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THE EFFECT OF NUTRIENT DEFICIENCIES ON PHOTOSYNTHESIS AND RESPIRATION IN SPINACH

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SUMMARY

Spinach plants were grown in nutrient-culture solutions containing reduced levels of all the macro- and micro-nutrient elements except cobalt and chlorine. The rates of photosynthesis (carbon dioxide fixation in the light expressed on a per unit chlorophyll or per unit fresh-weight basis) and respiration (carbon dioxide evolution in the dark expressed on a per unit nitrogen or per unit fresh-weight basis) for whole plants were measured using infra-red gas analysis techniques. Measurements were made when the plants displayed clear symptoms of deficiency relative to control plants.

All nutrient deficiencies except iron and molybdenum depressed photosynthesis when chlorophyll was the basis of calculation; manganese-, copper-, phosphorus- and potassium-deficient plants showed the greatest depression. Alternatively when photosynthesis was calculated on a fresh weight basis calcium was the only deficiency which had no affect. Similarly when respiration was calculated on a nitrogen basis all deficiencies except iron, molybdenum and nitrogen result in depressed rates but when respiration was expressed on a fresh-weight basis potassium deficiency resulted in enhanced respiration rates and nitrogen, phosphorus, sulphur, manganese, zinc and molybdenum deficiencies resulted in reduced respiration rates.

INTRODUCTION

A number of studies have been made of the effects of mineral nutrient deficiencies on carbon assimilation in plants. Experiments on different higher plants have established that deficiencies of manganese, potassium, molybdenum, nitrogen, magnesium and calcium all depress photosynthesis [4 6 7 11 15 16]. In the case of green algae it has been shown that deficiencies of manganese, potassium,

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phosphorus, nitrogen, magnesium, iron and sulphur all reduce photosynthesis [14]. With algae it has been possible to show that when the deficient element is resupplied in some cases there can be a rapid (1–2 hours) recovery of photosynthesis which preceeds the formation of new chlorophyll. Such recovery occurs in the cases of manganese, potassium and phosphorus deficiencies and it has been suggested that responses of this type occur with elements that play a direct role in photosynthetic reactions [14].

An extensive study on the effects of mineral nutrient deficiencies on the photosynthetic reactions of higher plant chloroplasts has been made using chloroplasts isolated from tomato and spinach plants [17 18]. In these experiments Hill activity, expressed on a per unit chlorophyll basis, was regarded as an index of the ability of chloroplasts to perform photochemical reactions. Chloroplasts isolated from plants deficient in most nutrient elements have an impaired ability for dye reduction. On a per chlorophyll basis manganese deficiency caused the greatest impairment while the chloroplasts of iron-deficient plants had the same ability to reduce dye as control chloroplasts. In this investigation chloroplasts from healthy and deficient plants were assayed over a range of light intensities up to saturation and from these results tentative conclusions were provided on whether particular deficiencies affected dark reactions or photochemical steps of the Hill reaction.

The aim of the present experiments was to determine what effects nutrient-element deficiencies have on overall carbon assimilation at the whole plant level. Gas analysis methods were employed and in this way data were obtained on the effects of mineral-nutrient deficiencies on both CO_2 fixation in the light (photosynthesis) and on CO_2 evolution in the dark (respiration). The one plant species (*Spinacea oleracea*) was used throughout so that the effects of different deficiencies could be compared one with the other. The CO_2 fixation results were expressed on the basis of chlorophyll present in the leaves in order to eliminate the complicating effects of various deficiencies on the formation of chlorophyll itself and to permit comparisons of the photosynthetic efficiency of the actual chlorophyll present in plants of differing nutritional status. The CO_2 evolution data were expressed per unit of insoluble nitrogen present in the plant as it was considered that this parameter is a better approximation of the overall metabolising mass of a plant than is dry or fresh weight.

