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CSIRO Division of Horticultural Research, Merbein, Victoria, Australia

Yield differences between Sultana clones related to virus status and genetic factors

by

R. C. WOODHAM, A. J. ANTCLIFF, L. R. KRAKE and R. H. TAYLOR[1])

Ertragsdifferenzen zwischen Sultana-Klonen in Beziehung zum Grad der Viruserkrankung und zu genetischen Faktoren

Zusammenfassung. — Ertragsdifferenzen zwischen Sultana-Klonen konnten teilweise durch Pfropfung übertragen werden, teilweise nicht. Der wichtigste pfropfübertragbare Faktor war die Rollkrankheit. Beim Vorliegen leichter Krankheitssymptome war der Traubenertrag in 3 von 6 Jahren um durchschnittlich 14 % verringert; bei stark rollkranken Reben war in allen 6 Jahren ein mittlerer Ertragsrückgang von 35 % zu verzeichnen. Die niedrigeren Erträge waren durch weniger Trauben je Rebe, in einigen Jahren bei starker Erkrankung auch durch weniger Beeren je Traube bedingt. Das Triebwachstum war bei schwerer Rollkrankheit im Frühjahr verzögert, und es wurde weniger Holz erzeugt; bei leichter Erkrankung war die Holzproduktion weniger rückläufig. Starke Erkrankung veränderte die Beerenfärbung nicht, weder bei frischen noch bei getauchten oder nichtgetauchten getrockneten Beeren. Die Zuckerkonzentration war sowohl bei milder wie bei starker Rollkrankheit nur geringfügig vermindert. Schwere Rollkrankheit mit zusätzlichem Fanleafvirus war in einigen Jahren mit einer weiteren Verringerung von Traubenertrag und Holzproduktion verbunden.

Die durch Pfropfung nicht übertragbaren Ertragsunterschiede erreichten annähernd 50 %. Die am schwächsten tragende Ausgangsrebe dieser Kategorie zeigte runzliges Laub und stärker abgeplattete Beeren; diese Merkmale waren nicht pfropfübertragbar. Nur diese eine Ausgangsrebe wies überhaupt deutliche morphologische Abweichungen auf.

Introduction

WOODHAM and ALEXANDER (1966) demonstrated differences in yield between Sultana vines (syn. Thompson Seedless, Sultanina) which were reproducible in vines propagated from them, but did not establish reasons for the differences at that time. Possible reasons would include genetic variation and differences in infection with virus[2]) diseases. This paper reports investigations into the virus status of the selections in the original trial and the effect of graft inoculating a high yielding selection with buds from a number of other selections.

Materials and methods

Status of the original selections

The 32 selections in the original trial were indexed for virus infection by:

(1) Mechanical inoculation of *Chenopodium quinoa* WILLD. plants with nicotine extracts of young leaves in early spring.

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[1]) Department of Agriculture, Melbourne, Victoria, Australia.

[2]) In this paper virus is used to refer both to known viruses and to graft transmissible agents of virus-like diseases of unknown etiology.

Table 1

Virus status of 16 pairs of Sultana selections, together with their mean yields of fresh fruit in the original experiment (WOODHAM and ALEXANDER 1966) · H = selected for high yield, L = selected for low yield; differences within the first 9 pairs were significant ($P<0.05$)

Grad der Viruserkrankung bei 16 Paaren von Sultana-Klonen sowie mittlerer Traubenertrag (Frischgewicht) des Originalversuches (WOODHAM und ALEXANDER 1966) · H = auf hohen, L = auf niedrigen Ertrag ausgelesen; die Ertragsunterschiede innerhalb der ersten 9 Paare waren signifikant ($P<0,05$)

Selection		Leafroll symptoms	Yield 1961—1964 kg/vine	Selection		Leafroll symptoms	Yield 1961—1964 kg/vine
C4	H	Nil	33.6	B2	H	Nil	27.7
	L	Severe	16.3		L	Nil	29.0
B4	H	Nil	31.8	B3	H	Mild	27.7
	L	Severe	21.3		L	Mild	24.5
D2	H	Nil	28.6	A3	H	Mild	25.9
	L	Severe	23.1		L	Mild	26.8
C3	H	Nil	29.0	C2	H	Mild	27.2
	L	Mild	20.0		L	Nil	25.9
C1	H	Nil	31.8	B1	H	Mild	25.4
	L	Mild	25.4		L	Nil	26.3
A2	H	Mild	26.3	D4	H	Severe	25.4
	L [1]	Severe	19.5		L [3]	Nil	19.1
A4	H	Mild	31.3	D3	H	Severe	24.0
	L [2]	Mild	15.9		L [3]	Nil	19.5
A1	H	Mild	25.4				
	L	Mild	18.6				
D1	H	Severe	22.7				
	L [3]	Severe	15.4				

