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DIURNAL VARIATIONS OF SOLUBLE SUGARS IN *EUCALYPTUS REGNANS*

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Key Word Index—*Eucalyptus regnans*; Myrtaceae; cambial saps; developing tissues; diurnal variations; soluble sugars; galactinol; myoinositol; raffinose; stachyose; water content.

Abstract—Diurnal variations in the amounts of soluble sugars in the developing stem tissues of *Eucalyptus regnans* were studied. The amounts of sucrose, of its hydrolysis products and of the other sugars estimated in the xylary tissues reached a maximum in the early-afternoon, early-morning and late-evening, respectively. In general, maximal amounts of the same sugars were reached a few hours earlier in the phloem tissues. Hydrolysis of sucrose was also observed and the overall variation in the concentration of sugars suggested the hydrolysis and synthesis of oligosaccharides. When the amounts of soluble sugars were expressed in terms of sap concentrations, the various sugars reached maximum values at the times indicated above, except in the case of fructose and glucose.

INTRODUCTION

MANY workers have investigated the translocated sugars in woody plants. Zimmermann[1] reported that sucrose, raffinose and stachyose occurred in many tree species. Franz and Meier[2] investigated the cambial sap of *Larix decidua* and isolated glucose, fructose, sucrose, myoinositol, coniferin and sequoitol; myoinositol was also found in the cambial sap of *Eucalyptus regnans*.[3] Oesch[4] and Stewart *et al.*[5] found galactinol as one of the components in the cambial saps of *Fagus silvatica* and *E. regnans*.

Daily changes in the sugar concentrations of sieve-tube saps of defoliated *Fraxinus americana* have been studied by Zimmermann.[6] Stewart *et al.*[5] have examined seasonal variations in the chemical composition of the cambial saps of *E. regnans* trees. As a sequel to this study, we now report on the diurnal changes of sugar concn in the same tissues from this eucalypt. Our main aim was to ascertain whether or not the changes are due to daily variations in the water content[7] of the tissues sampled.

RESULTS AND DISCUSSION

The cambial saps in the dividing and differentiating tissues of phloem and xylem were examined. The diurnal variations of water content[7] and the seasonal variations of sugars[5] in these tissues have been shown by statistical analyses to be significantly greater than would be expected from random variation between and within trees.

[1] ZIMMERMANN, M. H. (1957) *Plant Physiol.* **32**, 288.
[2] FRANZ, M. and MEIER, H. (1967) *Planta (Berl.)* **73**, 155.
[3] STEWART, C. M. and AUSTIN, S. C. (1963) *Nature* **197**, 203.
[4] OESCH, F. (1969) *Planta (Berl.)* **87**, 360.
[5] STEWART, C. M., MELVIN, J. F., DITCHBURNE, N., THAM, S. H. and ZERDONER, E. (1973) *Oecologia (Berl.)* **12**, 349.
[6] ZIMMERMANN, M. H. (1958) *Plant Physiol.* **33**, 213.
[7] STEWART, C. M., THAM, S. H. and ROLFE, D. L. (1973) *Nature* **242**, 479.

In the following discussion the concentration of sugars, except where otherwise indicated, is given in mg/100 mg of soluble dry matter. The diurnal variations of the concentrations of the estimated sugars are shown in Figs. 1–4. The variations in the concentrations of sugars were assumed to take place mainly within the sampled tissues, although Zimmermann[8] has shown that there is some variation in the sugar concentrations within the sieve-tube saps from *F. americana*. The sugars present in the cambial saps of *E. regnans* were fructose, glucose, galactose, sucrose, myoinositol, galactinol, raffinose and stachyose; the last two sugars were estimated together as total oligosaccharides.

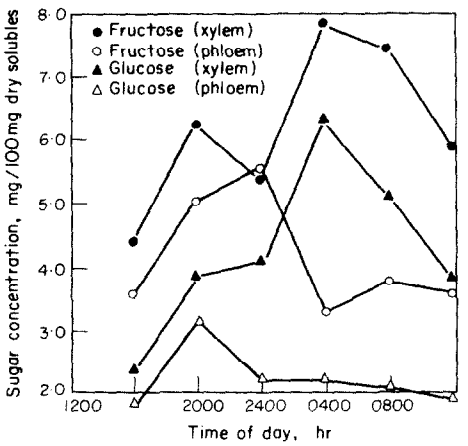

FIG. 1. DIURNAL VARIATIONS OF GLUCOSE AND FRUCTOSE IN STEM OF *Eucalyptus regnans*.

