Supplemental data

Downsizing in plants - UV induces pronounced morphological changes in the absence of stress

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Supplemental Table S1. Results from pairwise t-test for significance of treatment of cucumber seedlings as presented in Figure 8 of the main manuscript. A, Leaf adaxial epidermal flavonol content (LAEFC), as measured with the Dualex method; B, Total UV-absorbing pigments (TUAP), measured spectrophotometrically at 330 nm; C, Total antioxidant capacity (TAC) meausured as nmol Trolox equivalents. Measurements were performed on the 2nd true leaf of two-week old plants under UV-A-enriched (UVA) or UV-B-enriched light (UVB), respectively, and compared with the corresponding controls (C), for 0 (D0),1 (D1), 3 (D3), 5 (D5), 10 (D10) or 14 (D14) days. The number of plants analyzed were n = 15-18 for the UV-enriched treatments and n = 30-36 or the control treatment in (A); n = 9 for the UV-enriched treatments and n = 18 or the control treatment in (B); n = 3 for the UV-enriched treatments and n = 6 or the control treatment in (C).

A
LAEFC

Treatment	C-D0	UVA-D0	UVB-D0	C-D1	UVA-D1	UVB-D1	C-D3	UVA-D3	UVB-D3	C-D5	UVA-D5	UVB-D5	C-D10	UVA-D10	UVB-D10	C-D14	UVA-D14	UVB-D14
C-D0		p=0.232	p=0.200	p=1.000			p=0.178			p=0.089			p=0.013			p<0.001		
UVA-D0	n.s.		p=0.938		p=0.138			p=0.453			p<0.001			p=0.210			p<0.001	
UVB-D0	n.s.	n.s.				p=0.999			p<0.001			p<0.001			p=0.598			p<0.001
C-D1	n.s.	n.p.c.	n.p.c.		p=0.979	p=0.141	p=0.125			p=0.041			p=0.003			p<0.001		
UVA-D1	n.p.c.	n.s.	n.p.c.	n.s.		p=0.108		p=0.011			p<0.001			p=0.969			p<0.001	
UVB-D1	n.p.c.	n.p.c.	n.s.	n.s.	n.s.				p<0.001			p<0.001			p=0.587			p<0.001
C-D3	n.s.	n.p.c.	n.p.c.	n.s.	n.p.c.	n.p.c.		p<0.001	p<0.001	p<0.001			p=0.052			p<0.001		
UVA-D3	n.p.c.	n.s.	n.p.c.	n.p.c.	*	n.p.c.	****		p<0.001		p<0.001			p=0.038			p<0.001	
UVB-D3	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	****	****				p=0.004			p=0.005			p<0.001
C-D5	n.s.	n.p.c.	n.p.c.	*	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.		p<0.001	p<0.001	p<0.001			p<0.001		
UVA-D5	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	****		p=0.009		p<0.001			p<0.001	
UVB-D5	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	***	****	**				p<0.001			p<0.001
C-D10	*	n.p.c.	n.p.c.	***	n.p.c.	n.p.c.	n.s.	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.		p=0.016	p<0.001	p=0.015		
UVA-D10	n.p.c.	n.s.	n.p.c.	n.p.c.	n.s.	n.p.c.	n.p.c.	*	n.p.c.	n.p.c.	****	n.p.c.	*		p=0.089		p=0.003	
UVB-D10	n.p.c.	n.p.c.	n.s.	n.p.c.	n.p.c.	n.s.	n.p.c.	n.p.c.	**	n.p.c.	n.p.c.	****	****	n.s.				p<0.001
C-D14	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	*	n.p.c.	n.p.c.		p=0.107	p=0.004
UVA-D14	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	***	n.p.c.	n.s.		P=0.122
UVB-D14	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	***	n.s.	

