

USE OF CARBOHYDRATE RESERVES DURING GROWTH FROM CUTTINGS OF GRAPE VINE

By M. S. BUTTROSE*

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Summary

Changes in the carbohydrate status of cuttings of vines (*Vitis vinifera* cv. Sultana) from which plants were developed in a glasshouse were studied by dry weight measurements and analysis for sugars, starch, and hemicellulose. Treatments consisted of one-node cuttings and two-node cuttings (differing initial quantities of carbohydrate reserves) and one-node cuttings with shade (carbohydrate stress).

In all treatments the cane portions lost some 20% of their initial dry weight with a following rapid recovery by unshaded canes, and no recovery by canes of shaded plants during the observed 67 days after planting. Of the 20% loss in cane weight, roughly 1% was accountable for as sugar, 10% as starch, and 7% as hemicellulose. Where dry weight recovery took place starch accumulation was involved, suggesting carbohydrate excess at that time.

Plants as a whole lost weight until some 17 days after bud burst irrespective of treatment, at which time the approximate 50 cm² of leaf present must have been able to photosynthesize at a rate equivalent to whole-plant respiration.

Shoot plus root growth in the shaded treatment was greatly retarded after reserves were exhausted, and temporarily retarded in the one-node group compared with the two-node plants.

It is concluded that plants developed from cuttings of two internodes or longer were not subjected to any degree of carbohydrate stress which might affect physiological processes related to fruitfulness.

I. INTRODUCTION

Physiological studies on perennial fruiting plants in general, and the grape vine in particular, have usually been conducted with field plants or established potted plants. However, for growth-cabinet and glasshouse experiments in the study of some physiological processes of the vine it would be advantageous to use plants arising from cuttings of 1-year-old wood. Such plants differ from established ones in that reserves are not available for new shoot growth to the same extent, the establishment of a new root system necessarily involving depletion of such reserves. Some knowledge of how reserves are mobilized during establishment from cuttings would be useful in assessing the validity of data derived from the resulting plants.

Concerning physiological processes, there is a certain amount of evidence that fruit set of the grape vine, which occurs at roughly 2 months after bud burst, is influenced by carbohydrate status within the plant (Winkler 1929). Furthermore there is some indication that the carbohydrate status in the bud of the Sultana grape vine at a critical period of development is related to the extent of bunch primordia formation within the bud (May 1965). If these processes are studied in small plants it therefore becomes necessary to obtain some information on carbohydrate status during the first few weeks after bud burst of plants arising from 1-year-old cuttings.

*Horticultural Research Section, CSIRO, Glen Osmond, S.A.

No knowledge of this nature has been found documented in the literature, although several workers (notably Winkler and Williams 1945; Eifert, Panczel, and Eifert 1961) have studied carbohydrate changes in field plants or dormant cuttings. For the experiment reported in this paper it was postulated that carbohydrate status of the whole plant arising from a cutting would be reflected in changes occurring in the parent cane portion, and it is these changes which have been measured. The effect of initial carbohydrate supplies was studied by using canes of two different lengths, and the effect of increased demand on reserves by shading of plants. Changes in dry weight, sugars, starch, and hemicellulose were measured.

II. MATERIAL AND METHODS

(a) *Treatment of Cuttings*

Cuttings of 1-year-old canes of grape vine (*Vitis vinifera* cv. Sultana) were collected from an irrigated vineyard in August 1964, covered with paraffin wax, and stored in plastic bags at 4°C. In December, after removal of wax, cuttings were prepared for each of the following three treatments: (1) two-node cuttings; (2) one-node cuttings; (3) one-node cuttings, shaded. These cuttings incorporated buds 4–7 inclusively, counting from the base. For each treatment there were 100 cuttings which, after randomization, were assembled to 10 groups of 10 and the fresh weight of each group was measured. A calculated dry weight was obtained after determining moisture contents of corresponding samples which were dried at 60°C in a forced-air draught to constant weight.

To develop a root system before planting, cuttings were stood 5 cm deep in wet Perlite contained in a box insulated on sides and bottom and having a basal heating element. Temperature at the base of cuttings was held at 25°C, and the box was positioned in a room at 4°C so that buds remained dormant. The lower bud on two-node cuttings was amputated prior to rooting.

