Translocation of Sugars into Infected Cabbage Tissues During Clubroot Development

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Abstract. Sucrose, glucose, and inositol were the major sugars in cabbage hypocotyls infected by Plasmodiophora brassicae and in noninfected hypocotyls, based on paper, thin-layer, and gas-liquid chromatography. Small amounts of trehalose were tentatively identified in extracts from noninfected hypocotyls, whereas up to 20% this level occurred in extracts from infected hypocotyls. Inositol declined in the infected hypocotyl while glucose increased to about 4-fold the level in noninfected hypocotyls. Fructose and mannose concentrations increased about 2-fold in the diseased hypocotyls, whereas the galactose concentration was about one-third that of noninfected hypocotyls.

Translocation of 14CO2 sugar photosynthates into infected hypocotyls was more rapid than that into noninfected hypocotyls. The infected tissues also exported less sugar than the noninfected tissues. Sucrose was the major sugar translocated into the hypocotyls of both infected and noninfected plants.

Clubroot of cabbage (Brassica oleracea L. var. capitata L.), incited by Plasmodiophora brassicae Wor., is characterized by the formation of spindle-shaped root and hypocotyl galls which lead to stunted plant growth (17). The clubroot galls thus appear to act as metabolic sinks, competing with other host tissues for translocatable nutrients. In order to test whether preferential translocation of nutrients into the developing galls was occurring, rates of 14C sugar importation into the hypocotyls of infected and noninfected plants were studied after pulse feeding plants with 14CO2 in the light. Although the translocation of carbon into plant tissues infected by obligate parasites has been examined (6, 8, 11), few efforts have been made to determine the specific compounds that are translocated.

Materials and Methods

Feeding and Extraction Methods. Plants in 5 × 5 cm pest pots (5 plants/pot) were grown as previously described (19). Pots of infected or noninfected plants were exposed to 14CO2 enriched air (ca. 0.19 μCi/cm3) in a closed, illuminated chamber. Radioactive CO2 was produced by perchloric acid acidification of BaCO3 (5.0 mc/mmole). After 10 min, the chamber was opened and flushed with air. Immediately after feeding and at intervals thereafter, P. brassicae-infected or noninfected plants were withdrawn, washed free of soil, and 2-cm hypocotyl sections were harvested as previously described (14, 19). These sections and the corresponding plant tops (tissues above the cotyledons) were quickly weighed, frozen with liquid N2, and stored at −25°C until sugar extractions were made.

Sugars were extracted by placing frozen 14CO2-fed hypocotyls or freshly harvested hypocotyl sections in a 50 ml Servall Omni-mixer cup containing 25 ml of boiling 80% ethanol per g fresh wt and grinding for 2 to 3 min at full speed. Tops were similarly extracted with 40 ml of hot 80% ethanol per g fresh wt. The mixtures were further heated in a boiling water bath for 30 min. After cooling, the mixtures were filtered through Whatman No. 42 filter paper and the residues washed with 80% ethanol and discarded. Filtrates were evaporated to dryness at 40 to 45°C under reduced pressure, dissolved in 3 to 5 ml of water and extracted with 3 successive 10 ml volumes of petroleum ether. The water phases were then shaken for 2 to 3 hr at room temperature with 1 to 2 g of Bio-Rad Ag 501-X8 (D) mixed bed ion-exchange resin. The aqueous extracts were again filtered and clarified by centrifugation at 40,000g for 20 min. Supernatants were evaporated to dryness at 40°C, redissolved in water and stored at −25°C prior to analyses.

Sugar Analyses. Preliminary sugar separations were made using conventional paper and thin-layer chromatographic methods (13).

Sugars were separated as their trimethylsilyl ether (TMSE) derivatives (3) on an Aerograph model 1520 gas chromatograph equipped with dual columns and dual thermal conductivity and flame ionization detectors. Three stationary phases were

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used for gas-liquid chromatography (GLC), ranging from strongly polar to strongly nonpolar (EGS, QF-1 and SE-52, respectively). All stationary phases and supports were obtained from Applied Science Laboratories. Incorporated. Hexoses were successfully chromatographed on 3.2 mm by 1.52 m or 6.4 mm by 2.43 m columns of 14% ethylene glycol succinate (EGS) polyester on Chromosorb W by the procedure of Brower et al. (3). Di-, tri-, and tetrasaccharides were best resolved on the nonpolar SE-52 silicone stationary phase as described by Sweeley et al. (16).

