Pathways of Glucose Assimilation in *Puccinia graminis*

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[U-¹⁴C]-D-Glucose (1 and 20 mM) was added to glucose-grown suspension cultures of *Puccinia graminis*, and the sequence of movement of the label into metabolic intermediates was determined under steady-state conditions. Radioactive label moved most rapidly into cellular pools of free glucose, amino acids and phosphate esters of trehalose and glucose, and less rapidly into free fructose. No lag was detected in the movement of label into any of these compounds. Thus fructose was the first free carbohydrate synthesized from glucose. Radioactive label moved more slowly, and with an initial lag phase, into trehalose, glucitol and mannitol; the lag was more pronounced for mannitol than glucitol. Kinetic data and specific activity measurements to identify precursors suggest that trehalose was formed via trehalose phosphate, mannitol via fructose, and glucitol via both glucose and fructose. Steady-state specific activities of all free intracellular carbohydrates were much lower (15–50%) than that of the exogenous glucose, indicating that unlabelled carbon was entering these pools from storage reserves or other components from the culture medium.

INTRODUCTION

After infection of plants by rust fungi, photosynthates are rapidly converted to fungus-specific products such as sugar alcohols and trehalose (Smith *et al.*, 1969; Lewis, 1976). However, metabolic pathways for the synthesis and utilization of these compounds are experimentally difficult to determine in infected plant tissue. Axenic cultures present the simplest system to characterize fungal metabolism, and thus provide a rational basis for future investigations of parasitically growing mycelium (Maclean, 1982).

Some progress has already been made on the carbohydrate metabolism of *Puccinia graminis* in axenic culture. Thus, Maclean & Scott (1976) identified glucitol and ribitol as metabolites after growth on glucose; these compounds have rarely been reported as metabolites of other fungi (Pfyffer & Rast, 1980*a, b*; Maclean, 1982). Maclean & Scott (1976) suggested that glucitol might be formed by the direct reduction of glucose, as part of a novel pathway for the assimilation of glucose by this fungus. Experiments described in this paper have tested this hypothesis.

Several options are available when designing experiments to elucidate metabolic pathways. For example, the pathways of carbon assimilation in photosynthesis were determined firstly from [¹⁴C]carbon dioxide labelling experiments *in vivo*, followed by the detection and characterization of the necessary enzyme systems. Despite the obvious advantages of such kinetic labelling experiments in defining the direction of carbon flow during metabolism, there are surprisingly few reports of this type on the assimilation of exogenous sugars by fungi. Thus, a kinetic approach was adopted in this investigation, to detect the movement of label from exogenous [U-¹⁴C]-D-glucose through sequential pools of metabolites in *P. graminis*.

METHODS

Growth of cultures. Strain VIB of *Puccinia graminis* f.sp. *tritici*, isolated from race 126-ANZ-6,7 by Maclean & Scott (1970) was used for all experiments. The growth characteristics of this particular strain in axenic culture have been described by Maclean (1974). Liquid suspension cultures (unshaken) were maintained in the growth medium

of Howes & Scott (1973), viz. Czapek's minerals, 10 mM-tri-sodium citrate, 1% (w/v) Evan's peptone (Glaxo) and 2.1 mM-histidine-HCl at pH 6.0 with 212 mM-D-glucose. Cultures were fragmented and transferred to fresh medium (Howes & Scott, 1973) at intervals of 3 weeks and were removed for experiments 12 d after transfer. Asepsis was maintained in all experiments; stock cultures were maintained at 23 °C.

¹⁴C Labelling experiments. Cultures (400 ml) were pooled and filtered on a glass fibre disc (60 mm diam. Whatman GF/A) and washed four times with 150 ml glucose-free growth medium. The mycelium was resuspended in 150 ml growth medium containing lowered glucose concentrations (2 or 20 mM). Three 50 ml fractions were dispensed separately into 250 ml conical flasks, and incubated at 26 °C with gentle agitation for 18 h. Two of these flasks, A and B, were used to estimate the total carbohydrate pools of the fungus by GLC and the remaining flask, C, was used for ¹⁴C labelling experiments.

To estimate total mycelial carbohydrate pools, a 20 ml sample was taken from both flasks A and B at the beginning and end of each 3 h feeding experiment. Each sample was washed five times with 10 ml double distilled water at 4 °C by filtration on glass fibre discs (wash time under 30 s) and the mycelium and discs were placed in boiling 80% (v/v) ethanol prior to analysis by GLC.

