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Citrus Viroids: A re-emerging problem

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Introduction

Since the implementation of the Citrus Improvement Scheme (CIS) in 1984, pathogen-free propagation material has been supplied to citrus producers of southern Africa, which has been integral in establishing a healthy industry. However, isolated cases of viroid disease outbreaks were recently detected in commercial orchards. This is a troubling tendency and implies that there have been changes in propagation practices that are potentially detrimental to the health status of citrus orchards. Here we discuss two case studies where viroids were problematic in commercial orchards and the potential danger that these infections pose to adjacent orchards.

Viroid overview

Viroids are the smallest infectious agents of plants. They are un-encapsulated, single-stranded RNA molecules that induce a range of disease symptoms on citrus, as well as other hosts. The symptomology of a viroid infection is dependent on the host rootstock or scion. Viroids may be present as latent infections in tolerant hosts, which can serve as sources of inoculum from where it can be spread to sensitive hosts where it may cause disease. Citrus viroids (CVds) can only be transmitted mechanically via grafting with infected plant material or via contaminated tools, and are not seed- or insect-transmissible.

CVds identified thus far in South Africa include *Citrus bent leaf viroid* (CBLVd), *Hop stunt viroid* (HSVd) variants, *Citrus dwarfing viroid* (CDVd), *Citrus bark cracking viroid* (CBCVd) and *Citrus exocortis viroid* (CEVd). Not all of these viroids are noticeably pathogenic to citrus, but may induce effects such as stunting, either singly or in combination with other viroid species. Certain variants of HSVd (CVd IIb and CVd IIc) cause Cachexia disease, which affect mandarins and their hybrids (tangelos and tangors) and kumquats. This disease is characterized by discolouration, gumming and browning of phloem tissue and wood-pitting which results in unthrifty plants.

These HSVd variants also affect Rangpur lime, rough lemon, 'Palestine' sweet lime and 'Alemow' used as rootstocks by inducing fine-pitting reaction without discolouration, a disease originally referred to as Xyloporosis. Exocortis, the disease caused by CEVd, is characterized by bark scaling on sensitive rootstocks, such as trifoliate orange, some citranges and Rangpur lime. Stunting and reduced fruit yields are also associated with these viroid infections due to their detrimental effects on the trees. Additionally, trifoliate orange and some citrange rootstocks infected with CDVd manifested gum pockets and severe stunting was associated with the trifoliate rootstock in trials in South Africa.

Citrus Improvement Scheme

The main objective of the CIS is to supply the southern African citrus industry with true-to-type, pathogen-free propagation material. Subsequent to the establishment of the CIS and the elimination of viroids and other pathogens by means of shoot-tip grafting, there was a steady decrease in observed viroid infections as viroid-free orchards replaced older, infected blocks. However, despite the supply of viroid-free material to the industry, a number of disease outbreaks have occurred recently. The resurfacing of diseases associated with citrus viroid infections, reiterates the need for the use of clean propagation material supplied by the CIS and to determine the disease status of older orchards prior to top working to new cultivars.

Field studies

Many older orchards in southern Africa, mostly established prior to the implementation of the CIS, are contaminated by one or more citrus viroid. No detrimental symptoms are generally observed in these orchards, as they comprise viroid-tolerant scions and rootstocks. However, these asymptomatic infections remain sources of inoculum, which can be transmitted to sensitive hosts. With the practice of top working becom-

ing more common, an increase in viroid disease incidence has been observed. This occurrence is due to tolerant rootstocks that harbour viroid infections, being top worked with sensitive scions, such as the increasingly popular mandarin varieties. Alternatively, infected field-cut material was also used in some cases for propagation in nurseries or for top working.

In one incident, twig dieback and unthrifty plants were first noted in an orchard of a mandarin cultivar that was top worked to existing X639 rootstocks (Figure 1). Clear pitting and gumming were observed when removing bark windows on the main branches of the scion (Figure 2). These symptoms were typical for Cachexia disease. Samples were taken from these symptomatic trees as well as from adjacent orchard blocks to determine the extent of the probable infection. These samples were tested for viroids using molecular diagnostics and detection of a Cachexia-causing variant of HSVd (CVd Ilc) in the symptomatic mandarin trees confirmed the visual diagnosis. This result was surprising as this disease has not been a common occurrence in South African orchards for many years. Additionally, numerous other viroid strains were detected in both old and newer adjacent orchards, indicating contamination over time with various cultural practices where cutting tools were not properly sterilised and used between orchards.

The other case study was an incident where numerous stunted mandarin trees were scattered throughout a newly planted orchard on C-35 rootstocks (Figure 3). Investigation revealed that some of the mandarin propagation material was sourced from the CIS, but a proportion of the material was field cut from "healthy-looking" trees.

Leaves were sampled from stunted and normal trees in this orchard and tested using molecular diagnostics. CDVd was detected only in the stunted, and not in the normal sized trees. CDVd was therefore identified as the causal agent of the observed stunting, which is a known symptom of this viroid. This demonstrates, firstly, that the viroid



Figure 1. Twig dieback and poor tree condition of mandarin cultivar infected with a Cachexia causing viroid variant. **Figure 2.** Stem pits and gumming on a mandarin cultivar, typical of Cachexia disease.

infection on the field-source was previously masked by a tolerant rootstock and, secondly, that the recipient C-35 rootstock is sensitive for this viroid species.

Management of viroid infections

Unfortunately, there is no cure for viroids. As a standard practice and to prevent the spread of potential latent infections, growers should disinfect the blades of all pruning, hedging and harvesting tools with a 30% dilution of household bleach (3.5% sodium hypochlorite) solution. Prior to top working, it is advised that the rootstocks should first be indexed for possible viroid infections. Growers can contact CRI's molecular laboratory at chanel@cri.co.za for more information on sampling instructions and diagnostics of the rootstocks.

Prevention of viroid infections

Growers are strongly encouraged to only purchase trees from CIS certified nurseries and to request Tree Certificates, which is proof that the trees were made with certified budwood supplied by the Citrus Foundation Block.



Figure 3. Stunted mandarin tree on a C-35 rootstock infected with CDVd.