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Techniques for the Selection of Mild Isolates of Citrus Tristeza Virus

L. J. Marais and J. M. Kotzé

ABSTRACT. Pyrolysis-gas-liquid chromatography, peroxidase activity and oxygen uptake were used to differentiate between three different isolates of citrus tristeza virus. A positive correlation was found between the biological activity of the isolates and pyrolytic products, peroxidase activity and oxygen uptake.

Citrus tristeza virus (CTV) has been associated with serious losses of susceptible citrus, especially where the vector (*Toxoptera citricida* Kirk.) is endemic (2, 8). In South Africa CTV is becoming a cause for great concern in grapefruit orchards (6). In the past, control measures have been based on quarantine and the use of tolerant rootstocks (2). These methods have been ineffective for certain citrus types, e.g. grapefruit, with the result that additional control measures have had to be developed. Cross protection is being used with success in Brazil (2), and is the only method of control which appears feasible. The success of any cross protection program is based chiefly on the selection of suitable mild strains of the virus. This is a very time consuming and difficult task as CTV strains have been found to be host specific and exhibit geographical specificity. When screening is carried out for mild strains, a large number of mild strains have to be collected from different geographical areas and different scion-stock combinations and evaluated by tissue and vector transmission. The number of suitable strains eventually obtained this way is low (2, 9). Virus infection induces certain biochemical changes in plants, e.g. changes have been observed in respiratory and oxidative enzyme activity, free amino acids and total nitrogen and sugars (3). These changes have also been cor-

related with the biological activity of citrus exocortis viroid (CEV) isolates (4), while pyrolysis-gas-liquid chromatography (Py-GLC) has been used to differentiate between strains of CTV (7). The latter results were based on metabolic changes which took place in infected tissue.

This paper reports on the application of some metabolic changes in Mexican lime plants infected with three different isolates of CTV, and how, together with Py-GLC they may be useful in selecting mild strains for cross protection purposes.

MATERIALS AND METHODS

CTV isolates. Three isolates of CTV were obtained from three different geographical areas and designated as mild, moderate and severe on the basis of severity of symptoms in grapefruit seedlings and performance of orchard grown grapefruit trees (2, 9).

Indicator plants. Healthy, uniform 12-month-old Mexican lime seedlings grown from the same seed source, were inoculated at three different sites 3 cm apart on the stem with patch grafts of infected bark tissue. Four weeks later discs 1 cm in diameter were cut with a Bausch and Lomb leaf punch from the third leaf from the apex of the inoculated and healthy control seedlings. This was repeated at 14, 28 and 42 days thereafter. The composite sample of leaf discs was used in the studies on

oxygen uptake and enzyme activity.

Pyrolysis-gas-liquid chromatography (Py-GLC). A composite sample was prepared by stripping the bark off 20 similarly inoculated plants, freeze drying it, and grinding the bark into a fine powder in a Wiley mill (1). All experiments were repeated four times with a different composite sample. Py-GLC was carried out according to the method of Marais and Kotzé (7) at the termination of the experiment, using a glass column.

Oxygen uptake. The oxygen uptake was measured according to the method described by Kapur, Gumpf and Weathers (4).

Peroxidase activity. (P. A.) P. A. was determined by the method described by Loebenstein and Linsey (5).

RESULTS

Py-GLC. The pyrograms produced were unique to each isolate (figure 1). Comparison of the pyrograms shows qualitative similarities of the traces and numerous quantitative differences. Separation of the isolates using this technique was highly significant ($P = 0.01$) and the two dimensional plot (figure 2) indicates that

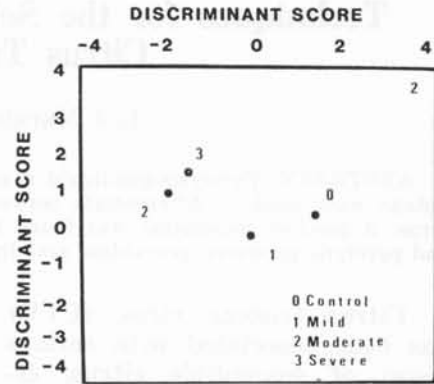


Fig. 2. Two dimensional plot of 24 samples of Mexican lime bark tissue infected with different isolates of CTV subjected to Py-GLC (● indicates a group centroid).

the moderate and severe isolates are somewhat similar regarding biological activity, but that the mild isolate differs considerably.

Oxygen uptake. The rate of oxygen uptake by leaf discs infected with the mild and moderate isolates of CTV differed from the control but not from one another 4 weeks after inoculation (figure 3) whereas leaf discs infected with severe isolate showed a greater increase after this period. During the 14 days that followed, the rate of oxygen uptake increased linearly. At the end of this period the rates