For the resolution of both mono- and disaccharides on a single column, the fluorosilicone stationary phase, QF-1, gave the best results. Columns 6.4 mm by 3.35 m or 3.2 mm by 2.43 m of 6% QF-1 on silanized and acid washed Chromosorb W were packed and preconditioned by conventional methods. The helium flow rate was 120 ml/min through the 6.4 mm columns and thermal conductivity detectors were used. Nitrogen at 25 ml/min was used with the 3.2 mm columns and the flame detectors were supplied with hydrogen at 30 ml/min and air at 380 ml/min. Injector block and detector temperatures with the QF-1 columns were 290° and 275° respectively and column temperatures were programmed as shown with the results.

All sugar identifications were based on co-chromatography of unknowns with known standards on at least 2 of the stationary phases. Anomeric sugars were also identified by ratios of their multiple peak areas. Sugars were quantitated by determination of peak areas (peak heights × one-half height widths). For sugars exhibiting multiple peaks, results are based on the area of 1 of the peaks. On QF-1, the second fructose peak in Fig. 3 was used for quantitation, because α-mannose and α-galactose overlapped the first peak. Glucose was determined from the β-peak in Fig. 3 because β-galactose overlapped the α-glucose peak. Mannose was determined from the β-peak because fructose overlapped the α-mannose peak. The QF-1 columns failed to resolve galactose from the other sugars. Galactose data were therefore taken from the EGS column separations (Fig. 2).

Since arabitol was not detected in extracts from noninfected or infected cabbage hypocotyls it was added as an internal standard to the vials containing unknowns. Standards containing the TMSE-sugars to be quantitated in the unknowns and arabitol were included with each day's runs. Concentrations of the unknowns were determined by the following equation:

\[
\frac{\text{Peak area}}{\text{unknown}} \times \frac{\mu g \text{ arabitol internal}}{\text{standard per vial}} \times \frac{\text{Peak area}}{\text{arabitol standard}} = \frac{\mu g \text{ arabitol internal}}{\text{unknown per vial}}
\]

Radioactive sugars chromatographed on the 6.4 mm QF-1 columns were collected from the thermal conductivity cell outlet ports in polyethylene liquid scintillation counting vials containing glass wool wetted with a few drops of benzene. Fifteen ml of Bray's solution (2) were then added to each vial and the radioactivity was determined in a liquid scintillation spectrometer. Recovery of 14C-sucrose and -glucose (New England Nuclear) by this method was 60% ± 6% in 3 trials with each sugar; 63% ± 4% of the recovered glucose counts were in the β-glucose peak.

**Results**

Sugar Analyses. Sucrose, glucose and trehalose were detected in extracts from *P. brassicae*-infected and noninfected cabbage hypocotyl extracts by cochromatography using paper and thin-layer chroma-

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Fig. 1. Silica gel G thin-layer chromatogram of sugars extracted from *Plasmodiophora brassicae*-infected or noninfected cabbage hypocotyls at 46 days after transplantation. A = 40 μg each of glucose, sucrose and trehalose; B = extract from ca. 25 mg infected tissue; C = extract from ca. 25 mg infected tissue + 20 μg each of glucose, sucrose, and trehalose; D = extract from ca. 25 mg noninfected tissue; E = extract from ca. 25 mg noninfected tissue + 20 μg each of glucose, sucrose and trehalose; F = 40 μg each of glucose, sucrose and trehalose. Silica gel G (250 μ) developed twice with *n*-butanol:acetic acid:water (3:1:1, v/v/v), detection with anisaldehyde-sulfuric acid (13).
cabbage hypocotyls (Fig. 2), but disaccharides were not successfully chromatographed. Fig. 3 shows the QF-1 column temperature program used and a chromatogram of standards of the major sugars which were found in cabbage hypocotyls. In addition to the major identified sugars, 13 minor peaks were observed in extracts from noninfected and infected hypocotyls (Fig. 4). Compounds comprising these peaks occurred in varying amounts in both noninfected and infected tissue extracts.

