Identification of stress in plants via femtosecond laser-induced fluorescence and steady-state absorption spectroscopy

MTV Workshop, 2021

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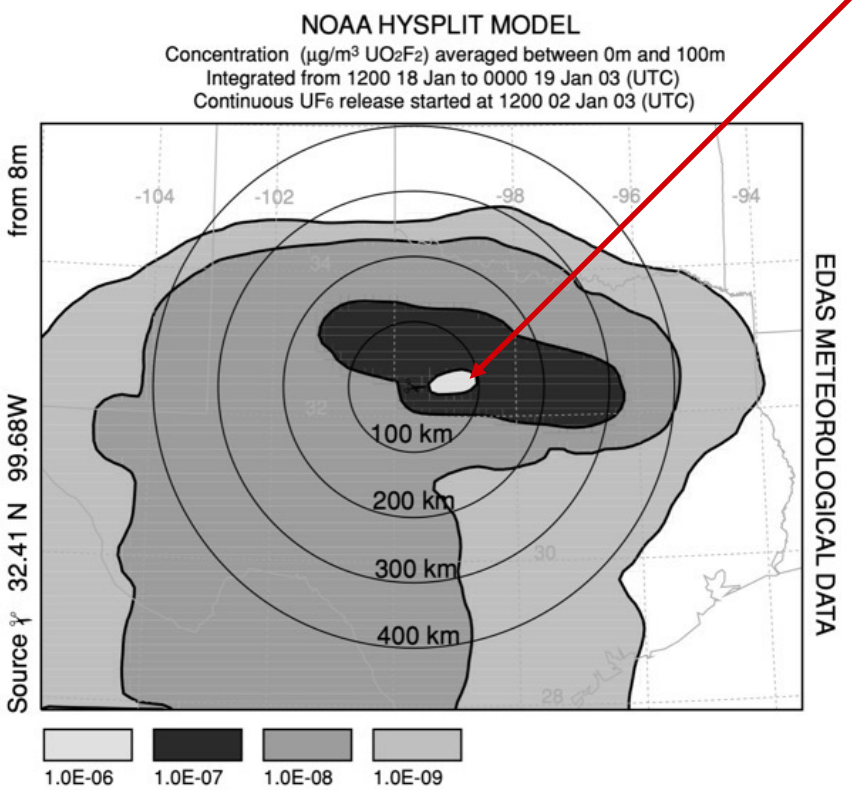
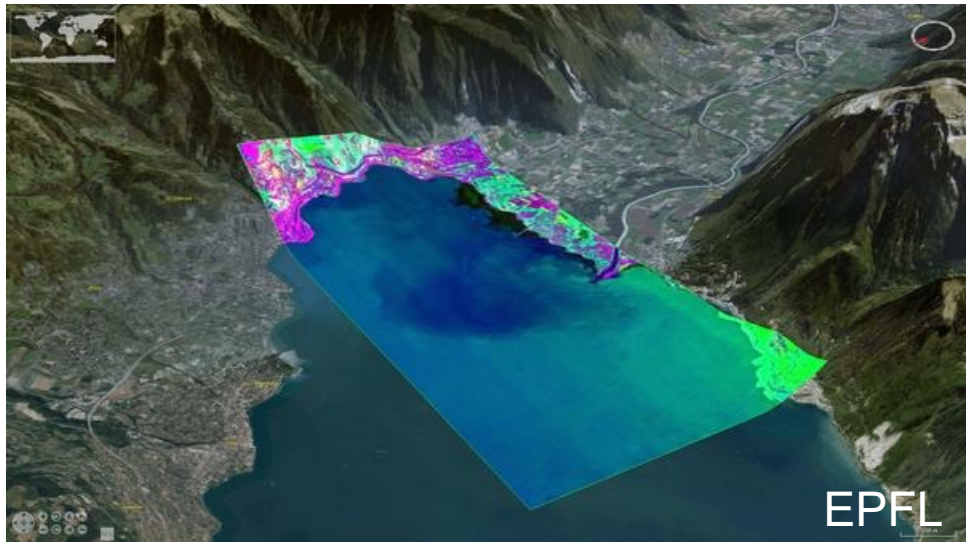
Introduction and Motivation

Cylindrical volume of 1 km height and 2 km radius contains enough UO_2F_2 to deposit 50 μM U.

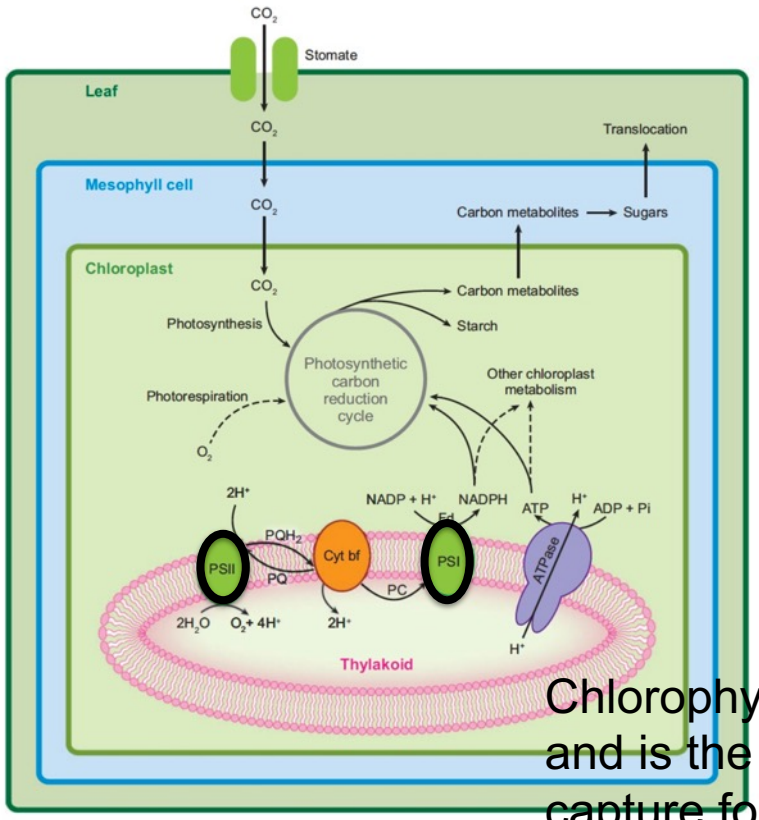




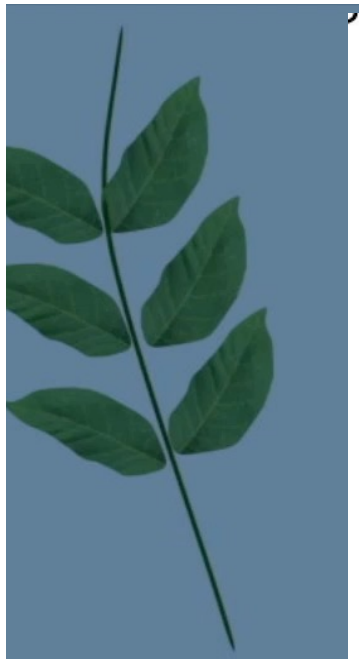
Emissions from centrifuge enrichment facilities may emit and deposit in the surrounding environment. Laser-based methods may be beneficial to detect low concentrations of nonproliferation relevant materials, like UO_2F_2, is necessary for monitoring enrichment activities.

