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Imaging fluorescence emission from chlorophyll for  
monitoring photosynthetic activity of plants

*Photronics Event 2012 is organised by Mikrocentrum and Photonics Cluster Netherlands  
empowered by the Dutch Ministry of Economic Affairs, Agriculture and Innovation.*





## Competence matrix

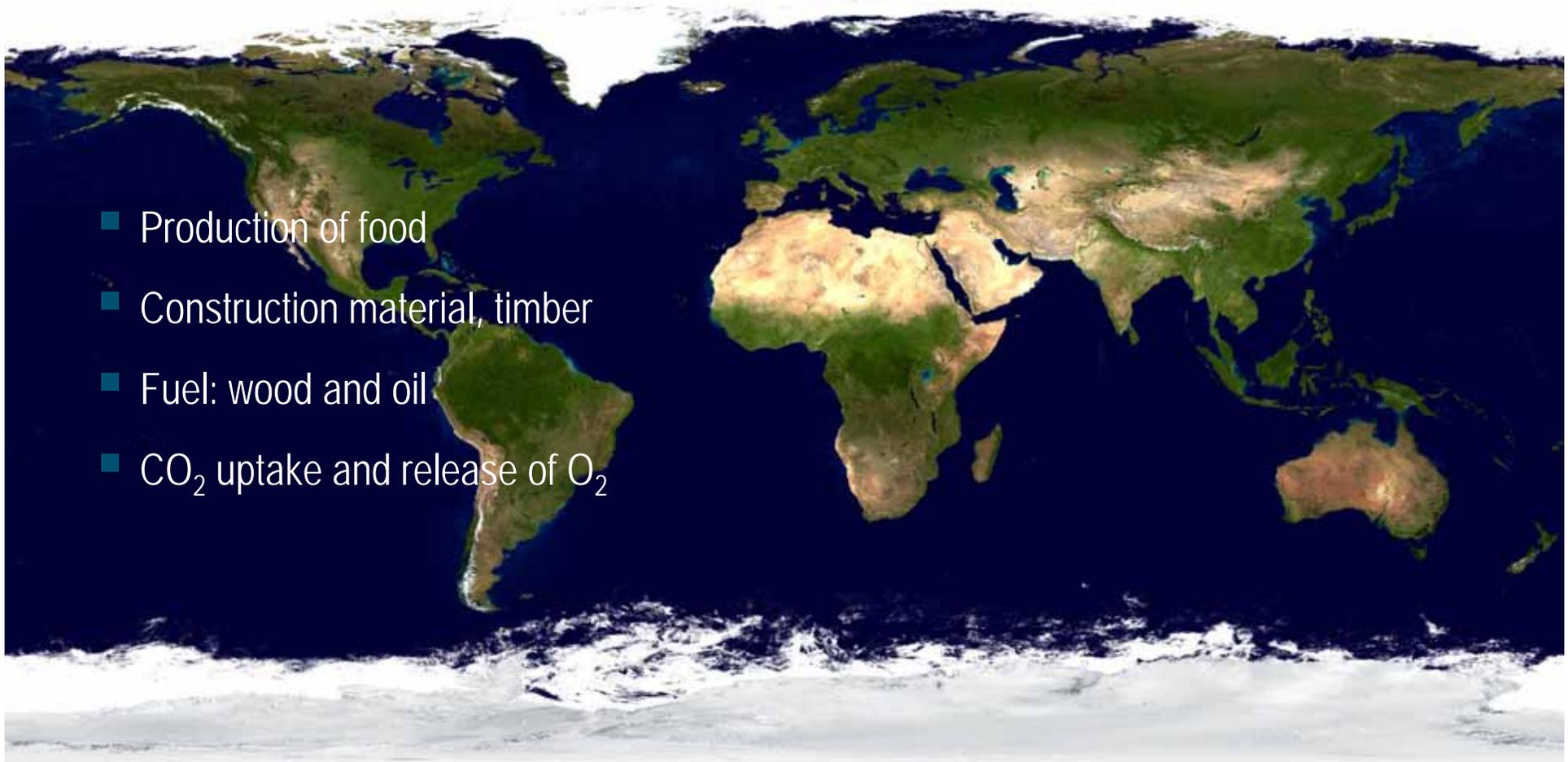
Sector	R&D	Technology	Application	Market
Photonics for green energy & environment	✓	✓	✓	
Lighting for well being				
Agriculture & food	✓	✓		
Solar fuels	✓	✓	✓	
Optical communication				
Business development, 'best practices'				
Photovoltaics		✓	✓	
Healthcare	✓	✓	✓	
Safety & security				
Lifescience (microscopy)	✓		✓	
Integrated photonics				
Solid state lighting technology			✓	
Metrology & sensing	✓	✓	✓	
Photonics technologies for machining				
Optical Design	✓	✓	✓	
Computer Vision	✓	✓	✓	

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# Photosynthesis: one of the most important processes

- Production of food
- Construction material, timber
- Fuel: wood and oil
- CO<sub>2</sub> uptake and release of O<sub>2</sub>