[U-¹⁴C]-D-Glucose (500–700 µCi; 260 mCi mmol⁻¹, 9.62 GBq mmol⁻¹) was added to flask C and samples (2–10 ml) were removed after various time intervals. Each sample of mycelium was washed with cold water and placed in boiling 80% ethanol, as for flasks A and B. To estimate the specific activity of the ambient [¹⁴C]glucose in the medium, a 0.5 ml sample was taken after 10 min incubation and centrifuged at 2000 g for 4 min. The mycelial pellet was discarded and the supernatant stored at –20 °C until analysed using methods described below. To estimate mycelial dry weight, two 4 ml samples were each filtered on to a pre-weighed Millipore filter (pore size 0.8 µm) previously dried for 30 h at 80 °C. These were washed once with 5 ml water, dried at 80 °C for 48 h and weighed.

Analysis of ¹⁴C-labelled extracts. All mycelial samples were homogenized in 80% ethanol in a Ten Broeck hand homogenizer, the insoluble material was separated by centrifugation (2000 g) and washed three times with 80% ethanol. Insoluble fractions were resuspended in 1 ml 80% ethanol and mixed with 10 ml of scintillant solution (4 g PPO l⁻¹, 50 mg POPOP l⁻¹, 33% (v/v) Triton X-100 in toluene) and the radioactivity was estimated; counts were standardized by the channels ratio method. The ethanol-soluble fractions were evaporated to dryness at 50 °C and 5 ml water added to each followed by 5 ml redistilled carbon tetrachloride to extract lipids; the aqueous and organic layers were separated and the process was repeated three times. The lipid fractions were pooled and evaporated to dryness in a scintillation vial, 10 ml scintillant solution was added and the radioactivity was estimated. The aqueous fraction was separated into neutral, anionic and cationic fractions by ion exchange chromatography as described by Neal & Beevers (1961). The fractions were evaporated to dryness, taken up in 1.5 ml water and 25 µl was removed for scintillation counting.

Neutral fractions were analysed by descending paper chromatography for 40–50 h on Whatman no. 1 paper with ethyl methyl ketone/acetic acid/boric acid-saturated water (9:1:1, by vol.) (Solvent 1, Rees & Reynolds, 1958). The solvent which had dripped from the end of each chromatogram was collected to estimate radioactivity in highly mobile compounds. In some instances, papers were washed prior to chromatography with each of 0.5% (w/v) EDTA, water and redistilled ethyl acetate. Radioactivity on the chromatograms was determined by cutting them into 5 mm strips and counting these directly in a detergent-free scintillant (4 g PPO l⁻¹, 50 mg POPOP l⁻¹ in toluene). It was sometimes necessary to rechromatograph samples, in which case strips were recovered from the scintillant, washed twice with toluene, dried and eluted with water. The eluate was evaporated to dryness after six successive additions of 2 ml methanol (to remove residual boric acid), and the sugars then dissolved in water.

Anionic fractions were initially analysed by descending paper chromatography for 30 h using ethyl acetate/acetic acid/formic acid/water (18:3:1:4, by vol.) (Solvent 2, Hough & Jones, 1962). Radioactive areas on the chromatogram were located with a Nuclear Chicago paper strip counter and those on or close to the origin (putative sugar phosphates and organic acids) were then eluted from the paper, evaporated to dryness and taken up in 200 µl alkaline phosphatase solution (20 units ml⁻¹, Sigma, *Escherichia coli* origin) in 25 mM-glycine/NaOH buffer pH 9.6, and incubated for 3 h at 37 °C. This was then fractionated into neutral and anionic compounds by passing through 2 g Dowex 1-X2 (100–200 mesh, acetate form). Neutral compounds were washed from the resin with water and anions subsequently eluted with 20 ml 2 M-formic acid. The neutral and anionic fractions obtained were then analysed by further paper chromatography as described above.

Cationic fractions were analysed by ascending paper chromatography (Whatman, no. 1) using phenol/water (3:1, v/v) (Solvent 3), and radioactivity was determined in 5 mm strips as above. Authentic standards were run on all paper chromatograms, and were detected by the following methods: (1) sugars, polyols and gluconate by the alkaline silver oxide method of Trevalyan *et al.* (1950); when necessary papers were pretreated with hydrofluoric acid to remove boric acid (Britton, 1959); (2) amino acids by the ninhydrin method (Harborne, 1973) and (3) organic acids with bromothymol blue (Harborne, 1973).

