The effect of MCPP on aspects of the metabolism of 17-day old seedlings of **Theobroma cacao,** variety F_3 Amazon*

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COMPENDIO

El cotiledón, hipocótilo y raíz de plántulas de 17 días de Theobroma cacao, *variedad F_3 Amazon, contenían lípidos, aminoácidos, maltosa, sacarosa, glucosa, fructosa, xilosa y almidón. El cotiledón almacenó la concentración más alta de lípidos, aminoácidos, almidón y carbohidratos totales. La aplicación de ácido 2,4-clorometil-fenoxi-propiónico (MCPP) sobre el cotiledón afectó la distribución de estas reservas en la plántula en el término de 48 horas. El almidón y los carbohidratos totales disminuyeron en el cotiledón con aumento concomitante en la raíz. Los lípidos desaparecieron rápidamente en el hipocótilo y la raíz a las 48 horas de aplicar MCPP al cotiledón. Los aminoácidos, por otra parte, aumentaron en los tres órganos de las plántulas tratadas con la acumulación más alta en el hipocótilo. La tasa de absorción de oxígeno por preparaciones mitocondriales de los tres órganos fue inhibida por el MCPP. Sin embargo, el herbicida en general aceleró las actividades de la oxidasa del ácido ascórbico en el hipocótilo y raíz de las plántulas antes de las 48 horas. Tuvo un efecto inverso sobre las actividades de la enzima en el cotiledón. La ATPasa de la raíz fue inhibida por el MCPP en tanto que sus actividades fueron aceleradas en los cotiledones e hipocótilos antes de las 48 horas.* -- Los autores

Introduction

THE application of 2, 4-chloro-methylphenoxy-propionic acid (MCPP) to the cotyledons of *Theobroma cacao* seedlings was reported by Olofinboba (9) to promote cambial activity of the hypocotyl. This resulted in the swelling of the hypocotyl followed by a suppression of growth. Some of these morphological changes were observed within 48 hours of herbicide application. The herbicide also suppressed leaf enlargement and dry weight. These effects were more pronounced on seedlings without expanded foliage leaves. MCPP had no significant effect on the root and stem if applied after the two-leaf stage of the seedling development. The rapidity with which the herbicide effected these morphological changes suggested a likelihood for some rapid metabolic changes in the affected seedling. The food reserves in cotyledons are required for the development of leaf and cambium in seedlings without expanded leaves. Rapid proliferation of cambial cells might result in changes in the respiratory rates.

This study was therefore designed to investigate changes in the respiratory activity as well as the distribution of amino acids, lipids, starch and of ethanol-soluble carbohydrates, in 17-day old seedlings within 48 hours of MCPP application.

Materials and methods

Seeds of *Theobroma cacao,* variety F_3 Amazon obtained from the Cacao Research Institute of Nigeria, Onigambari, Ibadan, were germinated directly in batches of three in sterile garden soil contained in polyethylene nursery cocoa bags. The bags were kept under a shed made of palm leaves to simulate the condition adopted in nurseries for raising the seedlings. The temperature in the shed varies widely as it naturally happens in the field. Seventeen-day-old seedlings, without expanded foliage leaves were used.

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Each cotyledon of the 17-day old seedlings was smeared with 0 1 ml of 10 ppm MCPP. The concentration of 10 ppm was selected from the experience in previous experiments (9). Other seedlings left untreated served as controls. Samples were taken immediately from control seedlings and further samples taken from both control and treated seedlings 24 and 48 hrs after treatment. The soil particles on the roots of the harvested seedlings were carefully washed in slow running tap water and the seedlings were then separated into roots, hypocotyls and cotyledons. Those for enzyme extraction were immediately chilled before further treatment while those for chemical analysis were dried to constant weight and then powdered in a coffee mill.

The methods used for the quantitative determinations of ethanol-soluble sugars, starch, lipids, amino acids and protein were those described by Olofinboba and Fasidi (10). Similarly the procedures used for the preparation of cell-free extract, measurement of respiration and the determination of adenosine triphosphate activity were those described by Fawole and Olofinboba (2).

