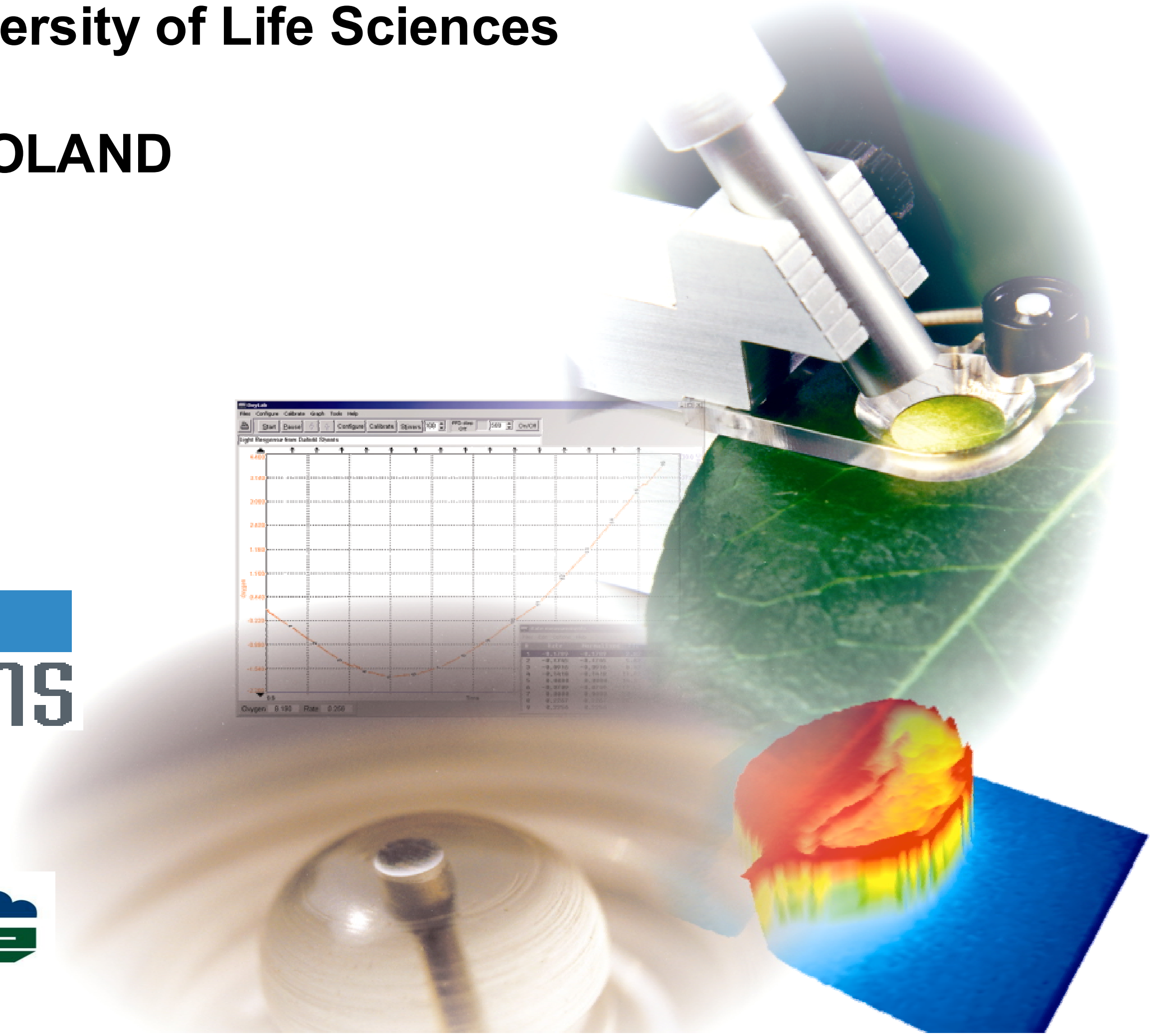
Chlorophyll *a* Fluorescence: a useful tool for biological, agricultural and aquatic research

Dr Hazem M. Kalaji

Warsaw University of Life Sciences

POLAND



bbe moldaenke

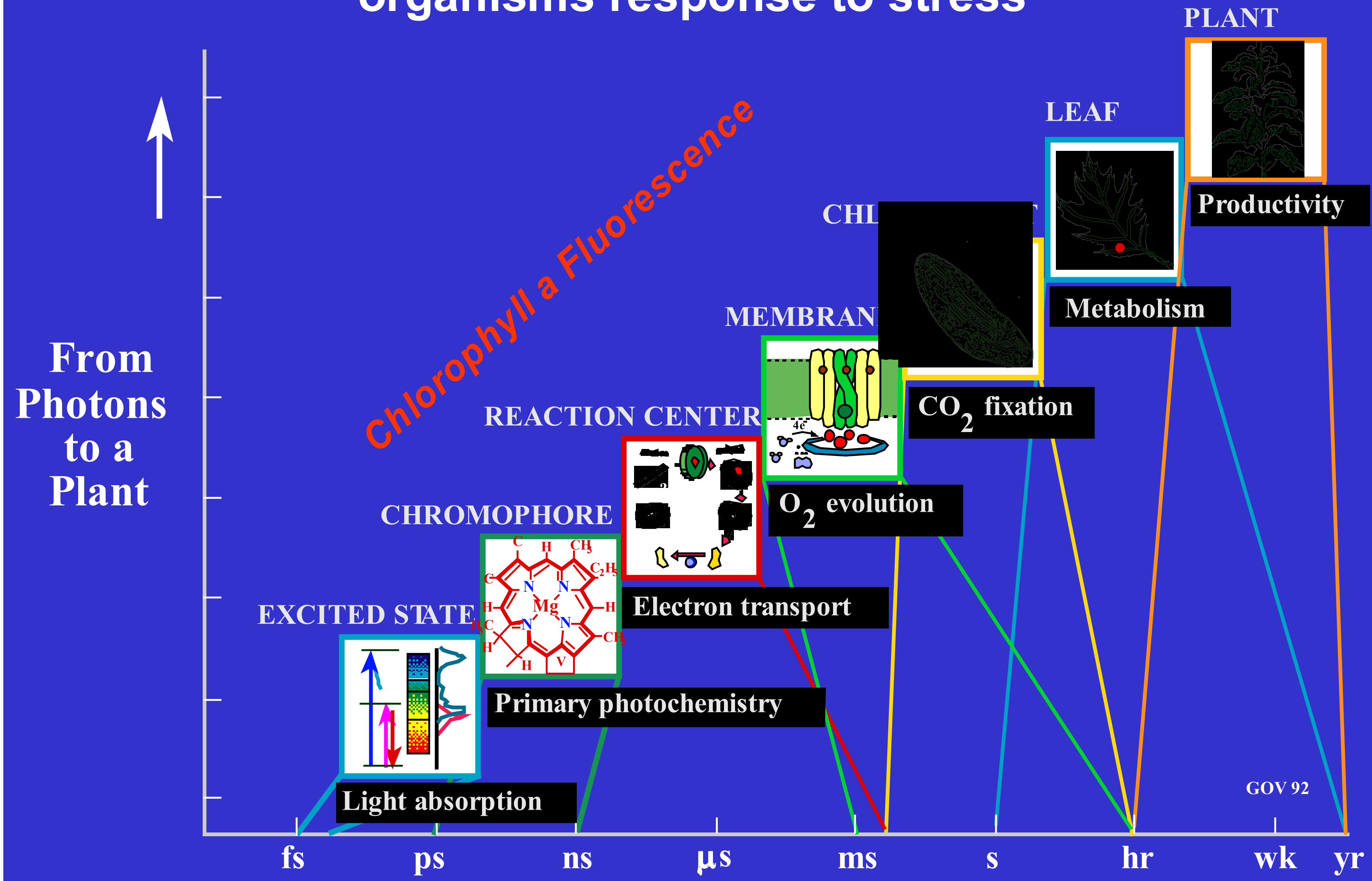
Hansatech Instruments

PP SYSTEMS

Photon Systems Instruments

Skye

Time-scales : understanding photosynthesizing organisms response to stress



Modified after Osmond and Chow, Aust J Plant Physiol 1988

ScienceDaily

Your source for the latest research news

Web address:
http://www.sciencedaily.com/releases/2008/10/081008100616.htm

Green Fluorescent Protein Pioneers Share 2008 Nobel Prize In Chemistry

ScienceDaily (Oct. 8, 2008) — The Royal Swedish Academy of Sciences has awarded the Nobel Prize in Chemistry for 2008 jointly to Osamu Shimomura, of the Marine Biological Laboratory and Boston University Medical School, Martin Chalfie of Columbia University, and Roger Y. Tsien of the University of California, San Diego "for the discovery and development of the green fluorescent protein, GFP."

The green fluorescent protein GFP consists of 238 amino acids, linked together in a long chain. This chain folds up into the shape of a beer can. Inside the beer can structure the amino acids 65, 66 and 67 form the chemical group that absorbs UV and blue light, and fluoresces green. (Credit: Image courtesy of Nobel Foundation)

Glowing proteins – a guiding star for biochemistry

The remarkable brightly glowing green fluorescent protein, GFP, was first observed in the beautiful jellyfish, Aequorea victoria in 1962. Since then, this protein has become one of the most important tools used in contemporary bioscience. With the aid of GFP, researchers have developed ways to watch processes that were previously invisible, such as the development of nerve cells in the brain or how cancer cells spread.

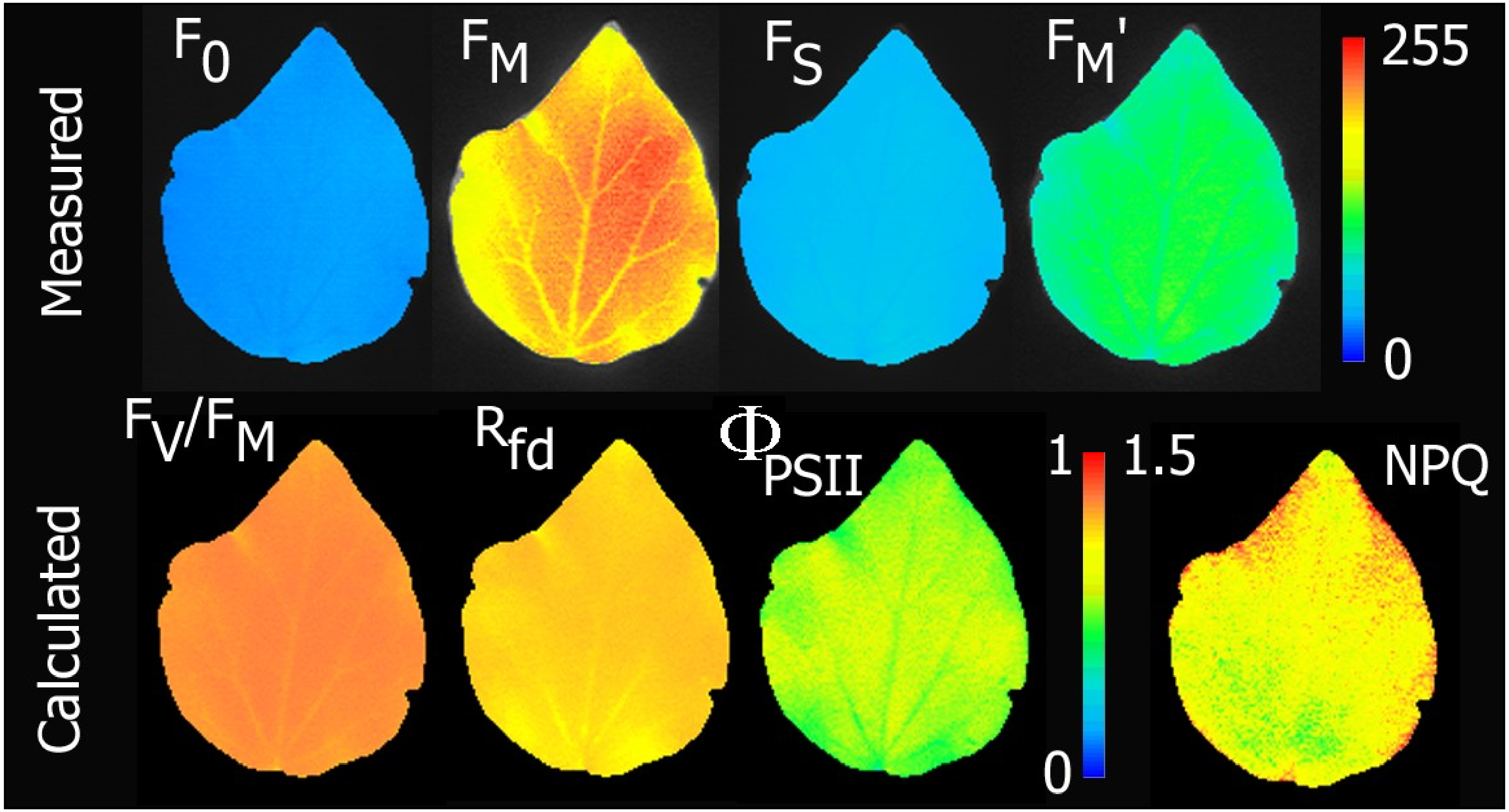
Tens of thousands of different proteins reside in a living organism, controlling important chemical processes in minute detail. If this protein machinery malfunctions, illness and disease often follow. That is why it has been imperative for bioscience to map the role of different proteins in the body.

Singapore Polytechnic students provide way for your plants to talk to you Hopefully you'll never kill another plant!

Transgenic plants emitting green fluorescence to signal their dehydrated state.

How might one provide plants with a means to communicate their needs to us? One way is **to 'teach' plants 'optical' sign language**. Using genetic engineering, transgenic plants containing a green fluorescent marker gene from a jellyfish have been developed and our Biotech students. These plants "light' up when subjected to dehydration stress.

Pixel-to-pixel arithmetic image operations



Plant Fluorescence Sensor

Aerodyne Research, Inc., Billerica, MA

INNOVATIONS COMMERCIALIZATION ACCOMPLISHMENTS APPLICATIONS
NASA SBIR SUCCESS

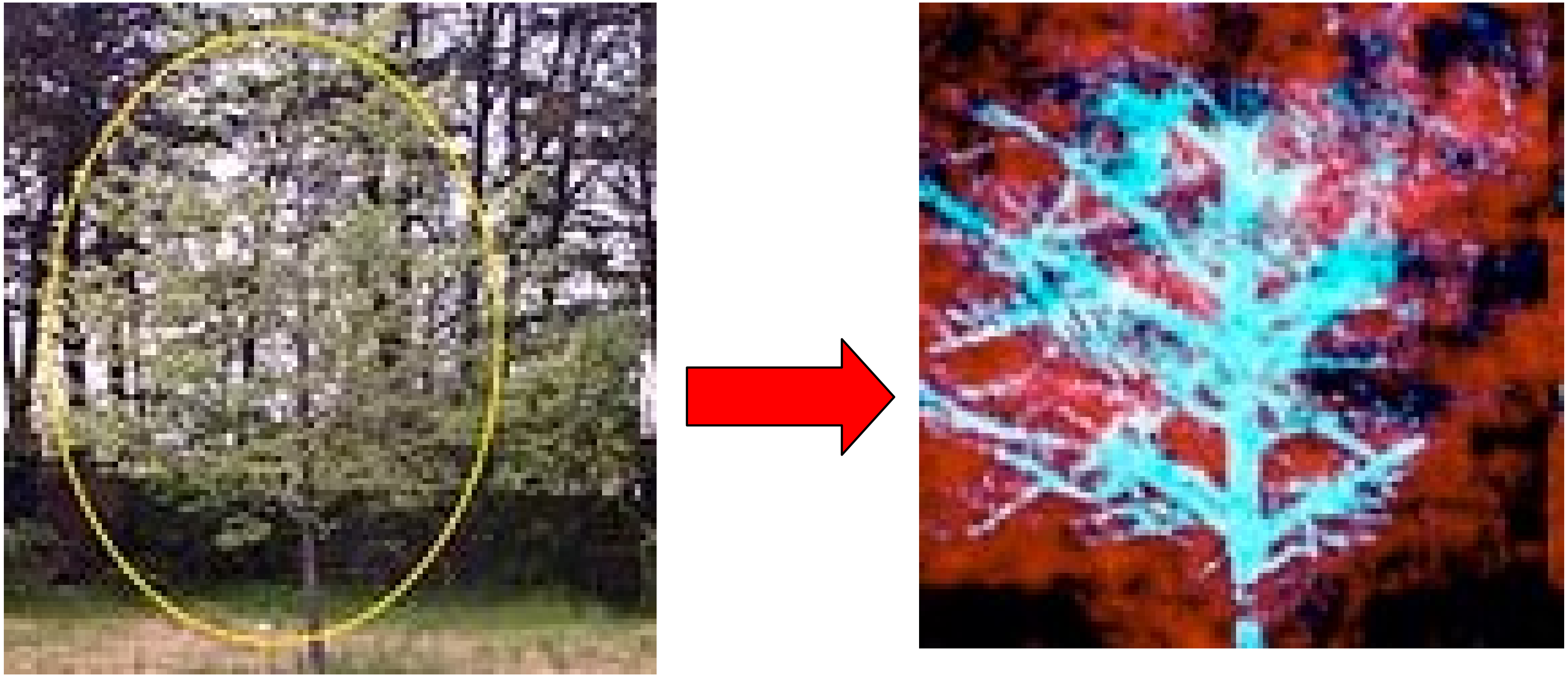
- Applications of immediate benefit to NASA, the USDA, and the private sector include: Vegetation characterization and monitoring (crop condition assessment); Damage assessment (in response to a variety of stressors); and,

Stennis Space Center
February 2000

- Publication: Kebabian, P.L., Theisen, A.F., Kallelis, S. and Freedman, A. (1998), A Passive Two-Band Sensor for Sunlight-Excited Plant Fluorescence, *Rev. Scien. Instrumen.,* in review.