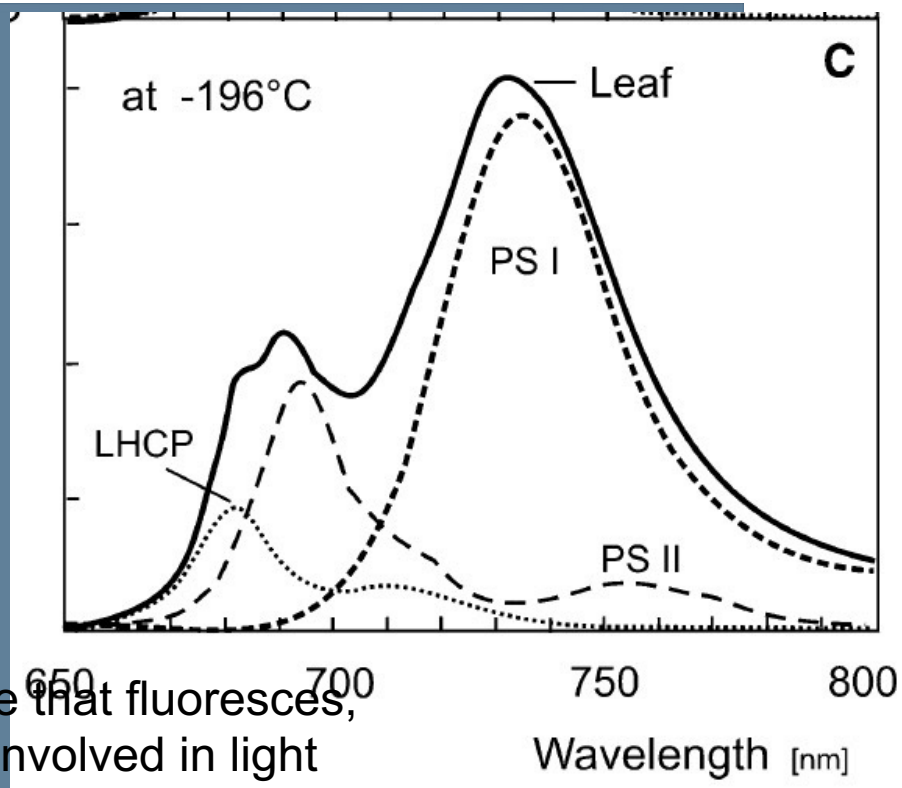
Mission Relevance: Develop remote, high sensitivity methods for in-field monitoring

Technical background: Plants as biosensors



Baker, Annu. Rev. Plant Biol., **113**, 89(2008).



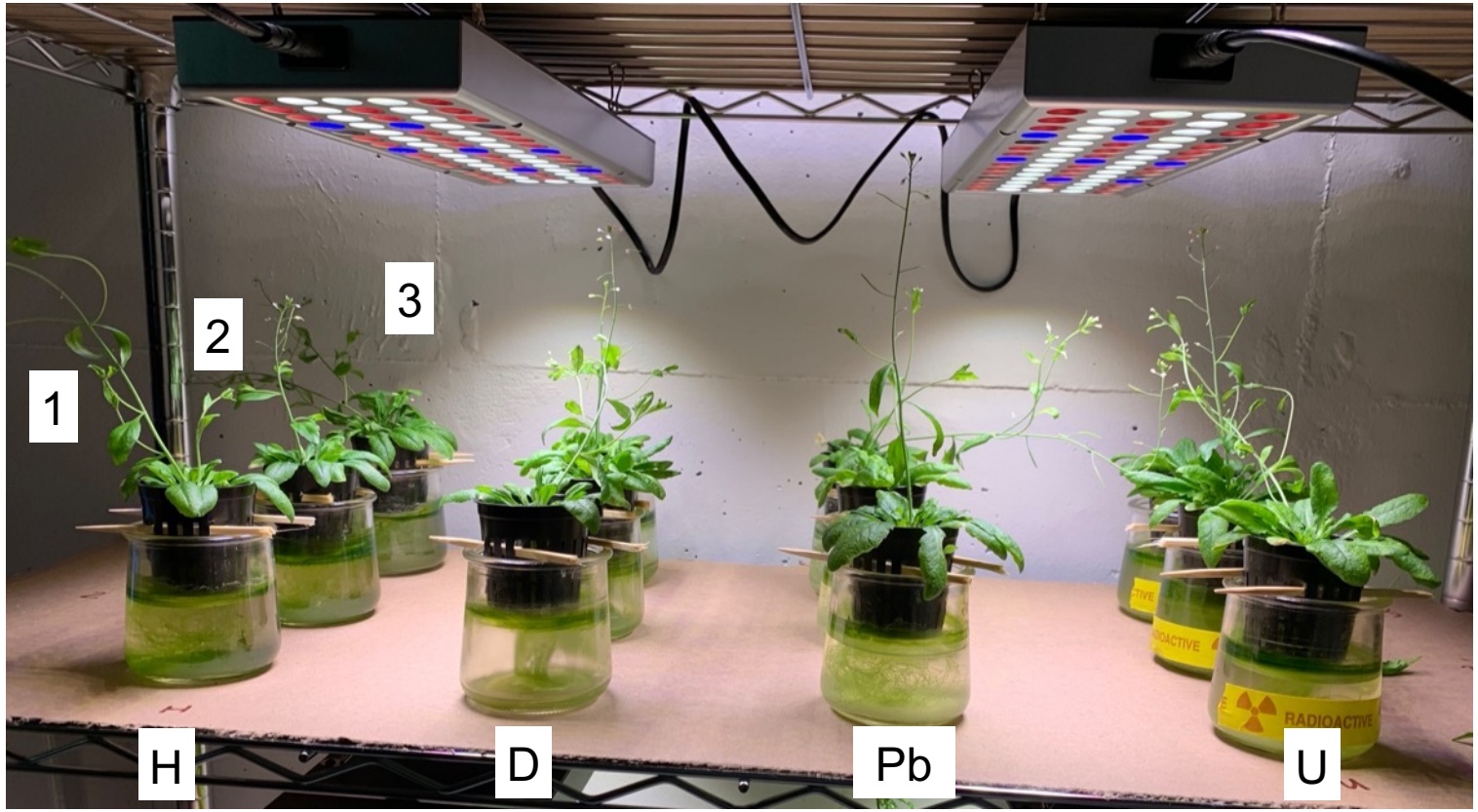


Chlorophyll is the molecule that fluoresces, and is the main molecule involved in light capture for photosynthesis.

Buschmann, Photosyn. Res., **92**, 261(2007).

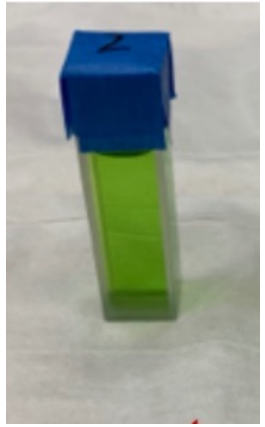
PSII reaction center called P680 (primarily fluoresces at 680 nm)
PSI reaction center called P735 (primarily fluoresces at 735 nm)

