Control of Stem Pitting of Grapefruit in Australia by Mild Strain Protection

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ABSTRACT. Trials to determine if mild strains of citrus tristeza virus (CTV) cross protect grapefruit against aphid-transmitted CTV severe strains were established at two climatically distinct sites in New South Wales. After 20 yr more than half the uninoculated (initially virus-free) control trees at Somersby (humid, coastal site) were unproductive with small fruit and severe trunk symptoms of stem pitting, as were the interplanted control trees inoculated with an aphid-transmitted severe strain of CTV. Only 10 of 117 mild strain inoculated trees showed marked deterioration of fruit quality resulting from infection by severe strains. By comparison at Dareton (hot, dry inland site) trees inoculated with the severe strain, although slightly smaller in size and less productive than the uninoculated or mild strain inoculated trees, remained in good health for 17 yr before the production of small fruit became a problem. These trials demonstrated the effect of climate on tristeza symptom expression and aphid transmission, and the benefits of mild strain protection.

Tristeza stem pitting has caused problems for growers of grapefruit (Marsh and Thompson cultivars) in New South Wales, Australia since the disease was first observed about 1932 (1). The greatest injury occurred in 1940 to 1950 when many trees 18-25 yr old developed dieback, trunk pitting, and small, deformed fruit (3). However, it was common to find occasional vigorous and productive trees in orchards where many trees were in various stages of deterioration. These vigorous healthy appearing trees were not free of the virus. Very mild vein clearing and pitting symptoms were produced when they were indexed on West Indian lime seedlings. Since these trees had remained apparently healthy for a number of years, under conditions of high aphid activity, it was postulated that the CTV strain complex resident in them must provide a high level of protection against the establishment of severe stem pitting. Attempts were made to exploit this cross protection.

Trials with Marsh grapefruit to assess the protective value of mild strains of CTV against natural infection by severe strains have been in progress in New South Wales for 30 yr (3, 2). The aim of these trials was to determine: (i) whether a mild strain of CTV confers protection to a grapefruit tree against severe strains of the virus under field conditions, and (ii) the most suitable budline carrying mild strain CTV for commercial propagation.

MATERIALS AND METHODS

Extensive surveys of grapefruit plantings (Marsh and Thompson cultivars) in New South Wales showed occasional trees with good vigour and fruit quality and free of stem-pitting symptoms in orchards where other grapefruit trees had been severely debilitated by stem-pitting. The healthy trees were selected for further studies and indexed on seedlings of West Indian lime.

Trial plantings

Two field trials were located on the central coast of New South Wales at the Gosford Horticultural Research Station (Somersby section), and one trial inland at the Agricultural Research and Advisory Station, Dareton. All trees were propagated in the field nursery at the Gosford Horticultural Research Station.

Somersby trial 1. Direct propagations of Marsh grapefruit were made from six indexed field trees onto rough lemon stocks (117 trees). The

indexed scion trees were carrying mild strain assemblages of CTV (3134, 3135, 3182, 3183, 3184, 3185). These trees were randomly interplanted with 24 trees carrying a severe CTV strain assemblage (3130). In this paper 'mild strain' refers to an assemblage of virus strains of CTV which give mild host reactions in grapefruit and West Indian lime. The trial commenced in 1954 and was terminated in 1979.

Somersby trial 2. Virus-free nucellar Marsh grapefruit trees on trifoliate orange and rough lemon rootstocks were tissue-inoculated with one of nine mild strains of CTV from field trees which showed no field symptoms and produced mild symptoms on West Indian lime seedlings (65 trees). These were interplanted with 11 uninoculated trees on the same two rootstocks. Direct propagations of the same symptom-free mild strain sources were also included in the trial (30 trees). The trial commenced in 1956 and was terminated in 1979.

Dareton trial. This trial was planted in 1959 and consisted of 15 trees each of virus-free nucellar Marsh, Thompson and Ruby grapefruit trees, and virus-free Marsh grapefruit trees tissue-inoculated with one of three selected mild CTV strains (3134, 3135, 3185). Distributed at random throughout the block were six Marsh grapefruit trees inoculated with the same severe strain assemblage of CTV (3130) as the Somersby trial. All trees were on rough lemon rootstock. The trial was terminated in 1980.

Climatic Conditions

The climate at Somersby is milder than at Dareton with less extreme summer heat, and a higher relative humidity during summer (October-April). On average, rainfall at Somersby is four times higher than at Dareton, with a higher incidence in the summer months. The cooling degree days at 12C (above which citrus growth occurs) are higher at Dareton from October to March. Cooling degree days at 28C (above which CTV would be inactivated (5)), occur at Dareton in January and February, but such conditions occur less frequently on the coast at Somersby.

