ACTION SPECTRA FOR THE ASSIMILATION OF $^{14}$C INTO LEAF SUGAR COMPONENTS OF SUGARCANE

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ABSTRACT

The effects of monochromatic light on the incorporation of $^{14}$C into leaf sugar components of sugarcane ($S. \textit{officinarum} \textit{L.}$, variety PR 980) were studied. A 4.8 m diffracted light spectrum was produced with a high-pressure xenon arc and 25 cm diffraction grating. Ten wavelengths of equal quantum flux were examined between 400 nm in the blue-violet and 710 nm in the far-red. Leaf sugars soluble in boiling 95% ethanol were isolated and identified by paper chromatography. Principal results were as follows: (a) a predominance of sucrose as the main recipient of $^{14}$C at all test wavelengths shorter than 710 nm; (b) a distinct action spectrum for sucrose with larger contributions from the orange and near-red regions, smaller but important contributions from the blue region, and a pronounced green depression; (c) an essentially constant proportion of $^{14}$C entering sucrose at all test wavelengths shorter than 710 nm; and (d) a predominance of d-raffinose as the principal recipient of $^{14}$C in far-red light. The importance of far-red light in the production of d-raffinose is interpreted in terms of a more efficient initial movement of $^{14}$C in sugarcane leaves.

INTRODUCTION

It is generally accepted that sucrose is both the principal photosynthate and transient form of sugar in leaves of sugarcane (Hartt, et al., Hatch and Glasziou, Hatch and Slack, Kortschak, et al.). Less certain is the stability of sucrose as it passes from its site of biosynthesis to conducting phloem en route to the leaf sheath and sink tissues. Transport roles for hexose and triose sugars, sucrose analogs, or various sugar derivatives in equilibrium with sucrose remain almost totally obscure (Wardlaw), as do the mechanisms themselves for vein loading and translocation. Moreover, present knowledge of leaf sugars and their movement stems mainly from white-light or natural-illumination experiments. Hence, the contributions of discrete spectral regions in determining sugar forms and their relative proportions preparatory to their efflux from the leaf are unknown. In the present study the effects of monochromatic light on the incorporation of radioactive carbon into different sugars in cane leaves were investigated.

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MATERIALS AND METHODS

Variety PR 980, an interspecific commercial hybrid, was used in conjunction with a biological spectrograph located at Washington State University, Pullman. A description of the spectrograph together with methods for plant propagation, plant treatment, and preparation and administration of $^{14}$CO$_2$ is detailed in prior publications (Alexander and Biddulph).

Principal features of the spectrograph include a high-pressure xenon arc and 25 cm diffraction grating with 1200 ruled lines/mm. A continuous diffracted-beam spectrum is attained from 225 to 775 nm. Test wavelengths of known range and intensity are readily administered to biological materials along a 4.8 m focal curve. In the present study, 10 wavelengths were examined between 400 nm in the blue-violet and 710 nm in the far-red. Quantum flux was equalized with the wavelength of lowest intensity, i.e., at 8.08 photons cm$^{-2}$ sec$^{-1}$, by regulation of amperage input to the xenon arc.

$^{14}$CO$_2$ generated from Ba$^{14}$CO$_2$ was administered as a standard dose of 44.8 $\mu$Ci to the apex of leaf +1 (bearing the uppermost visible dewlap). The latter was enclosed in a lucite chamber and equilibrated for 10 minutes. All light was excluded from the facility except the test wavelength centered on the blade’s midvein in a 10 cm monochromatic beam. Air flow was 1.5 l/min., air temperature was 22.5°C ($\pm$ 1°C), and total volume of the air-circulatory system was 0.589 l.

One hour was allowed for $^{14}$CO$_2$ assimilation, the remaining nuclide being trapped by diversion through Ba(OH)$_2$. The initial CO$_2$ concentration for all test wavelengths was approximately 330 ppm. Maximum consumption (at 620 nm) did not exceed 60 percent. Leaf samples, including lamina and midvein, were quick-frozen, lyophilized, and extracted with boiling 95% ethanol. The extractions were twice repeated. Residual extracts were combined and centrifuged at 5000 x g.