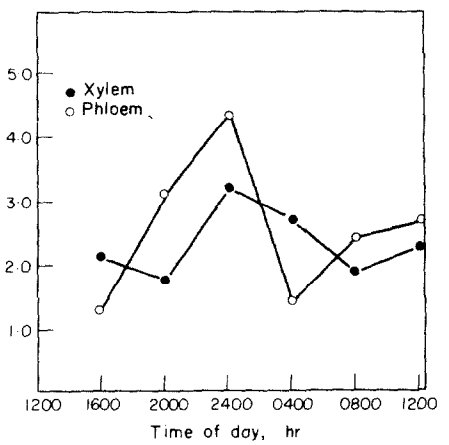

FIG. 2. DIURNAL VARIATION OF GALACTOSE IN STEM OF *Eucalyptus regnans*.

During the 24 hr sampling period, the concentration of fructose was consistently greater than that of glucose in the cambial saps. The concentrations of fructose, glucose and sucrose were greater in the developing xylem than in the developing phloem, but the reverse effect was observed for the concentrations of galactose, myoinositol, galactinol and the oligosaccharides.

In the sampled tissues, the sugar concentrations in most cases (except galactose, glucose and fructose) were greater in the early-evening (16:00–20:00 hr) than in the early morning (04:00–08:00 hr) period. The diurnal variation of water content in these tissues (samples used in this experiment) has been recorded[7] and it was found that early morning samples of the developing xylem tissues contained about twice as much H_2O as those collected during the late-afternoon, early-evening period. The results indicate that sugar concentrations in the saps are inversely related to the water content of the tissues; i.e. during periods of high water content, the sugar concentrations (sap basis) are low and vice versa. For example, in the developing xylem, maximum sucrose concentration (sap basis at 16:00 hr) occurs just before the time of minimum H_2O content (20:00 hr). Maximum concentrations (sap basis) for other sugars occurred at a later time.

Greater amounts of glucose and fructose occurred in the developing cells of the xylem than in those of the phloem during the period of minimum sucrose concentration (Figs. 1 and 3). The possibility of sucrose being converted to glucose and fructose during translocation across the developing tissues was investigated. When either fresh phloem or xylem tissues were incubated with sucrose-^{14}C, radioactive glucose and fructose were formed.

[8] ZIMMERMANN, M. H. (1957) *Plant Physiol.* **32**, 399.

However, if glucose-^{14}C or fructose-^{14}C was used as labelling sugar, radioactive sucrose was not observed. The failure to observe the synthesis of sucrose may be due to the absence or inactivation (during sampling) of a sucrose synthesizing enzyme in the cambial sap.[5] Gardner and Peel[9] investigated the transport of sucrose into the sieve tubes of *Salix viminalis* and suggested that the plasmalemma was impermeable to sucrose, but permeable to glucose and fructose. Sucrose was thought to be hydrolysed by a free-space invertase[9,10] to glucose and fructose before diffusing across the plasmalemma and into the sieve tube. In the present work, however, sucrose was observed in the developing tissues. Therefore, it is possible that sucrose either was able to diffuse across the sieve-tube plasmalemmas into the developing stem tissues or was resynthesized after leaving the sieve tubes. During translocation across the developing tissues some sucrose molecules undoubtedly break down to glucose and fructose. In the developing xylem rapid breakdown of sucrose occurred between 16:00 and 04:00 hr, whereas in the phloem breakdown occurred between 20:00 and 24:00 hr. The hydrolysis of sucrose was found to be associated with the maximum concentrations of glucose and fructose (Fig. 1). These results suggest the presence of a sucrose hydrolysing enzyme localized in the developing tissues of the phloem and of the xylem.

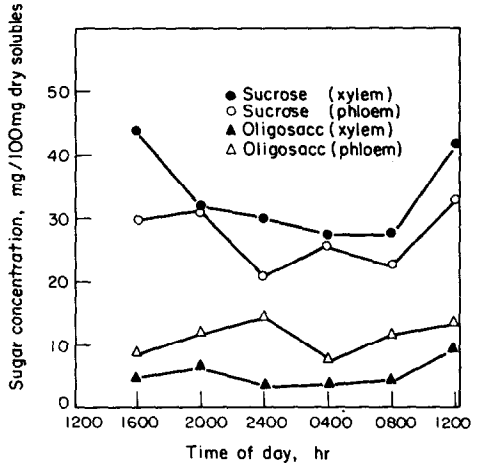

FIG. 3. DIURNAL VARIATIONS OF SUCROSE AND OLIGOSACCHARIDES IN STEM OF *Eucalyptus regnans*.