First systematic study of stress exposure with *arabidopsis thaliana*



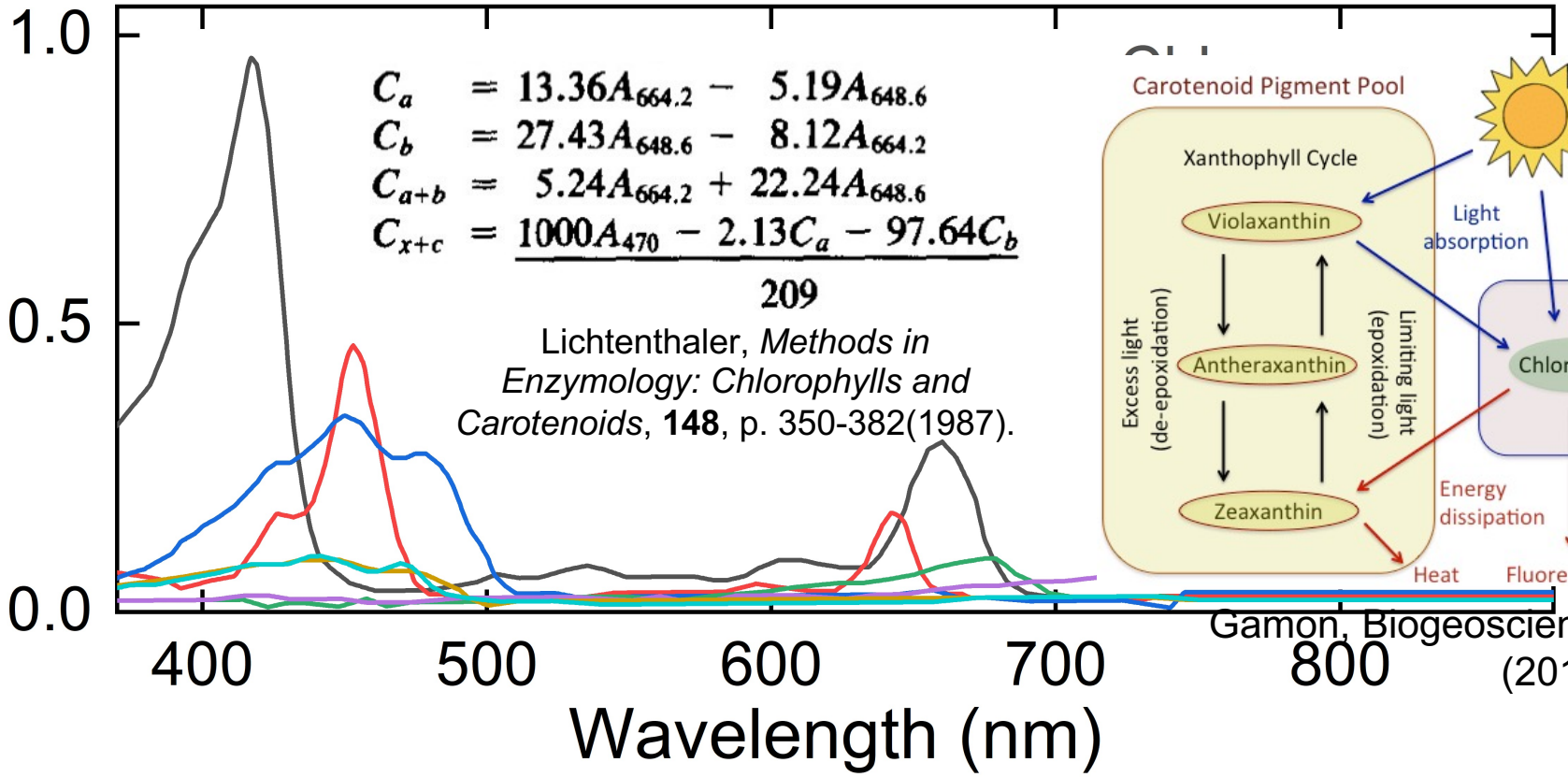
H: healthy (control)
D: drought
Pb: lead contaminated (500 μM)
U: uranium contaminated (500 μM)

Plants are 50 days old at time of exposure



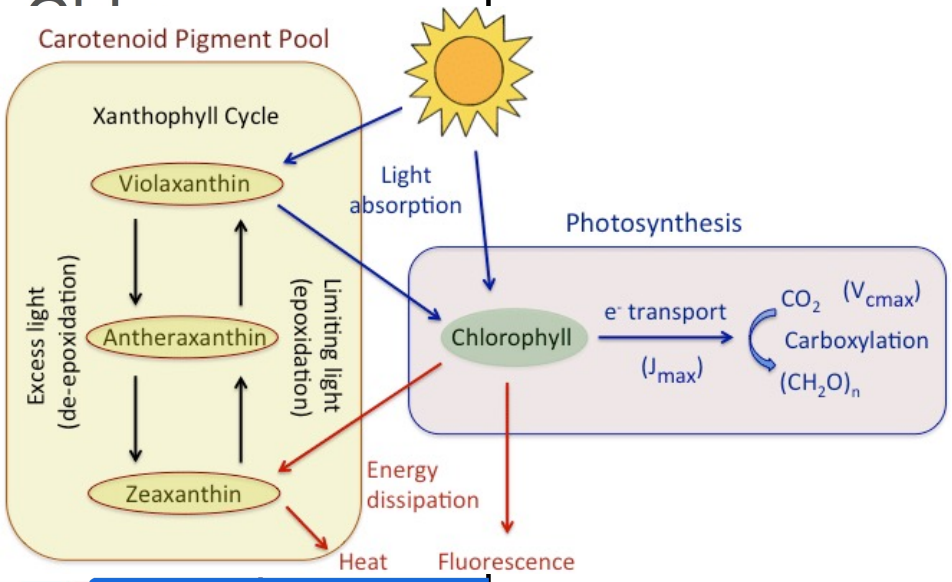
We use polar solvent extraction of leaves to draw pigments into solution.

Absorption spectroscopy informs how pigment content varies in different growth environments



$$C_a = 13.36A_{664.2} - 5.19A_{648.6}$$
$$C_b = 27.43A_{648.6} - 8.12A_{664.2}$$
$$C_{a+b} = 5.24A_{664.2} + 22.24A_{648.6}$$
$$C_{x+c} = \frac{1000A_{470} - 2.13C_a - 97.64C_b}{209}$$

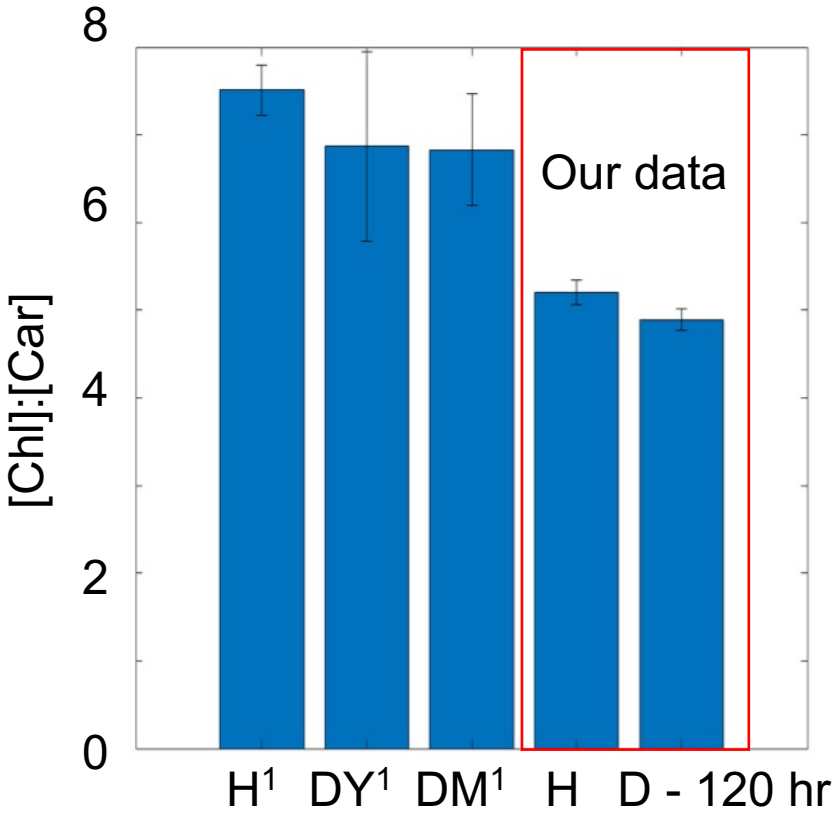
Lichtenthaler, *Methods in Enzymology: Chlorophylls and Carotenoids*, **148**, p. 350-382(1987).