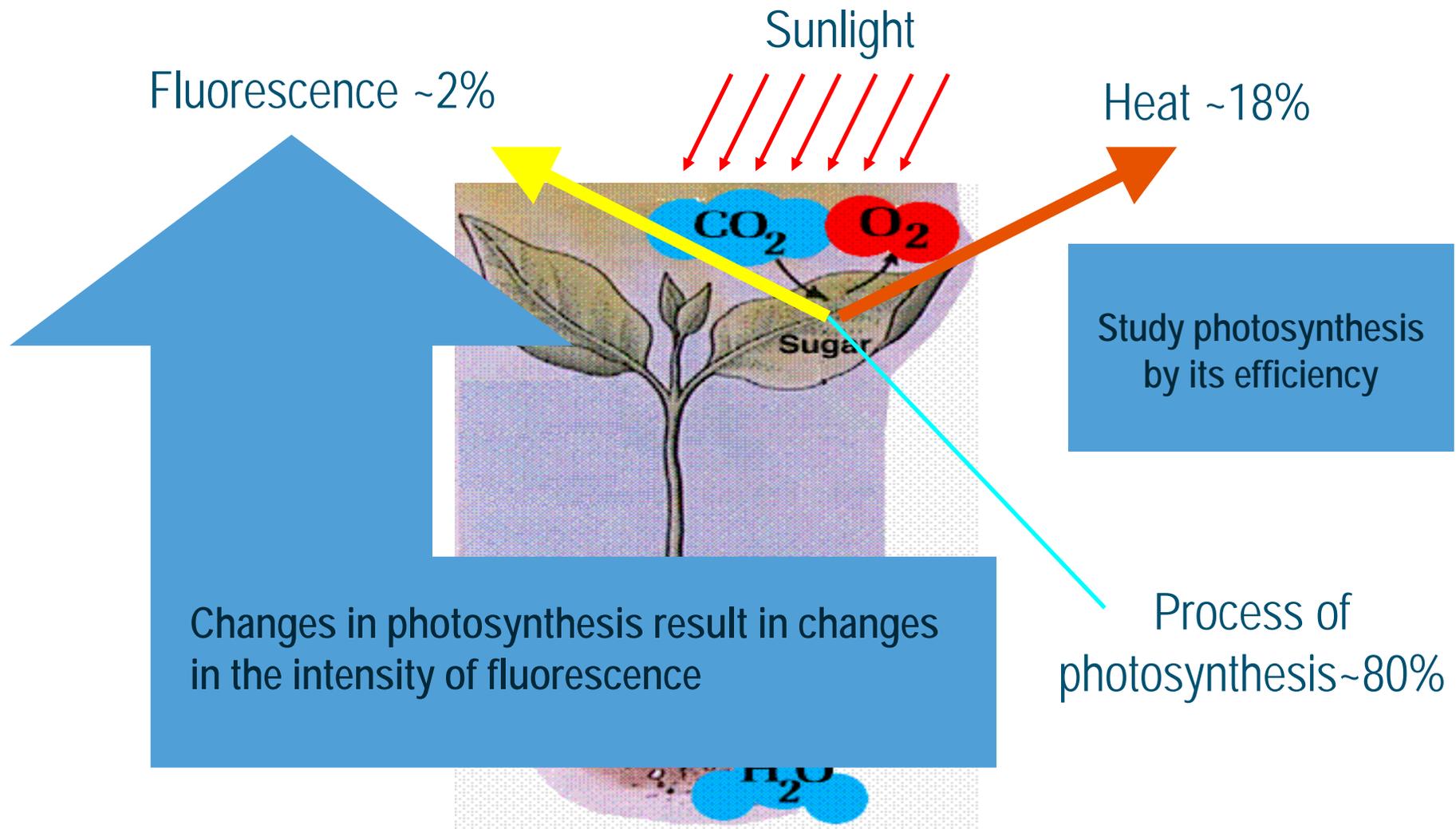


NOAA/USGS/MODIS/stockli/Tor Øra



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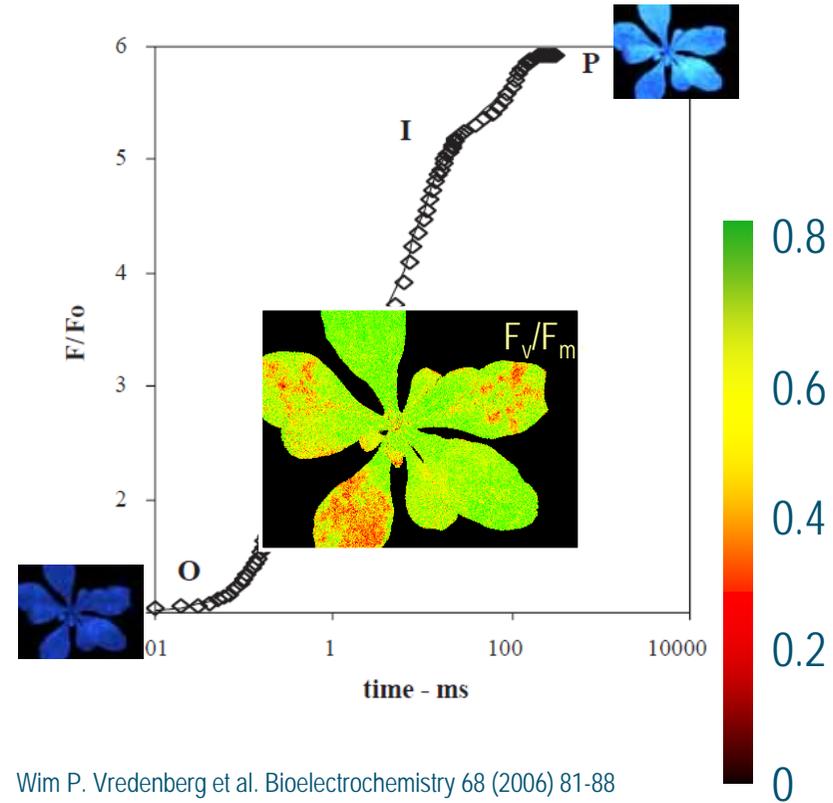
# Process of photosynthesis



# Principle of operation



More CF when photosynthesis is blocked



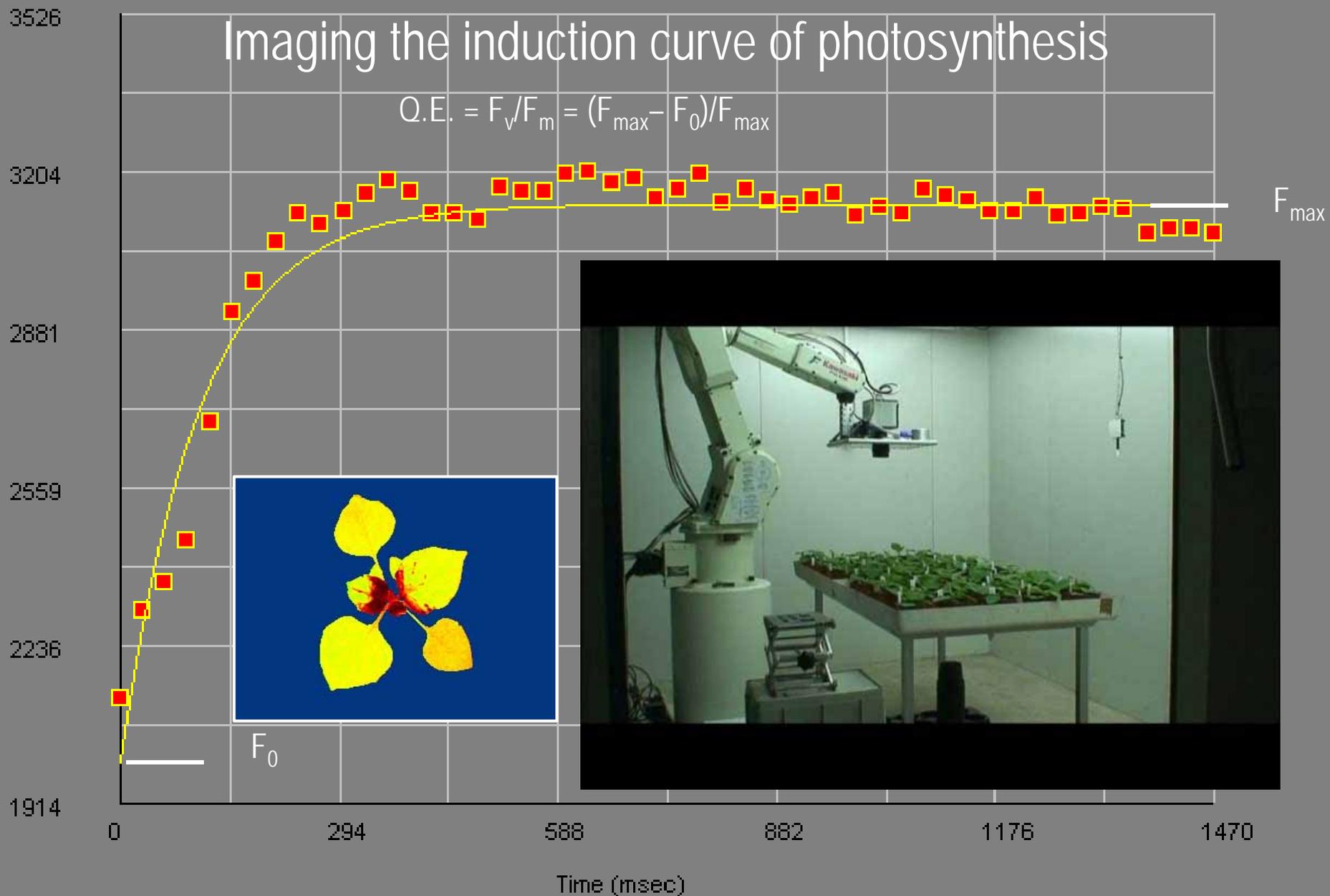
Wim P. Vredenberg et al. Bioelectrochemistry 68 (2006) 81-88

Colour table of  $F_v/F_m$

Chlorophyll fluorescence  
Intensity (a.u.)

