Overcoming the leaf cuticle as a barrier to foliar sprays

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Types of agrochemicals

Systemic: intended to cause effect within the plant (fertilizers, systemic insecticides, fungicides, bactericides)

External: intend to protect from external causes (insects, fungi, radiation)

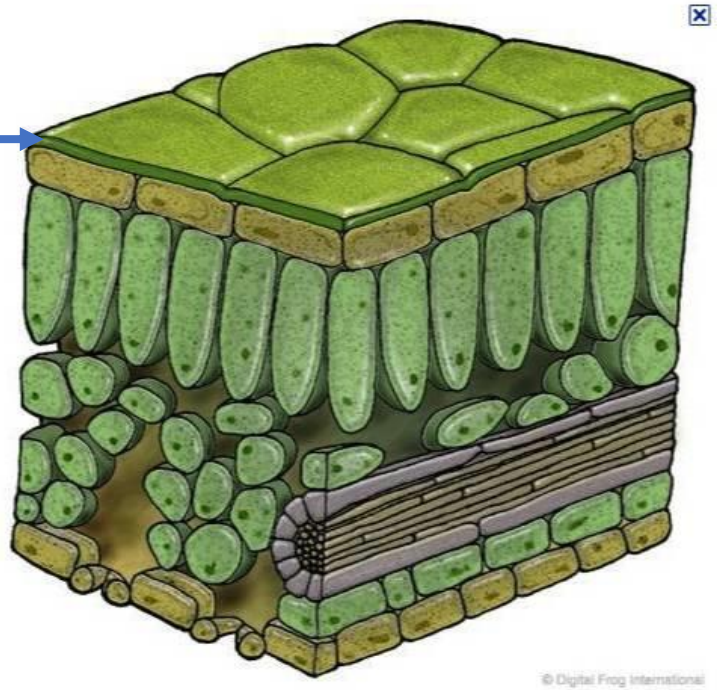
Most common applications methods for systemic agrochemicals are inefficient

1. **Ground applications** (excessive amounts needed, ground water contamination, affect ground biome)

2. **Foliar applications** (excessive amounts, short penetration time, washed off to the ground)

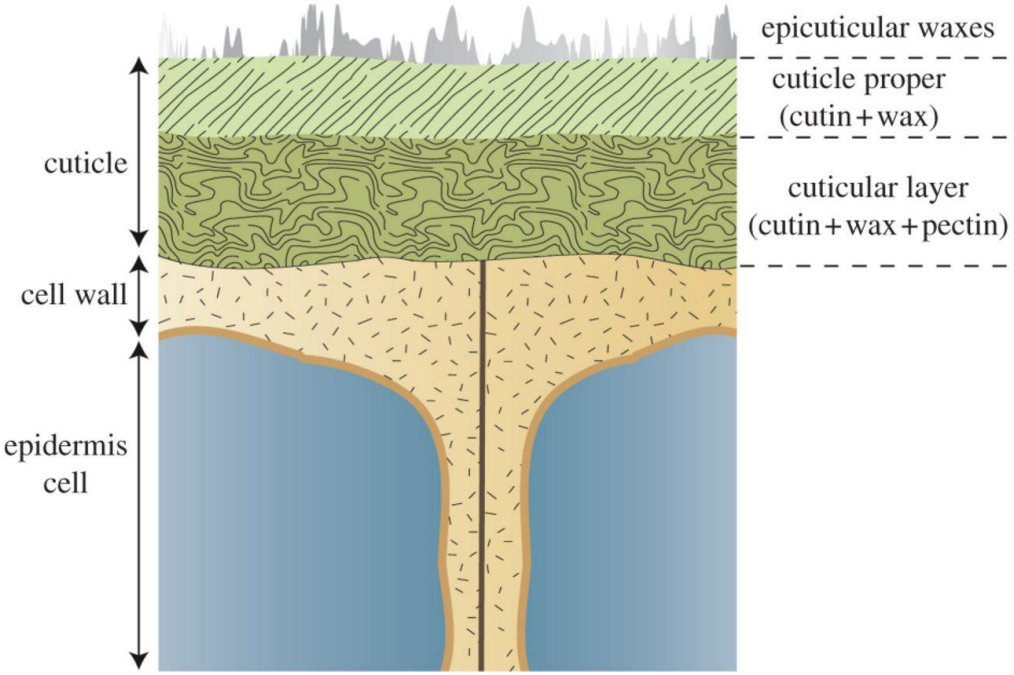
Penetration of foliar agrochemicals is Inefficient due to waxy cuticle

cuticle



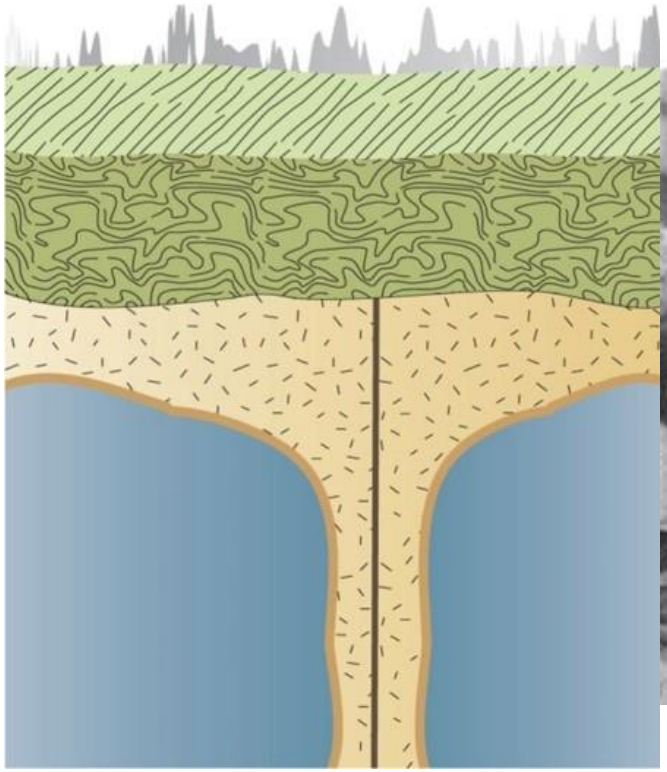
Plant leaf

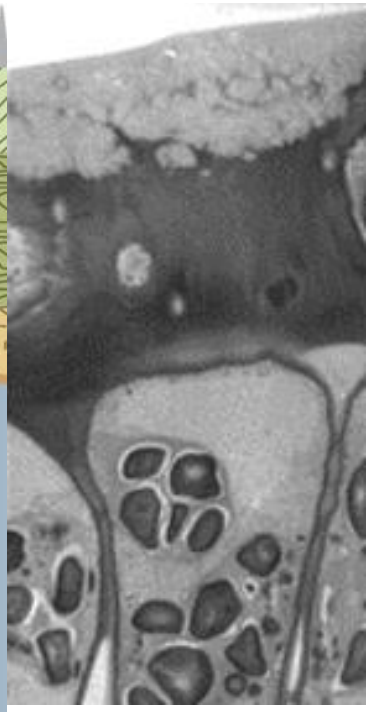
Cuticle: protective layer covering the surface of leaves and young stems. Composed of cutin and waxes. Mostly protect against desiccation.


epicuticular waxes
cuticle proper
(cutin + wax)
cuticular layer
(cutin + wax + pectin)
cuticle
cell wall
epidermis
cell

Cutin and waxes are hydrophobic
(water “impermeable or repellent)

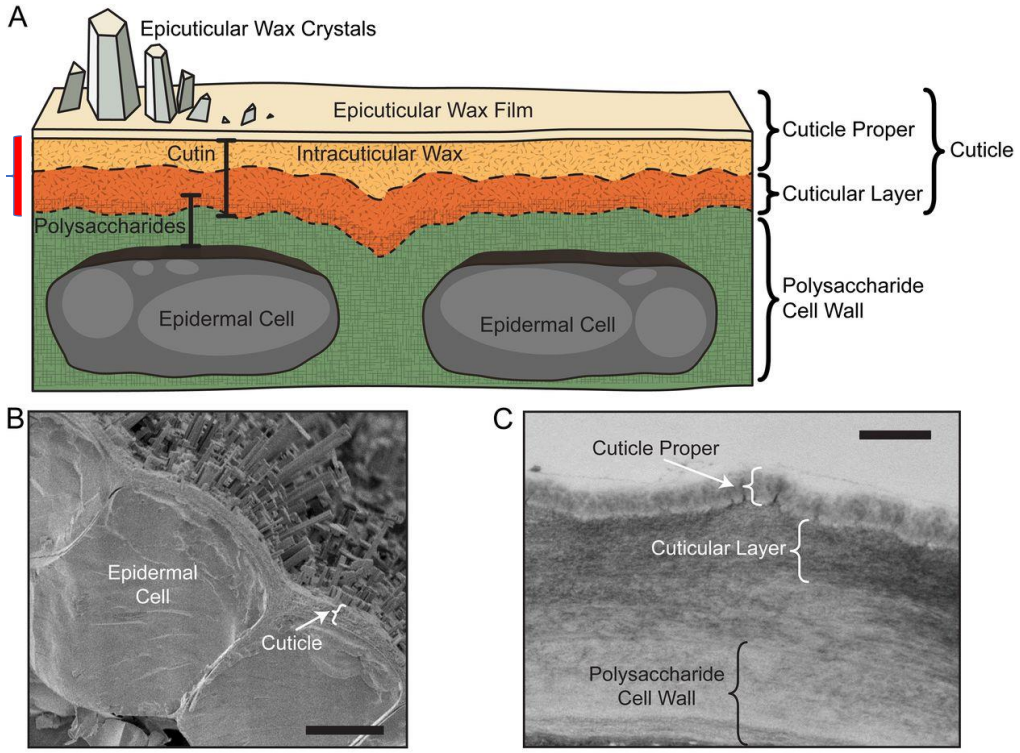
Aqueous solutions “bead up”