[1]) Induced leafroll reaction in Baco 22A.
[2]) Selection with puckered leaves and more oblate berries.
[3]) Infected also with fanleaf virus.

(2) Graft-inoculation tests using known vine indicators as the stock. The inoculated indicators together with corresponding uninoculated plants as controls were transferred to the field, except *Vitis rupestris* St. George plants, which were kept in glasshouse or shadehouse environments. All vines were observed during 3 years. The viruses tested for were fanleaf and fleck (St. George), leafroll (Cabernet Franc, Mission, LN33, Baco 22A), yellow speckle (Cabernet Franc, LN33, Mataro), summer mottle (Cabernet Franc, Mission) and corky bark (LN33).

Indexing showed that all 32 selections were infected with leafroll. Only 21 of the selections themselves showed typical "green vein" symptoms (UYEMOTO *et al.* 1978), 8 showing severe and 13 mild symptoms. One selection showing severe symptoms was the only one to induce a leafroll reaction on Baco 22A. All of the 21 selections showing symptoms induced leafroll symptoms on LN33 and Mission. The other 11 selections induced only occasional and inconclusive symptoms on LN33 and none at all on Mission, but always induced symptoms on Cabernet Franc, our most sensitive and reliable indicator for leafroll. No selection was infected with corky bark.

All selections were infected with yellow speckle, but none with fleck or summer mottle. 3 were infected with fanleaf virus and habitually showed vein banding symptoms (KRAKE and WOODHAM 1983). One low yielding selection showed a puckering in interveinal areas of older leaves in early spring. This leaf roughness and subsequent mild distortion remained obvious through to autumn, and the berries of this selection were noticeably more oblate than the normal oval type.

The data for the individual selections together with their yields in the original experiment are shown in Table 1. The design of the experiment (WOODHAM and ALEXANDER 1966) allowed for comparison of yields within but not between pairs.

Graft inoculation experiment

In the absence of any Sultanas known to be free of leafroll (WOODHAM and KRAKE 1978) and yellow speckle in Australia, selection C4H, since commercialized as clone H5 (ANTCLIFF and HAWSON 1974), was used as the test plant. Newly rooted cuttings of this selection were graft-inoculated in August 1966 with dormant chip-buds from selections C4L, B4L, C1L, A4H, A4L, D1L and C4H itself. The selections other than C4H were chosen to cover a range of yields, and the later observations and indexing showed (Table 1) that 3 had mild leafroll symptoms and 3 severe, 1 of the latter being infected also with fanleaf virus.

The site for planting the experiment was a Coomealla loam soil (PENMAN *et al.* 1939) on the Divisions's experimental farm at Merbein. The area had previously been planted with Sultana vines. An examination of 6 representative samples of soil at 15 cm depth for nematodes revealed large numbers of *Tylenchulus* spp., small numbers of *Paratylenchus* and *Pratylenchus* spp., and a few *Xiphinema americanum* in 2 samples. Consequently the area was fumigated with DD, 4 weeks before planting.

In November 1966 vines with live inoculum chips from each of the 7 sources, together with uninoculated C4H, were planted in randomized single-vine plots in 2 adjacent 8 × 8 latin squares. The vines were spaced 2.75 m apart in rows 3.35 m apart, and guard vines were planted at the ends and on each side of the 8 trial rows. In January 1967, the inoculum buds, all of which were still alive, were removed to prevent their growth.

In the following season the vines were trained on to a T-trellis providing 2 cane wires 0.25 m apart about 1 m above the ground and a single foliage wire a further 0.35 m from the ground. The vines were pruned according to vigour up to winter 1969, and thereafter balance-pruned to 84 buds (with a mean of 14 buds per cane) for the first 0.45 kg of prunings plus 12 buds for each additional 0.45 kg. The vines were furrow-irrigated and weeds were controlled by cultivation.

