Bot. Bull. Academia Sinica 25: 71-79 (1984)

EFFECTS OF *ALTERNARIA TENUISSIMA* ON THE COMPOSITION OF CARBOHYDRATES IN THE LEAVES OF *SOLANUM MELONGENA* L.

M. O. BASALAH

Department of Botany, College of Science
King Saud University, Riyadh, Saudi Arabia

(Received June 24, 1983; Accepted October 28, 1983)

Abstract

The major soluble carbohydrates extracted from leaves of *Solanum melongena* L. are fructose, sorbitol, glucose, inositol, sucrose and an unknown sugar. The content of fructose and glucose increased progressively with fungal attack. The sucrose level was higher in healthy leaves and lower in the first stage of infection. Sorbitol was found to increase with later stages of infection whereas inositol decreased with infection. An unidentified carbohydrate was detected in the late stages of infection. The results are comparable with those obtained for other fungi.

Introduction

The content of reducing sugars increased in leaves of *Solanum melongena* L. with attack of the pathogen *Alternaria tenuissima* (Kunza ex Pers.) Wiltshire while non-reducing sugars decreased and soluble amino and organic acids generally decreasad with the attack (Basalah *et al.*, unpublished results). Choudhri and Garg (1955) studied the metabolic changes in brinjal and noted that only susceptible varieties were affected by the disease. Plant pathogens induced changes in photosynthesis, respiration, soluble nitrogen compounds and some enzyme activities and decrease activity of growth regulating substances in plants (Diener, 1963; Goodman *et al.*, 1966; Kapur and Weathers, 1971; Barbara and Wood, 1972). Holligan *et al.* (1973) reported changes in the individual soluble carbohydrates in leaves of *Tussilago farfara* and *Poa pratensis* infected by the rust *Puccinia poarum*. Only a few reports have stated the effect of plant diseases on individual sugars.

In a previous paper (Basalah *et al.*, unpublished results) metabolic changes were noted in leaves of *Solanum melongena* L. attacked by *Alternaria tenuissima*.

These were, however, on total amounts of reducing sugars which were shown to increase with infection, while non-reducing sugars, amino acids and organic acids decreased with the attack.

This paper investigated further the changes of individual soluble reducing and non-reducing sugars.

Materials and Methods

Plant Material

Leaves of the egg plant (*Solanum melongena* L.) both healthy and with various degrees of attack with *Alternaria tenuissima* were collected from the fields of the Faculty of Agriculture, King Saud University, on 3 November, 1981. Leaf discs were sampled immediately after collection using a cork borer of 10 mm diameter. The following notation is used to describe the healthy and attacked leaf discs: H, discs of healthy leaf; D_1, D_2, D_3, D_4 and D_5, discs with necrotic area less than 25% about 25%, 25–50%, 50–75% and 75–100%, respectively.

Extraction Procedure and Anlysis of Exctracts

Leaf discs were extracted by boiling under reflux with methanol/chloroform/water (MCW, 12/5/3, v/v) with three changes of the solvent as previously described by Holligan *et al.* (1973). The water soluble portion was used for analysis and fractionated on ion-exchange resins for neutral fraction (carbohydrates) (Basalah, 1978).

Carbohydrates were identified by one-dimensional paper chromatography using two solvents: ethyl acetate/acetic acid/water (EAW, 11:3:3, Smith, 1960) and methyl ethyl ketone/acetic acid/boric acid (MAB, 9:1:1, Rees and Reynolds, 1958). Chromatograms were developed in silver nitrate-sodium ethoxide (Trevelyan *et al.*, 1950), and identified from their Rg values (Smith, 1960).

Identification of carbohydrates was confirmed and quantitive analysis made by gas-liquid chromatography, GLC; (Holligan and Drew, 1971), using a Pye series 204 column analytical gas chromatograph with flame ionization detectors and glass coulmns in conjunction with a recorder. Carbohydrates were converted to their trimethylsilyl (TMS) derivatives before analysis (Sweeley *et al.*, 1963) and the stationary phase used was 3% (w/v) SE 30 (Pye Unicam).