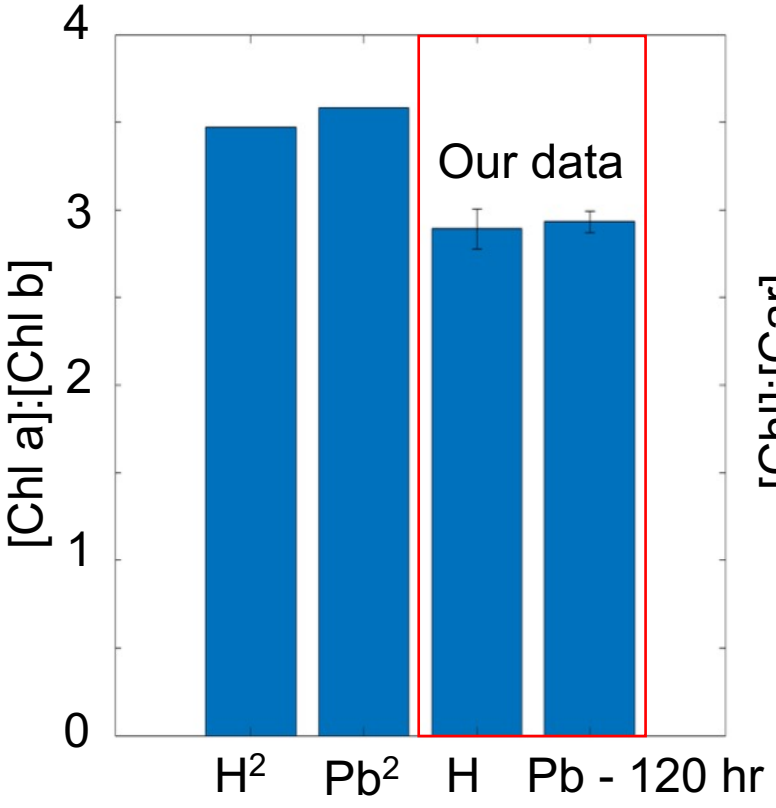


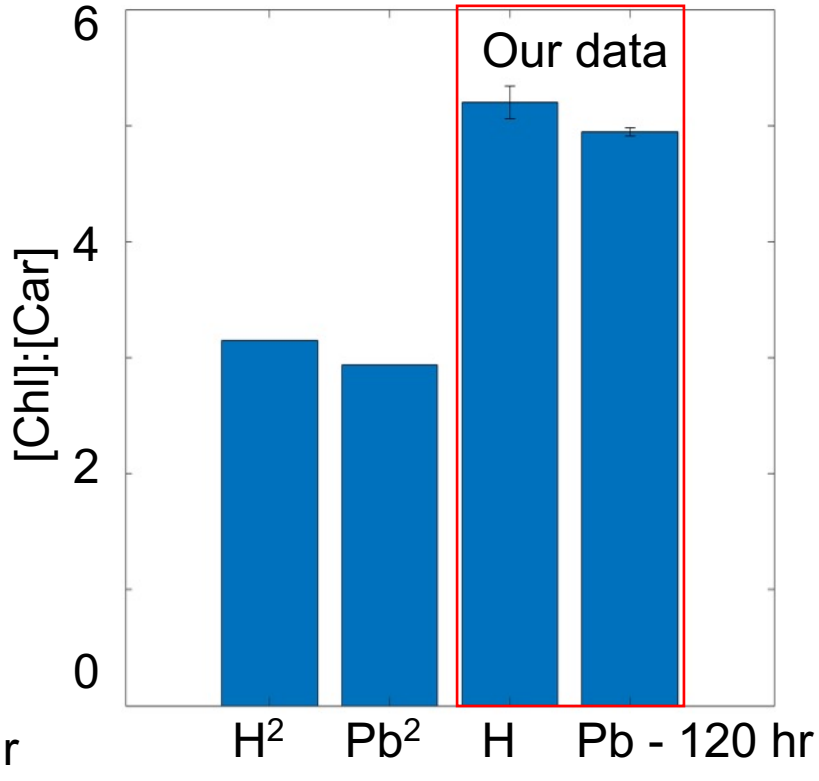
Gamon, Biogeosciences, 12, 4509-4523 (2015).

Carotenoids are heavily involved in stress remediation efforts, while chlorophyll content can be a signature of photosynthetic efficiency. Analysis of both can provide specifics about how the plant is responding to stress.

We observe similar change in pigment ratios for certain stresses to that of the literature







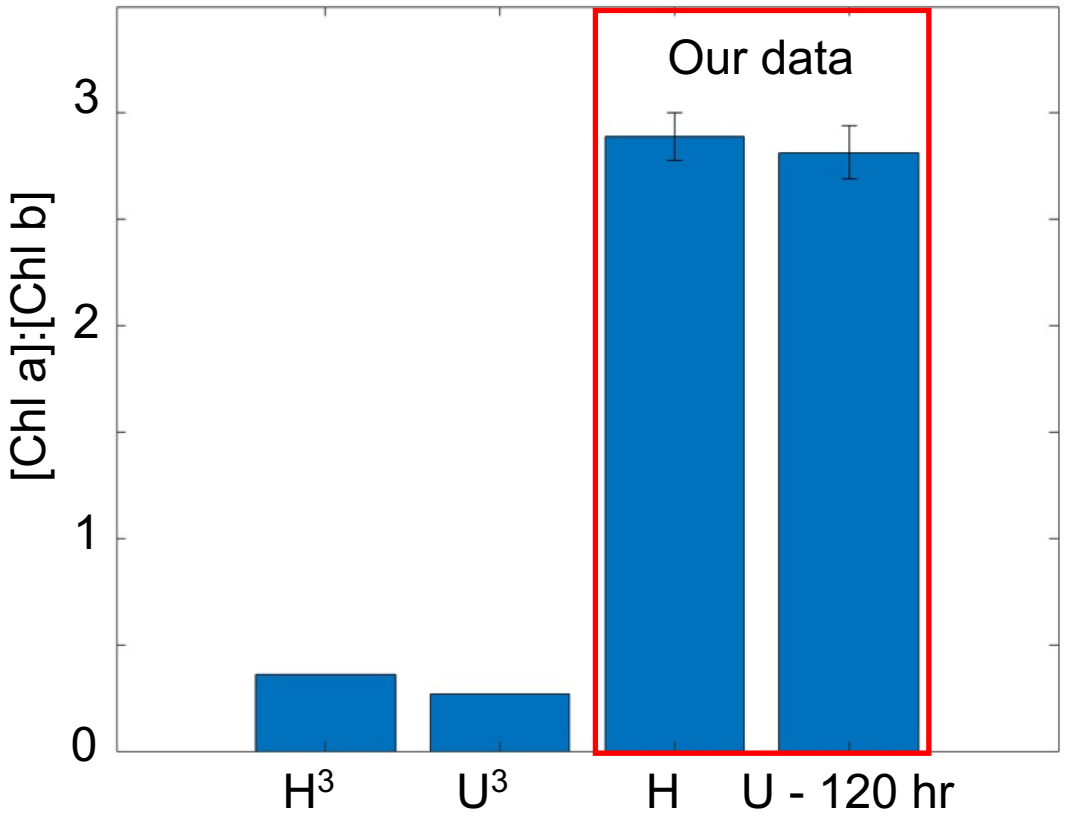
[1]Jung, Plant Science, **166**, 459(2004).
Arabidopsis thaliana, 28 days old
DY = drought, young leaves
DM = drought, mature leaves

