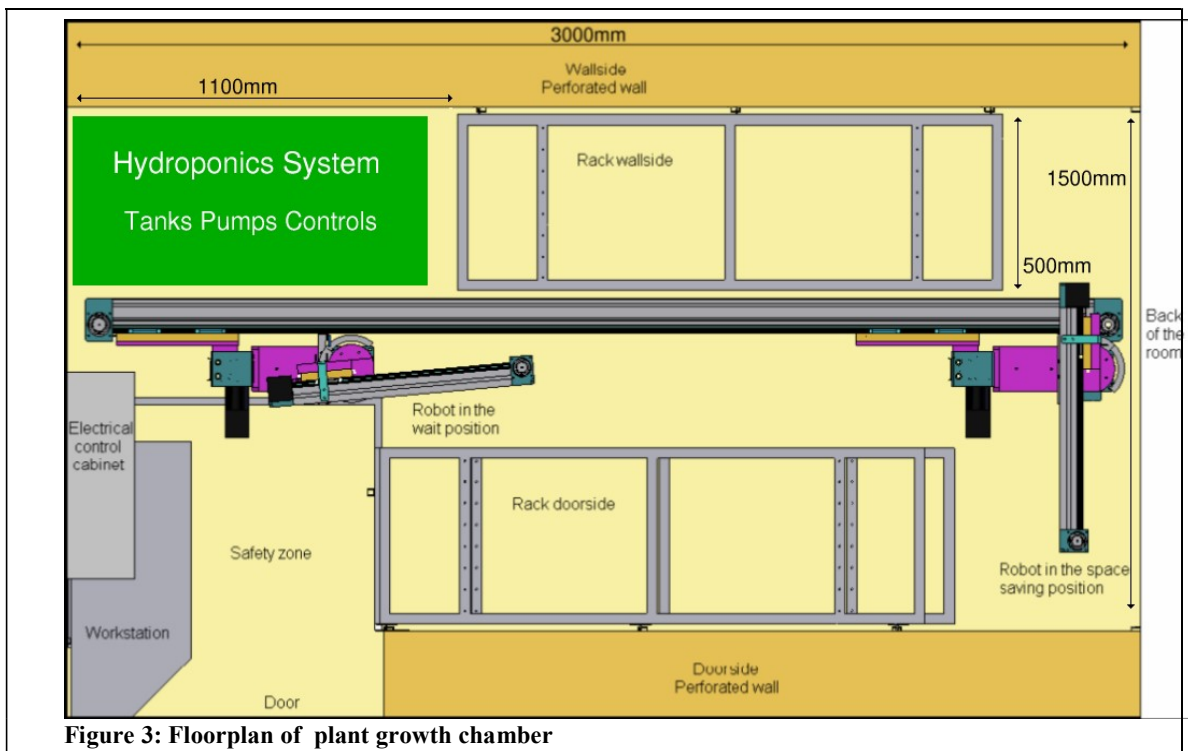


Figure 3: Floorplan of plant growth chamber

sensor (see below), each steering a dosing pump (Blackstone DL 1.5, <http://www.hannainst.be/>). The amount of added distilled water will be measured by a rotor element flow measure device ([www.honsberg.com](http://www.honsberg.com)).

All sensor outputs will be logged to a DL2e data logger (delta-T devices, [www.delta-t.co.uk](http://www.delta-t.co.uk)).