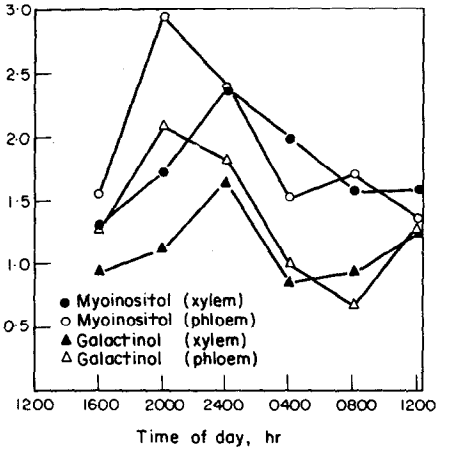

FIG. 4. DIURNAL VARIATIONS OF MYOINOSITOL AND GALACTINOL IN STEM OF *Eucalyptus regnans*.

Myoinositol concentration was higher than that of galactinol and the shapes of the curves in Fig. 4 indicate that the two components vary diurnally in a similar way. The presence of galactose and myoinositol in the tissues suggests that galactinol is synthesized in the cambial saps. In addition, both galactose and galactinol have been reported to be involved in the biosynthesis of the oligosaccharide stachyose.[11] The results also suggest that the synthesis and breakdown of the oligosaccharides occur concurrently. During early morning (24:00 hr), the maximum concentration of oligosaccharides in the phloem (Fig. 3) was preceded by those of galactinol and myoinositol (20:00 hr, Fig. 4), suggesting the synthesis of oligosaccharides. Also, the maximum concentration of the oligosaccharides

[9] GARDNER, D. C. J. and PEEL, A. J. (1971) *Phytochemistry* **10**, 2621.
[10] PEEL, A. J. and FORD, J. (1968) *J. Exp. Botany* **19**, 370.
[11] TANNER, W. and KANDLER, O. (1966) *Plant Physiol.* **41**, 1540.

in the xylem (12:00–16:00 hr, Fig. 3) was followed by maximum concentrations of galactose, myoinositol and galactinol (24:00 hr, Figs. 2 and 4), indicating breakdown of the oligosaccharides. Kandler[12] showed that galactinol was not translocated from the leaves and suggested that the minute amount of galactinol found in the stems of *Lamium maculatum* was formed from other translocated sugars. The galactinol found in the cambial tissues of *E. regnans* could also be derived from transported sugars such as the non-reducing oligosaccharides or their hydrolysis products.

Although the enzymes in the cambial saps were not investigated, the diurnal changes of the sugars studied suggest the presence of both synthetic and hydrolytic reactions. Such reactions are intimately related to the interconversions of sugars and cellular developments in the cambial zones.

EXPERIMENTAL

Collection of samples. Nine self-sown previously unsampled *Eucalyptus regnans* (F. Muell.) trees growing on Mt. Dandenong, Victoria, were sampled at 4-hr intervals. The soft tissues from the developing xylem and phloem surfaces were sampled by the technique of Stewart *et al.*[5,7] and were taken separately from 16:00 hr on 1 day to 12:00 hr on the following day. The samples were quick frozen and subsequently freeze-dried to give estimates of dry-matter and moisture contents. The dry tissues were then pooled to give composite phloem and xylem samples.

Analytical methods. The soluble sugars were extracted from the dry samples with 80% EtOH,[5] concentrated and separated by descending PC using *n*-BuOH–Me_2CO–H_2O (4:5:1) at 25° for 64 hr. With the aid of marking strips, the chromatograms were cut into 5 sections: A, B, C, D, E (in descending order). A contained the oligosaccharides and galactinol. B, myoinositol. C, sucrose. D, galactose and glucose and E, fructose and mannose. Mannose was added as an internal standard. The sections were eluted with dist. H_2O.

Sucrose was hydrolysed by refluxing with 1% oxalic acid for 1½ hr[13] and the oligosaccharides and galactinol by refluxing with 0·5 N H_2SO_4 at 100° for 4 hr. Hydrolysates were neutralized with 4 M KOH using phenol-red as indicator. The sugars were converted to the corresponding alditol acetates[14,15] and analysed by GLC. Sucrose, galactinol and the oligosaccharides were estimated as the sum of the hydrolysis products. Sugars concns were expressed as mg/100 mg dry solubles. The results can also be expressed as % sap concentrations by using the data of Stewart *et al.*[7]

Labelled compounds. Sucrose-U-^{14}C, glucose-U-^{14}C and fructose-U-^{14}C were obtained from commercial sources. Experimental details have been reported elsewhere.[5]

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[12] KANDLER, O. (1961) in *Harvesting the Sun—Photosynthesis in Plant Life* (SAN PIETRO, A. S., GREER, F. A. and ARMY, T. J., eds.), p. 131, Academic Press, New York.
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[14] CROWELL, E. P. and BURNETT, B. B. (1967) *Anal. Chem.* **39**, 121.
[15] BORCHARDT, L. G. and PIPER, C. V. (1970) *Tappi* **53**, 257.