(14), the only difference being that the apparatus was evacuated, and then the ethylene-containing mercuric perchlorate solution was drawn in. This procedure avoided possible losses by dissociation during evacuation of the system.

The following controls were run: As the tissue jars were set up, they were flushed with air drawn from a compressor with an intake well above the roof of the laboratory; at the same time, a volume of air equal to that of the twelve tissue chambers was drawn from the same source and passed through mercuric perchlorate solution. This solution was then tested manometrically to demonstrate freedom from ethylene. Similarly, a volume of oxygen equal to that used in the experiments was drawn from the same oxygen pressure cylinder and tested for purity. All solvents and reagents were freshly distilled or examined for residue by evaporation of a suitable aliquot. In no case was a possible contaminating amount of ethylene located.

Results

Manometric quantitative analysis showed that 13.5 kg of thistle leaves produced 10.8 ml (S.T.P.) of ethylene in four days in the first run. In the second run, 13.1 kg of leaves produced 6.0 ml of ethylene. Hence the total available was 16.8 ml; during the production of this amount, 400 l of oxygen were respired.

Melting points were obtained of the derivative N,N′-diphenylethylenediamine, of a known sample of this substance prepared from pure ethylene dibromide, and of a mixture of these. The values were determined simultaneously on the aluminum block of a Fisher-Johns melting point apparatus. All melted at 64.0–65.0°C (uncorrected), confirming the production of ethylene by detached leaves.

Summary

Using methods reported previously, ethylene was identified as a product of detached leaves of milk thistle (*Silybum marianum* Gaertn.).

Technical assistance by Mr. Carl Tucker, Mr. Milton Workman, and Mr. Fred D. Howard is gratefully acknowledged.

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METABOLISM OF SUCROSE AND RELATED OLIGOSACCHARIDES BY SPORES OF THE FUNGUS MYROTHECIUM VERRUCARIA[1]

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In an earlier study of invertase in spores of the fungus *Myrothecium verrucaria* it was postulated that metabolism of sucrose did not proceed through a hydrolytic pathway in spite of the presence of an excess of invertase (9). This was inferred from the equivalence of the measured invertase activity of living, metabolizing spores with that of killed spores. The present paper reports further studies related to the proposed non-hydrolytic mechanism. Experiments with the related sugars raffinose and melezitose and with turanose have also been carried out to compare the metabolic patterns with the structural relations of the sugars.

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Methods

Spores of the fungus *Myrothecium verrucaria* (QM 460) were obtained from cultures grown on a medium composed of distilled water—1000 ml, NH_4NO_3—3.0 gm, $MgSO_4 \cdot 7\,H_2O$—2.22 gm, KH_2PO_4—2.59 gm, K_2HPO_4—2.21 gm, Agar—15 gm. A sheet of filter paper served as carbon source (9). Washed suspensions of spores in 0.05 M $KHPO_4$ buffer at pH 6.25 were used in all work unless otherwise specified.

Respiration measurements were by conventional Warburg techniques at 30°C using 1.0 ml of spore suspension in the main compartment, 0.5 ml of substrate—usually 3%—in the side arm, and 0.2 ml 10% KOH in the center well. Rates of respiration are given as Q_{O_2} on a dry weight basis, i.e., μl O_2 per mg spores per hr or as μg sugar oxidized per mg spores per hr. To calculate this latter value a respiratory quotient of 1 was used. Actually, the R.Q. in sugar solutions increases from about 1.0 to about 1.2 after several hours. Thus the calculated quantities of sugar respired represent minimum values. Respiration of sugars in phosphate buffer is not significantly different from that in inorganic nutrient solution.

Carbohydrase activity was measured by determination of reducing sugars formed in suspensions incubated at 30°C on a reciprocal shaker with the dinitrosalicylic acid method of Sumner (14), or by specific determination of glucose using the glucose oxidase method of Keilin and Hartree (7). In all cases, rates of hydrolysis are reported in terms of μg reducing sugar (RS) formed per mg dry weight of spores per hour.

Total sugars were determined by hydrolysis with 2.5 *N* HCl at 60° for 10 minutes. After neutralizing with alkali, reducing sugars were determined as indicated above.

Assimilation is given as the increase in dry weight in μg per mg original dry weight per hr after incubation at 30°C on a reciprocal shaker. Aliquots of the suspensions were filtered through sintered glass crucibles of fine porosity, dried at 80°C, and weighed. Assimilation of sucrose by these spores is only slightly affected over a period of five hours by the inorganic composition of the medium, the rate being increased about 3% in phosphate buffer (pH 6.25), and about 9% in inorganic nutrient solution over that in distilled water.

Acid treatment of spores was effected by combining a suspension in distilled water with an equal volume of 0.2 *N* HCl, incubating at 30°C for twenty minutes, centrifuging, washing, and resuspending in buffer. Control samples received identical treatment except that equal quantities of distilled water were used instead of acid.

In any one experiment spores were obtained from cultures all of the same age. Unfortunately it was not practical to use spores of the same age in all experiments—most of the work being done with spores from cultures 8–30 days old. To a large extent the variations in absolute values reported in different tables can probably be ascribed to differences in spore age.

