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Functional Plant Biology

Supplementary Material

Both external and internal factors induce heterogeneity in senescing leaves of deciduous trees

Heta Mattila[A,B,*], *Sergey Khorobrykh*[A], *and Esa Tyystjärvi*[A]

[A]Molecular Plant Biology, University of Turku, Turku, Finland.

[B]Centre for Environmental and Marine Studies, University of Aveiro, Aveiro, Portugal.

[*]Correspondence to: Heta Mattila Molecular Plant Biology, University of Turku, Turku, Finland Email: h.mattila@ua.pt

Supplementary data:

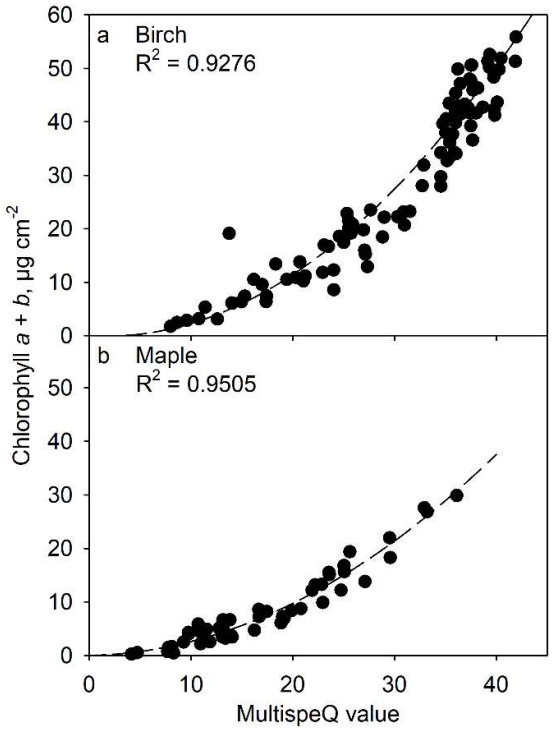

Figure S1. Calibration of optical (SPAD) chlorophyll measurements. Chlorophyll contents of birch (a) and maple (b) leaves were measured first with an optical method (MultispeQ) and then spectrophotometrically after extraction in DMF (Chlorophyll *a* + *b*, µg cm^{-2}). Symbols show individual measurements and lines show second-order polynomial equations fitted to the data (R^2 values of the fits are indicated). See Materials and methods for the equations.