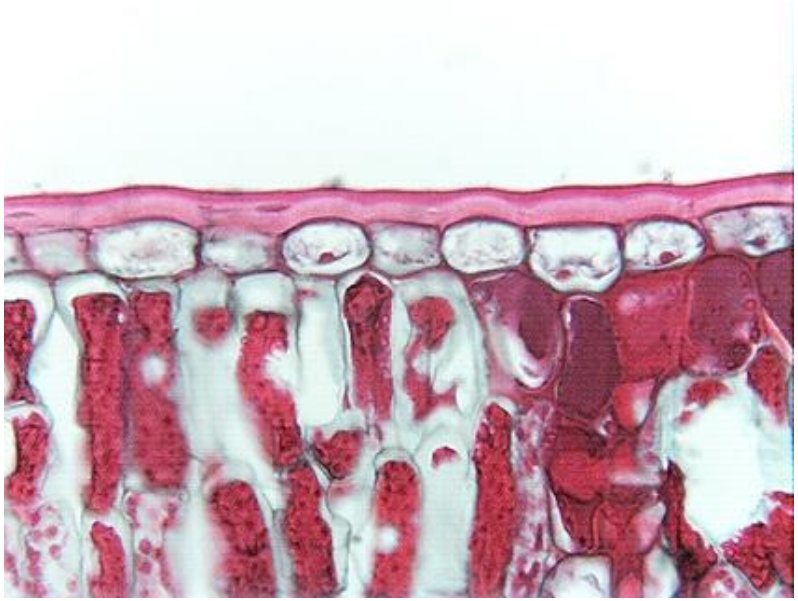


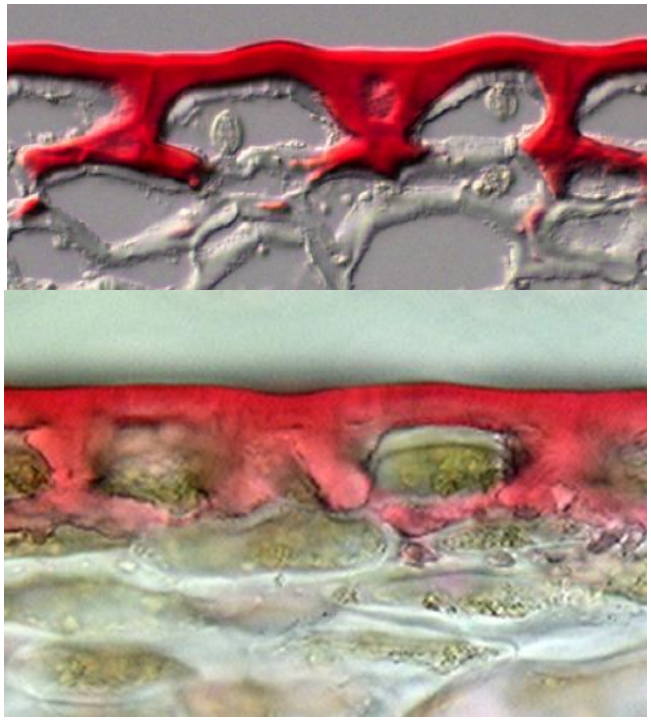


Citrus leaf

4 μm

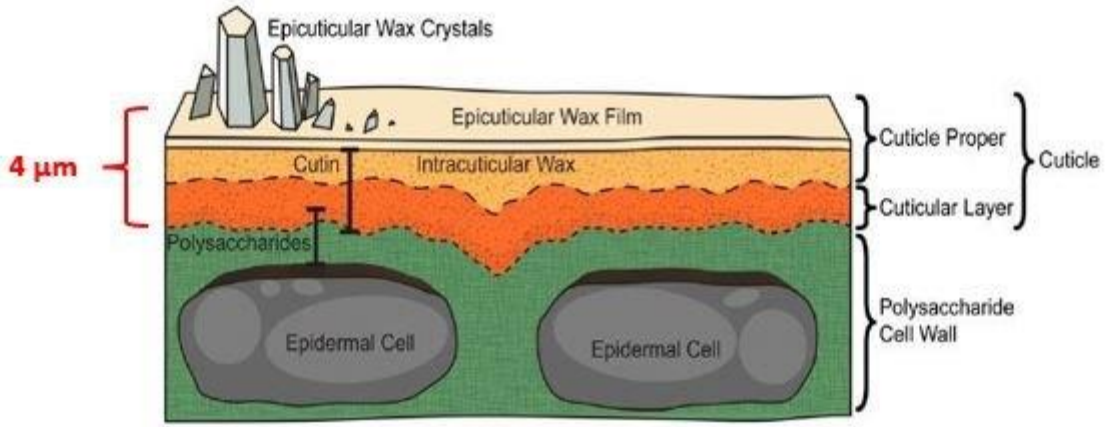

A
Epicuticular Wax Crystals
Epicuticular Wax Film
Cutin
Intracuticular Wax
Polysaccharides
Epidermal Cell
Epidermal Cell
Cuticle Proper
Cuticular Layer
Cuticle
Polysaccharide
Cell Wall
B
Epidermal
Cell
Cuticle
C
Cuticle Proper
Cuticular Layer
Polysaccharide
Cell Wall



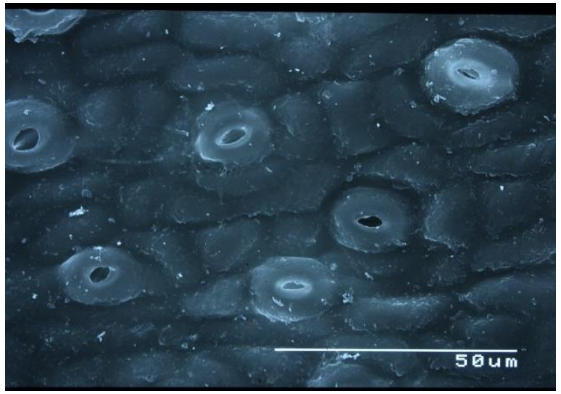


DENNIS KUNKEL MICROSCOPY/SCIENCE PHOTO LIBRARY

Thickness of *Citrus* leaf cuticle = 4 µm

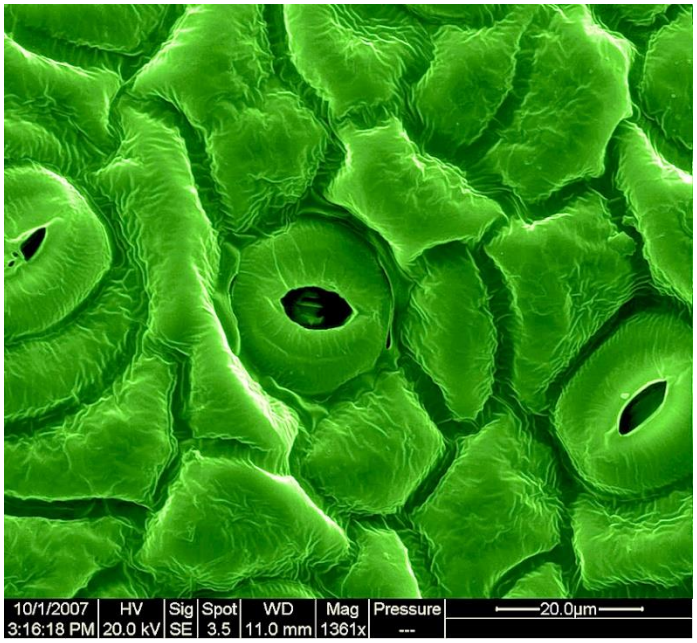


Penetration of solutes through stomata, cracks in the cuticle and through polysaccharide matrix

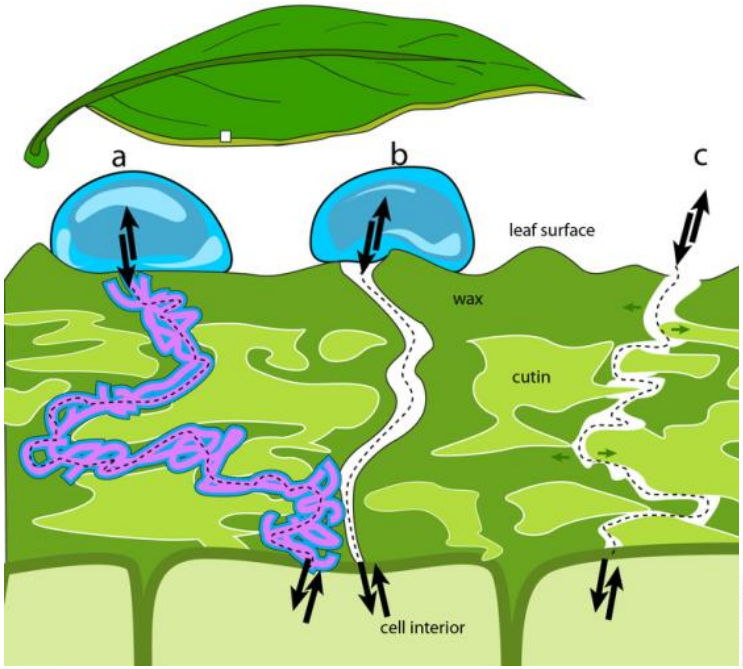


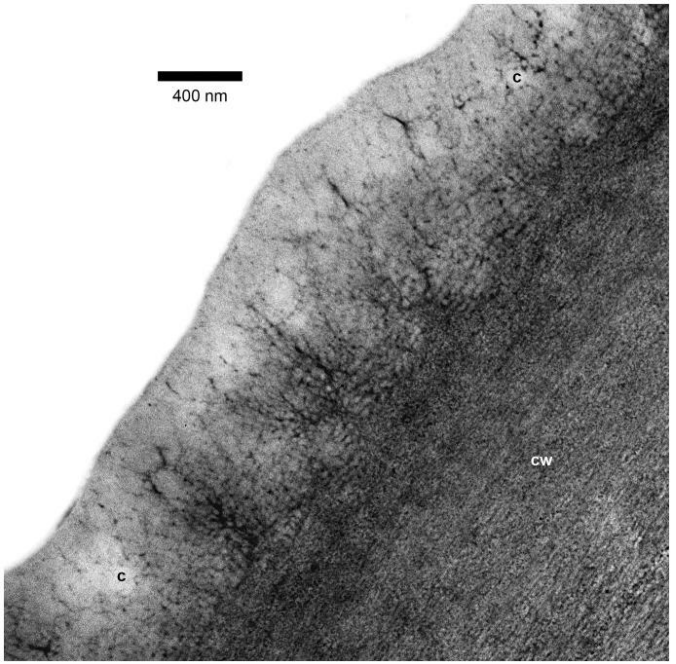
Brodribb T. J., Holbrook N. M. (2004); New Phytologist 162: 663-670