The number of inflorescences per vine was counted in spring and the weight of fresh fruit measured at harvest each season[3]) from 1970 to 1975. A sample of 100 berries per vine, 5 from each of 20 bunches selected at random, was taken at harvest for estimation of mean berry weight and sugar concentration of juice except in 1971 and 1973 when the fruit was badly damaged by rain. The number of berries per bunch for each vine was calculated from the yield, number of inflorescences and berry weight.

The weight of 1-year-old growth pruned from each vine plus an estimate of the weight of the canes retained was obtained in winter as a measure of growth during the preceding season. The number of buds retained on each vine was recorded. Time of bud burst was followed on all vines in one latin square in the 1976 season using the methods of ANTCLIFF and WEBSTER (1955).

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[3]) The growing season, which in the southern hemisphere covers parts of 2 calender years, is named by the year of harvest.

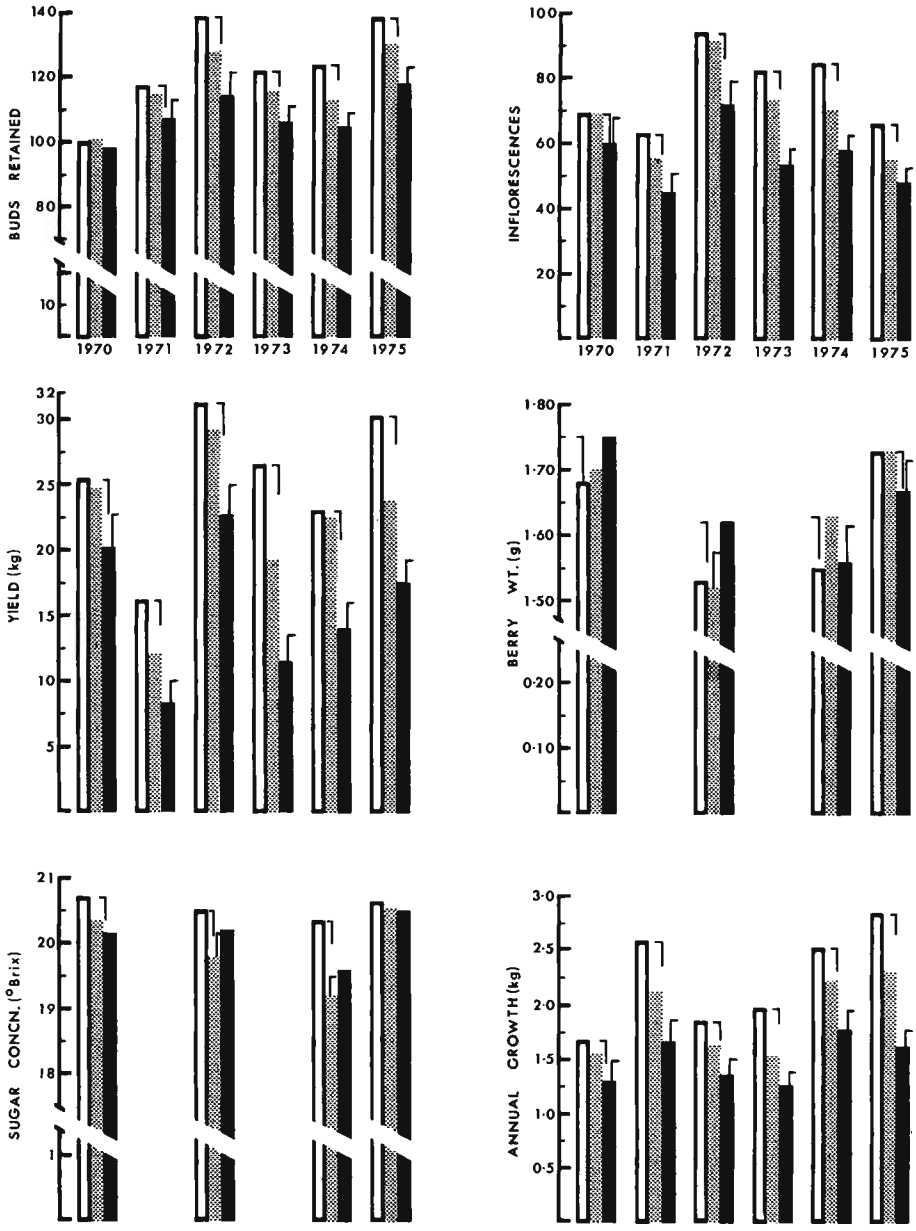

Fig. 1: Fresh fruit yield, yield components, weight of annual growth and number of buds retained from the no leafroll symptom group [1]) □, and from C4H inoculated with 2 groups of 3 vines showing mild ※ and severe ■ leafroll symptoms respectively. Data are annual means per vine for 6 seasons. Vertical bars represent the least significant difference (P = 0.01): ※ is used for differences between the no symptom group and either of the 2 symptom-groups and ※ for differences between the mild and severe symptom groups.

[1]) Combined from C4H self inoculated and uninoculated.

The external colour of fresh and dried fruit was measured in 1972 using a Hunterlab Colour Difference Meter model D35L (GRNCAREVIC and LEWIS 1973), standardised against a green standard, Serial No. 6240, for fresh fruit, and a yellow, Serial No. 6241, for dried fruit. 4 representative samples of 100 berries with a short length of pedicel attached were clipped from 20 bunches from each vine of the self-inoculated and 3 severe leafroll-inoculated treatments in one of the latin squares. The colour of the fresh fruit was measured on all samples and 2 samples from each vine were then dipped in commercial cold dip solution. The dipped and undipped samples were dried in forced draft ovens at about 50 °C until they appeared similar to commercially dried fruit.

All vines were inspected each spring and autumn for leaf symptoms associated with virus diseases. The vines inoculated with severe leafroll plus vein banding diseases were assayed on *Chenopodium quinoa* WILLD. in spring of the 1972, 1973 or 1974-seasons to confirm the presence of fanleaf virus. Inspections for symptoms of stem pitting or stem grooving (HEWITT 1973) were made in winter 1976 on 4 vines each of the self inoculated, the 3 severe leafroll inoculated and 1 mild leafroll inoculated treatments.

