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Permalink
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Journal
International Organization of Citrus Virologists Conference Proceedings (1957-2010), 14(14)

ISSN
2313-5123

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Publication Date
2000

DOI
10.5070/C534b6w7fn

Peer reviewed
Low Incidence of Huanglongbing Fruit Symptoms in Valencia Sweet Orange Trees in the Presence of a Population of Citrus Tristeza Virus

S. P. van Vuuren, J. B. van der Vyver, M. Luttig, and J. V. da Graça

ABSTRACT. Huanglongbing (causal agent is “Candidatus Liberibacter africanus”) (HLB) remains a limiting factor for citrus production in the cooler areas of Southern Africa notwithstanding present control measures. These include the establishment of new orchards with certified plant material, chemical control of the insect vector, Trioza erytreae, and the removal of infected plant material. Delta Valencia on Yuma citrange rootstock was planted in 1985 with GXI (greening cross-protecting isolate, formerly citrus dwarfing isolate 4 (CD 4)) as one of the treatments for dwarfing. HLB was first observed in 1988 and by 1990 fruit symptoms had increased to 100% in some trees which forced the termination of the dwarfing trial. Infected branches were removed by pruning and HLB continued to be monitored in 1991, 1993, 1996 and 1998 in the control trees and those with GXI. The percentage HLB fruit symptoms remained at a low level in the trees with GXI. Several attempts were made to identify the agent(s) that is present in GXI. Biological indexing was employed for citrus viroids, citrus tristeza, citrus psorosis, citrus impetratura and citrus tatter leaf. Only citrus tristeza virus (CTV) was found to be present in this isolate. Mass and single aphids were used to transfer the CTV component from GXI. The mass-aphid as well as the single-aphid sub-isolates reacted differently on Mexican lime, suggesting different strains within the GXI isolate. Differentiation between sub-isolates was also shown by amplifying the coat protein gene by the reverse transcriptase polymerase chain reaction (RT-PCR) followed by restriction fragment length polymorphism (RFLP) and single-strand conformational polymorphism (SSCP) techniques.
Glasshouse work was done in an attempt to identify the agent(s) that is present in GXI. Biological indexing was employed for citrus viroids, citrus tristeza, citrus psorosis, citrus impetratura and citrus tatter leaf (10). Except for citrus tristeza virus (CTV), none of the other agents was found to be present in this isolate.

Mass and single aphids were used to transfer the CTV component from GXI. The mass-aphid as well as the single-aphid sub-isolates reacted differently on Mexican lime and grapefruit, suggesting different strains within the GXI isolate (Table 2). Differentiation between sub-isolates was also shown by amplifying the coat protein gene by the reverse transcriptase polymerase chain reaction (RT-PCR) (9) followed by restriction fragment length polymorphism (RFLP) (6) and single-strand conformational polymorphism (SSCP) tech-

<table>
<thead>
<tr>
<th>Year</th>
<th>Control</th>
<th>GXI(CD4)</th>
<th>CD8</th>
<th>CD9</th>
<th>CD10</th>
</tr>
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<tbody>
<tr>
<td>1990</td>
<td>4'</td>
<td>0</td>
<td>35</td>
<td>29</td>
<td>14</td>
</tr>
<tr>
<td>1991</td>
<td>19</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1993</td>
<td>27</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1996</td>
<td>46</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td>26</td>
<td>11</td>
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</table>

*Infected branches were removed each year after HLB assessment.
Trees removed due to high HLB infection.

