CSIRO Division of Horticultural Research, Merbein, Victoria, Australia

Chemical composition of leaf tissues of Sultana vines grown in nutrient solutions deficient in macro/elements

by

D. McE. Alexander and R. C. Woodham

Introduction

Foliar diagnosis is widely used as a guide to optimum fertilizer practices for a range of horticultural crops (Chapman 1966) but the value of analytical data is dependent on knowledge of three factors: the change in composition of each element during the season for the particular foliage tissue sampled, the relative distribution between tissues, and some standards on which to base a comparison. The best time and best tissue for sampling are based on the first two factors while the third factor is generally based on results of either fertilizer trials or surveys related to plant performance (usually yield).

Effects of a complete range of deficient nutrients can be obtained only in artificial media because for practical reasons field fertilizer trials can at best vary in only a few of the elements essential for plant growth. Furthermore, in a given field situation, generally only one element is limiting so that investigations covering a wide range of situations are necessary to determine standards for all elements. For woody perennials under field conditions appreciable changes in chemical composition may not occur until treatments have been applied for several years so that the establishment of standards may be unnecessarily prolonged. Foliar diagnosis of mineral deficiencies in Vitis spp. is limited because not all elements have been studied adequately and because sampling procedures have not been standardized between workers in this field. Sampling times have generally been related to some phenological stage of development such as flowering, veraison or maturity because changes in chemical composition during the season have been reported for all elements studied (Ulrich 1942, Cook and Kishaba 1956, Shaulis and Kimball 1956, Beyers 1962) and because the rate of vine development varies between years. It is noteworthy that Chapman (loc. cit.) as recently as 1966 while proposing standardization of sampling methods has suggested more than one method of sampling.

The present study using Sultana vines was initiated to determine which leaf tissue was the most suitable for foliar diagnosis of nutrient deficiencies in the spring growth period.

Methods

One hundred and forty Sultana vines were selected for uniform time of bud burst from a batch of 256 struck from dormant two-node cuttings. When their roots were about 8 cm long (October 4) the vines were transferred to constantly aerated nutrient solutions. Initial solutions were ½ strenght complete nutrient based on Hewitt's Long Ashton Formula (Hewitt 1952) and concentrations were increased to full strength during the following two weeks. Iron dimethyldithiocarbamate (2.5 ppm) was added to the solutions to control root fungi. Five vines each with one shoot were established in each of twenty eight 30-litre drums. Nutrient solutions were kept between 20 and 25° C by immersing the drums in temperature-controlled

Composition (meq/l)										
Treatment	NO_3	NH_4	PO_4	K	Ca	Mg	Na	SO ₂	Cl	
Complete	15	1.5	4	5	10	3	2.5	3.2	2.5	
_N	0	0	4	5	10	3	3.7	10	7	
— P	15	1.5	0	5	10	3	4	4.5	4	
— К	15	1.5	4	0	11.8	3.5	4.7	3.7	1.5	
— Са	15	1.5	4	6	0	6	5.3	2	0.5	
Mg	15	1.5	4	5.3	10.5	0	4.2	3.2	2	
_s	15	1.5	4	5	10	3	4	0	7.2	

Table 1
Composition!) (m-equiv./l) of nutrient solutions

water baths. The shoots, from which inflorescences were removed, were exposed in the open to spring-summer ambient temperatures.

After one month (November 4), when shoots were about 20 cm long, treatments were applied by replacing the nutrient solutions with either a complete nutrient solution (control) or solutions wholly deficient in N, P, K, Ca, Mg, or S; each treatment was replicated four times. All solutions were renewed four weeks later. The compositions of macro-elements for each treatment are listed in Table 1.

One vine selected at random was removed from each drum at each of the sampling times November 17 and 28, December 6 and 22 except for the minus sulphur (—S) treatment which was discontinued on December 6. The —N, —K, and —Ca treatments were discontinued on December 22 and the final samples for —P, —Mg and control treatments were taken on January 4. The fresh weight of the shoot (stem and leaves) and dry weight of roots and of trunk were measured on each vine sampled. Both basal and recently matured laminae and petioles from each vine removed on November 28 (24 days after treatments started), December 6 and 22 were sampled for analyses; insufficient material was available on November 17.

All samples were analysed for P, K, Ca, and Mg, but because of insufficient sample N was determined only on laminae sampled on December 6 and 22. Results are reported on a dry weight (105° C) basis. Nitrogen (excluding nitrate) was determined by the Kjeldahl method, P by the molybdate blue method (A.O.A.C. 1965), and K, Ca, and Mg by atomic absorption spectroscopy using methods of Willis (1960 a, 1960 b), and David (1958) respectively.