Results

All graft inoculated C4H vines showed leafroll symptoms of corresponding severity to their inoculum source by the 3rd autumn (1969), thus 3 sources (C1L, A4H, A4L) induced mild leafroll symptoms and 3 sources (C4L, B4L, D1L) induced severe symptoms. The presence of fanleaf virus in all vines inoculated with severe leafroll plus vein banding diseases (D1L) was verified in all cases by positive assays on *C. quinoa*. Mild yellow speckle symptoms occurred on all treatments in several years, and the vines inoculated with D1L could be distinguished by an increased severity of yellow speckle symptoms in some years. Inspections in 1976 revealed no stem pitting, stem grooving or abnormal symptoms on the vine trunks examined. There was no sign of transmission of the leaf distortion or more oblate berry characters of selection A4L.

Analysis of yield and growth data indicated that there were significant differences between treatments. Sub-analysis for treatments showed that the differences were generally accounted for by differences between 3 groups, combining the treatments showing no leafroll symptoms, those with mild leafroll symptoms and those with severe leafroll symptoms. No significant differences were found within the first 2 groups and only for some variables in some seasons were there differences within the 3rd group between the treatment infected with fanleaf virus and the other 2 treatments. The results are therefore summarized in Fig. 1 by combining the treatments into these 3 groups.

The yield of fresh fruit from the mild leafroll symptom group was significantly less than that of the no symptom group in 3 of the 6 seasons while the yield of the severe symptom group was significantly less than that of each of the other groups in every season. The treatment with fanleaf virus yielded significantly less than the other severe leafroll symptom treatments in 2 seasons. Mean yields (kg per vine) over the 6 sea-

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Traubenertrag (Frischgewicht), Ertragskomponenten, jährliche Holzproduktion sowie Anzahl der verbliebenen Augen. □ = Gruppe ohne Blattrollsymptome, aus C4H, selbstinokuliert und nicht-inokuliert, kombiniert; ※ = C4H nach Inokulation mit 2 Gruppen von 3 Reben mit schwachen Blattrollsymptomen, ■ = C4H entsprechend mit stark blattrollkrankem Material inokuliert. Die Daten sind Mittelwerte aus 6 Jahrgängen. — ※ = Grenzdifferenz zwischen der symptomfreien Gruppe und den beiden rollkranken Gruppen; ※ = Grenzdifferenz zwischen der Gruppe mit schwachen und jener mit starken Symptomen. P = 0,01.

sons were 25.4 for the no symptom group, 21.9 for the mild leafroll symptom group and 15.7 for the severe symptom group. The mean for the fanleaf virus infected treatment was 14.0 kg per vine.