# Imaging the induction curve of photosynthesis

$$Q.E. = F_v/F_m = (F_{max} - F_0)/F_{max}$$



Example of visually no damage:  
detected damage in real time

Colour table of Q.E.



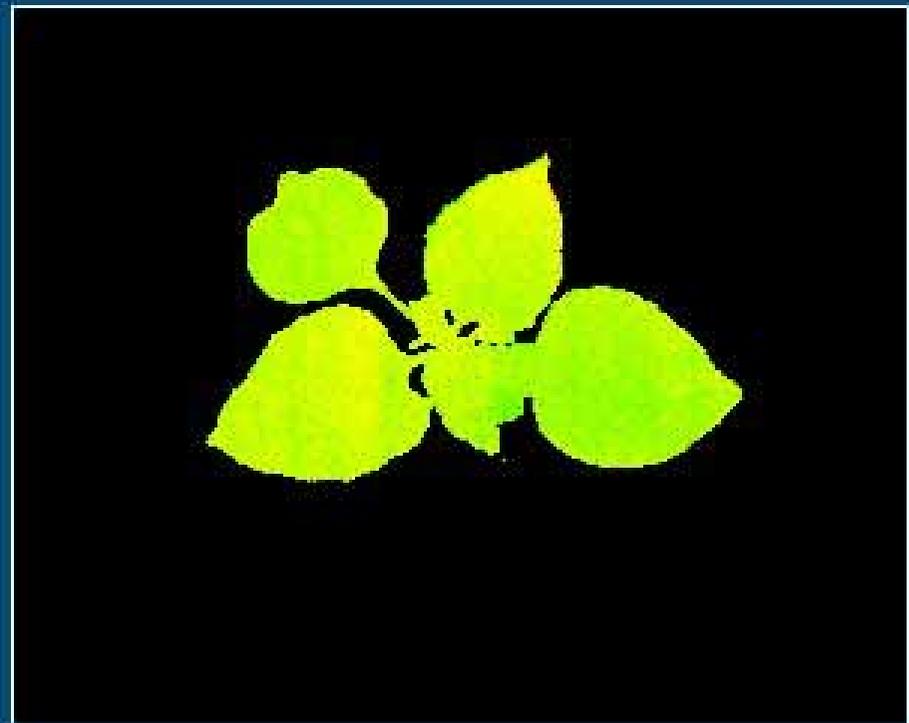
0 hrs



0 hrs

Sencor  
Metribuzin

0% 25% 50% 75% 100%



0 hrs



0 hrs

# Automated fluorescence imaging using a robot

## Develop herbicides with low active ingredient



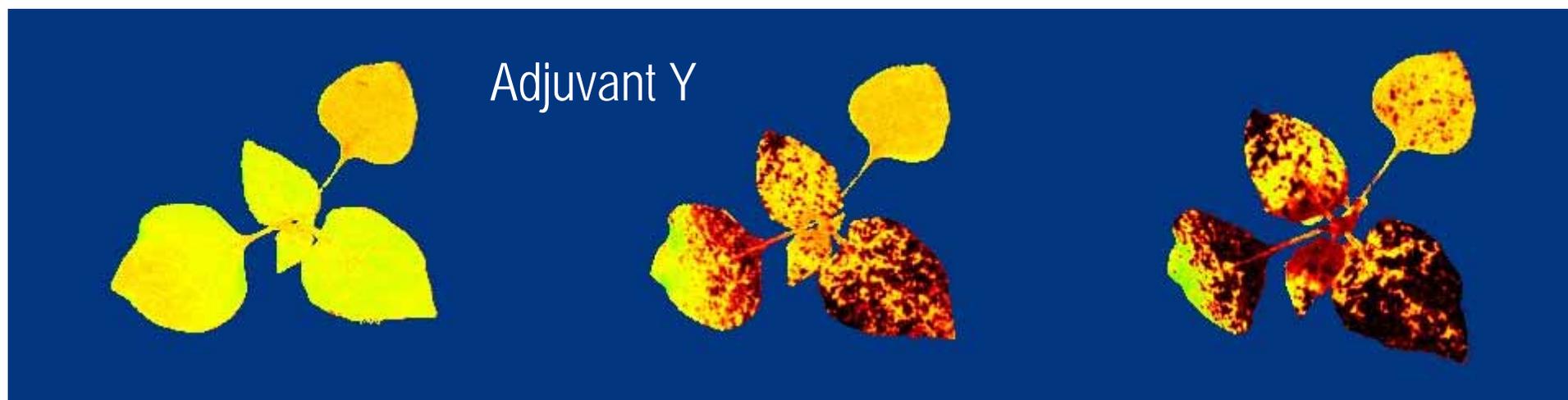
# Glyphosate 0.5 mM with two adjuvants, X & Y



