PRELIMINARY SURVEY OF CITRUS TRISTEZA VIRUS IN NEW ZEALAND

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SUMMARY

Citrus tristeza virus (CTV) isolates were collected during 1993 and 1994 from superior clonal budwood trees in the different citrus growing areas of New Zealand. Initial screening of these isolates under glasshouse conditions on Madam Vinous and Eureka lemon hosts showed distinct differences in the severity of seedling yellows and stem pitting symptoms of the different isolates. A milder form of the disease was produced by some sources of the virus, which either lacked both the seedling yellows and stem pitting symptom components such as the isolate from Miho satsuma, or lacked seedling yellows symptoms but had a mild form of stem pitting. These isolates will be further investigated for use in future mild strain cross protection trials.

Keywords: ELISA, Citrus tristeza virus, cross protection

INTRODUCTION

Citrus tristeza virus (CTV) is a phloem limited closterovirus, which represents the biggest threat to worldwide citrus production. By 1991 over 100 million trees had been destroyed by CTV quick decline (Roistacher 1992). CTV is spread in the field by aphids, different species of which transmit the disease with varying degrees of efficiency. The most efficient vector of CTV is the black citrus aphid (*Toxoptera citricidus*), which is widespread in New Zealand.

The most economically important symptom of the disease is the quick decline or death of orange, grapefruit and mandarin trees on sour orange rootstock. Virus infection in the scion causes dieback of the phloem in the sour orange rootstock below the bud union. A second symptom of the disease is a 'slow decline', where the trees decline in health over a period of years. This decline is accompanied by loss in productivity, but the trees do not necessarily die. A third common symptom is stunting where the virus does not kill the tree, but they do not grow. Some CTV strains cause stem pitting with symptoms ranging from numerous fine pits in the wood to deep furrows which give a bumpy appearance to the trunk and limbs of the tree, which is also accompanied by loss in productivity. The virus may also have a latent form which gives no symptoms. All the above symptoms have been detected in New Zealand citrus orchards. The seedling yellows or quick decline aspects of CTV disease can be controlled by the use of CTV tolerant rootstocks. Unfortunately, strains of CTV which cause severe stem pitting can cause losses even on CTV tolerant rootstocks. For these stem pitting strains of CTV, the use of mild strains for cross protection is currently the most effective control strategy.

Large numbers of citrus nursery trees were imported into New Zealand from Australia during the 1930's and 40's, during which time plant quarantine restrictions were almost non-existent. It is therefore likely that the New Zealand CTV strains were imported then and are similar to those present in Australia. We suspect that CTV strains have not caused major reductions in tree growth and yield in New Zealand orchards because of the use of tolerant scion/stock combinations, and because a degree of mild strain cross-protection is taking place. However, CTV is not limited only to certain scion/stock combinations, and our current state of bliss could be shattered by a similar situation to that which has occurred in Queensland, Australia (Owen-Turner

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1990). The careless introduction of an exotic orange stem pitting strain of CTV has resulted in the closure of the Queensland Budwood Scheme.

Mild strain CTV cross protection consists of inoculating plants with a vigorous mild strain of the virus to afford protection against attack by a severe strain (Lee *et al.* 1987). This has proven to be an economical and convenient means of reducing the effects of severe CTV in Australia, Brazil and South Africa (Muller and Costa 1968). Successful use of mild strain cross protection involves careful evaluation of specific host effects and protecting abilities. Mild strain selection is usually achieved by: (1) selection of mild isolates from healthy field trees in older orchards or by bud selection from milder reacting isolates on indicator plants; (2) selection by vector transmission and by selection from field trees or glasshouse plants which had previously been infected with CTV and exhibited seedling yellows symptoms but had recovered and lost this symptom. These isolates are then further evaluated in glasshouse screening tests and field experiments.

This paper reports the initial glasshouse screening of CTV isolates from superior clonal budwood trees in the New Zealand Citrus Budwood Scheme.

MATERIALS AND METHODS

CTV isolates used in this study were collected from field-grown superior clonal budwood trees in the New Zealand Citrus Budwood Scheme located in the Kerikeri, Bay of Plenty and Gisborne districts. Field isolates were maintained by graft inoculation to glasshouse-grown Madam Vinous sweet orange plants. It is important to note that the 'isolates' referred to in this study are highly likely to be a mixture of CTV strains and not a pure CTV strain.

