Citrus and Subtropical Fruit Research Institute, Nelspruit, South Africa

The Effect of Gentisic Acid on Activity of Peroxidase from Citrus aurantifolia Swing

L. J. van Lelyveld and S. P. van Vuuren

Author's address: Citrus and Subtropical Fruit Research Institute, Private Bag X11208, Nelspruit 1200 (South Africa).

Received July 21, 1986; accepted July 4, 1987

Abstract

The present study indicates that the peroxidase activity can be partly inhibited by gentisic acid and possibly thereby interferes with the mechanism of resistance to greening diseases of citrus.

Zusammenfassung

Der Einfluß von Gentisinsäure auf die Aktivität von Peroxidase aus Citrus aurantifolia Swing

Gezeigt wurde, daß Peroxidaseaktivität durch Gentisinsäure zum Teil gehemmt werden kann und daher möglicherweise störend in den Resistenzmechanismus gegenüber Greening Disease in Zitrusgewächsen eingreift.

The presence in citrus of the organism of greening disease was identified by chromatographical analysis (Schwarz 1965). A specific violet fluorescence was present in the albedo (mesocarp of the fruit) and bark of greening-affected material whereas this fluorescence was absent in apparently healthy material. A purified preparation of this substance was identified by Feldman (1969b) as a monoglucoside of gentisic acid (gentisoyl-B-D-glucose). Recently an HPLC method (Burger et al. 1984) confirmed the higher levels of gentisic acid in greening diseased citrus material than in healthy material but could not account for these results.

It was shown earlier (van Lelyveld and van Vuuren 1988), that the peroxidase (PO) enzyme activity in leaves of more tolerant citrus species and cultivars is
significantly higher than in leaves of more susceptible species or cultivars. The purpose of the present study was to establish if there is any link between gentisic acid concentration and PO activity.

**Materials and Methods**

Peroxidase was extracted from apparently healthy mature (2 to 3 months old) leaves of West Indian lime (*Citrus aurantifolia* (Christm.) Swing as previously described (VAN LEYVELD and VAN VUUREN 1988). After picking, the leaves were placed in a refrigerator (0 to 4°C) prior to extraction. Peroxidase activity was assayed in the presence and absence of gentisic acid using the method previously described (VAN LEYVELD and VAN VUUREN 1988). Assays were replicated four times.

10 mM gentisic acid standard (Merck, 2,5-dihydroxybenzoic acid) was made up and added to the incubating mixture at various volumes, that is: 0 = control, 5 μl = 7.706 μg; 10 μl = 15.413 μg; 20 μl = 30.826 μg; 30 μl = 46.239 μg; 40 μl = 61.652 μg.

Protein content of crude enzyme extract was determined by the method of LOWRY et al. (1951) as modified by LEGGETT-BAILEY (1962). Bovine serum albumin was used as standard.

**Results**

The peroxidase enzyme activity from apparently healthy West Indian lime leaves, was assayed in the absence and presence of various levels of gentisic acid and the results compared in Table 1.

**Table 1**

<table>
<thead>
<tr>
<th>Gentisic acid concentration</th>
<th>PO activity units*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.320</td>
</tr>
<tr>
<td>5 μl (7.706 μg)</td>
<td>6.620</td>
</tr>
<tr>
<td>10 μl (15.413 μg)</td>
<td>7.188</td>
</tr>
<tr>
<td>20 μl (30.826 μg)</td>
<td>6.541</td>
</tr>
<tr>
<td>30 μl (46.239 μg)</td>
<td>4.275</td>
</tr>
<tr>
<td>40 μl (61.652 μg)</td>
<td>3.800</td>
</tr>
<tr>
<td>I.S.D. P = 0.05</td>
<td>0.72</td>
</tr>
<tr>
<td>P = 0.01</td>
<td>1.00</td>
</tr>
<tr>
<td>CV = 8.074 %</td>
<td></td>
</tr>
</tbody>
</table>

All treatments replicated four times.

* Number of absorbance units min⁻¹ mg protein⁻¹.

Gentisic acid has a highly significant (P < 0.01) depressing effect on PO activity at 30 μl and 40 μl levels compared with the lower levels.

**Discussion**

Chemical changes in citrus after disease infection was demonstrated by FELDMAN (1969a). Higher levels of gentisic acid (hydrolized aglicone of gentisoyl-B-D-glucose) were present in greening positive than in apparently healthy
material (Burger et al. 1984). Genticis acid levels in different citrus species and cultivars are not known. Feldman and Hanks (1965) made a crude assessment of genticis acid levels in four citrus species and found higher levels in sweet orange (Citrus sinensis) and lemon (Citrus limon), which are susceptible to greening disease, than in grapefruit (Citrus paradisi) and 'Milam' (hybrid) which are more tolerant to the disease. According to the present results, PO activity would be lower in the first two species than in the latter. This is supported by the findings of Van Lelyveld and Van Vuuren (1988) which showed a higher PO activity in grapefruit compared with susceptible lemon and sweet orange.

The metabolism and reason for the increase in genticis acid after the appearance of greening symptoms is not clear at present and will have to be investigated. A question which arises is whether higher levels of genticis acid are the cause of disease symptoms or the result. Injection of genticis acid into healthy citrus did not induce greening symptoms (Van Vuuren, unpubl. data, De Lange, pers. comm.). From the present results it would appear that high levels of genticis acid in susceptible cultivars, may be the cause of lower PO activity, and thereby contribute to reduced resistance. By this implication it appears that genticis acid may have some effect on susceptibility through the PO enzyme activity.

To be able to understand the connection between PO activity and genticis acid concentration an investigation is now being made of the concentration of genticis acid in the albedo of the fruit and leaves as compared with the susceptibility of the different species and cultivars.

Literature


