The Use of Laser Light to Enhance Penetration of Antimicrobials into Citrus Leaves

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Penetration of externally applied agricultural solutions into citrus trees is hindered by the natural protective layers covering plant surfaces. At the present time, the battle against or Huanglongbing (or citrus greening) focuses on the possibility of antimicrobial substances. A possible solution to overcome the barrier imposed by the waxy cuticle is the use of laser technology. Laser light is capable of “melting” away microscopic “pin holes” on plant surfaces allowing for the penetration of applied substances. Using fluorescent molecules such as deoxyglucose and carboxyfluorescin, our investigation demonstrated the effectiveness of laser light technology in enhancing penetration of foliarily applied aqueous substances. We observed the movement of fluorescent deoxyglucose and carboxyfluorescin down the leaf in petiole within 4 hours and down the stem after 24 hours. The data presented in this communication demonstrated the usefulness of laser light in enhancing penetration of test substances into the leaf and movement through the tree.

Materials and Methods

Plant Material. Two-year-old ‘Valencia’ orange (Citrus sinensis L. Osbeck) trees on Swingle (Citrus paradisi x Pisonirus trifoliata) rootstock were kept in a greenhouse with natural lighting. When needed for experimental treatment, trees were brought to the laboratory.

Laser Treatment. Leaves were lasered using a low-energy carbon dioxide laser etching machine (model XY Mark-10; GPD Technologies, Peachtree City, Georgia) located at the University of Florida’s Citrus Research and Education Center in Lake Alfred, FL. The lasered area consisted of two successive rectangles (25 rows of alternating 13 and 12 depressions each for a total of 313 depressions per rectangle; Fig. 1). Each leaf was lasered once on each side of the mid-vein. Immediately after lasering, 10 µL of test solutions were manually smeared on each lasered area. After the solution dried off initially, water mist was applied and each leaf was covered with a zip-lock bag containing a piece of small, wet gauze.

To overcome the obstacles imposed by the cuticle in order to increase penetration of externally supplied substances, we propose the use of laser light. Laser light technology involves the use of low level light energy to disperse the cuticle creating microscopic and superficial indentations of approximately 250 µm (Etxeberria et al., 2009) that would allow penetration of applied substances. This technology has been approved by FDA for labeling fruits and vegetables (Patent #5,660,747 and Patent #5,897,797; FDA Docket # FDA-2007-F-0390). To test for the possible use in antimicrobial treatments, we used several phloem-mobile fluorescent substances, and demonstrated that laser light facilitates penetration, uptake and transport of substances into the phloem and throughout the tree.

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Table 1. List of fluorescent substances used to determine penetration and movement into lasered citrus leaves. Substances were individually applied onto laserd and control un-lasered citrus for 4 and 24 h. Fluorescence was followed using a Carl Zeiss Axion Scope A-1.

<table>
<thead>
<tr>
<th>Name</th>
<th>MW</th>
<th>Concentration</th>
<th>Ex/Em (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-NBDG</td>
<td>342.26</td>
<td>30 mM</td>
<td>465/540</td>
</tr>
<tr>
<td>(6) Carboxyfluorescein diacetate</td>
<td>460.39</td>
<td>4.85 mM</td>
<td>492/517</td>
</tr>
<tr>
<td>Dextran-Texas Red</td>
<td>3000</td>
<td>3.5 mM</td>
<td>595/615</td>
</tr>
<tr>
<td>Alexa-488</td>
<td>884.91</td>
<td>4.5 mM</td>
<td>495/519</td>
</tr>
<tr>
<td>Q-Dots 565, 15 nm</td>
<td>110,000</td>
<td>2 µM</td>
<td>405-525/565</td>
</tr>
</tbody>
</table>

**Microscopy.** Microscopic observations were made using a Carl Zeiss Axion Scope A-1 equipped with a Canon EOS Rebel T3i camera and a Carl Zeiss fluorescent stereoscope.

**Results**

**Lasered area.** For our study, we selected a dot matrix pattern that would maximize the amount leaf area exposed to applied substances (Fig. 1). Each pin-hole depression across the cuticle had an average diameter of 190 µm. With a pattern consisting of 313 pin-holes per rectangle, the exposed area totaled 17.78 mm² per leaf.

**Experimental solutions.** Table 1 presents all the solutions tested, their physical characteristics and concentrations applied. 2-NBDG rapidly penetrated mesophyll cells as depicted by the fluorescence emanating from the pin-hole depressions and surrounding area in Fig. 2A. By comparison, lasered leaves with no solutions added displayed cuticular fluorescence, but the central area remained opaque and there was not fluorescence in the interlasered spaces (Fig. 2B). Control un-lasered leaves supplied with 2-NBDG on the adaxial cuticle and rinsed before observation showed no fluorescence (dark field micrographs, not shown).

When treated leaves were viewed under fluorescence microscopy at low magnification 2 h after application, a strong fluorescence signal delineated minor and main veins (Fig. 3A). These results demonstrated that 2-NBDG was mobilized down from the treated area likely by phloem cells. A cross section of the petiole 4 h after treatment showed strong fluorescent signal within the vascular tissue (Fig. 3B).

In a separate experiment, a total of 5 treated leaves were treated and left on the tree for 24 h before observation. In these trees, portions of the bark over 30 cm below treated leaves showed fluorescence (Fig. 4A) compared to control trees (Fig. 4B) confirming the down flow of 2-NBDG through the phloem tissue in the direction of the bulk flow.

Carboxyfluorescin-diacetate, a cell permeant fluorescent compound (Aeschbacher et al., 1986), gave similar results to 2-NBDG. In contrast, artificial fluorescent “Q-dots” (15 nm CdSe/ZnS crystals), Dextran-TR (mw 3,000) and Alexa-488 (membrane impermeable fluorescent probe) did not move beyond the area of application.

**Discussion**

At the moment, there are no effective weapons in the fight against HLB. Because of the bacterial nature of the causing agent,
Without a system to help penetrate the plant body, the foliar use of antimicrobials alone can be futile. The data presented in this communication demonstrated the usefulness of laser light in enhancing penetration of test substances into the leaf and movement through the tree.

**Literature Cited**


