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An innovative approach to reduce copper load in horticulture

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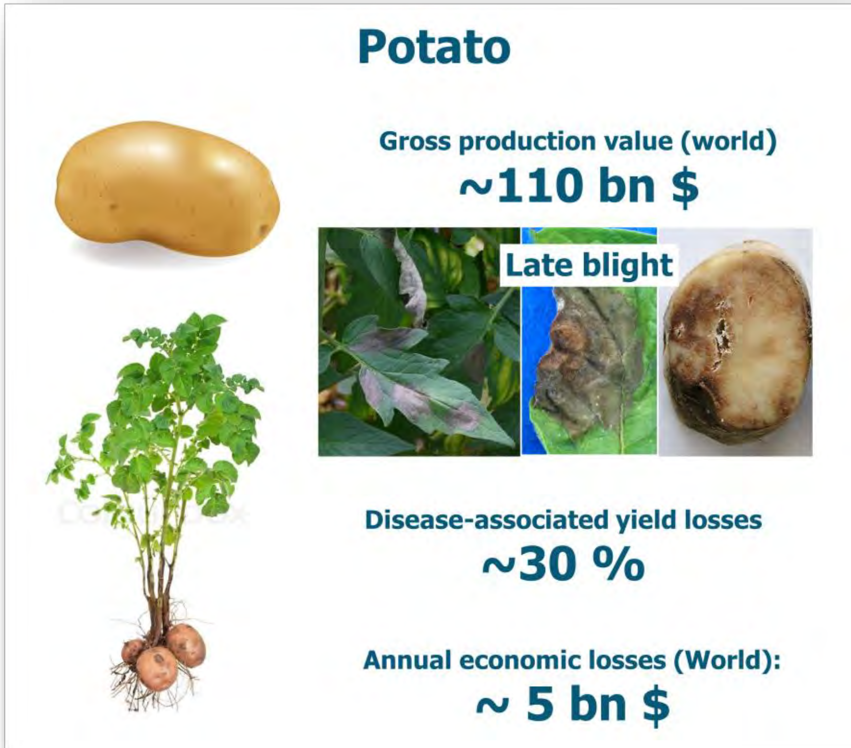
1. Background/problems
   - Plant protection (crop loss, rain wash-off, phytotox)
   - Copper fungicides (pros and cons)
2. Copper reduction approaches
3. Summary and outlook

Scenario: Crops vs Pathogens



Apple

Gross production value (world)
~ 52 bn $

Disease associated yield losses
~ 70%

Annual economic losses (world)
~ 30 bn $

Landwirtschaftkammer NRW & Calculated, (Data source: FAOSTAT (2014, 2017)

Cu2+ a.i. in agriculture

- Scottish farmer Sprayed Copper against Mildew (1861) (Smith and Secoy, 1976)

- Bordeaux/Burgundy mixture in Grape (1882/85) (Mason, 1928)

Dimitris Vlassis (www.alamy.com)

In Germany (since 150 years)

- Organic farming: 24% (~ 27 tons)
- Conventional farming: 76% (~ 85 tons)
- However, copper used in organic farming > conventional for grape, hop and potatoes. Apple almost similar. (Kühne et al., 2016 JKulturpflanzen, 2017 OrgFarming)

(Institu.inra.fr)

Cu in agriculture: pros and cons

Excess copper: (high dose or rain wash-off)

Harmful to most plants,

Plant beneficial microbiome

Soil microorganisms, and fauna

Water microorganisms, and fauna

Complete renunciation of copper as a pesticide is not practicable in organic farming.

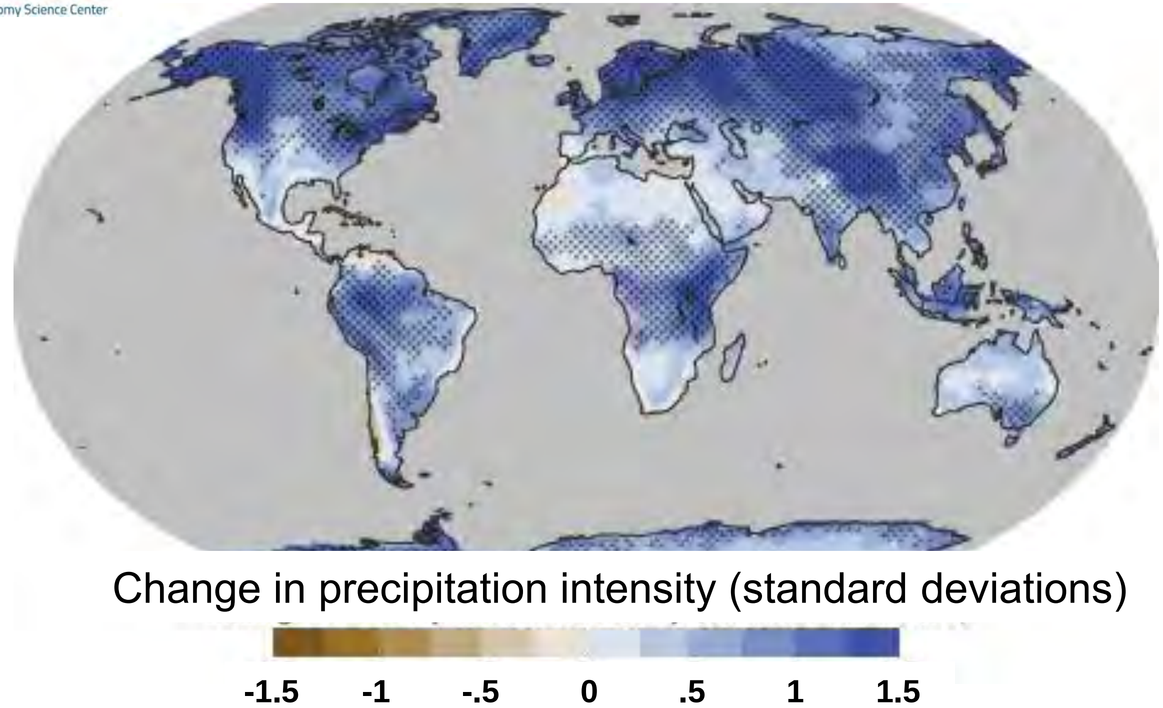
Production of several crops unprofitable.