Data collection and statistical analyses

Data from the Somersby and Dareton trials were co-analysed to determine the effect of climate on disease expression. The treatments at the two sites were not the same and only treatments common to both sites were included in the results. These treatments were inoculations of Marsh grapefruit on rough lemon rootstooks with mild CTV strains 3134 and 3135 and severe CTV strain 3130.

Treatment effects were examined by means of the following contrasts. Mild CTV strains 3134 and 3135 were compared to severe CTV strain 3130 (mild-severe) and mild strain 3134 was compared to the mild strain 3135 (between milds). Since both these trials lacked a true experimental error, all tests were performed using the variation between individual trees within treatments as error. As the trials were planted at different times, all comparisons were made on the basis of equal age of the trees.

Individual tree yields were recorded annually. Thirteen years of data from trees of corresponding age were analysed. Yields were log transformed prior to analysis to stabilise the variances. Fruit grading data for the ninth and nineteenth years after planting were analysed to examine any changes in fruit size distributions. The fruit was initially graded into five size categories, but for the analyses it was found necessary to combine classes four and five because of the low numbers of larger fruit, especially in trees inoculated with the severe CTV strain. The proportion of fruit in each of the remaining four classes was analysed using maximum likelihood methods with a logit link.

RESULTS

Twenty years after planting only 10 out of 117 trees direct propagated with mild strains at Somersby (trial 1) were showing any marked reduction in fruit size and/or quality, whereas eight of 11 uninoculated trees (trial 2) had declined in tree health and fruit size, after infection by aphid-transmitted severe strains of CTV. There was no breakdown in mild strain protection for strains 3183, 3134 and 3135 (Table 1). No marketable fruits were produced by trees with severe CTV strains, and the trees were stunted and heavily pitted.

There were no significant differences in yield and fruit size or stem pitting symptoms between direct propagation of mild strain sources and trees of two nucellar (virus-free) grapefruit budlines, tissue inoculated with those same mild strain sources.

In the Dareton trial 17 yr after planting, trees inoculated with severe CTV were still in good health, although they were smaller and less productive than uninoculated trees or trees inoculated with a mild CTV strain. Towards the end of the trial (21 yr), trees inoculated with the severe strain (3130) showed a marked reduction in vigour; defoliation and twig-dieback were evident in the tops of the trees, and the trees had a high proportion of small fruit. Uninoculated Marsh grapefruit trees at Dareton were obviously larger than trees inoculated with a mild strain and produced more fruit (Table 2). There were no differences in fruit size between uninoculated and mild strain inoculated trees.

Effect of severity of CTV strain and site on yield and fruit size

Annual yields. Trees inoculated with mild strain CTV strains outyielded trees inoculated with the severe strain in all years, and this difference increased with time. Figure 1 shows the plot of the regression coefficient for the contrast mild-severe for each of the 13 yr and there is a significant rise ($P<0.01$) in its value. This is complicated however, by an interaction of this contrast with sites

TABLE 1.
BREAKDOWN IN MILD STRAIN PROTECTION IN SOMERSBY TRIAL, AS EVIDENCED BY YIELD DATA OVER 4 YR (1970-1973).

Condition	Strain accession no.	Yield (kg) 1970	1971	1972	1973
Mean yield of trees of mild strain budlines where no breakdown in mild strain protection	3183	233	216	202	243
	3134	242	199	186	209
	3135	231	108	192	204
Mean yield of healthy trees of mild strain budlines where some breakdown occurred	3182	248	221	189	239
	3184	275	220	213	271
	3185	309	200	202	246
Yield of individual trees where breakdown in mild strain protection occurred	3182	73	113	82	142
	3182	113	168	132	185
	3184	63	176	124	183
	3184	159	—	100	165
	3185	186	172	162	174
	3185	0	45	90	0
	3185	154	141	131	146
	3185	269	190	137	128
	3185	318	218	164	190
Mean yield of trees inoculated with a severe strain of stem pitting	3130	31	56	90	63