After 4 weeks in the rooting box the cuttings were well calloused and approximately six roots of 1 cm length were visible on each cane. They were then planted in 8 cm porous earthenware pots filled with Perlite and placed in a glasshouse. The two unshaded treatments received all sunlight available, whereas the shading treatment consisted of a layer of cheesecloth 30 cm above the pots, allowing 25% of incident light to pass through, as measured by an EEL broad-spectrum light-meter. Pots were supplied liberally with half-strength Hoagland's solution each Monday, Wednesday, and Friday for the first 2 weeks, and thereafter with full-strength solution. All pots were watered freely twice each day with tap water.

The effect of prior rooting was observed by comparing the growth of a further two sets of 10 canes which were planted direct from cold storage into 8 cm pots, and which were harvested at the third harvest.

Harvests were made on removal from the rooting box, at 25 days after planting, and thereafter at 14-day intervals to give a total of five harvests (see legend to Fig. 1). Because carbohydrate analyses are so time-consuming, the number of samples had to be kept to a manageable total and only two samples were collected at each harvest. Thus on each occasion two of the groups of 10 plants were selected at random from

each treatment, and the Perlite washed from the root systems which were then blotted dry. Fresh weights of shoot, roots, and residual cane were measured for each group, followed by dry weight determinations after drying to constant weight at 60°C.

In order to obtain an approximate value for leaf area at earlier harvests, leaf area of two plants from each treatment, chosen to present a range of plant sizes, was determined by the method of leaf tracing and replica weighing. There was a straight-line relationship between leaf area and root plus shoot dry weight per plant, at least up to a total of 600 cm² per plant.

(b) *Total Sugar Assay*

Dry cane was milled to pass a 0.5-mm mesh and subsamples extracted at 78°C with 80% ethanol. After removal of alcohol, total sugars were assayed colorimetrically using the anthrone reagent according to the method of Loewus (1952).

(c) *Starch Assay*

Milled cane was further ground in a roughened glass mortar and pestle to a fine powder. Aliquots were completely extracted with 30% chloral hydrate solution at 80°C and the solubilized starch fraction collected by centrifugation. "Starch" was precipitated by the addition of acetone (75% final concentration) and its dry weight measured. One precipitate per treatment per harvest occasion was further treated with 1N H₂SO₄ at 100°C for 3 hr, and the resulting glucose determined colorimetrically using the Somogyi reagent. Starch contents were calculated from these values. As a check further aliquots of powdered cane were extracted with water at 100°C and starch determined colorimetrically after staining with iodine and reading optical densities at 680 mμ. It was found that the changes in relative values obtained by this method paralleled the changes in absolute values obtained by glucose estimation, from which it is concluded that the glucose estimated did in fact arise from starch.

(d) *Hemicellulose Assay*

Hemicellulose content of selected samples was determined according to a procedure based on that of Norris and Preece (1930). Powdered samples were extracted three times with 0.5% ammonium oxalate at 75°C followed by four extractions with 4% NaOH at room temperature. These latter extracts were combined and after acidification with acetic acid sufficient ethanol was added to give 80% final concentration, and the resulting precipitate dried and weighed as hemicellulose.

III. RESULTS

(a) *Dry Weight Changes of Whole Plants*

As the canes varied in diameter and internode length the initial dry weight of groups varied considerably. For this reason whole-plant dry weights are expressed as a percentage of the calculated initial dry weights of the respective cane sample, as shown in Figure 1. Each point is the mean of two values, which in turn are the mean of 10 plants. Any weight loss by the plant as a whole must be due to respiratory loss, and any weight gain due to a predominance of photosynthesis over respiration. From

Figure 1 it is seen that there was a considerable weight loss for all treatments during rooting and for up to 25 days after planting (harvest 2). As bud burst occurred approximately 8 days after planting, dry weight changes became positive about 17 days after new shoot growth began, and total dry weight returned to initial cane dry weight at 35–40 days after planting. Treatment differences became apparent only after this time, the rate of weight increase being markedly reduced by shading.