6 hr

30 hr

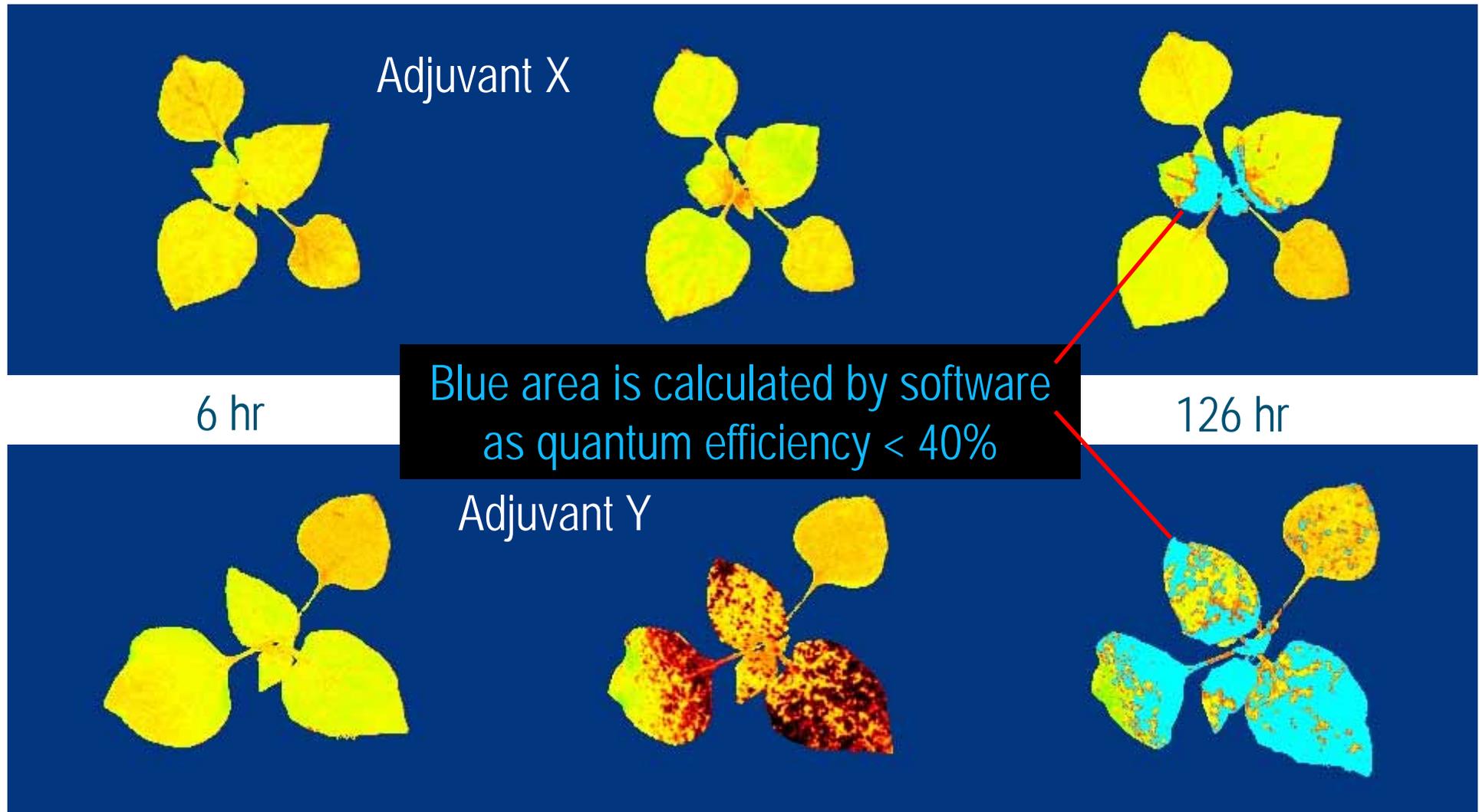
126 hr



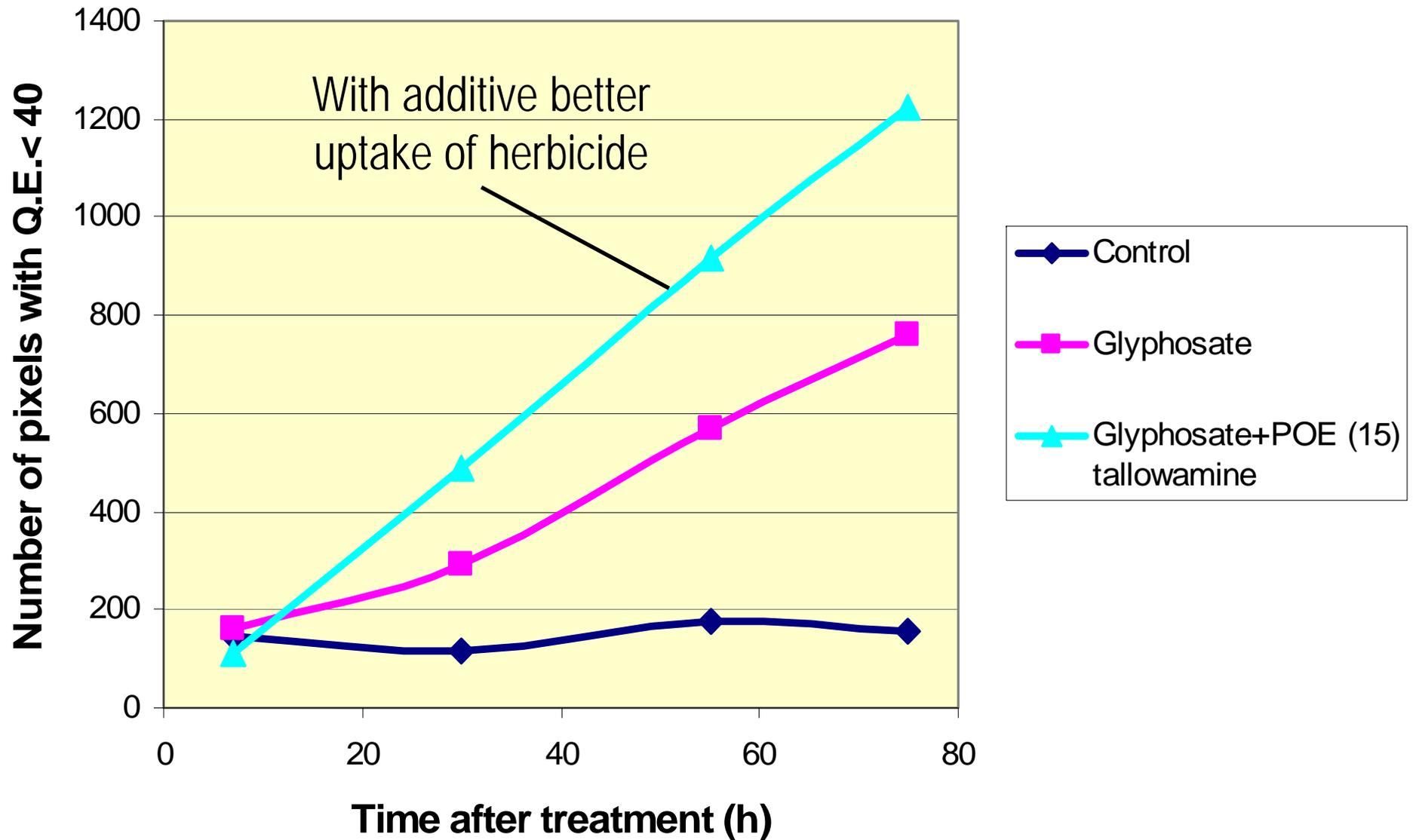
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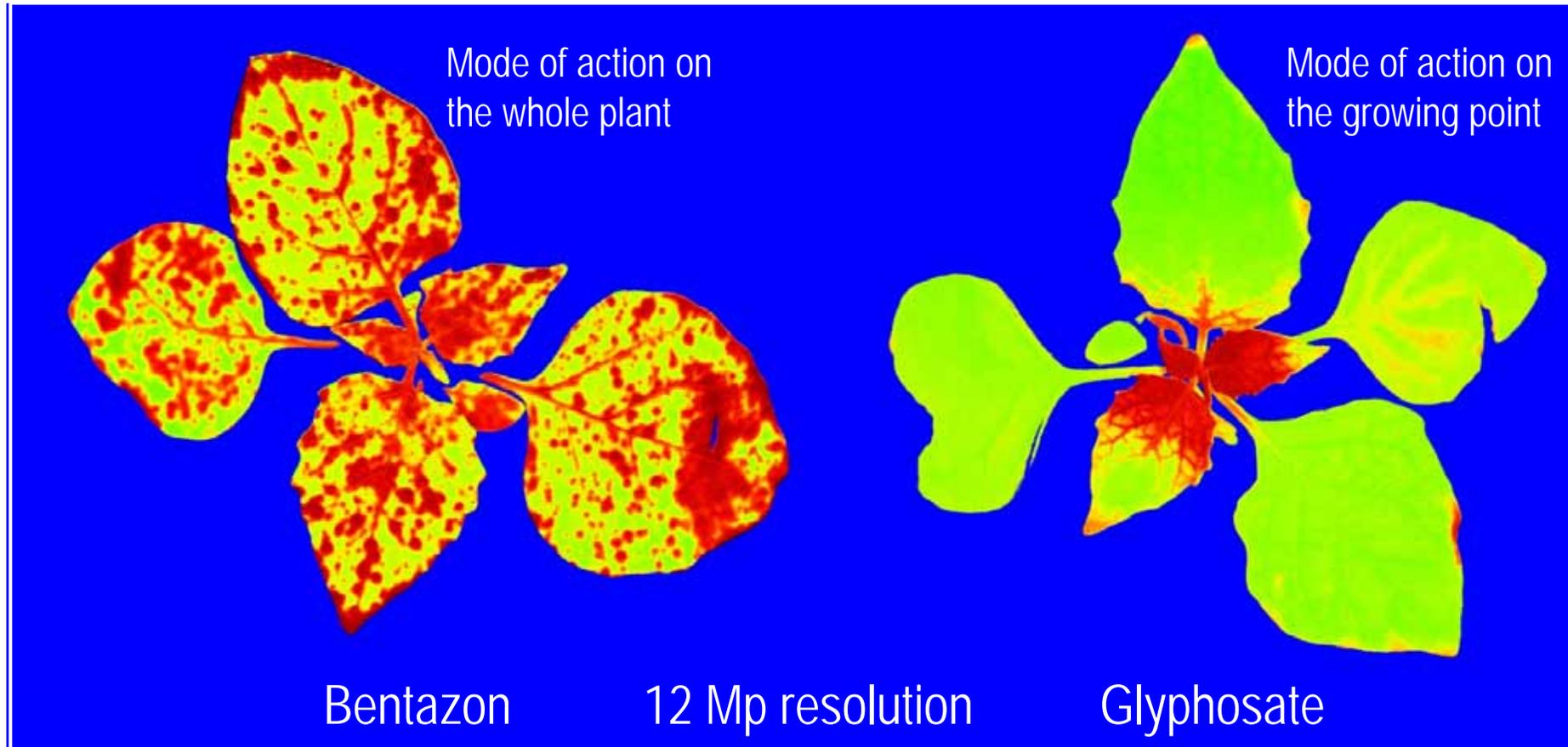
# Glyphosate 0.5 mM with two adjuvants, X & Y