TABLE 2.
ANNUAL YIELDS AND CUMULATIVE YIELD (1962-78) FOR MARSH GRAPEFRUIT TREES IN DARETON TRIAL

		CTV inoculation			
Year	Uninoc.	mild 3134	mild 3135	mild 3185	severe 3130
1962	30.4	22.2	24.7	23.2	22.7
1963	44.8	47.9	46.6	48.5	33.6
1964	114.3	86.5	97.3	102.4	73.7
1965	162.2	143.1	146.5	166.5	114.9
1966	117.6	78.9	92.2	89.9	62.0
1967	271.0	226.7	226.1	246.5	178.9
1968	217.6	201.5	220.6	163.1	152.3
1969	276.3	224.2	218.4	262.3	182.2
1970	233.6	276.3	271.4	133.2	181.5
1971	351.1	234.1	256.3	326.7	226.0
1972	69.7	70.8	72.0	66.3	26.2
1973	320.6	257.3	265.0	275.2	201.2
1974	190.3	241.4	235.1	215.3	158.6
1975	303.1	239.1	234.8	263.7	174.5
1976	175.3	198.5	211.2	187.5	148.2
1977	273.5	201.8	190.7	164.2	139.4
1978	280.3	240.4	239.7	235.1	145.7
Cumulative Yield (1962-78)	3431.7	2990.7	3040.0	3048.6	2221.6

($P<0.01$). The regression coefficient for this interaction (Fig. 2) decreased with time. This meant that the severe strain trees at Somersby were performing worse as time went on relative to those at Dareton.

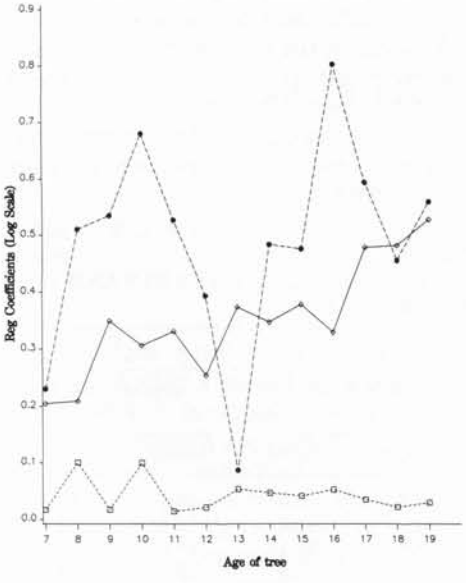

Fig. 1. Plot of the regression coefficients for contrasts: mild-severe strains ♦——♦, between mild strains □---□ and between sites ●---● with age of tree.

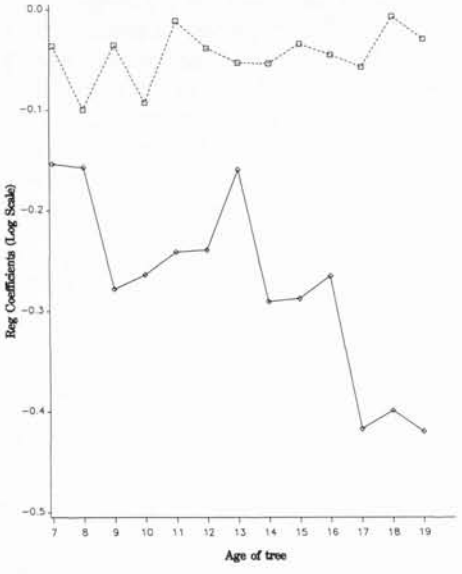

Fig. 2. Plot of the regression coefficient of contrasts: mild-severe strains ♦——♦ and between mild strains □---□ by site.

TABLE 3.
CUMULATIVE YIELD (LOG) TONNES FOR GRAPEFRUIT TREES CARRYING MILD (3134, 3135) AND SEVERE (3130) STRAINS OF CTV AT DARETON AND SOMERSBY

CTV strain	Dareton			Somersby		
	Log	SE	Tonnes	Log	SE	Tonnes
3134	.5666	.0093	(3.686)	.3576	.0083	(2.278)
3135	.5714	.0093	(3.727)	.2837	.0078	(1.922)
3130	.4732	.0132	(2.973)	.0305	.0066	(1.073)

There was a small but significant difference ($P<0.05$) between the mild strains since 3134 generally outyielded 3135, however this main effect remained relatively constant over the 13 yr (Fig. 1). Sites interacted with the treatment contrast between mild strains ($P<0.05$). The coefficients of this interaction for each yr are shown in Fig. 2. The coefficients are small and negative, indicating that trees inoculated with mild strain 3135 did not yield as well at Somersby as at Dareton.

There was a strong effect of sites ($P<0.001$), where trees at Dareton outyielded those at Somersby by a considerable amount except at year 13. Figure 1 plots the regression coefficients for the contrast Dareton-Somersby.

Cumulative yield. All effects were highly significant ($P<0.001$). There were large site by treatment contrast interactions. The means are given in Table 3. The yield from trees carrying the severe strain (3130) at Somersby was much less than that from similar trees at Dareton. Mild strain 3135 produced higher yields at Dareton than at Somersby, and this produced the significant interaction between mild strains and sites.