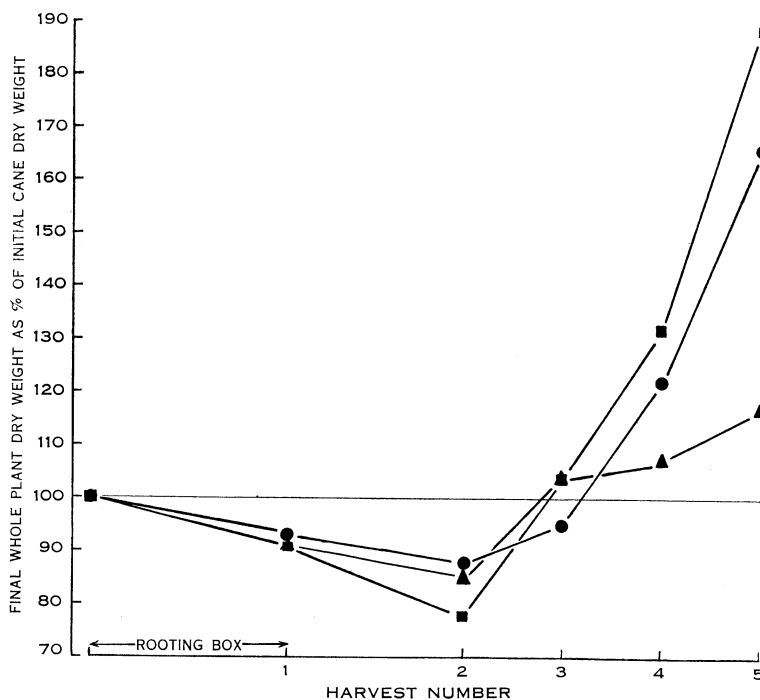


Fig. 1.—Changes in whole plant dry weight with time: ● two-node canes; ■ one-node canes; ▲ one-node canes, shaded. Canes were planted out at harvest 1, and harvests 2, 3, 4, and 5 were respectively 25, 39, 53, and 67 days after planting.

(b) Dry Weight Changes of Roots and Shoots

At planting the dry weight of roots was negligible, but subsequently constituted a similar proportion of the total root and shoot dry weight at any harvest irrespective of treatment, the proportion being 26% at harvest 2, 31% at harvest 3, 30% at harvest 4, and 37% at harvest 5. Thus changes in shoot weight were roughly paralleled by changes in root weight, and the two have been combined for the results shown in Figure 2. (The variability between replicates for any harvest occasion was greater for root and shoot dry weight than for values of percentage dry weight changes of canes. This information is included in Fig. 2.) Treatment effects were not visible until about harvest 3, after which there were considerable divergences. Thus it would appear that for a period between harvests 3 and 4 one-node cane plants were retarded in growth rate relative to two-node cane plants, and the shaded plants almost ceased

growth for a period, and up to the final harvest grew at a relatively slow rate. Two-node plants showed no evidence of a temporary fall-off in growth rate.

Where a root system was not established before bud burst, new growth was greatly retarded (Fig. 2), and no further consideration is given to this group.

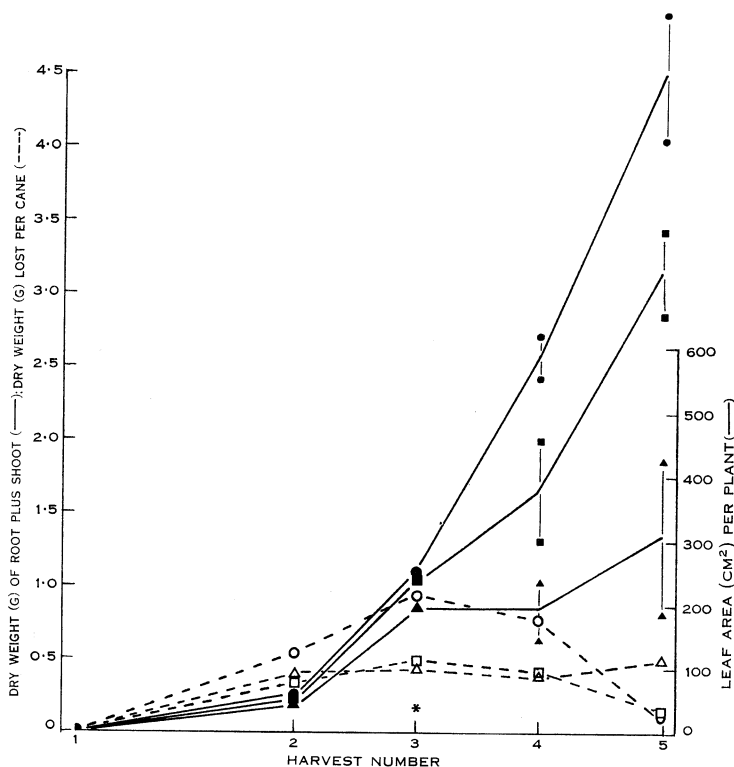


Fig. 2.—Changes in root and shoot dry weight, and in the amount by which cane dry weight fell below that at harvest 1, with time: ●, ○ two-node canes; ■, □ one-node canes; ▲, △ one-node canes, shaded; * unrooted canes. Leaf area was proportional to shoot and root weight up to 600 cm² and is indicated on the right-hand ordinate. Plant age as for Figure 1. Values for individual samples are shown for the last two harvests to indicate variability.