The activity of ascorbic acid oxidase (AA oxidase) was measured by determining the catalytic effect of the soluble fraction of the extract on the oxidation of ascorbic acid at 25°C (5). The rate of oxidation of ascorbic acid was measured by removing samples from the incubation mixture into 20 per cent metaphosphoric acid and then estimating the unoxidized ascorbic acid by titration against 0 01 per cent of 2,6-dichlorophenol indophenol (DCPIP). Since the volume of DCPIP used in titration is directly proportional to the quantity of ascorbic acid, the activity of AA oxidase was expressed as change in the volume of DCPIP/min/mg protein.

Table 1.—MCPP effect on the distribution of ethanol-soluble carbohydrates in seedlings of *Theobroma cacao*, variety F_3 Amazon (mg/g dry wt; percentage of total in parentheses).

		Ethanol-Soluble		Carbohydrates		
		Maltose	Sucrose	Glucose	Fructose	Xylose
	C	3 89±0.51 (58 85)	4 59±0 61 (62 62)	13 42±1 17 (35 13)	20 69±1 66 (53 49)	2 41±0.61 (37 25)
INITIAL	H	1.78±0 61 (26 93)	1 28±0 10 (17 47)	15 28±1 21 (40 00)	10 50±0.41 (27 15)	2.96±0 41 (45 75)
CONTROL	R	0 94±0 10 (14 22)	1 46±0 06 (19 91)	9 50±1 91 (24 87)	7 49±1 21 (19 36)	1.10±0 12 (17 00)
	CHR	6 61	7 33	38 20	38 68	6 47
	C	1 69±0.50 (29 47)	4 13±1 11 (58 26)	11 22±0 70 (30 85)	19 44±0 94 (48 91)	1 97±0 41 (32 25)
CONTROL	H	3 00±0 61 (53 20)	1 55±0 10 (21.87)	12 63±1 20 (34 72)	12.81±1 21 (32 23)	2.44±0 21 (39 94)
24 hr	R	0 95±0.20 (16 83)	1 41±0 13 (19 87)	12 53±0 41 (34 43)	7.50±0 65 (18 86)	1 70±0 41 (27 81)
	CHR	5 64	7 09	36 38	39 75	6 11
	C	3 46±0 10 (72 69)	4 74±0 17 (75 48)	20 62±1 40 (74 58)	11 82±1 40 (61 15)	0 90±0 66 (38.97)
TREATED	H	0 84±0.10 (17 65)	0 94±0 07 (14 47)	3 56±0 60 (12 88)	3 38±0.61 (17 49)	1 41±0 66 (61 03)
24 hr	R	0.46±0 22 (9 66)	0 60±0 11 (9 55)	3 47±0 90 (12 54)	4 13±0 55 (21 36)	0 00 (0 00)
	CHR	4 76	6 28	27 65	19,33	2 31
	C	2 42±0 10 (32 53)	3 69±0.16 (27 70)	12 34±2 41 (31 42)	17 60±1 71 (43 43)	2 10±0.91 (32 82)
CONTROL	H	3 81±0 30 (51 21)	2 41±0.14 (18 10)	14 22±1 70 (36 21)	13 59±1.99 (33 53)	2 64±1 41 (41 25)
48 hr	R	1 21±0 17 (16 26)	7 22±0.13 (54 20)	12 72±0 91 (32 37)	9 34±1 66 (23 04)	1 66±0 44 (25.93)
	CHR	7 44	13 32	39.28	40 53	6 40
	C	7.97±1 61 (97.55)	5.16±1 12 (98 10)	7 88±1 01 (17 52)	4 31±0 31 (13 25)	0 00 (0.00)
TREATED	H	0 20±0 07 (2 45)	0 10 (1 90)	3 44±0 70 (7.65)	0 66±0 04 (2 03)	1.27±0 11 (100 00)
48 hr	R	0 00 (0 00)	0 00 (0 00)	33 66±1 92 (74 83)	27 56±1.40 (84.72)	0 00 (0.00)
	CHR	8 17	5 26	44 98	32 53	1 27