Lead to reconversion to conventional production.

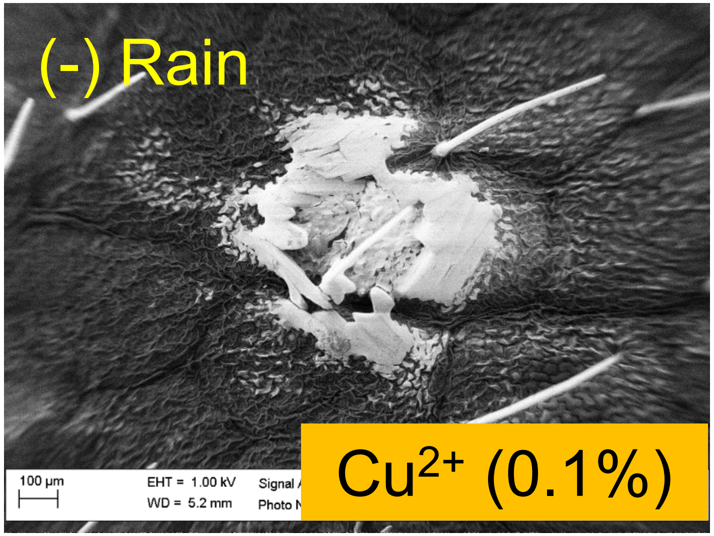
(Gitzel and Kühne, 2016, Kuhne et al., 2017).