Duplicate samples of each clarified extract were co-chromatographed with authentic sugars on Whatman No. 1 chromatography paper. They were irrigated in one dimension with butanol-pyridine-water (6:4:3 v/v). Sugars were located in one duplicate by silver nitrate staining (Dube and Nordin). The corresponding, unstained regions of the second duplicate were cut out and extracted three times with boiling 95% ethanol. Samples of the clarified extracts were evaporated to dryness in stainless steel planchets and monitored with a Nuclear Chicago Model D-47 gas-flow counter operating at 1100 v. Each sample was counted for 10 min. Activity values were corrected for background, instrument efficiency (40%), and self-absorption of the dried residues. Activity was computed as cpm mg$^{-1}$ of lyophilized tissue or as percentages of the total assimilated nuclide recovered per mg of tissue.

* *Saccharum officinarum* 11/16, *S. spontaneum* 3/16, and *S. sinense* 2/16.
The 1-hr exposure to $^{14}$CO$_2$ sufficed for a maximum of about 2 percent of the assimilated carbon to be translocated out of the leaf chamber at wavelengths shorter than 710 nm. Hence, the labeled sugars depicted in Figures 1 and 2 are involved either in temporary storage or the initial stages of transport.

The predominance of sucrose as the main $^{14}$C recipient is consistent with earlier reports identifying sucrose as the principal photosynthate of sugarcane (Hartt, et al.; Hatch and Slack). Further to this, its persistent dominance from the red to the blue-violet regions suggests that no spectrally sensitive, rate-limiting step exists in the long chain of biochemical reactions culminating in sucrose biosynthesis. To the contrary, the spectrum for sucrose (Fig. 1) conforms closely with published action spectra for net CO$_2$ assimilation in a range of higher plants (Balegh and Biddulph; Bulley, et al.; McCree). It conforms less closely with the net CO$_2$ assimilation spectra of Saccharum species in which maximum activity is found in the blue at 480 nm (Alexander and Biddulph).
Despite large differences in total $^{14}$C assimilation among different wavelengths, the proportion of $^{14}$C found in sucrose remains essentially constant from the red to the blue-violet regions (Fig. 2). An apparent trend toward increased unidentified carbohydrate in the blue-violet, from 437 to 400 nm, may reflect diversions of $^{14}$C from sucrose and d-raffinose. However, from present evidence it is doubtful whether monochromatic light can produce major changes in the sucrose eminence, either directly or at the expense of other leaf sugars. Moreover, while present analytical methods did not distinguish between mobile and stationary sugars, the small amounts of $^{14}$C found in d-fructose and d-glucose are consistent with the view that plants do not translocate reducing sugars (Trip, et al., Wardlaw). A lone exception on the proportionate dominance of sucrose is found in far-red light, at 710 nm, where d-raffinose trenchantly exceeds all other sugars produced at that wavelength (Fig. 2). Important implications of this feature include: (a) A possible far-red control of sucrose-linkage interconversions, and (b) a mechanism for increased transport efficiency in far-red light. Raffinose synthesis involves an elaboration or conversion of sucrose already formed, thereby diverting sucrose from storage or utilization in growth and respiratory processes.

The possibility of d-raffinose contributing toward sugar transport processes is not discounted. Both d-raffinose and its tetrasaccharide derivative d-stachyose are important transport sugars in a range of higher plants (Burley, Pristupa, Trip, et al.). Hatch and Glasziou point out that the $^{14}$C carrier in sugarcane may be a sucrose derivative assuming a rapid equilibrium with sucrose in which the derivative exists only in small amounts. Trip et al. found that the transport order of preference for $^{14}$C-labeled sugars in white ash and lilac places both d-stachyose and d-raffinose ahead of sucrose. To the extent that rate-limiting steps of vein loading and transport are better accomplished by a sucrose derivative, the role of d-raffinose in far-red light may be that of a $^{14}$C carrier offering more efficient movement in the early stages of sucrose transport.

REFERENCES


ESPECTRO DE ACCION PARA LA ASIMILACION DE CARBONO 14 DENTRO DE LOS COMPONENTES DE AZUCARES DE LA HOJA DE CANA DE AZUCAR

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RESUMEN

Fueron estudiados los efectos de la luz monocromática en la incorporación del C 14 dentro de los componentes de azúcares de las hojas de caña de azúcar. (S. officinarum L, variedad PR 980). Con un arco de xenón de alta presión, fue producida una difracción de la luz en el espectro de 4,8 m y una difracción de 25 cm de abertura.