Results

Qualitative Identification of Carbohydrates

Chromatograms of the neutral soluble fraction of healthy and infected leaves together with marker carbohydrates are shown in Figs. 1 and 2. The appropriate

Rg values are shown in Tables 1 and 2. It can be seen that the principal sugars present in healthy and all stages of infected leaves were fructose, glucose and inositol. Sucrose was present only in healthy and D_1 of infected leaves and disappeared in other stages. Sorbitol, on the other hand, was present in all stages of infected leaves but not in healthy leaves.

Spots 1 and 2 (Fig. 1) were characteristic of neutral extracts of reserve-storing tissues, i.e. fructosan oligosaccharides of less than approximately ten hexose units (Wain *et al.*, 1964; Basalah, 1978).

Quantitative Estimation of Carbohydrates

GLC was used for this purpose using TMS derivatives of carbohydrates. Fig. 3 shows GLC traces of the neutral soluble fraction of carbohydrates in

Table 1. *Principal carbohydrates of the soluble fraction in extracts of leaves of egg-plant (Solanum melongena L.)*

Reference	Carbohydrate	R_g values (E.A.W. solvent)						
		Marker carbohydrate	Leaf extract					
			H	D_1	D_2	D_3	D_4	D_5
A	Inositol	0.41	0.42	0.42	0.42	0.42	0.42	0.42
B	Sucrose	0.70	0.71	0.71	—	—	—	—
C	Glucose	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	Mannitol	1.067	—	—	—	—	—	—
D	Fructose	1.28	1.27	1.27	1.27	1.27	1.27	1.27
E	Sorbitol	—	—	—	1.48	1.48	1.48	1.48

Table 2. *Principal carbohydrates of the soluble fraction in extracts of leaves of egg-plant (Solanum melongena L.)*

Reference	Carbohydrate	R_g values (M.A.B. solvent)		
		Marker carbohydrate	Leaf extract	
			H*	D**
A	Inositol	0.154	0.154	0.154
B	Sucrose	0.301	0.300	0.300
C	Glucose	1.000	1.00	1.00
D	Fructose	1.65	1.64	1.65
	Mannitol	2.11	—	—
E	Sorbitol	2.40	—	2.40

*,** Helathy and diseased, respectively.

Table 3. *Relative retention times of peaks with respect to β-glucose peak ($RT_{\beta g}$ values) of the soluble carbohydrates present in ethanol soluble fractions of healthy and diseased leaves of egg-plant (Solanum melongena L.) analysed by GLC*

Values of $RT_{\beta g}$ listed are means of triplicate estimations.

Possible identify of carbohydrate with similar $RT_{\beta g}$ value	Mean $RT_{\beta g}$ in marker	Mean $RT_{\beta g}$ in sample
U_1*	—	0.718
Fructose	0.844	0.845
α-glucose	0.936	0.936
β-glucose	1.00	1.00
Sorbitol	1.015	1.015
Inositol	1.214	1.215
Sucrose**	1.895	1.895

* Unidentified compound present only in D_4 and D_5 leaves in significant quantities.

** Present only in healthy and D_1 leaves. Absent from D_2—D_5 leaves.

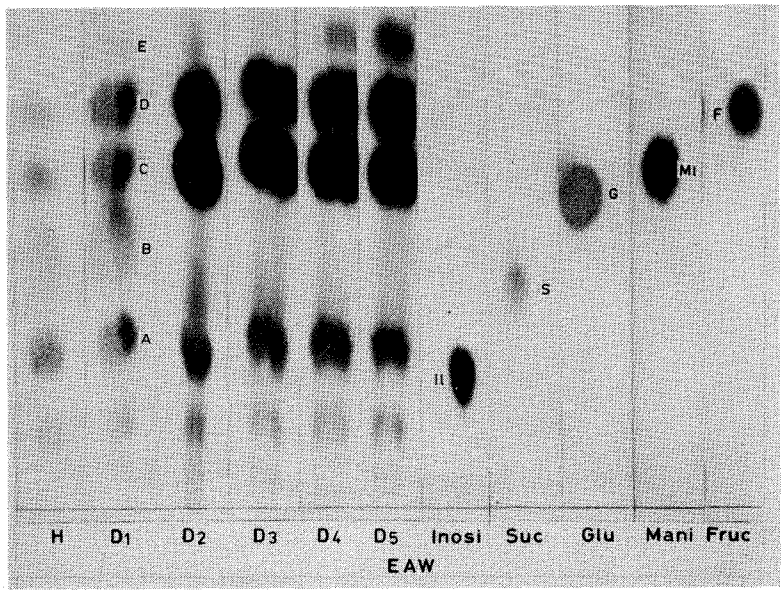
Table 4. *Quantitative determination of carbohydrate content of leaves of egg-plant (Solanum melongena L.) by GLC*

Values listed are means±SD of triplicate estimations.