Lengths of shoots were measured and the number of nodes were counted at weekly intervals from November 4 until December 23 when extensive lateral growth began.

Results and Conclusions

The vines in this experiment commenced growth in spring and were sampled 7 to 10 weeks after budburst. Results would therefore be applicable to about flowering time in the field.

Under field conditions this is a period of rapid change in chemical composition for some elements but treatment differences due to fertilizers are reflected in chemical levels of leaf tissues (ULRICH 1942, COOK and KISHABA 1956). However fertilizers applied at this time may influence the current crop and also the one in the

¹⁾ The following micro elements (concentrations in ppm) were added to all solutions: Fe 5, B 0.37, Zn 0.065, Mn 0.55, Cu 0.064, Al 0.009, Co 0.006, Ni 0.006 and Mo 0.019.

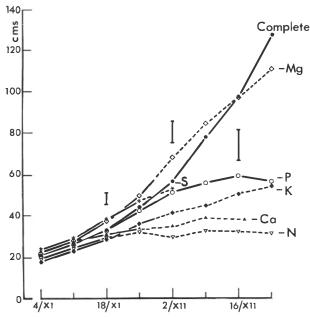


Fig. 1: Mean length (cm) of primary shoots of Sultana grown in complete nutrient and in solutions deficient in N, P, K, Ca, Mg, or S.

L.S.D. (P < 0.05) is shown by bars. — On November 4 each value was a mean of 20 vines; subsequently the number of replications was reduced by 4 at each of the following sampling times; November 17, November 28, December 6 and December 22.

following year because this period marks the start of inflorescence initiation (May and Antcliff 1963).

From the time treatments began foliar deficiency symptoms as described by Woodham and Alexander (1970) first appeared in the respective deficient treatments in 10 days for N, 20 days for Ca and S, and 23 days for K and Mg. Phosphorus deficiency in this experiment showed only as reduced shoot growth.

Mean shoot lengths of all vines in each treatment are shown in Fig. 1 and the mean weights of shoots (fresh weight) and of roots (dry weight) of the vines sampled at each sampling time are shown in Fig. 2 and 3 respectively. In each figure broken lines are used after the onset of symptoms. These figures indicate that all deficient treatments reduced vegetative growth. In contrast to other treatments the —P treatment reduced growth without foliar symptoms becoming evident while in the —Mg treatment symptoms occurred about 4 weeks before a reduction in shoot extension. Treatment effects on the number of nodes paralleled those for shoot length. Trunk (initial cutting) weights did not differ between treatments. It is of interest that shoot weights in the —N and —P treatments were more severely reduced than root weights whereas in the —Mg treatment the reverse occurred. Roots of plants in the —Ca treatment commenced to die from the tips within 7 days of treatments being applied. A complete collapse of the root system followed so that no increase in root weights occurred in this treatment (Fig. 3).

Nitrogen levels in basal and recently matured laminae are shown in Table 2, and P, K, Ca, and Mg levels in the 4 tissues sampled are shown in Fig. 4, 5, 6, and 7

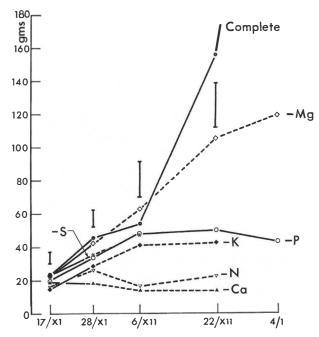


Fig. 2: Fresh weight (gm) of shoots of Sultana grown in complete nutrient and in solutions deficient in N, P, K, Ca, Mg, or S. L.S.D. (P < 0.05) is indicated by bars. Each value is a mean of 4 vines.

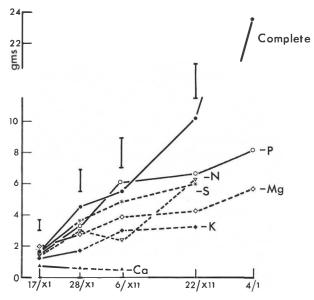


Fig. 3: Dry weight (gm) of roots of Sultana grown in complete nutrient or in solutions deficient in N, P, K, Ca, Mg, or S. L.S.D. (P < 0.05) is shown by bars. Each value is a mean of 4 vines.