Diez longitudes de onda de iguales cantidades de flujo fueron examinadas entre 400 nm en el azul violeta y 710 nm en el rojo lejano. Se aislaron los azúcares solubles contenidos en la hoja de caña por ebullición en etanol a 95% y se identificaron por cromatografía de papel. Los principales resultados obtenidos fueron los siguientes: a) una predominancia de sacarosa como el principal portador del C 14 en todos los tests de longitudes de onda menores de 710 nm; b) una distinta acción del espectro para la sacarosa con mayores contribuciones desde las regiones del naranja y rojo cercano y pequeñas pero importantes contribuciones desde la región del azul y una pronunciada depresión verde; c) una proporción esencialmente constante del C 14 que entra en la sacarosa en todos los tests con longitudes de onda más cortas de 710 nm y d) una predominancia de la d-rafinosa como el principal portador del C 14 en la luz rojo lejano. La importancia de esta luz en la producción de la d-rafinosa es interpretada aquí en términos de un movimiento inicial más eficiente del C 14 en las hojas de caña de azúcar.
OBSERVATIONS ON CANE RIPENING IN THE IRANIAN WINTER

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ABSTRACT

Cane grown in the nearly rainless months from April to October is ripened by withholding irrigation water and by the falling temperatures. The rate of improvement in juice quality may be retarded by winter rains, and reduced or temporarily halted as the temperatures fall near freezing, ripening resuming shortly after the return of more favorable conditions. Recently, ripening did not resume for nearly 7 weeks after a mild frost, but unexpectedly only after the first rains. This is in contrast to the usual effect of winter rains. Thus, there seems to be a parallel between the effects of mild and severe cold — promotive and inhibitory to ripening — and mild and severe drying-off.

INTRODUCTION

Commercial sugar cane in Iran is subject to an exceptional range of climatic conditions. The summer months have daily maximum temperatures over 45°C (a few days per year at 49-50°C, record extreme 53°C), and winter months commonly have several days of frost (average extreme minimum —3.5°C, record extreme —10.0°C). Because the 250 mm average annual rainfall occurs only in the cool months, November to April, all the crop is irrigated up to the beginning of the annual harvest in late October. For hastening the ripening of the first few fields scheduled for earliest harvest, some cane growth and yield is sacrificed by extending the irrigation intervals during late August and September, under temperatures in excess of 40°C. But since the harvest extends for five months or more, the bulk of the crop ripens in response to the falling temperatures in autumn and early winter.

Panje et al.1,2 discussed ripening in the cool, but relatively dry, winter conditions in Northern India, concluding that juice quality generally improves when minimum air temperatures are in the range 14°-7°C, and falls when the temperatures are in the range 5°-0°C, but there are differences between varieties, as would be expected. This paper is intended as a further contribution to such studies, noting also some effects of winter rains. Earlier papers, as by Sund,3,4 from Iran, and other workers from other areas, have been more concerned with the effects of severe freezes on subsequent cane quality, than with the effects of cold on ripening.
MATERIALS AND METHODS

The data herein are based on 20-stalk samples of standing cane taken weekly during the harvest from the same field blocks used for crop-logging during cane growth. The juices are expressed by Cuban mill after cutting and topping the stalks as for the commercial harvest. At the start of the season, 30-70 fields of each variety are represented by the averages, but fewer fields make up the average as the harvest proceeds.

There is a range of 2°C to 4°C in the minimum temperatures on cold nights in the crop area of about 10,000 hectares, and we harvest the coldest areas first before the frosts, if possible, to minimize the risk of damage to standing cane. When cane has been frozen and some deterioration occurs, as in the crop years 1971-72 and 1972-73, we harvest the most severely injured fields first. As the data for these poorest fields are removed, the average purity is raised by the less-injured fields remaining. But unless there are uninjured fields among them, improved juice purity does not necessarily represent an increase in sugar per hectare over the pre-frost level.

RESULTS AND DISCUSSION

Our experience has been that as long as the leaves remain green, and are not killed by frost, cane quality at Haft Tappeh improves fairly steadily through the winter and into the spring. The rate of ripening may be decreased temporarily in temperatures below 10°C, or by periods of rainy weather, and may be temporarily halted by temperatures below 7°C. There can be inversion and loss of purity below 2 to 3°C (some later recovery may be seen), and of course irreversible losses can follow if the cane is actually frozen.