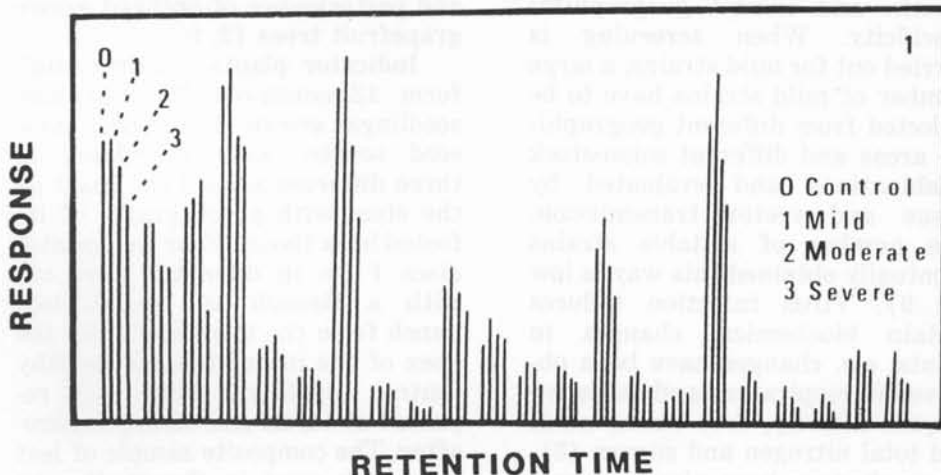


Fig. 1. Comparison of pyrograms of Mexican lime bark tissue infected with different isolates of CTV.

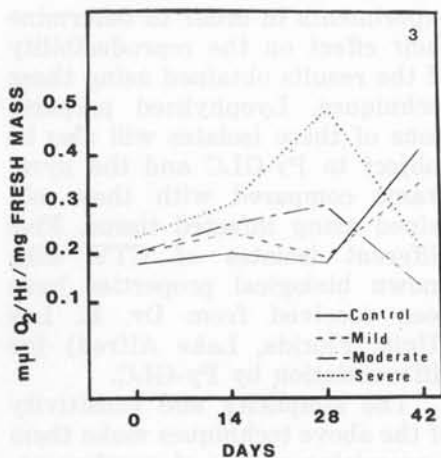


Fig. 3. Effect of three isolates of CTV on the oxygen uptake of Mexican lime leaf tissue.

of oxygen uptake of the leaf discs infected with the different isolates diverged from one another, that of the mild isolate following a rate similar to that of the uninfected control, while the rate of oxygen uptake of the leaf discs infected with the moderate and severe isolates continued to increase. After 28 days, the rate of oxygen uptake of the leaf discs infected with the severe isolate was significantly higher ($P = 0.01$) than that of the tissue infected with the moderate and mild isolates and the rate of oxygen uptake of the uninfected controls was lowest. The rate of oxygen uptake of the tissue infected with the moderate and severe isolates reached a maximum at 28 days after the initiation of the experiment, thereafter it declined rapidly, falling significantly ($P = 0.05$) below that of the leaf discs infected with the mild isolate and the uninfected controls. At the termination of the experiment the rate of oxygen uptake of the uninfected controls was highest followed by the tissue infected with the mild isolate. The pattern of respiration followed by the plants infected with the mild isolate was similar to that of the healthy plants in that the rate of

oxygen uptake was not affected to any great extent over a period of 42 days.

Enzyme activity. Throughout the experiment PA showed a positive correlation with virulence of the isolates (figure 4). PA in plants infected by the severe isolate was significantly higher ($P = 0.01$) than all other treatments. PA started decreasing in all plants from 28 days after the initiation of the experiment, this pattern continued until the termination of the experiment. The PA in the plants infected by the severe isolate remained significantly higher than in all other plants throughout the experiment.

DISCUSSION AND CONCLUSIONS

Py-GLC is a relatively new technique in the field of plant virology and its first application was used to diagnose fungal and viral diseases in plants (10). Its application in the differentiation of virus strains in infected plant material was demonstrated for the first time by Marais and Kotzé in 1979 who found the reproducibility of the pyrograms obtained to be very high. The results obtained using Py-GLC, PA and oxygen uptake support the findings of Kapur, Gumpf and Weathers (4) that

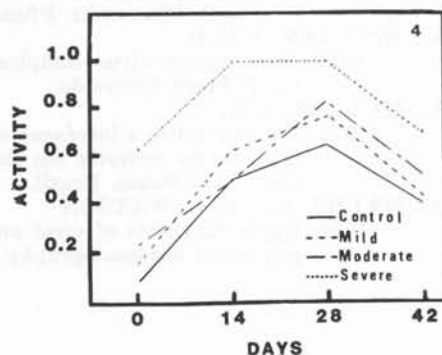


Fig. 4. Effect of three isolates of CTV on the PA of Mexican lime leaf tissue. (activity measured as sec/g fresh mass).

metabolic changes in tissue infected with viral diseases can be used to differentiate between different isolates of the micro-organism when refined and sensitive techniques are used. The extent of metabolic changes which took place were positively correlated with the biological activity of the isolates.

The sensitivity of Py-GLC can be enhanced by selecting the right type of column and column packing material. Oxygen uptake and PA should be measured under conditions optimum for virus infection and multiplication. Variables such as temperature, age of plant, citrus type (scionic combinations and seedlings) and a larger number of isolates will be included in further

experiments in order to determine their effect on the reproducibility of the results obtained using these techniques. Lyophilized preparations of these isolates will also be subject to Py-GLC and the pyrograms compared with those obtained using infected tissue. Five different isolates of CTV with known biological properties have been received from Dr. R. Lee (Univ. Florida, Lake Alfred) for differentiation by Py-GLC.

The simplicity and sensitivity of the above techniques make them a promising means of supplementing standard methods used in the selection of mild isolates of CTV for cross protection purposes.

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