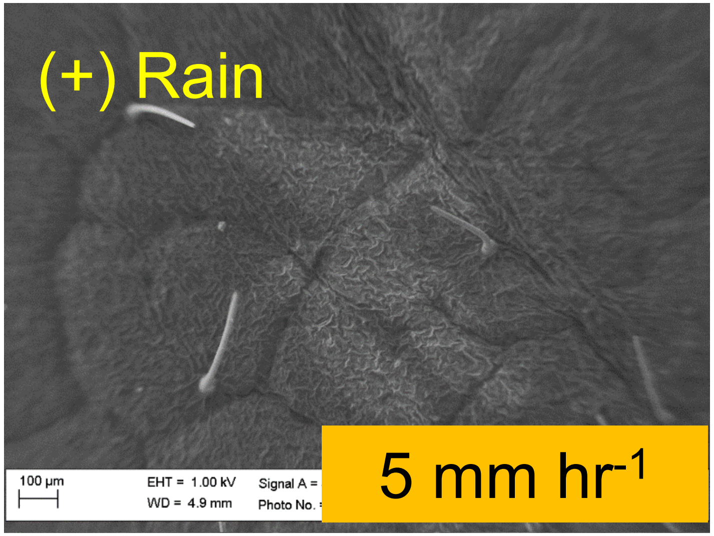
Germany:
3 kg Cu/ha

Problem 1: Rainfall vs. Rainfast

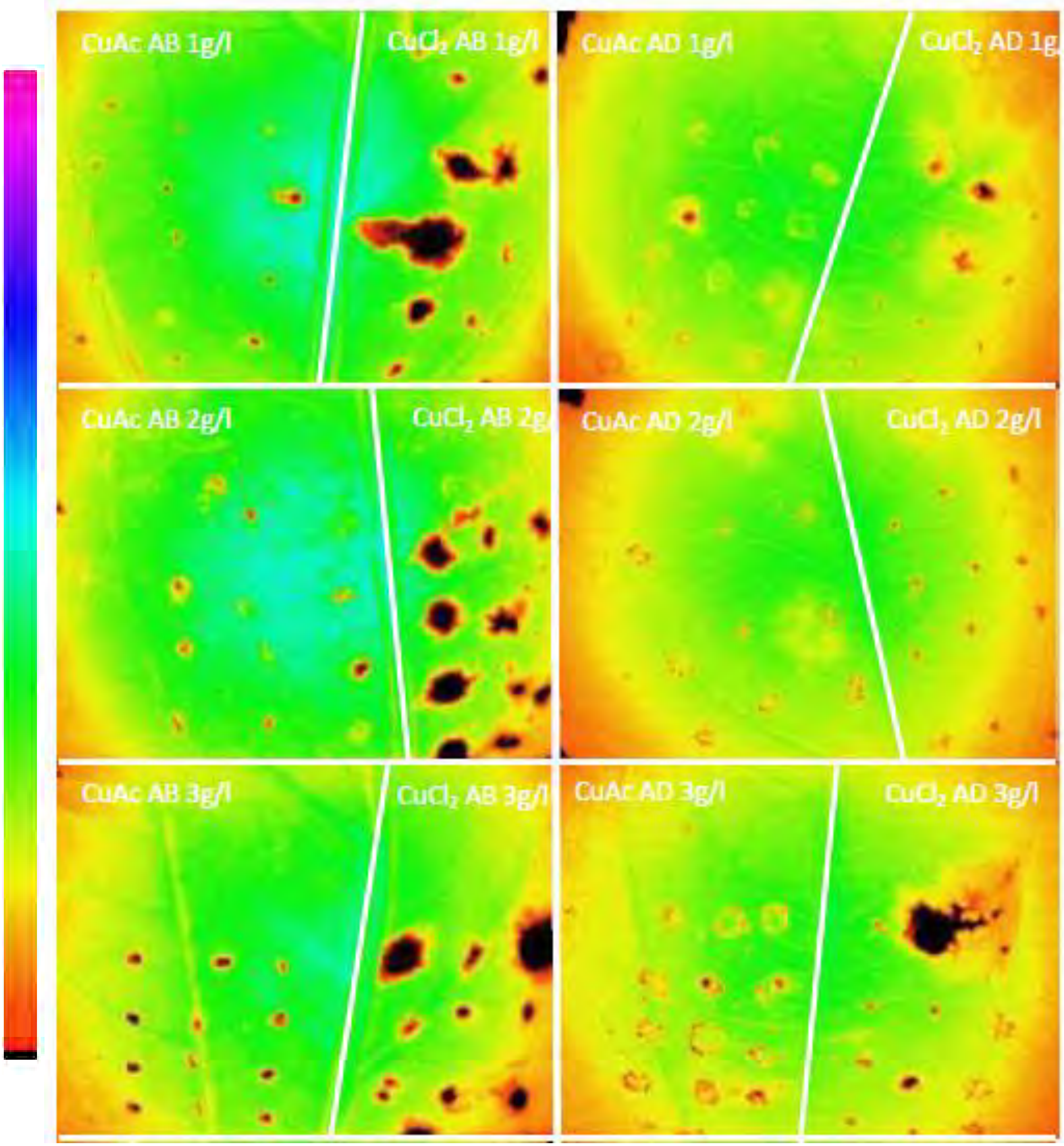


Projected increase in precipitation intensity by the end of 21st century (*adapted, IPCC AR4, earthobservatory.nasa.gov*)





Prob.2: Phytoxic/Dose-response (Cu2+)



Substance	Conc. (%)	Net Cu-load (g/L)
$CuCl_2$	0.22	1
$CuCl_2$	0.43	2
$CuCl_2$	0.64	3
$CuCl_2$	1.06	5
$CuCl_2$	2.1	10
$Cu(OOCH_3)_2$	0.27	1
$Cu(OOCH_3)_2$	0.56	2
$Cu(OOCH_3)_2$	0.86	3
$Cu(OOCH_3)_2$	1.42	5
$Cu(OOCH_3)_2$	2.84	10

Averaged photosynthetic efficiency for Cu-treatments on adaxial (AD) and abaxial (AB) apple leaves.

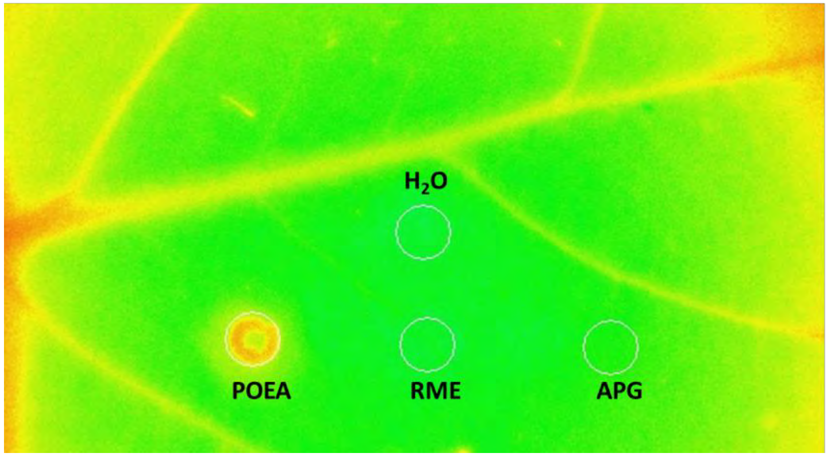
Color:
black = no photosynthesis,
purple = maximum photo.

