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Effects of thermotherapy and virus status on yield, annual growth and grape composition of Sultana

by

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Der Einfluß von Thermotherapie und Virusbefall auf Traubenertrag, Holzproduktion und Traubenqualität der Rebsorte Sultana

Zusammenfassung. — Der an Yellow speckle und Leafroll erkrankte ertragsstarke Sultana-Klon H5 wurde 5 Ernteperioden lang mit 6 wärmebehandelten H5-Klonen — 3 davon noch mit beiden Krankheiten, 3 nur noch mit Yellow speckle behaftet — sowie mit einem Thompson-Seedless-Klon (FV B6V1 HT91) aus Kalifornien verglichen.

Zwischen dem unbehandelten H5-Klon und dem Thompson-Seedless-Klon, der nur an Yellow speckle erkrankt war, wurden in keinem Jahr irgendwelche Unterschiede der erfaßten Meßgrößen festgestellt.

Der mittlere Traubenertrag der wärmebehandelten H5-Klone war bei den doppelt erkrankten Reben um 6 % verringert und bei den Leafroll-freien Reben nicht beeinflußt. Der Ertragsrückgang war mit einer geringeren Anzahl von Infloreszenzen verbunden.

Die 4 Versuchsgruppen zeigten keine Unterschiede bei jährlichem Holzzuwachs, Beerengewicht und kalkuliertem Traubengewicht sowie Zuckerkonzentration, pH und titrierbarer Säure des Beerensaftes.

Lange Perioden der Wärmebehandlung (196—338 d) ergaben keine Klone, die in ihrem Traubenertrag oder der Holzproduktion dem unbehandelten Ausgangsklon überlegen gewesen wären.

Abgesehen von den Leafroll-Symptomen der Blätter zeigten alle 6 wärmebehandelten H5-Klone keine erkennbaren morphologischen Abweichungen von ihrem Ausgangsklon; Entsprechendes gilt für den wärmebehandelten Thompson-Seedless-Klon.

Introduction

The debilitating effects of several grapevine virus [2]) diseases are well known. Recently, WOODHAM *et al.* (1984) reported that differences in yield between Sultana (Syn. Thompson Seedless, Sultanina) clones were partly due to strains of leafroll disease which produce typical 'green vein' symptoms illustrated by UYEMOTO *et al.* (1978), and partly to non transmissible genetic factors. They also indicated that selected high yielding clones of Sultana are infected with a combination of leafroll and yellow speckle diseases which reduced the annual growth and yield of cultivar Cabernet Franc, and suggested the yield of Sultana vines could possibly be further improved if these diseases were eliminated. As Sultana is by far the main multipurpose cultivar grown in Australia (MAY 1979), various attempts to produce a clone free of known virus diseases have been made using *in vivo* heat therapy.

The effects of thermotherapy on subsequent vine performance are not well documented (BOVEY 1980). To the authors' knowledge there are no reported effects from long term investigations of *in vivo* heat therapy particularly when mild strains of viruses are involved.

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[2]) Virus refers to known viruses and to graft transmissible diseases of unknown etiology.

This paper reports a comparison between the Sultana clone H5 and selected clones obtained by heat treatment of H5. The experiment investigated the effects of heat treatment on a clone infected with yellow speckle and a mild strain of leafroll, which does not produce "green vein" symptoms in Sultana, on yield, shoot growth and juice composition (sugar concentration, pH and titratable acidity). In this study H5 was also compared with another Sultana clone imported from the University of California, Davis in 1967 as a heat-treated Thompson Seedless clone.

Materials and methods

A high yielding Sultana clone, H5 (AC 70.8160, IKIN 1980) which is now widely used by industry (ANTCLIFF and HAWSON 1974), had been subjected in 1968 to constant heat treatment at 38 °C and several clones were produced by propagating shoot tips (GOHEEN *et al.* 1965) after varying periods of therapy. Each heat-treated explant had been multiplied and at least 4 plants of each were growing in the field. The original H5 was infected with yellow speckle plus an attenuated strain of leafroll which causes a yellowish blotching of basal leaves rather than the typical "green vein" symptoms (WOODHAM *et al.* 1983), but was free of fanleaf virus and of fleck, corky bark, summer mottle and enation diseases. Indexing of the heat-treated clones was started in 1970 or 1971. Results proved that leafroll had been eliminated from some but yellow speckle still remained in all of them. 6 clones, identified herein by the number of days under heat before propagation, were chosen to compare with untreated H5 in a field trial. This provided the following 7 treatments: untreated H5 infected with yellow speckle and leafroll; 3 heat-treated clones still infected with both diseases, namely 196, 311 [3]), 338; and 3 heat-treated clones with yellow speckle only, namely 255, 280, 311 [3]). In addition, the Thompson Seedless clone (FV B6V1 HT91) from California was included as the 8th treatment; this clone (IV 67.2166, IKIN 1980) had been heat-treated for 91 d and was infected with yellow speckle only.