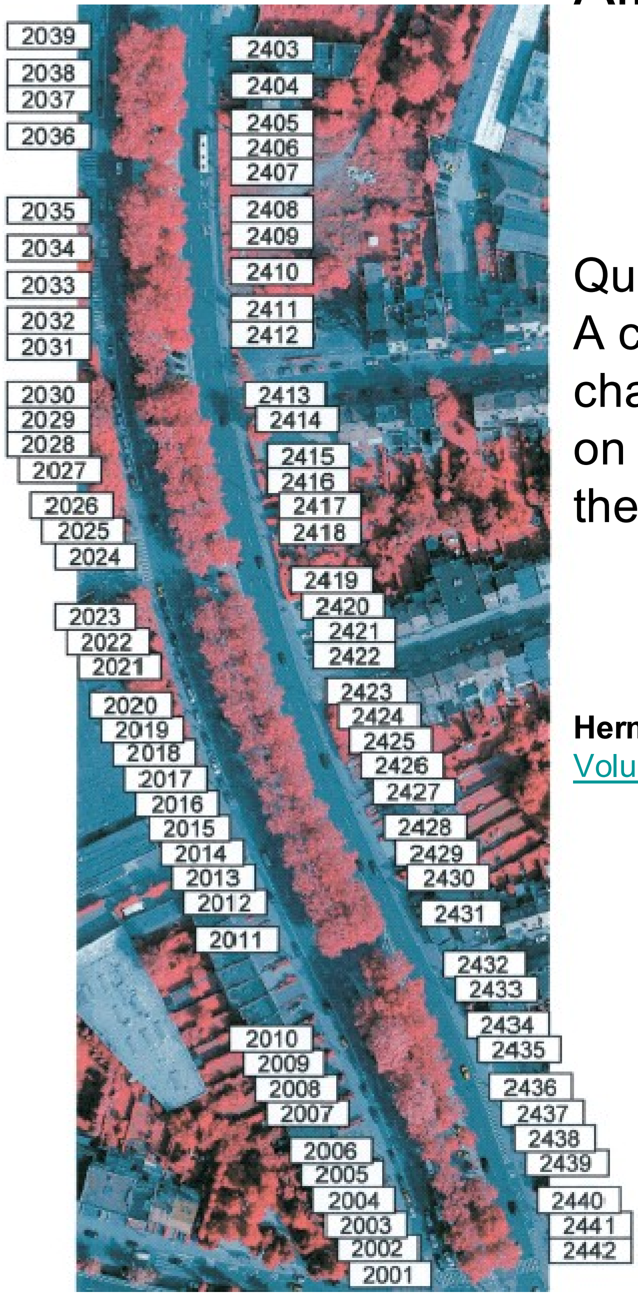
Remote Sensing of Vegetation Fluorescence

The FLuorescence EXplorer bla (**FLEX**) mission proposes to launch a satellite for the global monitoring of steady-state chlorophyll fluorescence in terrestrial vegetation



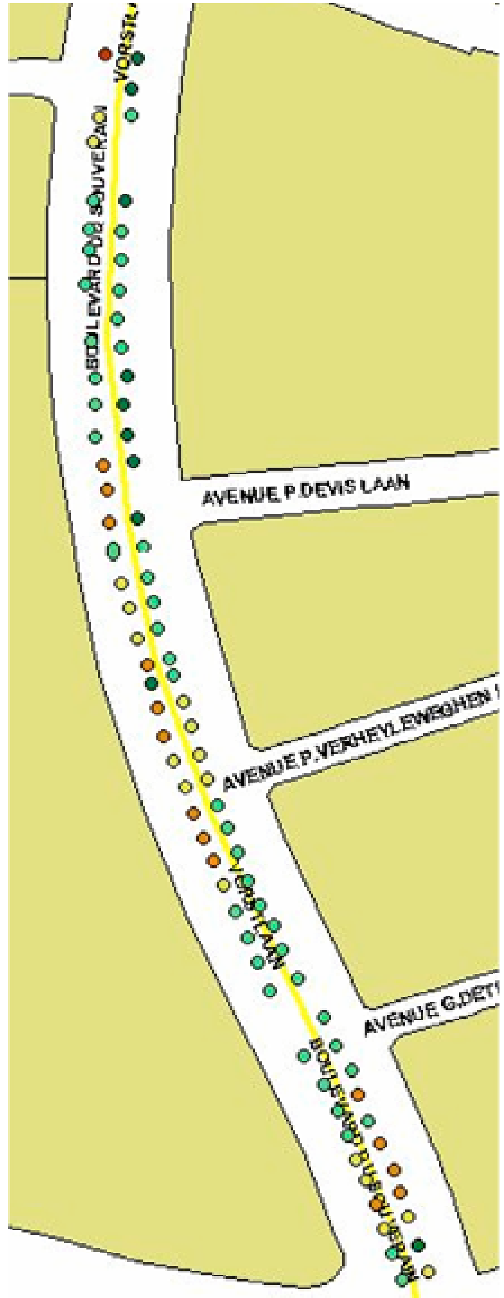
European Space Agency (http://www.esa.int/esaLP/SEM2VSBE8YE_index_0.html)

Airborne Remote Sensing



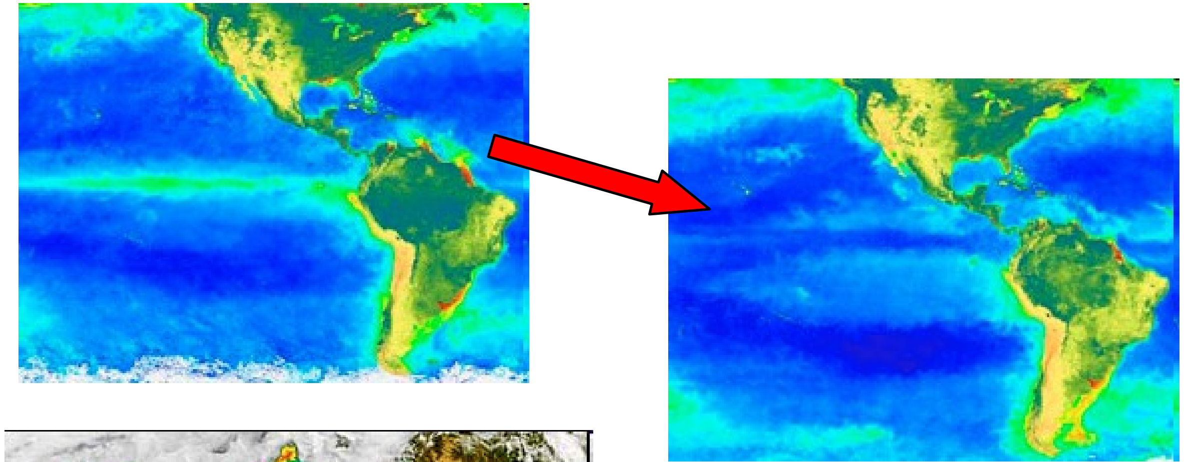
Quality assessment of urban trees:
A comparative study of physiological characterisation, airborne imaging and on site fluorescence monitoring by the OJIP-test

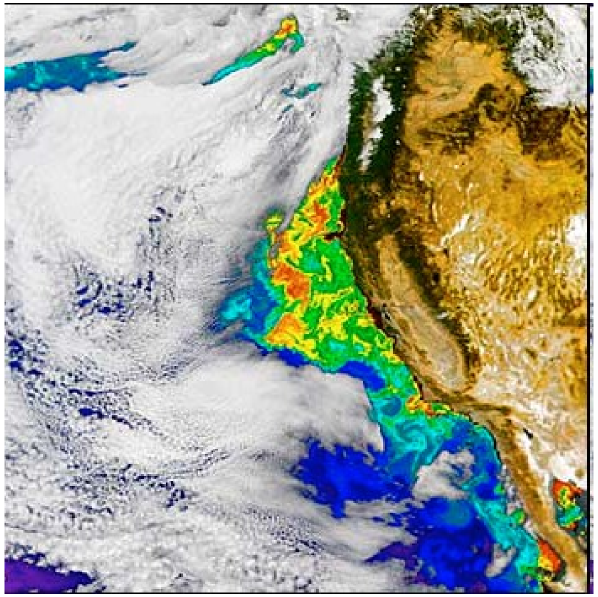
Hermans C et al. **Journal of Plant Physiology**
Volume 160, Issue 1, 2003, Pages 81-90

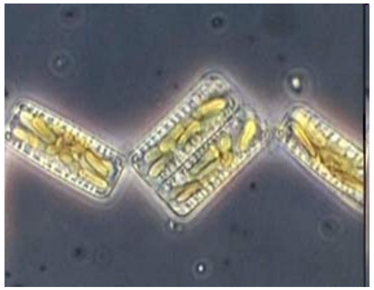


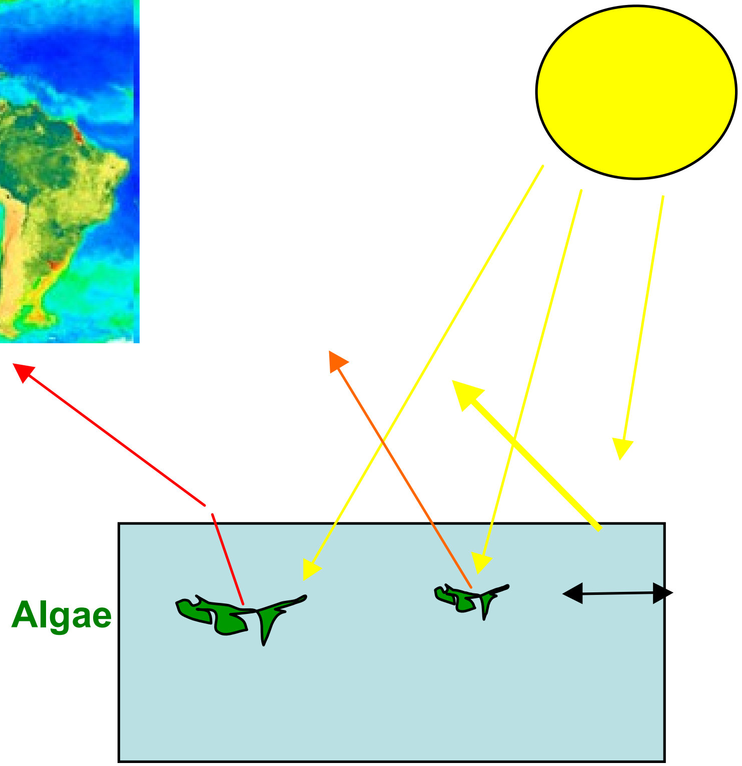
Pseudo-colour mapping of the deviation of the Performance Index relatively to the average PI by tree species in the park Leopold, Brussels

Using Chlorophyll Fluorescence Methods For Algae Vitality Detection as Biological Defense System



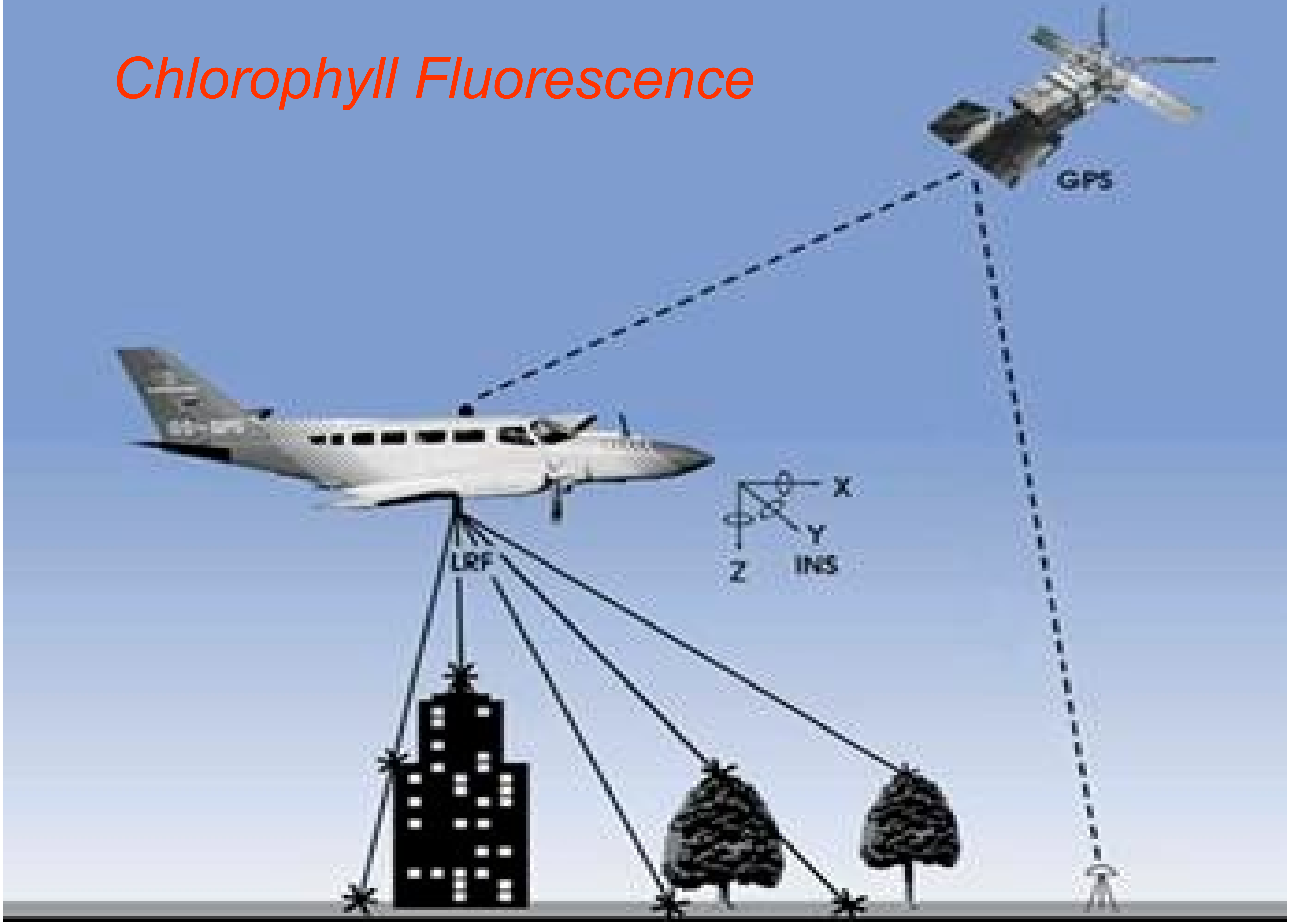




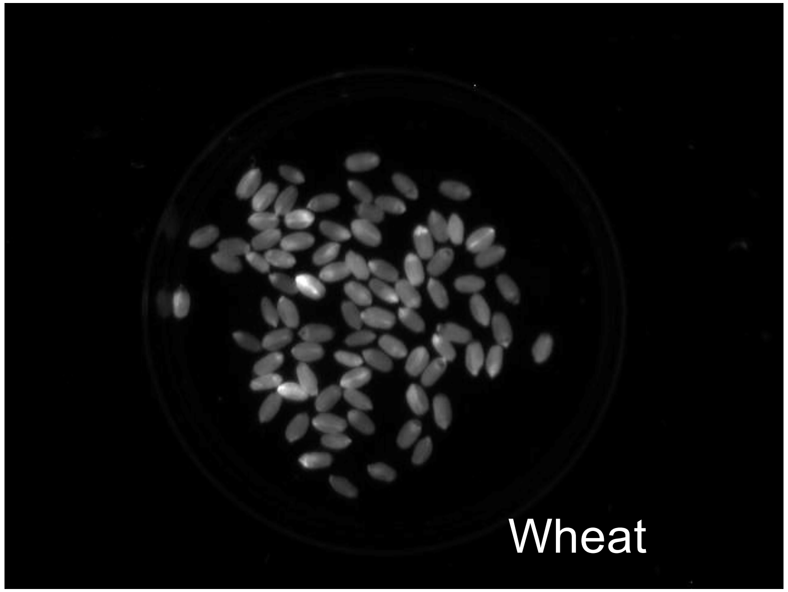


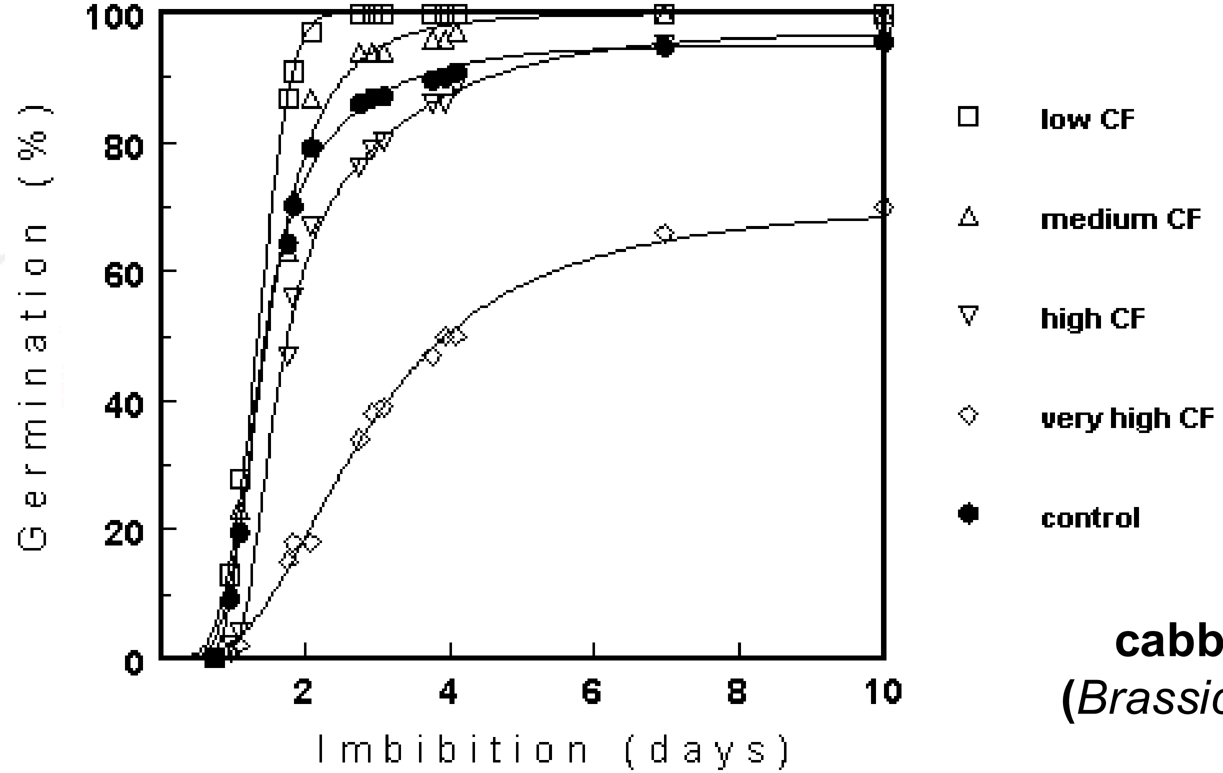
http://daac.gsfc.nasa.gov/oceancolor/scifocus/oceanColor/warming.shtml
http://oceancolor.gsfc.nasa.gov/

Chlorophyll Fluorescence



Wageningen University and Research Centre

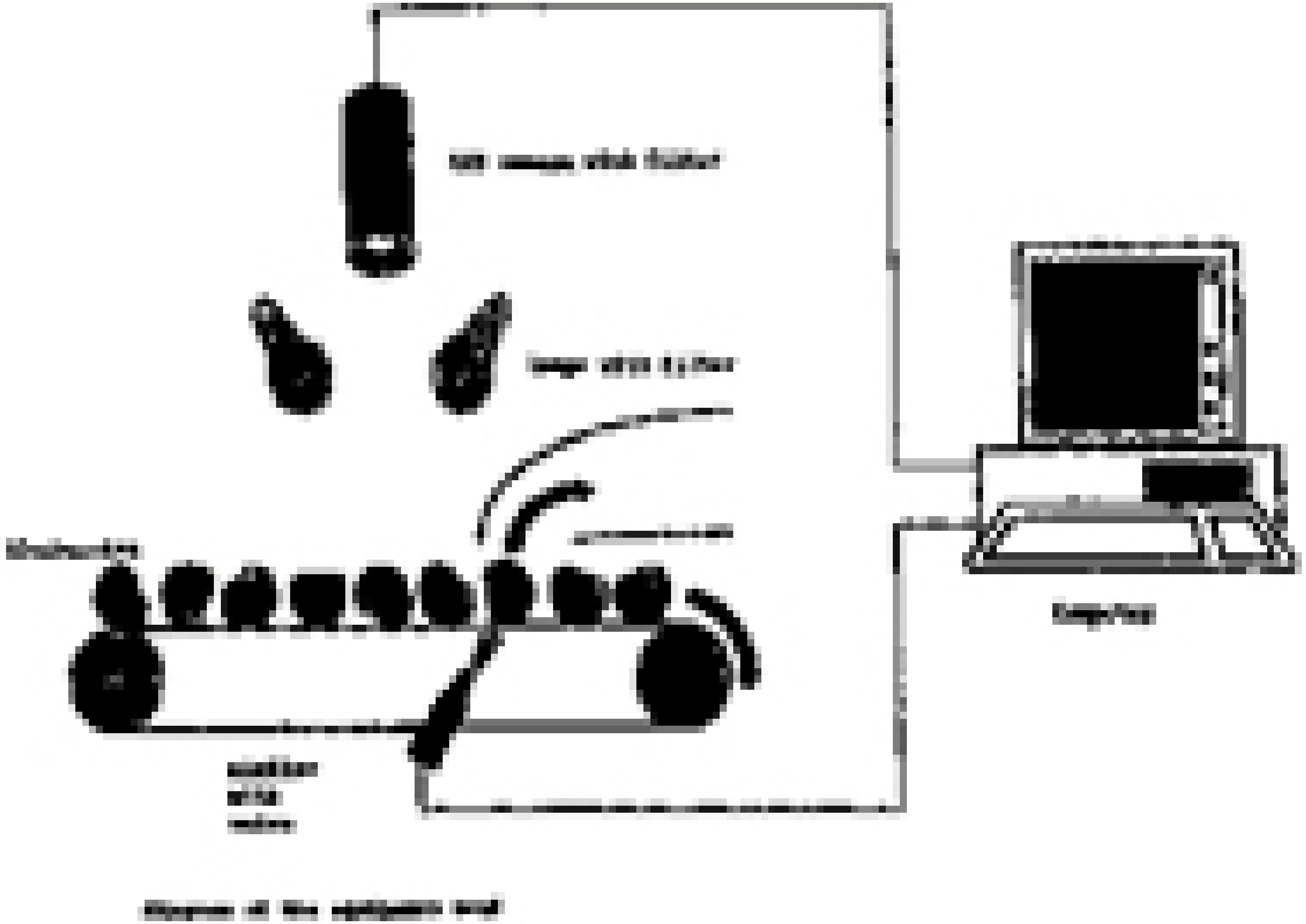




cabbage seeds
(*Brassica oleracea*)

H. JALINK et al., Sci. agric., Piracicaba, 55(Número Especial), p.88-93, agosto 1998

SORTING



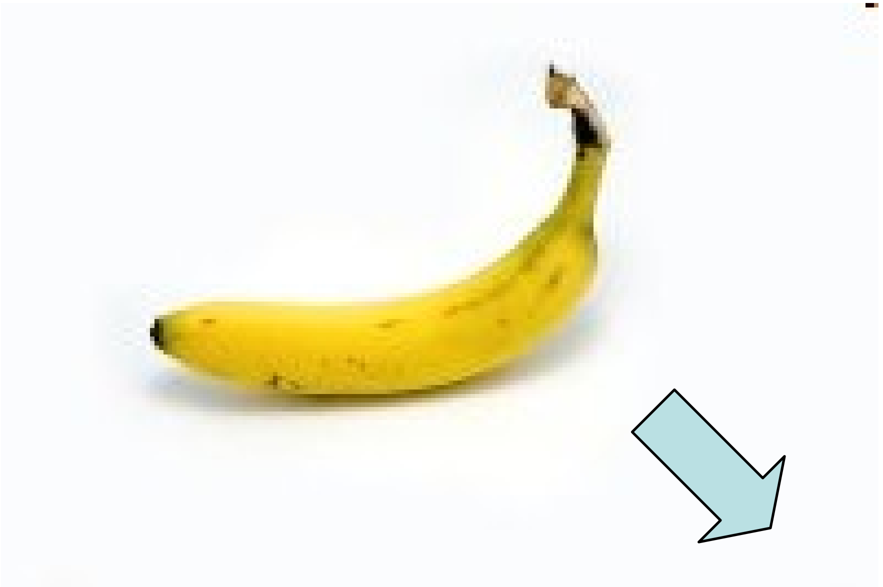
Jalink, Hendrik (NL); Schoor, Rob van der (NL); Bino, Raoul John (NL).