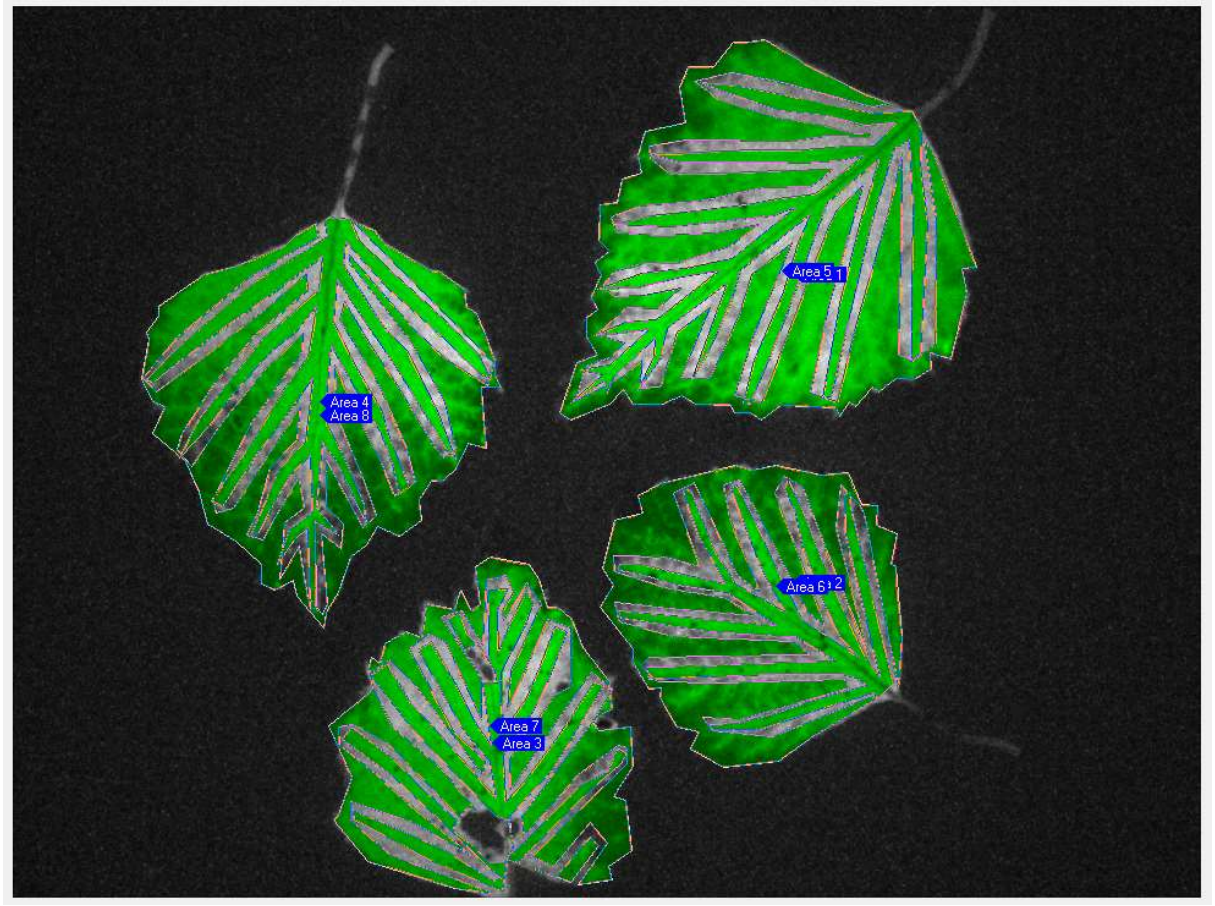

Figure S2. Selected areas of interests for the analysis of chlorophyll *a* fluorescence images from senescing birch leaves for Fig. 2. The underlaying grayscale image shows chlorophyll fluorescence. Black corresponds to background. Manually selected areas of interests are super-imposed on the fluorescence image with green colour and highlighted with blue labels. Two areas of interests are drawn on each leaf; areas close to main veins and areas between the main veins.

Figure S3. Photographs, taken on (a) 3/10/2022, (b) 22/10/2022, (c) 25/10/2022 and (d) 3/11/2022, of a senescing silver birch tree, used for the experiments in Fig. 1c–e. Note that more yellow leaves appear in (a) than in (b) because a slow progression of senescence during the first two dates.

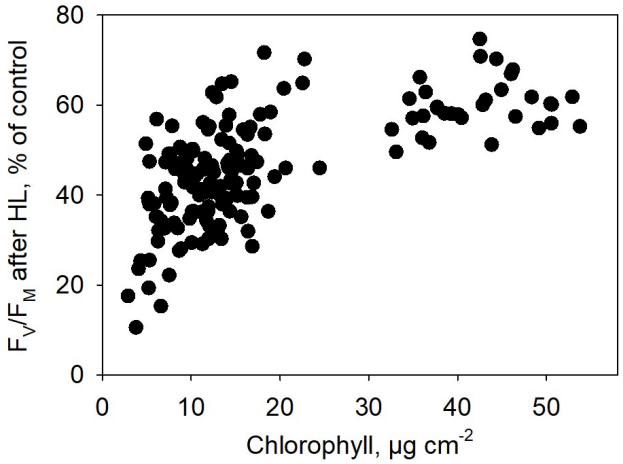

Figure S4. Remaining PSII activities, as percentages of the control (prior an illumination) F_V/F_M values, after a high light treatment (HL) plotted against the chlorophyll content of the leaf. The symbols show individual measurements. The data are from Fig. 1c–e.