The lower yields were mainly related to fewer inflorescences per vine, although in some seasons the severe leafroll symptom group also had fewer berries per bunch and a lower bunch weight. Detailed observations in the 1975 season indicated that differences in number of inflorescences were related to differences in the proportion of shoots with inflorescences rather than in the proportion of buds bursting.

Differences in mean berry weight were not consistently related to treatment. The severe leafroll symptom group had significantly larger berries in 1970 and 1972 but significantly smaller berries in 1975 than the mild and no symptom groups, which did not differ. In 1974 the mild symptom group had significantly larger berries than the severe and no symptom groups, which did not differ. In 1970 both symptom groups and in 1974 the severe leafroll symptom group had a significantly lower sugar concentration than the no symptom group while in 1974 and also 1972 the mild symptom group had a lower concentration than the severe; there were no other significant differences. In 1971 and 1973, when these variables were not measured because rain about 1 week before harvest caused considerable damage to the fruit, there were no obvious differences between the groups in the degree of damage.

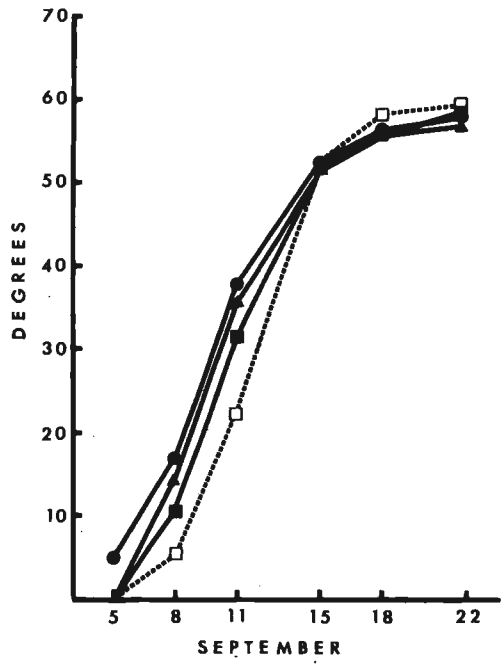

Fig. 2: Mean angular values for total buds burst at each date of observation in September 1975 for no leafroll symptom group ●, mild symptom group ▲, 2 treatments of the severe symptom group without fanleaf virus ■, and the treatment with severe symptoms + fanleaf virus □.

Mittlere Winkelgrade des Austriebs an den jeweiligen Beobachtungsterminen im September 1975. ● = symptomfreie Gruppe; ▲ = Gruppe mit leichten Blattrollsymptomen, ■ = 2 Varianten der Gruppe mit starken Blattrollsymptomen ohne Fanleafvirus, □ = Variante mit starken Blattrollsymptomen + Fanleafvirus.

Table 2

Mean Hunterlab Colour Meter values for fresh fruit and for fruit dried with and without dipping sampled from 8 replicates of 1 no symptom and 3 severe leafroll symptom treatments in the 1972 season

Mittlere Farbwerte (Hunterlab Colour Meter) frischer Beeren sowie getauchter und nichtgetauchter getrockneter Beeren · 8 Wiederholungen einer symptomfreien Variante und dreier Varianten mit starken Blattrollsymptomen · Jahrgang 1972

C4H inoculated with	No symptoms			Severe leafroll symptoms								
	C4H			C4L			B4L			D1L [1])		
Fresh fruit	L	−a	+b	L	−a	+b	L	−a	+b	L	−a	+b
	40.6	5.5	18.9	40.7	5.7	19.0	41.3	5.9	19.4	40.9	4.7	19.4
Dried fruit	L	+a	+b	L	+a	+b	L	+a	+b	L	+a	+b
— dipped	18.9	4.0	6.7	19.4	3.8	7.1	19.3	4.1	7.0	18.8	4.0	6.7
— not dipped	17.6	2.9	4.0	17.8	3.2	4.2	18.0	3.4	4.3	17.6	3.2	4.1

[1]) Infected also with fanleaf virus.
L measures lightness and varies from 100 for perfect white to 0 for black.
a measures redness when +, gray when 0 and greenness when −.
b measures yellowness when +, gray when 0 and blueness when −.