B

TUAP

Treatment	C-D0	UVA-D0	UVB-D0	C-D1	UVA-D1	UVB-D1	C-D3	UVA-D3	UVB-D3	C-D5	UVA-D5	UVB-D5	C-D10	UVA-D10	UVB-D10	C-D14	UVA-D14	UVB-D14
CKA-D0		p=0.813	p=0.797	p=0.040			p<0.001			p<0.001			p=0.061			p<0.001		
UVA-D0	n.s.		p=0.677		p=0.017			p<0.001			p<0.001			p=0.018			p=0.105	
UVB-D0	n.s.	n.s.				p=0.005			p<0.001			p<0.001			p<0.001			p=0.863
CKA-D1	*	n.p.c.	n.p.c.		p=0.689	p=0.156	p=0.044			p<0.001			p=0.414			p<0.001		
UVA-D1	n.p.c.	*	n.p.c.	n.s.		p=0.219		p<0.001			p<0.001			p=0.878			p<0.001	
UVB-D1	n.p.c.	n.p.c.	**	n.s.	n.s.				p<0.001			p<0.001			p=0.198			P=0.002
CKA-D3	****	n.p.c.	n.p.c.	*	n.p.c.	n.p.c.		p<0.001	p<0.001	p<0.001			p<0.001			p<0.001		
UVA-D3	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	****		p=0.008		p<0.001			p<0.001			p<0.001	
UVB-D3	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	****	**				p<0.001			p<0.001			p<0.001
CKA-D5	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.		p=0.003	p<0.001	p<0.001			p<0.001		
UVA-D5	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	***		P=0.002		p<0.001			p<0.001	
UVB-D5	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	****	***				p<0.001			p<0.001
CKA-D10	n.s.	n.p.c.	n.p.c.	n.s.	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.		p=0.121	p<0.001	p<0.001		
UVA-D10	n.p.c.	*	n.p.c.	n.p.c.	n.s.	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.s.		p=0.008		p<0.001	
UVB-D10	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	n.s.	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	****	**				p<0.001
CKA-D14	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.		p=0.098	p<0.001
UVA-D14	n.p.c.	n.s.	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.s.		p=0.007
UVB-D14	n.p.c.	n.p.c.	n.s.	n.p.c.	n.p.c.	***	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	****	**	