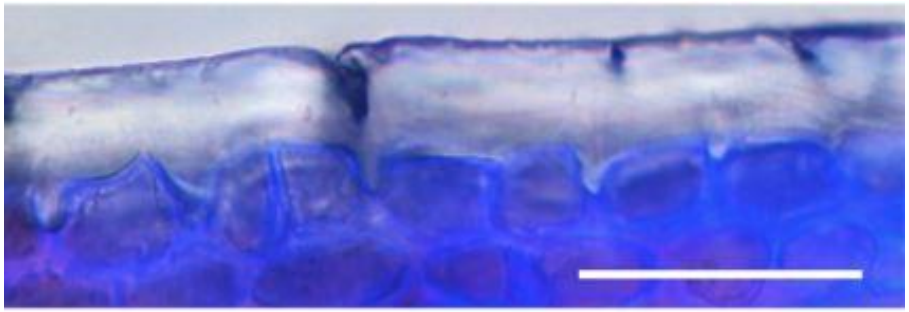
Stomates are not open all the time and geometry impedes movement of solutions to the leaf interior, short duration time.



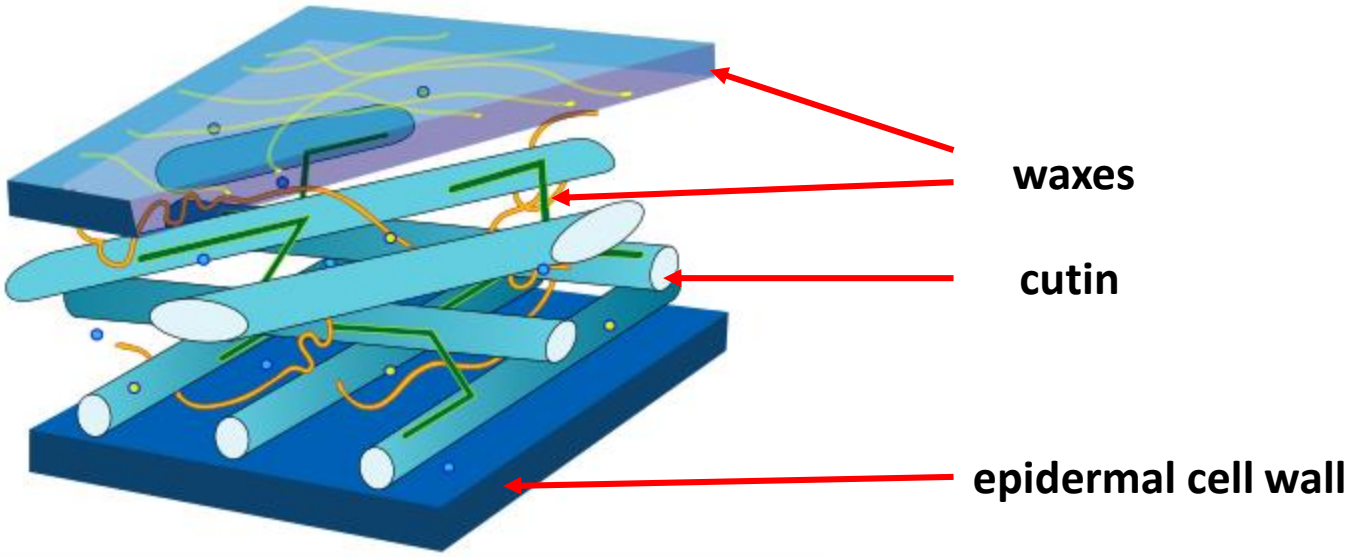
cracks in the cuticle







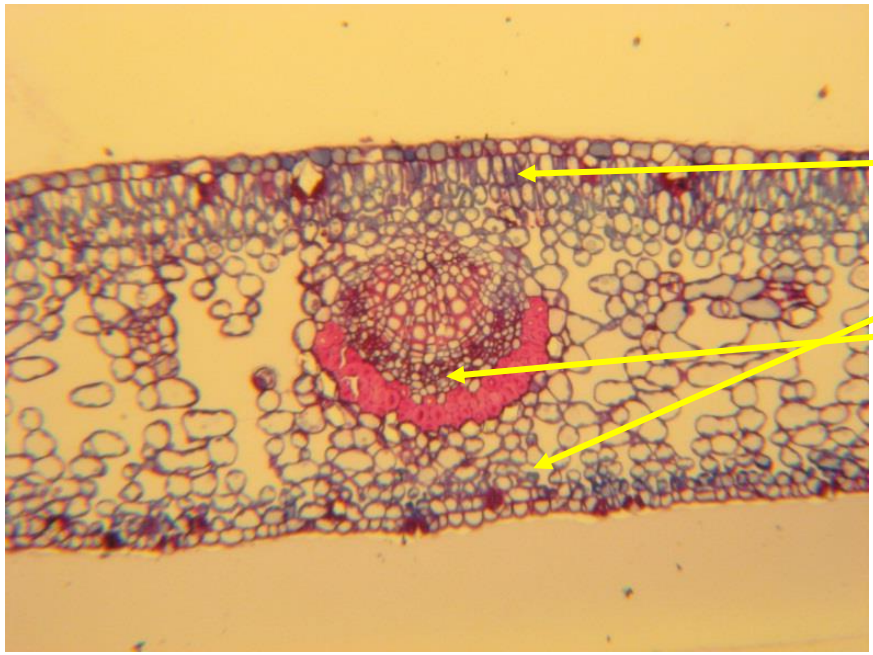
Cuticle



Cutin pores ≈ 3 Å (10^{-10} m)

Glucose = ≥ 15 Å

Citrus leaf



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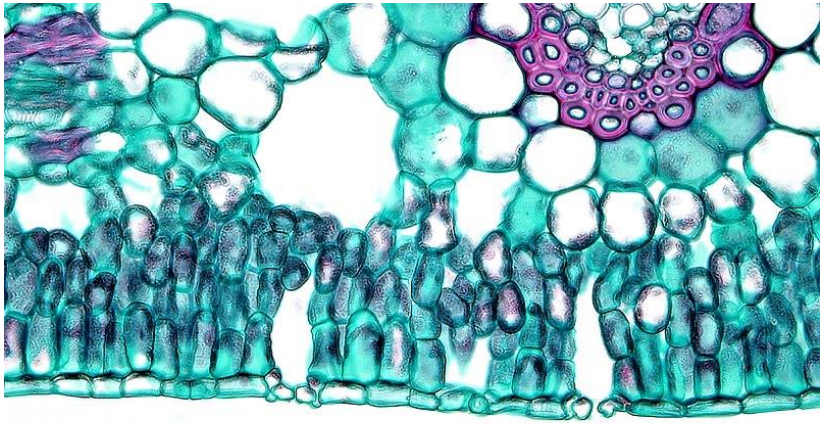
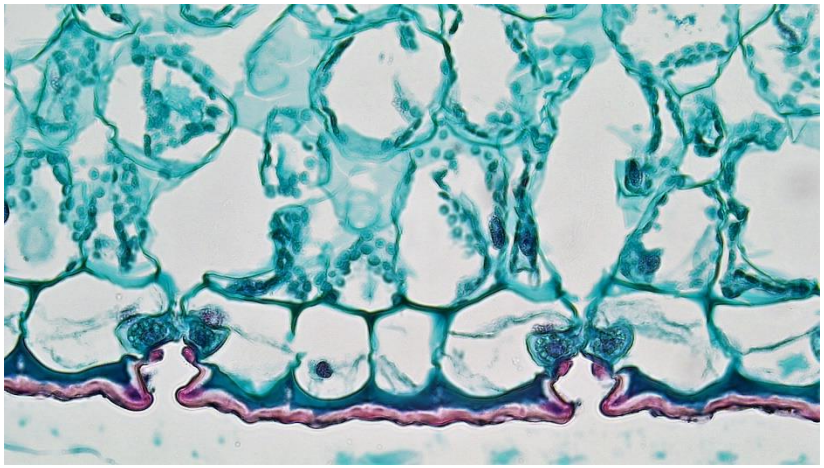
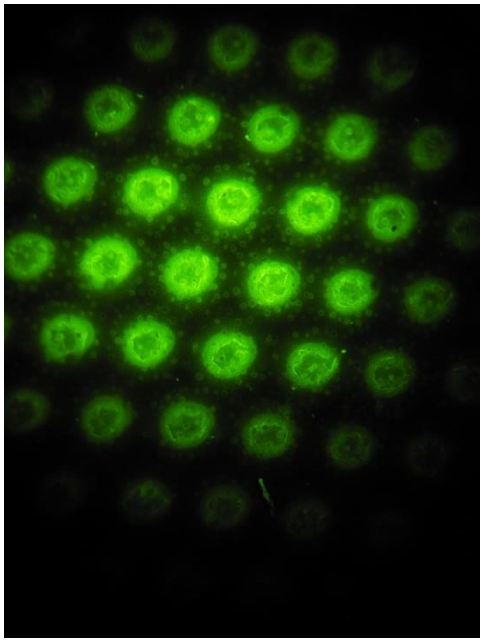


