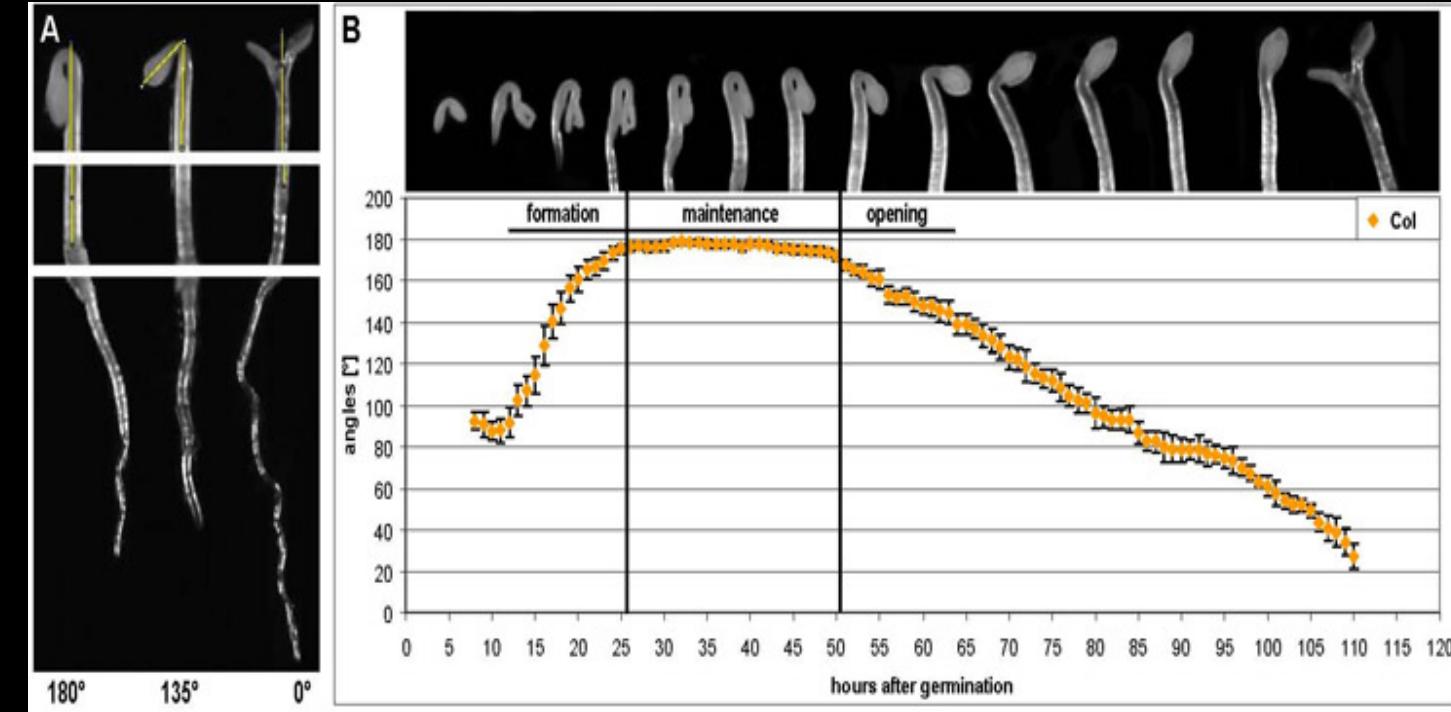


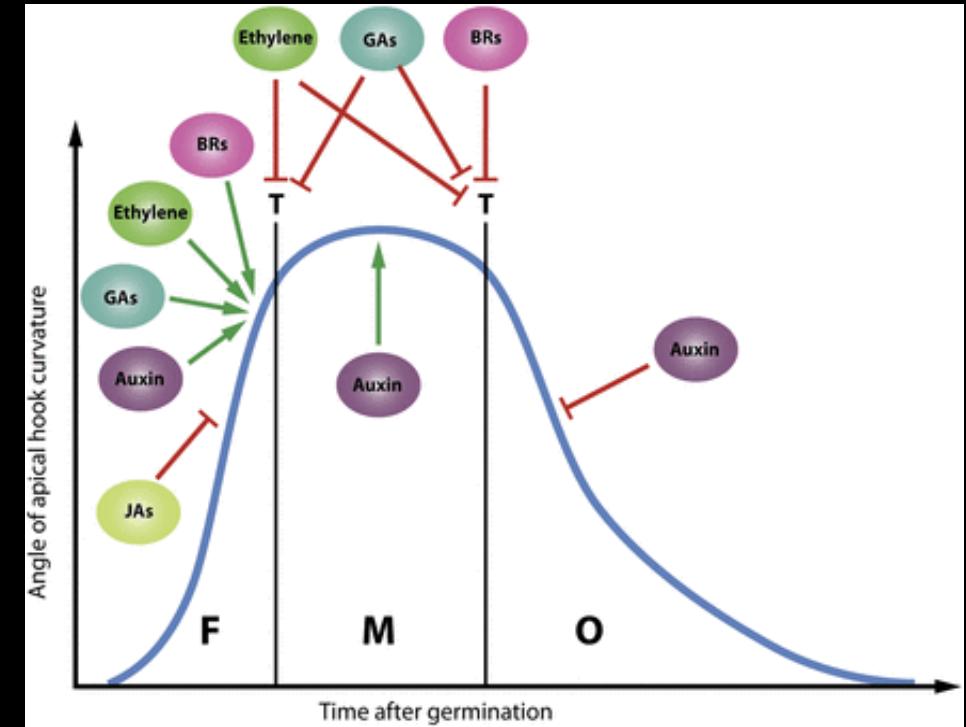
The role of the cuticle during apical hook development

Stéphanie Robert's group

The apical hook develops in a well-defined time frame

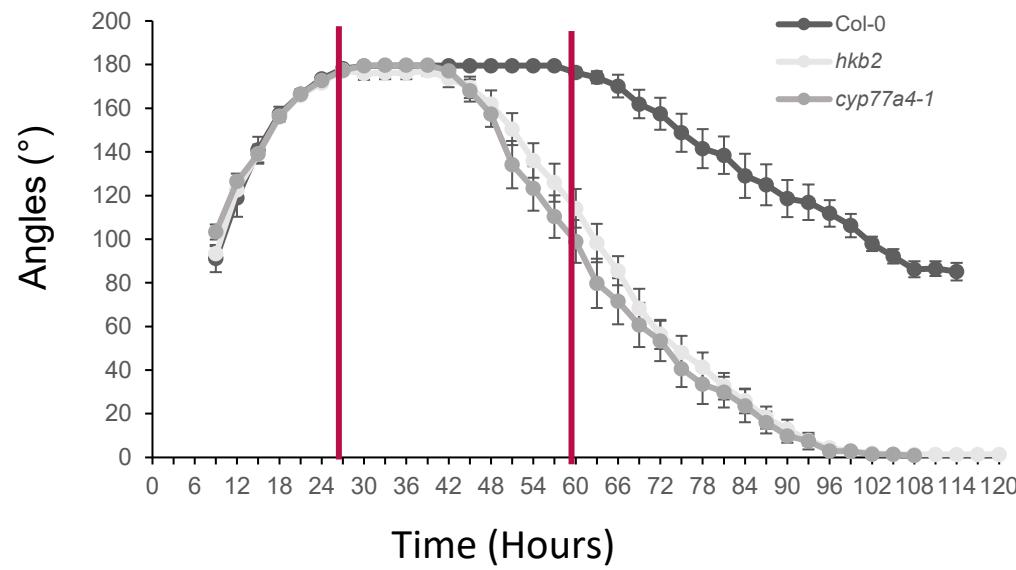


Žádníková et al., (2010)