C

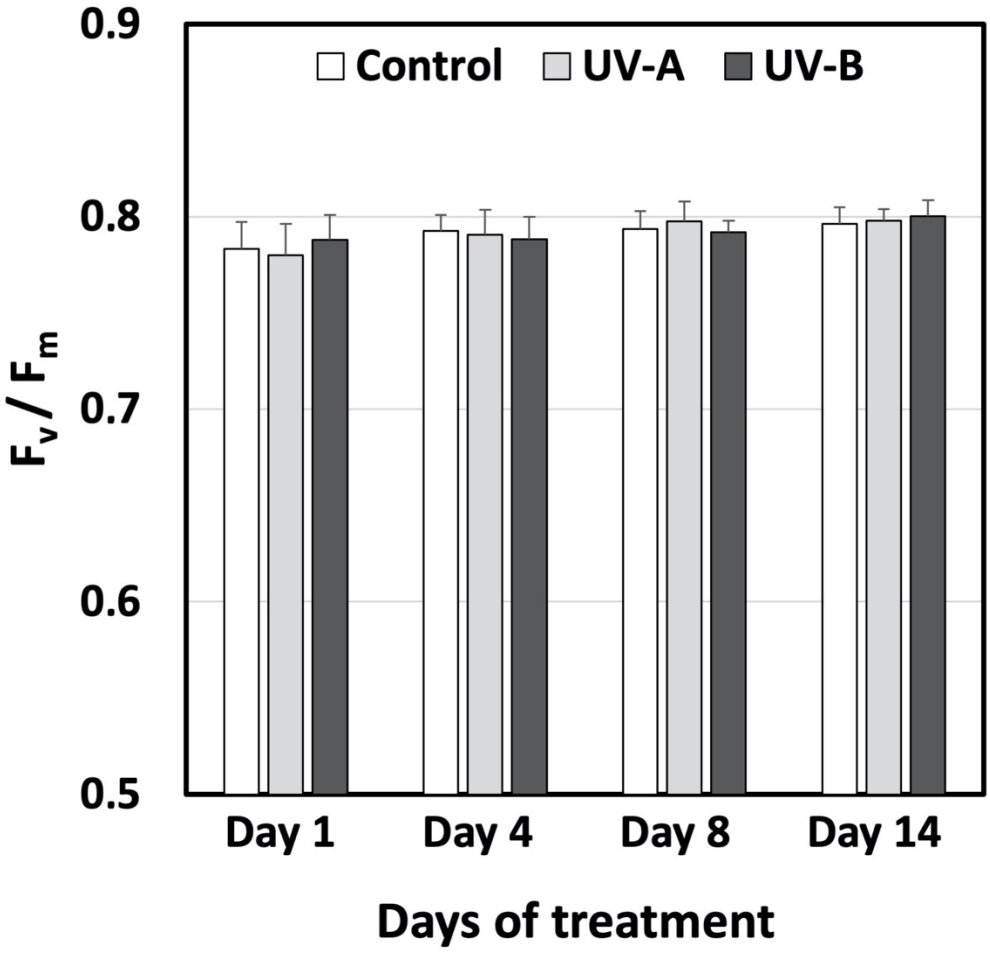
TAC

Treatment	C-D0	UVA-D0	UVB-D0	C-D1	UVA-D1	UVB-D1	C-D3	UVA-D3	UVB-D3	C-D5	UVA-D5	UVB-D5	C-D10	UVA-D10	UVB-D10	C-D14	UVA-D14	UVB-D14
C-D0		p=0.479	p=0.479	p=0.002			p=0.002			p<0.001			p<0.001			p<0.001		
UVA-D0	n.s.		p=1.00		p=0.062			p=0.001			p<0.001			p<0.001			p<0.001	
UVB-D0	n.s.	n.s.				p=0.007			p=0.002			p<0.001			p<0.001			p=0.001
C-D1	***	n.p.c.	n.p.c.		p=0.935	p=0.195	p=0.485			p=0.051			p<0.001			p<0.001		
UVA-D1	n.p.c.	n.s.	n.p.c.	n.s.		p=0.232		p=0.035			p=0.008			p<0.001			p<0.001	
UVB-D1	n.p.c.	n.p.c.	**	n.s.	n.s.				p=0.039			p<0.001			p<0.001			p<0.001
C-D3	***	n.p.c.	n.p.c.	n.s.	n.p.c.	n.p.c.		p=0.001	p<0.001	p=0.010			p<0.001			p<0.001		
UVA-D3	n.p.c.	***	n.p.c.	n.p.c.	*	n.p.c.	***		p=0.251		p=0.077			p<0.001			p<0.001	
UVB-D3	n.p.c.	n.p.c.	***	n.p.c.	n.p.c.	*	****	n.s.				p=0.004			p<0.001			p<0.001
C-D5	****	n.p.c.	n.p.c.	n.s.	n.p.c.	n.p.c.	*	n.p.c.	n.p.c.		p=0.022	p<0.001	p<0.001			p<0.001		
UVA-D5	n.p.c.	****	n.p.c.	n.p.c.	**	n.p.c.	n.p.c.	n.s.	n.p.c.	*		p=0.004		p<0.001			p<0.001	
UVB-D5	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	***	****	***				p<0.001			p<0.001
C-D10	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.		p=0.739	p=0.002	p=0-001		
UVA-D10	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.s.		p=0.002		p=0.017	
UVB-D10	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	***	***				p=0,014
C-D14	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	**	n.p.c.	n.p.c.		p=0,797	p=0.142
UVA-D14	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	*	n.p.c.	n.s.		p=0.323
UVB-D14	n.p.c.	n.p.c.	***	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	****	n.p.c.	n.p.c.	*	n.s.	n.s.	