(c) Dry Weight Changes of Canes

Results are presented in Figure 3. A greater percentage of dry weight was lost by one-node than two-node canes during rooting, due doubtless to a relatively greater proportion of their length being exposed to the higher temperature which would influence respiration rate. From planting to harvest 3, canes of all treatments lost weight at a similar relative rate, but between harvest 3 and 5 canes of the unshaded treatments increased in dry weight whereas the shaded ones remained low without change. Two-node canes increased in weight at the end at proportionately the same rate as one-node canes, probably due to their having a larger shoot system to supply carbohydrates. Absolute changes in weight, which are included in Figure 2

from planting time onwards, make it clear that on this basis one- and two-node canes differed considerably. Irrespective of treatment canes had lost between 20 and 22% of their initial dry weight by 40 days (harvest 3) after planting.

(d) *Changes in Carbohydrate Constituents*

Total sugars showed no pronounced changes over the course of the experiment (Fig. 4). Starch content, however, changed in a manner similar to cane dry weight, falling in all treatments during rooting and until the third harvest, with a final increase

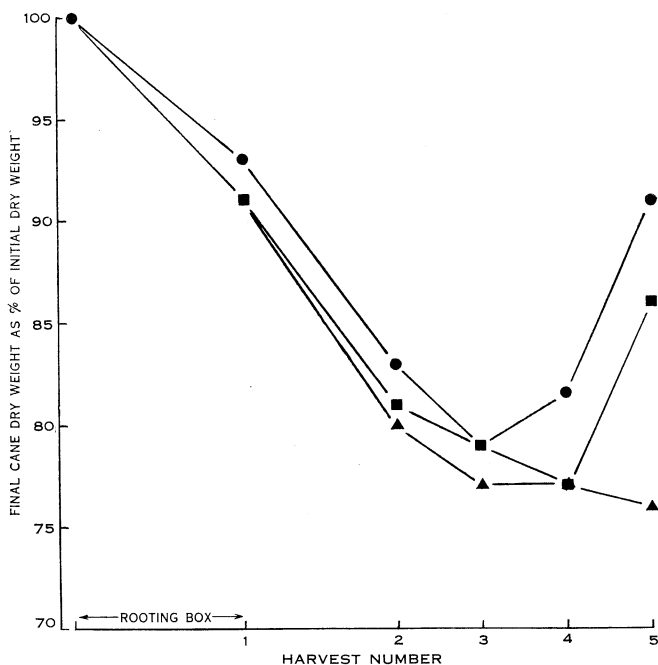


Fig. 3.—Changes in final cane dry weight with time: ● two-node canes; ■ one-node canes; ▲ one-node canes, shaded. Days from planting as for Figure 1.

in the unshaded treatments (more rapid in the two-noded canes) but no final change where shaded. The actual content fell from 12% to a little below 2% of initial cane dry weight.

Hemicellulose contents were determined (as a percentage of initial cane dry weight) on initial cane material and at harvest 3, and the results are given in the following tabulation:

| Initial | Hemicellulose | Harvest 3 | Hemicellulose |
|----------|---------------|-----------------------|---------------|
| Sample 1 | 17.8 | Two-node cane | 13.3 |
| Sample 2 | 17.4 | One-node cane | 11.9 |
| Sample 3 | 18.8 | One-node cane, shaded | 9.6 |
| Mean | 18.0 | | 11.6 |

There was a fall in hemicellulose content over this interval.

IV. DISCUSSION

As pointed out in Section II sample number per harvest was limited to two, hence small differences between treatments have not been considered in this paper, and the following discussion is confined to general features of the results not requiring the support of statistical analysis.