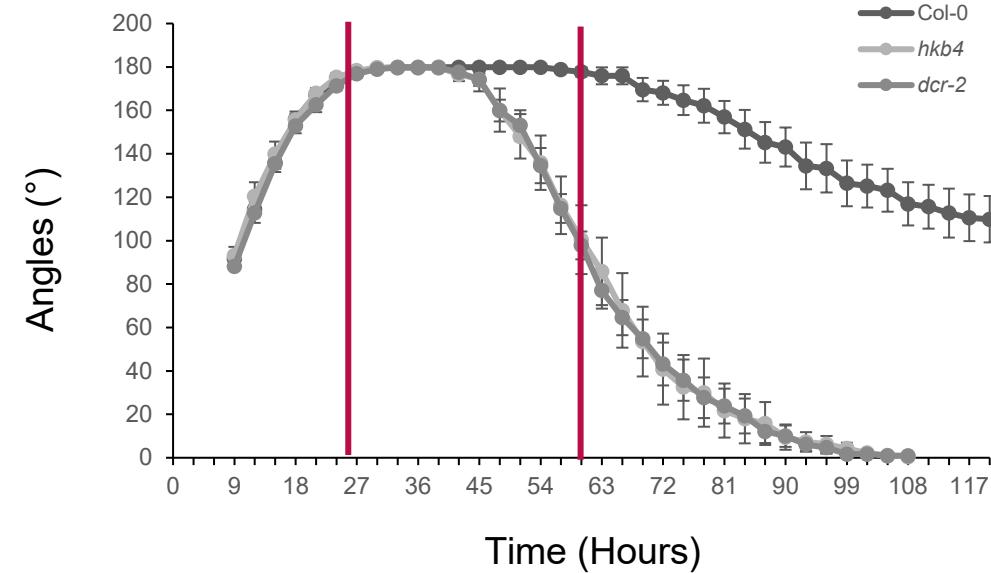


Van de Poel et al., (2015)