C = Cotyledon
H = Hypocotyl
R = Root
CHR = Cotyledon + Hypocotyl + Root

Results

The seedlings used in this experiment contained maltose, sucrose, glucose, fructose, xylose, starch, lipids and amino acids (Tables 1, 2 and 3). The most abundant carbohydrates were starch, fructose and glucose. The cotyledon of 17-day old seedlings contained the highest concentration of these reserves. It stored 55.60 per cent of starch; 49.68 per cent of total carbohydrates; 97.68 per cent of lipids and 48.08 per cent of amino acids. The hypocotyl contained 26.64 per cent starch; 30.47 per cent total cabohydrates; 1.60 per cent lipids and 28.85 per cent amino acids while the root had 17.76 per cent starch, 19.85 per cent total carbohydrate, 0.72 per cent lipids and 23.07 per cent amino acids.

The relative distribution of these reserves was affected by MCPP within 24 hrs of application (Tables 1, 2 and 3). Percentage accumulation of starch decreased in cotyledon from 55.60 per cent to 19.54 per cent within 24 hours of MCPP application but increased in both hypocotyl and root. Total carbohydrate also decreased in the cotyledon and hypocotyl with a concomitant increase in the root. The effects were more marked within 48 hrs. The percentage accumulation of starch in cotyledon dropped to 9.68 per cent while that of total carbohydrates dropped to 19.68 per cent. Accumulations of starch and total carbohydrate in roots were 60.16 per cent and 63.67 per cent respectively (Table 2).

The effect of MCPP was most marked on the distribution of lipids. The lipid content of cotyledons was

Table 2—MCPP effect on the distribution of starch and total carbohydrates in seedlings of *Theobroma cacao*, variety F_3 Amazon (mg/g dry wt; percentage of total in parentheses)

				Changes as per cent of initial control	
		Starch	Total carbohydrate	Starch	Total carbohydrate
INITIAL	C	31.31±4.21 (55.60)	76.31 (49.68)	—	—
CONTROL	H	15.00±1.60 (26.64)	46.80 (30.47)	—	—
	R	10.00±1.31 (17.76)	30.49 (19.85)	—	—
	CHR	56.31	153.60	—	—
	C	27.81±1.36 (52.47)	66.26 (44.78)	88.82	86.83
CONTROL	H	14.56±0.94 (27.47)	46.99 (31.76)	97.07	100.41
24 hr	R	10.63±1.00 (20.06)	34.72 (23.46)	106.30	113.87
	CHR	53.00	147.97	94.12	96.33
TREATED	C	11.25±1.61 (19.54)	51.89 (44.35)	35.93	68.00
24 hr	H	23.44±3.01 (40.72)	33.57 (28.69)	156.27	71.73
	R	22.88±2.11 (39.74)	31.54 (26.96)	228.80	103.44
	CHR	57.57	117.00	102.24	76.17
CONTROL	C	26.41±2.01 (47.78)	64.56 (39.79)	84.35	84.60
48 hr	H	16.74±1.66 (30.28)	53.41 (32.92)	111.60	114.12
	R	12.13±1.41 (21.94)	44.28 (27.29)	121.30	145.23
	CHR	55.28	162.28	98.17	105.63
TREATED	C	6.94±0.66 (9.68)	32.26 (19.68)	22.16	42.27
48 hr	H	21.63±2.11 (30.16)	27.30 (16.65)	144.20	58.33
	R	43.14±1.44 (60.16)	104.36 (63.67)	431.40	342.28
	CHR	71.71	163.92	127.35	106.72

C = Cotyledon. R = Root
H = Hypocotyl CHR = Cotyledon + Hypocotyl + Root

much more than that of either the hypocotyl or root in treated or control samples (Table 3). The level of lipids in the cotyledons and hypocotyls of control samples remained rather unchanged over the 48 hrs period, compared with the initial controls. During the same period, the level of lipids in roots of control plants steadily increased. On treatment of cotyledon with MCPP, the lipid content of each organ decreased compared with the levels in the controls. Such effect became most pronounced in the hypocotyls and roots where the lipid completely disappeared within 24 hrs of MCPP application.

The amino acid content of cotyledons was always higher than that of either hypocotyls or roots in the control samples (Table 3). This situation was changed when the samples were treated with MCPP even within 24 hrs when more than 50 per cent of the total amino acid content of the whole seedling was present in the hypocotyls. The relative distribution again changed within 48 hrs with the hypocotyl containing 43.24 per cent of total amino acids. The concentrations in the cotyledon and root increased to 32.43 per cent and 24.33 per cent respectively. The various organs of treated seedlings contained more amino acids than those of control seedlings.