Treatment	Sugars conc. (μg/mg dry wt.)					U_1*
	Fructose	α+β glucose	Sorbitol	Inositol	Sucrose	
Healthy	6.0±0.2	16.0±0.2	3.0±0.2	35.0±0.4	264.0±3.2	—
D_1	17.0±1.9	20.0±1.6	Trace	19.0±2.6	162.0±0.6	—
D_2	51.0±2.1	94.0±2.1	Trace	24.0±0.01	Absent	—
D_3	41.0±1.2	71.0±1.3	Trace	17.0±0.03	Absent	—
D_4	33.0±0.4	60.0±8.3	8.0±0.4	15.0±0.1	Absent	8.0±0.03*
D_5	20.1±0.3	36.0±1.6	15.0±0.6	9.0±0.6	Absent	14.0±0.11*

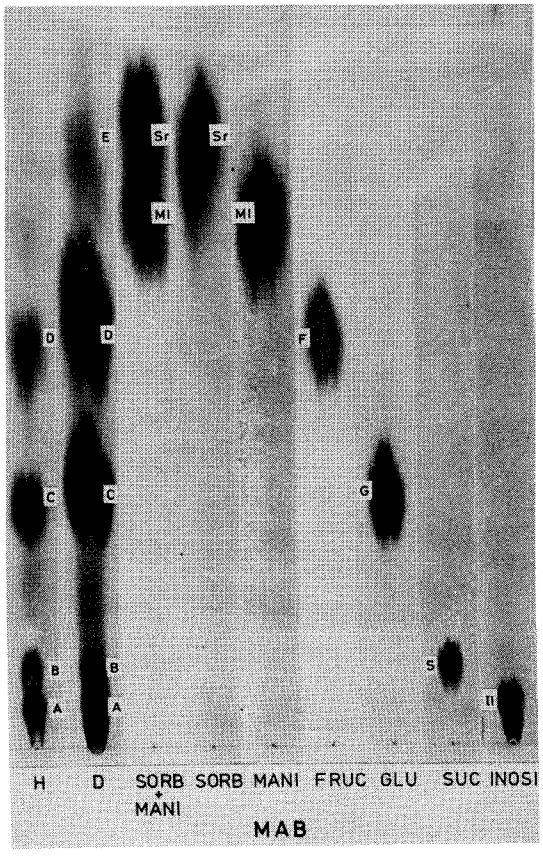
* Calculated as μg glucose equivelants $(mg\ DW)^{-1}$

healthy and diseased leaves. Identification of sugars in GLC traces was done by co-chromatography (Fig. 4) and by calculating their retention times with respect to β-glucose ($RT_{\beta g}$ values) which are listed in Table 3. Quantitative variation of the individual sugars in healthy and diseased leaves is shown in Table 4, and were obtained by reference to calibration curves of the different sugars from their peak heights.



↑

Fig. 1. Carbohydrates of neutral fractions of healthy (H) and infected (D_1—D_5) leaf extracts of egg-plant (E. A. W. solvent, silver nitrate/sodium ethoxide reagent). Inosi=Inositol, Suc =Sucrose, Glu=Glucose, Mani = Mannitol, Fruc = Fructose. (A—E refer to Table 1).



←

Fig. 2. Carbohydrate of neutral soluble fractions of healthy (H) and infected (D) leaf extract of egg-plant (M. A. B. solvent, silver nitrate/sodium ethoxide reagent). Sorb = Sorbitol, Mani = Mannitol, Fruc=Fructose, Glu=Glucose, Inosl=Inositol. (A—E refer to Table 3).