It is apparent from whole-plant dry weights that photosynthesis balanced respiration by, at the latest, 25 days after planting, or some 17 days after bud burst. At this time shoots carried approximately 50 cm² of leaf, and from more detailed leaf area studies in another experiment it is known that this area is achieved when the first leaf is just over half its final area, the second between one-third and one-half,

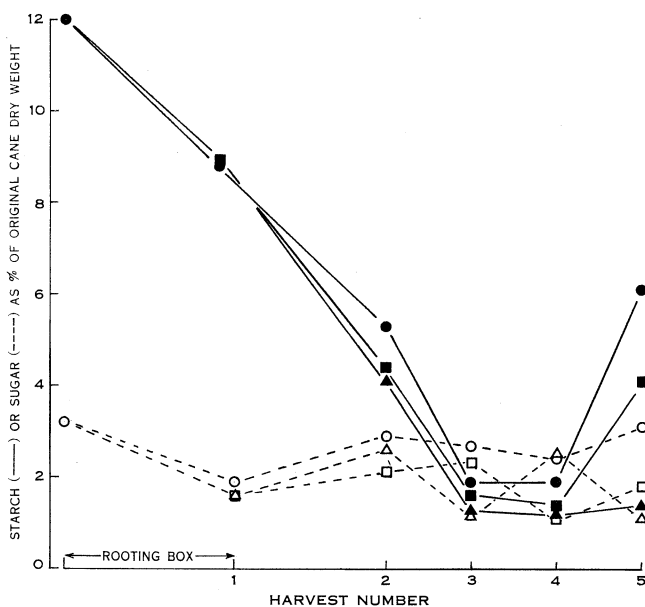


Fig. 4.—Changes in starch and sugar contents of parent canes with time: ●, ○ two-node canes; ■, □ one-node canes; ▲, △ one-node canes, shaded. Days from planting as for Figure 1.

the third about one-quarter, and the fourth leaf just beginning to expand. Thus a relatively small amount of immature leaf was able to meet respiratory needs of roots, parent cane, and shoot. It would be expected that with an increase in size of parent cane there would be a corresponding increase in respiration and consequently in requisite leaf area. There is some indication of this in Figure 1, where the curve for two-node plants shows a lag between harvests 2 and 3, compared with one-node plants, and there is a further indication in Figure 2 where it is seen that weight lost by two-node canes was greater than that lost by one-node canes between harvests 2 and 3, although leaf areas were comparable. It would furthermore be expected that under shade photosynthetic activity would be reduced and requisite leaf area increased, but the data obtained do not demonstrate such an effect.

The greater amount of reserve material present in two-node as compared with one-node plants had no effect on root and shoot growth up to 39 days after planting (harvest 3), by which time reserves were fully depleted in both cases. The question then arises as to the fate of the "extra" reserves in the two-node plants. It is apparent from Figure 2 that while root and shoot dry weight for both unshaded treatments rose by just over 0.8 g between harvests 2 and 3, dry weight lost by two-node canes was 0.39 g and by one-node canes was 0.15 g, so that the extra reserves used during the period amount to 0.24 g. The greater demand by the larger canes for respiratory substrate must certainly be one explanation for this use, and as cane respiration rates were not known some measurements were made of CO_2 evolution in the dark at 21°C (mean glasshouse temperature). A value of roughly 0.09 mg CO_2 /g fresh weight/hr was obtained, corresponding to some 0.37 g CO_2 for two-node cuttings for the period between the two harvests. This value is considered very high for woody tissue, and Winkler and Williams (1945) have pointed out that misleading results may be obtained in measuring cane respiration by means of CO_2 evolution, but it appears that respiratory needs of the cane may be of the same order as reserves utilized between harvests 2 and 3. However, the mention of two other possible reasons for the greater (absolute) reserve loss by two-node canes at this time is considered warranted. First, there may have been a temporary storage in roots and shoots, which could be one explanation for the superior growth of two-node plants between harvests 3 and 4. Secondly, photosynthesis may have proceeded at a slower rate in two-node plants when an alternative supply of carbohydrates (as reserves) was still available.

The initial starch content of 12% is, on the one hand, similar to the late autumn maximum contents of canes reported for example by Winkler and Williams (1945) and Eifert, Panczel, and Eifert (1961). These authors and others have found a marked fall in starch and a corresponding rise in sugars during winter under natural conditions, followed by a partial reversal to give a second starch peak in early spring. Furthermore, Eifert, Panczel, and Eifert (1961) have found the second starch peak even if cuttings are kept continuously at 4°C; it is possible that if canes are stored at this temperature till midsummer, as in the present case, the second peak is maintained. On the other hand the sugar level of 3% is much lower than midwinter (12%) or early spring values found by the same authors. It is possible that sugars are depleted in respiration during cold storage without the starch content being affected.