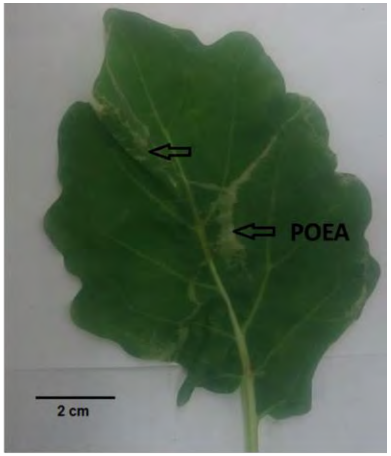
Prob.3: Additives on Cu-preparation

- Some agricultural adjuvants are known to alter the physico-chemistry of plant surfaces
  (Damato et al., 2017 CropProtection; Noga et al., 1986 Gartenbauwissenschaft)

  - induce phytotoxic effects
    (Jursik et al., 2013 RomanianAgrJ; Knoche et al., 1992 CropProtection)

  - alter epidermal transpiration and photosynthetic activity *(Raesch et al., 2018 PlantPhyBiochem)*





Cu-reduction approaches

Prevention

Crop management (Phytosanitation)

Breeding/ Genetic Engineering

Cure

Mechanical/ sensor technology (precision application)

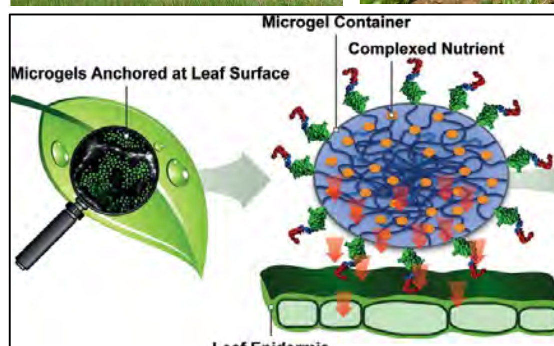
Alternative Preparations (- Cu)

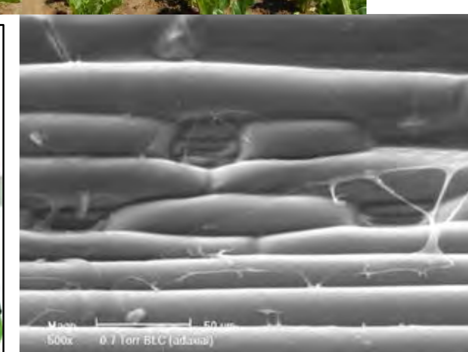
Biotechnological (Slow release/ rain-stability)

www.japantimes.co.jp

www.julius-kuehn.de

www.phenorob.de

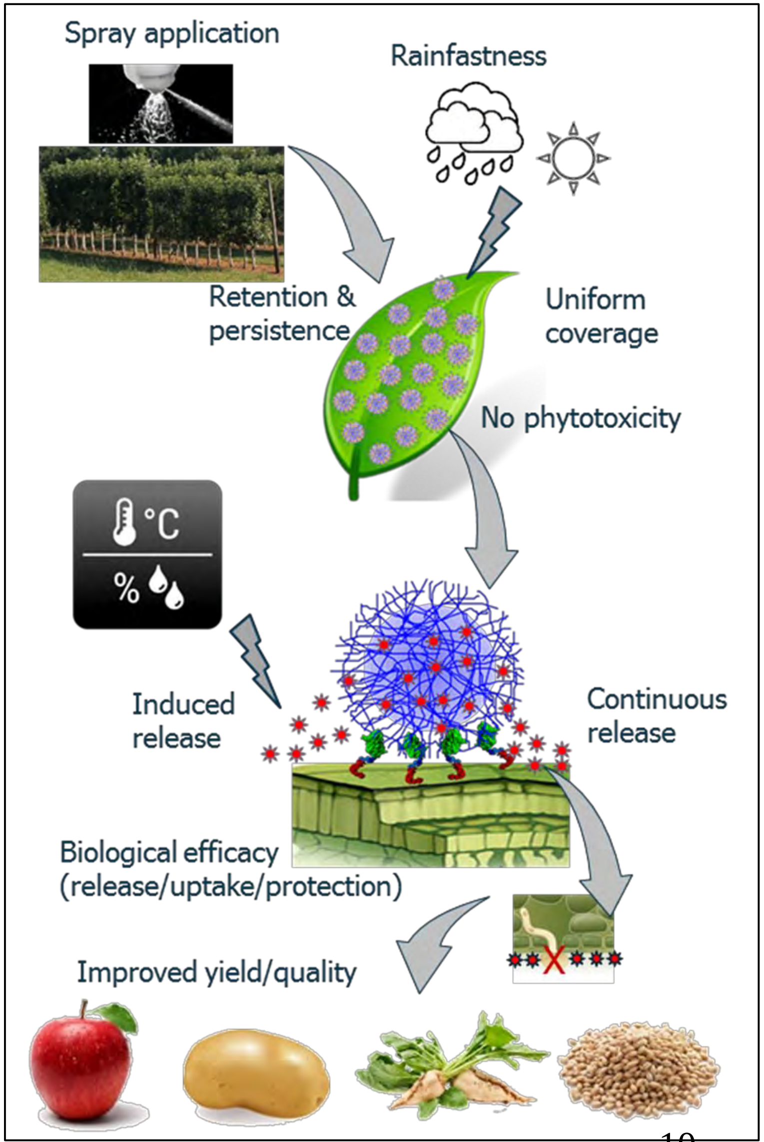




Maurer et al. (2017) Angew.Chem.Int.Ed

Slow release of Cu a.i.