Screening to find new molecular players regulating apical hook development



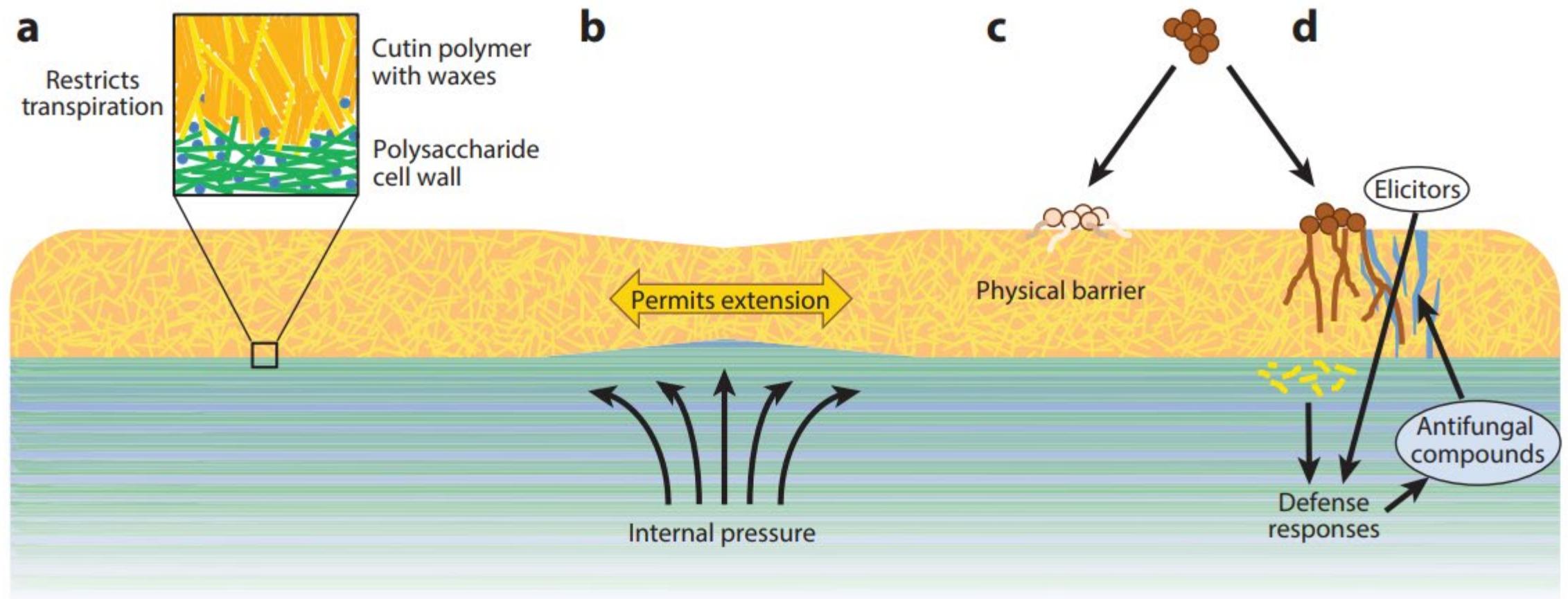
CYP77A4



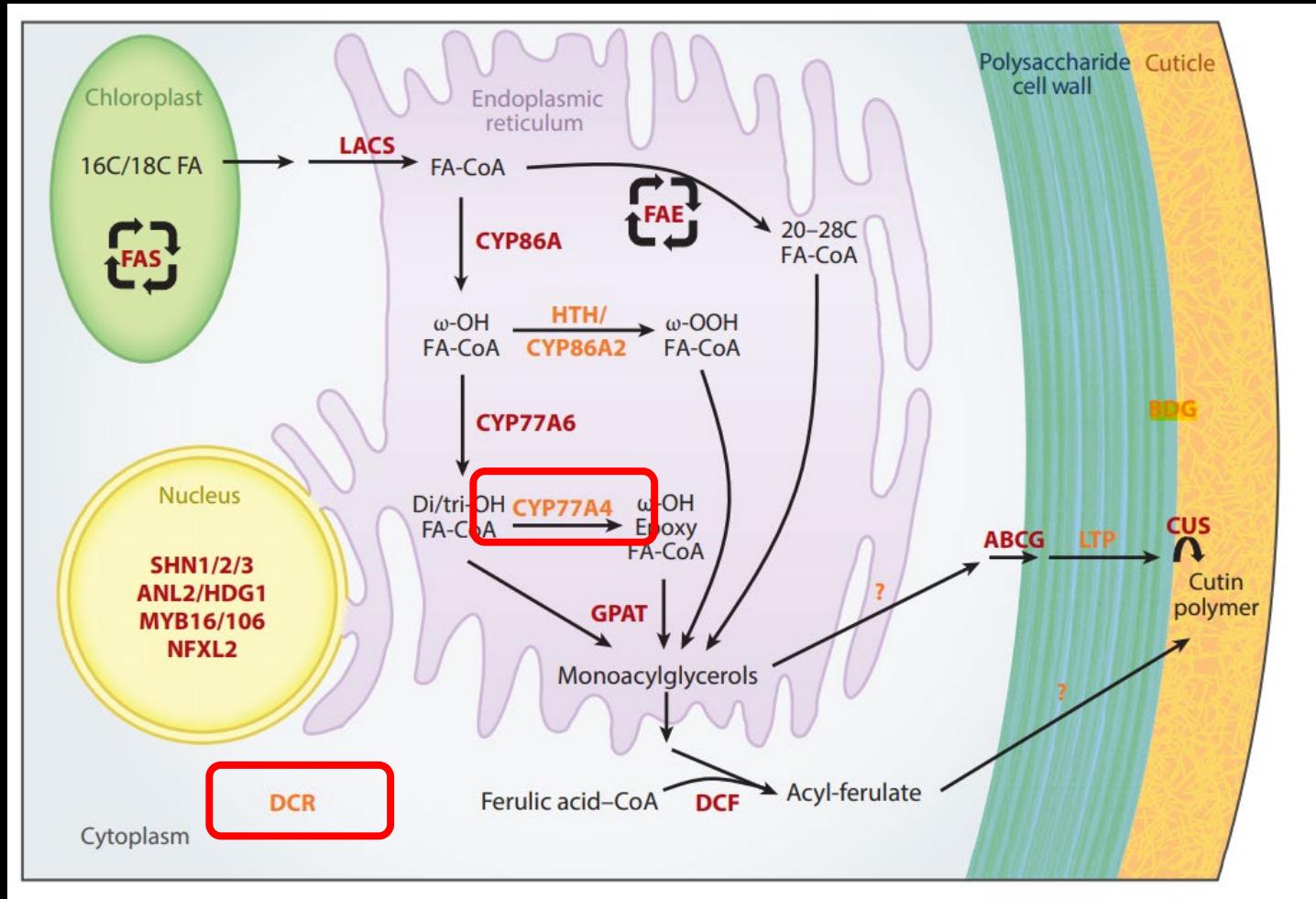
DCR

Cuticle deficient mutants displaying apical hook phenotype

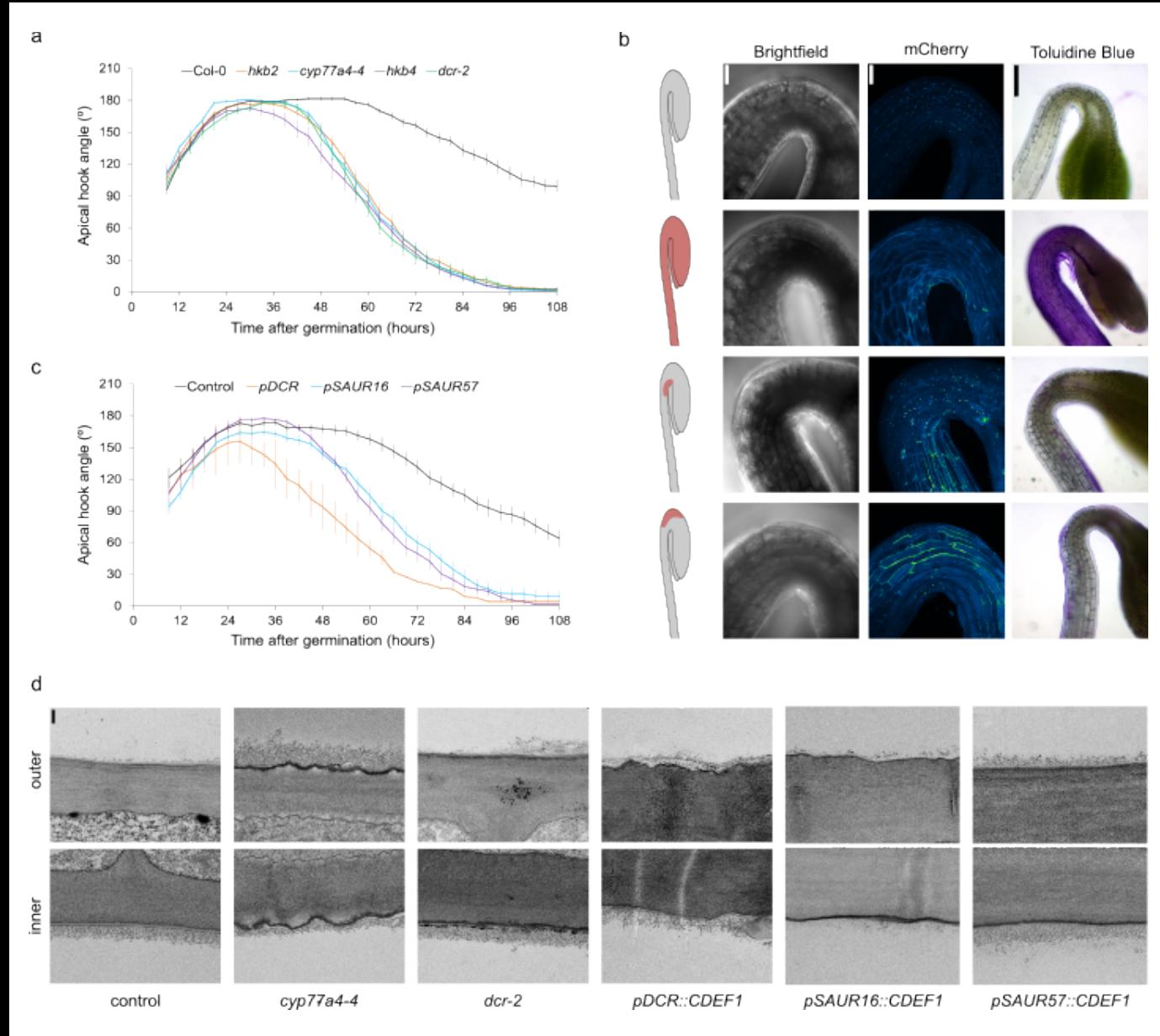
The cuticle acts as a barrier during biotic and abiotic stresses