The rate of respiration in the cotyledons when compared with the figures for the controls was inhibited by MCPP (Fig. 1). The low respiratory rate normally observed in the root was totally inhibited in the treated samples. It should be recalled that the seedlings used were 17 days old were root respiration might be normally low.

The activity of ascorbic acid oxidase appeared to be concentrated in the roots followed by the cotyledons, with the least amount in the hypocotyls (Fig. 2). The presence of MCPP in the cotyledons had no effect

Table 3.—MCPP effect on the distribution of lipids and amino acids in seedlings of *Theobroma cacao*, variety F_3 Amazon (mg/g dry wt; percentage of total in parentheses).

				Changes as per cent of initial control	
		Lipids	Amino acid	Lipids	Amino acids
INITIAL CONTROL	C	54.80±2.14 (97.68)	0.25±0.01 (48.08)	—	—
	H	0.90±0.01 (1.60)	0.15±0.04 (28.85)	—	—
	R	0.40±0.04 (0.72)	0.12±0.05 (23.07)	—	—
	CHR	56.10	0.52	—	—
CONTROL 24 hr	C	54.00±1.06 (95.58)	0.29±0.01 (50.00)	98.54	116.00
	H	1.00±0.14 (1.77)	0.20±0.03 (34.48)	111.11	133.33
	R	1.50±0.9 (2.65)	0.09±0.03 (15.52)	375.00	75.00
	CHR	56.50	0.58	100.71	111.54
24 hr TREATED	C	44.00±1.14 (100)	0.40±0.01 (25.48)	80.29	160.00
	R	0.00 (0)	0.80±0.02 (50.96)	0	533.33
	H	0.00 (0)	0.37±0.01 (23.56)	0	308.33
	CHR	44.00	1.57	78.43	301.92
CONTROL 48 hr	C	55.40±2.61 (94.06)	0.20±0.01 (42.55)	101.09	80.00
	H	1.10±0.14 (1.87)	0.15±0.03 (31.91)	122.22	100.00
	R	2.40±0.66 (4.07)	0.12±0.03 (25.53)	600.00	100.00
	CHR	58.90	0.47	104.99	90.38
TREATED 48 hr	C	41.20±2.14 (98.33)	0.36±0.06 (32.43)	75.18	144.00
	H	0.10±0.01 (0.24)	0.48±0.04 (43.24)	11.11	320.00
	R	0.60±0.04 (1.43)	0.27±0.03 (24.33)	150.00	225.00
	CHR	41.90	1.11	74.69	213.46

C = Cotyledon R = Root
H = Hypocotyl CHR = Cotyledon + Hypocotyl + Root

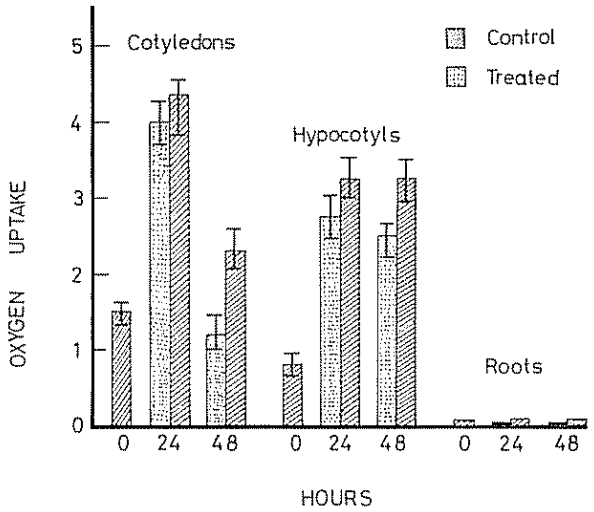

Fig 1 —*Oxygen uptake by mitochondrial preparations from cotyledons, hypocotyls or roots of* Theobroma cacao *treated or untreated with MCPP. Oxygen uptake has been expressed as mm³ oxygen/hr/mg protein*