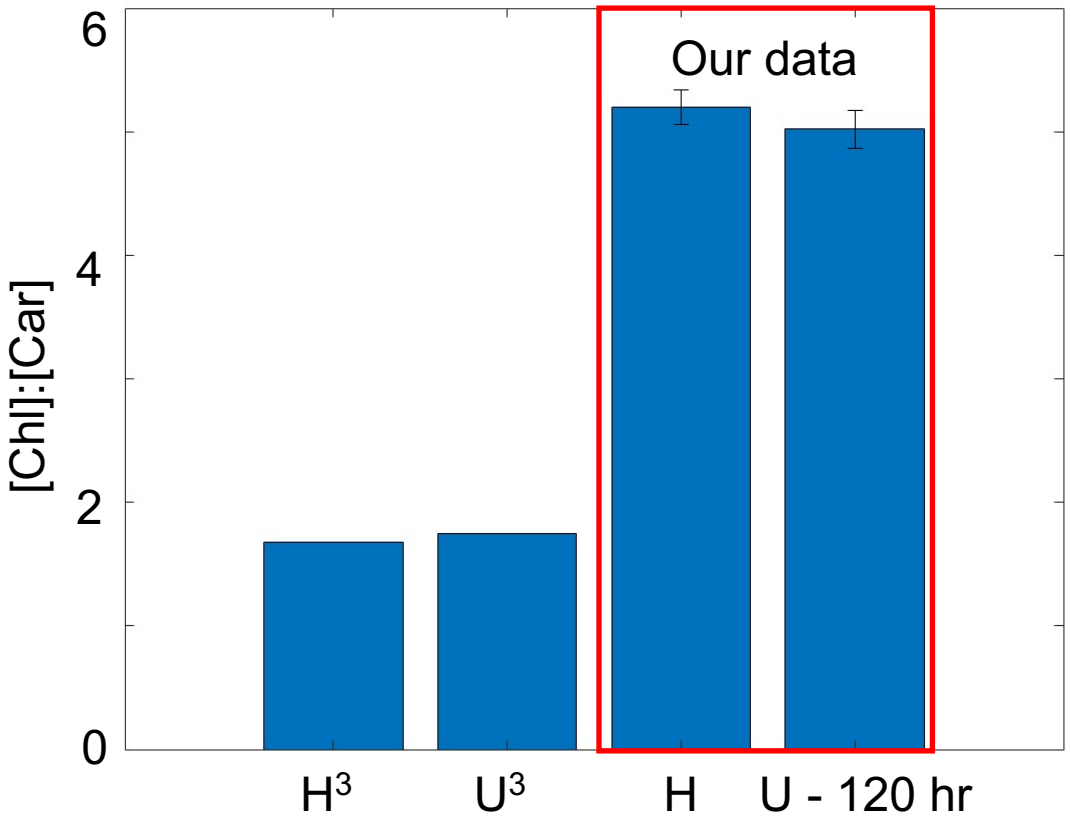
[2]Larbi, Funct. Plant Biol., **29**, 1453(2002).
Pb - 2 mM; *Beta vulgaris*, 28 days; 7 days after exposure

Uranium literature is more sparse, direct comparison needs further analysis

[3]Vanhoudt *et al.*, Environ. And Exper. Botany, **97**, 22(2014).

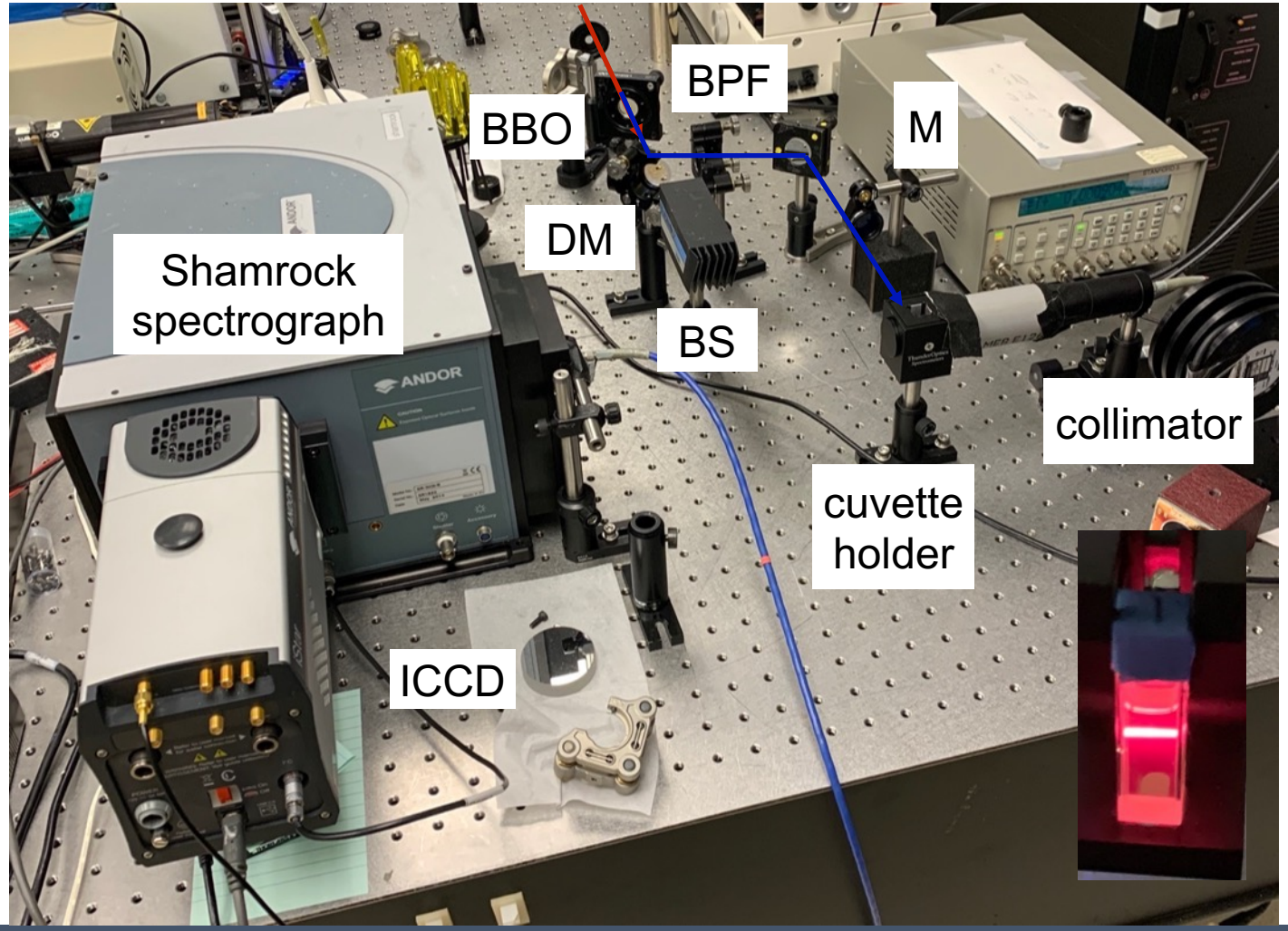
U - 50 uM; *arabidopsis thaliana*, 28 days; 96 hrs after exposure