## Using mask and counting glyphosate-pixels

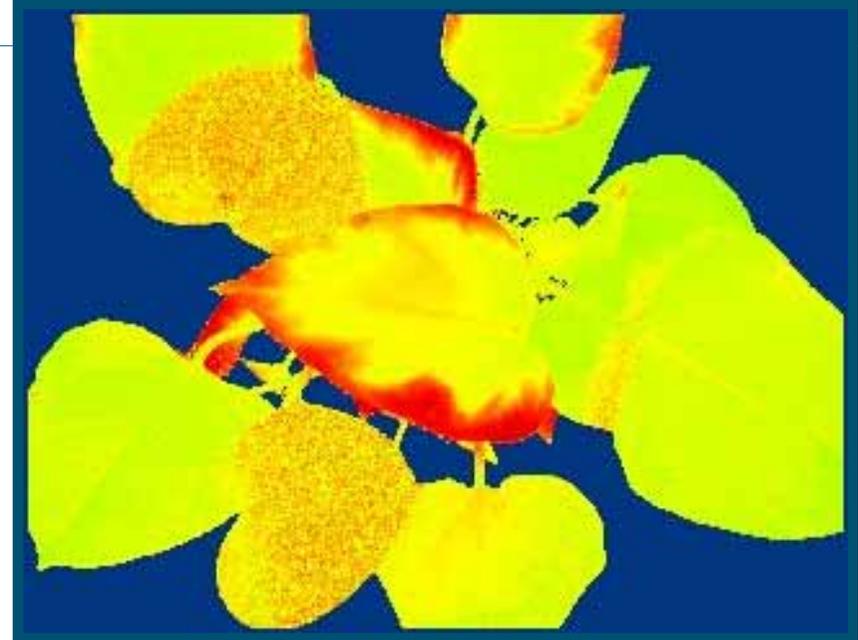


# Different mode of action of two herbicides



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Damage measured before observed by eye  
Take early precautions to prevent more damage