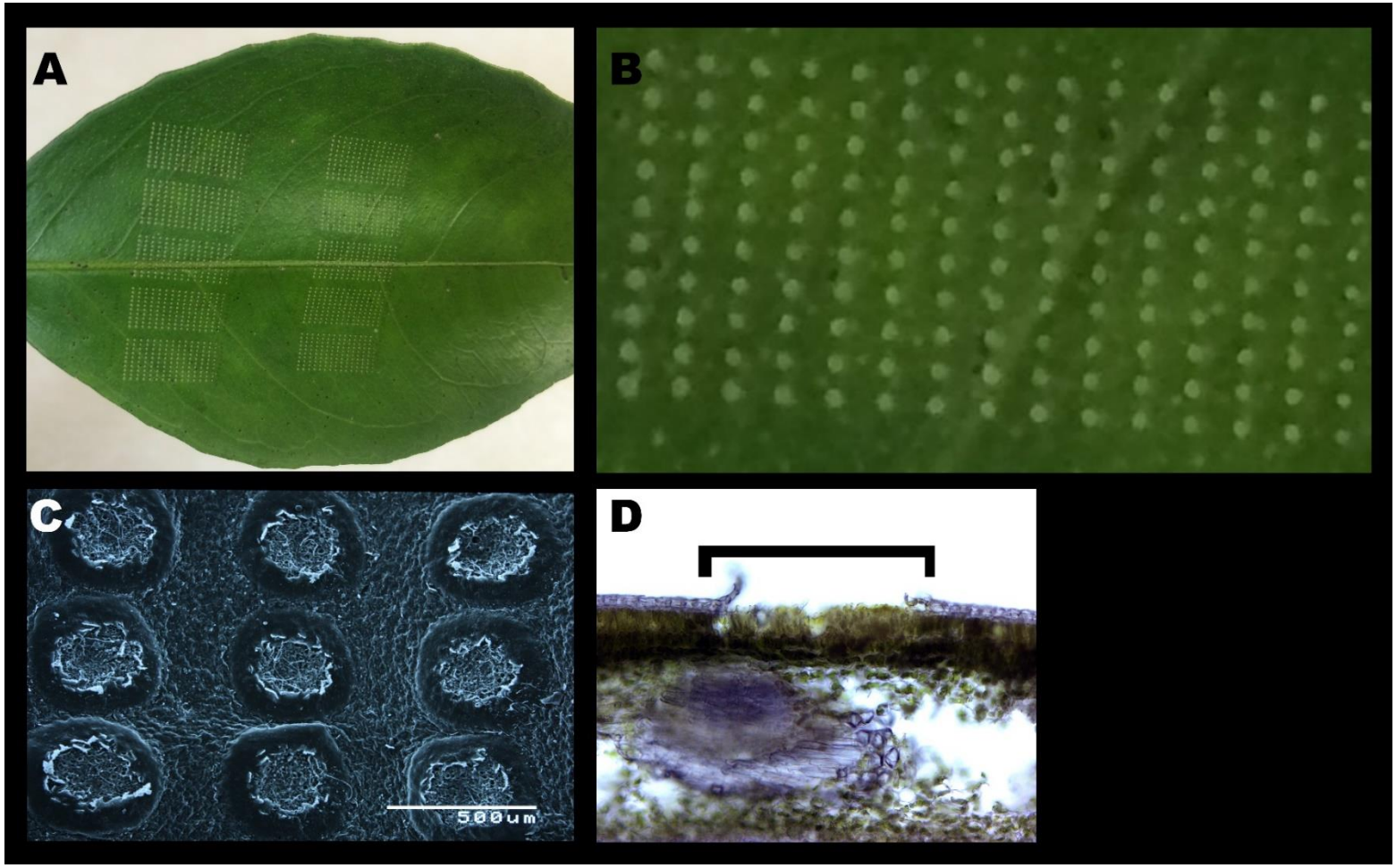


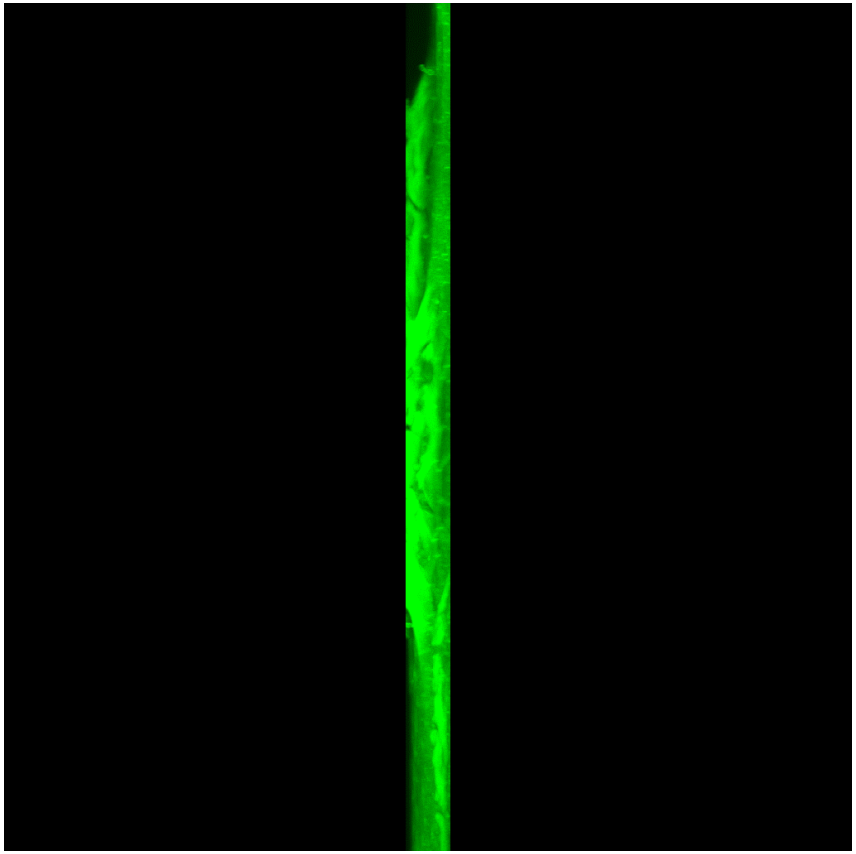
Laser light to overcome the cuticular barrier

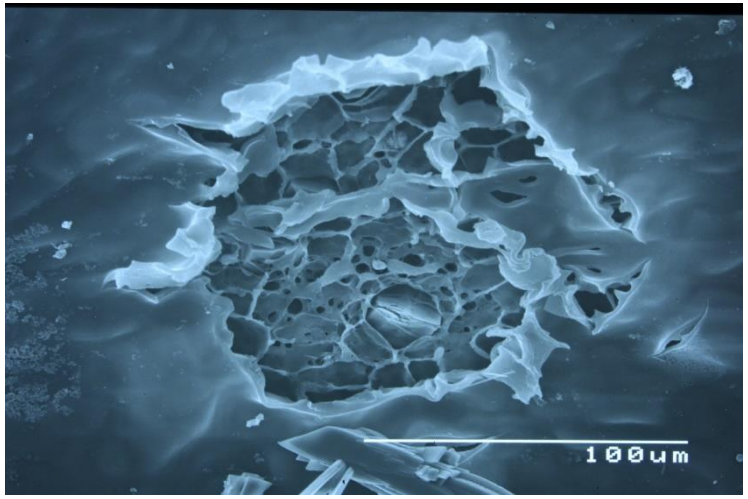
GPD Industries



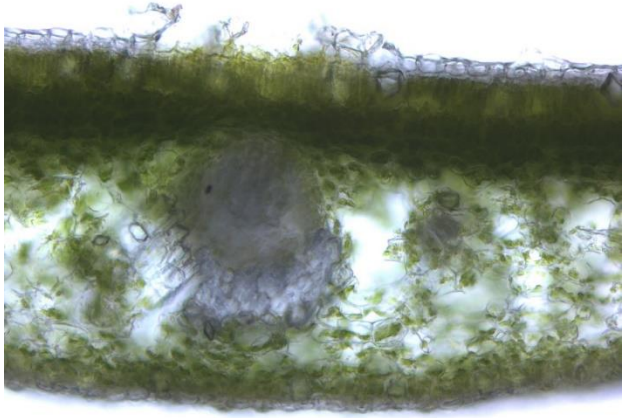
Light Amplification by Stimulated Emission of Radiation".


A
B
C
500um
D



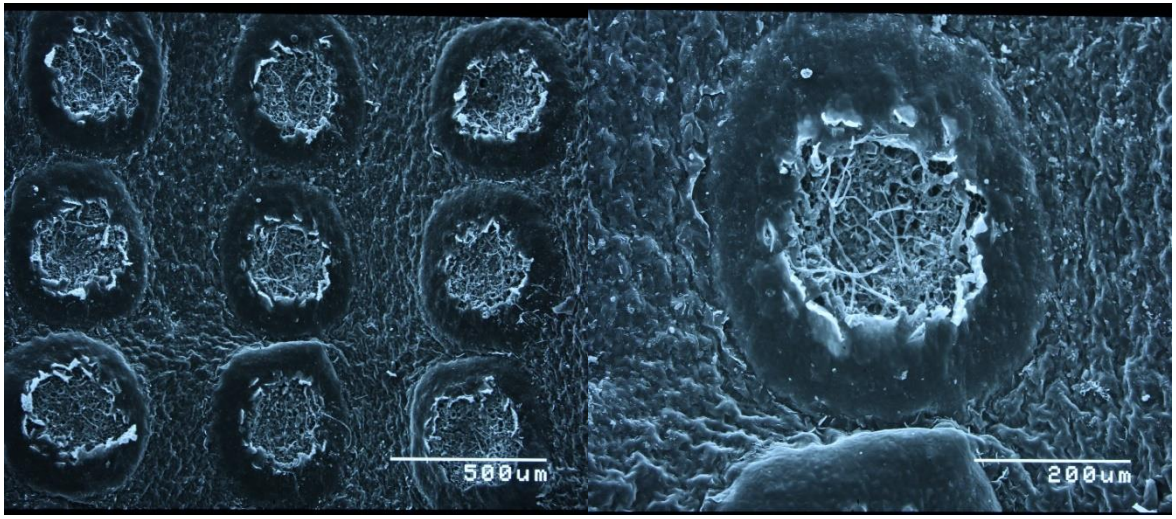


SEM



Cryo-sectioning

SEM images, citrus leaf

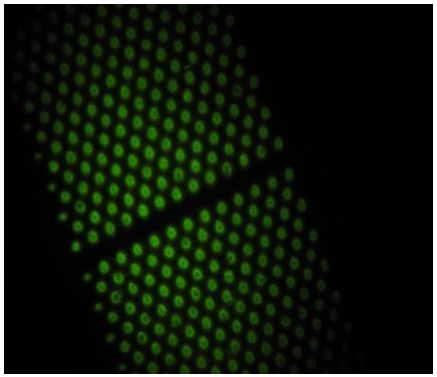


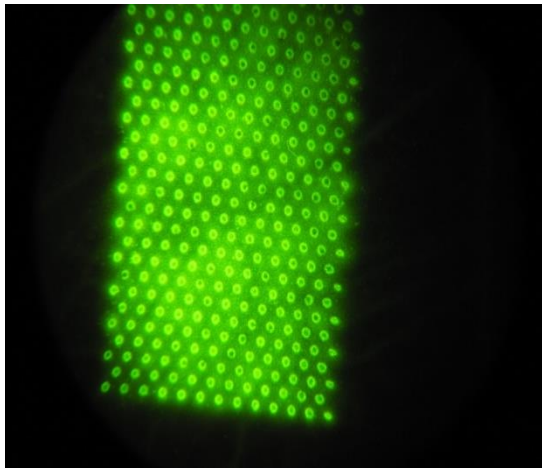
Laser technology for application of agrochemicals