All CTV isolates were graft-inoculated onto Eureka lemon and Madam Vinous sweet orange, as seedlings of the traditional indicator West Indian lime were not available. Results for only 19 of these cultivars are presented in this paper. The indicator seedlings were of uniform size and vigour and were planted singly in 150 mm plastic bags. Three bark patches from each mother plant were bark-graft inoculated onto each of three indicator plants; for each set of three inoculated indicator plants an extra seedling was left uninoculated. The indicator plants were held in an aphid-proof glasshouse with a temperature range of 18°C to 28°C. Plants were watered and fertilised by overhead fertigation.

Symptoms of chlorosis on Eureka lemon were ranked on a scale of 0 (no chlorosis) to 5 (very severe chlorosis). Symptoms of stunting on Eureka lemon and Madam Vinous sweet orange were recorded as grams fresh mass/plant expressed as a proportion (%) of the combined uninoculated controls. Symptoms of vein clearing and stem pitting on Madam Vinous were visually ranked on a scale of 0 (no symptoms) to 5 (very severe symptoms). All symptoms were measured 4 to 5 months after plant inoculation.

Preparation of lyophilised plant extracts and ELISA by double antibody sandwich ELISA using polyclonal antiserum supplied by Sanofi (Sanofi Phyto-Diagnostics, F-33501 Libourne Cedex, France), were performed according to the methods of Garney and Cambra (1986). Each ELISA plate contained three sets of controls: extracts from a known severe CTV strain (TP8/71), extracts from healthy CTV-free plants, and extraction buffer alone. Test samples were duplicated on each plate to give two readings. The ELISA plates were read manually using a visual scale of 0 (nil reaction) to 5 (intense colour reaction).

RESULTS

Polyclonal ELISA showed that all cultivars tested, except for Encore mandarin were infected with CTV (Table 1). Chandler pummelo showed very low virus titre, while Berris lime, Silverhill satsuma, Miho satsuma, Seminole tangelo and Parent Washington Navel (ex Gisborne) indicated moderate virus titre. The remaining cultivars gave severe or very severe reaction in the ELISA indicating high or very high virus titre.

Biological indexing results are shown only for cultivars from the Kerikeri district

TABLE 1: CTV field isolate strain severity detected in 30 commercial citrus clones in New Zealand.

Citrus cultivar	Location	ELISA ¹	Biological Indexing				
			Madame Vinous			Eureka Lemon	
			Fresh mass % of control	Vein clearing ²	Stem Pitting ²	Fresh mass % of control	Seedling yellows
Pummelos, Grapefruit, Lemons and Limes							
Chandler pummelo	Kerikeri	+	117.5	0.0	0.0	107.1	0.3
Cutler Red							
NZ grapefruit	Tauranga	+++++					
Golden Special							
NZ grapefruit	Tauranga	+++++					
Tahiti lime	Kerikeri	+++++	84.5	0.7	0.7	116.7	0.0
Bearrs lime	Kerikeri	+++	93.9	0.3	0.3	90.1	1.0
Meyer lemon	Gisborne	+++++					
Villa Franca lemon	Tauranga	+++++					
Mandarins							
Silverhill satsuma	Kerikeri	+++	50.9	1.0	1.0	40.7	2.3
Thorny (Willowleaf)	Kerikeri	+++++	63.8	3.3	1.7	1.5	5.0
Richards Special	Kerikeri	+++++	65.9	3.0	1.3	8.2	3.7
Miho satsuma	Kerikeri	+++	91.7	0.0	0.0	98.4	0.0
Okitsu satsuma	Kerikeri	+++++	64.5	2.0	0.0	73.4	1.3
Miyagawa satsuma	Kerikeri	+++++	93.9	0.3	0.0	59.9	2.3
	Gisborne	+++++					
Clementine	Kerikeri	+++++	40.8	2.3	2.3	3.9	5.0
Encore	Tauranga	-					
Ponkan	Kerikeri	+++++					
Tangelos and Tangors							
Ugli tangor	Kerikeri	+++++	59.5	2.7	2.0	17.2	3.3
Dweet tangor	Kerikeri	++++	56.6	2.7	3.3	8.5	4.7
Ortanique tangor	Kerikeri	+++++	65.2	2.7	2.0	11.2	2.3
Seminole tangelo	Tauranga	+++					
Oranges							
Sanguinelli	Kerikeri	++++	48.7	3.7	1.7	7.7	4.3
Seville	Kerikeri	+++++	101.7	1.0	0.0	69.1	1.0
Harward Late	Kerikeri	+++++	43.3	2.3	2.0	26.7	2.0
Waiono Navel	Kerikeri	++++	68.8	2.0	2.0	6.0	4.0
Johnson Navel	Kerikeri	+++++	56.6	2.0	3.3	6.5	4.0
Ryan	Kerikeri	++++	53.7	1.0	1.7	10.6	3.3
Parent Navel	Tauranga	++++					
	Gisborne	+++					
Washington Navel (Brown)	Gisborne	+++++					
SED ³			6.5	0.5	0.5	5.6	0.6