Table I

Effect of Azide on Metabolism of Sucrose

		Sugar metabolized or hydrolyzed μG/MG × HR	
		$-NaN_3$	$+NaN_3$ (5×10^{-3} M)
Respiration	(1)	24	0
Assimilation	(2)	70	14
Sugar metabolized	(1+2)	84	14
Invertase activity		389	389

(Duration of experiment 4 hours; 6.5 mg spores/ml; inorganic nutrient solution.)

Table II

Effect of Method of Blocking Metabolism on Hydrolysis of Sucrose and Raffinose by M. verrucaria Spores

Treatment	Rate of hydrolysis *		Relative hydrolysis	
	Sucrose	Raffinose	Sucrose	Raffinose
Control	307	30	1.00	1.00
Azide (5×10^{-3} M) ..	307	67	1.00	2.23
Toluene	327	74	1.06	2.46
Merthiolate (0.25%)	307	73	1.00	2.43
Anaerobic (N_2) ...	287	67	0.93	2.23

(0.05 M PO_4 buffer pH 6.25; 1.5 mg spores/ml; 1% sugar.)

* μg/mg × hr—from slopes of linear curves; corrected for autolytic release of reducing sugars which was significant only where toluene added.

Experimental Results

Metabolism of Sucrose: It can be reasoned that if sucrose were metabolized via invertase, then the invertase activity measured with suspensions of metabolizing spores should be less than that of dead spores or of spores whose assimilatory mechanisms are blocked by inhibitors. The magnitude of this difference should approximate the rate of metabolic utilization of the products of hydrolysis. Measurements summarizing certain phases of the metabolism of spores in solutions of sucrose in the presence and absence of sodium azide are presented in table I. The invertase activity of the suspension has not been affected by the azide, although respiration and assimilation have been almost completely inhibited. The sugar metabolized, as calculated from the increase in dry weight and from the respiration data, can be accounted for within the limits of experimental error by the decrease in sucrose remaining in the solution (unpublished). If sucrose were metabolized solely through the mediation of invertase, then blocking assimilation and respiration with azide could be expected to increase the production of reducing sugars by an amount equal to the rate of utilization of these

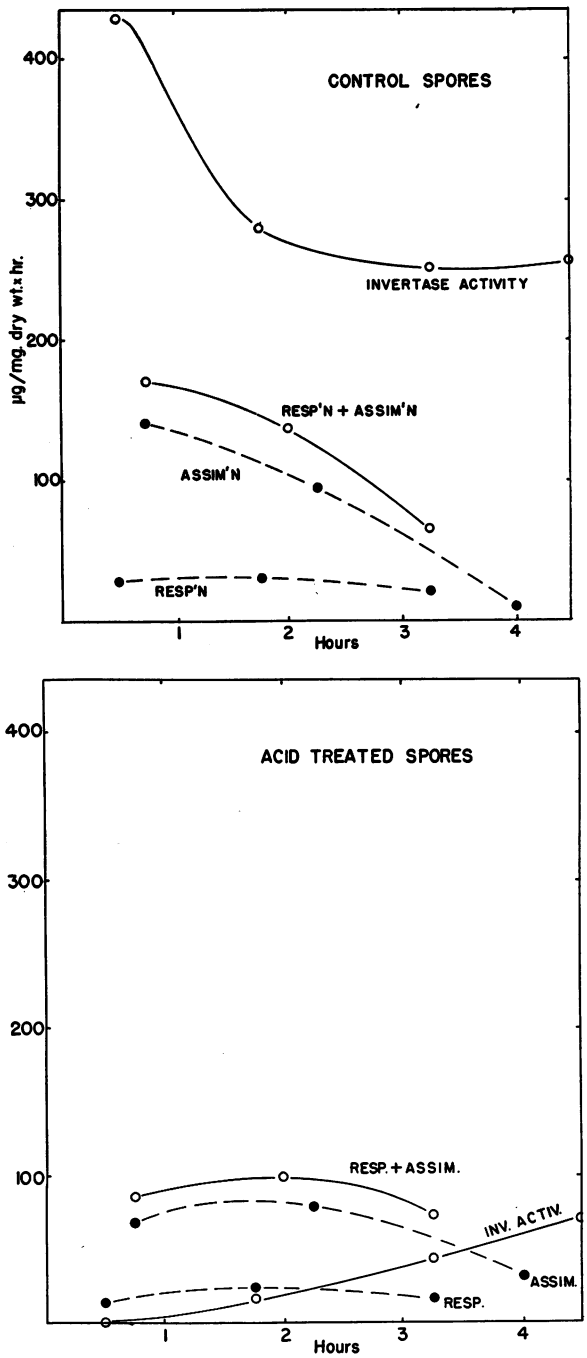

FIG. 1. Metabolism and hydrolysis of sucrose. A. Control spores. B. Acid treated spores. (Data in terms of µg sugar metabolized or hydrolyzed per mg dry wt of spores per hour.)

sugars—in other words, invertase activity in the presence of azide should be approximately 483 instead of 389. It can be inferred that sucrose enters the metabolism of the cell by some means other than through invertase action. Other methods of blocking metabolism—toluene, merthiolate, anaerobiosis—also fail to increase the rate of sucrose hydrolysis (table II). Heating to 60°C, a treatment which kills the spores but does not inactivate the invertase, does not increase the measured invertase activity.