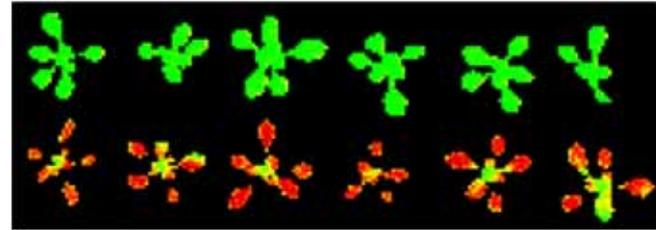


Anthurium

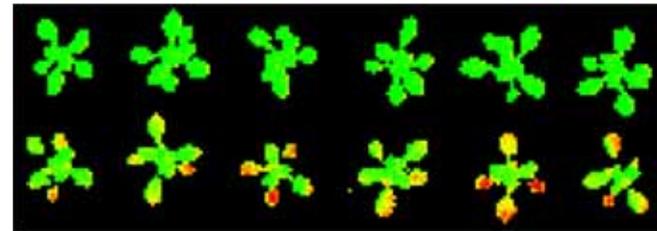


# Damage by high light depending on cultivar

Screen the plants for high light from the sun



Cultivar 1



Cultivar 2

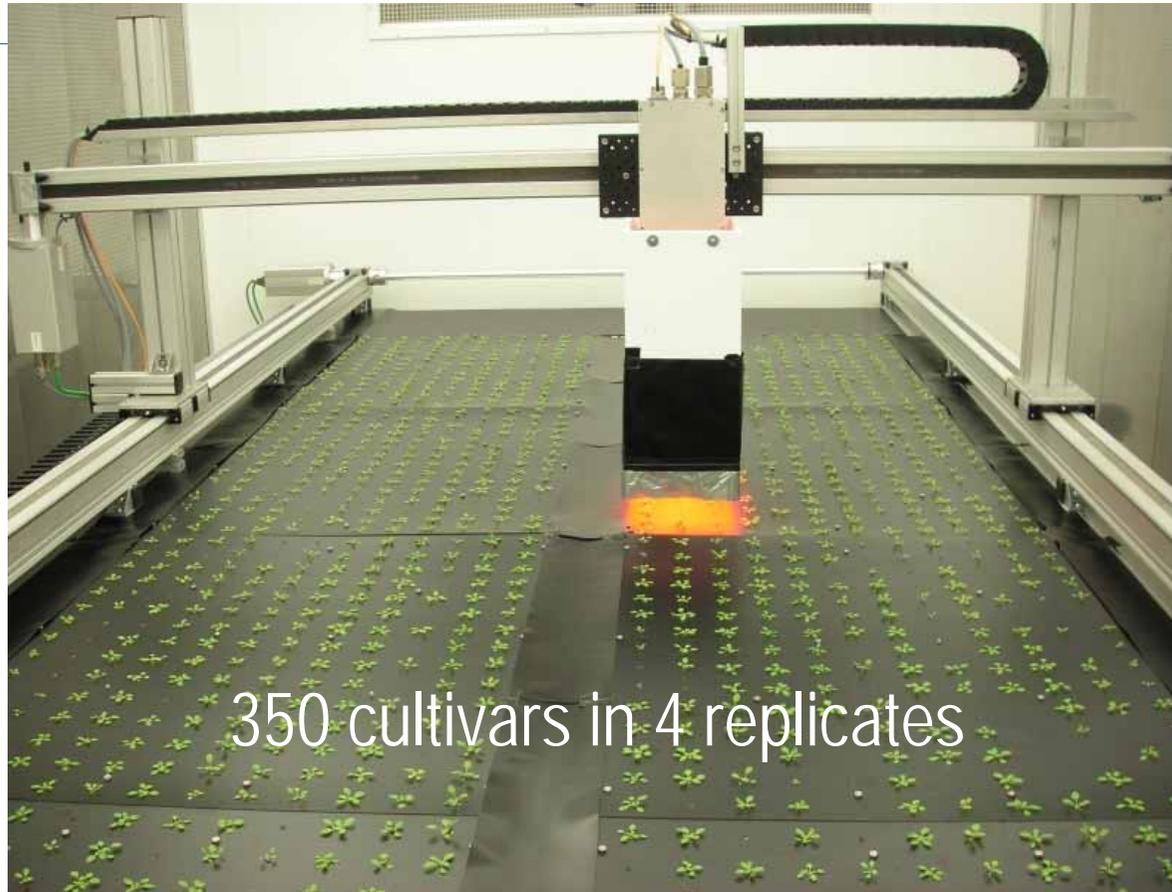


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# PhD project: phenotyping 1400 *Arabidopsis* plants

## Correlate genetic information with plant properties

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# XY-table: software to analyse on plant properties

CF-Image Analysis Software      Wageningen UR Greenhouse Horticulture      Version 4.3 (c) 2010 by RvdS

Open      Mask      Mask level: 1900      Display:  Fluorescence image      Stretch: 4.0      Save Data      Smooth:  1x1

Pos: 0074      No: 0104      Mask Image: 3       Intensity curve      Fv/FM: 13      Save Image       3x3

Grab: 30      No Edge       Fv/Fm raw image      TAU: 90      Save Image      ROI XYplot       5x5

Show: 30       Auto mask       Del noise       Centre mask       Show Histogram      INFO

meanstd: 37.9±6.6      ymax: 162      sum: 5178      FvFM:  Raw       Fitted

min: 1.6%      max: 65.1%      Hist of:  Mask       ROI1       ROI2

0%      100%

Opened file with time stamp: 11 nov 2010 17:18  
D:\My Project\Experiments\XY system\Plot screen\y Data\data T11 Exp01\SP\_E0001P0074N0104.DAT

Total Image:  
Mask: 5195  
msk/im: 6.8 %  
dark FL: 996  
(30) FL: 13041

raw data:  
i0: 0  
im: 0  
fv/im: 0

fitted curve:  
i0: 0  
im: 0  
fv/im: 0 %  
tau: 0 ms  
r2: 0

Per Pixel:  
Xp: 318  
Yp: 51  
FL: 0

raw data:  
i0: 0  
im: 0  
fv/im: 0.0 %

fitted data:  
i0: 0  
im: 0  
fv/im: 0.0 %  
tau: 0 ms

all pixels:  
fv/im: 37.8 %  
s.d: 6.9  
s.e: 0.1

Calculate and locate Fv/FM range:  
Fv/FM range: 0 - 50      Pixels: 5127      Area [%]: 98.7  
 Show in image       Invert in image

ROI 1 (CTRL-key) white square  
 Roi visible      position: [0,0,0,0]       Auto mask:  Fixed      mask size: 0  
 Size fixed      roi size: 0      pixels in range: 0  
area of mask [%]: 0

	Mean	Raw	Fitted	St.dev	St.err
i0	0	0	0	0	0
im	0	0	0	0	0
fv/im [%]	0	0	0	0	0
tau (ms)	n.a.	0	0	0	0

ROI 2 (SHIFT-key) blue square  
 Roi visible      position: [0,0,0,0]       Auto mask:  Fixed      mask size: 0  
 Size fixed      roi size: 0      pixels in range: 0  
area of mask [%]: 0

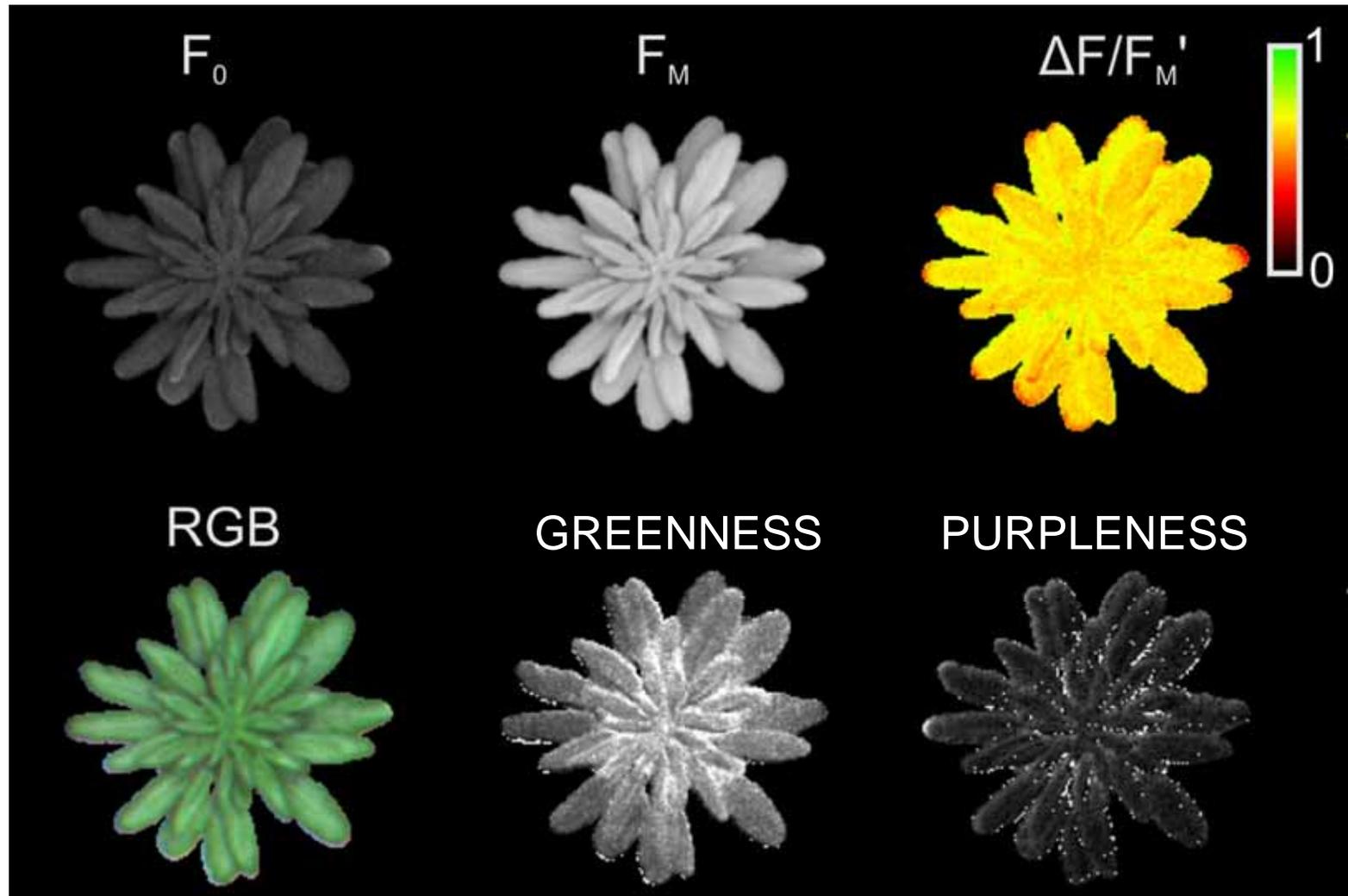
	Mean	Raw	Fitted	St.dev	St.err
i0	0	0	0	0	0
im	0	0	0	0	0
fv/im [%]	0	0	0	0	0
tau (ms)	n.a.	0	0	0	0