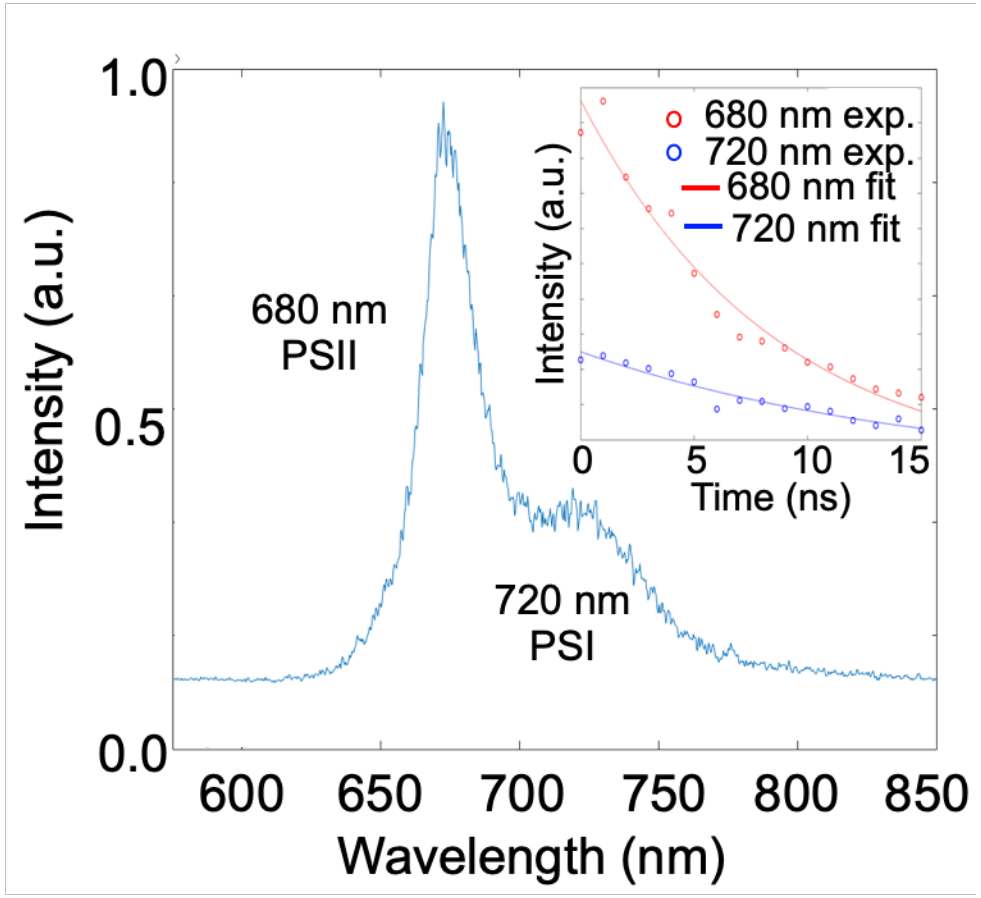
We record time-dependent fluorescence spectroscopy via ultrafast laser excitation

390 nm
<10 uJ
120 fs pulse duration
1 kHz rep. rate

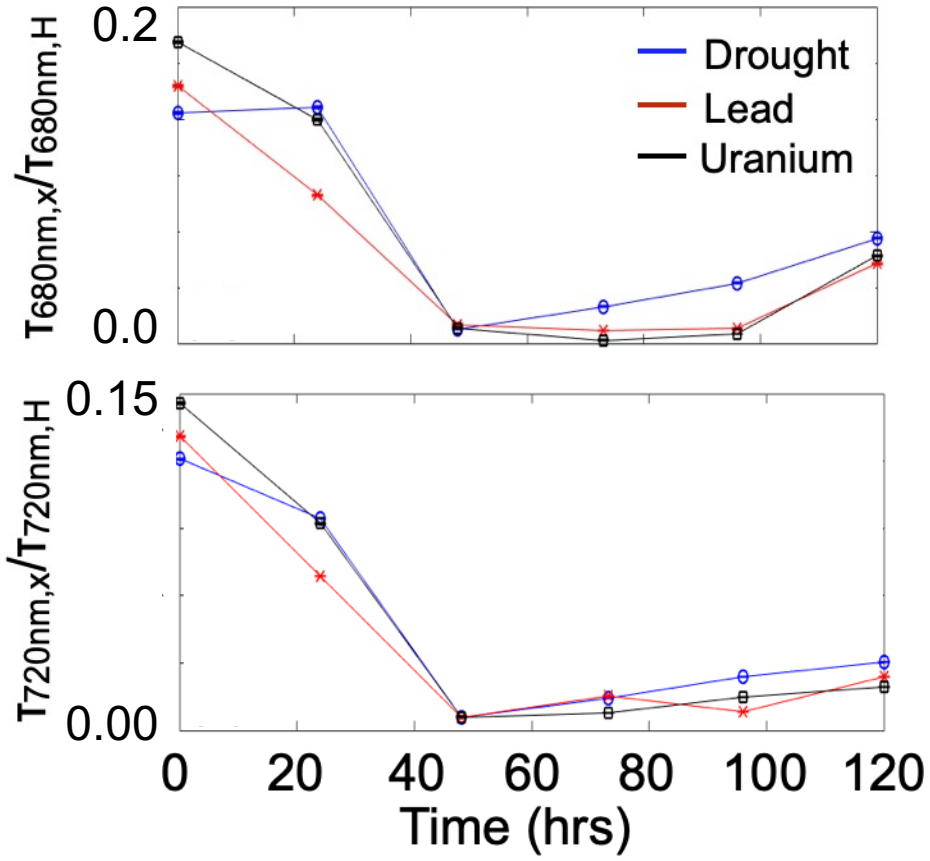


BPF: 390 nm band pass filter
BS: beam stop
DM: dichroic mirror

We observe an overall decrease in fluorescence lifetime at 48 hours after stress exposure