Wageningen University and Research Centre

Determining quality of fruit and berries and apparatus for sorting

Ripe bananas look blue in UV light, 22 Oct, 2008

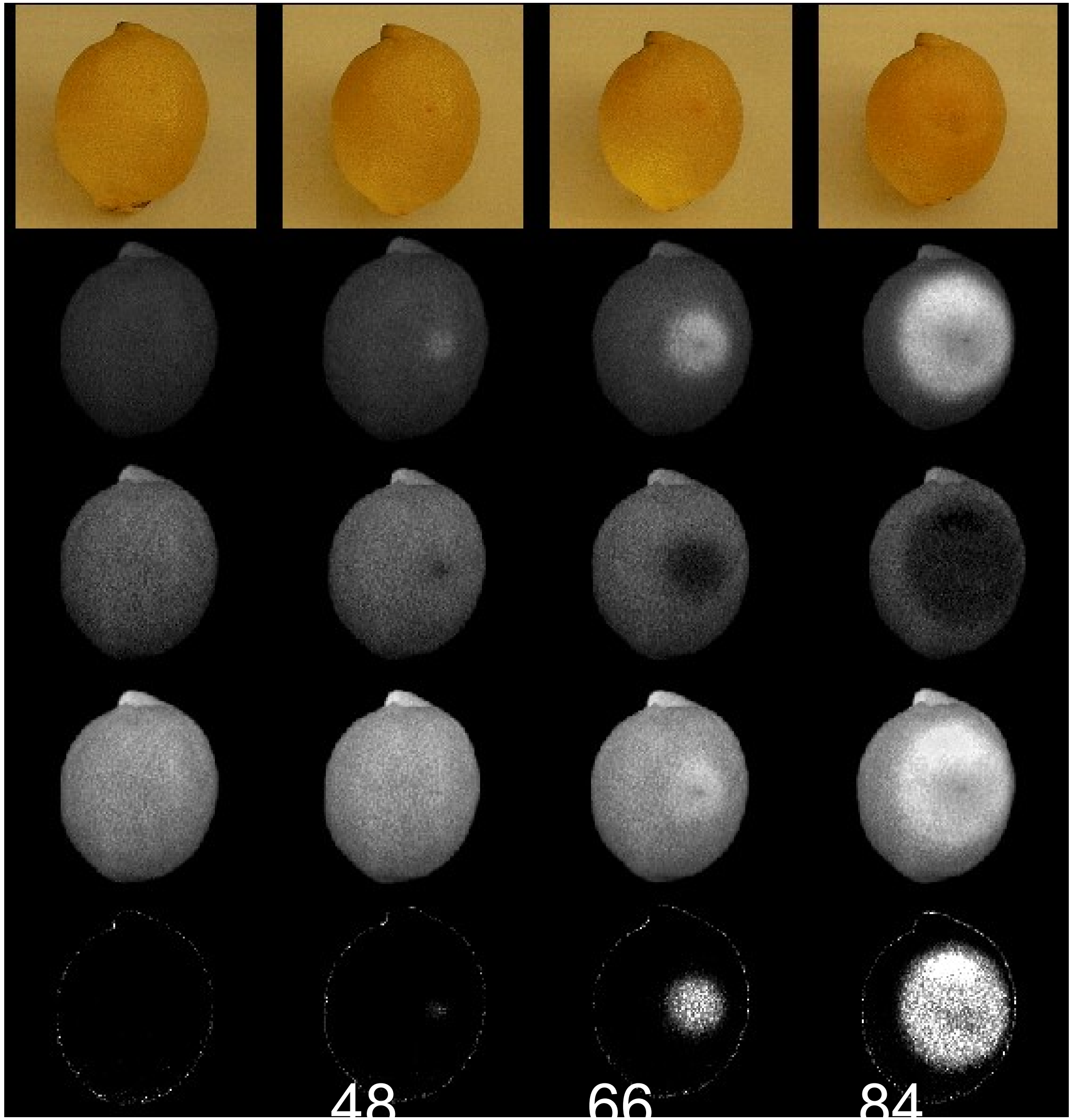
(University of Innsbruck Prof. *Bernhard Krautler*)



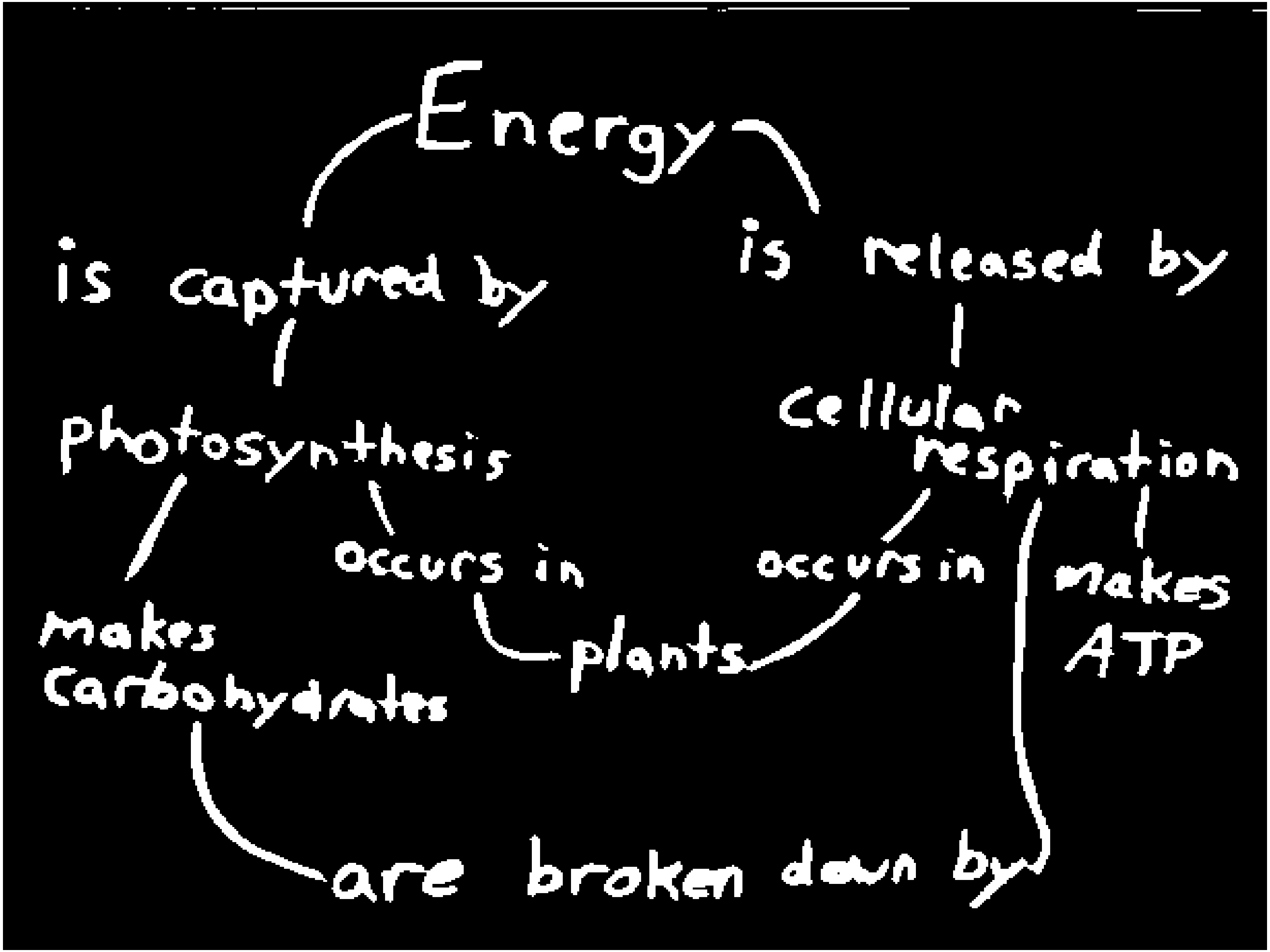
Green mold infection of lemons

Color photographs

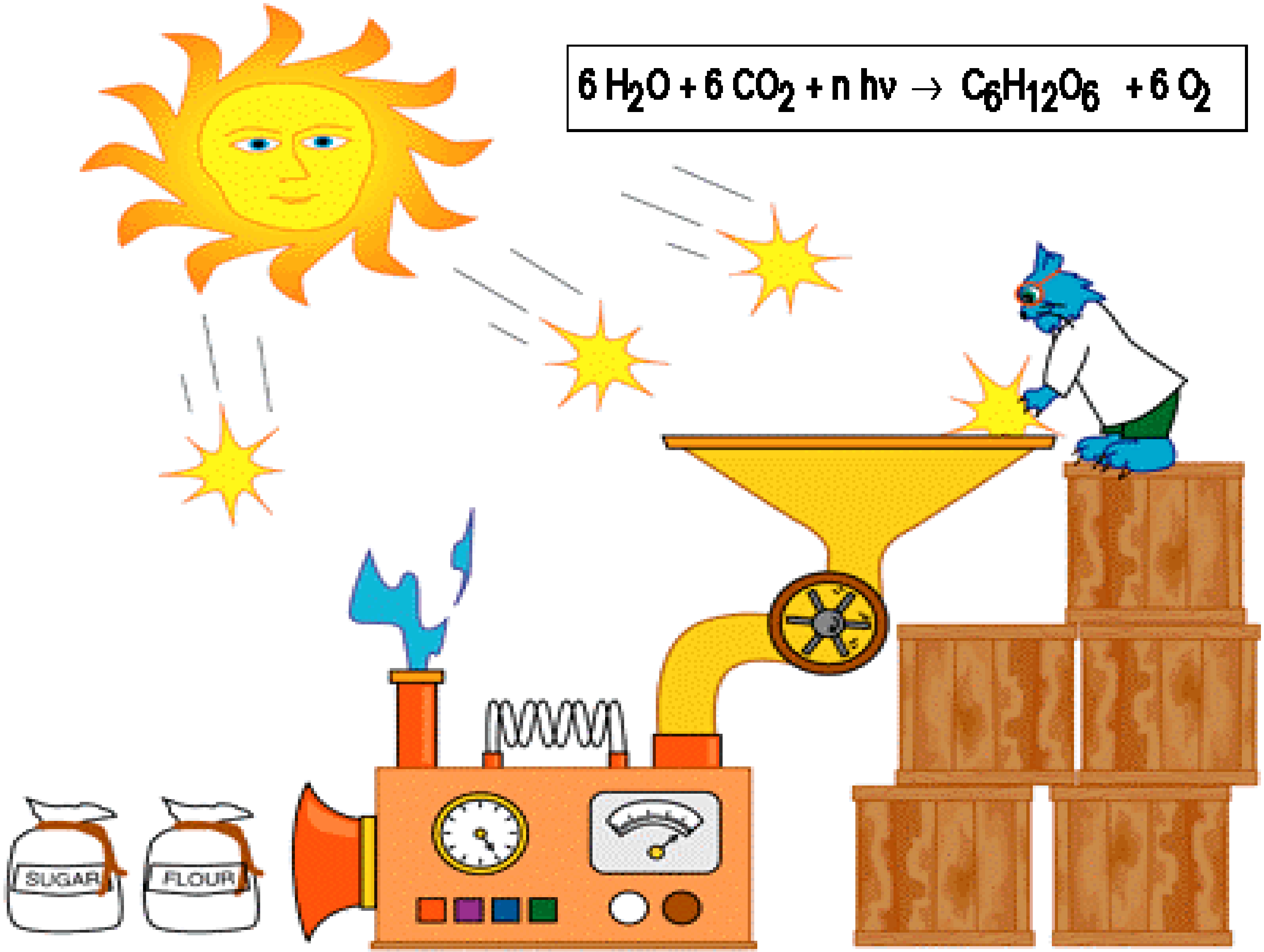
Fluorescence

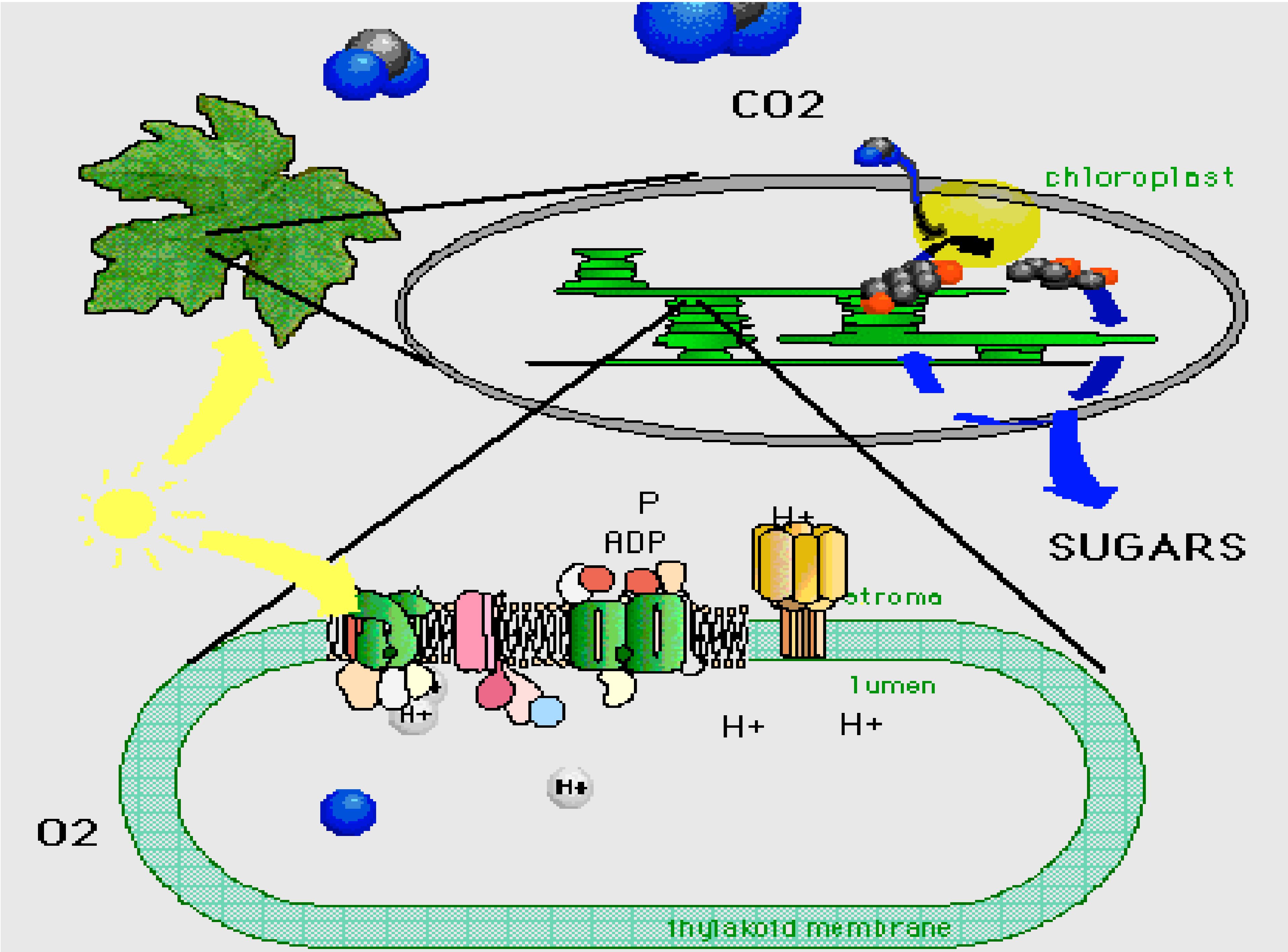


Photon Instruemnts, Bio-Sphere2, Tuscon AZ, Nov.29, 2001


Energy
is captured by
is released by
photosynthesis
cellular respiration
makes
carbohydrates
occurs in
occurs in
plants
makes
ATP
are broken down by

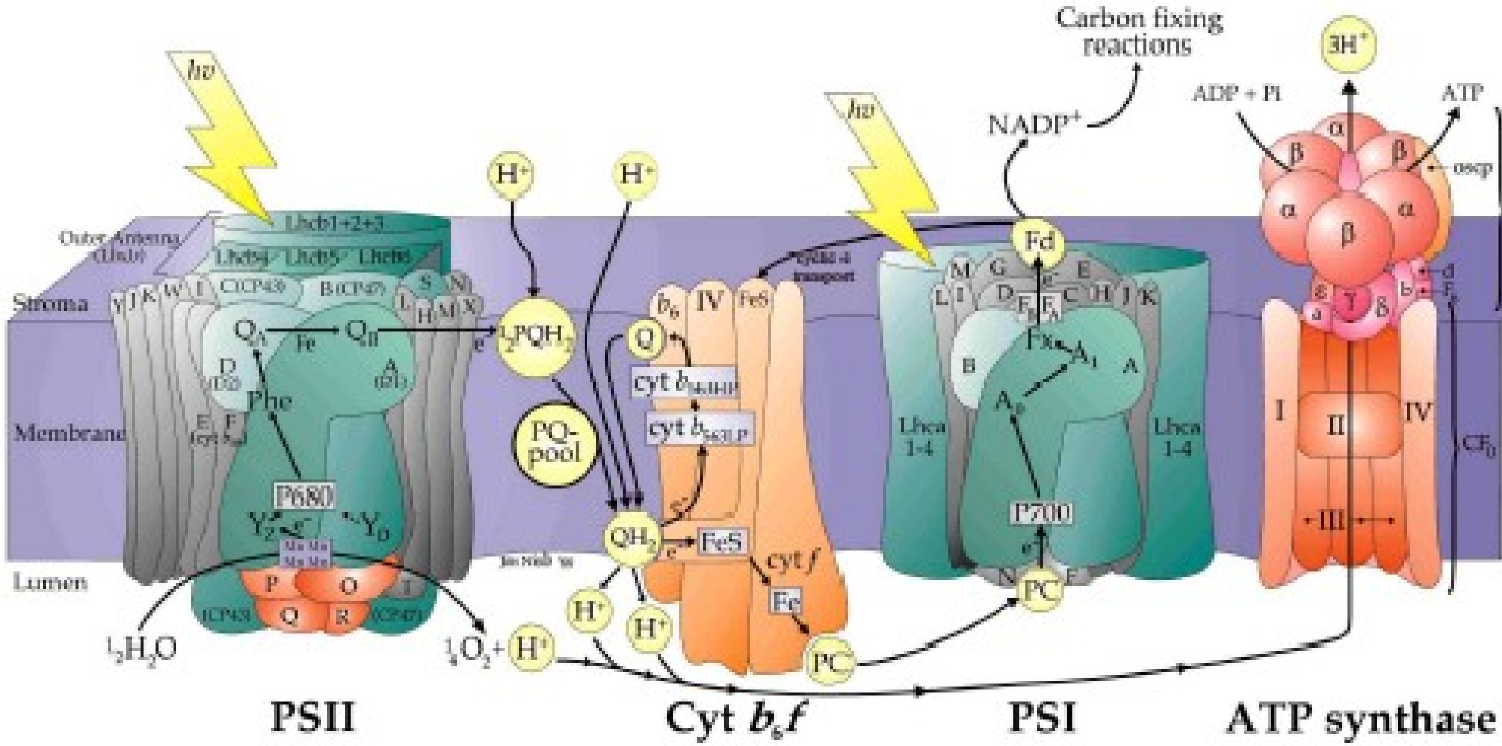
$$6\,H_2O + 6\,CO_2 + n\,h\nu \rightarrow C_6H_{12}O_6 + 6\,O_2$$