A useful technique to develop further evidence on the role of invertase in sucrose metabolism would be a specific inhibitor for the enzyme. While no such agent is available, it is possible to completely inactivate the invertase of *M. verrucaria* spores by incubation with HCl at ca pH 1.0 without significantly affecting the viability of the spores or their rate of germination (10). Data showing the effect of acid treatment on various metabolic activities are presented in figure 1 A, B. No invertase activity in the treated spores is evident prior to one hour, but by two and one-half hours reducing sugars can be detected. This gradual increase in activity represents synthesis of invertase rather than reactivation (10). In spite of the extremely low invertase in the acid-treated spores, assimilation and oxidation of sucrose proceed at relatively rapid rates. The differences between the respiration and assimilation curves for control and acid-treated spores can be ascribed to acid injury, since other data show glucose metabolism as well as endogenous respiration to be similarly affected. In control spores the invertase activity is more than twice as great as to account for the combined respiration and assimilation. For the acid-treated spores, however, the utilization of sugar greatly exceeds the production of reducing sugars. In calculating these values it has been assumed that in the oxidation of sugar an R.Q. of 1.0 maintains, and also that all of the increase in dry weight is due to assimilation of sugar. Correction of this latter value to account for absorption of buffer components is very small and would be more than offset by the correction of the amount of sucrose oxidized calculated for an R.Q. of 1.2, which is closer to the actual value. The obvious conclusion is that sucrose can be metabolized at a rate approaching normal without the participation of invertase. In the experiment from which these data were taken the spores were washed to remove the acid. In other experiments the acid has been neutralized by adding buffer. Essentially the same results are obtained in both methods. In the experiment depicted in figure 1, invertase activity was measured without the addition of any poisons. It could be argued that the measured activity is not real, and that most of the reducing sugars formed are being metabolized. This point was checked in a similar experiment in which toluene was added periodically to a number of aliquots to follow the course of synthesis of invertase after acid treatment. Summarizing the data at two hours we find that metabolism of sucrose is twice the hydrolysis. Significant invertase activity cannot be demonstrated in acid-treated spores incubated with toluene, merthiolate, azide, ether or subjected to heat treatment (unpublished).

Further evidence relating to the path of metabolism of sucrose is found in a comparison of the rates of oxidation and assimilation of sucrose with that of its constituent sugars (fig. 2). The data lend strong

support to the concept of direct metabolism of sucrose since this sugar is respired and assimilated faster than its constituent sugars, single or combined.

One of the pathways by which sucrose can be metabolized, at least in certain organisms, is by phosphorolytic cleavage in which sucrose is split into glucose-1-phosphate and fructose by the enzyme sucrose phosphorylase in the presence of phosphate (4). This reaction is competitively inhibited by glucose (1). The action of several concentrations of glucose on the oxidation of sucrose and of fructose by spores is shown in table III. Low concentrations of glucose (1%) increase respiration on sucrose, while in the presence of higher concentrations the respiration is below that with sucrose alone. Fructose, however, does not suppress respiration on sucrose. In the presence of 10% glucose the respiration on sucrose is lowered to about that with glucose plus fructose. Without sucrose, respiration is more rapid as glucose

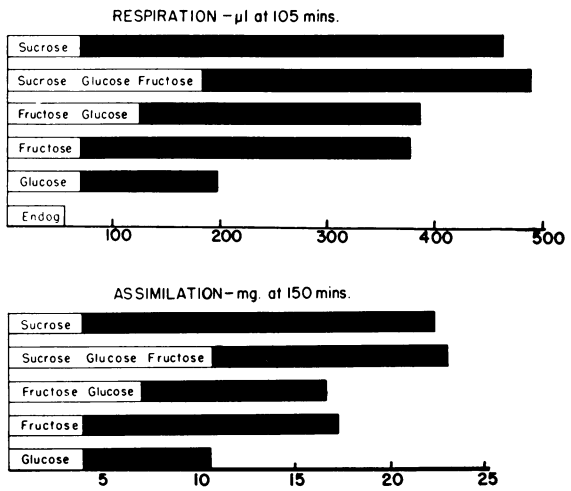

FIG. 2. Metabolism of sucrose and constituent sugars by spores. (Total concentration of sugar = 2% for assimilation; 1.33% for respiration.)

concentration is increased. High glucose increases somewhat the respiration on fructose.

Doudoroff *et al.* (2) have shown that sucrose phosphorylase is also capable of effecting arsenolysis of sucrose, the glucose-1-arsenate decomposing spontaneously into glucose and arsenate. The effect of arsenate on respiration with sucrose and related sugars is shown in table IV. Arsenate inhibits respiration on all of the substrates tested, but to varying degrees. It appears significant that the greatest inhibitory effect is found with sucrose, and furthermore that in the presence of arsenate, respiration on sucrose is equal to that on glucose + fructose. Endogenous respiration is stimulated slightly.