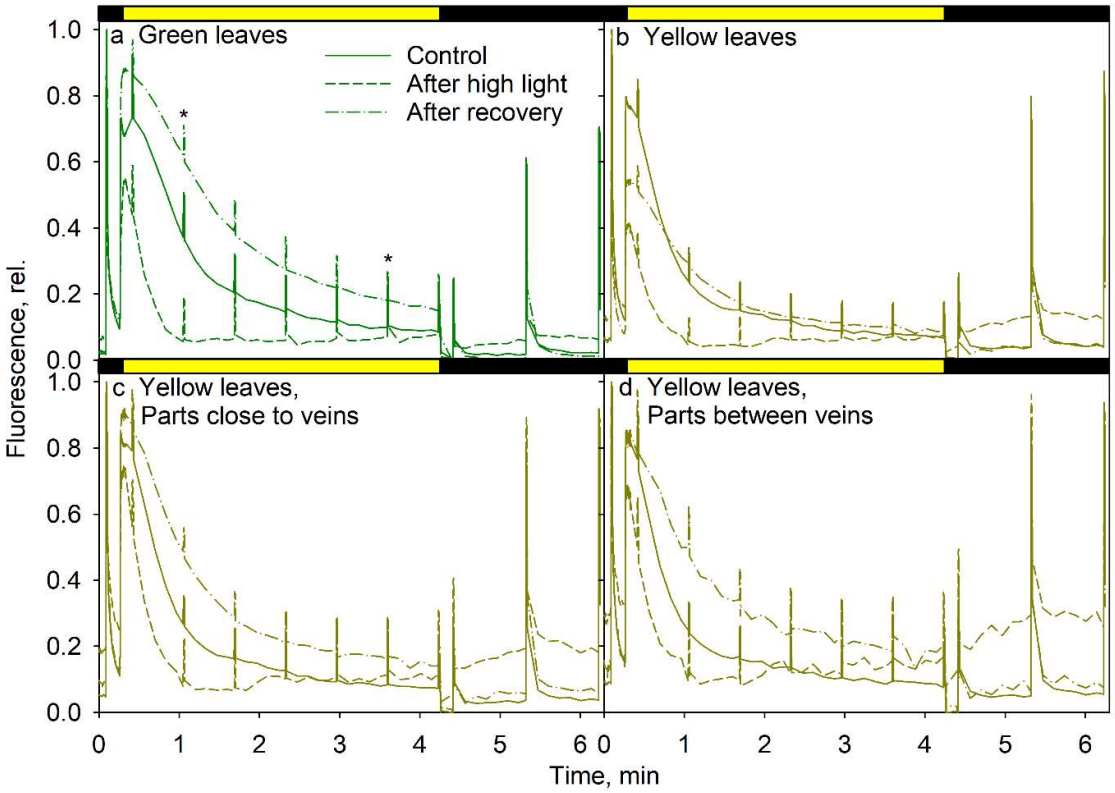

Figure S5. Fluorescence traces measured from green (a) and senescing (yellow; b–d) birch leaves. In the case of yellow leaves, fluorescence values were averaged over whole leaves (b), and also over leaf parts close to main veins (c) and over leaf parts between veins (d). See Fig. S2 for examples. The imaging protocol is shown above the figures; black horizontal bars indicate darkness (only a weak measuring beam on) and the yellow bars indicate actinic illumination (red light of PPFD ~265 µmol m^{-2} s^{-1}). In addition, 10 saturating pulses were fired to calculate F_V/F_M, NPQ and qL; the asterisks in (a) indicate the time points for NPQ measurements (Fig. 2). Before the fluorescence imaging, leaves were kept at least 30 min in the dark. After the control measurements, leaves were illuminated for four (green leaves) or two (yellow leaves) hours (PPFD 1300 µmol m^{-2} s^{-1}; After high light), after which they were let to recover over-night (14 h) under low light (PPFD ~10 µmol m^{-2} s^{-1}; After recovery). Leaves were collected on 14/10/2020, from four birch trees (one green leaf and one yellow leaf per tree). Lines show averages and from four independent measurements.