Experimental Solutions

1. Fluorescent deoxy-glucose (NBDG).
2. Carboxyfluorescin-DA (CF).

Lasered leaf after NBDG application



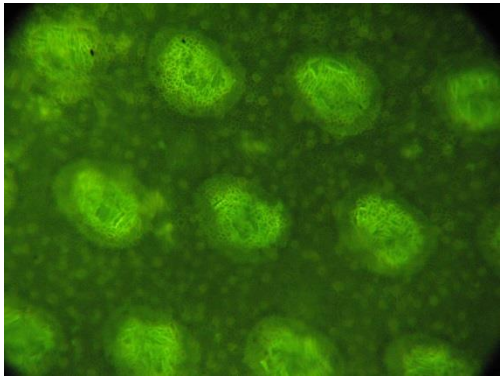


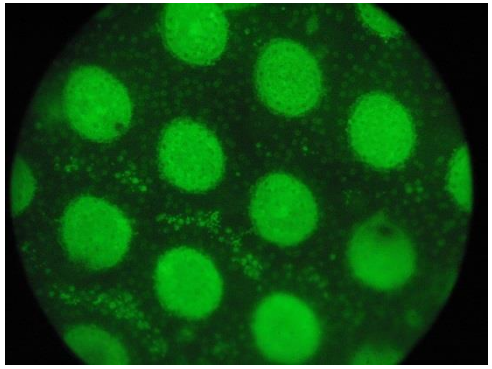
Control **NBDG**

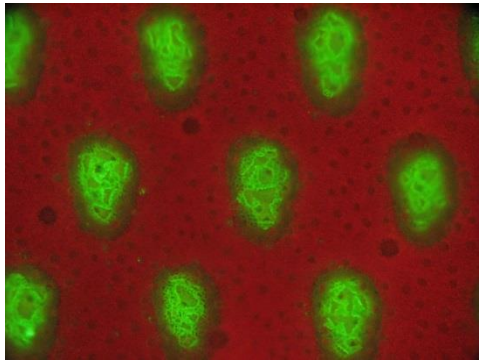
30 min

(Laser abrasions of ~ 250 μm)