In view of the indications of some of the preceding data that a sucrose phosphorylase may be concerned in sucrose metabolism by these spores, several unsuccessful attempts to demonstrate the enzyme were made. The presence of both phosphatase and invertase in the spores interferes with the measurement of

TABLE III

EFFECT OF GLUCOSE AND FRUCTOSE ON RESPIRATION OF MYROTHECIUM VERRUCARIA SPORES IN PRESENCE OF SUCROSE

SUBSTRATE			Q_{O_2}*		RELATIVE Q_{O_2}*	
SUCROSE	GLUCOSE	FRUCTOSE	EXP. 1	EXP. 2	EXP. 1	EXP. 2
1%	0%	0%	16.9	16.5	1.00	1.00
1	1	0	18.1	18.1	1.07	1.09
1	10	0	14.5	15.0	0.86	0.91
1	25	0	14.1	...	0.83	...
1	0	10	...	19.8	...	1.2
0	0.5	0.5	...	14.3	...	0.87
0	10	0.5	...	15.9	...	0.96
0	1	0	11.5	11.2	0.68	0.68
0	10	0	12.3	14.4	0.73	0.87
0	25	0	13.9	...	0.82	...

(In buffer—0.05 M PO_4 at pH 6.25; Exp. 1—16 mg spores/vessel; Exp. 1—10 mg spores/vessel.)

* Not corrected for endogenous respiration.

phosphorylase. In experiments with whole spores, acid treatment was used to destroy the invertase. No phosphorylase activity could be demonstrated by measuring phosphate uptake of these spores in the presence of sucrose and phosphate when incubated under toluene. This may have been due to the fact that the phosphatase of the spores is not inactivated by acid treatment. Attempts to extract phosphorylase from ground spores following the procedure of Hassid *et al.* (4) in which fractional precipitation with $(NH_4)_2SO_4$ is employed were equally unsuccessful.

NATURE OF INVERTASE: Spores of *M. verrucaria* hydrolyze both sucrose and raffinose readily, yet do not hydrolyze melezitose, melibiose, nor turanose (tables V, VII and unpublished data). Glucose cannot be detected in the raffinose hydrolyzates. It is inferred that the enzyme in the spores is a fructosidase rather than a gluco-invertase (12). Several types of experiments were carried out to obtain data relating to the possibility that separate enzymes were involved in the hydrolysis of raffinose and sucrose by the spores. These experiments (unpublished) included: relative rates of hydrolysis of both substrates by spores and by spore extracts; determination of pH optimum for hydrolysis of both substrates; effect of

TABLE IV

EFFECT OF ARSENATE ON RESPIRATION

SUBSTRATE	µL O_2 IN 60′		INHIBITION
	CONTROL	+ 10^{-3} M ARSENATE	
Sucrose	326	189	42%
Glucose + fructose ..	259	183	29
Glucose	155	137	12
Fructose	215	171	20
Raffinose	118	103	13
Endogenous	34	43	−26

(12 mg spores/vessel; phthalate buffer pH 5.9; total sugar concentration—1%).

TABLE V

EFFECT OF ACID TREATMENT ON METABOLISM OF SUGARS BY MYROTHECIUM VERRUCARIA SPORES

SUBSTRATE	TREATMENT	μG SUGAR PER MG SPORES PER HR				
		METABOLISM			HYDROLYSIS	
		OXIDATION *	ASSIMILATION	OXIDATION + ASSIMILATION	− TOLUENE	+ TOLUENE †
Sucrose	Control	19	77	96	306	338
	Acid treat.	13	46	59	33	7.4
Raffinose	Control	9	33	42	129	204
	Acid treat.	4.8	7.8	13	8.6	6.3
Melezitose	Control	3.4	18	21	0	14
	Acid treat.	4.9	24	29	5.7	6.9
Glucose	Control	11	63	72	....	....
	Acid treat.	8	30	38	....	....

(Data are for 4 hours incubation with substrates.)
* Oxidation values calculated on basis of RQ = 1; not corrected for endogenous respiration.
† Values for hydrolysis under toluene are not corrected for autolysis.

raffinose and sucrose on resynthesis of the enzyme in acid-treated spores, activity being measured against both substrates. No evidence was encountered contradicting the assumption that a single enzyme is involved.

METABOLISM OF RAFFINOSE: Raffinose is respired and assimilated at rates approximately half those for sucrose and is hydrolyzed by metabolizing spores at about 40% of the rate for sucrose (table V). If metabolism is blocked or if killed spores are used, the apparent rate of raffinose hydrolysis is increased markedly. This increase is approximately equal to the rate of sugar utilization as calculated from the rates of respiration and assimilation (table II).

Acid treatment of spores renders them unable to hydrolyze raffinose and almost completely suppresses metabolism of this sugar (table V). Absorption of raffinose from the medium does not occur (table VI).

TABLE VI

METABOLISM OF SUGARS BY ACID-TREATED SPORES

SUBSTRATE	RESPIRATION	TOTAL SUGAR IN SOLUTION		
		INITIAL	1 HR	3 HR
	μl O_2 in 3 hr	*mg/ml*	*mg/ml*	*mg/ml*
Raffinose	54	0.80	0.86	0.82
Sucrose	216	1.12	0.76	0.0
Glucose	124	1.09	0.90	0.38
Eudogenous ...	42	...	...	...