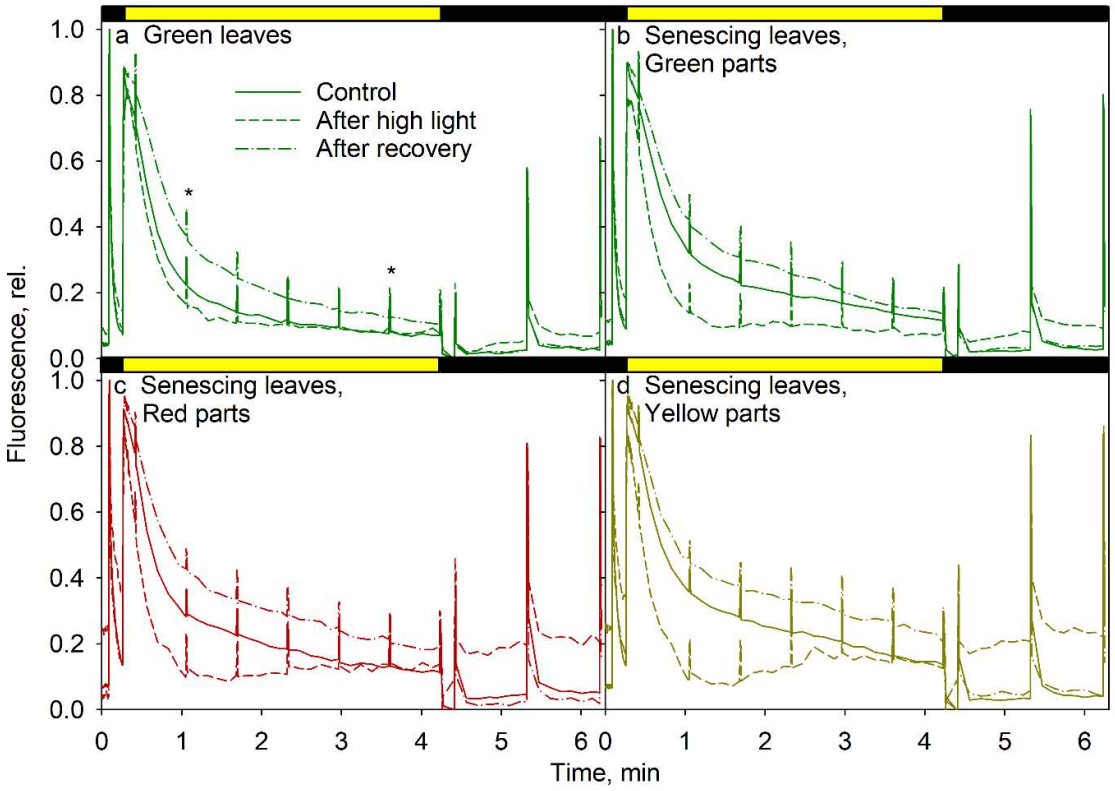

Figure S6. Fluorescence traces measured from green (a) and senescing (b–d) maple leaves. In the case of senescing leaves, leaf sections were manually annotated as green (b), red (c) or yellow (d). The imaging protocol is shown above the figures; black horizontal bars indicate darkness (only a weak measuring beam on) and the yellow bars indicates an actinic illumination (red light of PPFD ~235 µmol m^{-2} s^{-1}). In addition, 10 saturating pulses were fired to calculate F_V/F_M, NPQ and qL; the asterisks in (a) indicate the time points for NPQ measurements (Fig. 3). Before the fluorescence imaging, leaves were kept at least 30 min in the dark. After the control measurements, leaves were illuminated for two hours (PPFD 1300 µmol m^{-2} s^{-1}; After high light), after which they were let to recover over-night (14 h) under low light (PPFD ~10 µmol m^{-2} s^{-1}; After recovery). Leaves were collected on 12–19/10/2020, from three maple trees (one green leaf and at least one senescing leaf per tree). Lines show averages from at least three independent measurements.

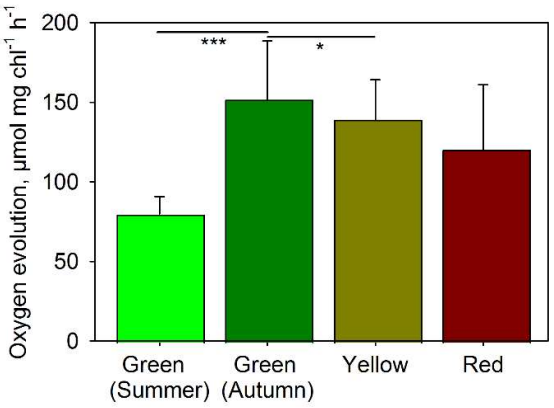

Figure S7. Light-saturated (maximum) oxygen evolution capacity of PSII in the presence of an artificial electron acceptor (0.5 mM 2,6-dimethoxybenzoquinone), measured from thylakoids isolated from fully green leaves (collected either during summer or autumn, as indicated) and from yellow or red senescing leaves of maple. All samples contained the same amount of chlorophyll. Statistically significant differences between the indicated groups are highlighted with asterisks.