In each season the severe leafroll symptom group produced significantly less 1-year-old growth than the mild group, and this in turn produced significantly less than the no symptom group in each season except the 1st. The decrement for severe leafroll (20—40 %) was about twice that for mild leafroll. In 3 seasons the treatment with fanleaf virus produced significantly less growth than the other severe leafroll symptom treatments, the mean difference over 6 seasons being 17 %. Because of the balanced pruning, these differences led to differences in numbers of buds retained for the following season, although the formula used meant that the latter differences were smaller on a percentage basis.

Each season shoot growth of the severe leafroll symptom group, but not of the mild group, was obviously depressed (by about 50 %) for the first 2 to 3 weeks after bud burst. In most seasons differences could still be seen at flowering, about 8 weeks after bud burst, and in some seasons for a further 4 weeks. Time of bud burst observations in the 1976 season (Fig. 2) indicated that there was a delay of a day or so in the severe leafroll symptom treatments without fanleaf virus and a further similar delay when fanleaf virus was also present. However the final percentage bud burst was similar for all treatments and the differences in shoot growth observed would seem to be mainly due to differences in rate of growth. More limited observations in the 1975 season showed similar trends.

The colour data for fresh and dried fruit in the 1972 season are shown in Table 2. While, as expected, dipping led to a marked difference in the colour of the dried fruit, there were no differences in colour related to the presence of severe leafroll symptoms or fanleaf virus.

The differences in yield between the inoculation treatments did not necessarily follow the differences in yield between the selections used for inoculation. Data for the 2 cases where valid comparisons can be made (Table 3) show striking divergence. Selection A4L yielded only about half as much as A4H but this large difference was not

Table 3

Mean yields (kg of fresh fruit per vine) of 2 pairs of high and low yielding Sultana selections, and of C4H inoculated with these selections

Mittlere Erträge (kg frische Trauben/Rebe) bei zwei Paaren stark- und schwachtragender Sultana-Klone und bei C4H nach Inokulation mit Material dieser Klone

Selection		Source vines		Propagules, 1961—64		C4H inoculated with selection, 16 vines 1970—75
C4	H	18.2	mean of 13 years	33.6	mean of 7 pairs	26.3
	L	8.6		16.3		17.0
A4	H	15.0	mean of 7 years	31.3	mean of 4 pairs	22.4
	L	8.6		15.9		21.6

transmitted to C4H by grafting. Similarly C4L yielded a little less than half as much as C4H, but inoculating C4H with C4L reduced its yield by only a little more than a third. That is, there may be as much as 25 % of the lower yielding capacity of C4L which is not transmitted by grafting. While only such corresponding pairs could be validly compared in the original selection trial, it is interesting to note that C1L also appeared to have an appreciably lower yield than A4H (Table 1), but did not depress the yield of C4H to any greater extent than A4H in the inoculation trial, the mean yield of C4H inoculated with C1L being 21.7 kg.

Discussion

The results presented show that differences in yield between Sultana clones may be partly due to factors which are graft transmissible and partly to factors which are not. In the inoculation trial the most important graft transmissible factor was leafroll. Mild symptoms were associated with a yield reduction of 14 %, severe symptoms on their own with 35 % and combined with fanleaf virus 45 %. As the high yielding test plant C4H is itself infected by a strain of leafroll which induces symptoms on Cabernet Franc, these results confirm the lack of cross protection between leafroll isolates reported by GOHEEN and HEWITT (1964). On the other hand we found yield differences of up to 50 % which were not transmitted by grafting (Table 3). The virus observations on the original selections (Table 1) suggest that there may be more differences of this type. In the C2 and B1 pairs there was no difference in yield between the selection with mild leafroll symptoms and the selection with no symptoms instead of the expected 14 % decrease, and D2L, with severe leafroll, yielded only 19 % less than D2H instead of the expected 35 % less. Similarly ANTCLIFF (1973 b) found that a clone of Cabernet Sauvignon expressing mild leafroll symptoms yielded significantly more than a clone which has indexed free of known virus diseases. Evidence for a genetic difference in berry weight between Sultana clones has already been reported by ANTCLIFF (1973 a). The very low yield of clone A4L was accompanied by obvious morphological differences which were not graft transmissible, but no other such differences between clones were noted.