						Га	ble 2	2					
Nitrogen	content				,				-		laminae	of	vines
		gr	own i	n co	mplete c	or d	leficie	nt nu	trient so	lutions			

				Trea	tment			
Sampling date	Complete	-N	-P	-K	-Ca	-Mg	-S	L.S.D. (P < 0.05)
Basal laminae								
December 6	3.43	1.58	2.78	3.34	2.65	2.95	3.02	0.47
December 22	3.06	1.31	2.23	2.78	n.d.1)	2.61	2.75	0.38
Recently matu	red laminae							
December 6	4.00	1.79	3.00	3.26	3.14	3.73	3.78	0.40
December 22	4.41	1.32	2.71	3.81	n.d.1)	3.03	4.04	0.63

¹⁾ n.d. = not determined.

respectively. Deficiency symptoms of each element occurred only in the respective deficient treatments although the level of each element was influenced by more than one treatment. The chemical analyses covered the period during which initial deficiency symptoms for each element occurred except those for N for which insufficient sample was available at the appropriate time. The —N treatment values relate to severely N-deficient plants because the earlier samples analysed were taken 3 weeks after symptoms appeared. At this stage basal and recently matured laminae contained less than 1.8% N. A level associated with the onset of symptoms would therefore be higher than 1.8% but less than 2.2%, the level in basal laminae for the —P treatment on December 22 because these vines did not show symptoms.

The P, K, Ca, and Mg levels of each tissue sampled in the respective deficient treatments were markedly reduced before the onset of visual symptoms. This decrease appeared to be closely related to the retardation of shoot growth except for the —Mg treatment.

The P level was severely reduced by both —P and —Ca treatments. However, where P levels are low a comparison of Figs. 4 and 6 indicate that P or Ca deficiency can be distinguished by either a Ca analysis of basal petioles or a P analysis of basal laminae. Phosphorus deficiency is indicated by a low ratio of P content of basal petioles to that of basal laminae; in the present experiment this ratio was lowest (about 0.5) for the —P treatment.

Besides low K or Mg levels deficiencies of K or of Mg are also clearly identified by the ratio of K to Mg values in basal petioles. Vines with ratios less than 1 (Table 3) showed K deficiency symptoms while those with ratios greater than 2.3 did not. Vines with ratios greater than 28 showed Mg deficiency symptoms while those with ratios less than 12 did not. Data of Cook and Goheen (1961) for Zinfandel and of Shaulis and Kimball (1956) for Concord indicate that during the season the K level in leaf tissues decreases while the Mg level increases so that the ratio changes with time. However, ratios calculated from the data of Shaulis and Kimball (loc. cit.) were lowest for K deficient vines.

Five criteria on which the selection of the most suitable sampling tissue for diagnostic purposes could be based are (I) a minimum absolute level, (II) a maximum ratio of control to treatment level, (III) that the level must be specific to deficiency of a particular element (specificity), (IV) the tissue with the earliest onset of low levels following nutrient stress and (V) a minimum coefficient of variation. The coefficient

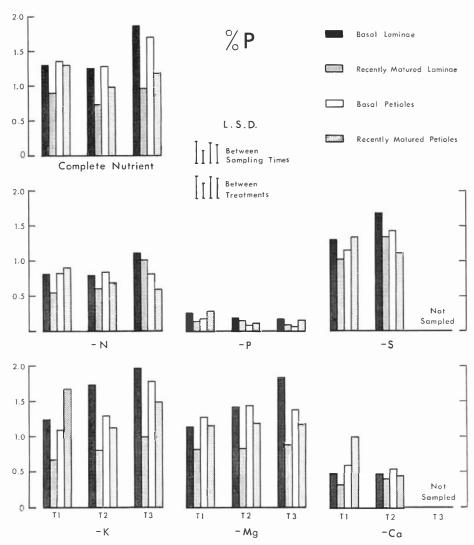


Fig. 4: Phosphorus content (% dry matter) of basal and recently matured laminae and petioles sampled at three times from Sultana grown in complete nutrient or in solutions deficient in N, P, K, Ca, Mg, or S.

T1 = November 28. T2 = December 6, T3 = December 22. L.S.D. (P < 0.05) for the respective tissues between sampling times for each treatment, and between treatments at each sampling time. Replication $\times 4$.

of variation determined over all treatments was in the range from 10 to 30 per cent, except for Mg in both basal and recently matured laminae, for which it was about 5 per cent. It varied inconsistently between elements and sampling times for each tissue and except for Mg was of little value for selecting an optimum sampling tissue. There were insufficient analyses during the early stages in the present experiment to establish which tissue was the first to be depleted. The tissues that would be selected by each of the other three criteria are listed in Table 4.