¹ ELISA reaction scored visually. Colour reaction: +++++ (very intense) to - (no reaction).² Symptom severity ranked on a scale of 0 (no symptoms) to 5 (severe symptoms)³ Standard Error of the Difference.

as these isolates were inoculated in December 1993 and symptom expression had developed. CTV complex severity can be determined on the basis of symptom development in biological indicator plants. Symptoms in Eureka lemon include seedling yellows chlorosis and growth retardation while symptoms in Madam Vinous sweet orange include stem pitting, vein clearing and growth retardation.

Biological indexing results from 19 cultivars are presented in Table 1. Out of the 19 cultivars only Miho satsuma mandarin did not produce symptoms of seedling yellows or stunting in Eureka lemon, nor symptoms of vein clearing or stem pitting in Madam Vinous. There was, however, a small reduction in growth in Madam Vinous. ELISA results for this cultivar demonstrated a moderate virus titre. These results therefore suggest that this cultivar contains a very mild CTV strain. CTV from Tahiti lime was the only isolate which did not elicit a seedling yellows reaction in Eureka lemon. This strain did, however, produce mild stem pitting and growth retardation in Madam Vinous, which suggests that Tahiti lime contains a mild CTV stem pitting strain. No symptoms of stem pitting were found in Madam Vinous inoculated with bark from either Chandler pummelo, Okitsu satsuma, Miyagawa satsuma or Seville orange. However these cultivars all elicited mild to moderate seedling yellows symptoms in Eureka lemon. Tahiti lime, Bearrs lime and Seville orange also all elicited mild seedling yellows symptoms.

The most severe seedling yellows symptoms were detected in samples from Thorny and Clementine mandarins and Sanguinelli orange. These isolates also induced moderate stem pitting and vein clearing symptoms in Madam Vinous. The most severe stem pitting symptoms were detected in the isolate from Dweet Tangor.

DISCUSSION

Data presented here show that most citrus cultivars in New Zealand are infected with a complex of CTV strains showing wide biological diversity in their severity of reaction. CTV exists as a large number of distinct strains. Field isolates may be mixtures of several distinct CTV strains, and in field-grown trees where aphid transmission is possible, a pure strain rarely or never exists (Fraser and Broadbent 1979). Both the host plant and the vector aphids affect the strain complex present and the ability to transmit all or part of the strain complex.

The results we have obtained are consistent with this finding in that isolates from clonal selections in a single orchard block elicited vastly different symptoms in both the biological indicator plants and ELISA. Cultivars selected in this block included Chandler pummelo, Tahiti and Bearrs limes, Thorny mandarin, Sanguinelli and Seville oranges, Dweet tangor and Waiono Navel (Table 1). Although the ELISA does not indicate biological diversity, the results demonstrated possible differences in the virus titre within the field trees. Tahiti lime appears to have voided the seedling yellows symptom component of the virus, while Chandler pummelo, Bearrs lime and Seville orange had modified this component. Chandler pummelo and Seville orange also appear to have voided the stem pitting component. Thorny mandarin had the most severe seedling yellows component, while Dweet tangor isolate contained the most severe stem pitting component. These results are consistent with those of McLean (1977), who found that species like sour orange, lemon and grapefruit, which are sensitive to the seedling yellows component can, under field conditions, either selectively exclude or modify the seedling yellows component during aphid transmission.

From this initial study, there appear to be several candidate strains which meet some of the criteria for a mild protective strain, namely, have a high virus titre and show only mild symptoms on host plants (Lee *et al.* 1987). The most promising selection is from Miho satsuma, which although it only had a moderate virus titre, did not elicit either seedling yellows, stem pitting or vein clearing symptoms in the indicator plants. The other potential candidates are isolates from Tahiti lime, Bearrs lime and Seville orange. It remains to be established if these isolates are single strains, are able to rapidly invade all new growing tissue, are readily transmitted by aphids, and are stable and not prone to mutation.

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