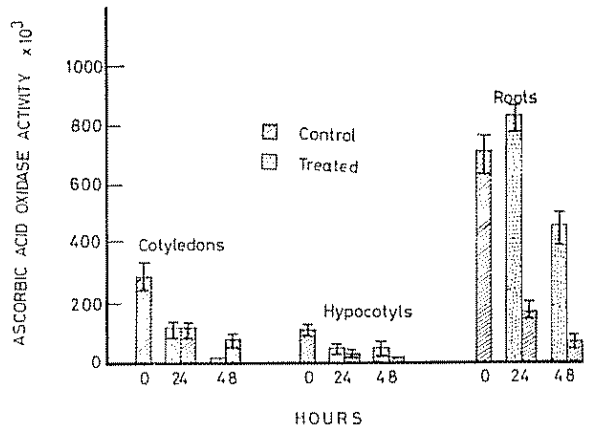

Fig 2 —*Activity of ascorbic acid oxidase in the cotyledons, hypocotyls and roots of* T. cacao *treated or untreated with MCPP. Enzyme activity was expressed as change in the vol of DCPIP/min/mg protein*

during the first 24 hrs of application but became significantly inhibitory in 48 hrs. Compared with the data for controls, the herbicide had no inhibitory effect on the enzyme of the hypocotyl even in 48 hr. In fact MCPP actually stimulated the enzyme in the hypocotyl and the root.

ATPase activity was concentrated in the roots as in the case with ascorbic acid oxidase. The soluble fraction contained more ATPase than the mitochondrial fraction of cotyledon, hypocotyl or root extracts. MCPP had no effect on the already low enzyme activity of the cotyledon mitochondria of *T. cacao* (Fig. 3). This was in contrast to the soluble enzyme which was initially stimulated and later declined in 48 hr, but to a level still higher than that of the control (Fig. 3). The effect of MCPP on the hypocotyl mitochondria ATPase was similar to that in the cotyledon. However, the activity of the soluble enzyme was lowered during the first 24 hr of treatment but increased above the control level within 48 hr (Fig. 3). In the roots, MCPP stimulated both mitochondrial and soluble ATPase. However, while in the mitochondria the stimulation was steady, in the soluble enzyme it was reduced, albeit to a level still much higher than that of the control.

Discussion

The cotyledon of 17-day-old seedlings of *T. cacao* is the main storage organ for carbohydrates, lipids and amino acids. The reserves are used for the initial growth of the seedling which at this stage of growth has no green foliage leaves. The distribution of the reserves within the seedling was greatly influenced within 48 hr and sometimes within 24 hr of application of MCPP on the cotyledons.

Starch and total carbohydrate decreased in the cotyledon with subsequent increase in the hypocotyl and more in the roots. The abundance of a reserve in any part of a seedling may depend on the rate of either its utilization or hydrolysis and eventual translocation to other parts of the seedling. The total amounts of either starch or total carbohydrate in treated and control seedling within 48 hr were not significantly different from the initial control concentration. MCPP did not appear to have significantly affected the utilization of these reserves within the seedling. It, however, affected the redistribution of the reserves. Starch is not a translocable reserve in plants. MCPP must have accelerated its hydrolysis before the products were translocated from the cotyledons to other parts especially the root.

Maltose, an intermediate product of starch hydrolysis accumulated in cotyledons smeared with MCPP. It thus appears that even though MCPP accelerated starch hydrolysis, it inhibited the complete breakdown of maltose to glucose. The inhibition was however not complete as evidenced by an accumulation of glucose, starch and fructose in the root. The hydrolysed products of starch were probably moved within 48 hrs into root where resynthesis of starch occurred from the glucose molecules. The high concentration of fructose and glucose and the complete disappearance of sucrose in root of treated seedling suggests rapid hydrolysis of sucrose in the root.

Lipids are the most abundant food reserve in the cotyledon (Table 3) and the concentration was relatively high in the hypocotyl and root of 17-day-old seedling. The lipids, however, completely disappeared from the hypocotyl and root within 24 hrs of MCPP application on the cotyledon. The total amount of lipids in treated seedling within 24 hrs was less than that in the controls. The utilization of lipids was therefore influenced by MCPP. The fact that the level of lipids declined in all three organs when seedlings were in contact with MCPP might also suggest a participation of the glyoxylate cycle in the metabolism of the seedlings. This may be more so in the roots and hypocotyls than in the cotyledons.