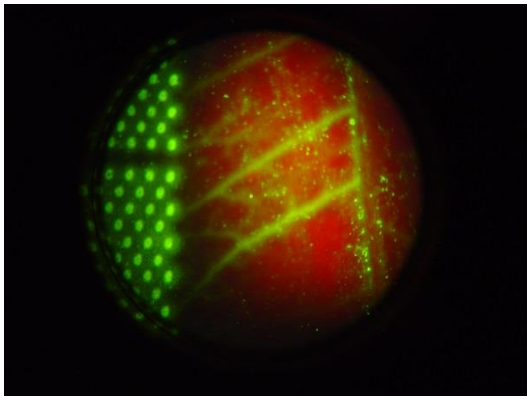
NBDG, CF and Alexa-488 after 30 min

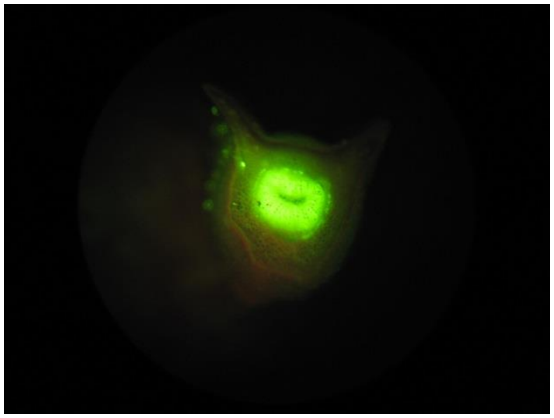


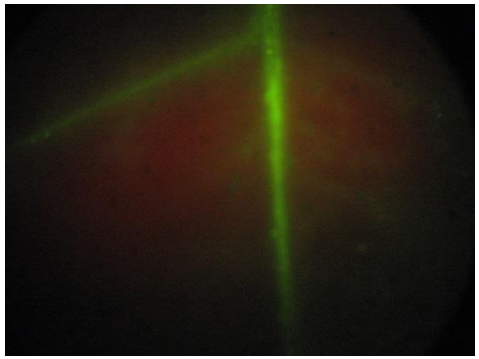


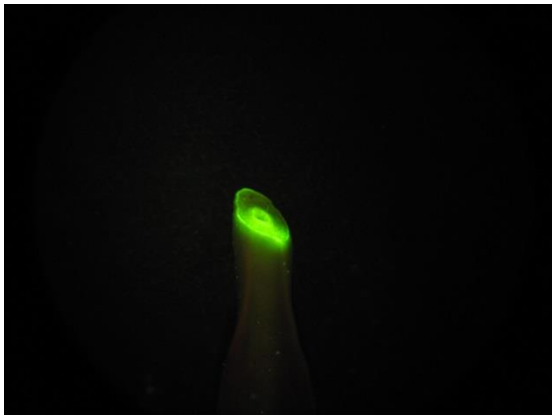


NBDG Treated leaf 4 hrs . Blade and petiole

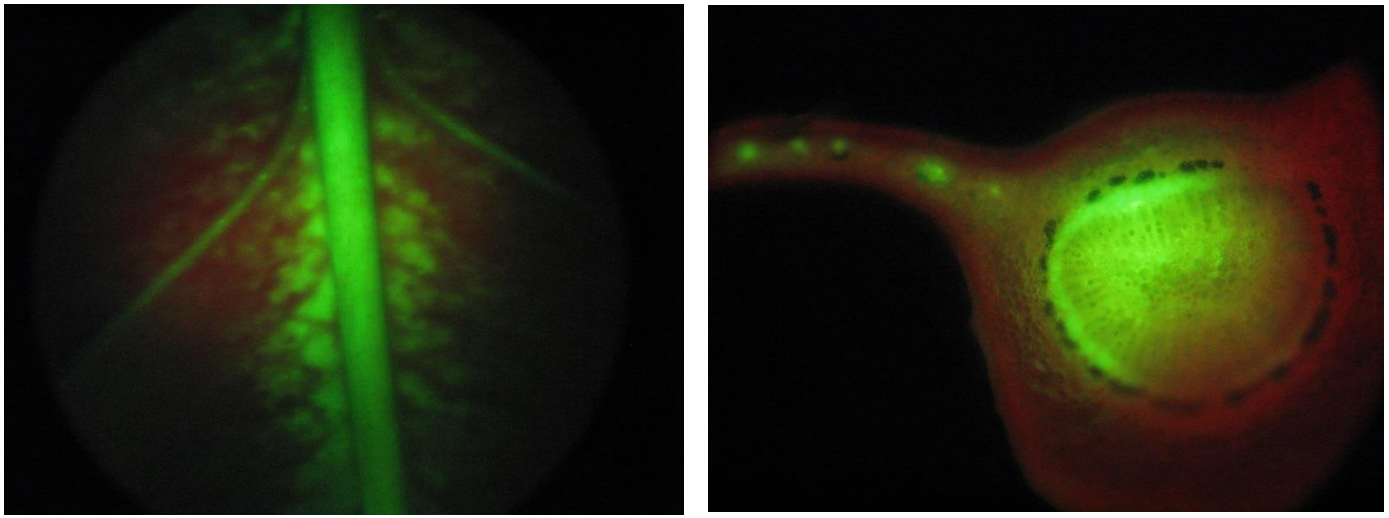




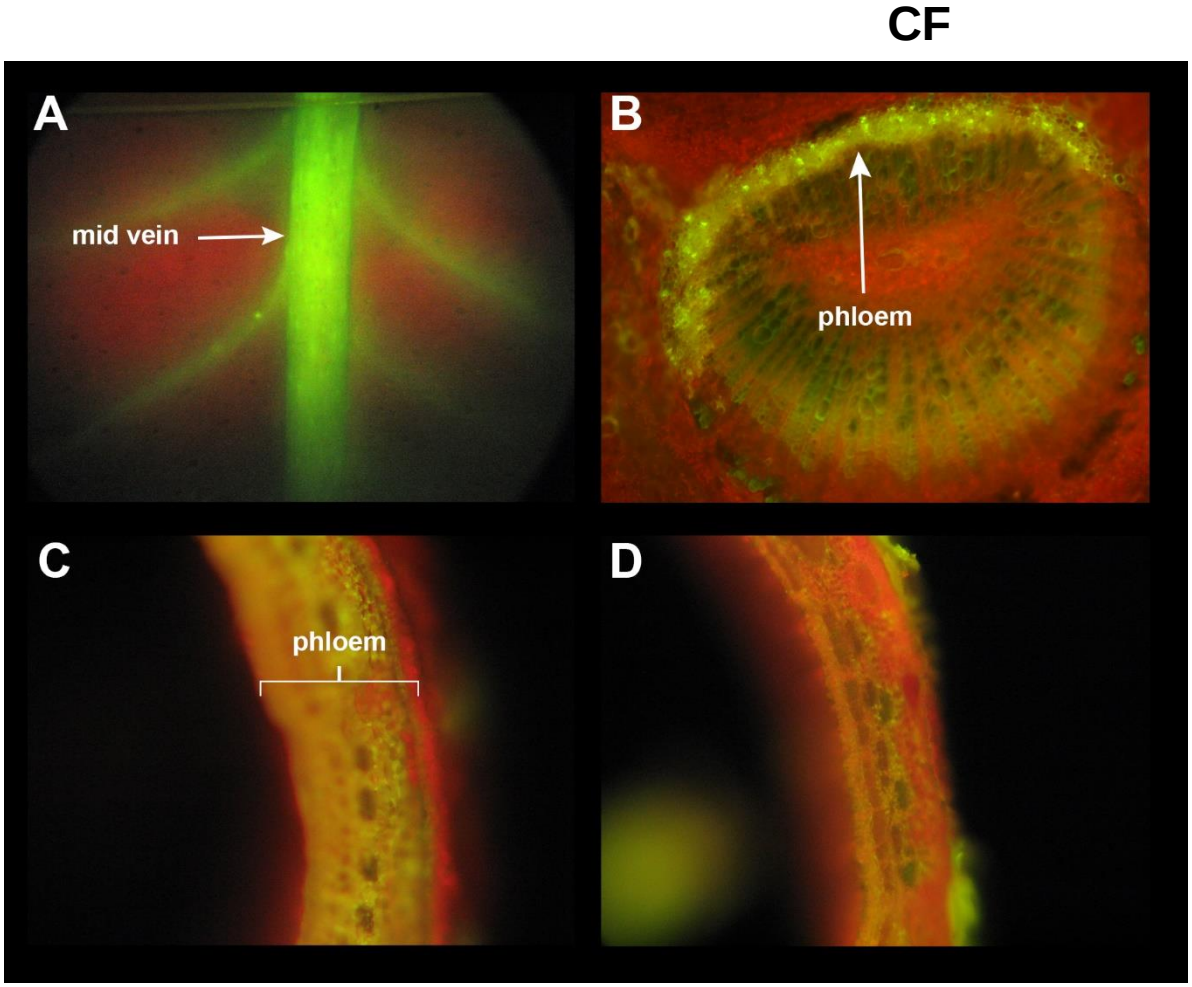




Lasered leaf, NBDG



Penetration over 4,000 times depending on lasered surface area


CF
A
mid vein
B
phloem
C
phloem
D

Compound	m.w.	Conc.	λ Ex/Em	λ
Fluorescent deoxy-glucose, 2-NBDG	342	30 mM	465/540	+
Carboxyfluorescein	376	5 mM	492/517	+
Ethidium bromide	394	1 mM	285/605	-
Carboxyfluorescein-SE	460	4.9 mM	492/517	+
Lucifer Yellow	522	1 mM	428/536	-
Lysine-Tamra	559	9 mM	545/575	+
Penicillin-Bocillin FL	661	3 mM	504/511	+
Trehalose-FITC	744	3 mM	492/517	+
Alexa Fluor-488	884.9	4.5 mM	495/519	-
ATP-Bodipy Fl	933.3	5 mM	504/513	+
Calcein Green-AM	995	1 mM	488/515	-
Vancomycin-Bodipy Fl	1723.35	100 mM	504/510	-
Dextran-Texas Red	3,000	3.5 mM	595/615	-
PAMAM dendrimers generation-4-Alexa 488 (ø = 4.5 nm)	14,215	0.2 mM	495/519	+
Lysine-tRNA (Bodipy Fl)	26.5 kD	1 mM	502/510	-
Quantum dots (Qtracker) 565 (ø = 10 nm)	~110,000	2 µM	405-525/565	-

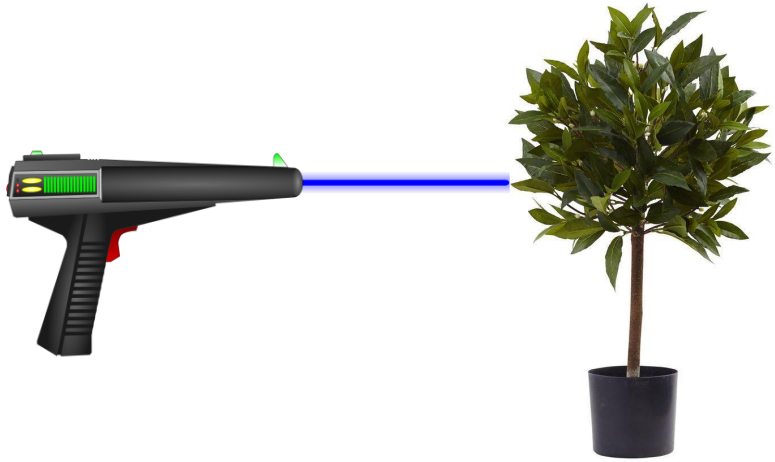
How to improve existing technology?

(IP and patent belong to Premier LTD)