Whether hemicellulose of vine canes can function as a reserve carbohydrate is still controversial. Winkler and Williams (1938) concluded from extensive analyses of plant parts throughout the year that hemicellulose content remained constant, whereas Afrikjan, Marutjan, and Saakjan (1954) and Eifert, Panczel, and Eifert (1961) concluded that it can fall due to mobilization. The fall in hemicellulose content found in the present experiment, representing some 6.4% of initial cane dry weight, speaks strongly for it being classed as a reserve material, although under normal field conditions it may not be mobilized. As starch loss accounts for a little over 10% of initial cane dry weight, sugar loss for a further 1%, the 6.4% value for hemicellulose brings the total loss accounted for to nearly 18% of initial cane dry weight. It remains for more extensive analyses than have been attempted here to complete the balance sheet.

Between the last two harvests canes of unshaded treatments had started to increase in weight, and this raises the question of whether the growth of shoots and roots was carbohydrate-limited at this relatively early stage. Canes of course also undergo growth with callousing and secondary thickening, and it was found that at the final harvest there was up to 1 mm thickness of new wood over a limited portion at the base of two node canes, and to a lesser extent in unshaded one-node canes. This accounts for part of the cane weight increase. However, by the end of the experiment the unshaded canes had stored starch, and as this is looked upon purely as a reserve substance, playing no active part in cell metabolism, it would appear that carbohydrates were indeed in excess of requirements.

In viticultural practice cuttings are usually considerably longer than those employed in this experiment, and it is a common observation that length of cuttings is without influence on shoot growth, although no experimental data to confirm this belief have been encountered. There is one report (Tleuz 1940) that two-node cuttings give rise to better plants than those with one-node, which is confirmed by the present results, indicating that when cuttings are sufficiently short there is an influence of length on root and shoot growth. However, a more extensive study would be needed to show whether this influence persisted for canes longer than two internodes.

The short period of possible carbohydrate stress undergone by one-node plants, but which two-node plants apparently avoided, falls at a time which cannot be far removed from the period (approx. 10 weeks after bud burst) when fruitfulness of Sultana vines is influenced by weather (Baldwin 1964), and this is also roughly the time of fruit-set determination. For this reason a two- or three-node cutting may be preferable to one-node cuttings as a source of experimental plants. In this regard, however, it is impossible to generalize because variations in cane thickness will involve corresponding variations in quantities of reserves per internode, and as the shading treatment clearly showed environmental conditions under which the plants are grown can be critical. It should be stressed on the one hand that shoots developing on these plants were not as vigorous as those on established plants, and thus not directly comparable, but on the other hand it has been found (Buttrose, unpublished data) that the buds developed on such shoots are fruitful.

V. ACKNOWLEDGMENT

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VI. REFERENCES

- AFRIKJAN, B. L., MARUTJAN, S. A., and SAAKJAN, R. G. (1954).—Forms of reserve carbohydrate in vine. [In Russian.] *Dokl. Akad. Nauk SSSR* **96**: 1195–6. [cf. *Hort. Abstr.* **25**: 264 (1955).]
- BALDWIN, J. G. (1964).—The relation between weather and fruitfulness of the sultana vine. *Aust. J. Agric. Res.* **15**: 920–8.
- EIFERT, J., PANCZEL, M., and EIFERT, A. (1961).—Änderung des Stärke- und Zuckergehaltes der Rebe während der Ruheperiode. *Vitis* **2**: 257–65.
- LOEWUS, F. A. (1952).—Improvement in anthrone method for determination of carbohydrates. *Analyt. Chem.* **24**: 219.
- MAY, P. (1965).—Reducing inflorescence formation by shading individual sultana buds. *Aust. J. Biol. Sci.* **18**: 463–73.

- NORRIS, F. W., and PREECE, I. A. (1930).—Studies on hemicelluloses. I. The hemicelluloses of wheat bran. *Biochem. J.* **24**: 59–66.
- TLEUZ, G. I. (1940).—The propagation of vines by means of short cuttings having one or two buds each. [In Russian.] *Bjull. Kazah. Naucno-issled. Inst. Zemled. im. Akad. V. R. Viljjamas.* No. 7–8. pp. 22–9. [cf. *Hort. Abstr.* **14**: 537 (1944).]
- WINKLER, A. J. (1929).—The effect of dormant pruning on the carbohydrate metabolism of *Vitis vinifera*. *Hilgardia* **4**: 153–73.
- WINKLER, A. J., and WILLIAMS, W. O. (1938).—Carbohydrate metabolism of *Vitis vinifera*: Hemicellulose. *Plant Physiol.* **13**: 381–90.
- WINKLER, A. J., and WILLIAMS, W. O. (1945).—Starch and sugars of *Vitis vinifera*. *Plant Physiol.* **20**: 412–32.