<table>
<thead>
<tr>
<th>Isolate or subisolate</th>
<th>Seedling growth (mm)</th>
<th>Molecular characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mexican lime&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Marsh grapefruit&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>GXI-original</td>
<td>385&lt;sup&gt;b&lt;/sup&gt; bc</td>
<td>105&lt;sup&gt;c&lt;/sup&gt; bc</td>
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<tr>
<td>GXI-CTV1&lt;sup&gt;x&lt;/sup&gt;</td>
<td>185&lt;sup&gt;a&lt;/sup&gt;</td>
<td>203&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>GXI-CTV2&lt;sup&gt;v&lt;/sup&gt;</td>
<td>403&lt;sup&gt;c&lt;/sup&gt;</td>
<td>196&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>GXI-CTV3&lt;sup&gt;v&lt;/sup&gt;</td>
<td>120&lt;sup&gt;abc&lt;/sup&gt; ab</td>
<td>155&lt;sup&gt;c&lt;/sup&gt; abc</td>
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<tr>
<td>GXI-CTV4&lt;sup&gt;v&lt;/sup&gt;</td>
<td>265&lt;sup&gt;a&lt;/sup&gt; a</td>
<td>97&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>GXI-CTV5&lt;sup&gt;v&lt;/sup&gt;</td>
<td>365&lt;sup&gt;a&lt;/sup&gt; a</td>
<td>209&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>GXI-CTV6&lt;sup&gt;v&lt;/sup&gt;</td>
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<td>97&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>194&lt;sup&gt;abc&lt;/sup&gt;</td>
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<tr>
<td>GXI-CTV9&lt;sup&gt;v&lt;/sup&gt;</td>
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<td>173&lt;sup&gt;c&lt;/sup&gt; abc</td>
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<td>GXI-CTV10&lt;sup&gt;v&lt;/sup&gt;</td>
<td>340&lt;sup&gt;a&lt;/sup&gt; a</td>
<td>126&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Figures in each column which are followed by the same letter do not differ significantly at the 5% level (LSD).
<sup>b</sup>Similar RFLP (Fig. 1) and SSCP (Fig. 2) bands for each isolate are indicated by the same number.
<sup>c</sup>Positive seedling yellows reaction.
<sup>d</sup>Mass aphid transfers.
<sup>e</sup>Single aphid transfers.
Biological differences were detected among sub-isolates and between the original isolate and sub-isolates transmitted by mass aphids. Molecular characterization techniques (12) revealed even greater variation among the sub-isolates. It appears that at least five groups exist among the single aphid transmissions (A) and single aphid transmissions (B) of the GXI-CTV coat protein gene. A. Mass aphid transmissions are in lane 1: pUC19 digested with HindII; lane 2: GXI-CTV1; lane 3: GXI-CTV2; lane 4: GXI-CTV3; lane 5: GXI-CTV4; lane 6: GXI original. B. Single aphid transmissions are in lane 1: pUC19 (HindII) marker; lane 2: GXI original; lane 3: GXI-CTV5; lane 4: GXI-CTV6; lane 5: GXI-CTV7; lane 6: GXI-CTV8; lane 7: GXI-CTV9; lane 8: GXI-CTV10. Marker bands ranging in size from 1419, 517, 396, 214, 75 and 65 base pairs. Restriction digests were separated on 4% agarose gels.

Electrophoresis under non-denaturing conditions was performed at room temperature at 300V for 2h in 8% acrylamide gels with 5% glycerol. Gels were stained with silver nitrate.
sub-isolates and their abilities to protect against HLB, singly or in combination, will be investigated.

Apart from cross protection by mild virus isolates of the same virus, several cases have been reported where protection was afforded by non-related viruses as well as non-related pathogens. Koizumi and Sasaki (8) reported cross protection against CTV, a closterovirus (1), by citrus vein enation virus, a luteovirus (4). Apple mosaic virus in plum trees was suppressed by the presence of plum dwarf virus (5). In the first cross protection studies to control tomato mosaic virus, a mild strain of tobacco mosaic virus was used (7). The inoculation of citrus viroids in sweet orange induced greater resistance to Phytophthora infection (11).

The presence of a virus or biological control agent may protect against fungal infection (13, 15) or may promote infection (14). Chen et al. (3) rarely found the HLB bacterium and CTV in the same plant cell. The reasons for these phenomena are unknown but may be the result of chemical changes in the plant cell, the production of protective substances on the surface of the host or the production of substances in the plant tissue which prohibit entrance or multiplication of the pathogen (2, 11, 15).

Several formal and commercial trials have been initiated for further evaluations of GXI as a protector against HLB as well as against severe CTV. Investigations into the characteristics of the sub-isolates are also continuing.

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