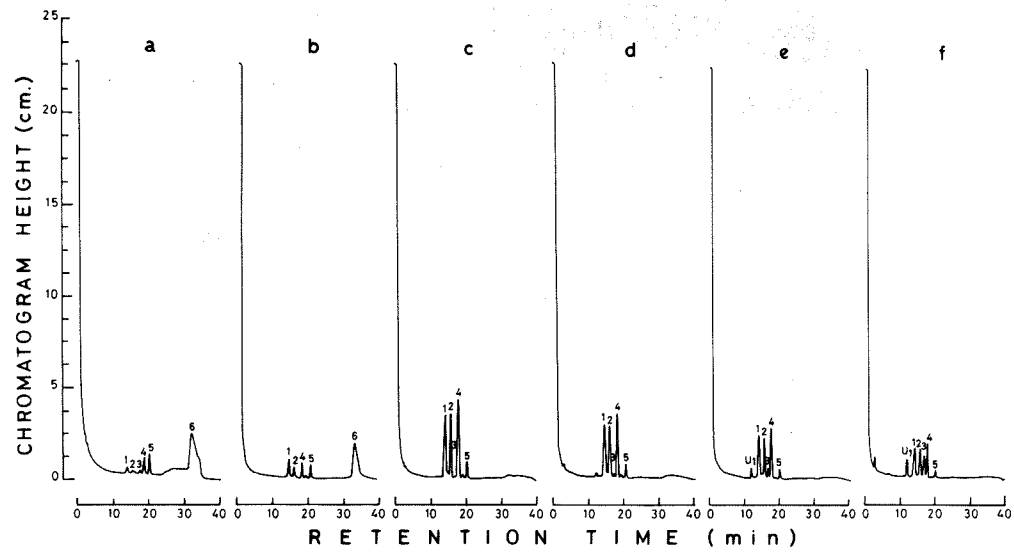


Fig. 3. Analysis by gas-liquid chromatography of TMS derivatives of soluble carbohydrates in healthy and diseased leaves of egg-plant. Temperature programm: 140-290°C at 4°/min, attenuation 4×10^3. Abbreviations: a=Healthy, b=Diseased-1, c=Diseased-2, d=Diseased-3, e=Diseased-4, f=Diseased-5.

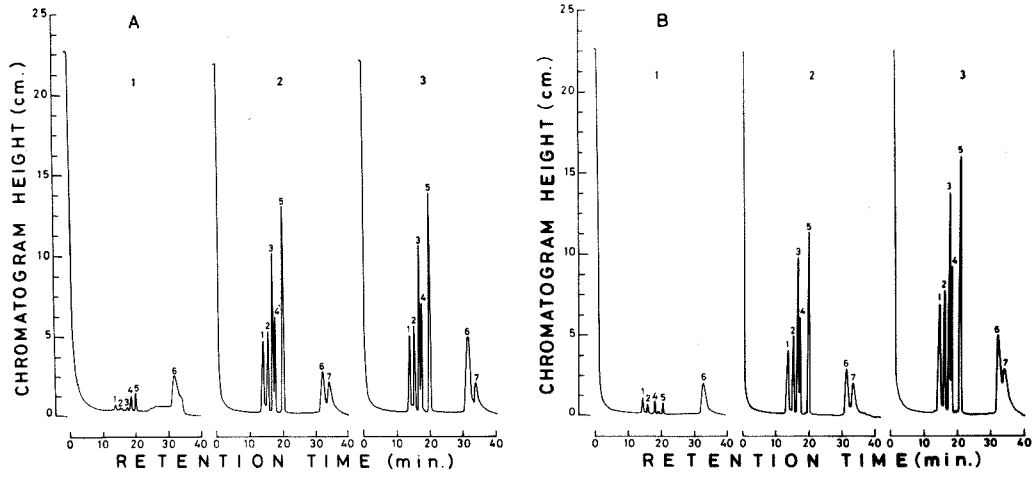


Fig. 4. Analysis by gas-liquid chromatography of TMS derivatives of soluble carbohydrates in healthy and diseased leaves of egg-plant. Temperature programme: 140-290°C at 4°/min, attenuation 4×10^3. Abbreviations: A_1=Healthy, A_2=Marker, A_3=Healthy+Marker; 1=Fructose, 2=α-glucose, 3=Sorbitol, 4=β-glucose, 5=Inositol, 6=Sucrose, 7=Trehalose; B_1=Diseased 1, B_2=Marker, B_3=Diseased 1+Marker.

