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: protective layer covering the surface of leaves and young stems. Composed of cutin and waxes. Mostly protect against desiccation.
Cutin and waxes are hydrophobic (water "impermeable or repellent")
Aqueous solutions “bead up”
Citrus leaf
A. Epicuticular Wax Crystals

Cutin
Intracuticular Wax
Polysaccharides

Epidermal Cell

Cuticle Proper
Cuticular Layer
Polysaccharide Cell Wall

B. Epidermal Cell
Cuticle

C. Cuticle Proper
Cuticular Layer
Polysaccharide Cell Wall
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

Photosynthetic cells

Phloem
Laser light to overcome the cuticular barrier

Light Amplification by Stimulated Emission of Radiation".
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

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

(PDS = phytoene desaturase)