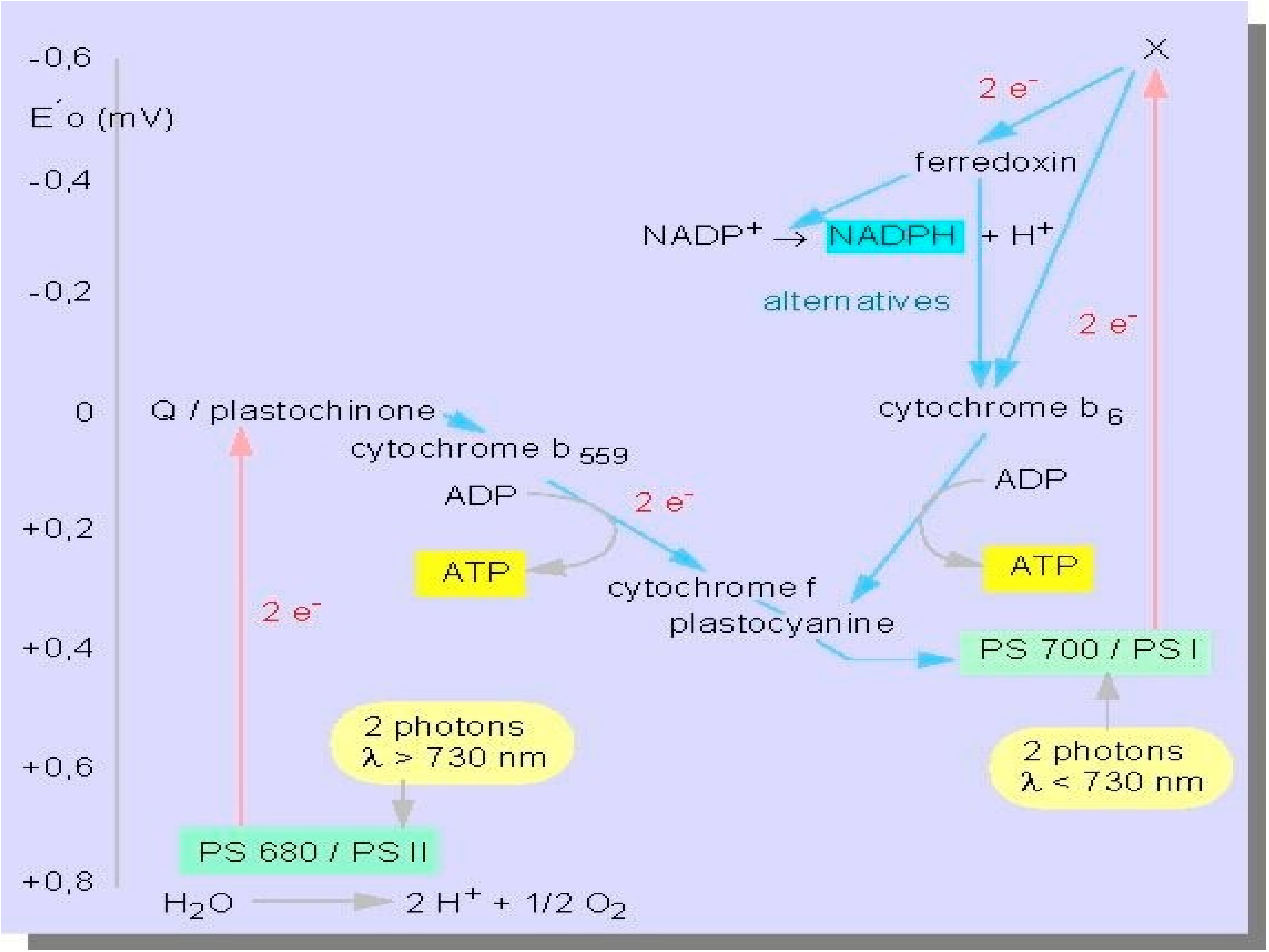




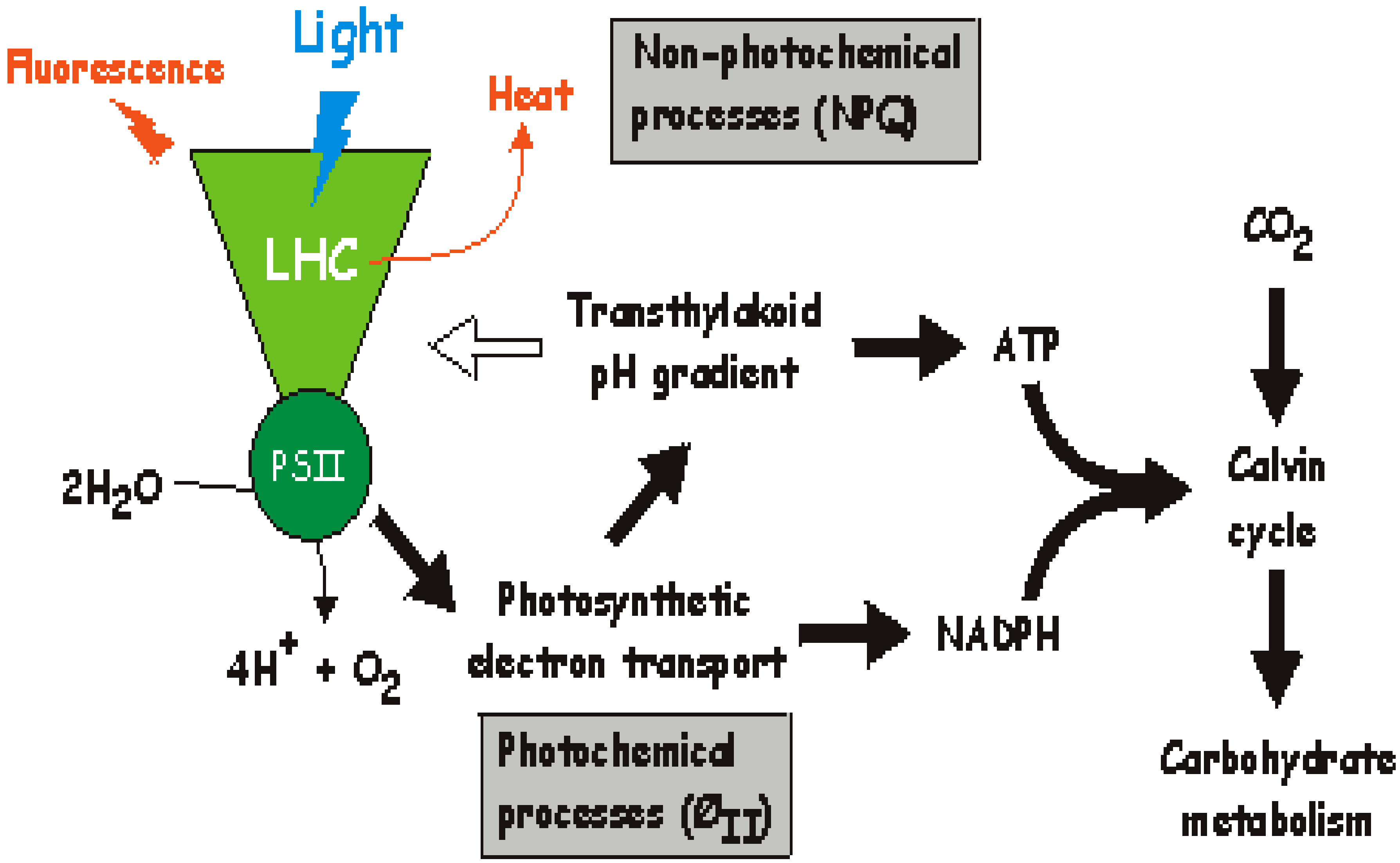
CO2
chloroplast
P
H+
ADP
SUGARS
stroma
lumen
H+
H+
H+
H+
O2
thylakoid membrane

...embedded in a membrane.





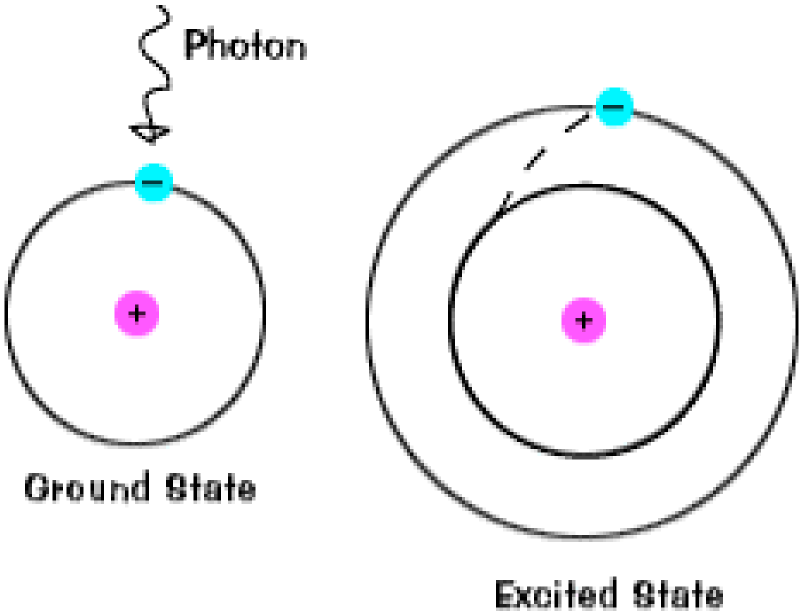
-0,6
E´o (mV)
-0,4
-0,2
0
+0,2
+0,4
+0,6
+0,8
X
2 e⁻
ferredoxin
NADP+ → NADPH + H+
alternatives
2 e⁻
cytochrome b 6
Q / plastochinone
cytochrome b 559
ADP
2 e⁻
ATP
cytochrome f
plastocyanine
ADP
ATP
2 e⁻
PS 700 / PS I
2 photons
λ > 730 nm
2 photons
λ < 730 nm
PS 680 / PS II
H2O → 2 H+ + 1/2 O2



Fluorescence
Light
Heat
Non-photochemical processes (NPQ)
LHC
PSII
$2H_2O$
$4H^+ + O_2$
Transthylakoid pH gradient
ATP
CO_2
Calvin cycle
Photosynthetic electron transport
NADPH
Photochemical processes (Φ_{II})
Carbohydrate metabolism

What is fluorescence ?

When light strikes chlorophyll molecules, absorbed quantum rises an electron from a ground state to an excited state. Upon chlorophyll de-excitation to ground state, a small portion of the excitation energy is dissipated as red fluorescence (690 nm).



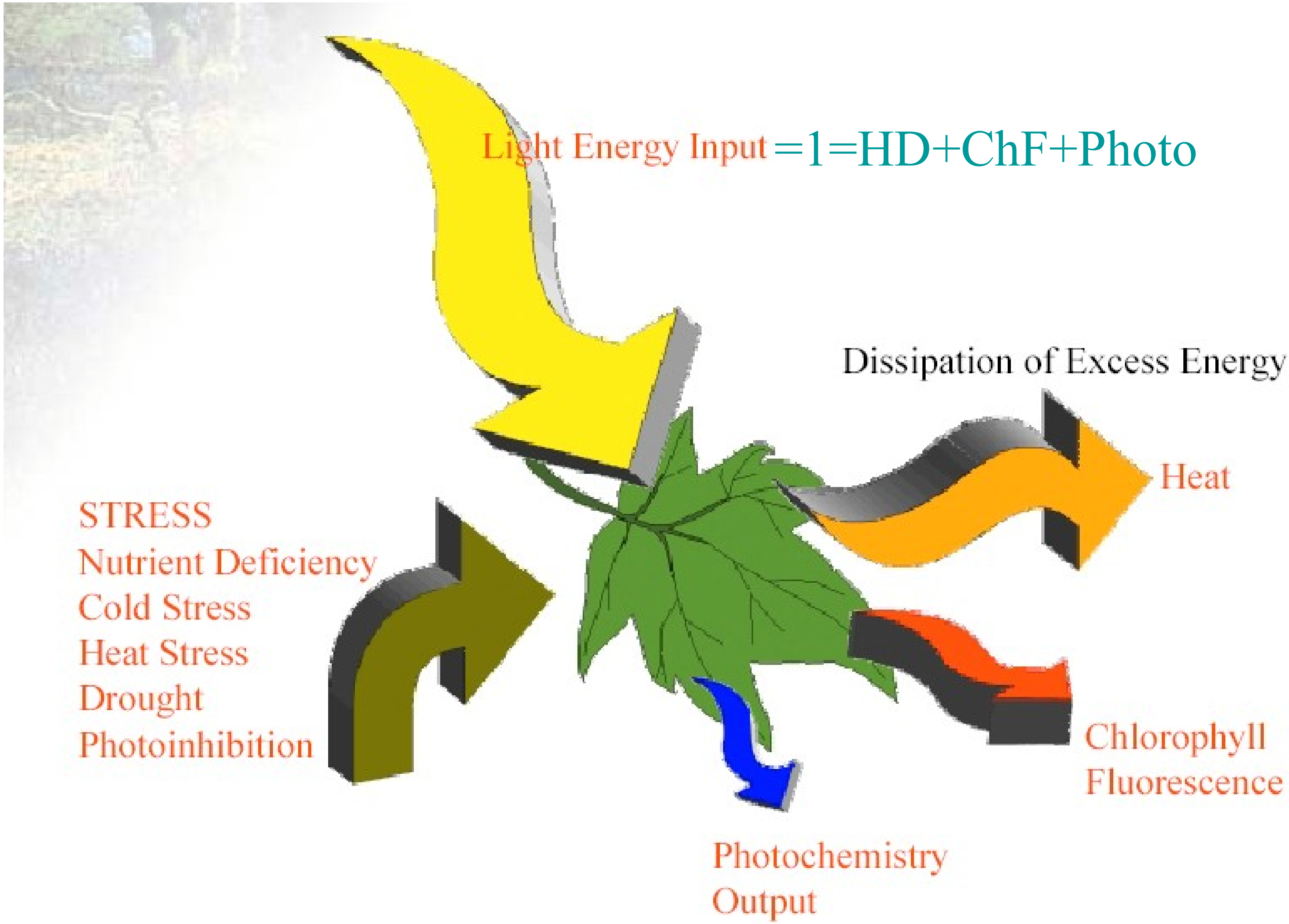
• Chlorophyll fluorescence is a **re-emission of light energy** absorbed by chlorophyll molecules.

UNITS:

relative (r.u.)

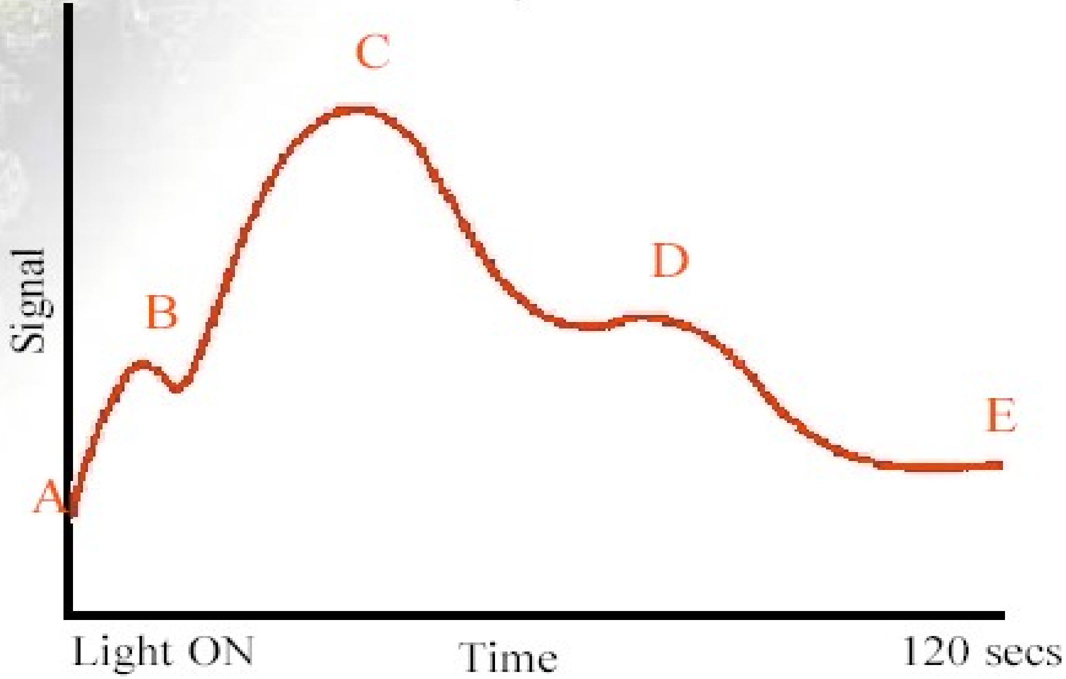
arbitrary (a.u.)

mv


Light Energy Input =1=HD+ChF+Photo
Dissipation of Excess Energy
Heat
STRESS
Nutrient Deficiency
Cold Stress
Heat Stress
Drought
Photoinhibition
Chlorophyll
Fluorescence
Photochemistry
Output

Fluorescence Induction Analysis (Kautsky Effect).

If a sample is **dark adapted** the PSII electron acceptor pool is gradually re-oxidised to a point where all of the PSII reaction centres are capable of undertaking photochemistry. Illumination induces a fast (approx. 1 s) polyphasic **rise in fluorescence** which is followed by a slow (approx. 2 min) fluorescence decline to a steady state level of fluorescence. This induction phenomena is often referred to as the **Kautsky Curve**.

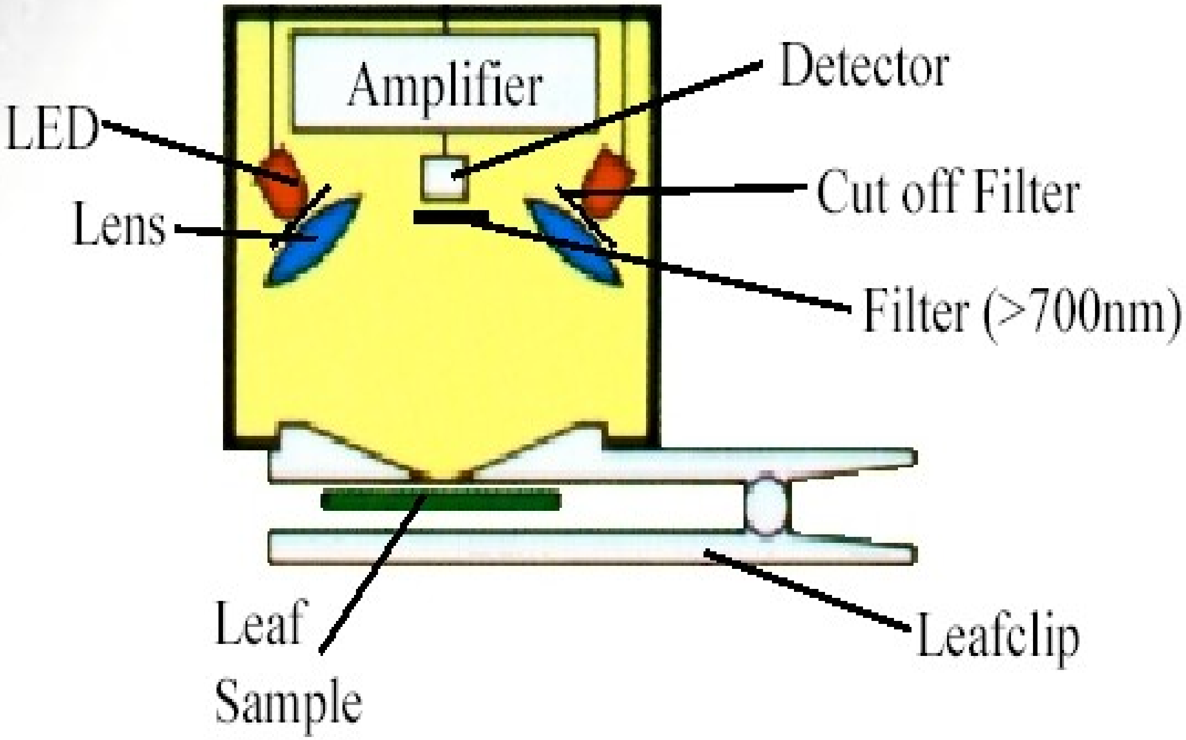


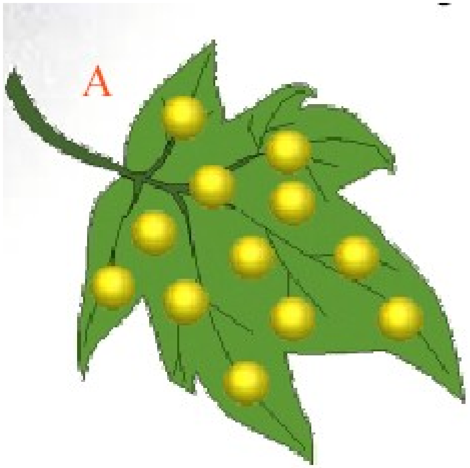
Kautsky and Hirsch (1931)

The reverse relation between Pn and ChF

Continuous Excitation Systems.