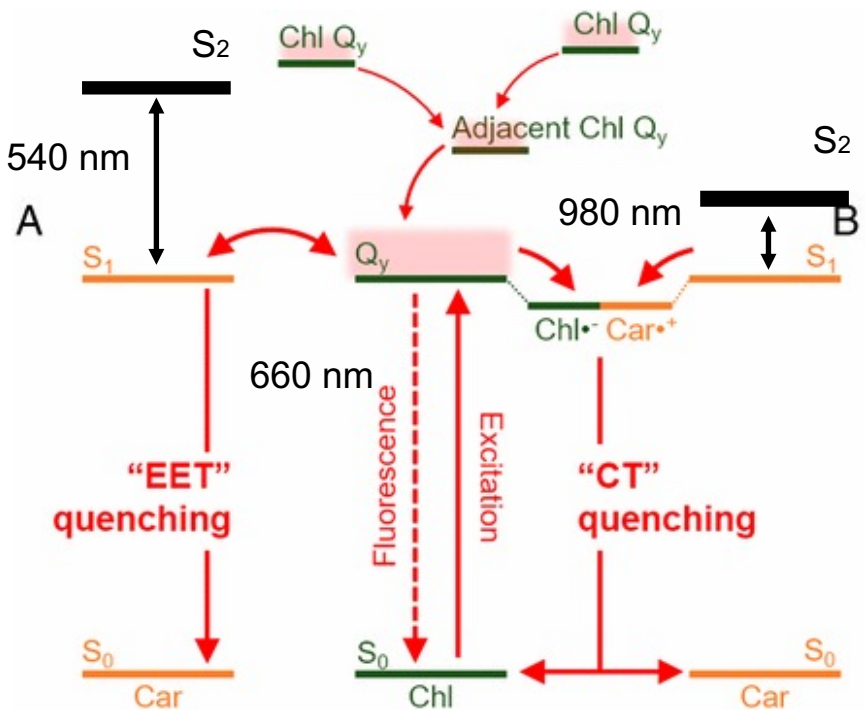


X denotes stress
H = healthy

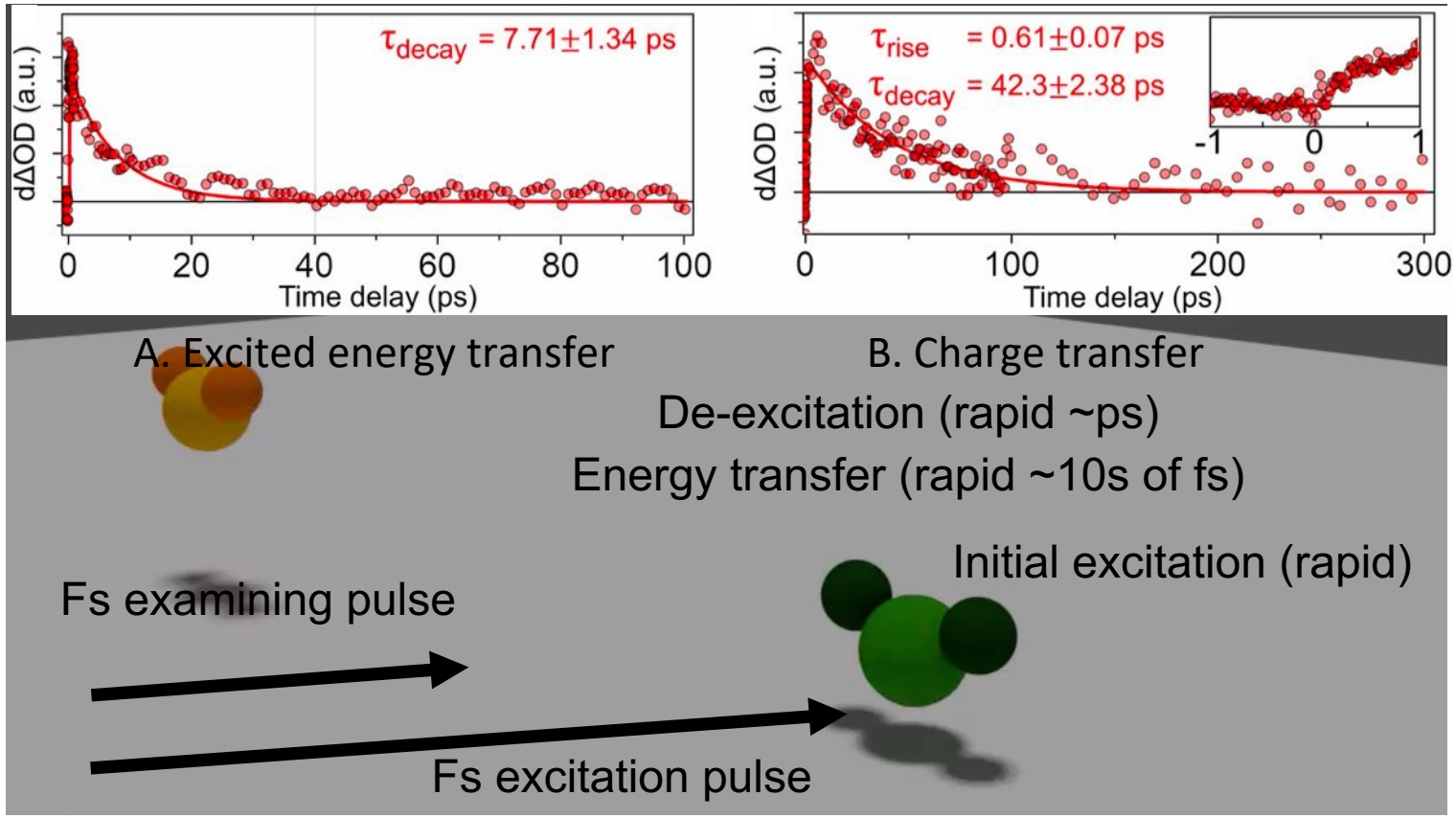


While our results are promising, we plan to explore more sensitive spectroscopic techniques

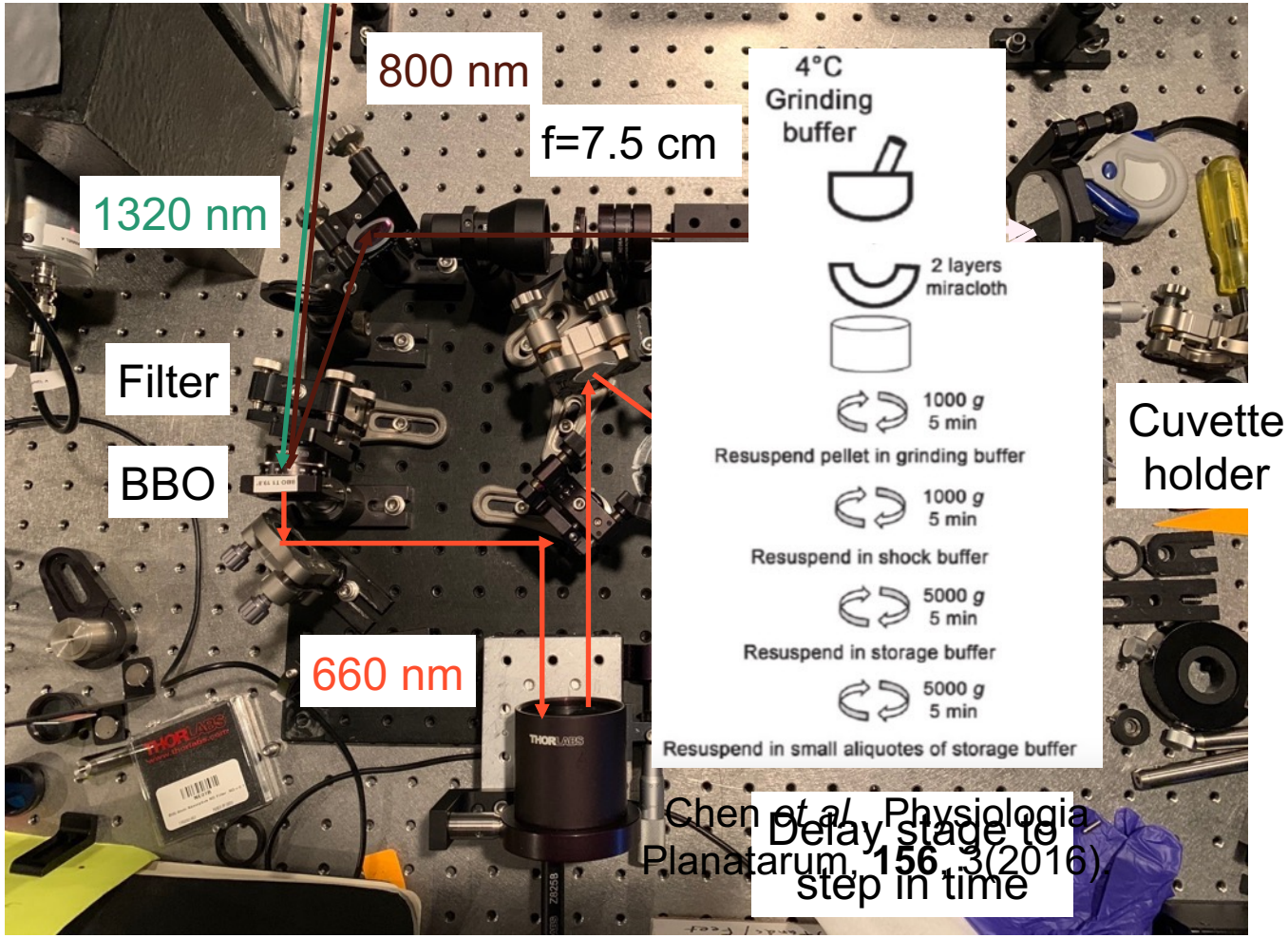
Park *et al.*, PNAS, **116**(9), 3385-3390(2018).



Non-photochemical quenching mechanisms is driven by energy transfer to and from Car and Chl a.