(5.5 mg acid-treated spores/ml; phosphate buffer pH 6.25.)

The metabolism of sucrose and raffinose differs also in the effect of arsenate on respiration with these sugars as substrates (table IV). Arsenate inhibits sucrose respiration markedly and raffinose respiration only slightly. Effects of glucose on raffinose respiration could not be tested with this organism, since glucose is respired more rapidly than is raffinose.

At this stage in the investigation the data were all consistent with the hypothesis that raffinose was metabolized hydrolytically via invertase. If this were valid, then only one-third of the raffinose molecule—the fructose portion—should be utilized. Melibiose should, therefore, accumulate in the medium since no melibiase has been detected in the spores (unpublished). Under conditions of normal growth more than one-third of the total sugar is utilized. Experiments designed to effect complete utilization of available sugar—i.e., dense spore suspensions and low concentrations of sugar—showed that melibiose is absorbed very slowly under these conditions (fig. 3). The data are thus not inconsistent with the original hypothesis.

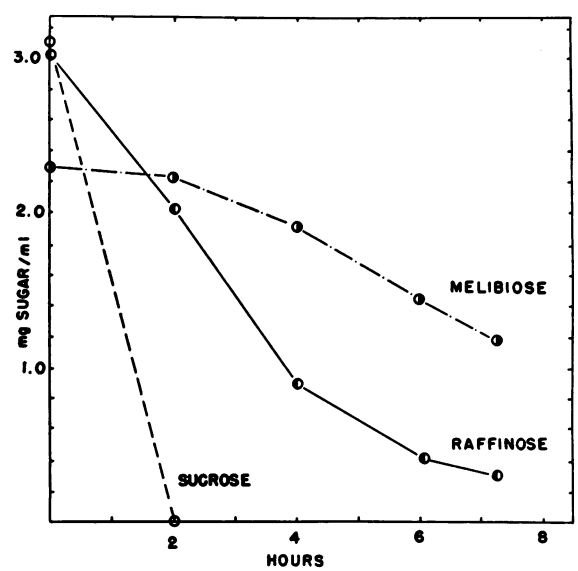

FIG. 3. Absorption of sugars by spores (ca 12 mg dry wt spores/ml; PO_4 buffer pH 6.25; data in terms of total sugars determined after acid hydrolysis).

METABOLISM OF MELEZITOSE: Melezitose is oxidized by *M. verrucaria* spores at rates significantly above

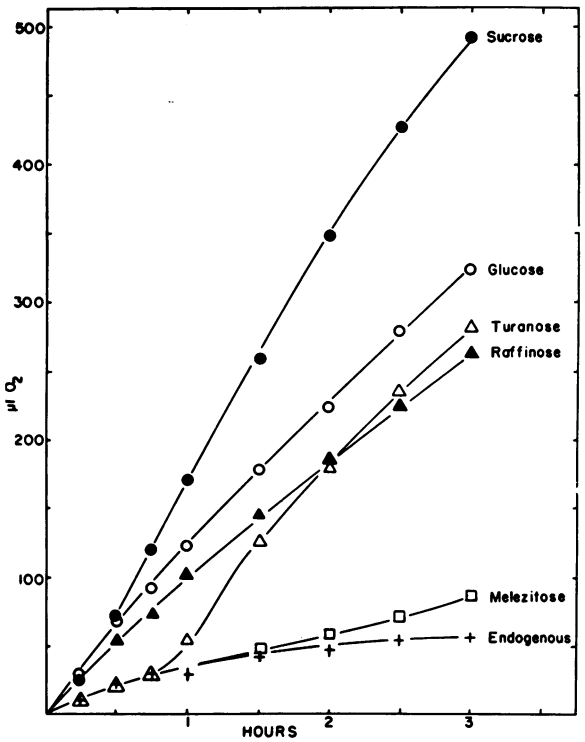

FIG. 4. Course of respiration of spores with various sugars. (5.1 mg spores/ml; 1% sugar; 0.05 M PO_4 buffer pH 6.25; 1.5 ml total volume in vessels.)

endogenous levels only after a lag of about an hour, the rate increasing gradually (fig. 4) and is assimilated slowly (table V). Measurable hydrolysis of this sugar does not occur, however. The small amount of reducing sugar noted in table IV where the spores were incubated under toluene with substrate is due to autolytic release of sugars. Acid treatment of the spores does not decrease metabolism of this sugar, but actually increases it slightly (table V).

TABLE VII

METABOLISM OF SUCROSE AND RELATED SUGARS

SUBSTRATE	OXIDATION	ASSIMILATION	OXIDATION + ASSIMILATION	HYDROLYSIS
	$\mu g/mg \times hr$	$\mu g/mg \times hr$		
Turanose	17	94	111	0
Melezitose ..	4.7	10	15	0
Sucrose	26	126	152	..
Endogenous ..	2.4	...	...	..

(Sugars at 1%; phosphate buffer pH 6.25; duration of experiment—2 hours; 4.35 mg spores/ml.)

