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Serological Diagnosis of *Citrus psorosis virus* and *Citrus tristeza virus* Using Flower Parts

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ABSTRACT. *Citrus psorosis virus* (CPsV) and *Citrus tristeza virus* (CTV) isolates from citrus species of different origin were tested at flowering time by DTBIA and ELISA, using ovaries, or petioles and leaves. Reagents were commercial antibodies to CPsV, and commercial antibodies and kits for CTV. Compared to petioles or leaves, the use of flower parts (ovary and pistil) gave better reactions in DTBIA and ELISA. Ovary prints from CPsV- and CTV-infected plants stained uniformly and intensely, whereas no reaction was observed with prints from comparable healthy tissue. Ovaries could be processed fresh or after up to 1 yr storage at -20°C. Printed membranes could be stained immediately or processed after 1 yr using the same types of buffer without apparent loss of sensitivity.

Index words. *Citrus psorosis virus*, *Citrus tristeza virus*, detection, DTBIA, ELISA, monoclonal antibodies, ovary.

Tristeza and psorosis are severe diseases of citrus with a worldwide distribution (11, 12). As alternatives or additional to biological indexing, simple and rapid procedures such as ELISA and DTBIA are now being utilized (1, 2, 3, 5, 6, 7, 8, 9, 13) to detect the causal viruses, and commercial kits make it possible to test a large number of samples with high sensitivity and specificity.

The disease agents, *Citrus tristeza virus* (CTV) and *Citrus psorosis virus* (CPsV) have differing distributions in the host plant, so different tissues are used for diagnostic assays, i.e. young shoots and leaf petioles for CTV (3) and mature leaves for CPsV (5). Sampling should be done in Autumn and Spring when the temperature is 18-24°C and the virus concentration is highest. Recently, flower parts have been successfully used for detection of both viruses using ELISA and DTBIA (7, and unpublished information).

At flowering time, closed flowers and mature leaves were sampled from two separate collections of citrus genotypes of different origin, mainly Mediterranean, infected by CTV and CPsV respectively. The citrus types covered sweet oranges, mandarins, grapefruits, lemons, pummelo, kumquat and *Citrus excelsa*. These collections were

maintained in insect-proof screen-houses and have been biologically and serologically characterized (4, 5, 13, and unpublished information). Samples of 50 flowers and leaves were collected from each source, and subsamples were stored at 4°C, -20°C or -70°C. These were tested, respectively, at daily, weekly and monthly intervals.

For CTV, leaf petioles as well as flower parts (pistils and ovaries) were tested by ELISA and DTBIA. With CPsV, ELISA was used on pistils and mature leaves while DTBIA was only applied to the ovaries; this was because DTBIA proved not to give a consistent signal with CPsV-infected leaves (7) although with later and very recently reported experiments (9) it has been found useful also for young shoots and leaves. Similar tissues from the corresponding healthy species were used as controls.

ELISA assays were done with commercial monoclonal antibodies specific to CTV (Domaines Royales, UCP, Morocco) and to CPsV (Agritest-Italy; 10). Extracts were prepared by grinding different types of tissue (0.5 g of mature leaves or petioles and one pistil) in the extraction buffer (1:10 dilution). Each sample was assayed in two wells. Optical densities were measured at 405 nm

in a Titertek Multiscan photometer. The results were based on the mean absorbance values of the two sample wells, which were considered positive when three or more times higher than the healthy controls.

DTBIA was carried out using the Plant Print Diagnostics kit (Spain) for CTV; for CPsV the conjugated monoclonal antibodies of Agritest (Italy) were used, with 0.45 μm nitrocellulose membranes (BioRad). The blotted membranes were allowed to dry at room temperature before being analyzed. About 20 additional blotted membranes were also stored dry and tested monthly. After blocking with BSA, the alkaline phosphatase-conjugated antibodies were added. The membranes were then rinsed in washing buffer and developed using the substrate. The color reaction was stopped by washing with tap water and, after drying at room temperature, the membranes were examined with a 10 or 20 \times lens.

All samples from CTV- and CPsV-infected sources were positive using both serological techniques (data not shown). In ELISA, higher values were obtained, with CTV, from pistils than from petioles, and with CPsV, from pistils than from mature leaves.

With DTBIA, purple-stained areas appeared in the ovary prints of all CTV- and CPsV- infected sam-

ples whereas control blots were virtually unstained, confirming the ELISA results. With CTV, the localization and distribution of the stain was intense and homogenous in blots from ovaries (Fig. 1), whereas petiole imprints were less colored (not shown).

No differences were noted in the use of fresh or frozen flower parts for ELISA or DTBIA; however, leaves could not be frozen, and could be stored at 4°C for only a week. In contrast, blotted membranes could be kept for at least 1 yr before processing, as previously described (3, 8).

Our results indicate that CTV- and CPsV-infected flowers are better antigen sources than other tissues (leaf petioles and leaf blades) for both ELISA and DBTIA. Flowers can easily be collected in the field without damaging the trees, and can be stored for long periods without deterioration; moreover each virus can be consistently detected in a single ovary.

We conclude that CTV and CPsV can be rapidly and reliably detected by DTBIA using ovaries. For large scale surveys in the field, a few flowers can be collected from each tree for fresh printing and storage at -20°C. However, in most cases, only one flower is sufficient for successful blotting. Blotted membranes or frozen pieces can be readily processed throughout the year.

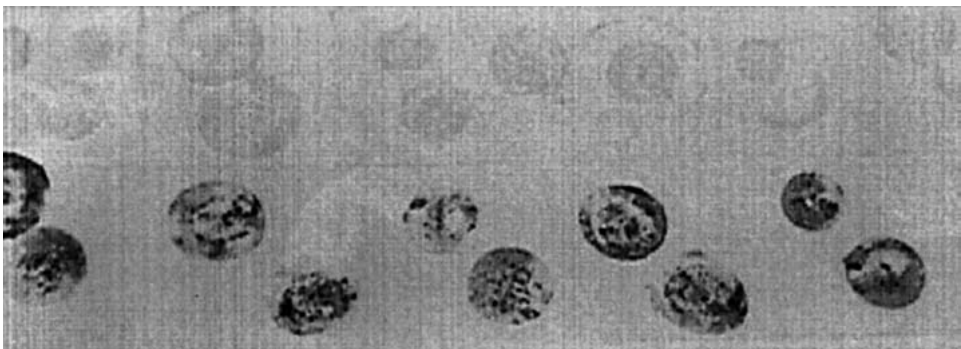


Fig. 1. Membrane printed with ovary sections of healthy plants (two upper rows), and *Citrus tristeza virus*-infected plants.

Although flowers gave best results, these are only available for brief periods. At other times CTV can be detected by DBTIA using leaf petioles, stem sections or fruits, whereas ELISA using mature leaves is needed for CPsV.

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