PA scale: 0 - 100 [%]      Blue background Fv/FM      FL scale: 0 - Max intensity

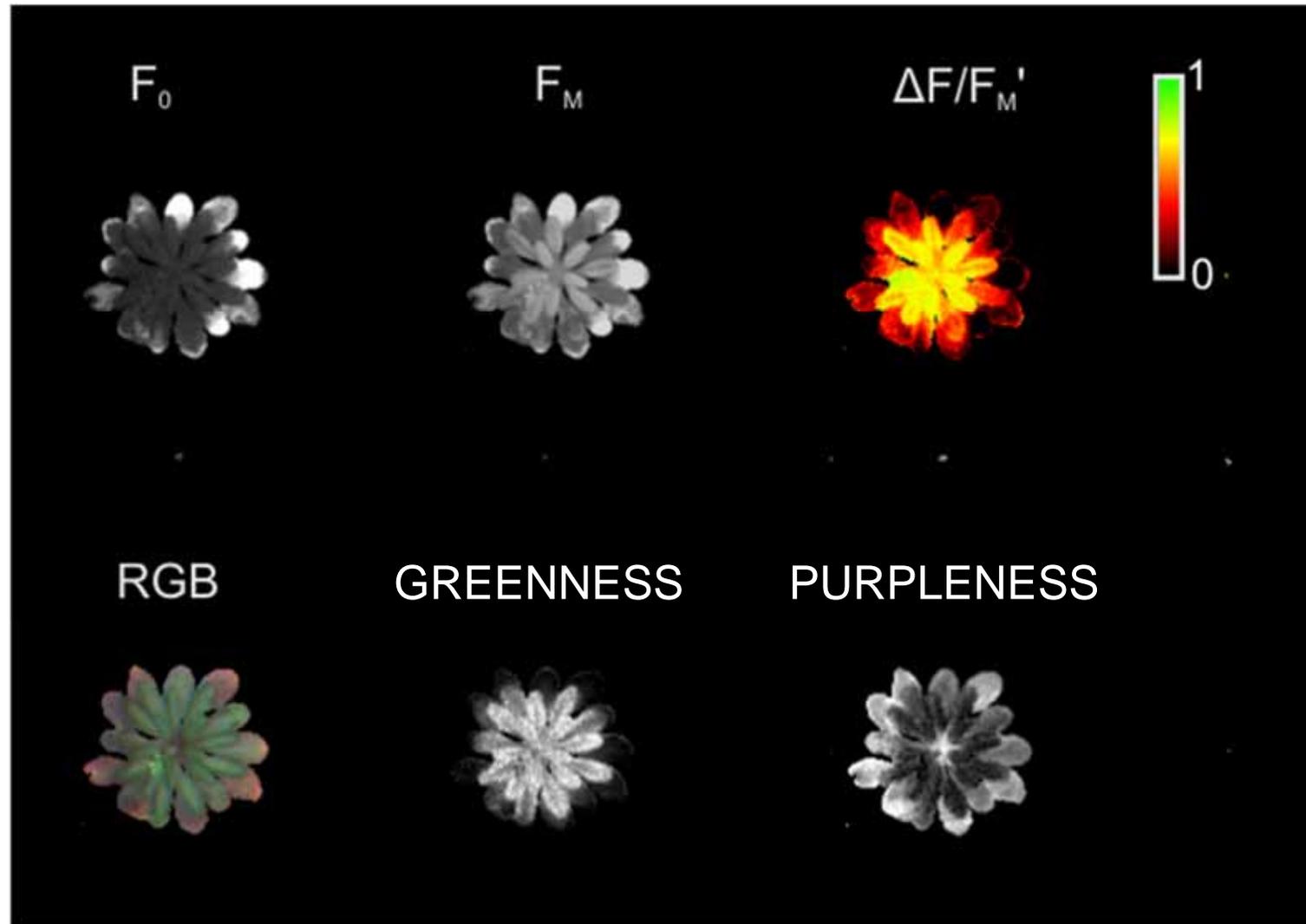
14 nov 10 20:52      320x240      File type: CF-LED Camera version 3 (Single puls)



# XY-table: *Arabidopsis* under normal phosphate



# XY-table: low phosphate



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# Applications of CF-Technology

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- Herbicide action: optimization of formulation, adjuvants
- High light damage: depends on cultivar and climate conditions
- Screening on diseases: susceptible or resistant cultivars with respect to different
- Screening on nutrition: phosphate or salinity (coastal area)
- Screening on drought: example on Hedera plants