Cambial activity is intense in the hypocotyl after MCPP application (9) and this involves an increase in cell number of the hypocotyl. The formation of new

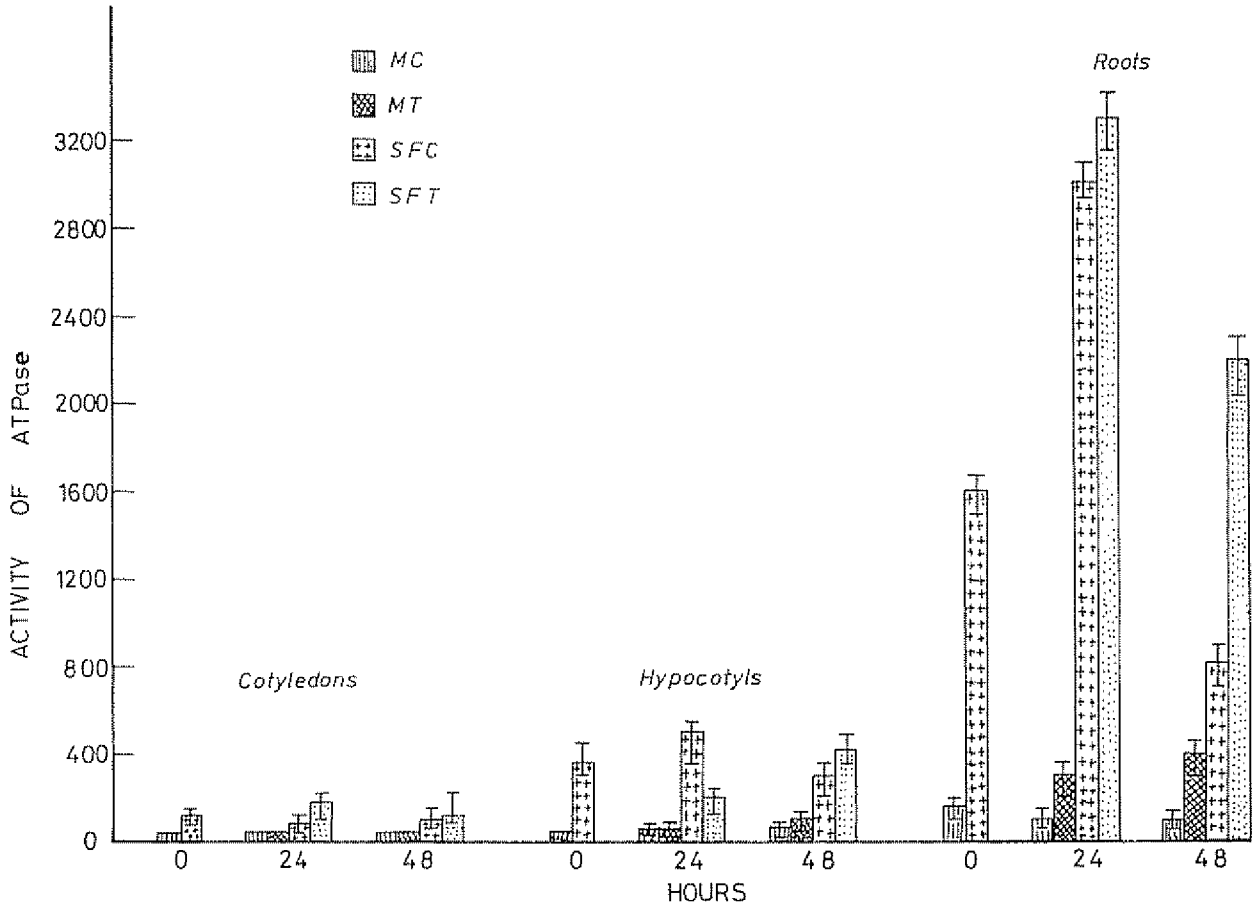

Fig. 3.—*Activity of ATPase in the cotyledons, hypocotyls and roots of* T. cacao *treated or untreated with MCPP. Enzyme activity was expressed as µg phosphorus released/min/mg protein*

MC = mitochondria control; MT = mitochondria treated; SFC = soluble fraction control, SFT = soluble fraction treated

protoplasm requires the utilization of amino acids. The high concentration of amino acids in the hypocotyl of treated seedlings might be needed for building new protoplasm of the rapidly dividing cells of the swollen hypocotyl. MCPP influenced the utilization of lipids while it affected the hydrolysis of starch and protein and the redistribution of the hydrolytic products.