Legend

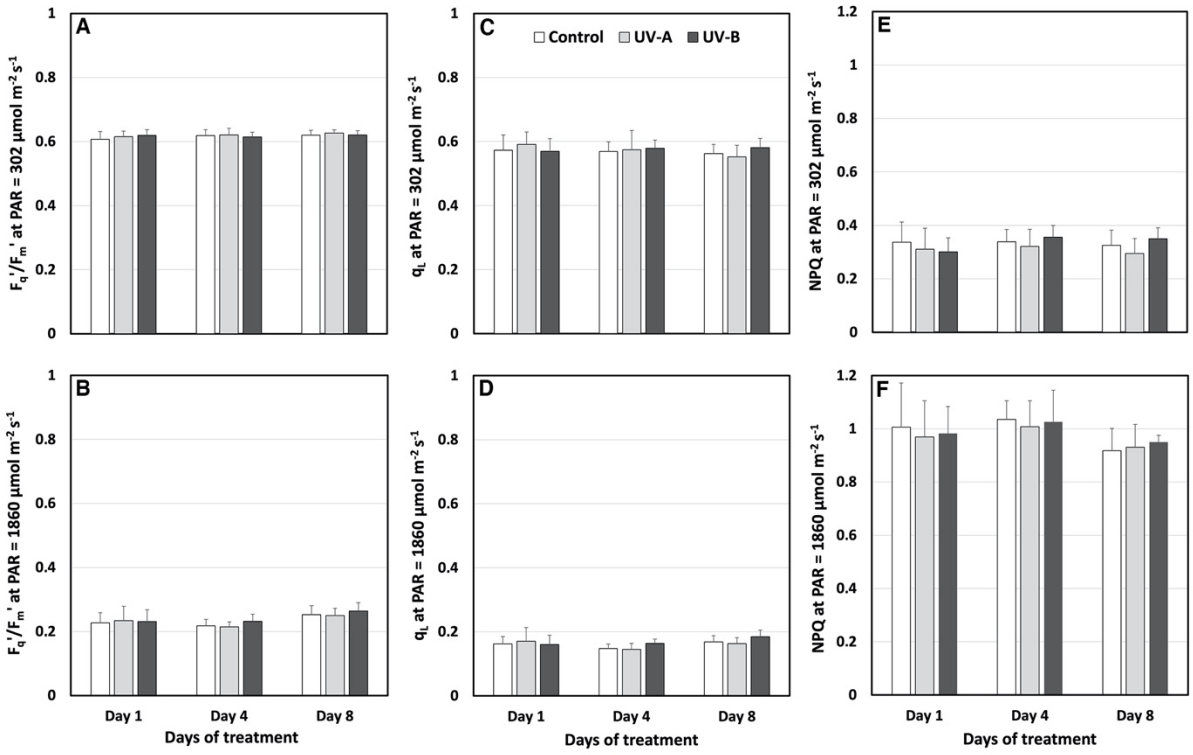
*	0.01≤p<0.05	****	p<0.001
**	0.005≤p<0.01	n.s.	not significant
***	0.001≤p<0.005	n.p.c.	non-physiological comparison

Supplemental Figure S1.