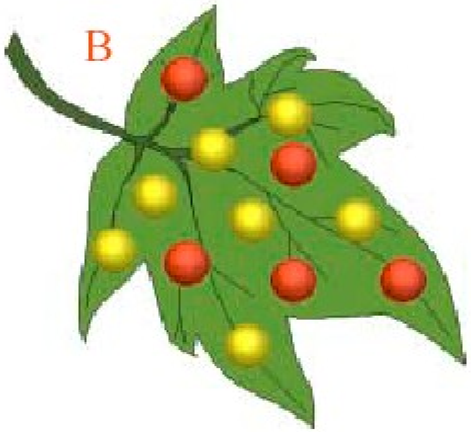
Optical filtering is used to separate the actinic light which illuminates the sample from the fluorescence signal. In such systems the sample **must be shielded from ambient light** to prevent the red / far-red component of daylight interfering with the measurement.





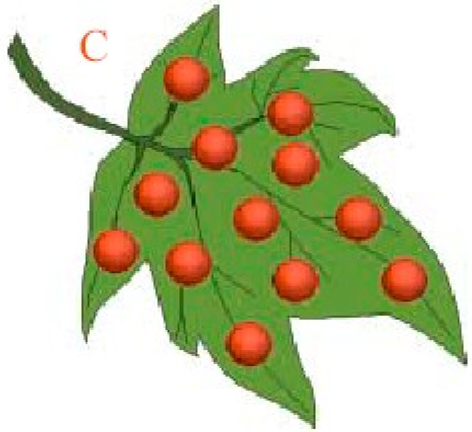
Dark Adapted State.

All electron acceptors fully oxidised & available to receive light energy.



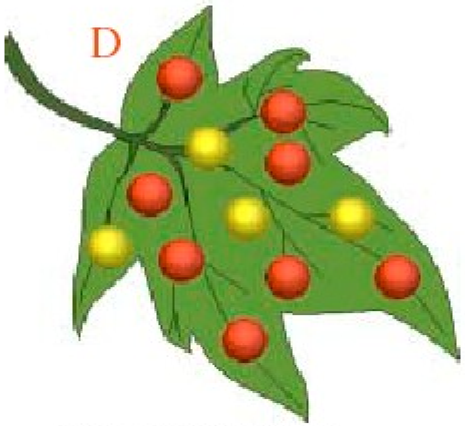
Intermediate State.

Some electron acceptors Reduced by light & no longer available for photochemistry.



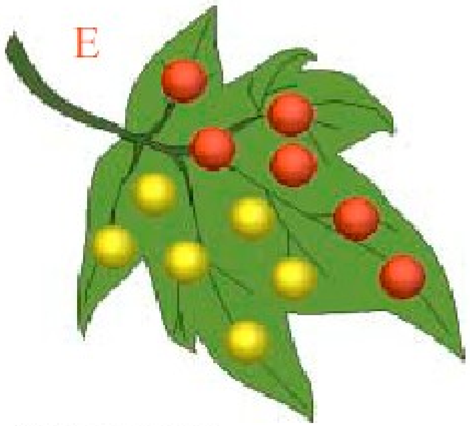
Light Saturated State.

All electron acceptors reduced by light & no longer available for photochemistry.



Quenching State.

Re-reduction of electron acceptors occurs as energy proceeds to photochemistry, acceptors are again available for photochemistry.



Steady State.

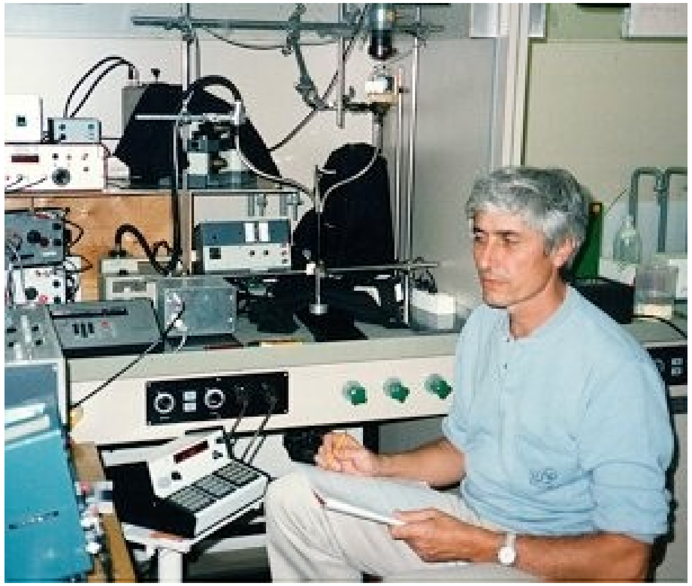
Equilibrium is established between energy input / dissipation processes and photochemistry.

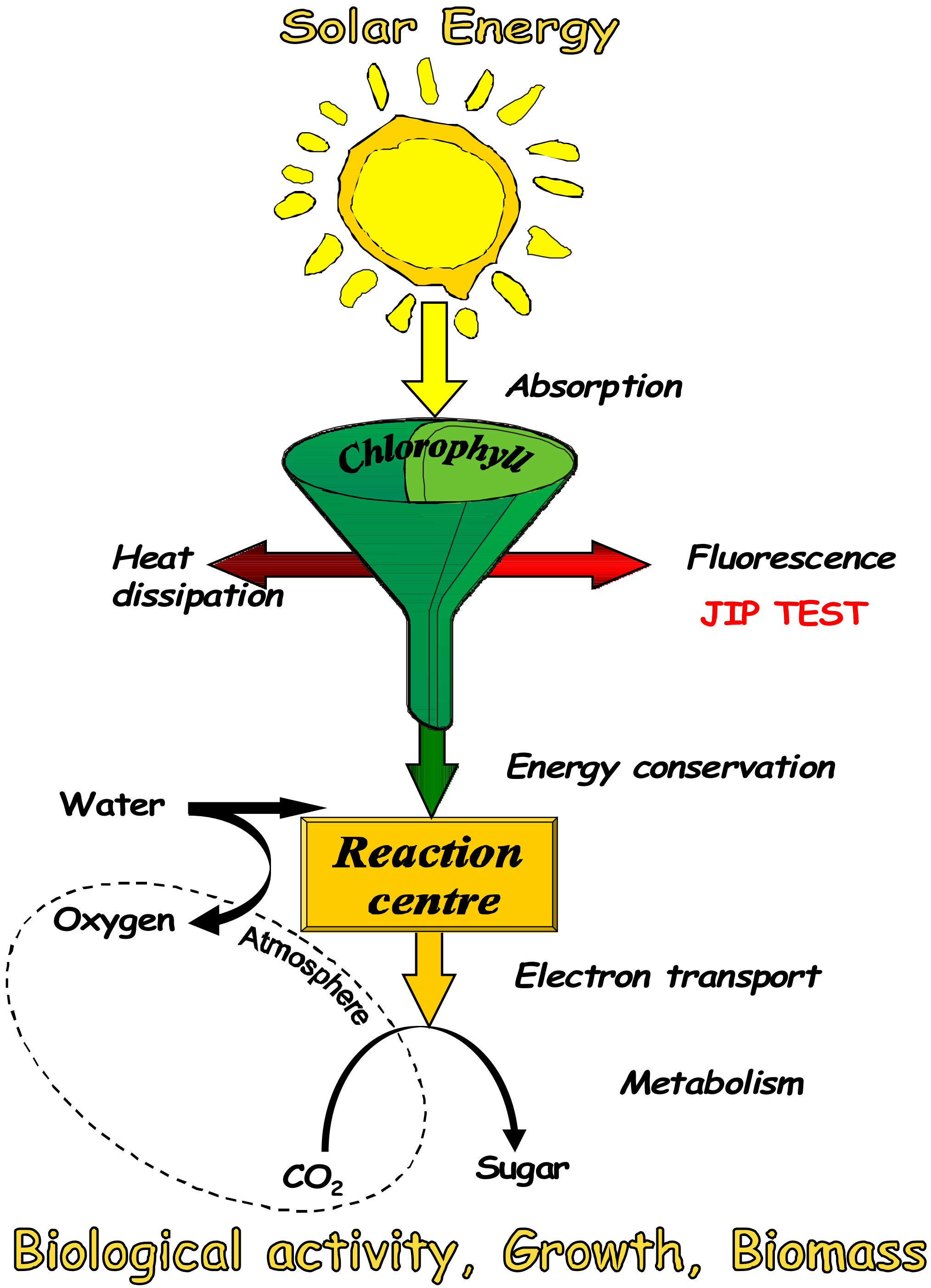
Oxidised electron acceptors

Reduced electron acceptors

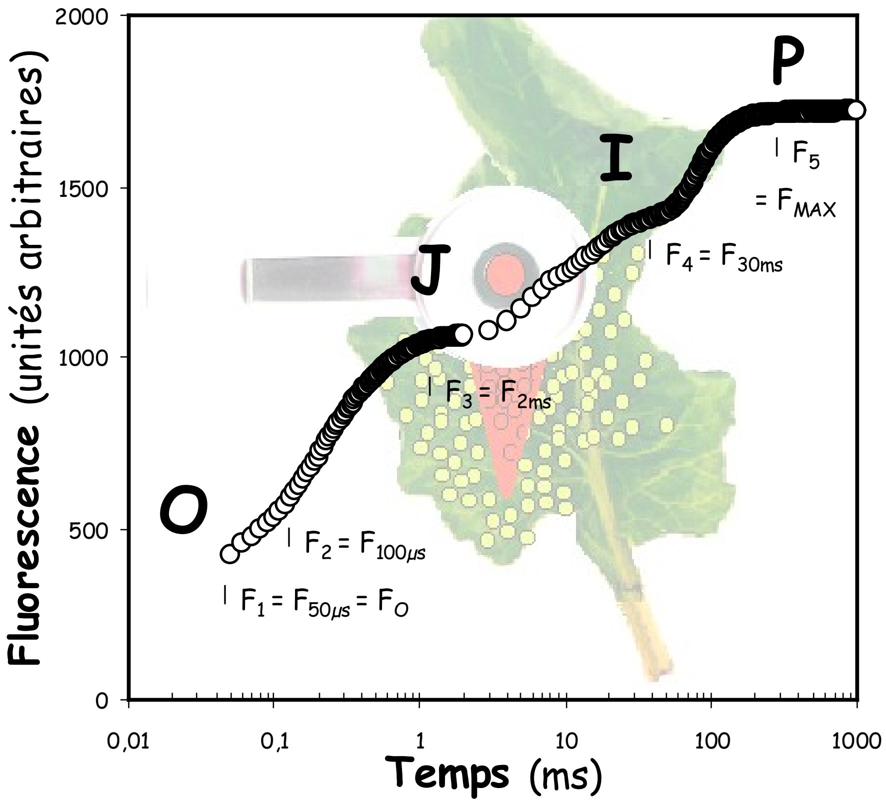
Prof. Reto STRASSER

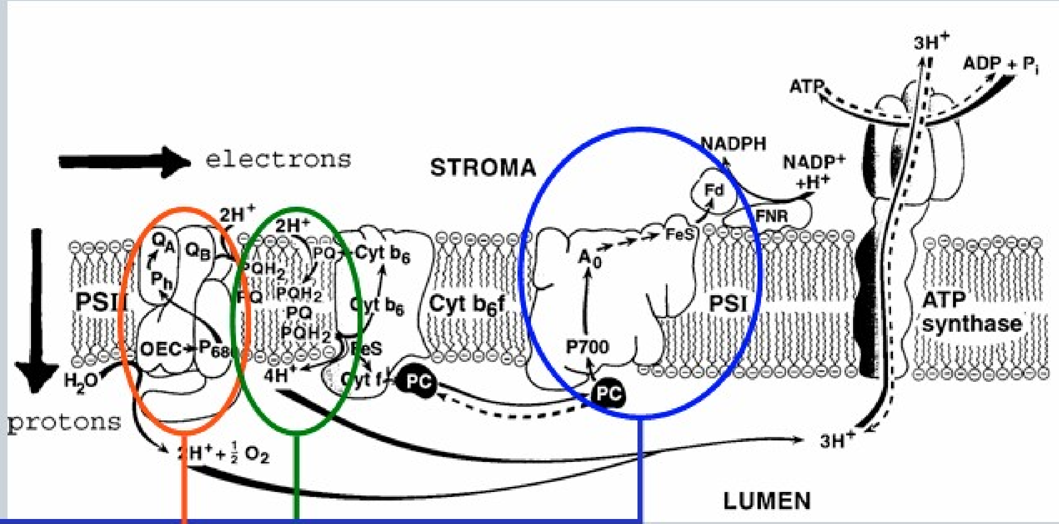
Bioenergetics Laboratory-
University of Geneva, Switzerland

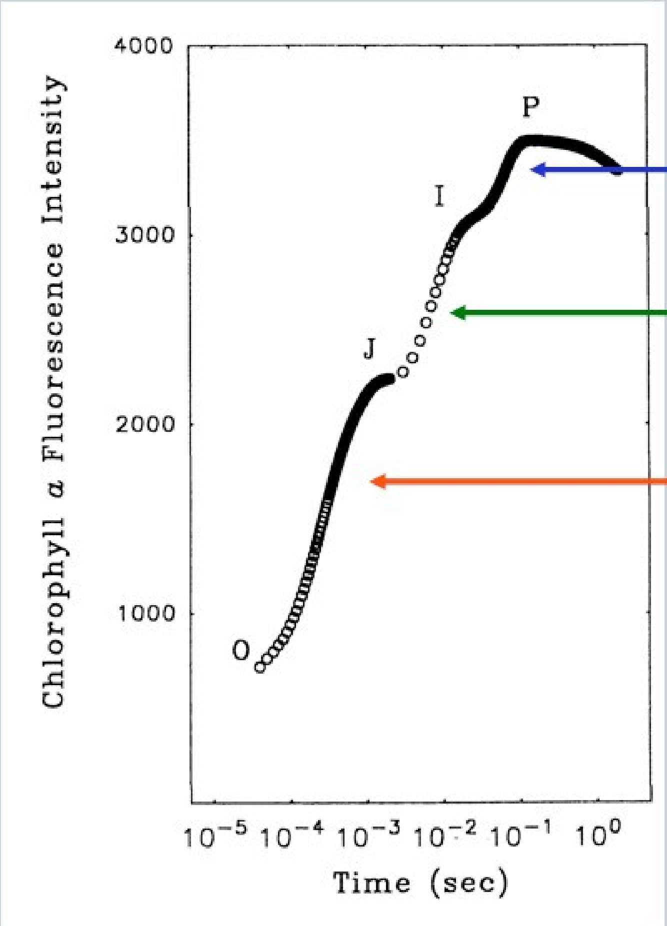




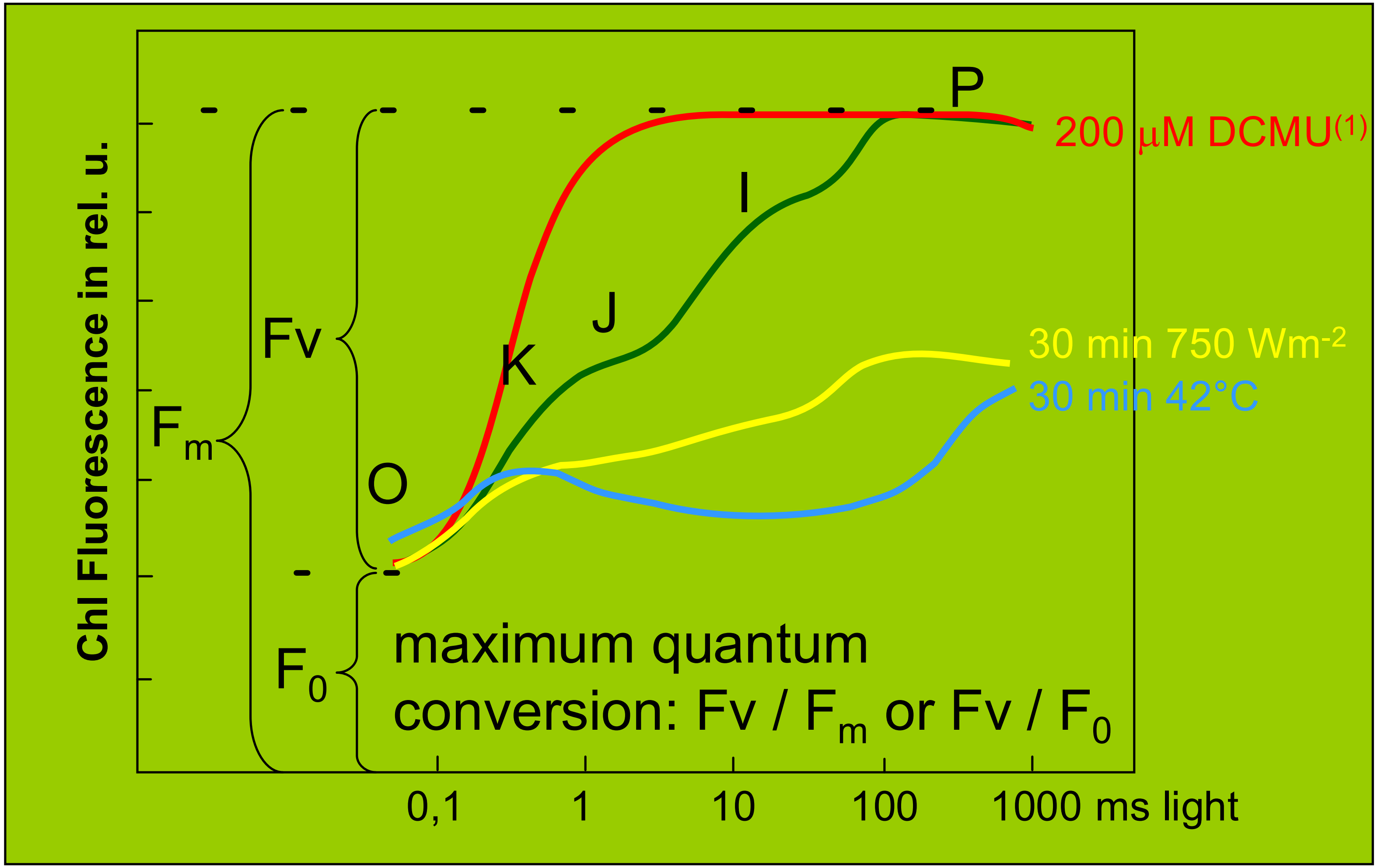
The primary signal : the fluorescence curve (Strasser, 1995).





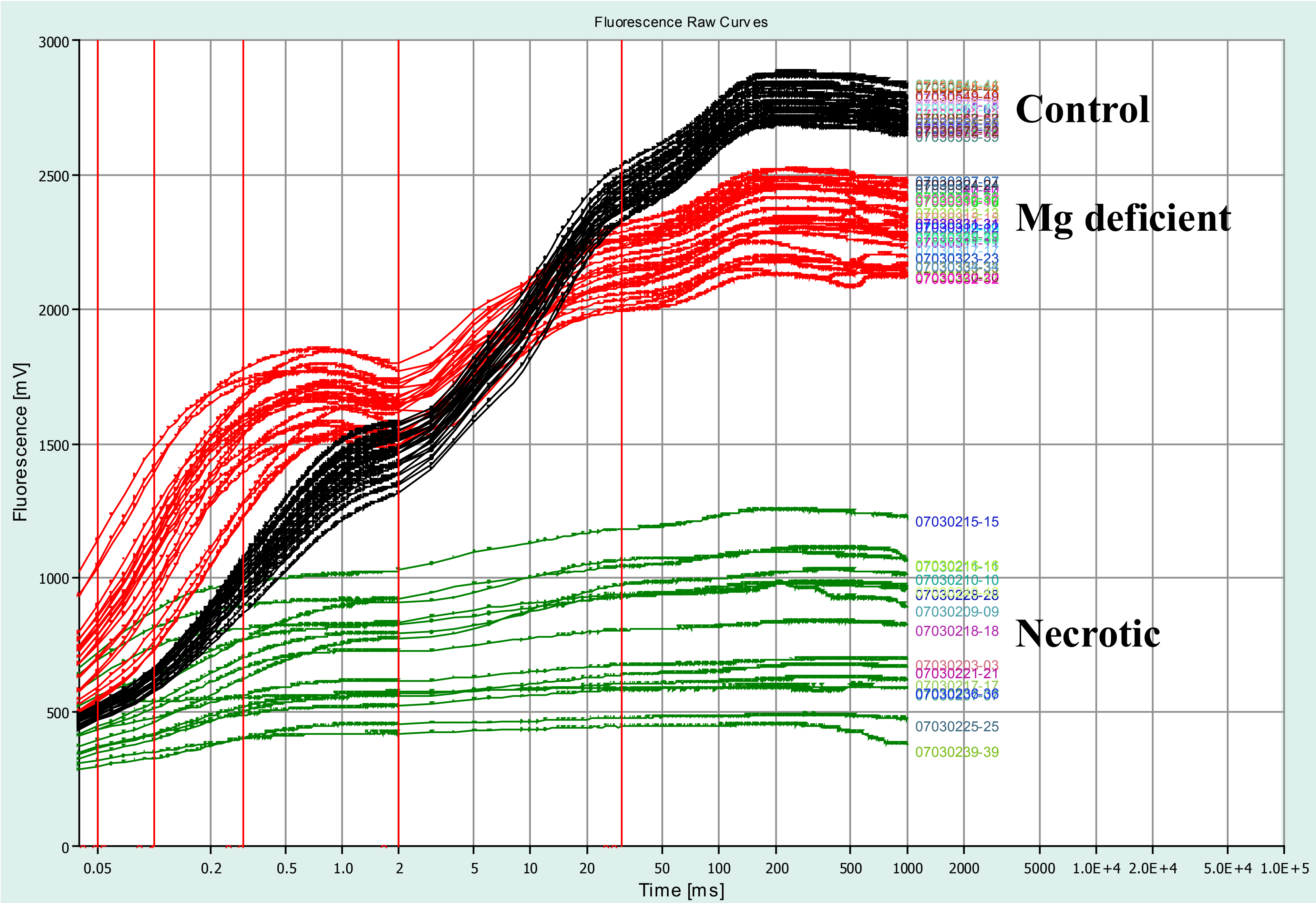


A simplified interpretation of the relationship between OJIP-transient and electron acceptor pools of the electron transport chain.