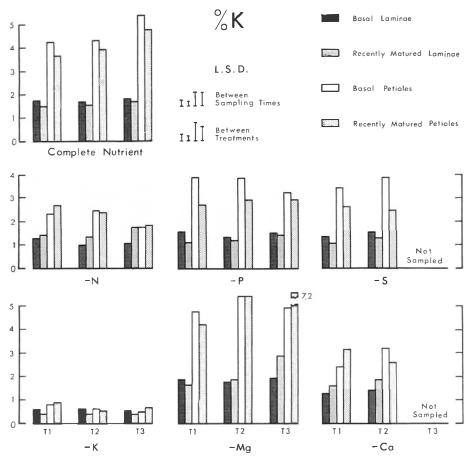


Fig. 5: Potassium content (0 / 0 dry matter) of basal and recently matured laminae and petioles sampled at three times from Sultana grown in complete nutrient and in solutions deficient in N, P, K, Ca, Mg, or S.

T1 = November 28, T2 = December 6, T3 = December 22. L.S.D. (P < 0.05) for the respective

T1 = November 28, T2 = December 6, T3 = December 22. L.S.D. (P < 0.05) for the respective tissues between sampling times for each treatment, and between treatments at each sampling time. Replication $\times 4$.

 $$\rm T~a~b~l~e~3$$ Ratio of K/Mg contents in basal petioles of vines grown in complete or deficient nutrient solutions

			Г	reatme	nt		
Sampling date	Complete	-N	- P	-K	-Ca	-Mg	-S
November 28	7.5	4.0	11.3	0.9	6.0	28	5.6
December 6	7.1	3.4	9.1	0.6	9.1	31	6.0
December 22	11.2	2.3	8.8	0.3	_	35	_

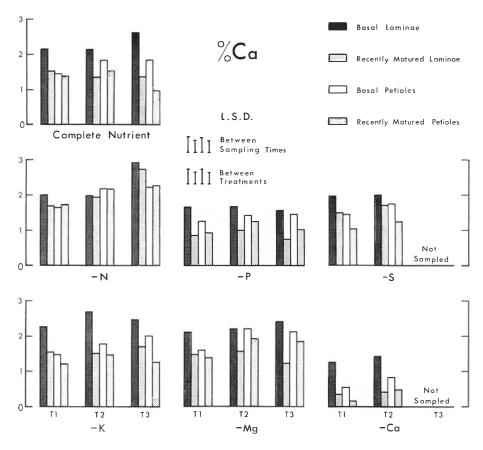


Fig. 6: Calcium content (%) dry matter) of basal and recently matured laminae and petioles sampled at three times from Sultana grown in complete nutrient and in solutions deficient in N, P, K, Ca, Mg, or S.

T1 = November 28, T2 = December 6, T3 = December 22. L.S.D. (P < 0.05) for the respective tissues between sampling times for each treatment, and between treatments at each sampling time. Replication $\times 4$.

It is concluded that of the four tissues sampled basal petioles are the most satisfactory single tissue to sample for diagnosing deficiencies of P, K, Ca, or Mg. However, when concerned only with the Mg status, analyses of recently matured laminae more clearly distinguished between deficiencies of Mg and of Ca or P (Fig. 7). In this study basal petioles were not analysed for N but other work by the authors (unpublished) and data of Cook and Kishaba (1956) indicate that this tissue could also be used to diagnose N deficiency.

The chemical contents of P, K, Ca, aand Mg in basal petioles associated with deficiencies together with the contents of control vines are listed in Table 5. Control values apply to vines with an abundant supply of the respective elements and may represent luxury levels.

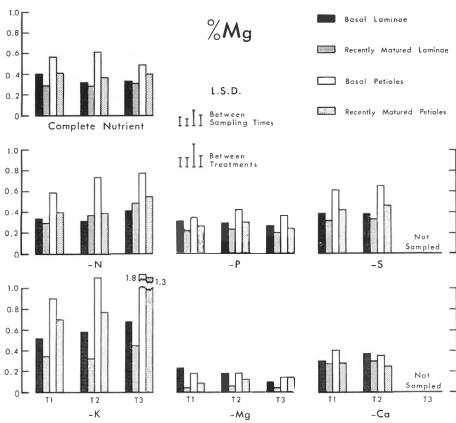


Fig. 7: Magnesium content (0 / 0 dry matter) of basal and recently matured laminae and petioles sampled at three times from Sultana grown in complete nutrient and in solutions deficient in N, P, K, Ca, Mg, or S.