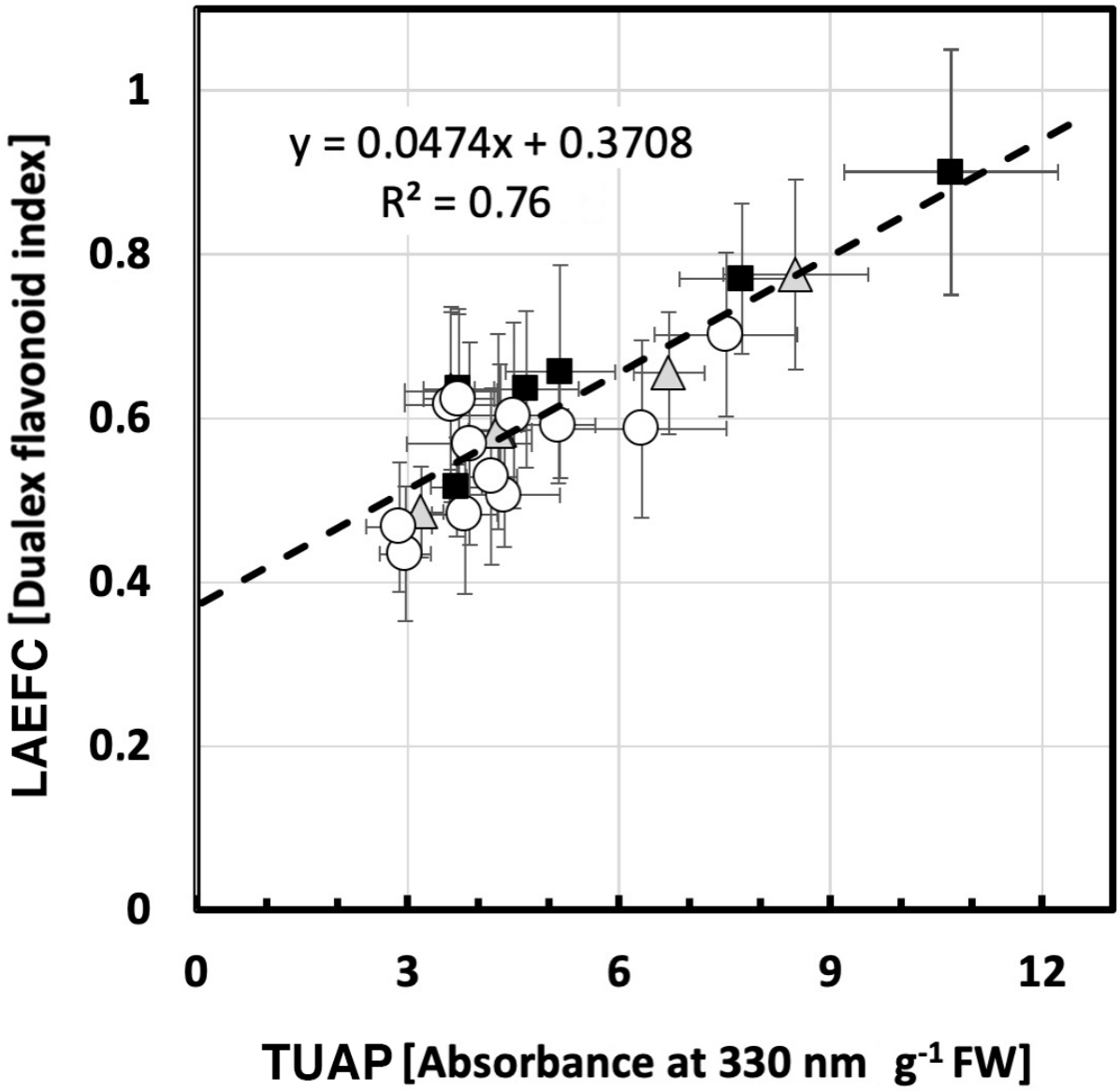
Supplemental Figure S1. Maximum photochemical efficiency (F_v/F_m) measured in cucumber plants grown under different light conditions. The response of F_v/F_m measured on the youngest well-developed leaf after 30 min dark adaptation leaf was measured on days 1, 4, 8, and 14 days after commencement of UV exposure to UV deficient control (white columns), UV-A-enriched (light grey columns), or UV-B-enriched (dark grey columns) light. The data represent mean values ± SD with n = 9 for UV treatments and n = 18 for controls.