Quantitative analysis of soluble carbohydrates by GLC. Compounds soluble in 80% ethanol were extracted from mycelium and lipids were removed as described above. Aqueous extracts or media samples were then deionized by shaking for 10 min with mixed ion exchange resins (1 g each of Bio-Rad AG 50 hydrogen form and Amberlite IR-

45 free base form). Beads of resin were removed by centrifugation, washed three times with water and the supernatants pooled. Each deionized extract was divided into two fractions of equal volume. One fraction was evaporated to dryness, redissolved in 0.425 ml redistilled pyridine and trimethylsilyl (TMS) ethers of soluble carbohydrates (Sweeley *et al.*, 1963) prepared by addition of 50 μ l hexamethyldisilazane followed by 25 μ l trimethylchlorosilane. Suitable standards were prepared similarly and all vials were left overnight at 26 °C. GLC of the TMS ethers was performed on a Shimadzu gas chromatograph model GC-6AM with a glass column (1.5 m $\times$ 3 mm i.d.) of 2% SE52 on dimethylchlorosilane-treated acid-washed Chromosorb W (mesh size 100–120, Johns-Manville), attached to a flame ionization detector. The following programme was used, N₂ carrier gas at 30 ml min⁻¹, injection port temperature at 260 °C and the column temperature increased at 6 °C min⁻¹ from 115 °C to 290 °C. TMS ethers were quantified by comparing peak heights with those of standards. Isomeric hexitols were quantified by GLC of acetate derivatives using the remaining half of the deionized extracts. The extract was reduced to 2 ml and 2 g Dowex 1-X8 (hydroxide form, 50–100 mesh) was added and the mixture was incubated at 100 °C with constant agitation for 20 min. Similar treatment of standards showed that this degrades and removes any aldose or ketose sugars present in the extract (Maclean & Scott, 1976). The resin was separated and washed with water three times by centrifugation, the supernatants pooled, reduced to dryness and the residue of polyols redissolved in 250 μ l each of acetic anhydride and pyridine, and the mixture left overnight at 37 °C. Samples were then evaporated to dryness at 55–60 °C *in vacuo* and redissolved in 100 μ l redistilled carbon tetrachloride. GLC was performed on a glass column (3 m $\times$ 3 mm i.d.) containing a stationary phase of Celite (100–120 mesh) coated with a mixture of 0.5% SE52 and 6.0% OV275, a nitrogen flow rate of 40 ml min⁻¹, injection port temperature of 250 °C and column temperature increasing at 20 °C min⁻¹ from 140 °C to 225 °C, then isothermal at 225 °C. Polyol acetates were quantified by comparison of peak areas with those of standards.

RESULTS

Overall distribution of radioactivity from [¹⁴C]glucose

Analysis of the medium by GLC showed that during the 18 h preincubation, less than 5% of the initial 20 mM-glucose was utilized, whereas 2 mM-glucose was reduced to 1.05 mM. Thus, labelling experiments were effectively carried out in either 1 or 20 mM glucose. Uptake rates after addition of label were linear: 0.078 and 0.51 nmol (mg dry wt)⁻¹ min⁻¹ for mycelium in 1 and 20 mM glucose, respectively.

Because the kinetics of labelling of the major metabolite pools were very similar with both glucose concentrations (Fig. 1) they are described together. [¹⁴C]Glucose was rapidly metabolized to anions and cations, each of which constituted 16–20% of the total ¹⁴C assimilated after only 2 min. The proportion of label located in cations increased considerably during the first 20 min with a concomitant reduction in the label present in neutral compounds; little change was observed thereafter. In later samples 37–38% of the total incorporated radioactivity was recovered in neutral compounds and 30–34% in cations. However, the proportion of label in anions declined very slowly to approximately 12% after 3 h. Insoluble

Fig. 1. Distribution of radioactivity between classes of compounds extracted from *P. graminis* after incubation in (a) 1 mM exogenous [¹⁴C]glucose or in (b) 20 mM exogenous [¹⁴C]glucose (see Methods for experimental details). ■, Compounds insoluble in 80% ethanol. The following compounds soluble in 80% ethanol: ○, neutral compounds; ●, cations; □, anions.

compounds constituted less than 3% of the total incorporated radioactivity after 2 min, followed by an increase to 15–17% by 3 h. Label in lipids never exceeded 1% of the total.