Biosynthesis, transport and polymerization



Plant cuticle integrity is essential for proper apical hook development



Control is p35S::PIP2::GFP

Plant cuticle integrity is essential for proper apical hook development

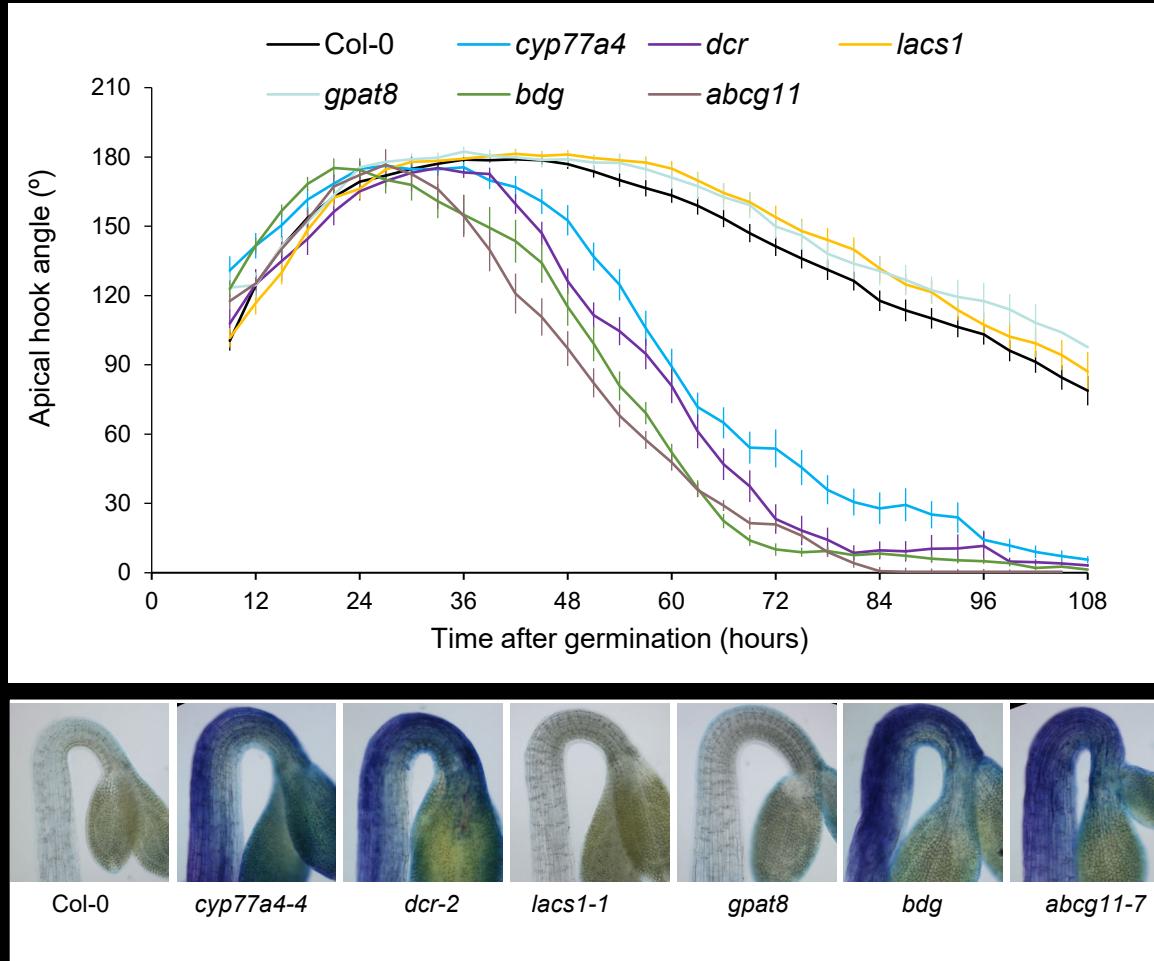
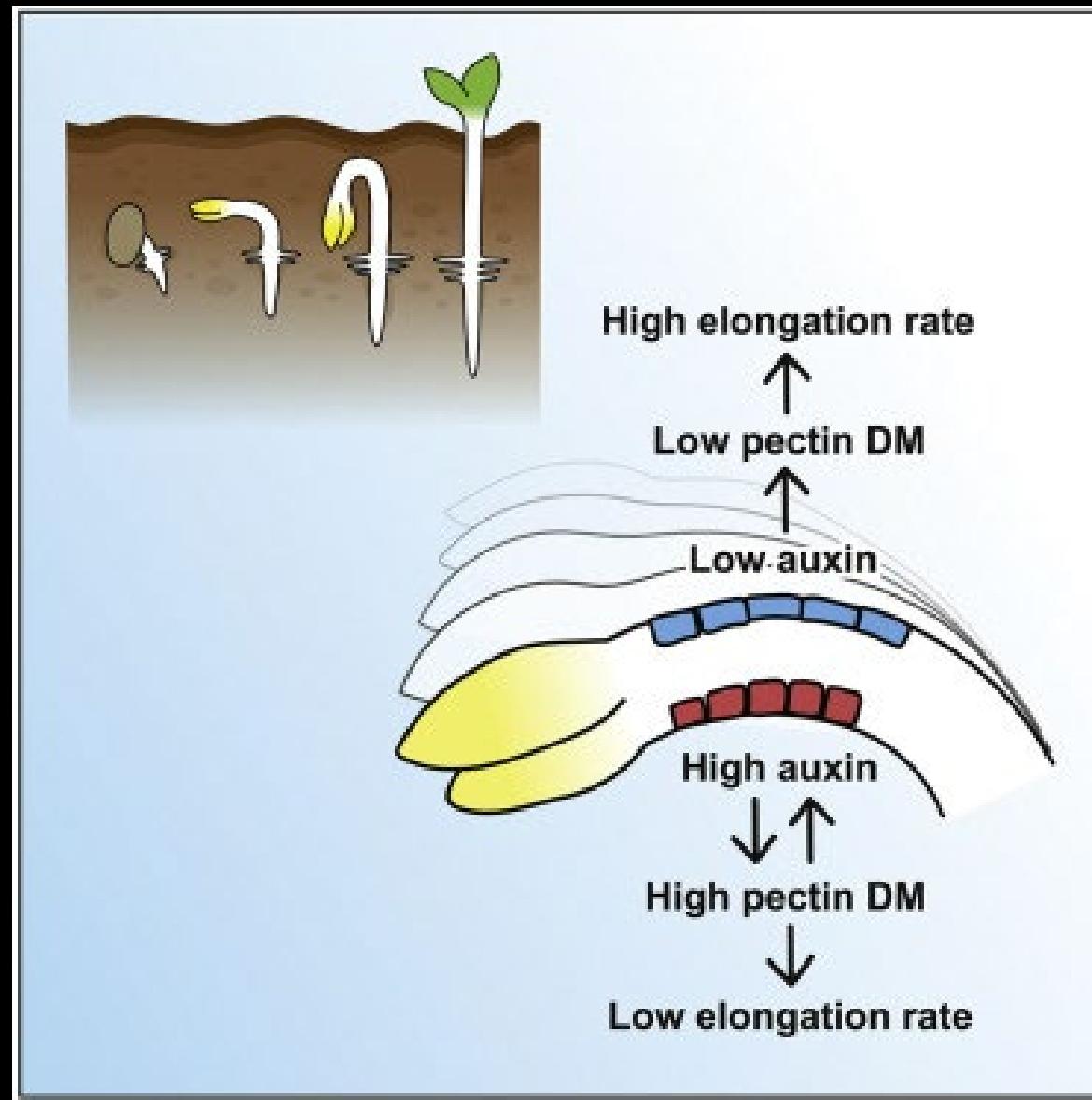


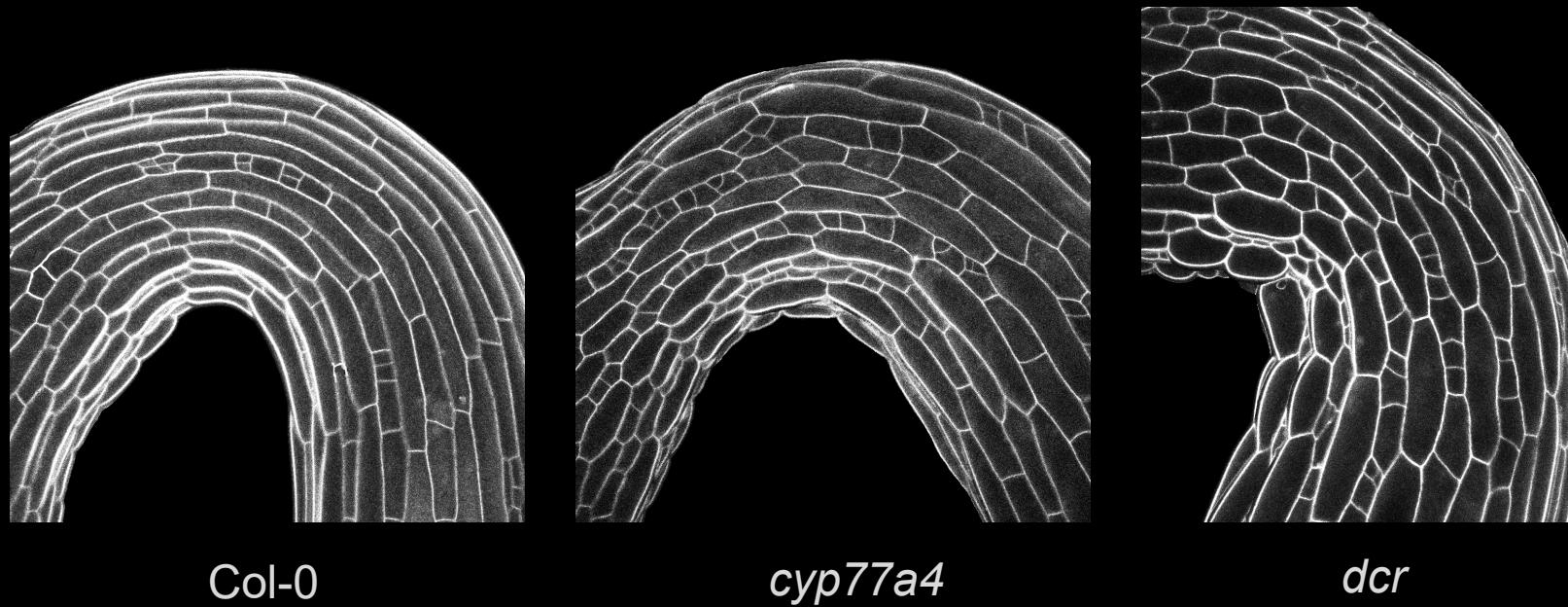
Figure Supp 1

- *Cuticle integrity is essential for apical hook maintenance*

The apical hook development



dcr and *cyp77a4* phenotypes show an increase of smaller, more isotropic cells with strange pattern of divisions