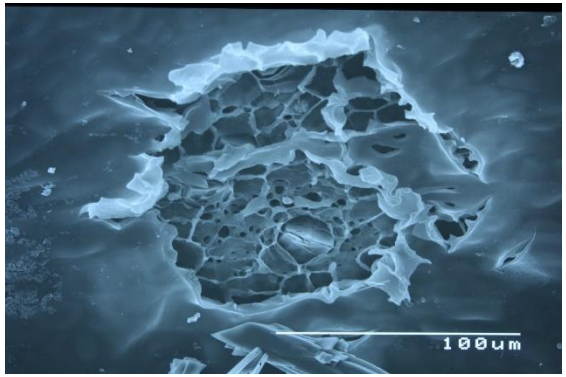
Lab UV laser, CO2 laser

Focal distance and perforate the cuticle

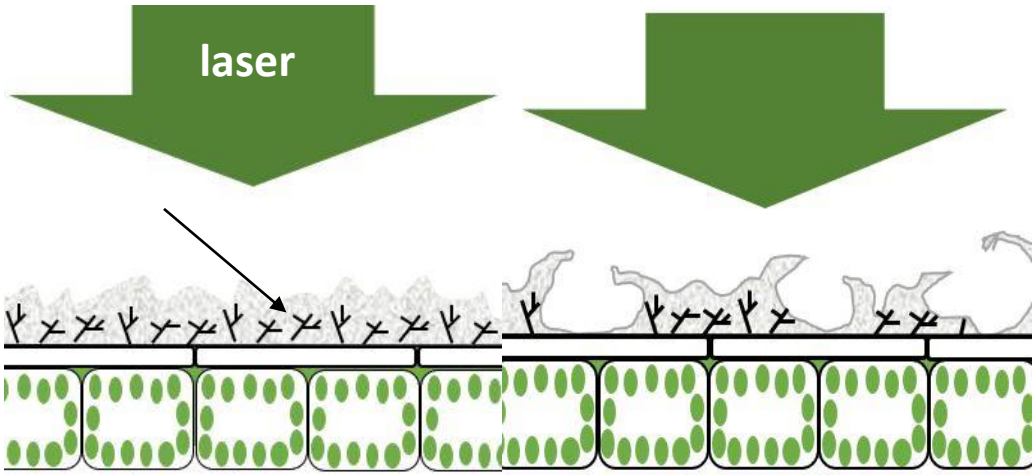
Specific focal distance



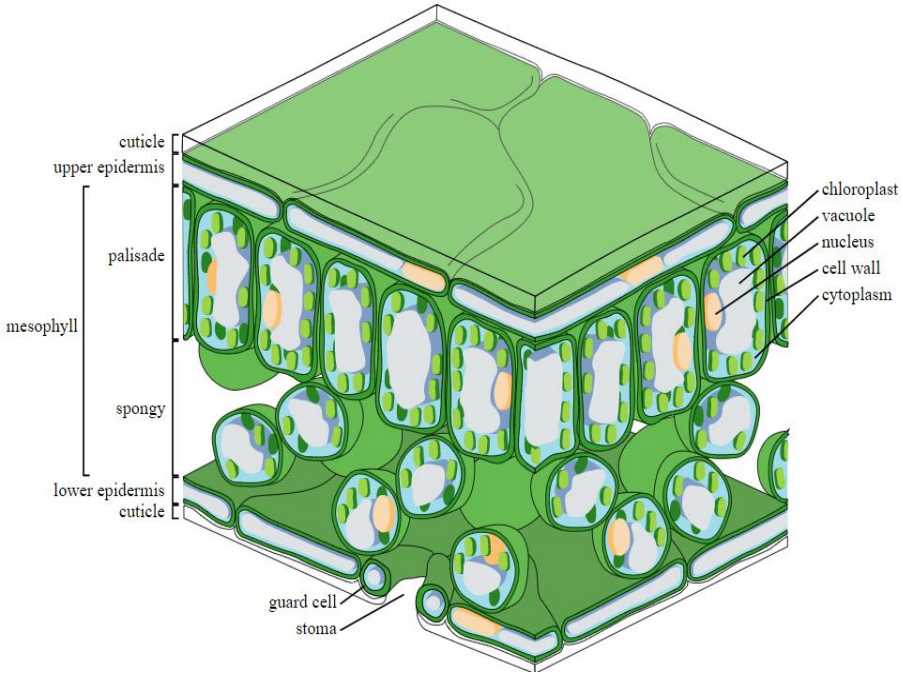
Perforate the epidermis

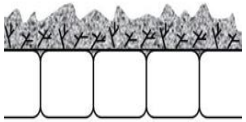


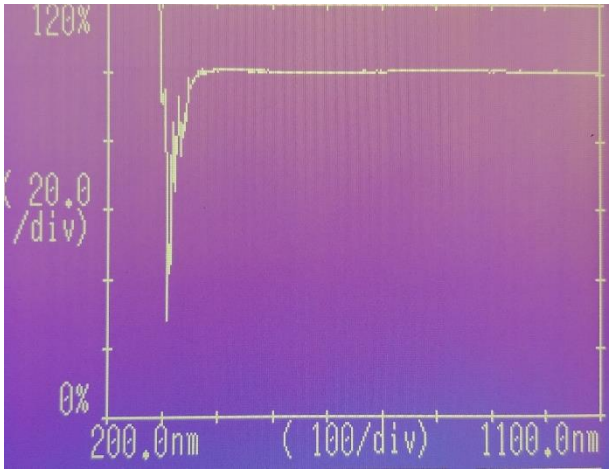
wax exfoliation



Structure and optical properties of citrus leaf

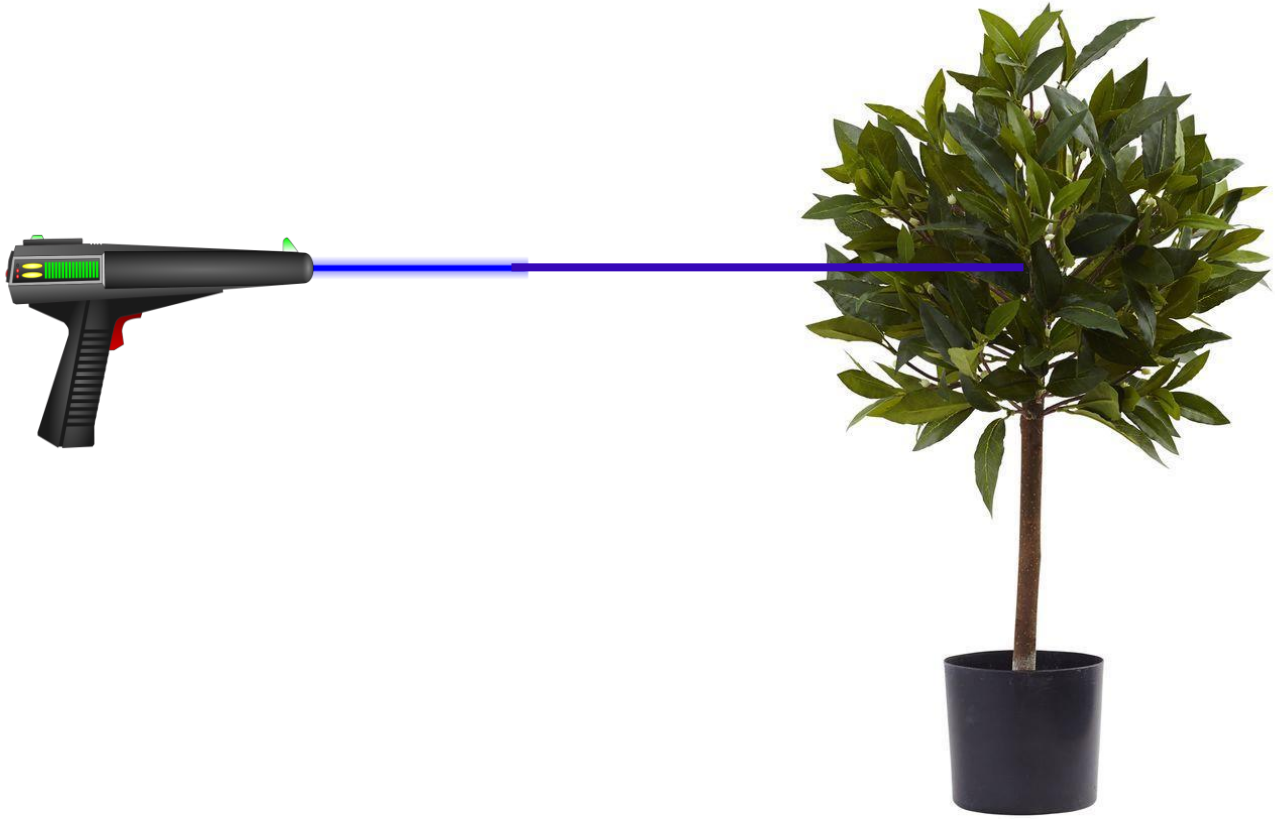


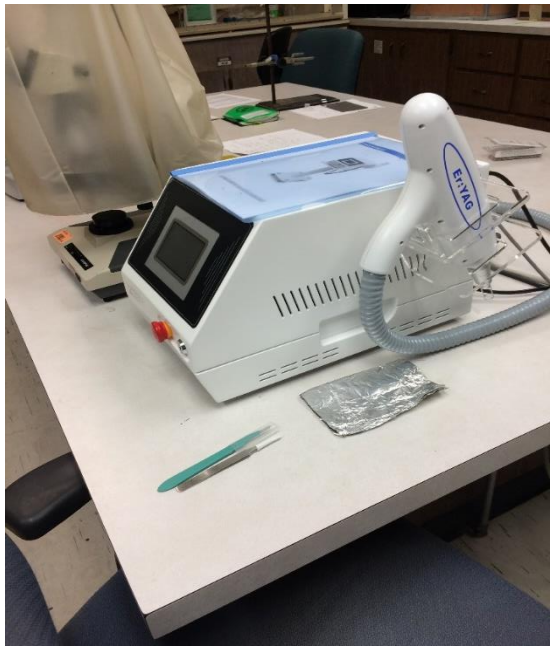




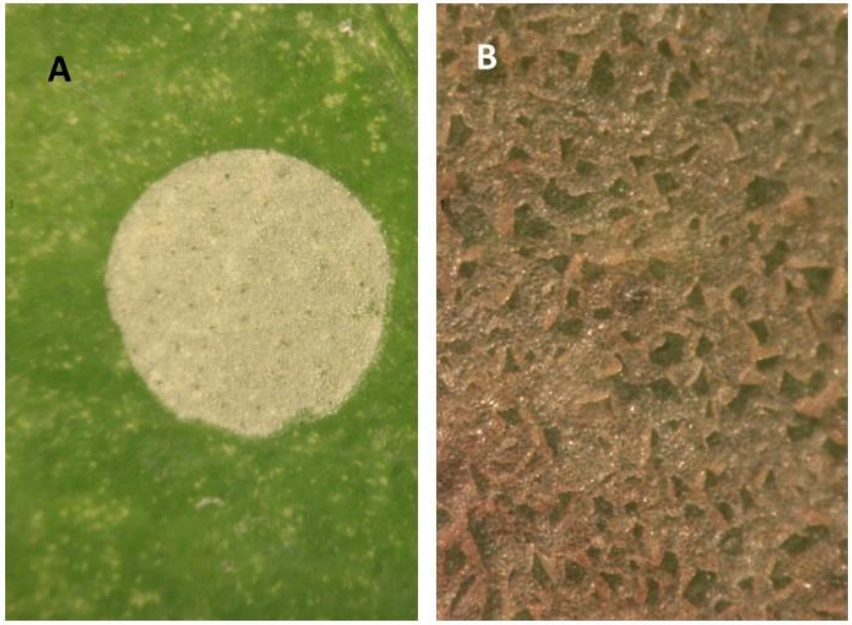
Transmission spectrum of wax extracted from citrus leaf