Incorporation of radioactivity into neutral sugars and polyols

Chromatography of neutral fractions on unwashed Whatman no. 1 paper using Solvent 1 clearly separated trehalose, glucose and fructose, and gave a single spot of putative hexitols (Fig. 2a). Although the putative hexitols (shaded region in Fig. 2a) were not as mobile as adjacent standards of D-mannitol and D-glucitol, analyses by GLC indicated that both were present in the mycelium. The putative hexitols were eluted, and rechromatographed on prewashed papers in the same solvent system after removal of boric acid from the sample and the addition of 100 µg each of unlabelled D-mannitol and D-glucitol as cold carriers, thus demonstrating label in mycelial mannitol and glucitol (Fig. 2b). Negligible radioactivity was detected in compounds more mobile than hexitols.

Analysis of neutral sugars and polyols showed that after 2 min, more than 50% of total incorporated label was present in glucose (Fig. 3) with smaller proportions in fructose (5–6%), trehalose (2–4%) and hexitols (0.9–1.5%). The proportion of label in glucose then dropped rapidly reaching 3% after 3 h, whilst the label in fructose increased during the first 10 min to 7–8%, followed by a decline to 2% after 3 h. After a short lag (see below), label increased rapidly in trehalose and glucitol, reaching a maximum of 18–23% in trehalose and 10–12% in glucitol after

Fig. 2. Chromatographic separation of ethanol-soluble neutral compounds from extracts of *P. graminis* after 20 min incubation in 1 mM [^{14}C]glucose. (a) Initial chromatographic separation using unwashed Whatman no. 1 paper and Solvent 1. (b) Radioactivity in the shaded peak of (a) was eluted and rechromatographed using the same solvent on washed Whatman no. 1 paper. The positions of authentic standards run on the same chromatograms are indicated by the arrows.

Fig. 3. Distribution of radioactivity between ethanol-soluble carbohydrates after incubation of *P. graminis* in (a) 1 mM exogenous [^{14}C]glucose or (b) 20 mM exogenous [^{14}C]glucose (see Methods for experimental details). $\circ$, Glucose; $\bullet$, fructose; $\square$, glucitol; $\blacksquare$, mannitol; $\triangle$, trehalose.

1 h. Less label was incorporated into mannitol, with an eight- to fifteenfold excess of glucitol after 10 min, and a threefold excess after 3 h.

GLC analyses (cf. legend to Fig. 4) showed no significant changes in the concentrations of any sugar or polyol during the experiment, indicating that labelling was carried out under essentially steady-state conditions. No significant changes were detected in the specific activities of intracellular glucose at any time point, with mean values ($\pm$ s.d.) of 13.4 ± 3.0 d.p.m. ng^{-1} and 4.4 ± 2.7 d.p.m. ng^{-1} for mycelium incubated in 1 and 20 mM glucose, respectively. Specific activities of all other sugars and polyols increased more slowly and eventually approached values similar to intracellular glucose (Fig. 4a, b), and thus reached only 12 and 47% of the respective specific activities of exogenous glucose.

Specific activities of fructose increased more rapidly than glucitol, mannitol and trehalose and showed no lag period (Fig. 4). However, the kinetics of ^{14}C incorporation into glucitol, mannitol and trehalose showed an initial lag (Fig. 4c, d) during which the specific activities of glucitol and trehalose rose more rapidly than mannitol. After about 30 min the specific activities of mannitol increased considerably and reached values similar to those of glucitol.

Incorporation of radioactivity into anions

After chromatography of anionic fractions in Solvent 2, almost all of the label remained near the origin except for a trace amount which migrated to the same position as gluconic acid. After phosphatase treatment of compounds near the origin, predominantly neutral compounds were released at early time points, whereas after about 80 min anions began to predominate (Fig. 5). Chromatography of neutral compounds released by phosphatase revealed most radioactivity in trehalose (>85%), with traces in glucose and occasionally fructose; results from the 2 min sample (Fig. 6a) are typical. Thus, the kinetics of ^{14}C incorporation into neutral sugar phosphates (Fig. 5) are essentially incorporation into trehalose phosphate.

Rechromatography of anions retaining charge after phosphatase treatment showed most radioactivity remained in positions similar to TCA cycle intermediates (citric, malic and fumaric acids, Fig. 6b, c); no attempts were made to confirm the identity of these compounds. However, earlier samples (e.g. 10 min, cf. Fig. 6b) yielded a peak with identical mobility to gluconic acid, indicating a phosphate ester of gluconate.