METABOLISM OF TURANOSE: Turanose is respired and assimilated rapidly, although not as fast as sucrose (fig. 4, table VII), yet no hydrolysis can be detected by intact spores nor by spores in the presence of merthiolate or toluene. The shape of the respiration curve is suggestive of an adaptive mechanism. To determine whether an adaptive hydrolytic enzyme is formed, spores were incubated with turanose for three hours and were then tested for turanase activity after adding toluene to kill the cells. No hydrolytic activity was found. Arsenate inhibits respiration of turanose, yet with other disaccharides such as maltose (and also trehalose and cellobiose) stimulation occurs (fig. 5). This stimulation may be ascribed to effects upon endogenous respiration. Respiration on turanose is apparently suppressed by high concentrations of glucose—i.e., in the presence of turanose-glucose mixtures, respiration decreases at high glucose concentrations (table VIII). In this experiment the glucose or buffer was added from a second side arm at 100 minutes after adding the turanose, at which time the spores were respiring rapidly in linear fashion.

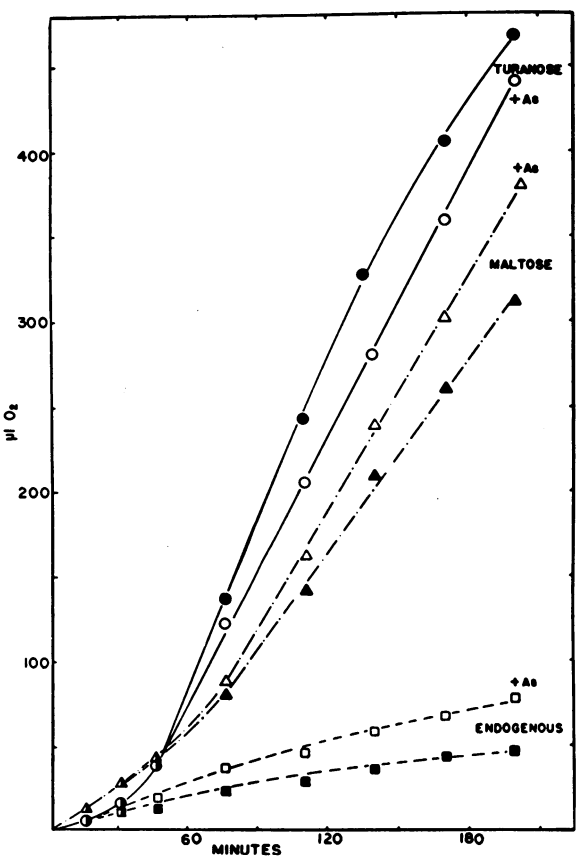

FIG. 5. Effect of arsenate on respiration of spores with turanose and maltose (10^{-3} M arsenate; 0.05 M phthalate buffer pH 6; 8.3 mg spores/ml; 1% sugar; 1.5 ml total volume in vessels).

DISCUSSION

Before proceeding with the interpretation of the data it should be pointed out that the spores of *M. verrucaria* do not germinate under the conditions and duration of experimentation employed in this study. The germination of these spores has been considered previously (8, 11).

TABLE VIII

EFFECT OF GLUCOSE ON TURANOSE RESPIRATION

SUBSTRATE		Q_{O_2}*	RELATIVE Q_{O_2}
TURANOSE	GLUCOSE		
1%	0%	13.5	1.00
1	1	19.2	1.43
1	10	16.4	1.22
0	1	12.8	0.95
0	10	13.6	1.01

(10 mg spores/vessel; phosphate buffer pH 6.25.)
* Not corrected for endogenous respiration.

Metabolism of sucrose may be initiated by preliminary hydrolysis with invertase or by some non-hydrolytic pathway such as phosphorolysis, polymerization reaction (5, 6), or other unknown system/s. The presence of a great excess of invertase over the metabolic requirements of the cell, coupled with metabolic systems capable of utilizing glucose or fructose, would be expected to indicate a hydrolytic pathway. This is the situation in *M. verrucaria* spores, yet evidence from several types of experiments indicates this conclusion to be invalid.

If sucrose were metabolized via invertase, then the invertase activity of a suspension of cells as measured by the appearance of reducing sugars in the suspending medium should be a function of the metabolic activity of these cells, since the products of hydrolysis are being utilized in metabolism. Reduction of metabolism should produce a corresponding increase in reducing sugars in the medium, provided they are free to diffuse from the cells and that the conditions employed for blocking metabolism do not affect the actual activity of the enzyme. Experiments reported here have shown that blocking metabolism by a variety of techniques produces no increase in invertase activity of the spores when sucrose is substrate. Because of the reported surface location of the invertase (10), we need not be concerned with possible restrictions in the exit of sugars from the cells. In experiments with *Aspergillus luchuensis* spores (unpublished) blocking metabolism increases the measured invertase activity to the predicted magnitude. Results of additional studies are entirely compatible with a postulate of hydrolytic metabolism of sucrose via invertase with these spores.

Further evidence indicating non-hydrolytic metabolism of sucrose is found in comparing the rate of metabolism on sucrose with that on its products of hydrolysis. Both respiration and assimilation are greater on sucrose than on glucose-fructose mixtures.