No focal distance

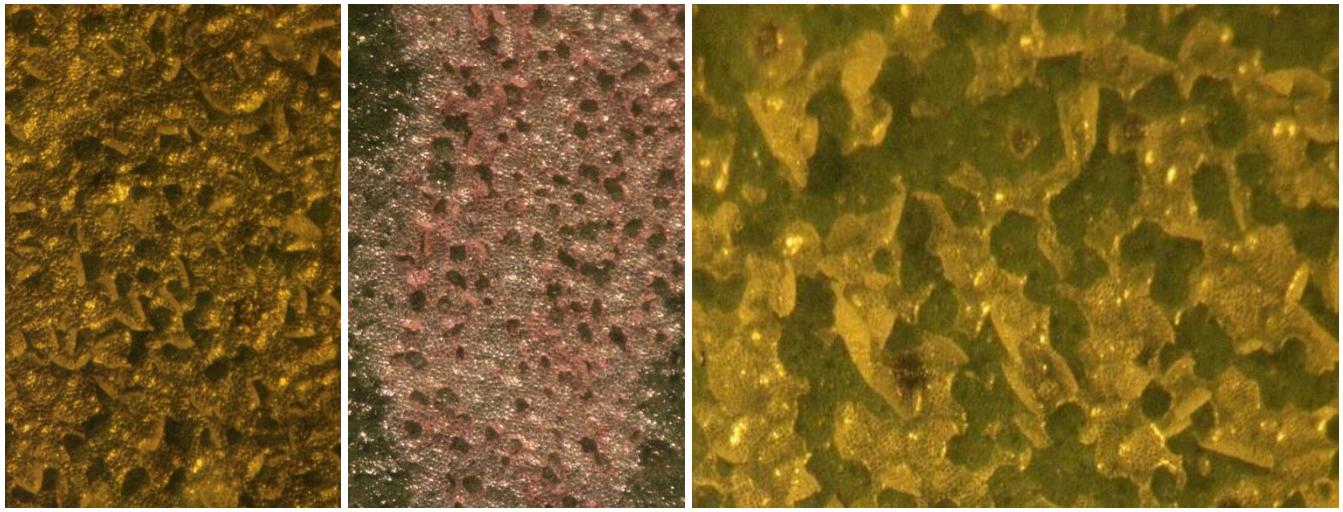




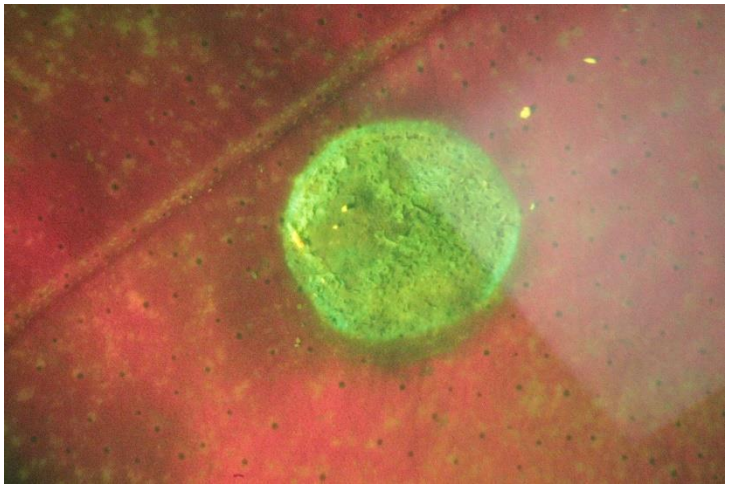
1064 nm

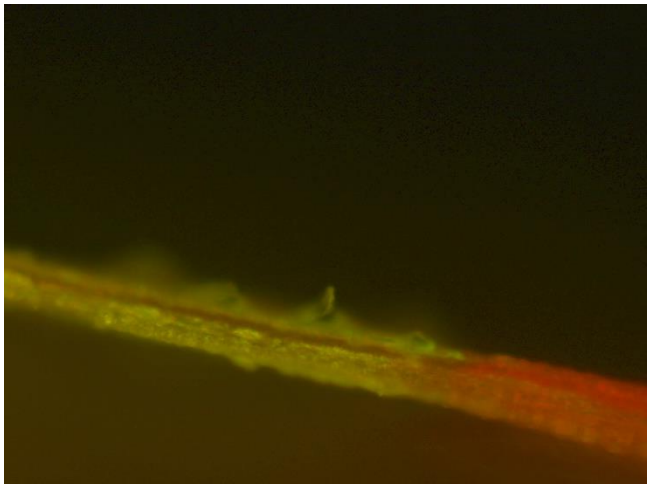

A
B

Wax exfoliation



dsRNA- 1



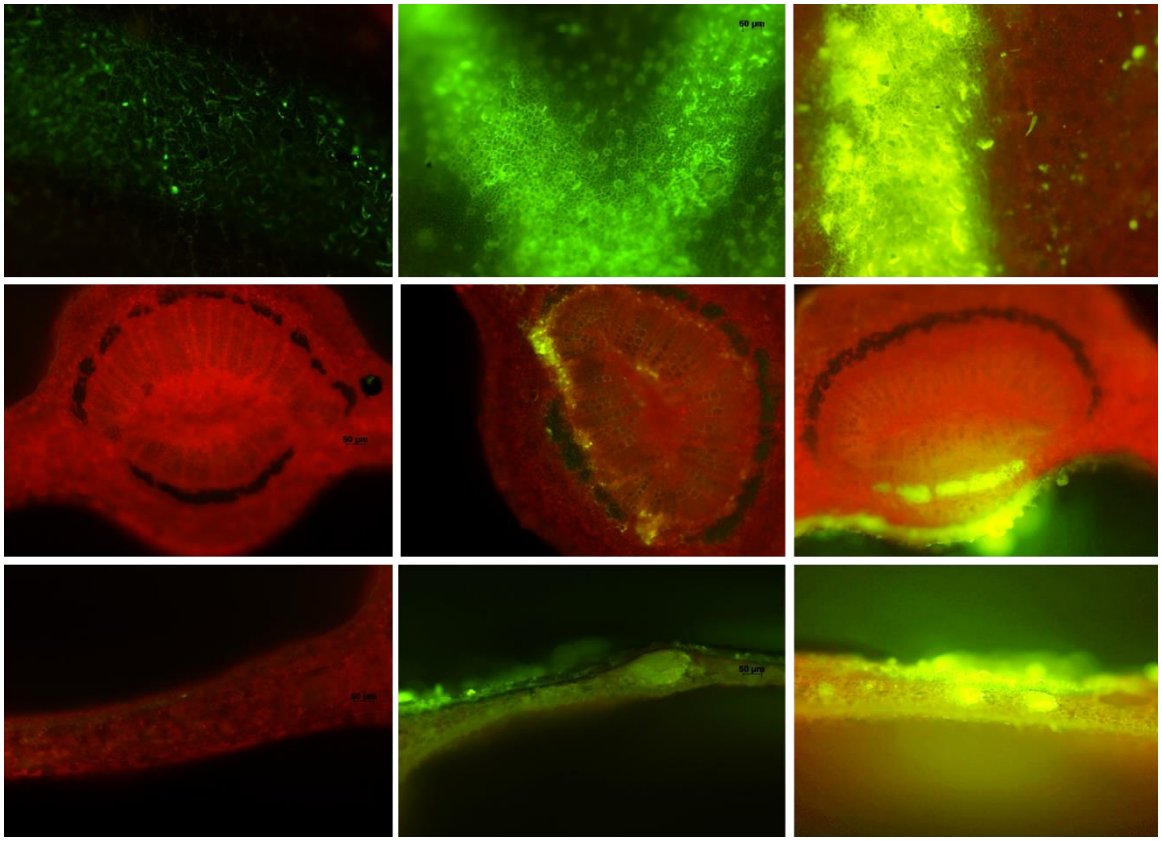


Fluorescent deoxyglucose

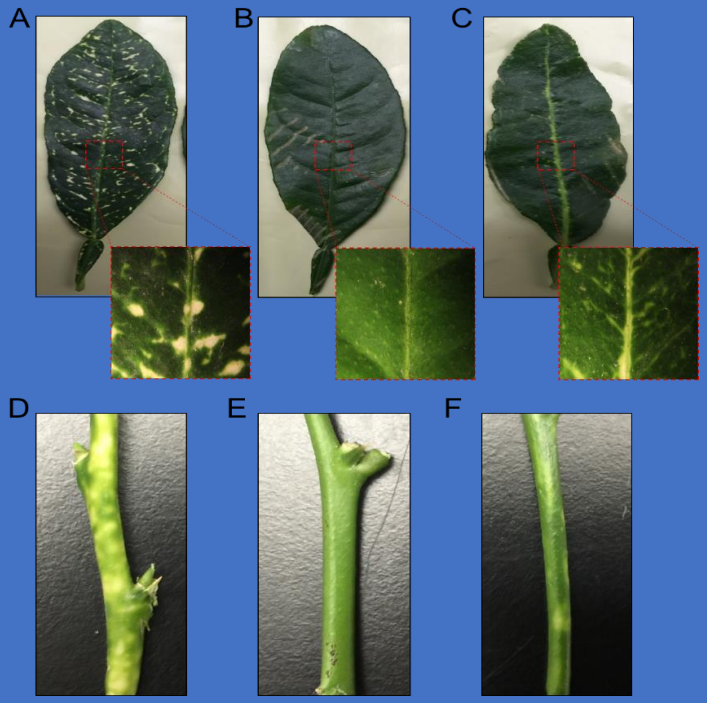
Challenges:

1. Adapting the technology to the
2. Seal the abrasion
3. Integrating sprays (potential mixes)
4. Timing

Delivery of dsRNA to citrus phloem through laser micro-puncture

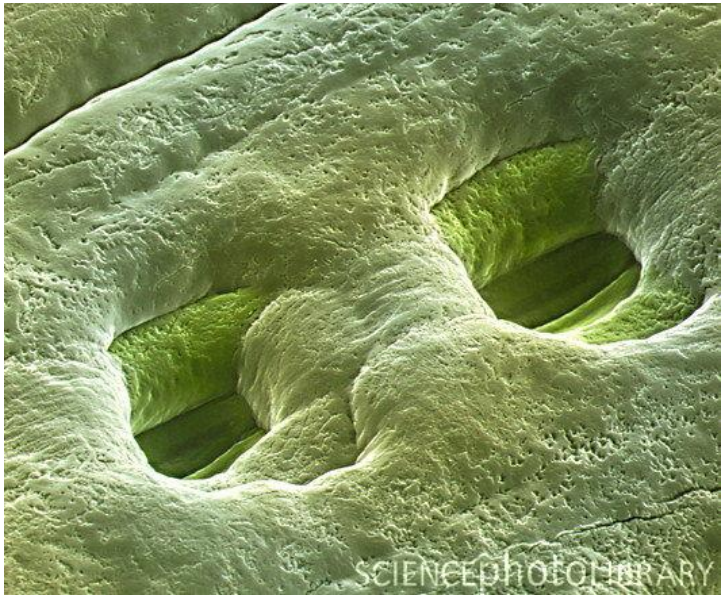


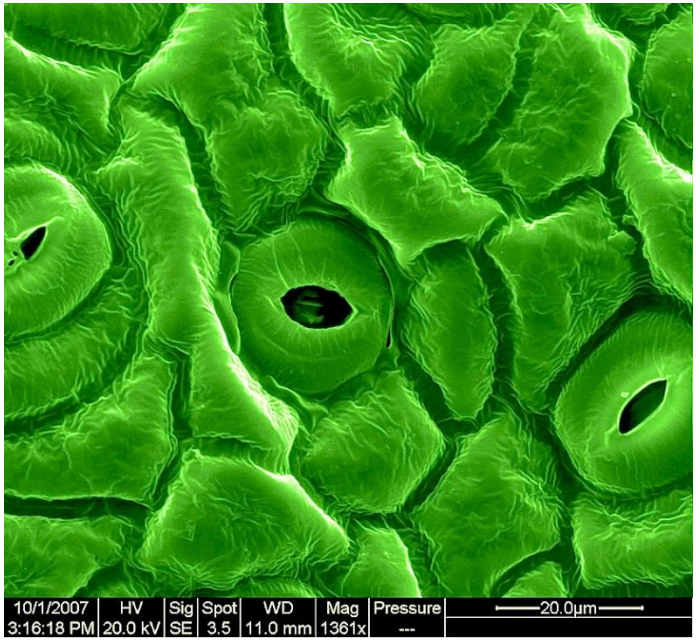
Water NBDG CY-3 labeled dsRNA-PDS



CTV-*t*PDS inoculated, dsRNA-*GFP*, dsRNA-*PDS* laser-delivered

(PDS = phytoene desaturase)


SCIENCEPHOTOLIBRARY


10/1/2007
3:16:18 PM
HV
20.0 kV
Sig
SE
Spot
3.5
WD
11.0 mm
Mag
1361x
Pressure

20.0µm