Sugars of the raffinose series were chromatographed on the SE-52 columns run isothermally at 250°C and 280°C. Traces of raffinose were present in the extracts from both noninfected and infected hypocotyls, but stachyose was not detected.

Clubroot galls were beginning to form on the hypocotyls of seedlings 17 days after transplanting into P. brassicae-infested soil. At this time, concentrations of sucrose and fructose were higher and concentrations of galactose and inositol were lower on a fresh weight basis in the infected tissue extracts compared to noninfected tissue extracts (table 1). A marked increase in the compound tentatively identified as trehalose occurred in the extracts from infected tissues at 25 and 38 days after transplanting.

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**Fig. 2.** Chromatogram of TMSE-sugars in an extract from infected hypocotyl tissue at 23 days after transplanting. Sample was injected onto EGS column run isothermally at 140°C for 4 min, then programmed at 4°C/min to 190°C.

A non-reducing compound which co-chromatographed with trehalose was also detected in the noninfected tissue extracts by chromatographing ca. 10 times the amount of extract used in Fig. 1.

Using GLC, the EGS stationary phase gave the best resolution of monosaccharides extracted from 240

**Fig. 4.** QF-1 chromatograms of arabinol internal standard with TMSE-sugar extract from noninfected (upper) or from Plasmopora brassicae-infected hypocotyls (lower). Tissues harvested at 25 days after transplanting.
An unknown sugar in the noninfected tissue extracts was also identified as trehalose on the basis of cochromatography with commercial trehalose standards on the QF-1 and SE-52 columns when varying temperature programs were run. Concentrations of this compound remained approximately constant in the noninfected hypocotyl extracts (table I). Glucose and fructose concentrations were higher in infected than non-infected hypocotyl extracts whereas the galactose concentration was lower. Sucrose remained relatively constant in the infected tissue extracts but increased in the noninfected tissue extracts (table I).

Sugar Translocation. When 14C02 was supplied to cabbage seedlings, radioactivity in the hypocotyl sugar fractions per g tops accumulated more rapidly in infected than in noninfected hypocotyls (Fig. 5, upper). On the basis of top and hypocotyl weights, however, radioactivity of sugars in infected hypocotyls was lower than that of the noninfected until about 1 hr after the 14C02 pulse (Fig. 5, lower).

In order to determine which sugars were translocated into infected and noninfected hypocotyl sections, sugars were extracted from hypocotyls at various times after a 14C02 pulse. The sugars were separated as their TMSE derivatives on a 6.4 mm QF-1 column, collected, and their radioactivities determined. Radiochromatograms showed some counts in the solvent peak and some in the hexoses (Fig. 6), but most of the radioactivity was recovered in peaks corresponding to sucrose and the unknown peak X-10. Forty to 55% of the radioactivity applied to the columns was recovered in identified sugars and 70 to 89% of this was in sucrose.

Sucrose contained from 10-∞ times the total detectable radioactivity of any other sugar in infected or noninfected hypocotyl extracts at zero time after a 10 min 14C02 feeding (Fig. 7, lower). Sucrose always contained the highest specific radioactivity (Fig. 7, upper) whereas little radioactivity was present in the hexoses until 15 to 30 min after termination of the 14C02 feeding. Specific activities of the other sugars were 0.1 or less the sucrose values immediately after termination of the 14C02 feeding but then increased. Very low radioactivities (<1 cpm/ug) were detected in raffinose from hypocotyls of infected and noninfected seedlings at 1 hr after a 14C02 pulse.