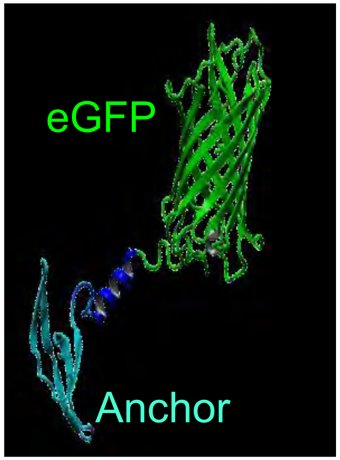
- Cu-ions are gradually released from microgel formulation.
- Continuous release provides residual protection against plant pathogens.
- Slow release of copper ions reduces risks of phytotoxicity to plant tissues. (Rosenberger, 2012, Scafolds Fruit Journal).
- Coupling of anchor peptide may enable stability against rain.



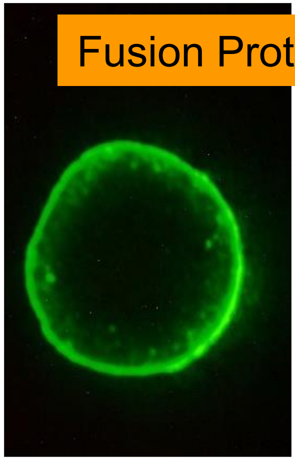
Anchor peptides (APs) ?

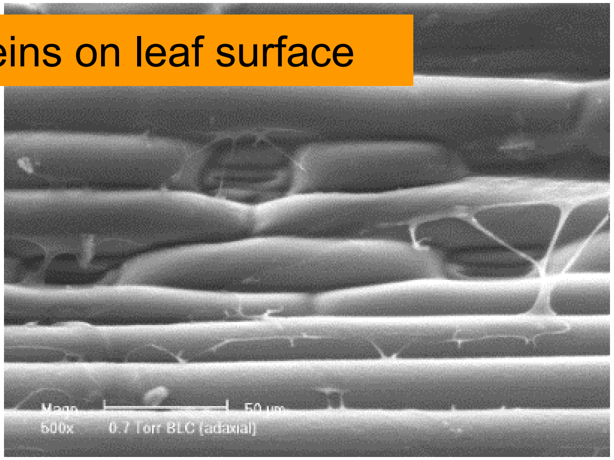
Anchor peptides are functional proteins, which promote binding to the surfaces. *(Ruebsam et al., 2017 Polymer (116):124-132)*

- bifunctional peptides
- biological production
- fused with marker, eGFP (enhanced green fluorescence proteins)