Supplemental Figure S2.



Supplemental Figure S2. Time courses of photosynthetic parameters measured in cucumber plants grown under different light conditions. The response of F_q'/F_m' (the operation efficiency of PSII) (A, B), q_L (the fraction of open PSII) (C, D), and NPQ (heat dissipation measured as non-photochemical quenching) (E, F) measured on the youngest well-developed leaf and followed by exposure to actinic light at low (302 µmol m^{-2} s^{-1}) (A, C, E) and high (1860 µmol m^{-2} s^{-1}) (B, D, F) PAR. to UV deficient control (white columns), UV-A-enriched (light grey columns), or UV-B-enriched (dark grey columns) light. The data represent mean values ± SD with n = 9 for UV treatments and n = 18 for controls.

Modelling the relationship between leaf flavonoid content and antioxidant capacity



Supplemental Figure S3. The monophasic relationship between LAEFC and TUAP. The graph shows the simple linear relationship relationship assumed in Eqn. S1 between LAEFC and TUAP. Open circles denote UV-deficient control treatment, grey triangles denote treatment with UV-A-enriched light and black boxes denote treatment with UV-B-enriched light. The data represent mean values ± SD. N = 15-18 for LAEFC UV-enriched treatments and 30-36 for control treatments; n = 9 for TUAP UV-enriched treatments and 18 for control treatments.

LAEFC vs. TUAP

To ascertain to what extent the methods used for LAEFC and TUAP leaf flavonoid content describe the same physiological process within the plant leaf (i.e. that the two different pools

of flavonoids measured by these techniques where directly proportional to each other), two models were adapted to describe the relationship between the methods. In Supplemental Figure S1, a simple linear relationship between read-outs of the two analytical methods was assumed, without taking into account the type of treatment (UV-A- or UV-B-enriched) or leaf age. The linear relationship was found to be:

$$\hat{y} = 0.37 + 0.047X, \qquad \text{(Eqn. S1)}$$

where $\hat{y}$ is the estimated expected LAEFC Dualex read-out and x is the value when TUAP method is used. The R^2 of this linear fit was 0.76. In the second model, the dependence between the results of the LAEFC and TUAP methods was assumed to be due also to treatment (UV-A- or UV-B-enriched, or control) and leaf age (see main text Eqn. 1). A small influence of leaf age was found with a R^2 of this fit was 0.84, indicating that the second model is better in explaining the dependence between LAEFC and TUAP. Also, the residual plots show a random pattern on both sides of 0 (Supplemental Figure S2A and S2B), thus justifying the model assumptions. Thus, as the tissue aged, there was a tendency for the first model (Supplemental Eqn. S1) to exhibit increased residuals (deviations between true and estimated values), indicating that leaf age is also a determinant of the relationship between LAEFC and TUAP.

TAC vs. LAEFC

The dependence between the TAC and the LAEFC assays, was first studied assuming two simple linear relationships, without taking into account the type of treatment (UV-A- or UV-B-enriched), but dividing up the samples in those for younger leaves (≤ 5 days of exposure time, i.e. ≤ 19 days after sowing) and those from older leaves (≥ 10 days of exposure time, i.e. ≥ 24 days after sowing) (see Main Text Eqs. 2 and 3).

We also applied a second model to describe the results of the LAEFC measurements with the TAC method as an explanatory variable and also including explanatory variables treatment (UV-A- or UV-B-enriched) and leaf age. However, in this case we did not find any statistically significant proof for this assumption, and instead obtained a straight line dependence with the following equation:

$$\hat{y} = 0.34 + 0.08X, \qquad \text{(Eqn. S2)}$$

with an R^2 value 0.70. The intercept at 0 TAC now gave a value of 0.34 in the LAEFC parameter. The plot of the residuals for the second model is shown in Supplemental Figure S2C, again exhibiting a random pattern on both sides of 0 indicating that this model is