Table S1. Retention times and areas (related to chlorophylls) of peaks associated with different carotenoids (recognized based on their absorption spectra), detected with HPLC from thylakoids, isolated from fully green leaves (collected either during summer or autumn, as indicated) and from yellow or red senescing leaves of maple. neo = neoxanthin, vio = violaxanthin, ant = antheraxanthin and zea =zeaxanthin. N.d. = not detected. N.a. = not available.

Peak	**Retention time, min**	**Green (summer)**	**Green (autumn)**	**Yellow**	**Red**	**Yellow, % of Green**	**Red, % of Green**
1	4.1	0.0011	0.0012	0.0019	0.0031	154	248
2	5.8	0.0009	0.0011	0.0012	0.0014	112	123
3	6.5	0.0027	0.0024	0.0028	0.0040	113	164
4	6.7	0.0038	0.0054	0.0070	0.0115	129	211
5 (neo)	7.3	0.0673	0.0681	0.0640	0.1195	94	175
6 (vio)	9.7	0.0732	0.0523	0.0622	0.1759	119	**337**
7	10.7	0.0065	0.0037	0.0074	0.0186	203	**508**
8	11.6	0.0005	0.0007	0.0008	0.0021	117	**320**
9	12.1	0.0022	0.0056	0.0053	0.0117	95	**210**
10 (ant)	12.5	0.0121	0.0015	0.0034	0.0176	228	**1194**
11 (lutein)	13.8	0.2409	0.2928	0.2889	0.4726	99	161
12 (zea)	14.0	0.0156	n.d.	n.d.	0.0145	n.a.	n.a.
13 (zea)	14.2	0.0112	0.0162	0.0190	0.0223	117	138
14	16.1	0.0012	0.0008	0.0018	0.0015	216	182
15	16.3	0.0048	0.0041	0.0036	0.0045	86	108
16	16.5	0.0026	n.d.	n.d.	n.d.	n.a.	n.a.
17	16.7	0.0072	n.d.	n.d.	n.d.	n.a.	n.a.
18	16.9	0.0131	n.d.	n.d.	n.d.	n.a.	n.a.
19 (Chl *b*)	17.3	0.2310	0.2435	0.2170	0.2132	89	88
20	18.1	0.0034	0.0010	0.0240	0.0442	2319	4272
21	18.5	0.0080	0.0004	0.0070	0.0101	1637	2370
22	18.7	0.0022	n.d.	0.0132	0.1123	n.a.	**n.a.**
23 (Chl *a*)	19.0	0.7690	0.7565	0.7830	0.7868	104	104
24	19.3	0.0005	n.d.	0.0066	0.0034	n.a.	n.a.
25	19.5	0.0013	n.d.	0.0030	0.0115	n.a.	**n.a.**
26	19.8	n.d.	n.d.	0.0010	0.0062	n.a.	**n.a.**
27	20.3	n.d.	0.0002	0.0023	0.0047	1323	**2680**
28	20.4	n.d.	0.0003	0.0040	0.0104	1475	**3850**
29	20.6	n.d.	n.d.	0.0045	0.0164	n.a.	**n.a.**
30	20.8	n.d.	0.0002	0.0031	0.0096	1640	**5048**
31	21.0	n.d.	0.0007	0.0009	0.0010	144	150
32	21.5	n.d.	0.0042	0.1545	0.2001	3695	4785
33	22.0	0.0030	0.0028	0.0031	0.0004	110	14
34	22.3	n.d.	n.d.	0.0054	n.d.	n.a.	n.a.
35	22.4	n.d.	n.d.	n.d.	0.0076	n.a.	n.a.
36	23.8	n.d.	0.0003	0.0078	0.0106	2598	3513
37	24.8	n.d.	n.d.	0.0020	n.d.	n.a.	n.a.
38	26.7	0.0271	0.0225	0.0322	0.0100	143	44

39	27.3	0.0654	0.0503	0.0650	0.0747	129	148
40	27.6	0.0078	0.0034	0.0072	0.0083	212	244