From Table 4, it is clear that the two hexoses, fructose and glucose, increased initially and then decreased with increase of infection in leaves. On the other hand, sucrose behaved in the opposite manner. It was present in high amounts in healthy leaves, then in D_1, it dropped and disappeared completely in D_2 to D_5. Inositol quantity was highest in healthy leaves and gradually decreased with stages of infection. Sorbitol was present in small quantities in healthy leaves. Then only trace amounts of it were present in D_1, D_2 and D_3. The highest quantities of sorbitol were obtained with the highest stages of infected leaves (D_4 and D_5). Also characteristic of the last two stages of infection (D_4 and D_5) was the presence of an unidentified carbohydrate noted U_1 in Fig. 3 and Table 4 with its quantity highest in D_5.

Discussion

Sugar alcohols were reported to be accumulated in sites of fungal infection (Holligan *et al.*, 1973, 1974; Whipps and Lewis, 1981). In the present study, sorbitol was found to increase with the later stages of infection and its value was highest in the last stage of infection. This supports the findings of Fung (unpublished, cited by Lewis, 1976) who reported accumulation of sorbitol in *Poa pratensis* infected by *Puccinia graminis*. Levels of inositol were reported in the present study to decrease with infection. This is in agreement with the fact that inositol is commonly required as a growth factor for fungi (Holligan *et al.*, 1973). Biochemical investigation have been carried out on fungal translocation of carbohydrates and it has usually been found that the major compounds moving are polyols, principally mannitol (Lewis and Harley, 1965; Smith, 1967; Milne and Cooke, 1969). In fact, there is reason to believe that polyols have a major function in this regard in the majority of symbiotic and parasitic fungi (Lewis and Smith, 1967; Smith *et al.*, 1969). The present study is in agreement with this since sorbitol is shown to increase with the infection.

The unidentified carbohydrate (U_1) shown in the present study appeared in the latest stages of infection with its highest content in the last stage of infection.

Two reducing sugars, fructose and glucose, were found in healthy leaves, increased initially and then decreased with fungal attack. This was opposite to the behaviour of the non-reducing sugar, sucrose. This supports the findings of Long and Cooke (1974) on accumulation of the hexoses and decrease of sucrose in *Senecio squalidus* leaves infected with *Albugo tragopogonis* (Pers.) S.F. Gray. Content of the sucrose was highest in healthy leaves and less at the early stage of infection, but disapeared at later stages of infection. It can be postulated that invertase enzyme activity increases with fungal attack and that sucrose is then readily converted to its constituents, fructose and glucose. This is supported by

Long and Cooke (1974) and Whipps and Lewis (1981) who stated that the amount of sucrose varies and is commonly influenced by the activity of invertases. However, it has been reported that powdery mildew on sugar beet (*Beta vulgaris*), led to an inhibition of photosynthetic carbon dioxide assimilation and a shift in the products from sucrose to amino acids (alanine, glutamate, aspartate). These observations provide an explanation for the report that sugar beet plants infected with powdery mildew have reduced capacity to form sucrose. In addition, there is a reduction in the activity of enzymes that led to production of organic acids (Magyanosy *et al.*, 1976).

Holligan *et al.* (1973) reported a general increase in the two hexoses, glucose and fructose, with infection and also fructans increased. Sucrose, however, was not shown to decrease with infection as in the present study, but it was also shown to increase.

Literature Cited

Barbara, D.J. and K.R. Wood. 1972. Virus multiplication, peroxidase and polyphenol oxidase activity in leaves of two cucumber (*Cucumis sativus*) cultivars inoculated with cucumber mosaic virus. Physiol. Plant Path. **2**: 167-173.

Basalah, M.O. 1978. Hormone directed transport of ^{14}C-labelled assimilates in *Dahlia variabilis*. Ph.D. thesis, University of Sheffield, England.

Choudhri, R.S. and O.K. Garg. 1955. Effect of "Little-leaf" disease on the growth and physiology of brinjal (*Solanum melongena* L.). Indian J. Hortic. **12**: 180-187.

Diener, T.O. 1963. Physiology of virus-infected plants. Ann. Rev. Phytopath. **1**: 197-218.

Goodman, R.N., Z. Kiraly, and M. Zaithn. 1966. The Biochemistry and Physiology of Infectous Plant Diseases. D. Van Nostrand Co., Inc., pp. 187-281.