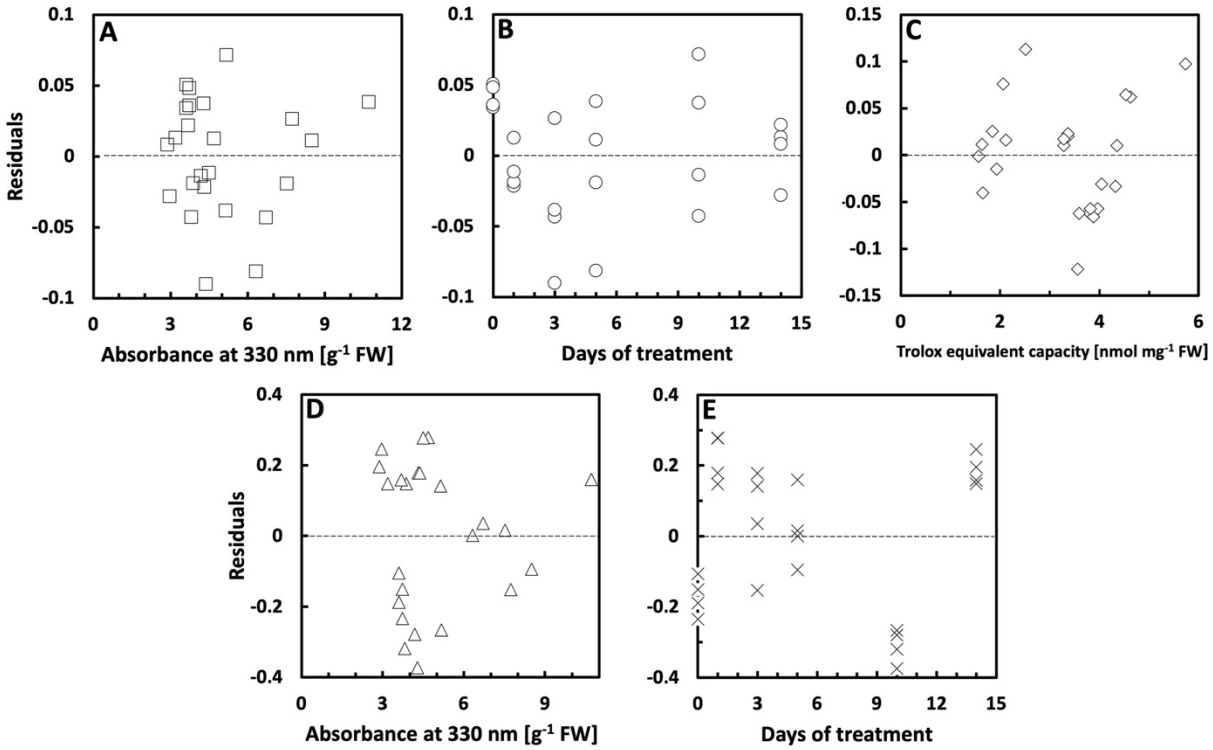
sufficient to explain the data. Thus, we could not conclude with statistical significance whether a leaf age effect was explaining the correlation between the data or not.

TAC vs. TUAP

The dependence between TAC and TUAP was then studied. In Eqs. 4 and 5 and Figure 9B of the Main Text, we first assumed two simple linear relationships between TAC and TUAP with $R^2 > 0.91$ in both cases. Also, in the second model, where involvement of both treatment effects and leaf age, were considered. A clear effect of tissue age was seen on the dependence between the TUAP and TAC assays. In this case, the estimated equation became:

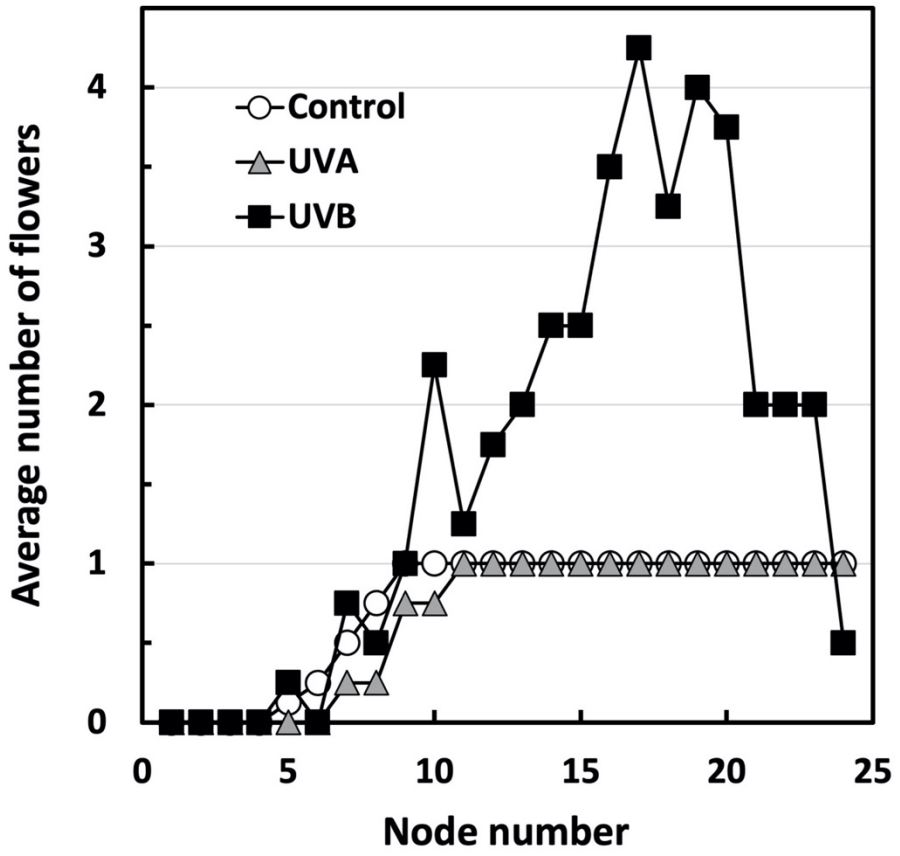
$$\hat{y} = 2.07 + 0.39X - 0.13X3, \qquad \text{(Eqn. S3)}$$

again with a high factor $R^2 = 0.96$. The residual plots (Supplemental Figures S2D and S2E) corroborate this choice of model. Thus, leaf age has to be considered when comparing results of assays for leaf total flavonoids and anti-oxidative capacity.



Supplemental Figure S4. Residual (ε) plots for models of correlation between the three methods used to determine LAEFC, TUAP, and TAC. See also Figure 9 of the main text and Supplementary Figure S3. A) Residual plot for the OD330 variable of the correlation of between LAEFC and TUAP; B) Residual plot for the leaf age variable of the correlation between LAEFC and TUAP; and C) Residual plot for the TAC variable of the correlation of between LAEFC and TAC; D) Residual plot for the TUAP variable of the correlation of between TAC and TUAP; E) Residual plot for the leaf age variable of the correlation between TAC and TUAP.

Supplemental Figure S5



Supplemental Figure S5. Average number of flowers in each leaf node in cucumber plants that had been exposed to UV-A-enriched, UV-B-enriched non-UV supplemented growth light as seedlings. Seedlings treated with UV-enriched light were exposed during 4 h for 14 days and then transferred to a commercial or semi-commercial greenhouse for fruit production. In one of the four studies carried out during three years, plants pre-exposed to UV-B-enriched light showed increased number of flowers in leaf nodes 10 to 23, whereas control plants or plants pre-exposed to UV-A-enriched light had only one flower per leaf node in nodes 11 to 24. n=12 for control plants, n= 4 for plants pre-exposed to UV-A- or UV-B-enriched light.