Experiments using acid-treated spores substantiate the postulated nonhydrolytic metabolism. Willstätter and Lowry (15) have used this type of data to postulate direct fermentation of sucrose by yeast. Spores treated with 0.1 *N* HCl, or other acids, no longer display invertase activity, yet no comparable effects on cell viability nor on general metabolic activity are found. This is ascribed to the surface location of the enzyme (10). Study of sucrose metabolism in such acid treated spores has shown that respiration and assimilation are affected only slightly—to about the same extent as with a glucose substrate. Since the rate of metabolism on sucrose greatly exceeds hydrolysis, it is presumed that metabolism must proceed via some other pathway. While resynthesis of invertase does occur during the period of incubation with sucrose, this activity is inadequate to account for the high rate of metabolism.

These observations cannot be interpreted on a basis involving hydrolysis by invertase as the first step in the metabolism of sucrose. While the possibility of a pathway for the metabolism of disaccharides other than one mediated by hydrolytic carbohydrases had been recognized for many years, it was not until the work of Doudoroff and his colleagues that a definite mechanism was established through demonstration of a sucrose phosphorylase. Several reviews have appeared recently summarizing the status of our concepts and information regarding the significance and occurrence of direct metabolism of carbohydrates (2, 3, 4, 5, 6). In a critical evaluation of the evidence regarding direct fermentation of sucrose, maltose and lactose by yeast, Gottschalk (3) has refuted the direct schemes proposed by others primarily on the basis of pH relations, or lack of relation, of the cell with its environment and upon consideration of permeability of the cells to the sugars employed. He concludes that there is no evidence contraindicating a preliminary hydrolytic step. To explain the data presented here on a basis of preliminary hydrolysis requires several assumptions to be made to interpret each of the various lines of evidence. In brief, these assumptions would be: (1) postulation of an intracellular invertase whose activity cannot be demonstrated in control or acid-treated spores whose metabolic activity is blocked or destroyed by a variety of treatments; (2) this enzyme must be inhibited by glucose but not by fructose; (3) arsenate must block penetration of sucrose or inhibit the intracellular invertase; (4) following a brief lag in control spores, and a more extended one in acid treated spores (unpublished), sucrose must penetrate more rapidly than glucose and fructose. Space precludes a detailed analysis of the data on the basis of these assumptions which, in fact, do not permit completely satisfactory interpretations. We must infer that a non-hydrolytic pathway is much more probable. In seeking a mechanism for non-hydrolytic metabolism in *M. verrucaria* spores, the mediation of a sucrose phosphorylase appeared probable. While efforts to extract such an enzyme or to demonstrate phosphorolytic activity of intact cells—viable or under toluene—have been unsuccessful, certain indirect evidence is suggestive of the participation of this enzyme. The studies of Doudoroff *et al.* (1) have shown that sucrose phosphorylase is fundamentally a transglucosidase which is inhibited competitively by glucose. It would be anticipated, therefore, that glucose should suppress metabolism of sucrose by spores if sucrose phosphorylase is involved. Data presented verify this—high concentrations of

glucose suppress respiration to the same level as glucose-fructose mixtures. This would be the predicted behavior since blocking sucrose phosphorylase should shift the metabolism to invertase, the latter enzyme being present in more than adequate amounts. Other studies of Doudoroff (2) have shown that arsenate can be substituted for phosphate in the cleavage of sucrose by sucrose phosphorylase. In this case the glucose-arsenate formed hydrolyzes spontaneously, releasing free glucose. Thus if a comparable sucrose phosphorylase is involved in *M. verrucaria* spores, arsenate should suppress sucrose metabolism to the same level as with glucose-fructose mixtures, since only glucose and fructose are available, arising from either sucrose phosphorylase or from invertase activity. This prediction has been realized in measurements of the effects of arsenate on respiration on sucrose. Arsenate has no effect on the invertase activity of the spores.

Following this reasoning, it might be anticipated that metabolism of sucrose should be lowered to the level with glucose-fructose mixtures if phosphate is omitted from the medium. Experiments have shown, however, that sucrose metabolism is not affected by the presence or absence of phosphate. Since 1.4% of the dry weight of the spores is phosphorus, it must be presumed that this is adequate for the postulated mechanism. Indeed, the reserves of all inorganic nutrients are adequate to satisfy the metabolic requirements for at least five or six hours since assimilation and respiration on sugars are not greatly influenced by omitting nitrogen, phosphate, magnesium, sulfate or potassium.

We must now explain the more rapid rate of metabolism on sucrose than on a mixture of glucose and fructose. Assuming that a phosphorolytic mechanism is involved, both glucose-1-phosphate and fructose are present within the cell or are available to it if the enzyme is at the cell surface. Since invertase is also present, glucose is also available. Thus cells suspended in sucrose are presumably metabolizing all three of these sugars. The more rapid metabolism on sucrose than on glucose plus fructose is thus ascribed, hypothetically, to the additional metabolite glucose-1-PO_4. It must be assumed further that the glucose-1-PO_4 is formed within the cell since attempts to duplicate this effect by adding glucose-1-phosphate extracellularly were unsuccessful (unpublished). Presumably permeability of the cell to the phosphorylated sugar is limiting, since the endogenous respiration is increased only very slightly when this compound is added.