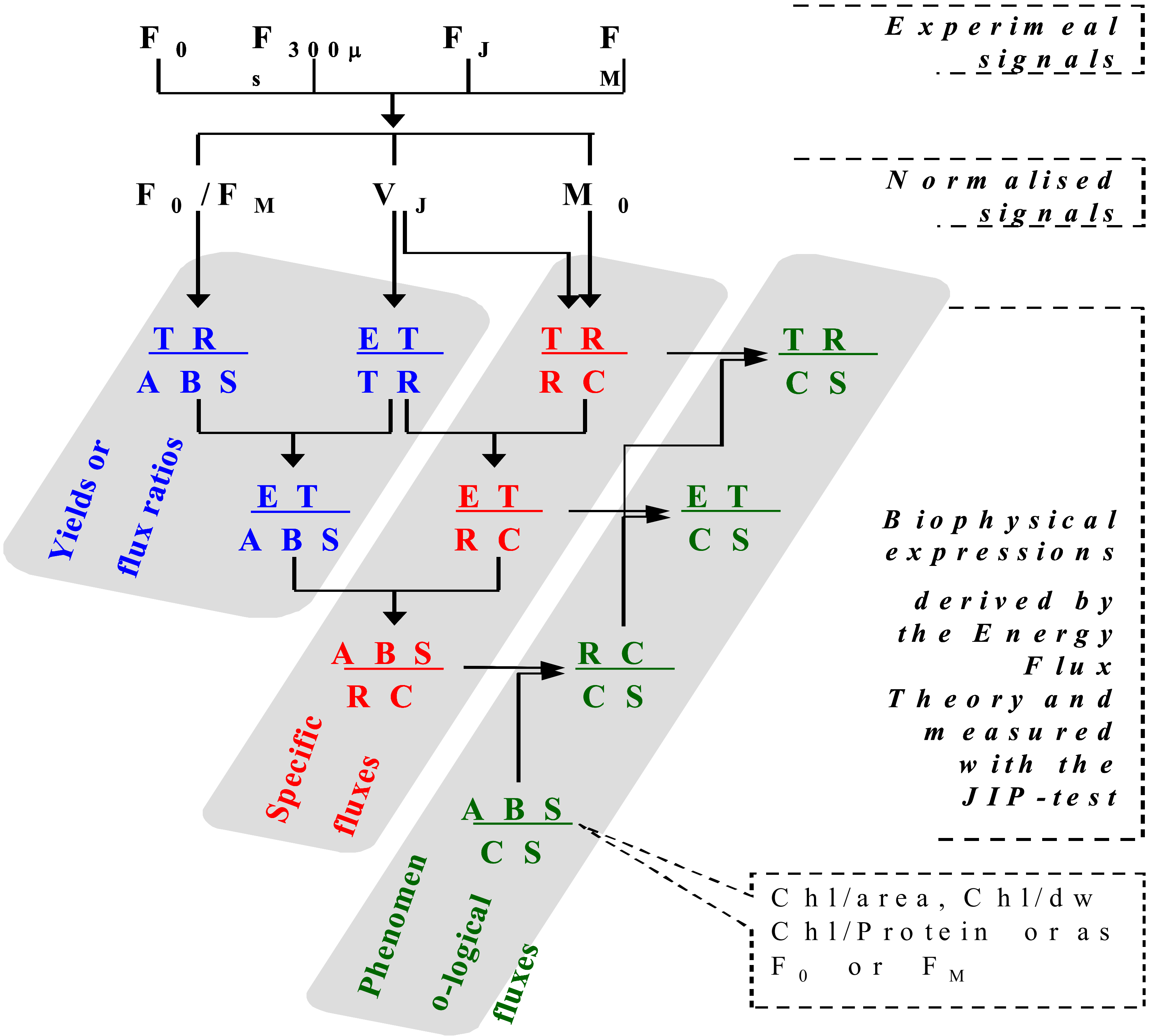


Srivastava A, Strasser RJ (1999) in: Crop Improvement for Food Security (Behl RK et al. eds.) SSARM, HISAR, pp 60-71

[1] Haldimann P, Strasser RJ (1999) Photosynthesis Research 62: 67-83



Fluorescence Raw Curves
Fluorescence [mV]
Time [ms]
Control
Mg deficient
Necrotic
07030215-15
07030210-10
07030209-09
07030218-18
07030221-21
07030225-25
07030239-39



Experimeal signals
Normalised signals
Biophysical expressions derived by the Energy Flux Theory and measured with the JIP-test
F0
Fs 300µ
FJ
FM
F0/FM
VJ
M0
TR/ABS
ET/TR
TR/RC
TR/CS
ET/ABS
ET/RC
ET/CS
ABS/RC
RC/CS
ABS/CS
Yields or flux ratios
Specific fluxes
Phenomeno-logical fluxes
Chl/area, Chl/dw
Chl/Protein or as
F0 or FM

Derived JIP-test parameters table

Technical parameters

Slope at the origin of the fluorescence rise	M_O	$= (F_{300\mu s} - F_O) / (F_M - F_O)$
Relative variable fluorescence at 2 ms	V_J	$= (F_{2ms}/F_O) / (F_M - F_O)$

The specific fluxes (expressed per RC - reaction center)

Absorption, per RC	**ABS/RC**	$= (M_O/V_J) / ((1-F_O/F_M))$
Trapping at time zero, per RC	**TRo/RC**	$= M_O/V_J$
Dissipation at time zero, per RC	**DIo/RC**	= (ABS/RC) - (TRo/ABS)
Electron transport at time zero, per RC	**ETo/RC**	$= (M_O/V_J)(1-V_J)$

The phenomenological fluxes (expressed per CS – cross section of the leaf tissue)

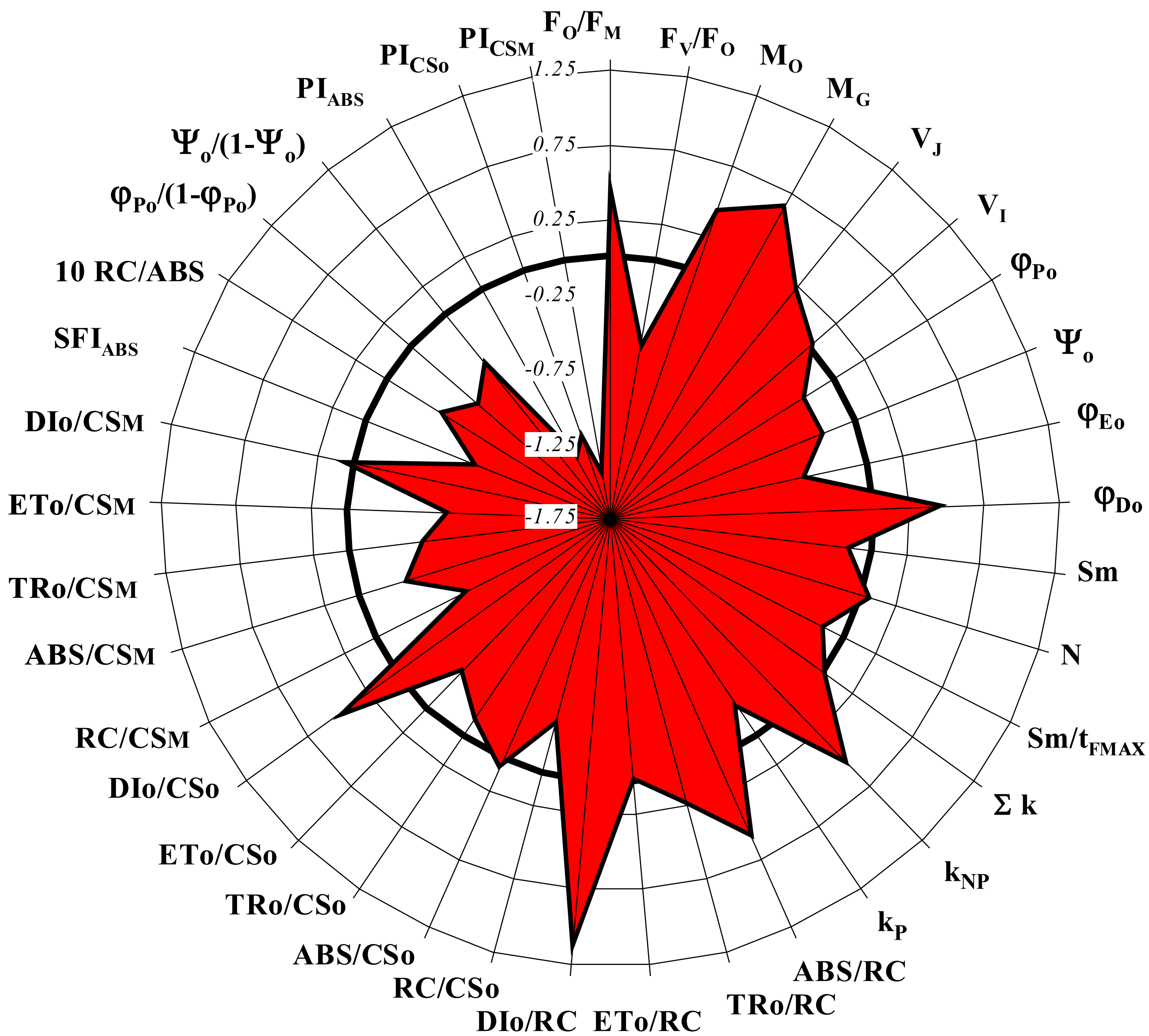
Absorption, per CS	**ABS/CS**	$= (TR_O/ABS) / (ABS/CS)$
Trapping at time zero, per CS	**TRo/CS**	= (TRo/ABS) (ABS/CS)
Dissipation at time zero, per CS	**DIo/CS**	= (ABS/CS) - (TRo/CS)
Electron transport at time zero, per CS	**ETo/CS**	$= (M_O/V_J)(1-V_J)$

The yields (or fluxes ratios)

Maximum quantum yield of primary photochemistry	φ_{Po}	$= TRo/ABS = (F_M - F_O) / F_M$
Probability that a traped exciton moves an electron further than Q_A^-	Ψ_o	$= ETo/TRo = 1 - V_J$
Probability that an absorbed photon moves an electron further than Q_A^-	$\varphi_{Eo} = \varphi_{Po}\Psi_o$	= (TRo/ABS) (ETo/TRo) $= ETo/ABS = (1-F_O/F_M)(1-V_J)$

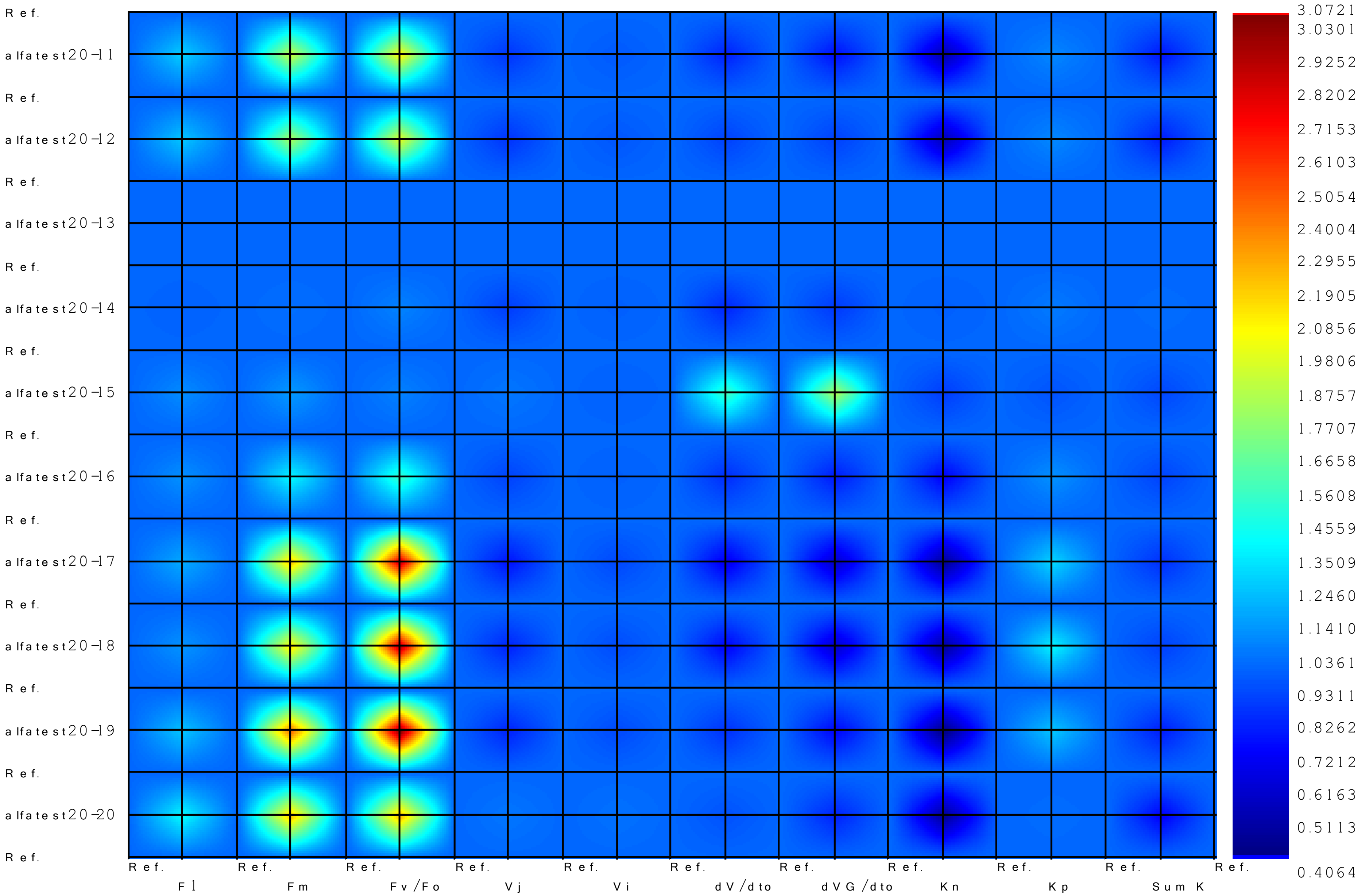
Vitality Indexes

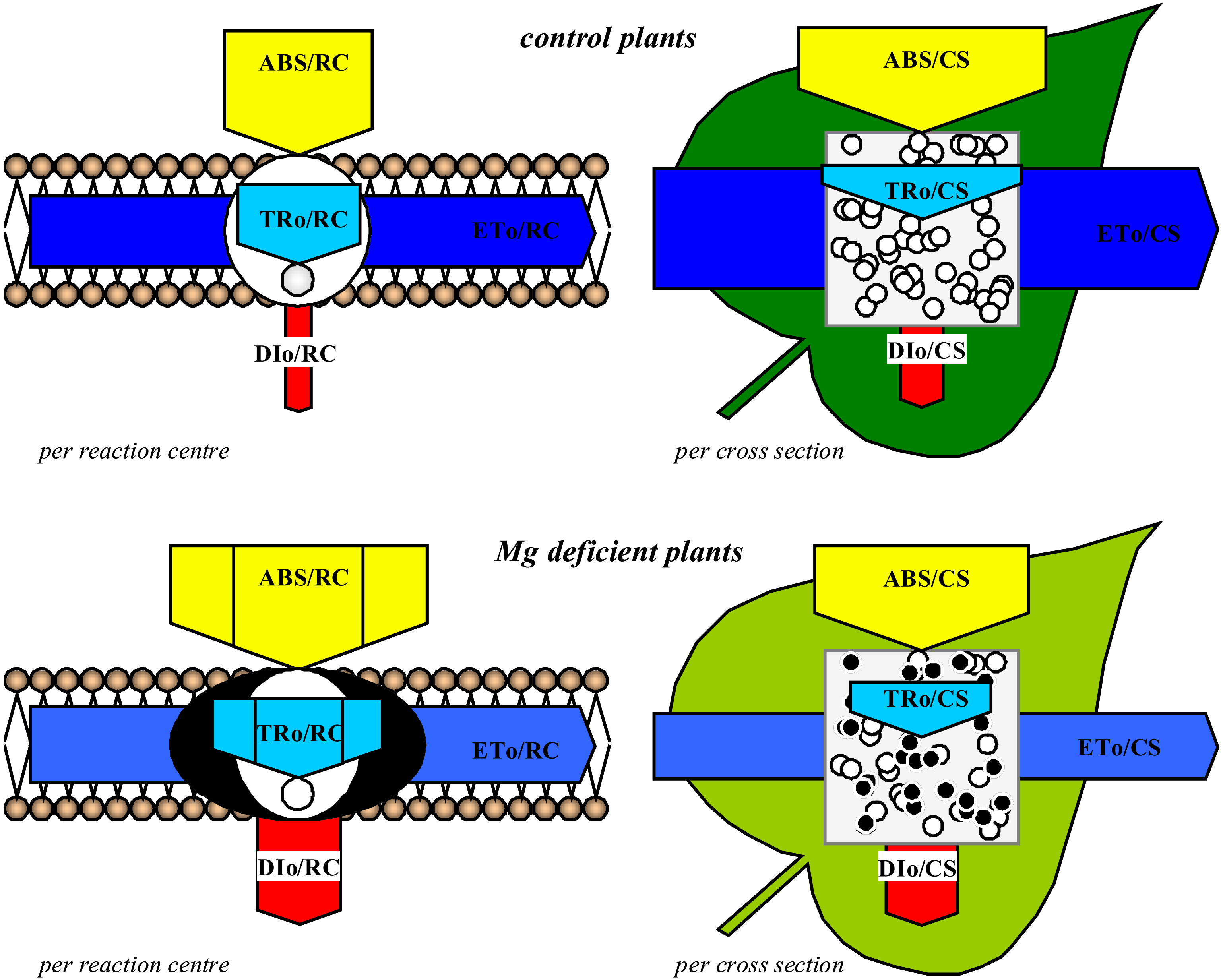
Density RCs per chlorophyll	**RC/ABS**
Conformation term for primary photochemistry	$(\varphi_{Po}/(1-\varphi_{Po})) = TRo/DIo = F_O/F_M$
Conformation term for the thermal reactions (non light depending reactions)	$(\Psi_o/(1-\Psi_o)) = ETo/(dQ_A^-/dt_0)$
Performance Index	$PI_{ABS} = [RC/ABS]\,[\varphi_{Po}/(1-\varphi_{Po})]\,[\Psi_o/(1-\Psi_o)]$
Driving force on a chlorophyll basis	$\Delta F_{ABS} = \log [PI_{ABS}]$