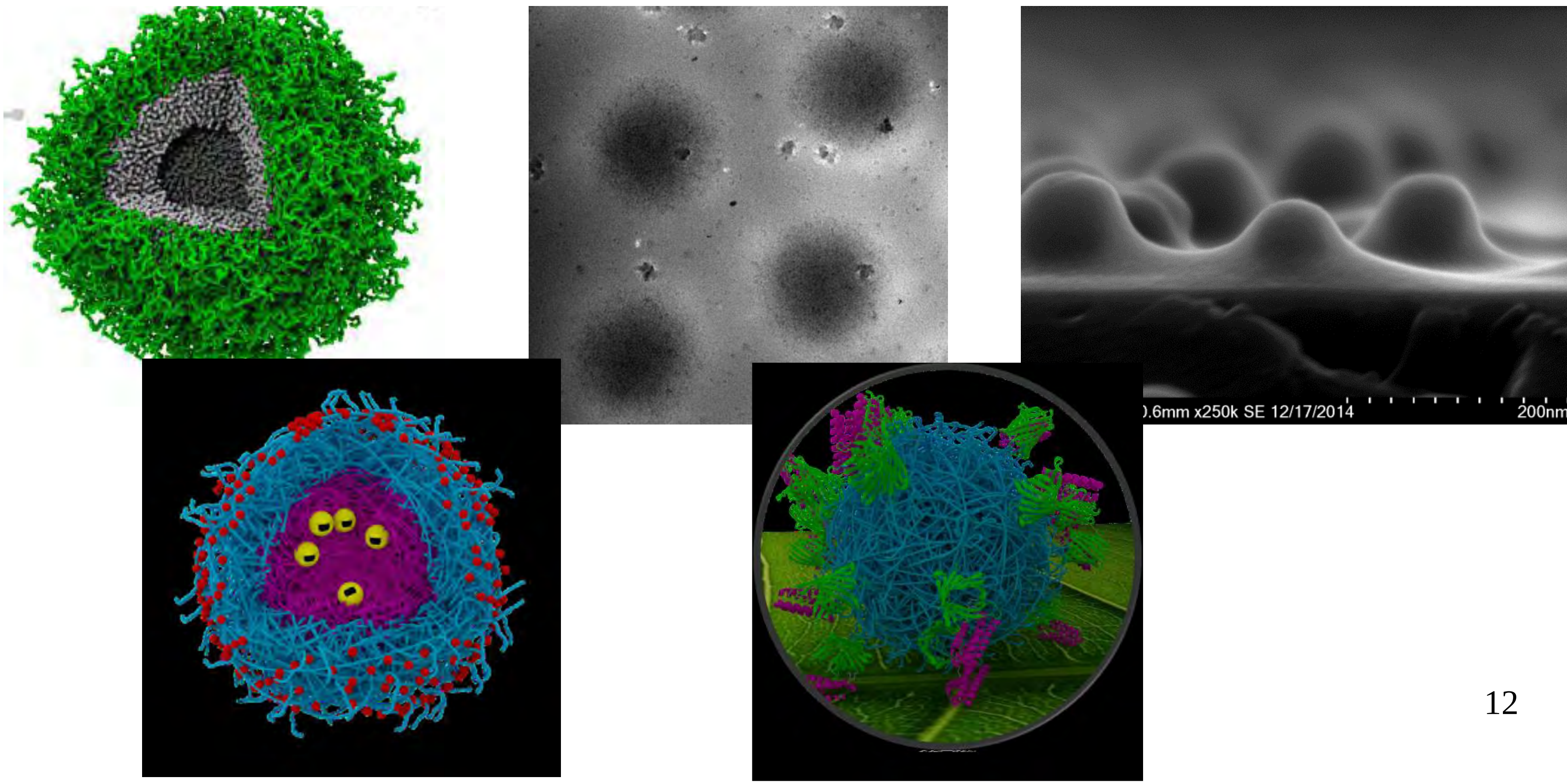
Fusion Proteins on leaf surface





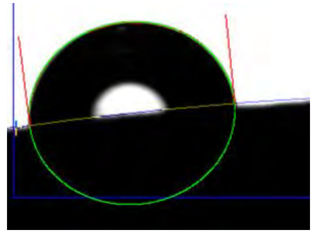
Microgel container (MG) ?

- Biodegradable, hydrophilic polymer
- Copper loaded
- fused with eGFP-anchors

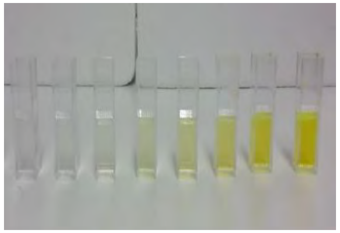


Experimental approaches: up-scaling interaction

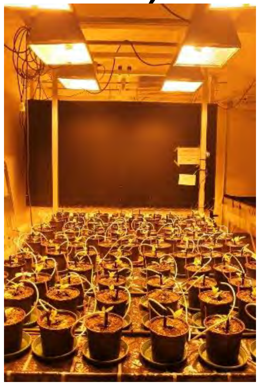
1. Lab scale:

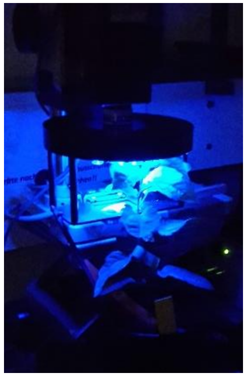


- Chl, wax, CA, ST, penetration or uptake



2. Controlled growth chamber: (-rain, ±UVaB, < PM)



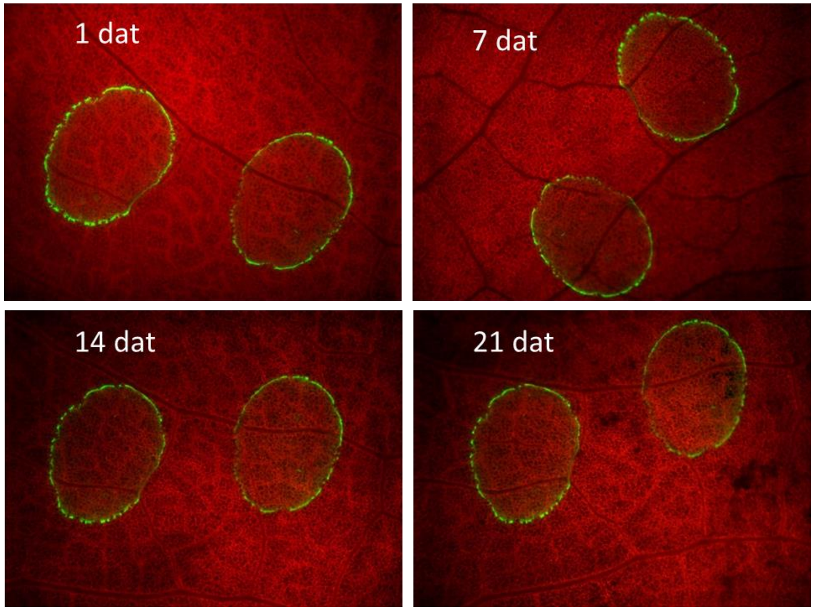


- Distribution, retention, UV-stability, phytotox,
- biological efficacy and rainfastness