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

Parameter	Sensor
<b>In air:</b>	

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CO <sub>2</sub>	WMA4 from PPSystems measuring range 0-2000ppm
O <sub>2</sub>	WMA4 from PPSystems – attached electrochemical cell measuring range 0-25%
Light	Quantum Sensor SKP 215 (Skye instruments)
Temperature	Vaisala Humicap®
Humidity	Vaisala Humicap®
Ethylene	ETD-300 from Sensor Sense
Weight gully	Load cells (MP40 from Sartorius)
<b>In solution:</b>	
Temperature	NTC 10K sensor (1 in each feeding tank)
O <sub>2</sub> offline – manual measurement*	HI-914604 dissolved oxygen meter with HI 76407/4F electrode (Hanna Instruments)
EC	HI700-221-2 Controller + HI7639 sensor and Pt100 T-sensor (Hanna Instruments)
pH	pH 500-1212 Controller + HI6101405 sensor and Pt-100 T-sensor (Hanna Instruments)

\* Manual measurement and data transfer

### 1.5. Air temperature and relative humidity

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

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: Vapour tight

Data signal output: RS-232  
 Controllable from: LabView

### 1.6. Ethylene

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

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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 litre 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, the company recommends annual calibration.

Preliminary measurements on sampled air indicated 36 ppbv ethylene in the measuring room and 24 ppbv in outside air.

### 1.7. Biomass accumulation

Two gully's will be fixed on load-cells (MP40, [www.sartorius.com](http://www.sartorius.com)) to obtain continuous weight readings from which biomass accumulation over time will be calculated.

### 1.8. Condense water quantification

The condense water from the cooling section of the ventilation system will be quantified using a tipping bucket gauge Rain-o-matic Typ 100.054 ([www.pronamic.com](http://www.pronamic.com)). The collected water will be fed into the replenishment tank for the hydroponics system.

### 1.9. CO<sub>2</sub> - control

An OMRON E5CN-Y2ML PID controller ([omron-industrial.com/](http://omron-industrial.com/)) + mass flow meter is installed for CO<sub>2</sub> supplementation from a compressed gas bottle.

### 1.10. Alarms

Liquid level monitoring devices will be fitted on all employed reservoirs to avoid overflow or abnormal low levels in case of malfunction of the controlling system. Stock solution tanks will only be fitted with a low-level alarm. All level measurements and controls are steered by a Mitsubishi Melsec Compact PLC (<http://www.mitsubishi-automation.com/>)

### 1.11. Off-line measurements

To complement and calibrate the online measurements, a set of off-line measurements will be carried out. The methods encompass both non-destructive and destructive approaches.

#### 1.11.1. Dissolved oxygen

On the recollected water line from each circuit (just before re-entry of the solution into the stirred mixed tank), a measuring cuvette is provided to allow measurement of dissolved oxygen representative of the root conditions at the end of the gullies.

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### Data acquisition system and management

#### *1.11.2. Destructive sampling*

Fresh and dry weight will be determined on sample plants at weekly intervals during crop growth and at the end of the experiment. See TN 89.52 for a detailed schedule. An available drying oven and analytical balance will be used.

Composition of the nutrient solution and of sample tissues will be determined in an external laboratory at the end of the growing period.

To corroborate the output of the online sensors with histological and molecular-genetic data, fluorescence microscopy and quantitative polymerase chain reaction (qPCR) will be applied. A high quality fluorescent light microscope, (Axiovert 200 from Zeiss: [www.zeiss.com/micro](http://www.zeiss.com/micro)) will be used for histological examinations of root and leaf morphology. A qPCR machine (IQ 5 Multicolor Real-Time PCR Detection System, [www.bio-rad.com](http://www.bio-rad.com)) will be used to quantify stress-induced gene expression.

The data from the online sensors sent to the DL2e data logger will be converted to (semi) on-line graphical displays.

The NI-Labview program captures thermal, video and chlorophyll fluorescence images. These images will be converted into time-lapse sequences and organized using an adapted freeware javascript user-configurable image cataloguing application that provides a web-interface to the collected data on a per-experiment basis, allowing easy data viewing and retrieval.

Analysis of the organized image data will be done offline using ImageJ (<http://rsb.info.nih.gov/ij/>), in a second step analysis will be made semi-automatically using ImageJ and Matlab (<http://www.mathworks.com/>). Labview could be used in a further step to have online display of data analysis just after image capture. However it is most important to first validate and test the measurements on a tightly user-controlled basis.

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