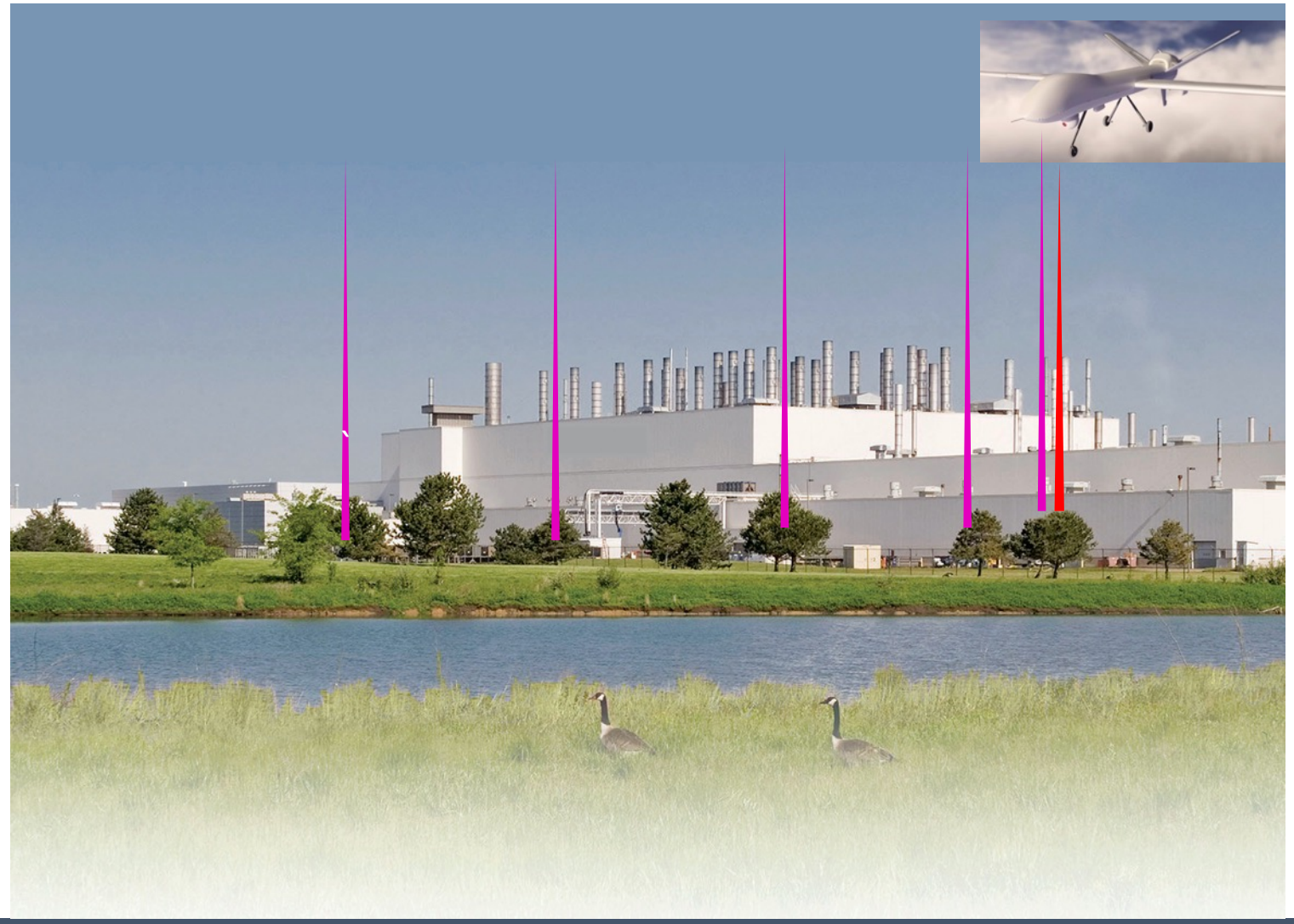


Next Steps: Transient absorption experiment set up for studying stress response in *a. thaliana*



Chen *et al.* Physiologia Planatarum, **156**, 3(2016)

Impact: Development of remote, in-situ environmental monitoring for identification of nuclear nonproliferation relevant materials



https://www.thinplytechnology.com/markets/uav-drones

J. Walsworth, *U.S. Plant took subaru on new journey*, web (2018).

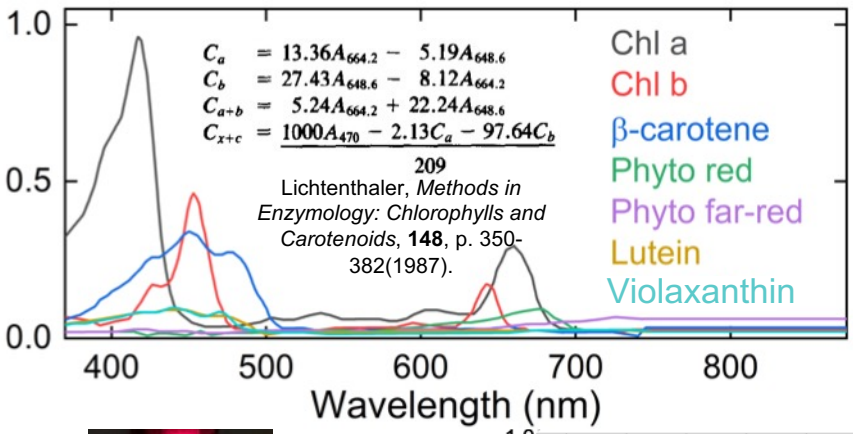
MTV Impact: Workshops and networking

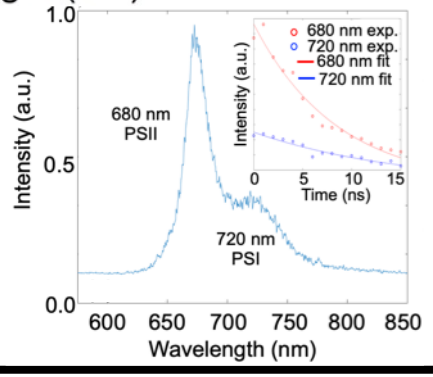
I have been able to participate in workshops over the past 4 years, meeting with other students and scientists which has influenced my PhD work.

Last year, I met Dr. Laura Tovo from SRNL. She is now a member of my dissertation committee.

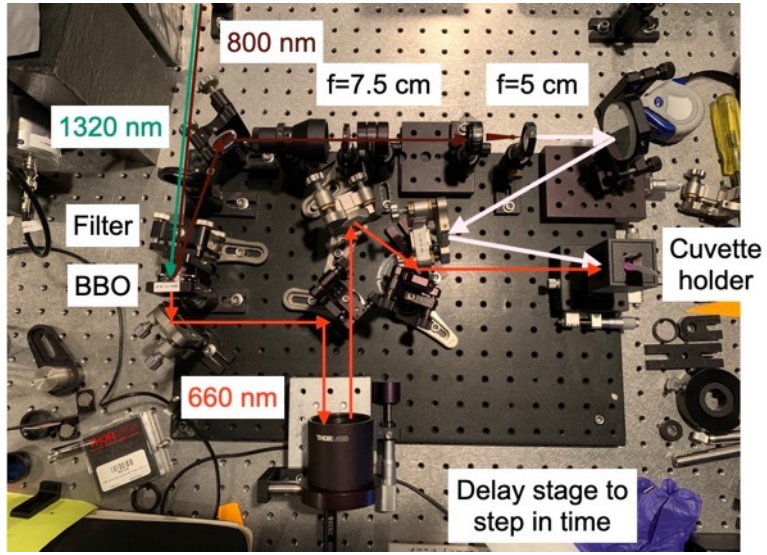
Conclusion: fluorescence is promising as an in-field method, however further work is needed to identify how U affects plants

Absorption and fluorescence are promising for in-field monitoring





Implement higher sensitivity method to understand how stress induces changes in optical properties



Acknowledgements

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