Cuttings from at least 2 vines of each of the 8 clones were struck in winter 1973 and transferred to containers. Because of variable growth in spring, insufficient vines of suitable vigour were available for field planting and the plants were maintained in containers in a shadehouse. Cuttings from each clone were again propagated in winter 1974. In spring, 16 vines from each treatment in both the 1973 and 1974 cutting-groups were visually selected for uniformity based on the vigour and health of shoot growth in an attempt to minimise variation in future vine vigour within and between treatments. The 8 treatments were randomised as single-vine plots in 4 adjacent 8 × 8 latin squares. To eliminate possible variation due to age of plant, the first 2 squares contained vines from the 1973 group, with the later cuttings in the other squares.

The vines were planted in spring 1974 in soil classified as Tiltao loamy sand (described by NORTHCOTE 1951) on the CSIRO experimental farm at Koorlong. They were spaced 3.05 m apart in rows 3.05 m apart, and 2 guard vines were planted at the ends of the 8 rows. The vines were trained on a T-trellis providing 2 cane wires 0.3 m apart about 1 m above the ground with a single foliage wire a further 0.3 m from the ground. Each winter the vines were pruned according to visual vigour. The vines were irrigated by overhead sprays and weeds were controlled by cultivation. Nitrogen (sulphate of ammonia or urea) was broadcast annually at the rate of 22 kg N/ha immediately before irrigations in early spring and again in early summer. In case fruit set might have been

[3]) 2 clones were produced after 311 d of heat therapy; leafroll had been eliminated from one of them.

affected by zinc deficiency, a suspension of zinc sulphate was applied as a foliage spray (ALEXANDER and WOODHAM 1964) to all measured vines at the first sign of flowering in seasons [4]) 1980 to 1983. To provide a visual comparison, the guard vines were not sprayed. Immediately preceding the first application of zinc in spring 1979, three representative samples of laminae and of corresponding petioles taken from opposite the bunch on single-bunch shoots were analysed for zinc content by the method outlined in AOAC (1975, p. 22). The samples were dried at 105 °C.

The number of inflorescences per vine was counted in spring and the weight of fresh fruit measured at harvest each season from 1979 to 1983. The first crop would have been in 1978 but data were not obtained because a hail storm in spring destroyed most of the inflorescences. 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 (°Brix) and pH of the juice; in seasons 1982 and 1983 titratable acidity (g/l as tartaric acid) was also determined. The mean bunch weight and the number of berries per bunch for each vine were calculated from measured variables.

The weight of 1-year-old growth pruned from each vine plus an estimated weight of the canes retained was obtained in winter as a measure of growth during the preceding growing season. The canes retained each season ranged from 6 to 10 per vine; these were adjusted to a mean of 16 buds per cane and recorded.

All vines were inspected each autumn for leaf symptoms associated with leafroll and yellow speckle.

The respective measurements were subjected to analyses of variance.

Results

Analyses of the annual data showed that there were some significant differences between treatments in some seasons but no differences between untreated H5 and the Thompson Seedless clone in any measured variable. Sub-analysis of yields for the 7 H5 treatments indicated that the differences and trends were generally accounted for by differences between 3 groups: untreated H5 with yellow speckle and leafroll, combining the 3 heat-treated clones with both diseases, and combining those with yellow speckle only (i.e. leafroll was eliminated).

The only significant difference within either of the latter 2 groups was found in 1980 when 1 of the clones with both diseases yielded less than the other 2 and there were no significant interactions between seasons and the 7 treatments. The results over 5 seasons are therefore summarised in the table by presenting the mean annual data for each of these 3 groups and for the Thompson Seedless clone. In both heat-treated H5 groups, the variability of yield from the respective mean was about ±2 %.

The yield of fresh fruit from the heat-treated H5 group with yellow speckle and leafroll was significantly less than that from the other 3 groups. However, yields of heat-treated H5 free of leafroll and of heat-treated Thompson Seedless, free of leafroll, did not differ from that of untreated H5 with both diseases.

The number of inflorescences for the heat-treated H5 group with both diseases was significantly fewer than that for untreated H5 and Thompson Seedless vines, while that for H5 vines free of leafroll was less than for Thompson Seedless and almost so for untreated H5.

The weight of 1-year-old growth did not differ between any of the groups.

Occasional significant differences between the 8 treatments in mean berry weight, calculated mean weight of bunches and sugar concentration were found in some sea-

[4]) The growing season, which covers parts of 2 calendar years, is named by the year of harvest.