Fruit size. There was a marked difference in the behaviour of the mild and the severe CTV strains at the two sites. At year nine, both the treatment contrasts (mild-severe; between mild strains) with respect to fruit size, were highly significant ($P<0.01$) and so was the difference between sites ($P<0.05$). Table 4 gives the estimated percentages in each of the four fruit size classes for each treatment at year 9. There were far more small fruit at Somersby on the 3130 (severe strain) trees than on similarly inoculated trees at Dareton. The mild strain trees at Somersby produced fruit of a similar size to trees at Dareton. As a check the data were re-analysed using just two classes, the very small and the rest. This gave the same results, indicating that the primary difference was the proportion of very small fruit.

TABLE 4
ESTIMATED PERCENTAGE OF FRUIT IN EACH SIZE CLASS AT YEARS 9 AND 19 AT DARETON AND SOMERSBY.

Fruit size class	Year after planting	CYV strain					
		Mild strain 3134		Mild strain 3135		Severe strain 3130	
		Dareton	Somersby	Dareton	Somersby	Dareton	Somersby
1. >150	9	3.9	1.0	8.2	3.4	16.1	69.4
	19	1.9	1.2	3.2	1.0	29.6	41.8
2. 150-125	9	20.4	14.0	32.9	28.1	40.8	24.3
	19	20.6	21.8	11.2	16.8	49.5	34.5
3. 113-80	9	27.9	35.9	30.2	39.6	26.3	5.2
	19	48.6	63.4	51.8	63.7	19.2	19.5
4. <72	9	47.8	49.0	28.7	28.9	15.7	1.2
	19	28.9	13.6	33.8	18.9	1.6	4.2

By year 19, there were no treatment interactions with sites as the proportions of fruit in the size classes were similar at both sites. There were big differences between sites ($P<0.01$) and treatments ($P<0.01$). Table 4 show the expected percentages in the four size classes. In reanalysing, using the two classes very small and the rest, the major cause of the differences was shown to be the disproportionate number of small fruit.

Reduction in fruit size for trees inoculated with the severe strain of CTV was far greater at Somersby. Mild strain inoculated trees at Dareton had a higher proportion of very small fruit than at Somersby, but there was no difference between the two mild strains.

DISCUSSION

The mild CTV strains used to protect grapefruit in the trials reported in this paper were selected from vigorous and productive grapefruit trees in orchards severely debilitated by stem pitting.

Comparison of the trials at Dareton and Somersby show the need for evaluating the protective capacity of mild strains at sites favourable for symptom expression and the activity of the aphid vectors. Breakdown in mild strain protection was less in the hotter inland site than on the coast. A comparison of behaviour of grapefruit trees carrying severe stem pitting (CTV 3130) in the Somersby and Dareton trials demonstrates this modifying effect of climate. At Somersby, no marketable fruit was produced by trees inoculated with severe strains—the trees were very stunted and heavily pitted, whereas at Dareton the trees were almost as large as mild-strain trees, pitting was slight or absent, and cropping and fruit size, though slightly less than that of the mild strain trees, was not significantly so up to year 17.

An acceptable degree of protection was obtained at both sites (2), but this has been less complete at Somersby than at Dareton. Deterioration of originally virus-free trees at Somersby started to show up at the age of 8 yr but there was no breakdown in protection in the trees inoculated with mild strains until the eleventh year of the trial. After 22 yr, 35% of the protected trees at Somersby were showing signs of deterioration in fruit size and tree vigour, compared with 90% of those originally virus-free. The degree of protection varied with the mild strain used. At Dareton there was little evidence of stempitting in trees at 18 yr.

There was no decline in tree health or fruit size of uninoculated Marsh grapefruit trees at Dareton and by the termination of the trial after 21 yr, these were carrying mild strains of CTV as evidenced by their West Indian lime seedling reactions. In a comparable time period, eight of 11 uninoculated trees at Somersby had declined in tree health and fruit size after infection by aphid-transmitted severe strains of tristeza. These results are presumably influenced not only by the effect of climate on tristeza symptom expression but also by aphid activity. The principal vector of tristeza in Australia, the black citrus aphid, *Toxoptera citricidus*, is more frequent in coastal than in inland districts presumably because mild, moist conditions on the coast and an abundance of succulent new citrus foliage are more favourable for aphid activity (4). However the more likely explanation is that mild strains of CTV were spreading in the Dareton trial and the severe strain interplanted in the trials was not the source of inoculum.

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