The respiratory rate of the cotyledons was higher than that of hypocotyls and roots in either treated or untreated samples (Fig. 1). At the same time, there was a reciprocal relationship between oxygen uptake and ascorbic acid oxidase activity in all the three organs (Figs. 1 and 2). Mapson and Moustafa (7) suggested that ascorbic acid oxidase probably plays a minor role in the total respiration of seedlings. It may well be that most of the contribution of the enzyme activity to respiration was concentrated in the root. Such contribution became enhanced by MCPP, especially during the first 24 hr of application of the herbicide.

The ATPase of *Theobroma* appeared to be concentrated in the soluble fraction (Fig. 3). In some earlier studies on the subcellular localization of the enzyme it was found to be most abundant in the cell wall fraction (1, 4) and in some cases in the mitochondria (3, 12). It is noteworthy that ATPase activity in roots was several times higher than that in either cotyledons or hypocotyls (Fig. 3). If *Theobroma* ATPase merely hydrolyses ATP to ADP and inorganic phosphate one would expect an acceleration of respiration as happens similarly in the castor bean endosperm (12). It must be noted however that the enzyme could also be associated with ion transport (11). There is evidence that ATPase can effect the cleavage of both pyrophosphate linkages of ATP in animals (6) and in higher plants (8). In that case the ATP molecule is converted to adenylic acid (AMP) and pyrophosphate. This should not be taken as a rule, however, as Forti (3) has shown definitely that ADP is not further hydrolysed by the ATPase of pea mitochondria. The present data for *Theobroma* show that respiratory activity was lowest in roots which may suggest that the cleavage of ATP in this case goes beyond ADP production.

The present study indicated that the absorption and translocation of MCPP occurred through the cotyledon of *Theobroma*. The herbicide rapidly moved from the cotyledon to the hypocotyl and root where it effected noticeable morphological changes within 48 hr. The seedlings used in the study were without expanded

foliage leaves The cotyledons of transplanted *Theobroma* seedlings remain physiologically active long after transplantation from the nursery to the field. Even at such advanced stage of development, absorption and translocation of herbicide can still occur through the cotyledon. The results of this study emphasised the adverse effects the application of MCPP could have on the normal nutritional status and respiration of *Theobroma*. These disturbances of normal metabolism invariably lead to disturbed growth rate There is therefore a great need that herbicides like MCPP that are readily absorbed and translocated within *Theobroma* seedlings are not recommended as weed killers for as long as such seedlings still retain physiologically active cotyledons.

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Summary

The cotyledon, hypocotyl and root of 17-day old seedlings of *Theobroma cacao,* variety F_3 Amazon, contained lipids, amino acids, maltose, sucrose, glucose, fructose, xylose and starch. The cotyledon stored the highest concentration of lipids, amino acids, starch and total carbohydrates Application of MCPP on the cotyledon affected the distribution of these reserves in the seedling within 48 hr. Starch and total carbohydrates decreased in the cotyledon with concomitant increase in the root. Lipids completely disappeared in hypocotyl and root within 48 hr of applying MCPP on cotyledon Amino acids, on the other hand, increased in the three organs of treated seedlings with the higest accumulation in the hypocotyl. The rate of oxygen uptake by mitochondrial preparations from the three organs was inhibited by MCPP. However, the herbicide generally accelerated the activities of ascorbic acid oxidase in the hypocotyl and root of seedlings within 48 hr. It had a reverse effect on the activities of the enzyme in the cotyledon The ATPase of root was inhibited by MCPP while its activities were accelerated in the cotyledons and hypocotyls within 48 hr.

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