Field studies of a novel virus-like agent with commercial citrus varieties in California

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Methodology

I. Field trial establishment at UCR

• 12 commercially popular rootstock/scion (R/S) combinations (Table 1).
• 15 trees per R/S combination.
• 6/15 trees per R/S combination graft-inoculated (GI) with CYVaV. Monitored for CYVaV symptoms.

Table 1. Rootstock/Scion (R/S) combinations included in the UCR field trials.

<table>
<thead>
<tr>
<th>No.</th>
<th>Citrus variety name (Scion/Rootstock)</th>
<th>Abbreviated Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Limoneira 8A Lisbon Lemon/Rubidoux Trifoliate</td>
<td>LAV/RT</td>
</tr>
<tr>
<td>2</td>
<td>Limoneira 8A Lisbon Lemon/Mandarinha</td>
<td>LAV/Mn</td>
</tr>
<tr>
<td>3</td>
<td>Parent Washington Naval/Carrizo Citrange</td>
<td>PNC/Cc</td>
</tr>
<tr>
<td>4</td>
<td>Biggold Giulietta (Shiranu) Mandarin/Carrizo 5R</td>
<td>BMGC</td>
</tr>
<tr>
<td>5</td>
<td>Limoneira 8A Lisbon Lemon/West Indian Citrange</td>
<td>LAV/WIC</td>
</tr>
<tr>
<td>6</td>
<td>Cara Cara Navar/Carrizo Citrange</td>
<td>CCC/Cc</td>
</tr>
<tr>
<td>7</td>
<td>Biggold Giulietta (Shiranu) Mandarin/Rubidoux Trifoliate</td>
<td>BMG/RT</td>
</tr>
<tr>
<td>8</td>
<td>Cara Cara Navar/Rubidoux Trifoliate</td>
<td>CCC/RT</td>
</tr>
<tr>
<td>9</td>
<td>Tangi Mandarin/Rubidoux Trifoliate</td>
<td>TM/RT</td>
</tr>
<tr>
<td>10</td>
<td>Mito Wake Safari/Carrizo Citrange</td>
<td>MWC/Cc</td>
</tr>
<tr>
<td>11</td>
<td>Tangi Mandarin/Carrizo Citrange</td>
<td>TM/Cc</td>
</tr>
<tr>
<td>12</td>
<td>Parent Washington Naval/Opal Trifoliate</td>
<td>PNC/RT</td>
</tr>
</tbody>
</table>

II. CYVaV screening by RT-qPCR

• CYVaV infection status evaluated at 6m, 12m, and 24m timepoints post graft-inoculations.
• Leaf/budwood samples collected from each tree, high-throughput DNA extraction, followed by RT-qPCRs targeting CYVaV, citrus vein enation virus (CVEV), and citrus tristeza virus (CTV).

III. CYVaV pollen transmission assay

Spring 2022: Pollen collected from CYVaV-infected and untreated trees. Tested for CYVaV by RT-qPCR.

In-field hand pollinations (n=229): Limoneira 8A Lisbon Lemon, Biggold Giulietta (Shiranu) Mandarin.

Successful fruit set harvested and tested during winter 2023.

IV. Fruit harvest and qualitative analyses

Winter 2023 – first harvest of field trial.

• Fruit qualitative analyses performed at Lindcove Research and Extension Center (LREC) Fruit Quality Lab.

Results

I. CYVaV screening by RT-qPCR

Figure 1. Two-year (November 2022) post CYVaV graft-inoculation screening of the UCR field trial. Left: RT-qPCR testing summary. 17/71 (~24%) GI trees tested CYVaV-positive, and all 15 trees tested GIVaV-negative. Right: CYVaV foliar symptoms observed in infected Limoneira 8A Lisbon Lemon (top right) and Tango Mandarin (bottom right) trees.

II. CYVaV pollen transmission assay. CYVaV was detected by RT-qPCR in pollen from CYVaV-infected GI trees and not in pollen from untreated trees. With this pollen, 229 flowers were hand-pollinated in the field trial in 2022 (Figs. 2A and 2B). Treatments included:

a. CYVaV-positive pollen on untreated tree flowers.
b. CYVaV-negative pollen on CYVaV-positive tree flowers.
c. CYVaV-negative pollen on untreated tree flowers.

26 hand-pollinations resulted in successful fruit set by winter 2023, when they were harvested, collected, (Fig. 2C), and tested for CYVaV. All fruits from treatments ‘a’ and ‘b’ tested CYVaV-negative.

Fruits from treatment ‘b’ tested CYVaV-positive.

III. Fruit harvest and qualitative analyses. In February 2023, all field trial trees were manually harvested. Overall, there were no significant differences in fruit quantity per tree between GI and untreated trees across all R/S combinations (Fig. 3). Fruit qualitative analyses were performed with 24 samples of 10 fruits per treatment (2 samples per treatment; GI and untreated trees). Parameters studied included: rind colorimetry, firmness, rind texture and packline height/width, rind thickness, juice volume (Fig. 4B), juice weight (Fig. 4C), and juice acid content. Overall, fruits collected from GI and untreated trees across the 12 R/S combinations were morphologically similar (Fig. 4A) and did not have any significant qualitative differences.

Conclusions

• A field trial was established at UCR with 12 commercially popular citrus R/S combinations. CYVaV graft-inoculations performed, and trees being monitored for infection and symptom development.
• Limoneira 8A Lisbon Lemon and Tango Mandarin currently the most compatible citrus varieties for CYVaV colonization.
• CYVaV detected in pollen collected from infected field trees and used for hand-pollinations. Based on our results, there was no transmission of CYVaV from infected pollen to untreated flowers (and corresponding tree).
• Harvested fruit showed no significant differences in yield or qualitative parameters between GI and untreated trees for all 12 R/S treatments.

References and Funding

• Moreno et al., 2008. Molecular plant pathology, 9(2), pp.251-268.
• Munir et al., 2018. Microbial Ecology, 76, pp.192-204.
• Kwon et al., 2021. Frontiers in microbiology, 12, p.681930.

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