F_O/F_M
F_V/F_O
M_O
M_G
V_J
V_I
φ_Po
Ψ_o
φ_Eo
φ_Do
Sm
N
Sm/t_FMAX
Σ k
k_NP
k_P
ABS/RC
TRo/RC
ETo/RC
DIo/RC
RC/CSo
ABS/CSo
TRo/CSo
ETo/CSo
DIo/CSo
RC/CSM
ABS/CSM
TRo/CSM
ETo/CSM
DIo/CSM
SFI_ABS
10 RC/ABS
φ_Po/(1-φ_Po)
Ψ_o/(1-Ψ_o)
PI_ABS
PI_CSo
PI_CSM
1.25
0.75
0.25
-0.25
-0.75
-1.25
-1.75

Fluorescence JIP-Test parameters map




control plants
ABS/RC
TRo/RC
ETo/RC
DIo/RC
per reaction centre
ABS/CS
TRo/CS
ETo/CS
DIo/CS
per cross section
Mg deficient plants
ABS/RC
TRo/RC
ETo/RC
DIo/RC
per reaction centre
ABS/CS
TRo/CS
ETo/CS
DIo/CS
per cross section

SPIRODELA OLIGORRHIZA

- **THE USE OF PSII ACTIVITY OF *SPIRODELA OLIGORRHIZA* PLANTS AS AN INDICATORE FOR WATER TOXCITY**

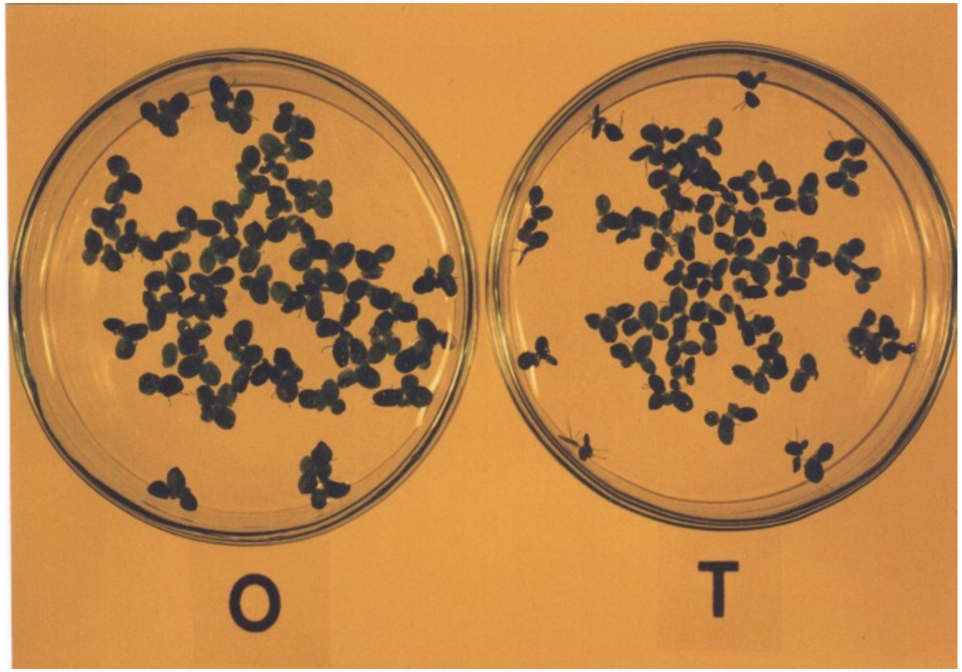
Z. Romanowska-Duda, M. Hazem Kalaji, Reto J. Strasser

Photosynthesis: Fundamental Aspects to Global Perspectives, 2005, International Society of Photosynthesis

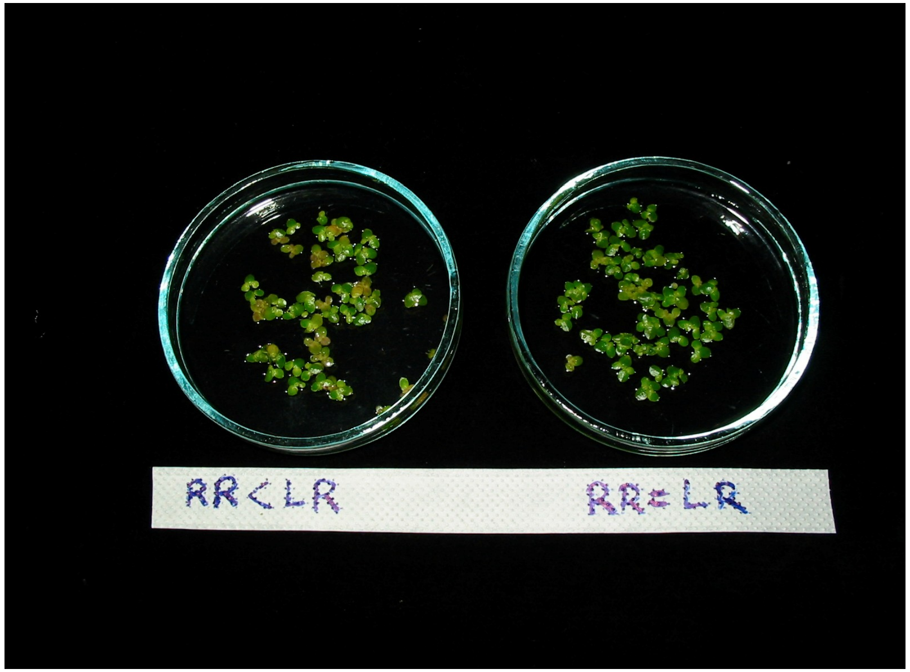
- The effect of different toxic extracts on several sites of the PSII of *Spirodela oligorrhiza* water plants was studied. Plants were grown for 6 days in a medium with two microcystins extracted from blue-green algae (MC-RR=MC-LR and MC-RR<MC-LR) of the following concentrations: 0, 5,10, 25, 50, and 100 µg/l.

- Each microcystin is designated a name depending on the variable amino acids which complete their structure. The most common and toxic microcystin-LR contains the amino acids Leucine (L) and Arginine (R) in variable positions.

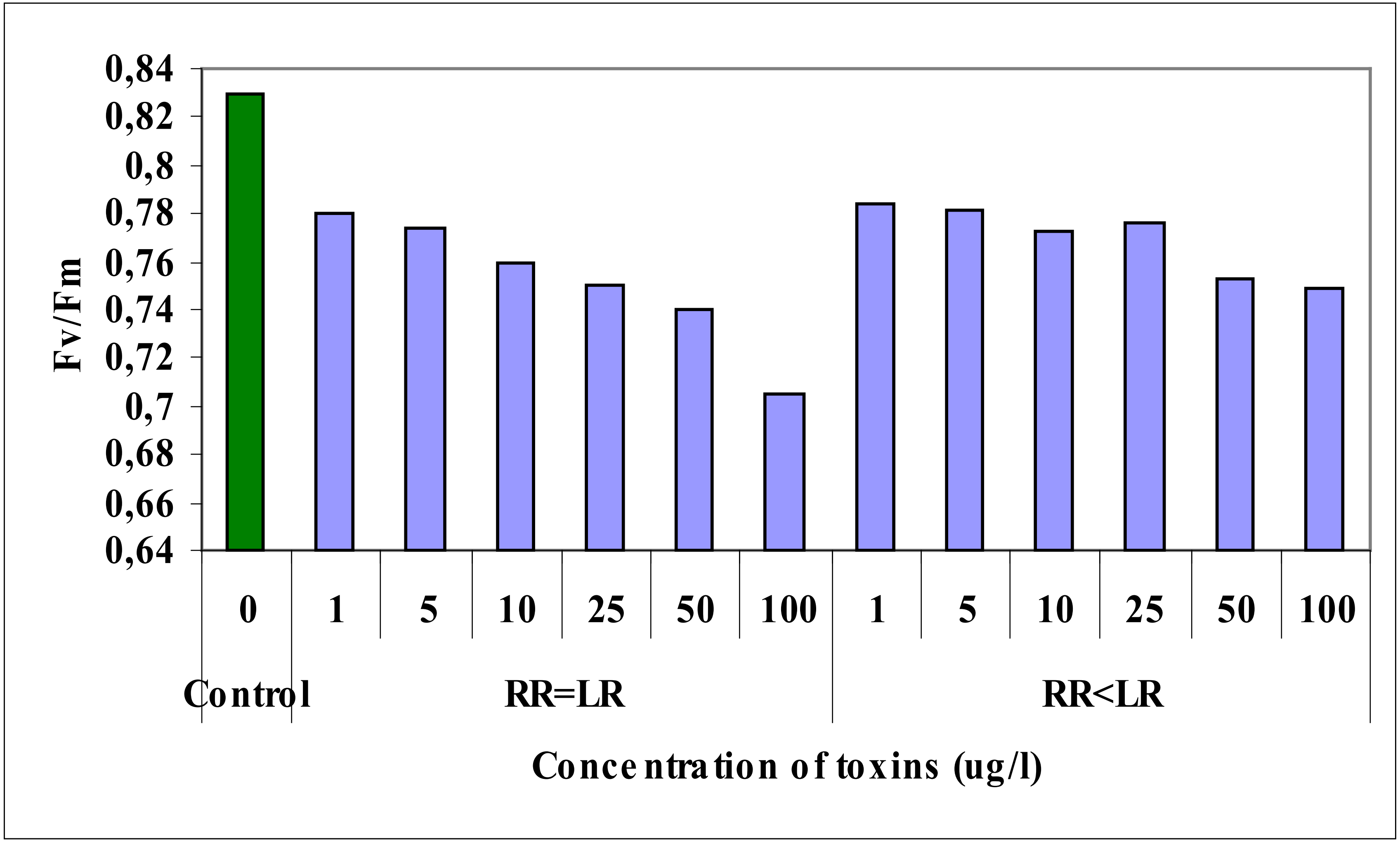
Toxins



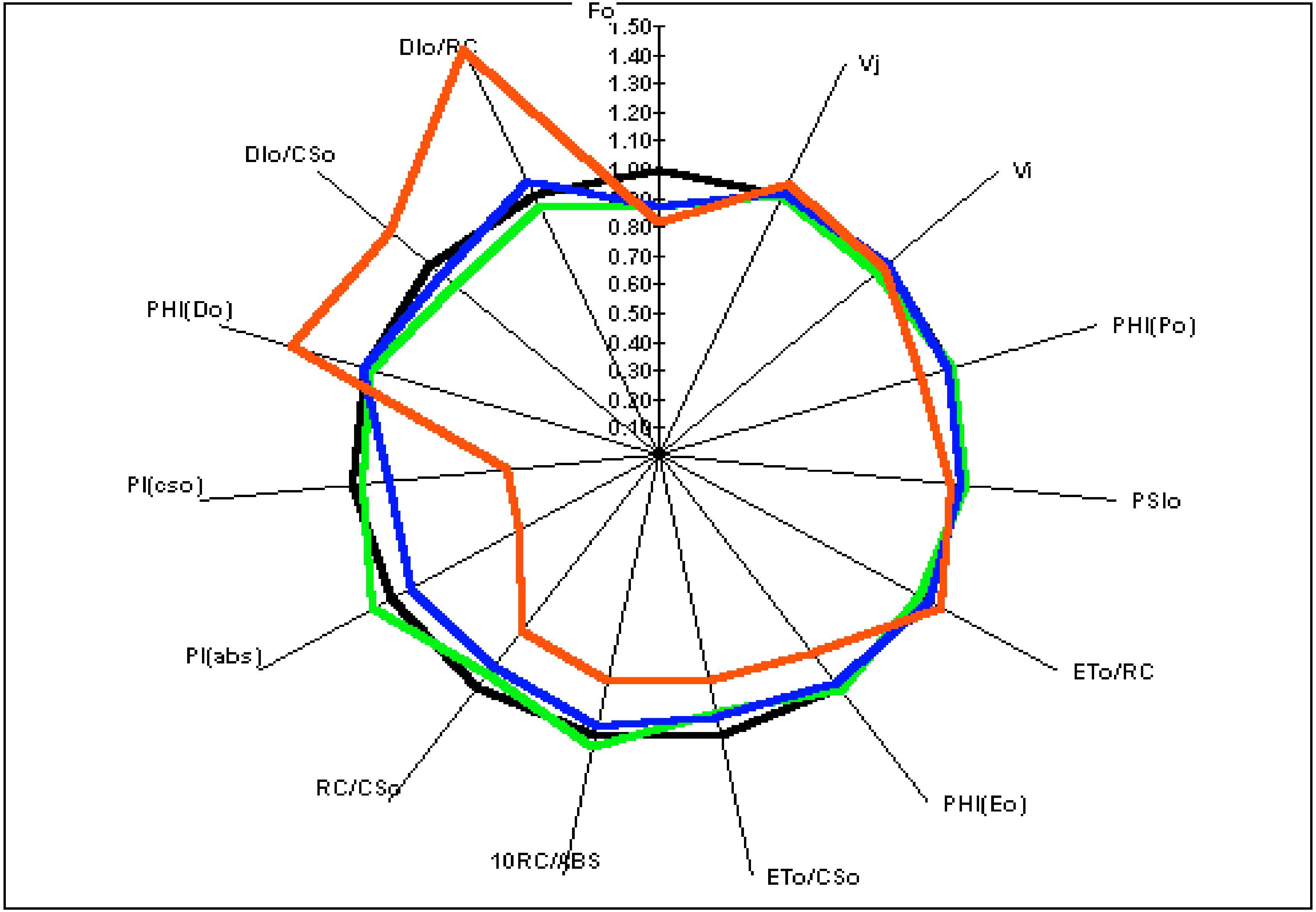
Spirodela oligorrhiza without (0) and with toxin MC-LR (T)



Spirodela oligorrhiza with toxins: MC-RR and MC-LR



Fv/Fm
0,84
0,82
0,8
0,78
0,76
0,74
0,72
0,7
0,68
0,66
0,64
0
1
5
10
25
50
100
1
5
10
25
50
100
Control
RR=LR
RR<LR
Concentration of toxins (ug/l)



Control 5μg L^{-1} 25 μg L^{-1} 100 μg L^{-1}

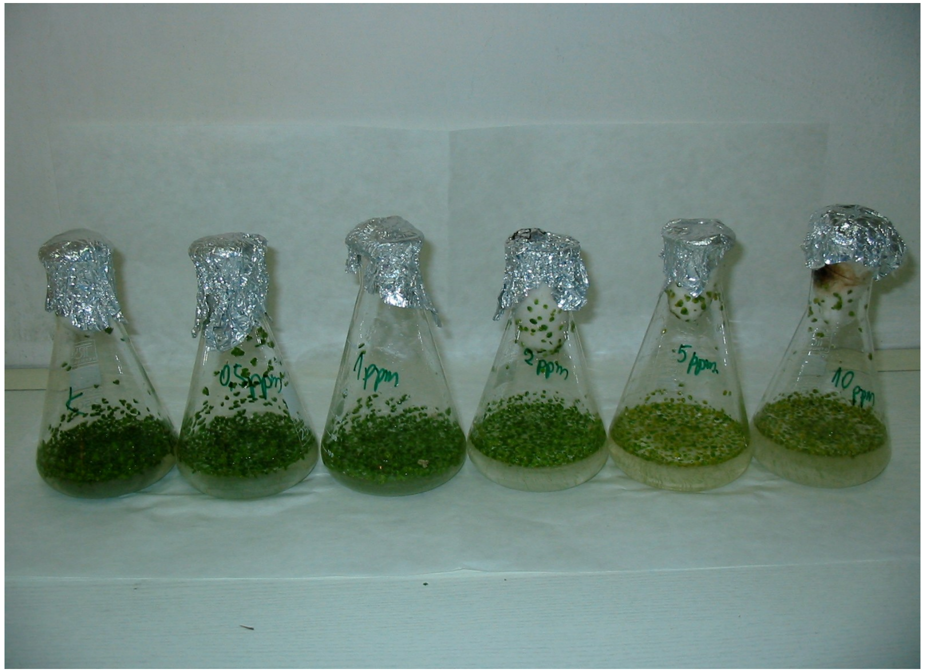
- **JIP-Test technique as biosensor for early detection of heavy metals effects on water plants (*Spirodela oligorrhiza*)**

Hazem M. Kalaji, Z. Romanowska-Duda, Reto J. Strasser

BIOLOGICAL LETT. 2005, 42(2): 191

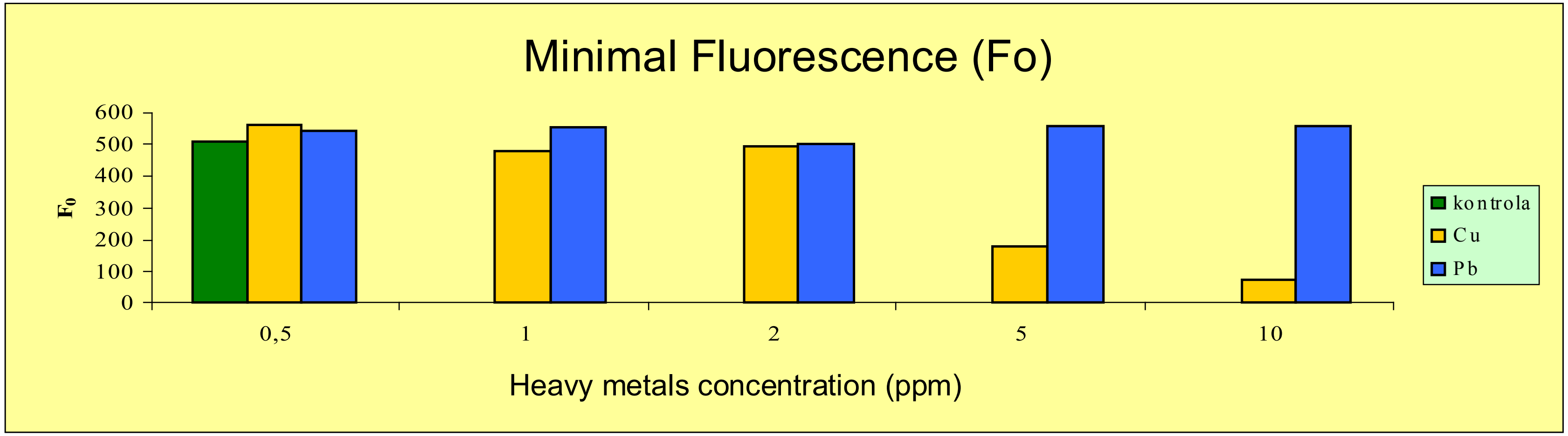
Spirodela oligorrhiza plants were grown under optimal conditions on a growth medium with or without the addition of heavy metals (Cu,Pb) ions during 24 hours to growth medium in the range from 0 to 10 ppm

