

with those based on symptomatology including leaf mottle.

DIFFERENTIATION OF CITRUS TRISTEZA VIRUS ISOLATES IN SPAIN

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Analysis of double stranded RNA (dsRNA) was used to differentiate Spanish isolates of citrus tristeza virus (CTV). DsRNA analysis of a collection of biologically characterized isolates representative of the different citrus areas in Spain, kept at the IVIA, showed up to 7 different profiles, one of them being shared by 75% of the isolates. Correlation between dsRNA profile and pathogenic characteristics was not observed.

In a field survey, 137 CTV-infected trees of different varieties were sampled from 53 orchards and analyzed for dsRNA content. Up to 16 dsRNA profiles differing by the number and/or position of subgenomic bands were detected. One of these profiles, identical to the most abundant in the IVIA collection of isolates, was found in more than 50% of the trees analyzed and in some citrus areas it was found in about 95% of the samples. The highest diversity of dsRNA profiles was found in the oldest citrus areas where CTV has been spreading for many years and mutation of the virus and repeated infections have likely occurred in many trees. In these areas several dsRNA profiles were often found in the same plot and, in one case, up to 6 different profiles were detected in a single plot. When some of these isolates were transmitted by *Aphis gossypii* the new isolate often showed a different dsRNA profile. Variations in the dsRNA profile and sometimes in the pathogenic characteristics were also observed when some CTV isolates were passed through certain hosts, or sub-cultured by graft-inoculation from a host recently infected by aphid or by graft-inoculation. In one case, a very severe strain was sub-cultured from a mild field isolate.

The results obtained indicate a great diversity of virus strains in the field and the presence of several strains in many CTV isolates. This might explain the difference in the rate of decline often observed between neighbour trees of the same age and scion/rootstock combination. The presence of severe strains hidden in mild isolates is an additional threat for the citrus industry.

INDEXING OF CITRUS VIROIDS

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Mandarin trees grown in the Mediterranean are vulnerable to viroid induced diseases. Scion cultivars are sensitive to cachexia-xyloporosis, and rootstocks (*Poncirus trifoliata* and citrange hybrids) are sensitive to exocortis. Availability of reliable indexing methods is critical to implement sanitation, quarantine and certification programs, and to identify field trees suspected of being infected with viroids.

Viroid indexing as routinely performed at IVIA involves:

a) Graft inoculation on the indicator Arizona citron (*Citrus medica* L.) 861-S1 grafted on Rough lemon rootstock (*Citrus jambhiri* Lush.); b) incubation of inoculated indicators at 28-32°C; c) observation on symptoms after 3 months; d) viroid analysis by sPAGE. The method combines the properties of biological and biochemical techniques, and permits the reliable detection of all the citrus viroids studied (including the cachexia-xyloporosis agents) in a single assay. The method is more sensitive and shorter than the conventional indexing. When the inoculated citrons are incubated under less than optimal conditions, similar levels of sensitivity can be consistently achieved using longer incubation periods.

Assays were performed to study the possibility of detecting viroids directly from field trees known to be infected with the complex viroid isolate (E-117). Bark yielded more viroid than young succulent stems and leaves, regardless of the age of the tissue. Extractions done during the Summer (July-August) gave better yields than those performed during Fall (November-December) and Spring (March-May). The sensitivity of sPAGE analysis on bark tissues collected during the Summer months, from clementine (*C. clementina* Hort. ex Tan.) and lemon (*C. limon* (L.) Burm. f.) grafted on citrange carrizo, was comparable to that achieved by analysis from inoculated citrons. Good detection levels were also achieved in 'Navelate' sweet orange (*C. sinensis* (L.) Osb.) grafted on *Poncirus trifoliata*, and seedling trees of Alemow (*C. macrophylla* Wester), Palestine sweet lime (*C. limettioides* Tan), tangelo Orlando (*C. paradisi* Macf. x *C. tangerina* Hort. ex Tan.) and Parson's Special mandarin. Unexpectedly difficulties were found in detecting viroids from 'Navel' and 'Navelina' trees growing on citrange carrizo rootstock. The CEVd usually found as high titers in the others species was virtually undetectable in most of the samples tested.