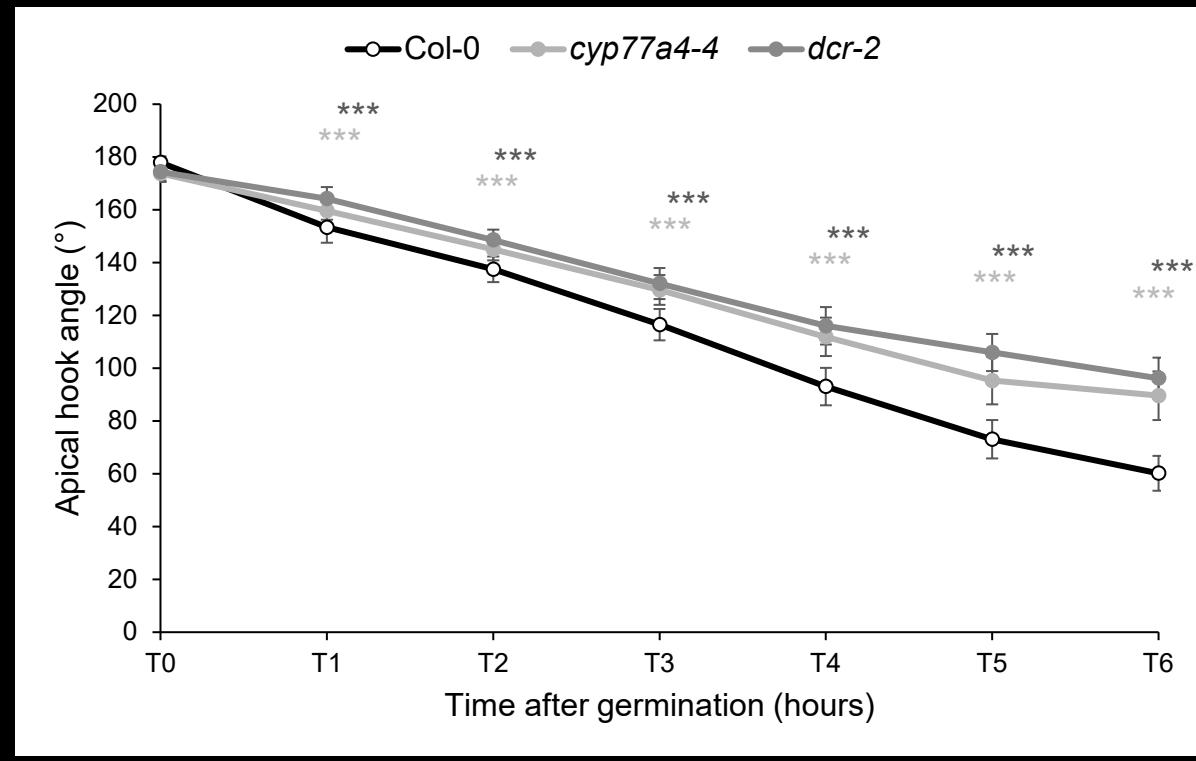
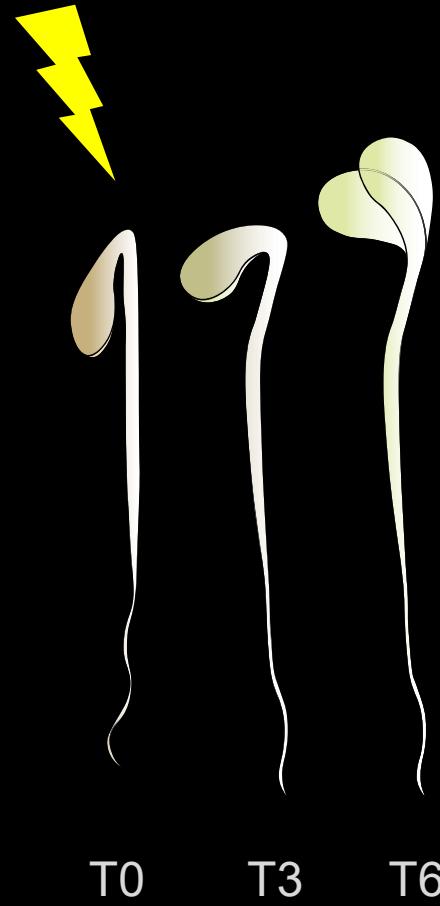
Seedlings stained with PI during maintenance phase.

The signal from the epidermis was extracted by using SurfCut in ImageJ

Raggi et al., unpublished

- *Cuticle integrity is essential for apical hook maintenance*
- *Cell division or elongation?*

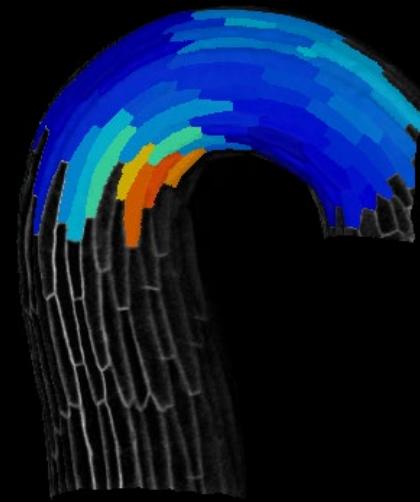
Cuticle mutants open slower in response to light indicating a defect in cell elongation



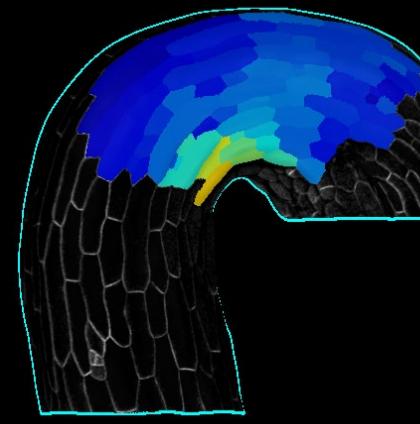
During the maintenance phase the mutants displayed increased inner/outer growth ratio but no increased rate of cell division

- Z-stacks for each seedlings were acquired at T0 (24 hours after germination) and T8 (32 hours after germination)
- Image processing
- For each cell, the rate of growth was projected on the T0 labelled image