Fresh fruit yields, inflorescence numbers, and weights of 1-year-old growth for a heat-treated clone of Thompson Seedless from California and for untreated Sultana H5 and heat-treated clones of H5 · Data for each group are the means over 5 seasons (1979—1983)

Traubenertrag (Frischgewicht), Anzahl der Infloreszenzen und Gewicht des 1jährigen Holzes bei einem wärmebehandelten Klon von Thompson Seedless aus Kalifornien, dem unbehandelten Sultana-Klon H5 und den wärmebehandelten H5-Abkömmlingen · Die Werte jeder Gruppe sind über 5 Vegetationsperioden (1979—1983) gemittelt

Clone	Virus	Yield kg/vine	No. of inflorescences	Growth kg/vine
Thompson Seedless – 91	YS	31.0	79.0	2.38
Untreated H5	YS + LR	30.4	77.4	2.37
Heat-treated H5 – 196, – 311, – 338	YS + LR	28.5	73.1	2.29
Heat-treated H5 – 255, – 280, – 311	YS	30.7	74.7	2.30
LSD P = 0.05 A		1.5	2.8	0.22
LSD P = 0.05 B		1.1	2.0	0.11

LSD A is used for the differences between either untreated H5 or Thompson Seedless and each heat-treated H5 group, and LSD B for differences between the 2 heat-treated H5 groups.
YS = yellow speckle; LR = leafroll.

sons but none of these variables was consistently related to any treatment, and there were no differences between the 4 groups. The actual sugar levels rarely differed by more than 0.5 °Brix, thus corresponding yields of dried fruit would closely follow those of fresh fruit. The pH and titratable acidity of juice did not differ significantly between the groups in any season.

Fruit set on vines sprayed with zinc was not visibly improved in any season. However, the laminae and petiole levels of zinc found in the spring before zinc application (50 and 57 ppm respectively on a dry matter basis) were much higher than those indicative of zinc deficiency (Christensen *et al.* 1978).

Inspections for virus symptoms in autumn usually distinguished the untreated and the 3 heat-treated H5 clones with yellow speckle plus leafroll from the 3 heat-treated H5 clones and the Thompson Seedless clone free of leafroll. The former 4 clones, in most seasons, expressed a yellowish blotching of basal leaves that is associated with leafroll (Woodham *et al.* 1983). Yellow speckle symptoms were detected on most vines of all clones in some seasons. Except for the leafroll symptom, the 6 heat-treated H5 clones did not differ from their untreated source clone in any other morphological way.

Discussion

The results presented show that none of the 6 heat-treated clones of Sultana H5 proved superior to their original source clone which is now widely planted in Australian vineyards. On the contrary heat treatment produced some lower yielding clones. In comparison with untreated H5 the yield of fresh fruit and 1-year-old growth were not affected by elimination of the attenuated strain of leafroll. However, in the continued presence of both yellow speckle and leafroll yield, but not annual growth, was reduced from those of the 2 groups by about 6 %. The lower yield was related to fewer inflorescences which was not due to the retention of fewer buds.

Effects of heat treatment could be due to elimination of viruses or to possible induced mutations either in the virus or host. Our results suggest two possible reasons for the yield responses following heat treatment of H5, either (1) the leafroll strain present in H5 does not reduce yield but the agent was altered by heat treatment, or (2) there was an adverse effect on the yellow speckle agent which was offset by the elimination of leafroll.

As the heat-treated H5 clones were selected at random based on their residual virus status, one could speculate that heat therapy does not invariably produce better clones especially when, as in our case, the source vine is infected with attenuated viruses or those thought to have little effect on vine performance. For such diseases and in areas of no natural spread, the expensive, long term nature of thermotherapy and of the subsequent comparisons between original and heat-treated material may not be warranted.

In our experiment there remains the possibility that the pruning method used (a maximum of 160 buds per vine retained annually) may have prevented the maximum production of some treatments and thus masked potential yield or growth differences. This is under investigation by comparing certain pruning methods on these vines.

Using known *in vivo* heat therapy methods we have been unable to eliminate yellow speckle from 12 other heat-treated explants of H5, from explants of other Sultana clones and of other cultivars, which indicates the heat stability of the yellow speckle agent. BARLASS *et al.* (1982), however, have successfully regenerated plants free of yellow speckle, and of other virus diseases, by culturing fragmented shoot apices; this or similar techniques could well prove better than heat therapy for producing improved vine material.

Our results support the view of BOVEY (1980) who emphasised that the quantity and quality of production from virus-infected and healthy clones obtained by selection or heat treatment should be compared before their distribution to industry.

Summary

A high yielding Sultana clone, H5, infected with yellow speckle and leafroll diseases was compared over 5 harvests with 6 heat-treated H5 clones, 3 of which still contained both diseases and 3 from which leafroll had been eliminated, and also with a Thompson Seedless clone (FV B6V1 HT91) from California.

There were no differences between the untreated H5 clone and the Thompson Seedless clone which was infected with only yellow speckle for any of the measured variables in any season.

The mean yield of heat-treated H5 clones was reduced by 6 % in those which still contained both diseases and was not affected in those from which leafroll had been eliminated. The yield reduction was related to fewer inflorescences.

The mean weight of annual shoot growth, berry weight, calculated bunch weight, sugar concentration and pH and titratable acidity of juice, did not differ between the 4 groups.

Long periods of heat treatment (196—938 d) produced no clones with yields or annual shoot growth superior to the untreated source clone.

Except for expression of leaf symptoms associated with leafroll, all 6 heat-treated H5 clones showed no obvious morphological differences from their original source clone, nor did the heat-treated Thompson Seedless clone.

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