Fig. 4. Specific activities of intracellular pools of carbohydrates during the assimilation of 1 mM (*a* and *c*) and 20 mM (*b* and *d*) exogenous [^{14}C]glucose (see Fig. 1 and Methods for experimental details). Pool sizes [$\text{mg (g dry wt)}^{-1}$] estimated by GLC are indicated in parentheses for 1 and 20 mM glucose, respectively. $\circ$, Glucose (0.37 and 0.67); $\bullet$, fructose (0.22 and 0.49); $\square$, glucitol (2.06 and 3.30); $\blacksquare$, mannitol (0.54 and 1.09); $\triangle$, trehalose (4.26 and 8.75). Specific activities of exogenous glucose at the start of the radiolabelling period were 140 d.p.m. ng^{-1} (1 mM), and 9.6 d.p.m. ng^{-1} (20 mM), respectively.

Fig. 5. Distribution of radioactivity between phosphate esters of neutral sugars ($\circ$) and other anions ($\bullet$) during the assimilation of 1 mM exogenous [^{14}C]glucose (see Fig. 1, Methods and Results for experimental and analytical details).

Fig. 6. Chromatographic analysis of ethanol-soluble anions extracted from mycelium after incubation in 1 mM [^{14}C]glucose. Radioactivity was detected using a Nuclear Chicago strip counter after development in appropriate solvent systems (see Methods). (a) Neutral compounds remaining after phosphatase treatment of the total anion fraction obtained 2 min after adding the label. (b) and (c) Compounds which retained anionic charge after phosphatase treatment of the total anion fractions obtained 10 min (b) and 110 min (c) after adding the label. Solvent 1 was used for (a) and Solvent 2 for (b) and (c). The positions of authentic standards run on the same chromatograms are shown. 'Tricarboxylic acid (TCA) cycle intermediates' represents succinic, fumaric and malic acids, each of which produced a broad spot over the position indicated.

Incorporation of radioactivity into cations

At each time point investigated more than 80% of the label in cations was located in two major peaks on chromatograms, corresponding to (i) glutamic and/or cysteic acids and (ii) alanine and/or glutamine (Table 1). The peak corresponding to glutamic and/or cysteic acids showed a decrease after 10 min indicating turnover, whereas the peak corresponding to alanine and/or glutamine continued to increase, suggesting accumulation into a storage pool.

DISCUSSION

Results obtained during this investigation indicate that low (1–20 mM) concentrations of D-glucose are assimilated by pathways involving phosphorylation, isomerization and reduction as primary metabolic events.

Table 1. *Conversion of [¹⁴C]glucose to amino acids*

The fungus was incubated in 1 or 20 mM [U-¹⁴C]-D-glucose as described in Fig. 1, and samples taken for analysis as described in the Methods.

Amino acid	Percentage of total cellular radioactivity					
	1 mM [¹⁴ C]Glucose			20 mM [¹⁴ C]Glucose		
	Incubation time (min):			Incubation time (min):		
	2	10	110	2	10	110
Aspartic acid	1.7	1.8	0.5	ND	ND	0.1
Glutamic plus cysteic acid	6.3	13.8	5.7	5.4	10.9	5.7
Alanine plus glutamine	9.6	16.2	23.2	8.8	15.9	28.2
Valine plus methionine	1.3	1.8	1.3	1.9	2.3	0.8
Other cations	0.6	0.4	1.2	0.2	0.3	0.1
Total cations	19.5	34.0	31.9	16.3	29.4	34.9

ND, Not detected.

Assimilation via phosphorylated intermediates

Quantitatively, phosphorylation of glucose appears to be the major primary step in the assimilation of glucose because metabolites of hexose phosphates contained considerable amounts of assimilated radioactivity at the earliest times after adding exogenous [¹⁴C]glucose. Thus, 2 min after adding label, trehalose phosphate, free trehalose, and amino acids contained about 80% of the radioactivity present in metabolites of glucose. Because phosphates of glucose and fructose contained only traces of radioactivity they constituted small, rapidly turning-over pools, which, like the intracellular pool of free glucose, became saturated with label within 2 min of adding [¹⁴C]glucose. The lack of a lag of incorporation of label into trehalose phosphate or amino acids shows that pools of sugar phosphates quickly reached isotopic equilibrium.