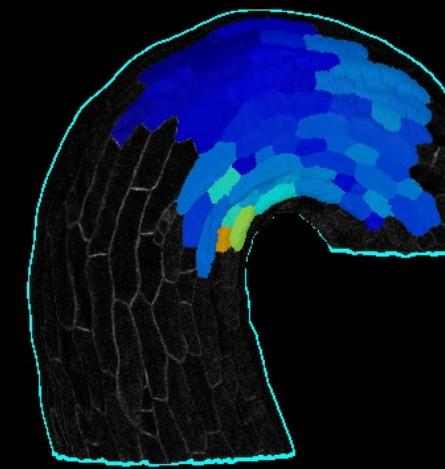
growth - ratio



Col-0



cyp77a4-4



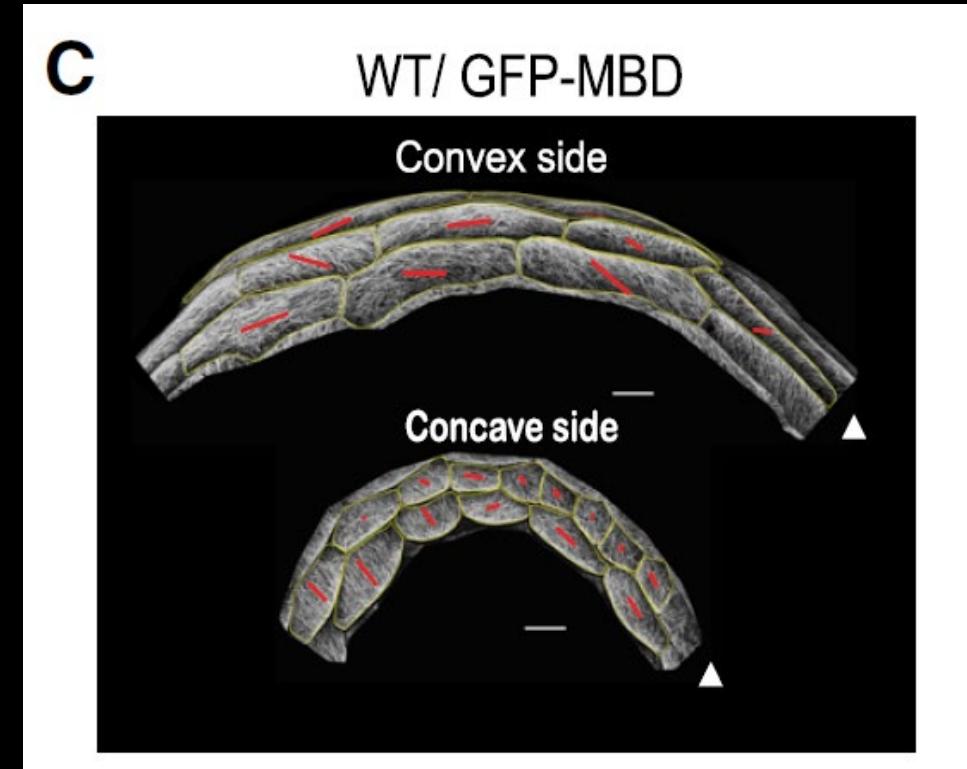
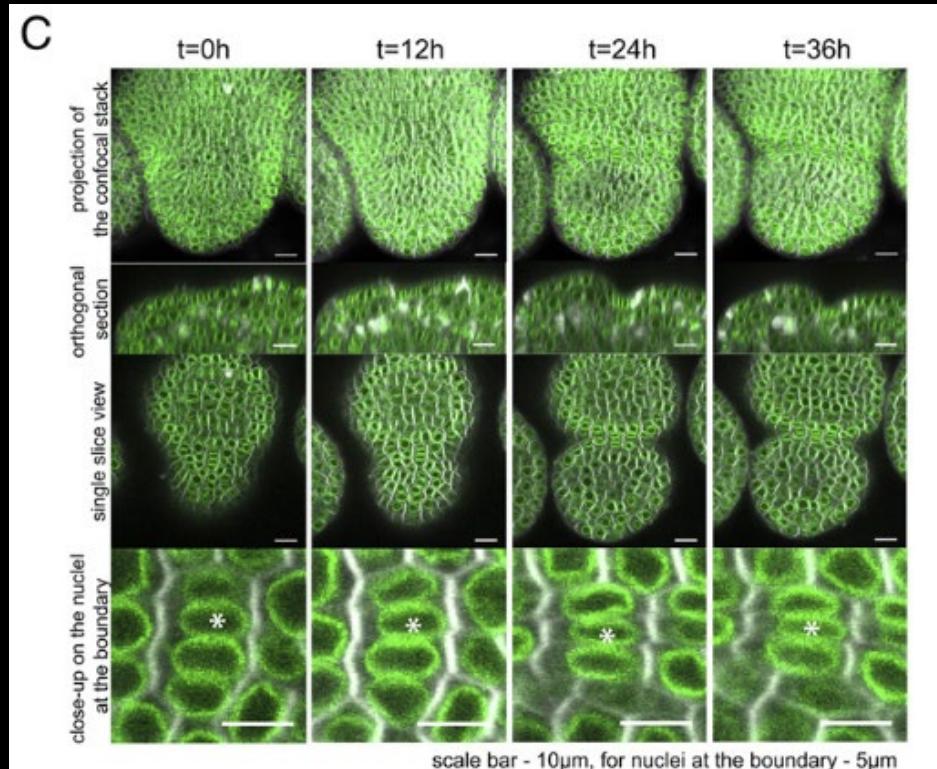
dcr-2

- *Cuticle integrity is essential for apical hook maintenance*
- *Cuticle sustains the differential growth by maintaining the anisotropic growth*

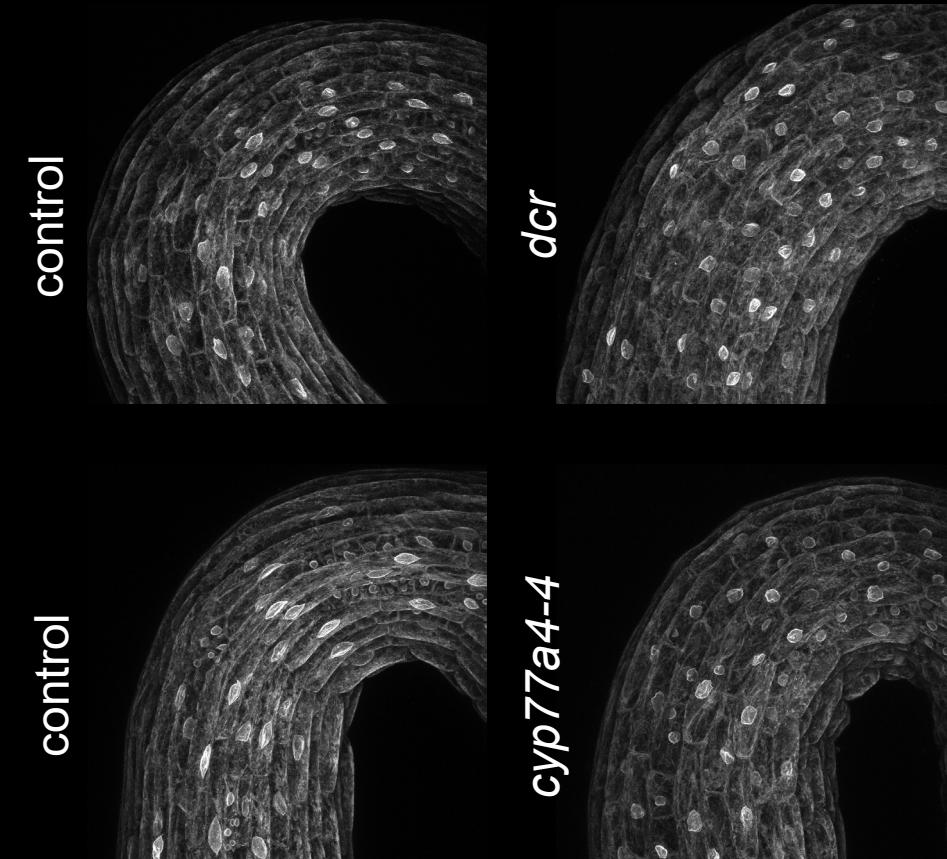
Cuticle and differential cell elongation

Mechanical effect?