Holligan, P.M. and E.A. Drew. 1971. Routine analysis by gas-liquid chromatography of soluble carbohydrates in extracts of plant tissues. II. Quantitative analysis of standard carbohydrates, and the estimation of soluble sugars and polyols from a variety of plant tissues. New Phytol. **70**: 271-297.

Holligan, P.M., C. Chen, and D.H. Lewis. 1973. Changes in the carbohydrate composition of leaves of *Tussilago farfara* during infection by *Puccinia poarum*. New Phytol. **72**: 947-955.

Holligan, P.M., E.M.M. McMgee, and D.H. Lewis. 1974. Quantitative determination of starch and glycogen and their metabolism in leaves of *Tussilago farfara* during infection by *Puccinia poarum*. New Phytol. **73**: 873-879.

Kapur, S.P. and L.G. Weathers. 1971. Some metabolic changes in *Lycopersicum esculantus* plants infected with potato spindle tuber virus. Phytopath. **61**: 1322.

Lewis, D.H. 1976. Interchange of metabolites in biotrophic symbiosis between Angiosperms and fungi. *In* N. Sunderland (ed.), Perspectives in Experimental Biology, Vol. 2, Pergamon Press, Oxford, pp. 207-219.

Lewis, D.H. and J.L. Harely. 1965. Carbohydrate physiology of mycorrhizal roots of beech. II. Movement of sugars between host and fungus. New Phytol. **64**: 256-269.

Lewis, D.H. and D.C. Smith. 1967. Sugar alcohols (polyols) in fungi and green plants. I. Distribution, physiology and metabolism. New Phytol. **66**: 143-184.

Long, D.E. and R.C. Cooke. 1974. Carbohydrate composition and metabolism of *Senecio squalidus* L. leaves infected with *Albugo tragopogonis* (Pers.) S.F. Gray. New Phytol. **73**: 889-899.

Magyarosy, A.C., P. Schurmann, and B.B. Buchanan. 1976. Effect of powdery mildew infection on photosynthesis by leaves and chloroplast of sugar beets. Plant Physiol. **57**: 486-489.

Milne, L. and R.C. Cooke. 1969. Translocation of ^{14}C-glucose by ***Rhizoctonia solani***. Trans. Brit. Mycol. Soc. **53**: 279-284.

Rees, W.R. and T. Reynolds. 1958. A solvent for the paper chromatographic separation of glucose and sorbitol. Nature **181**: 767-768.

Smith, D. (ed.). 1960. Chromatographic and Electrophoretic Techniques, Vol. I, Chromatography. Heinemann, London.

Smith, D., L. Muscatine, and D. Lewis. 1969. Carbohydrate movement from autotroph to heterotroph in parasitic and mutalistic symbiosis. Biol. Rev. **44**: 17-90.

Smith, S.E. 1967. Carbohydrate translocation in orchid mycorrhizas. New Phytol. **66**: 371-378.

Sweeley, C.C., R. Bentley, M. Makita, and W.W. Wells. 1963. Gas-liquid chromatography of trimethylsilyl derivatives of sugars and related substances. J. Am. Chem. Soc. **85**: 2497-2507.

Trevelyan, W.E., D.P. Proctor, and J.S. Harrison. 1950. Detection of Sugars on paper chromatograms. Nature **166**: 444-445.

Wain, R.D., P.P. Rutherford, E.W. Weston, and C.M. Griffiths. 1964. Effects of growth regulating substances on inulin storing plant tissues. Nature **203**: 504-506.

Whipps, J.M. and D.H. Lewis. 1981. Patterns of translocation, storage and interconversion of carbohydrates. ***In*** P.G. Ayres (ed.), Effects of Disease on the Physiology of Growing Plant. Cambridge University Press, Cambridge, pp. 47-83.

真菌 *Alternaris tenuissima* 對茄葉部碳水化合物組成分的影響

M. O. BASALAH

沙烏地阿拉伯利亞德沙烏地王大學理學院植物學系

茄葉部的主要可溶性碳水化合物是果糖、葡萄糖、蔗糖、花楸醇 (sorbitol)、肌醇 (inositol)，以及一種未知醣類。眞菌 *Alternaris tenuissima* 感染增加茄葉部的果糖、葡萄糖含量。蔗糖量以健康葉最高，而以第一階段感染者較低。花楸醇量隨眞菌感染程度而增加，但肌醇量反而減少。一種未鑑定的碳水化合物出現於感染後期。