The year 1970-71 brought essentially a “green winter”, only 23 hours of frost, minimum —1.8°C, and juice purities rose throughout the harvest (Fig. 1, upper). Although CP 48-103 usually has a higher juice purity in the early season, NCo 310 will often overtake it, and did so in this year. The rate of improvement was at times reduced by lower temperatures (CP 48-103 has seemed less sensitive and faster to recover than NCo 310), and by the cold winter rains (NCo 310 has seemed less sensitive), but ripening continued two or three weeks after such interruptions. The overall percentage of refined sugar obtained was a very satisfactory 10.5% on the net cane for the season.
In 1971-72 (Fig. 1, center), ripening in November was a little retarded by early rains, 73 mm in contrast to 4 mm in the year before. Ripening was stopped by the first frost occurring shortly before Christmas, and there were several further nights giving 58 hours of frost in all, with an extreme minimum of $-4.0^\circ$C on 20 January, one month later. NCo 310 was more severely affected, and the most injured fields were removed first. There was also some decline of quality in the last few fields in the warmer days at the end of the harvest. However, the chief limitation on the percentage
of refined sugar, 8.5% for the season, was the January frost and subsequent juice deterioration.

Fig. 1, lower, shows data from an even less favorable year. In 1972-73, early ripening was delayed by temperatures below 10°C and 7°C two and three weeks earlier than in the previous year. And although the first frost occurred again shortly before Christmas, there were several nights of killing frosts, to a minimum of -3.5°C on 27 December. The year’s extreme minimum of -4.2°C occurred on 8 January, and there were 142 hours of frost overall. This permitted a refined sugar recovery of only 7.5% overall, for the season.

Data for the year 1972-73 are shown in Fig. 2. The earliest frosts on record killed leaves over much of the plantation shortly after the harvest began. However, the extreme minimum of -2.8°C on 8 November occurred mostly in the areas taken for early harvest. Subsequent frosts were less severe, and so degradation of frozen cane was not a problem — it was more a matter of limited opportunity for photosynthesis since so much foliage had been injured in November. With a total of 54 hours of frost, the overall refined sugar recovery was only 8.5% for the year, although juice purities went to 90% toward the end of the season.

It was interesting that in this season there was almost no rain on the standing crop until 18 December and 10 days later. Since irrigation had been stopped in latter October, the cane had become fairly dry. Commonly, ripening would resume in a few weeks after a mild frost when the cane has not been frozen, but in this season ripening did not resume in the long dry period which followed. When the drought was broken, the juice purity rose sharply, and ripening continued, in contrast to the pattern usually experienced after a cold winter rain.

**FIGURE 2.** Juice purities in Cuban mill samples from crop log blocks.
CONCLUSIONS

We conclude that there is some parallel in the effects of a mild vs. a severe cold, and mild vs. a severe "drying-off".

That is —

Ripening is promoted by moderately cool weather, and
Ripening is promoted by moderate drying-off, but
Ripening is interrupted by severely cold weather, and
Ripening is interrupted by too severe a drying-off.

REFERENCES


OBSERVACIONES SOBRE LA MADURACION DE LA CAÑA DE AZUCAR DURANTE EL INVIERNO IRANIO.

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RESUMEN

La caña de azúcar que crece en los meses de menor precipitación, que van de Abril a Octubre en el Hemisferio Norte, es ayudada a madurar por una parte mediante la supresión paulatina del riego y por otro lado mediante la disminución natural de la temperatura.

El ritmo del mejoramiento en la calidad del jugo puede ser retardado por las lluvias de invierno y estar reducido o temporalmente detenido cuando las temperaturas descienden a un valor cercano a las heladas, reasumiéndose el aumento de azúcares un corto tiempo después de retornar las condiciones climáticas más favorables.

Recientemente la maduración no se recuperó por aproximadamente siete semanas después de una helada suave, sino inesperadamente después de las primeras lluvias. Esto está en contraste con el efecto usual de las lluvias invernales. Asimismo pareciera haber un paralelismo entre los efectos de las heladas suaves y severas — como promotoras e inhibidoras de la maduración — y el de una disminución de la humedad del suelo suave y severa.