The trisaccharides, raffinose and melezitose, both contain sucrose (fig. 6). In raffinose a galactose residue is attached to the glucose portion of the sucrose moiety, the galactose-glucose linkage being the same as that in melibiose. Raffinose can thus be considered as a combination of sucrose and melibiose, with the glucose being common to both disaccharides. In melezitose an extra glucose is linked to the fructose portion of the sucrose in the same manner as in the disaccharide, turanose. Melezitose can thus be considered as a combination of sucrose and turanose, with the fructose group being common to both sugars. The composition of these oligosaccharides and the enzymes responsible for their hydrolysis are indicated in figure 6. Data presented here indicate that of the four enzymes which may be concerned in the hydrolysis of these sugars, only fructo-invertase is present in *M. verrucaria* spores in measurable quantities. In spite of the absence of enzymes which can hydrolyze melezitose or turanose, both these sugars are metabolized —the former slowly, the latter quite rapidly. Such data are strongly indicative of non-hydrolytic metabolism of these two sugars. It is interesting to note in this connection that the rate of hydrolysis of raffinose by spores is increased if metabolism is blocked, and that the magnitude of increase is about equal to the rate of utilization in metabolism. These observations are all consistent with the postulated non-hydrolytic metabolism of sucrose. It is possible that the same enzyme—a transglucosidase—may be responsible for the metabolism of sucrose, turanose and melezitose, all of which have an exposed α-glucopyranose group in contrast to the internal location of this group is raffinose. It must be emphasized that while circumstantial evidence points toward a sucrose phosphorylase, failure to detect this enzyme may be suggestive of some other pathway.

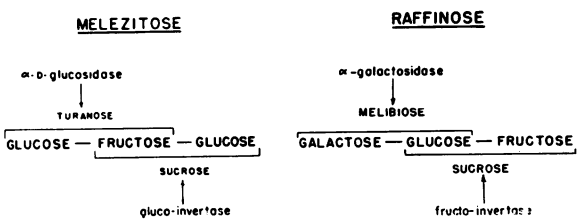

FIG. 6. Structures and enzymic mechanisms of hydrolysis of the non-reducing trisaccharides, raffinose and melezitose (after Pigman and Goepp, 1948).

SUMMARY

(1) Data from four types of experiments indicate that sucrose is metabolized by a non-hydrolytic system in spores of the fungus *Myrothecium verrucaria* even though invertase is present in excess of the metabolic requirements. The evidence available is consistent with the postulate that sucrose metabolism is mediated by a sucrose phosphorylase, although attempts to demonstrate such an enzyme were unsuccessful.

(2) Melezitose and turanose are not hydrolyzed by the spores and thus appear to be metabolized by a non-hydrolytic system.

(3) Evidence indicates that raffinose is metabolized via invertase, the enzyme being a fructosidase.

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PEANUT AND OIL PALM FOLIAR DIAGNOSIS INTERRELATIONS OF N, P, K, Ca, Mg[1]

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Foliar diagnosis is now extensively used as a research tool to evaluate the nutritional status of plants. Its definition and name, "diagnostic foliaire," was first used in France by Lagatu and Maume (8) in their pioneer work, although many other workers had already analyzed plant materials to determine the nutritional requirements of cultivated plants.

In Sweden, Lundegårdh (9) established some fundamental concepts regarding the techniques of plant analysis in his extensive researches on oats and in the United States the important work of Thomas (19) attracted attention to foliar diagnosis. A comprehensive historical review of the subject has been given by Goodall and Gregory (7) and more recently by Ulrich (10).

Since the French literature on this subject is not readily available in English-speaking countries it may be well to mention recent work done in France. Maume *et al.* worked on grape vines (11, 12, 13) and on corn (14); Bouat, Renaud, and Dulac worked on olive trees (1); Franc de Ferriere worked on grape vines (5), corn, and potatoes (6). Also, many papers can be found in *C. R. Académie des Sciences* and *C. R. Académie d'Agriculture.*

This paper deals with the results of foliar diagnoses obtained with the peanut (*Arachis Hypogaea* L.) and the oil palm (*Elaeis Guineensis* Jacq.) in French Africa. The work reported for the peanut plant is based on the physiological researches of Burkhardt and Page (3) and of Prevot (15), who laid down the principles of leaf sampling for this plant in Africa (16), and on the many field experiments conducted in Africa during 1950 and 1951 (17, 18). For the oil palm we started with the basic work of Chapman and Gray (4), whose techniques we applied at our research stations in 1951. Lack of space prevents us from giving complete results of the experiments, but we shall briefly describe a typical experiment and summarize the other results.

Methods

The experiments were conducted at twelve different locations in French Africa (fig. 1), with climatic and ecological conditions ranging from those of Sénégal to those of French Moyen-Congo, which provided a good basis for a generalization of some of the conclusions.

The leaf sampling techniques for the peanut are those described by Prevot and Ollagnier (17) and for

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