This trial, like those of OVER DE LINDEN and CHAMBERLAIN (1970) and LEGIN (1972), used genetically uniform material inoculated from particular diseased vines. Many observations have been made on pre-existing plantings by comparing diseased and nearby apparently healthy vines, which would allow confounding of transmissible and non transmissible effects (see, for example, the bibliography of HEWITT and BOVEY 1979). In a comparison of 182 Sultana clones selected for yield, ANTCLIFF *et. al.* (1979) found that clones with mild leafroll symptoms yielded about 15 % less than clones with no symptoms, agreeing well with the yield decrease found to accompany mild leafroll symptoms in the inoculation trial. Selection for yield would have eliminated any serious non-transmissible effects such as found with selection A4L, and any other non-transmissible differences may have averaged out over the large number of clones.

The consistent depression of annual growth associated with both mild and severe leafroll symptoms resulted in fewer buds being retained at pruning. Over the 6 seasons about 12.4 % less buds were retained on vines of the severe symptom treatments than on those without symptoms. The results of ANTCLIFF (1965) suggest that this would have reduced yield by about 7.5 %, much less than the 38.3 % reduction actually found. The slower growth in spring associated with leafroll symptoms agrees with the findings of GOHEEN and COOK (1959) for other cultivars in California.

The decrease in sugar concentration associated with leafroll symptoms was very small compared to the much larger differences sometimes found for other cultivars (e.g. OVER DE LINDEN and CHAMBERLAIN (1970). This may be partly due to the favourable conditions for sugar accumulation during the ripening period for Sultanas at Merbein.

The absence of any difference associated with leafroll symptoms in colour of fresh fruit is contrary to the findings of GOHEEN and HEWITT (1964), who reported that fruit from leafroll infected Thompson Seedless was more yellowish than fruit from healthy vines. The absence of any difference in colour of dried fruit would indicate that the adoption of selected Sultana clones (ANTCLIFF and HAWSON 1974) should not make any difference to the character of Australian dried sultanas. WOODHAM and KRAKE (1978) found that the incidence of typical leafroll symptoms in non-clonal Sultana vines in major settlements along the Murray River ranged between 80 and 98 %. Even the selected clones are infected with a combination of a very mild strain of leafroll and yellow speckle, which affects the growth and yield of the cultivar Cabernet Franc (WOODHAM *et. al.* 1983). Possibly the growth and yield of Sultana vines could be further improved if these diseases were eliminated. In the meantime the reliability of symptom expression in Sultana of the more virulent strains of leafroll facilitates the propagation of vines free of these strains, and as there is no evidence of natural spread of leafroll in Australia such propagation would clearly improve production per unit.

Summary

Differences in yield between Sultana clones were found to be partly transmissible by grafting and partly not. The most important graft transmissible factor was leafroll, mild symptoms being associated with yield reduction in 3 out of 6 seasons with a mean overall of 14 %, and severe symptoms in all 6 seasons with a mean of 35 %. The lower yields were related to fewer bunches per vine and in some seasons for severe leafroll with fewer berries per bunch. Shoot growth was delayed in spring with severe leafroll and less total annual growth was produced. There was a smaller reduction in total annual growth with mild leafroll. Severe leafroll did not alter the colour of the fruit,

either fresh, or dried with or without dipping. Sugar concentration was only slightly reduced when either mild or severe leafroll was present. Fanleaf virus in addition to severe leafroll was associated with a further reduction in yield and growth in some seasons.

Yield differences not transmissible by grafting ranged up to about 50 %. The lowest yielding source vine which did not transmit reduced yield by grafting also showed puckered leaf and more oblate berry characters which were not transmitted by grafting. This was the only source vine showing any obvious morphological differences.

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R. C. WOODHAM
A. J. ANTCLIFF
L. R. KRAKE
CSIRO Division of Horticultural Research
Merbein, Vic, 3505
Australia

R. H. TAYLOR
Department of Agriculture
P. O. Box 4041
Melbourne, Vic, 3001
Australia