T1 = November 28, T2 = December 6, T3 = December 22. L.S.D. (P < 0.05) for the respective tissues between sampling times for each treatment, and between treatments at each sampling time. Replication $\times 4$.

 $$\operatorname{T}$$ a ble $\,4$ Optimum sampling tissue based on three different criteria for selection

Element	Min. absolute levels	Criteria for selection Max. ratio (control to treatment level)	Specificity
N ^t)	$\mathrm{BL}^2)$	RML²)	Both tissues satisfactory
P	BP^2)	BP	BP
K	RML	BP	All tissues satisfactory
Ca	RML or RMP	RML or RMP	BP
Mg	RML	RML	RML

¹⁾ Only basal and recently matured laminae were analysed.

²) BL = Basal laminae, BP = Basal petioles, RML = Recently matured laminae, RMP = Recently matured petioles.

Table 5

Chemical	content	**					petioles ete nutrie	associated ent	with	deficiency
		Treatme	nt	Deficie	nt le	evel	Rang complete r			

Treatment	Deficient level	Range in complete nutrient')
—_P	0.1	1.3—1.7
—K	0.8	0.9—5.4
—Ca	0.5	1.4—2.3
-Mg	0.2	0.5—0.6

¹⁾ This range may include luxury levels.

Summary

Sultana vines, grown for one month after budburst in complete nutrient solution, were then grown in solutions deficient in N, P, K, Ca, Mg, or S for periods ranging from one to two months.

Shoot and root weights were obtained from vines at five sampling times. The first time was associated with nitrogen deficiency symptoms in the nitrogen deficient treatment and the second time with potassium, calcium, magnesium, and sulphur deficiency symptoms in the respective deficient treatments. In the phosphorus deficient treatment foliar symptoms were not observed but shoot growth was reduced. Shoot lengths were measured weekly.

Contents of P, K, Ca, and Mg in basal and recently matured laminae and petioles, and of N in laminae are discussed in relation to the choice of sampling tissue for diagnostic purposes.

It is concluded that the most satisfactory single tissue for diagnosing deficiencies of P, K, Ca, or Mg is basal petioles.

Concentrations (per cent dry matter) in basal petioles of 0.1% P, 0.8% K, 0.5% Ca, and 0.2% Mg were associated with either the onset of deficiency symptoms or of reduced shoot growth.

Acknowledgements

The authors wish to thank Mr. R. Birtwistle of CSIRO Division of Mathematical Statistics for statistical analyses of results and Messrs. J. M. Eder, I. A. Kneebone and R. E. Palmer for technical assistance.

References

Anonym, 1965: Official methods of analysis 10th Ed., A.O.A.C., Washington, D.C.

Beyers, E., 1962: Diagnostic leaf analyses for deciduous fruit. J. Agricult. Sci. S. Africa 5, 315—329. Chapman, H. D., 1966: Diagnostic criteria for plants and soils. Berkeley, Univ. Calif.

- COOK, J. A. and GOHEEN, A. C., 1961: The effect of a virus disease, leaf roll, on the mineral composition of grape tissue and a comparison of leafroll and potassium deficiency symptoms. Amer. Inst. Biol. Sci. 8, 338—354.
- and Kishaba, T., 1956: Petiole nitrate analysis as a criterion of nitrogen needs in California vineyards. Proc. Amer. Soc. Hort. Sci. 68, 131—140.

Hewitt, E. J., 1952: Sand and water culture methods used in the study of plant nutrition. C.A.B. Tech. Commun. 22.

- MAY, P. and ANTCLIFF, A. J., 1963: The effect of shading on fruitfulness and yield in the sultana. J. Hort. Sci. 38, 85—94.
- SHAULIS, N. and KIMBALL, K., 1956: The association of nutrient composition of Concord grape petioles with deficiency symptoms, growth and yield. Proc. Amer. Soc. Hort. Sci. 68, 141—156.
- ULRICH, A., 1942: Potassium content of grape leaf petioles and blades contrasted with soil analyses as an indicator of the potassium status of the plant. Proc. Amer. Soc. Hort. Sci. 41 204—212
- Willis, J.B., 1960 a: The determination of metals in blood serum by atomic absorption spectroscopy. III. Sodium and potassium. Spectrochim. Acta 16, 551—558.
- , 1960 b: The determination of metals in blood serum by atomic absorption spectroscopy.
 I. Calcium. Spectrochim. Acta 16, 259—272.
- WOODHAM, R. C. and ALEXANDER, D. McE., 1970: Foliar symptoms of malnutrition in the Sultana vine. CSIRO Austral. Div. Hort. Res., Tech. Commun. 1.

Eingegangen am 6. 10. 1969

D. McE. Alexander CSIRO Div. of Horticult. Research Merbein, Vic. 3505 Australia