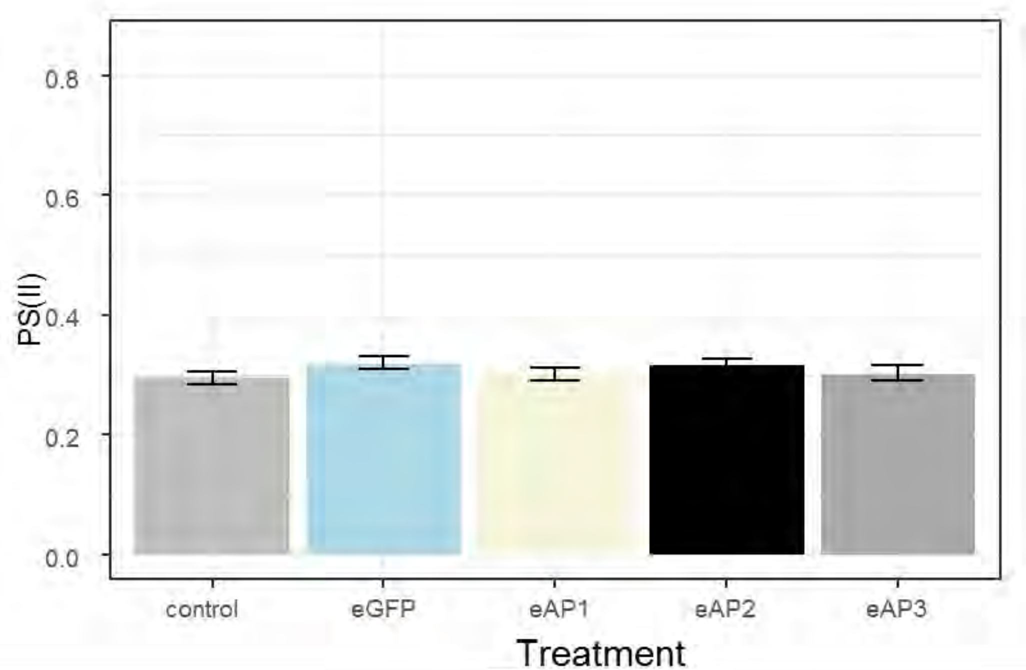
3. Field condition (+CKA): (± rain, +GlobRad, +UVAb, > PM)

- Distribution, Rad, UV,Temp-stability, phytotox,
- biological efficacy and rainfastness