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Table I. Concentrations of Sugars Extracted From Noninfected and Plasmodiophora brassicae-infected Cabbage Hypocotyls at Various Times After Transplanting

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Concentration of sugars</th>
<th>17 Days after transplanting</th>
<th>25 Days after transplanting</th>
<th>38 Days after transplanting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Noninfected</td>
<td>Infected</td>
<td>Noninfected</td>
<td>Infected</td>
</tr>
<tr>
<td></td>
<td>mg/g fresh wt tissue</td>
<td></td>
<td>mg/g fresh wt tissue</td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>15.4</td>
<td>23.1</td>
<td>10.6</td>
<td>24.2</td>
</tr>
<tr>
<td>Galactose</td>
<td>29.0</td>
<td>1.0</td>
<td>9.0</td>
<td>3.2</td>
</tr>
<tr>
<td>Mannose</td>
<td>4.5</td>
<td>1.5</td>
<td>1.5</td>
<td>2.8</td>
</tr>
<tr>
<td>Glucose</td>
<td>30.8</td>
<td>32.0</td>
<td>32.8</td>
<td>82.0</td>
</tr>
<tr>
<td>Inositol</td>
<td>147.0</td>
<td>114.0</td>
<td>172.0</td>
<td>34.1</td>
</tr>
<tr>
<td>Sucrose</td>
<td>172</td>
<td>305</td>
<td>256</td>
<td>368</td>
</tr>
<tr>
<td>Trehalose</td>
<td>112</td>
<td>78</td>
<td>91</td>
<td>256</td>
</tr>
</tbody>
</table>

1 Data are averages of 2 replicated extractions and analyses using GLC. Columns were 3.2 mm EGS for galactose and 3.2 mm QF-1 for the remaining sugars. Dual flame detectors were used.
**Discussion**

Sucrose and glucose were the major sugars present in cabbage hypocotyls based on PC, GLC, and TLC data and these sugars were more concentrated in extracts from *Plasmodiophora brassicae*-infected than noninfected tissues early in gall development (table I). The later increase of sucrose in the noninfected hypocotyls to levels above those of the infected tissues was also noted by Yukawa (21), but not in our earlier report (19). This is probably because our previous results were expressed on a tissue dry weight basis, whereas the present data are based on fresh weights. Because noninfected hypocotyls contain a lower percentage of water than infected tissues (19), data expressed on a fresh weight basis increases relative values for the former.

A sugar tentatively identified as trehalose was present in large amounts in infected tissue extracts (table I). This accumulation is thought due to the growth of the parasite, since trehalose is a common storage sugar in obligately parasitic fungi (4, 5, 20) and is found in extracts of *P. brassicae* resting sporangia (unpublished data). Trehalose was apparently also detected in extracts from noninfected tissues by GLC (Fig. 4) and when large amounts of extracts were run on TLC plates. This was not expected because trehalose has been reported from only 1 higher plant (1). It is possible that the low levels of this compound in extracts from noninfected tissue may have been contributed by microbial surface contaminants.

The declining levels of inositol in infected tissues may indicate utilization by the parasite since this cyclitol is required by many fungi (7).
The more rapid translocation of 14C sugars from leaves into the infected hypocotyls as compared with healthy plants (Fig. 5, upper) demonstrates the existence of a "sink" effect resulting from infection by *P. brassicae*. The apparent lag in sugar translocation into infected hypocotyls when calculated on a hypocotyl fresh weight basis (Fig. 5, lower) is considered a result of the ca. 10-fold greater weight of infected hypocotyls than noninfected (19). The nearly exponential curves for radioactive sugar import into the infected tissues (Fig. 5) indicate an accumulation of sugars in the clubroot galls, whereas the asymptotic curves for the noninfected tissues suggest that they were exporting sugars at rates approaching those of import.

Sucrose exhibited the highest total and specific radioactivity of the hypocotyl sugars after a 10 min 14CO2 feeding (Fig. 6 and 7). This, together with the lag in increase of radioactivity of the hexoses and inositol (Fig. 7) indicates that sucrose is the major sugar translocated into infected and noninfected cabbage hypocotyls as in many other plants (10, 15, 18). The presence of clubroot galls therefore appears to alter sugar translocation in the cabbage plant quantitatively but not qualitatively. Sugars of the raffinose series are translocated in some plants (9, 10); however they do not seem of importance in cabbage since only low concentrations of raffinose were detected in extracts and this sugar was very weakly radioactive in the 14CO2 feeding experiments.

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Literature Cited