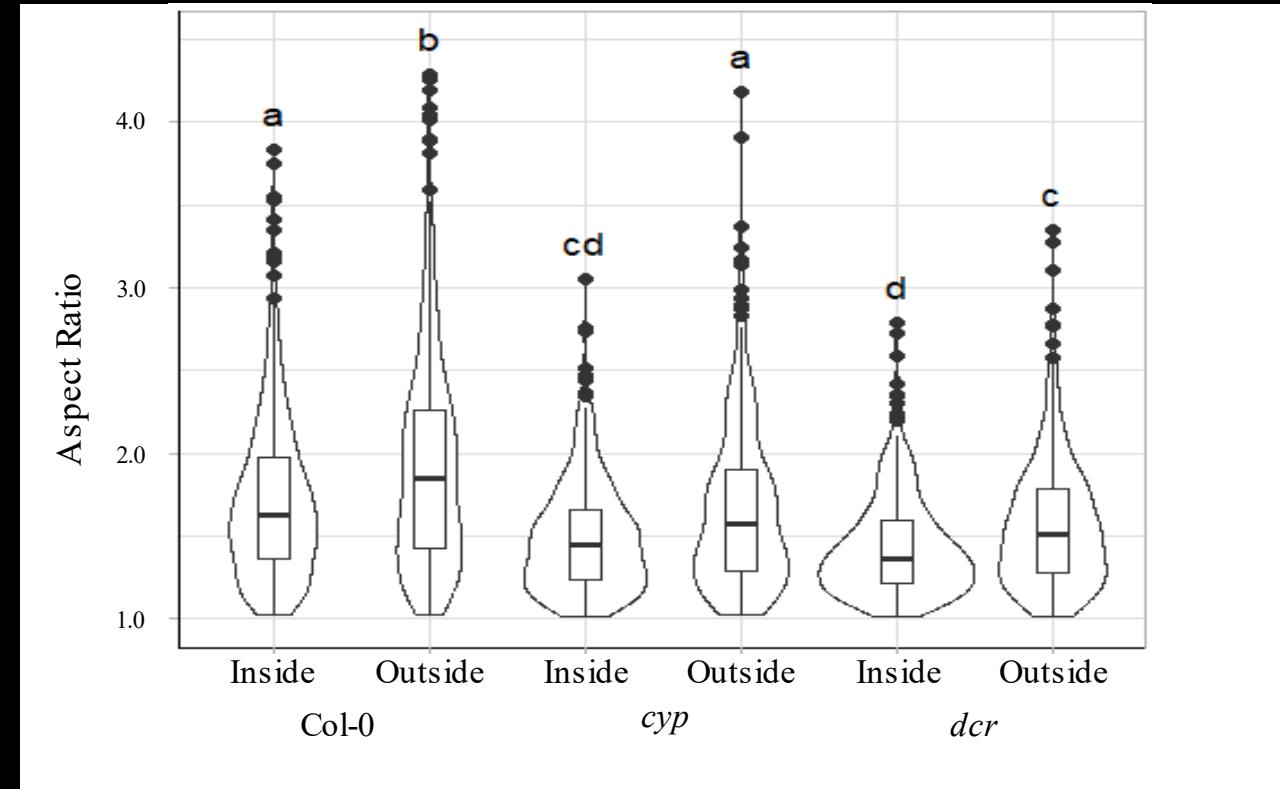
Lines for mechanical stress read-out



cyp77a4-4 and *dcr-2* display increased nuclei circularity throughout the apical hook



35S::SUN1::YFP
nuclear marker reporter



The alignment of cortical microtubules differ in the cuticle defective mutants



Control

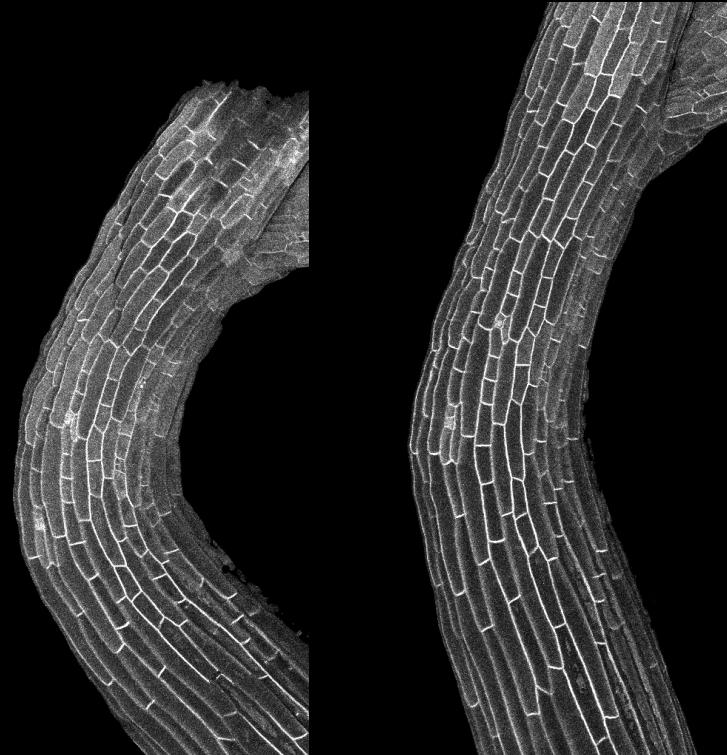
cyp77a4-4

dcr-2

Representative pictures. PRELIMINARY DATA

Hemamshu Ratnakaram

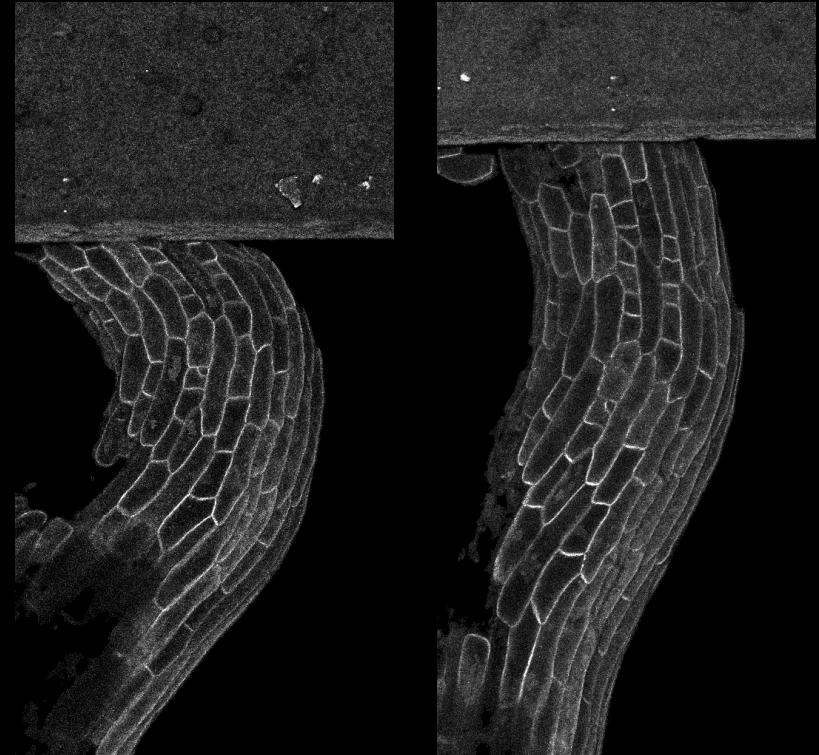
Extensometer to investigate the contribution of the cuticle to tissue elasticity