Assimilation via fructose

The rapid appearance of label in free fructose would be difficult to detect in infected plant material, because fructose of host origin would obscure fungal fructose. The kinetic data were consistent with two possible routes of synthesis of fructose: (1) by direct isomerization from glucose as demonstrated in prokaryotes (Suekane *et al.*, 1978; Chen *et al.*, 1979) or (2) from fructose-6-phosphate by phosphatase activity. The pathway of fructose synthesis via glucitol demonstrated in animal systems (Hers, 1960; Touster & Shaw, 1962) can be discounted because label accumulated in fructose prior to glucitol (cf. Figs 3 and 4). Synthesis of fructose via sugar phosphates would waste energy with no apparent advantage to the fungus. However, aerial hyphae of *Melampsora lini* after 4 h incubation in specifically labelled glucose showed labelling of fructose consistent with some synthesis via the pentose phosphate pathway (Mitchell & Shaw, 1968). Further research is necessary to elucidate pathways of fructose synthesis in rust fungi.

Synthesis of glucitol and mannitol

Despite many analyses of carbohydrates in rust fungi by previous workers, few have detected glucitol, and careful attention to technique is necessary to resolve glucitol from mannitol, even in defatted and deionized extracts (Fig. 2), as discussed by Maclean (1982).

Kinetic data are consistent with glucitol being synthesized from both glucose and fructose, but with mannitol being synthesized only from fructose. Fructose took up label without lag and prior to each hexitol (Fig. 4). The role of fructose as a precursor of glucitol is evident from the lag in movement of label into glucitol, which would not be expected if glucitol was only synthesized from the more rapidly labelled glucose pool. However, at early time points label moved into glucitol prior to mannitol (Fig. 4), indicating an additional precursor pool such as glucose that was labelled at a faster rate than fructose. The accumulation of 2-deoxyglucitol after feeding 2-deoxyglucose (Manners *et al.*, 1981) is also consistent with glucose being a precursor of glucitol.

Synthesis of trehalose

Using cell-free extracts of *Saccharomyces cerevisiae*, Cabib & Leloir (1958) showed trehalose synthesis via glucose-6-phosphate, UDPG and trehalose phosphate. Our *in vivo* experiments with *P. graminis* are consistent with this pathway, because trehalose phosphate accepted label prior to trehalose. Trehalose phosphate constituted the largest pool of sugar phosphates, and the time required to saturate this pool accounts for the lag observed in the synthesis of trehalose (Fig. 4). Total label in free plus phosphorylated trehalose accumulated linearly with time, indicating a common origin from small, rapidly turning-over pools of glucose phosphates and sugar nucleotides.

Sources of carbon in sugar and polyol pools

Because steady-state specific activities of cellular pools of sugars and polyols were much lower than specific activities of exogenous glucose, a large flow of unlabelled carbon also entered these pools. Possible sources of this unlabelled carbon include exogenous peptone or citrate, or endogenous reserves such as lipids or glycogen. Pathways for mobilizing carbon into free sugars from any of the above sources might have been induced after transfer from 212 mM-glucose (growth medium), and during the 18 h incubation in the lower concentrations necessary for providing high specific activity [^{14}C]glucose to the fungus. The proportion of unlabelled to labelled glucose entering the sugar and polyol pools was greater with 1 mM than with 20 mM exogenous glucose, but the kinetics of ^{14}C assimilation into all metabolites were almost identical at each concentration. Therefore, regardless of the source of unlabelled carbon in sugar and polyol pools, the pathway of assimilation of exogenous glucose appears to be the same.

The rapid movement of carbon from exogenous [^{14}C]glucose to amino acids suggests that when *P. graminis* is maintained on low concentrations of glucose, there may be concurrent counterflows of carbon through glycolysis and gluconeogenesis. This would result in the operation of a number of futile cycles (Hue, 1981), and thus waste ATP. If pools of trehalose are turned over, as is suggested by the specific activity of trehalose tending towards that of intracellular glucose, this also would result in wastage of energy, because the two molecules of ATP required for the synthesis of trehalose from free glucose are not recouped during its catabolism by trehalase (Elbein, 1974), or trehalose phosphorylase (Marechal & Belocopitow, 1972). Such metabolic inefficiency could be a contributing factor to the requirement of *P. graminis* for high glucose concentrations for optimal growth (cf. Maclean, 1974).

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