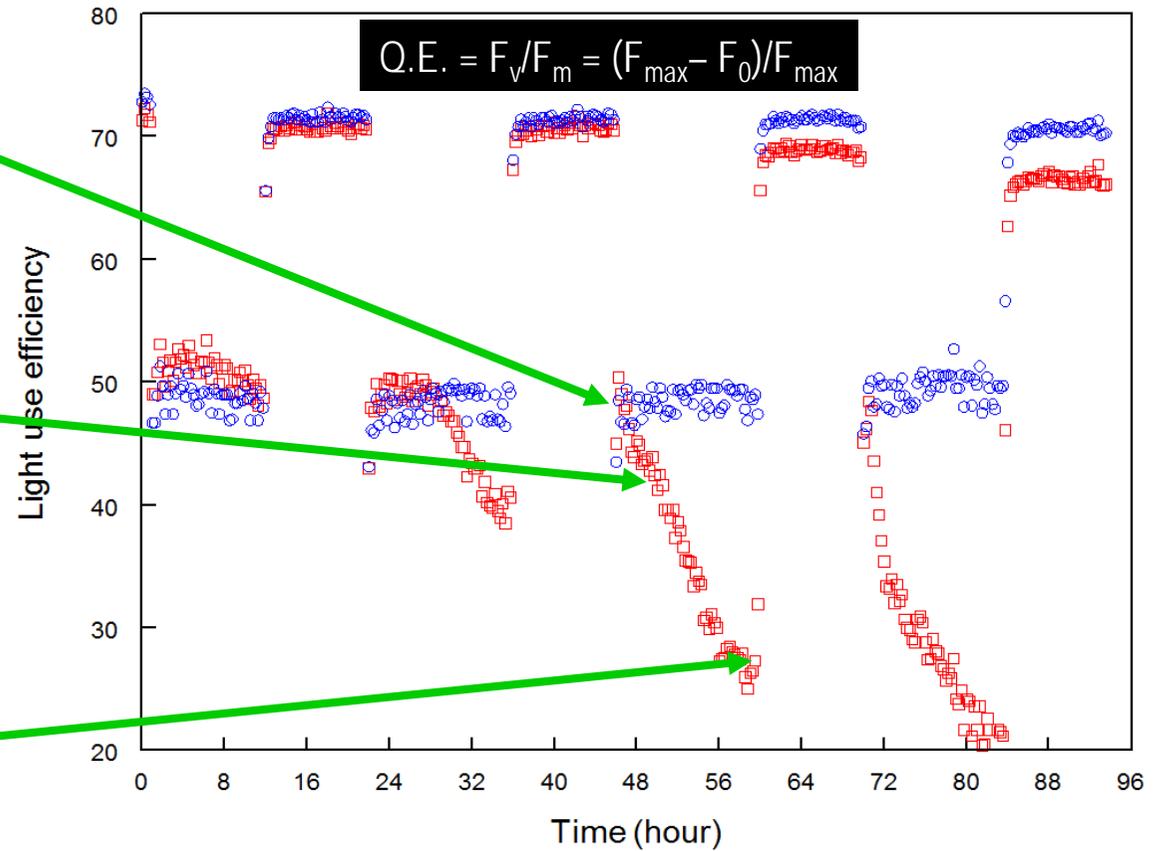
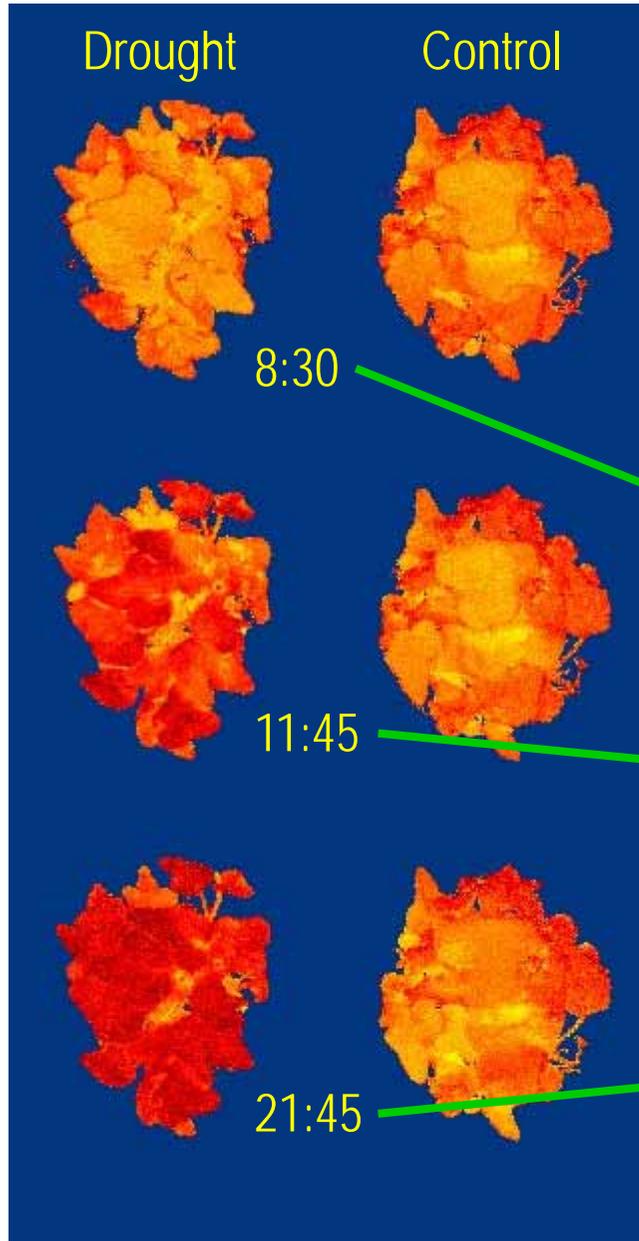
# Drought experiment on plants



- Two Hedera plants followed in time
- Every 15 min a measurement
- Day/night 14/10 hours
- One is drying out
- The other one is the control



# Change in photosynthesis due to drought stress



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# Summary on fluorescence imaging

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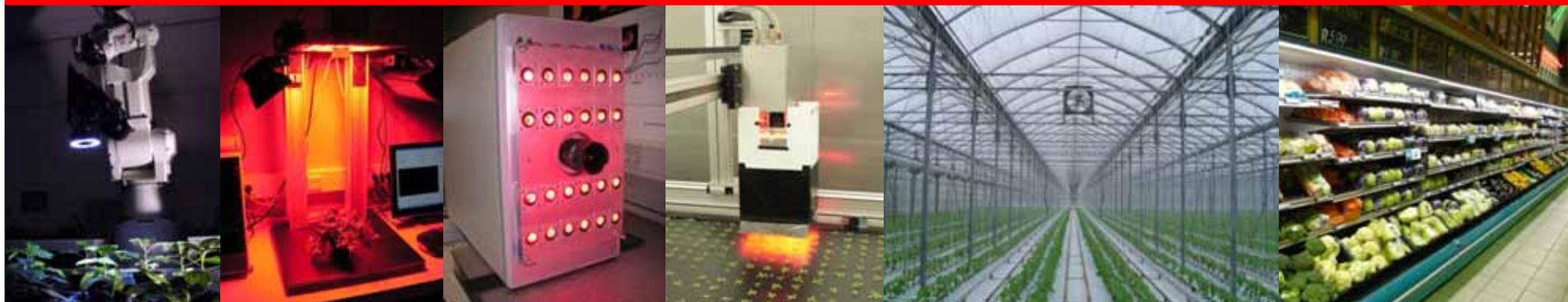
- New equipment developed for imaging the photosynthesis using chlorophyll fluorescence technology
- Functioning of photosynthesis can be visualized on an  $F_v/F_m$  image
- Factors that influence the photosynthetic apparatus, like herbicides, light stress, phosphate deficiency and drought, show up in the  $F_v/F_m$  image; by eye usually no stress can be observed
- Plants can be monitored continuously at different scenarios using the XY-Phenotyper facility: information on  $F_v/F_m$ , anthocyanin and chlorophyll distribution



# Coming soon



Thank you for your attention



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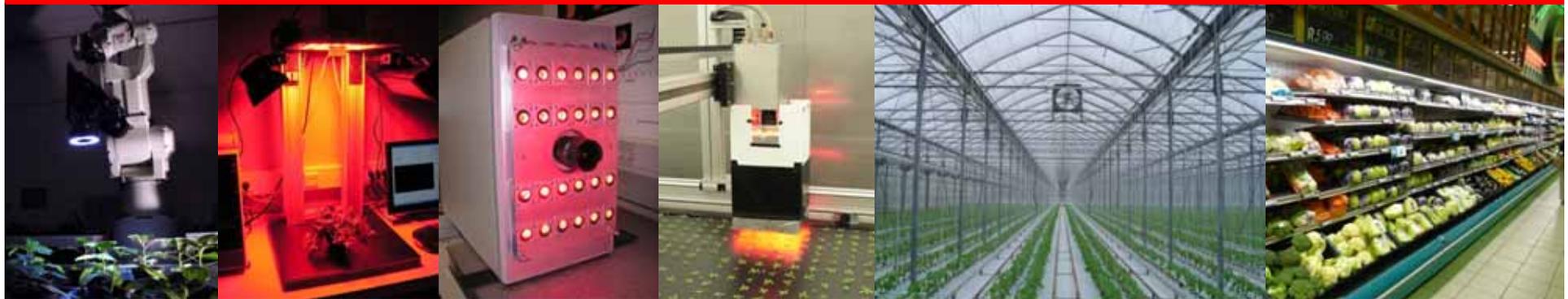
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