0 mN

4 mN

control

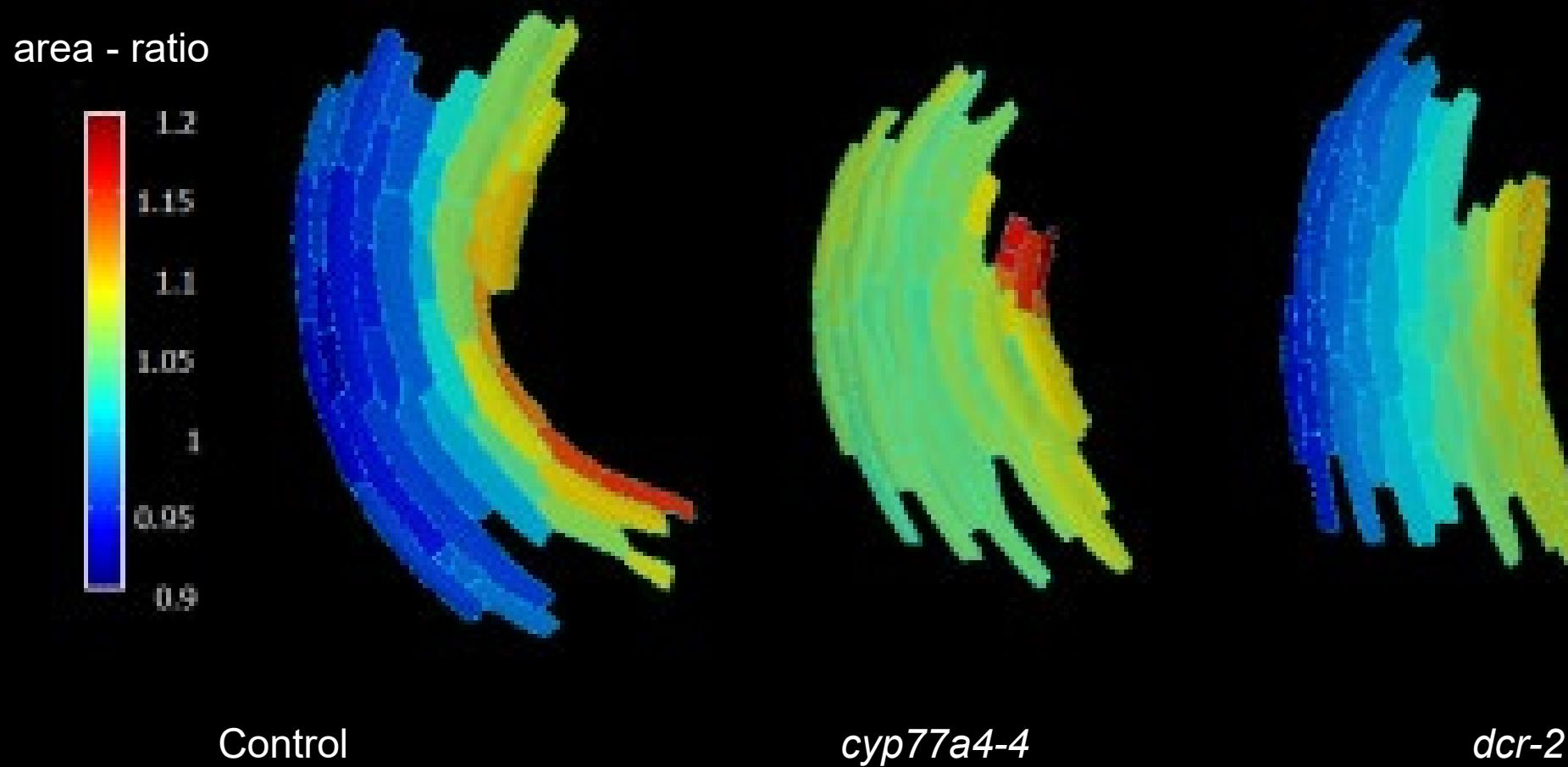


0 mN

4 mN

dcr-2

The cuticle mutants display increased deformation compared to the control



AFM measurements on live seedlings.

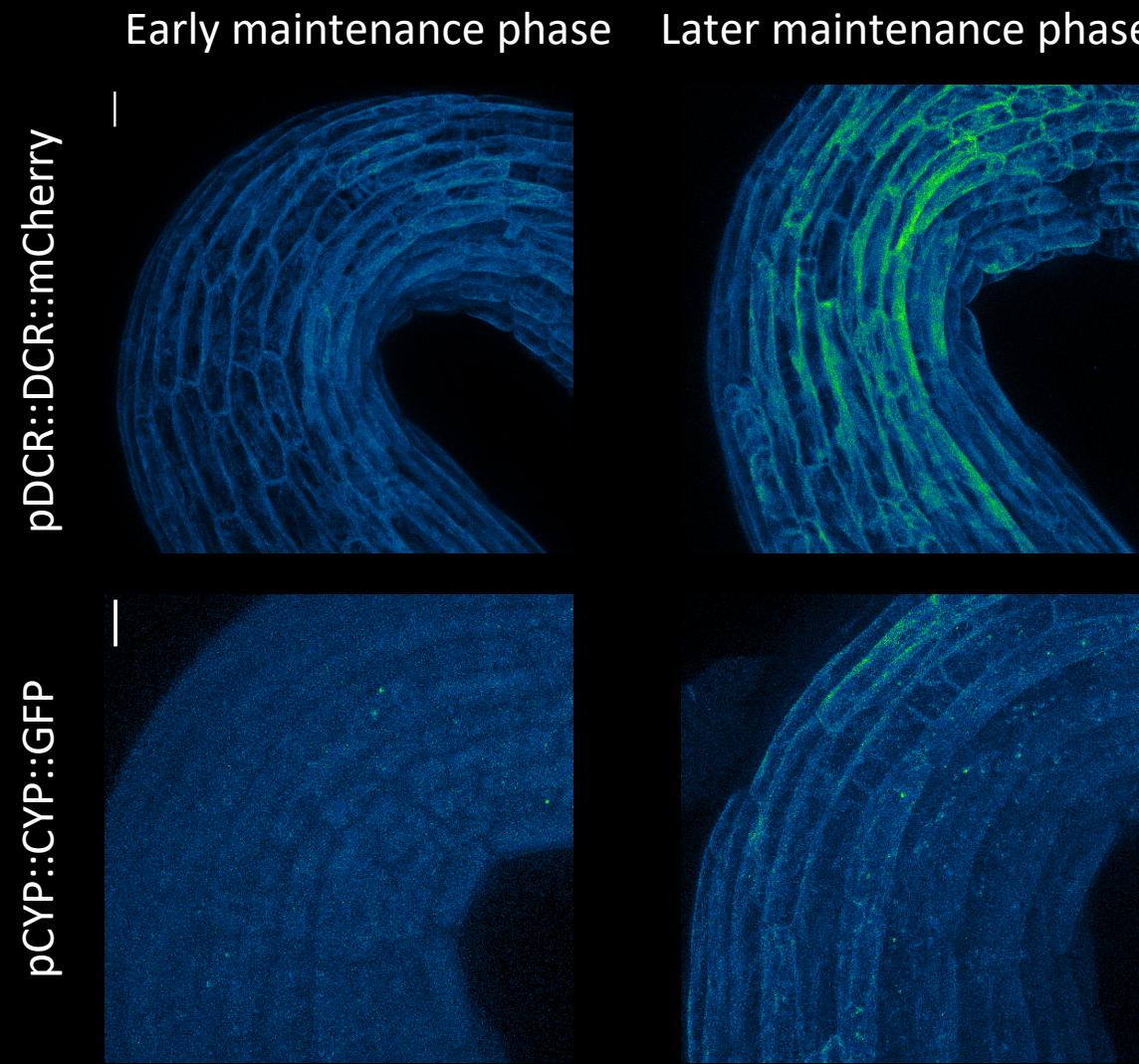
Preliminary measurements on the hook periclinal cell wall indicate that **cuticle mutants have increased young's modulus.**

- *Cuticle integrity is essential for apical hook maintenance*
- *Cuticle sustains the differential growth by maintaining the anisotropic growth*
- *Loss of cuticle integrity affects the organ / tissue mechanical properties*

Why maintenance phase?

Developmental program?

DCR protein levels during apical hook development



Scale bar 20um

- *Cuticle integrity is essential for apical hook maintenance*
- *Cuticle sustains the differential growth by maintaining the anisotropic growth*
- *Loss of cuticle integrity affects the organ / tissue mechanical properties*
- *Cuticle deposition is developmentally regulated to increase cuticle “levels” during the maintenance phase*