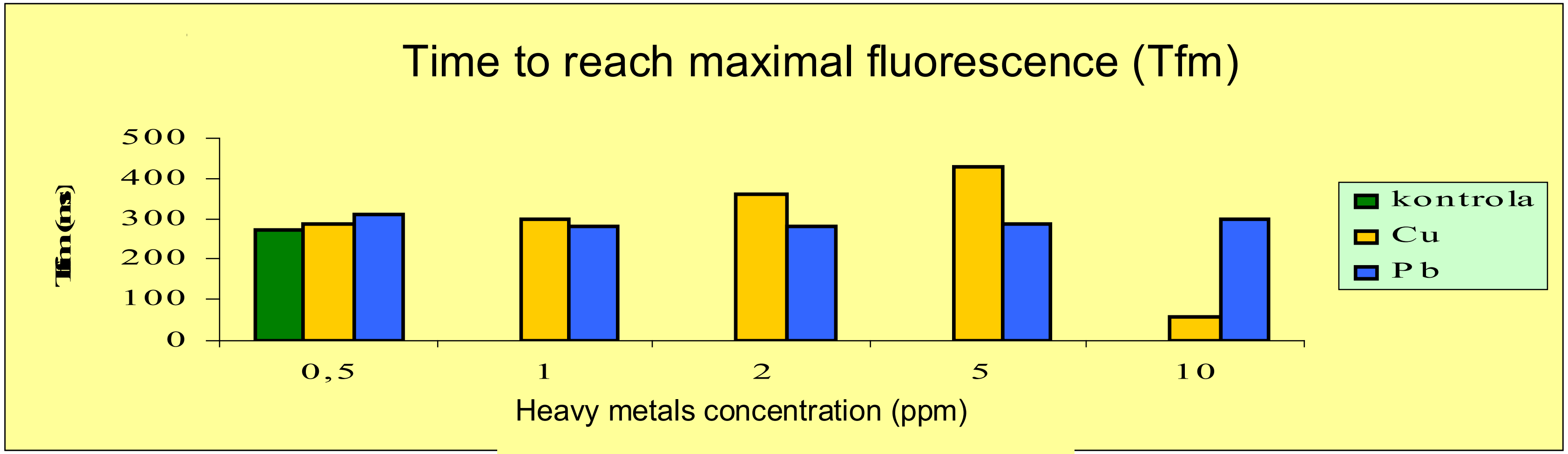
Heavy metals

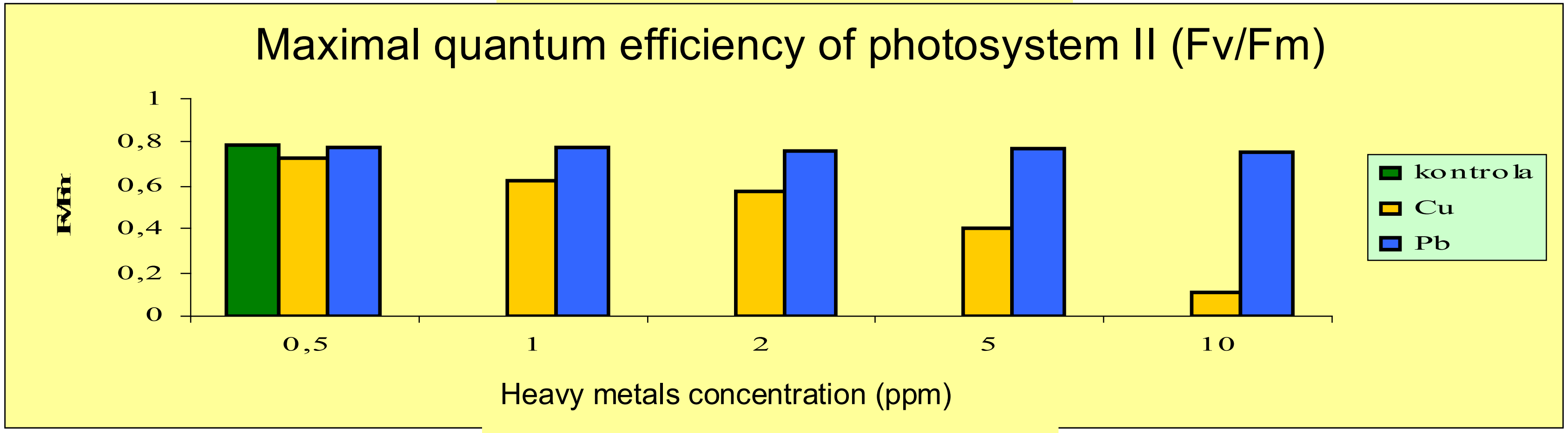


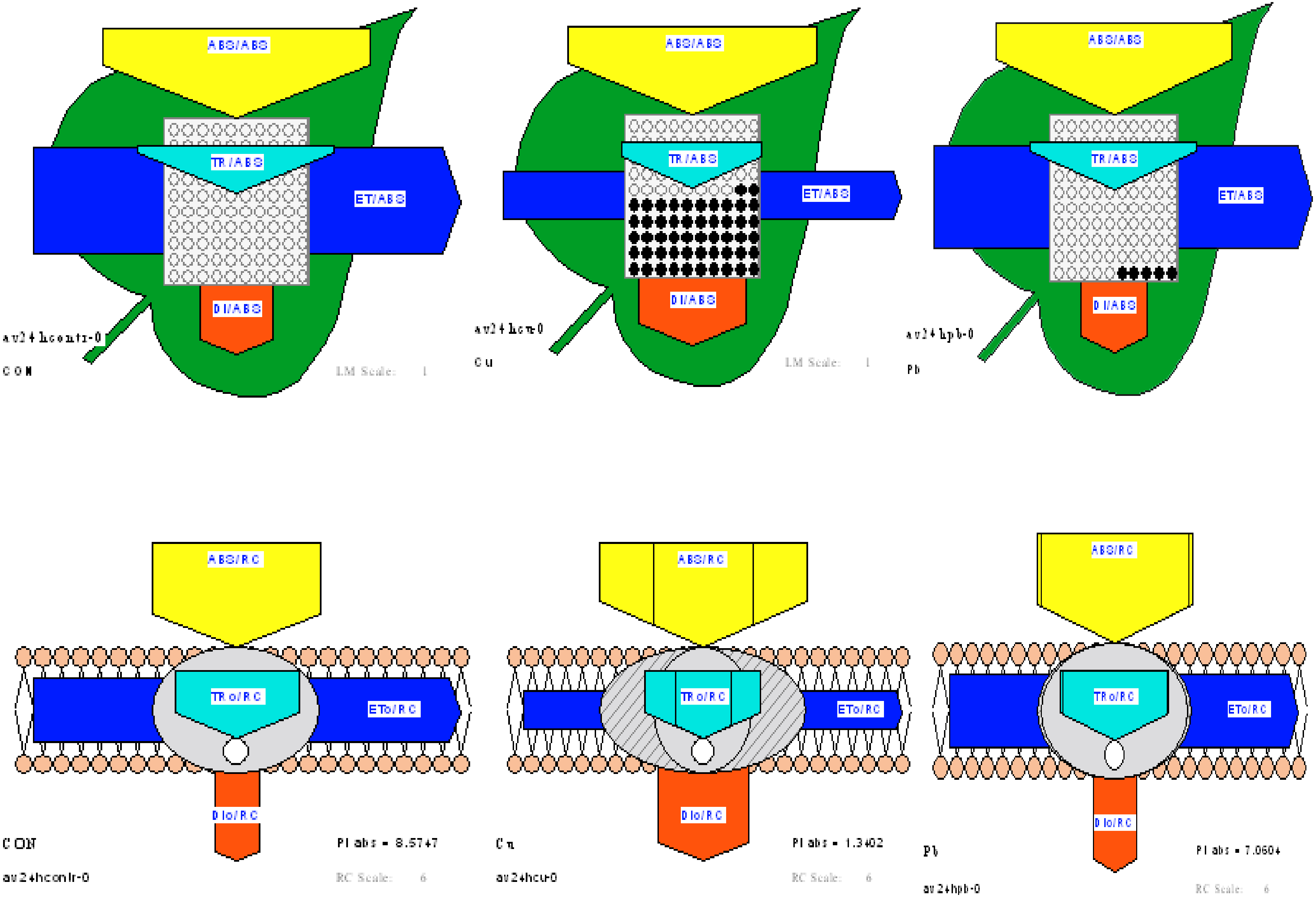
Spirodela oligorrhiza with different concentrations of Cd

Spirodela oligorrhiza with different concentrations of Cu


Minimal Fluorescence (Fo)
F0
600
500
400
300
200
100
0
0,5
1
2
5
10
Heavy metals concentration (ppm)
kontrola
Cu
Pb


Time to reach maximal fluorescence (Tfm)
Tfm (ns)
500
400
300
200
100
0
0,5
1
2
5
10
Heavy metals concentration (ppm)
kontrola
Cu
Pb


Maximal quantum efficiency of photosystem II (Fv/Fm)
1
0,8
0,6
0,4
0,2
0
0,5
1
2
5
10
Heavy metals concentration (ppm)
kontrola
Cu
Pb


ABS/ABS
TR/ABS
ET/ABS
DI/ABS
av2+hcontr-0
CON
LM Scale: 1
ABS/ABS
TR/ABS
ET/ABS
DI/ABS
av2+hcu-0
Cu
LM Scale: 1
ABS/ABS
TR/ABS
ET/ABS
DI/ABS
av2+hpb-0
Pb
ABS/RC
TRo/RC
ETo/RC
DIo/RC
CON
PI abs = 8.57+7
av2+hcontr-0
RC Scale: 6
ABS/RC
TRo/RC
ETo/RC
DIo/RC
Cu
PI abs = 1.3+02
av2+hcu-0
RC Scale: 6
ABS/RC
TRo/RC
ETo/RC
DIo/RC
Pb
PI abs = 7.060+
av2+hpb-0
RC Scale: 6

A. Abiad	Tf(max)	Area	PHI(Po)	N	Kn	Kp	ABS/RC	TRo/RC	ETo/RC	DIo/RC	PI(abs)	PI(cso)
Control	280.00	37968.00	0.79	54.18	0.58	2.13	2.48	1.95	1.13	0.53	20.47	7575.20
Low Temp.	180.00	23074.00	0.74	37.21	0.58	1.62	2.79	2.06	0.88	0.74	7.52	3428.21
High Temp.	10.00	241.00	0.17	2.48	1.25	0.26	8.25	1.41	0.50	6.84	0.14	92.50
Low PAR	240.00	27327.00	0.78	39.38	0.55	1.96	2.62	2.05	1.15	0.58	17.30	6901.14
High PAR	180.00	24297.00	0.75	39.89	0.59	1.81	2.79	2.10	1.01	0.68	10.10	4211.83
NaCl	280.00	37520.00	0.78	51.33	0.55	1.92	2.50	1.94	1.11	0.56	18.67	7579.82
+Cd	2.30	4.00	0.01	2.67	1.87	0.01	357.12	2.00	0.67	355.12	0.00	0.05
+Pb	270.00	28968.00	0.76	49.97	0.63	2.04	2.74	2.09	1.18	0.65	15.24	5700.25
-Ca	500.00	32216.00	0.76	54.71	0.64	2.09	2.64	2.02	1.13	0.62	15.56	5695.33
-S	300.00	33335.00	0.76	57.19	0.64	1.99	2.69	2.03	1.14	0.65	14.78	5631.14
-Mg	800.00	32002.00	0.76	54.98	0.65	2.02	2.66	2.02	1.09	0.64	13.70	5136.49
-K	240.00	27417.00	0.75	46.52	0.61	1.85	2.78	2.09	1.04	0.69	10.78	4378.43
-N	280.00	33377.00	0.77	51.39	0.60	2.02	2.56	1.97	1.07	0.59	15.58	5935.98
-P	300.00	31029.00	0.76	54.03	0.63	1.97	2.78	2.11	1.08	0.67	11.91	4585.04
-Fe	700.00	33326.00	0.76	57.28	0.65	2.00	2.66	2.01	1.07	0.65	13.16	4974.25

A. Aswad	Tf(max)	Area	PHI(Po)	N	Kn	Kp	ABS/RC	TRo/RC	ETo/RC	DIo/RC	PI(abs)	PI(cso)
Control	280.00	36384.00	0.78	56.99	0.62	2.20	2.52	1.96	1.12	0.55	18.68	6614.07
Low Tepm.	280.00	34961.00	0.76	53.51	0.60	1.91	2.55	1.94	0.96	0.61	12.22	4863.88
High Temp.	280.00	35779.00	0.78	51.16	0.57	1.98	2.50	1.94	1.09	0.56	17.67	6927.66
Low PAR	400.00	25449.00	0.73	44.23	0.62	1.71	2.81	2.06	1.13	0.75	11.92	5114.84
High PAR	290.00	34503.00	0.76	54.93	0.63	1.95	2.54	1.92	1.00	0.62	13.12	5089.51
+NaCl	240.00	31018.00	0.77	48.13	0.59	2.01	2.62	2.02	1.10	0.60	15.45	5931.65
+Cd	1.60	2.00	0.00	0.89	1.63	0.01	272.10	1.33	0.00	270.77	0.00	0.00
+Pb	270.00	19955.00	0.75	40.31	0.67	1.97	3.01	2.25	1.08	0.76	9.06	3425.85
-Ca	250.00	23991.00	0.75	46.84	0.69	2.04	2.82	2.10	1.06	0.71	10.57	3866.94
-S	270.00	25633.00	0.75	46.22	0.66	1.93	2.75	2.05	1.01	0.70	10.40	4024.06
-Mg	290.00	22510.00	0.73	43.67	0.67	1.86	2.88	2.11	1.07	0.76	9.75	3842.41
-K	240.00	23338.00	0.76	37.11	0.61	1.96	2.60	1.98	0.96	0.62	11.60	4511.78
-N	260.00	32456.00	0.76	51.03	0.59	1.90	2.68	2.05	1.17	0.63	16.12	6495.84
-P	290.00	28513.00	0.76	46.39	0.61	1.94	2.68	2.05	1.03	0.64	12.16	4780.81
-Fe	500.00	19433.00	0.74	35.11	0.66	1.91	2.73	2.03	0.95	0.70	9.39	3653.76

Ident.		Area	PHI(Po)	N	Kn	Kp	ABS/RC	TRo/RC	ETo/RC	DIo/RC	PI(abs)	PI(cso)
Control	280	34943	0.7771	45.0091	0.5184	1.8072	2.4846	1.9308	1.261	0.5538	26.4151	11358.493
Low Temp.	500	24238	0.7331	42.2287	0.5999	1.6473	2.9041	2.129	1.3172	0.7751	15.3432	6827.724
High temp.	800	28298	0.4168	147.4415	0.8945	0.6392	5.8254	2.428	1.3808	3.3974	1.6177	1054.7404
Low PAR	400	21593	0.7087	46.5002	0.6532	1.589	3.2969	2.3365	1.3374	0.9604	9.8773	4405.2758
High PAR	800	14089	0.4346	145.0563	1.6779	1.2895	6.1357	2.6666	1.5237	3.4691	1.6698	562.7226
NaCl	190	3028	0.191	21.1113	0.7402	0.1747	9.4177	1.7988	0.76	7.6189	0.1833	200.3469
+Cd	2.9	3	0.0071	6	3.5714	0.0257	563.3803	4	2	559.3803	0.0001	0.0278
+Pb	280	24034	0.7297	47.1263	0.5889	1.5897	3.3294	2.4295	1.5255	0.8999	13.6803	6279.2577
-Ca	290	10741	0.5237	54.5661	1.0515	1.156	4.8309	2.5299	0.8432	2.301	1.1375	515.2875
-S	290	30034	0.7405	52.287	0.6313	1.8018	2.7577	2.0421	1.2953	0.7156	17.9506	7377.6966
-Mg	260	15343	0.6545	43.1603	0.8511	1.612	3.3051	2.1632	1.1281	1.1419	6.246	2535.876
-K	300	5550	0.3223	51.0335	1.139	0.5417	8.074	2.6023	0.8921	5.4717	0.3072	182.784
-N	500	17891	0.5934	62.6154	0.7716	1.1259	4.5355	2.6914	1.3546	1.8441	3.26	1718.02
-P	280	30346	0.7306	57.1547	0.6477	1.7561	2.9079	2.1245	1.3089	0.7834	14.9641	6225.0656
-Fe	280	15122	0.6238	47.8492	0.7937	1.316	3.987	2.4871	1.2657	1.4999	4.3097	2042.7978

Ident.	Tf(max)	Area	PHI(Po)	N	Kn	Kp	ABS/RC	TRo/RC	ETo/RC	DIo/RC	PI(abs)	PI(cso)
Control	280	34623	0.7812	44.1621	0.5198	1.8555	2.454	1.9171	1.2118	0.5369	24.9952	10522.979
Low Temp.	290	29471	0.7442	44.7572	0.5698	1.6574	2.6652	1.9834	1.2422	0.6818	18.2903	8212.3447
high Temp.	500	10402	0.2459	90.7406	0.8688	0.2833	10.0395	2.4687	1.3521	7.5708	0.3932	341.2976
Low PAR	300	6002	0.304	32.7747	0.6711	0.2932	8.1371	2.4737	1.2287	5.6634	0.5298	549.4026
High PAR	270	34799	0.7605	49.139	0.5359	1.7012	2.6348	2.0038	1.254	0.631	20.1499	9007.0053
NaCl	600	22560	0.7037	51.21	0.7776	1.8471	2.9193	2.0543	1.135	0.865	10.0454	3827.2974
+Pb	230	6449	0.3936	38.8454	0.8446	0.5482	7.1314	2.8069	1.3734	4.3245	0.8719	626.0242
-Ca	220	4733	0.5825	35.4467	1.7182	2.397	4.3585	2.5388	0.8987	1.8197	1.7541	426.2463
-S	290	30407	0.7013	71.0522	0.7215	1.694	3.2387	2.2713	1.4441	0.9674	12.6551	5239.2114
-Mg	280	4580	0.4186	51.7342	1.8939	1.3634	5.9636	2.4964	0.9941	3.4672	0.7988	245.2316
-K	260	2573	0.2573	72.1261	2.4272	0.8408	11.5483	2.9714	1.0091	8.5769	0.1542	47.1852
-N	400	30533	0.7	62.7014	0.6579	1.5351	3.1214	2.185	1.3842	0.9364	12.9209	5891.9304
-P	270	25655	0.7184	51.4319	0.6609	1.6865	3.0334	2.1792	1.3071	0.8542	12.6073	5370.7098
-Fe	240	1427	0.1972	38.1039	2.2936	0.5635	11.645	2.2964	0.8545	9.3486	0.125	43.75

Stress Identifying

IF:

Tf(max)	280 - 294
Area	> 37968.00
PHI(Po)	0.79 - 0.77
Kn	< 0.58
Kp	< 2.13
ABS/RC	> 2.48
TRo/RC	1.81 - 1.95
ETo/RC	> 1.13
DIo/RC	< 0.53
PI	>20.47

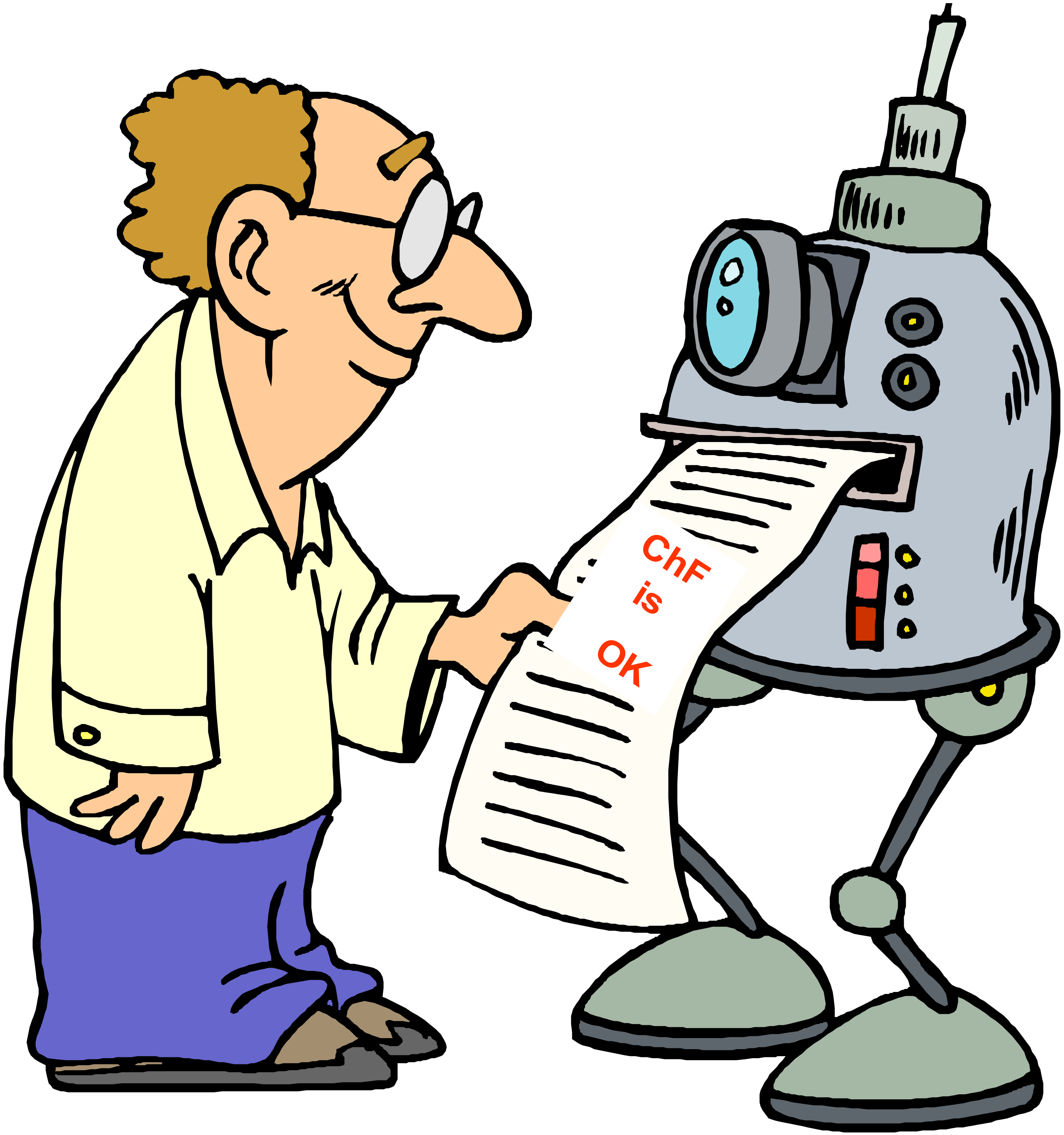
Heavy metals

IF:

Tf(max)	290 - 299
Area	37968.00
PHI(Po)	0.79 - 0.77
Kn	0.58 – 0.66
Kp	< 2.13
ABS/RC	< 2.48
TRo/RC	1.81 - 1.95
ETo/RC	1.13- 1.25
DIo/RC	< 0.53
PI	20 - 30

Toxic

Instruments Development


ChF
is
OK