Retention and phytotoxic potential



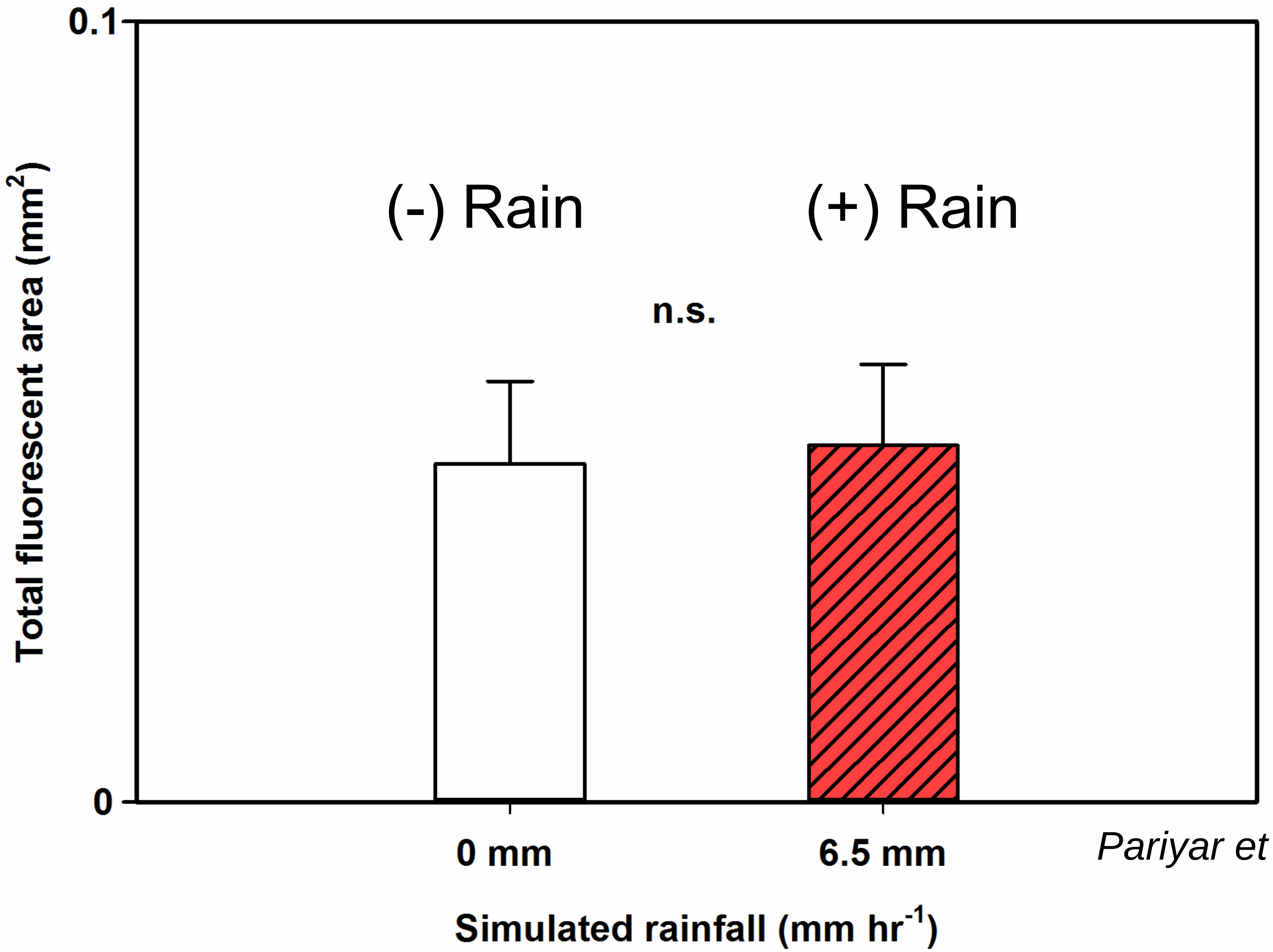
eGFP-AP2 retention on apple leaf



PAR = 100, dat = 0
ANOVA(PS(II) ~ treatment: N = 32, $P > 0.05$)

➢ The eGFP-anchor was quite stable on apple leaf and has no negative effect on photosynthetic activity.

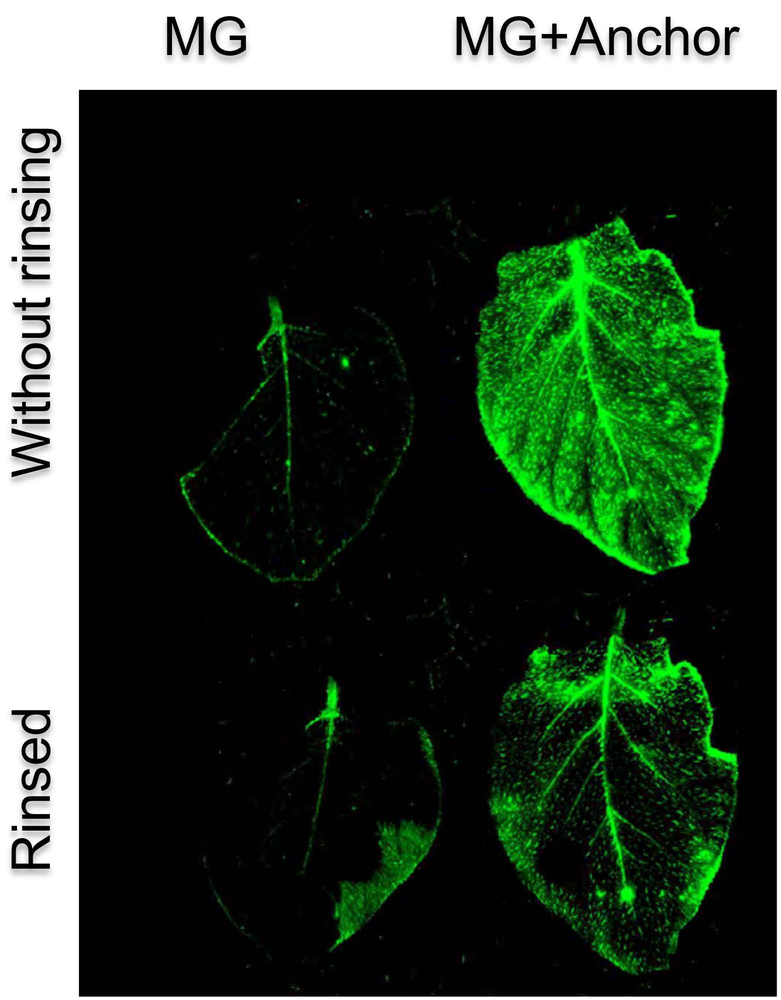
Rain wash-off



Pariyar et al. (submitted)

- The AP eGFP-anchor withstand simulated heavy (6.5 mm hr^{-1}) rainfall.

Wash off: microgel + anchor



- Microgel-anchor remained in significant amount after leaf washing on potato leaf.

Summary and outlook

1. Anchor peptides as adhesion promoter:

- did not impair photosynthetic activity.
- did not show phytotoxic potential
- increased stability against simulated rainfall.

2. The adhesion promoter can be used in a novel Cu2+ loaded microgel container to enhance slow release and stability of Cu-preparation on plant surfaces.

3. Next: Cu-preparation biological activity and stability on plant surfaces.

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Thank you for your attention !

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