METHODS

(a) *Water culture methods*

Spinach plants (*Spinacea oleracea*) were routinely grown in nutrient cultures in a growth cabinet in which the temperature was maintained at 22°C. The plants were given a 12-hour day with a light intensity of approximately 3000 ft candles. Seeds were germinated in vermiculite in the dark at 21°C and after 14 days the seedlings were transferred to nutrient cultures. The plants were grown in six inch diameter plastic pots fitted with disposable plastic liners. Each pot held 8 plants and contained 2.5 l of solution. Plant age was measured as the time from transplanting to nutrient cultures.

Purification and preparation of the nutrient solutions was as previously described [17]. The water used to prepare cultures deficient in copper (and appropriate controls) was passed over a column containing Chelex-100 chelating resin (Bio-Rad Laboratories) to remove contaminating copper. It was necessary to provide supplementary levels of most of the nutrient elements to obtain plants in the size range 1–5 g fresh weight. Supplementary levels added to the approparite deficient nutrient solutions were N 0.56 mM, K 0.028 mM, P 0.01 mM, Ca 0.1 mM, Mg 0.02 mM, S 0.01 mM, B 0.0014 mM, Fe 0.00037 mM and Mn 0.000018 mM.

The Merbein experiments used plants grown in a glasshouse. The glasshouse temperature was maintained at approximately 25°C by day and 20°C by night and growth rates were similar to those obtained in the controlled environment cabinet.

(b) *Measurement of photosynthetic rates*

Most of the measurements of gas exchange were made in a perspex cuvette 15 cm diameter and 23 cm high which was maintained at 21.5 $\pm$ 0.2°C by immersion in a water bath at approximately 8°C. Precise temperature control was obtained by a heating strip located inside the cuvette and regulated by a contact thermometer and relay. Temperature was recorded using a thermistor sensor placed near the plant leaves. The air in the cuvette was stirred vigorously by means of a small electric fan inside the cuvette.

Illumination for studies of photosynthesis was provided by 5 Philips Photolita Type SM 250V 300W lamps arranged in a circle around the cuvette. This arrangement of lights permitted illumination of intact spinach plants so that the leaves of a number of plants could be simultaneously exposed to direct light. The lights were normally supplied with 220 V at which voltage the light intensity from one light inside the cuvette was approximately 8,000 foot candles (EEL photoelectric illuminance meter) or 3.5 $\times$ 10^5 erg/cm^2/sec (YSI Kettering Radiometer).

Outside air was passed into the cuvette through the root chamber which contained water. In this way the roots were aerated and the atmosphere of the cuvette was saturated with water vapour. On leaving the cuvette the airstream was passed through anhydrous calcium chloride, anhydrous magnesium perchlorate and a scintered glass disc before being assayed for carbon dioxide content using a Uras 1 infra red gas analyser (Hartmann and Braun,

Frankfurt). Flow rates were measured with a Rotameter (ROTA, Oeflingen). The air flow rates and quantity of plant material were varied so that photosynthesis depleted the CO_2 concentration by approximately 0.01%. The measuring cuvette held between 1 and 4 plants depending on their size and age. Respiration was measured as the rate of gas exchange in the dark. For calculations of photosynthesis it was assumed that the same rate of respiration occurred both in the light and in the dark.

A number of experiments were conducted at Merbein, Victoria, using the infra red gas analysis assembly described in detail previously [10]. With this apparatus it was possible to separately record gas exchange rates for either the whole plant or alternatively for only the aerial portions. The cuvette in this case was illuminated by a combination of mercury vapour and incandescent lamps and could be maintained at temperatures of 20–29 $\pm$ 0.1°C.

(c) *Chemical analyses*

Plants were homogenized in a Serval Omnimix in 80% acetone. The homogenate was filtered through Whatman No. 1 filter paper, the filtrate assayed spectrophotometrically for chlorophyll a and b [2]. The acetone residues were assayed for nitrogen following Kjeldahl digestion using a Markham still.

EXPERIMENTAL RESULTS

(1) *Effect of light, temperature and* CO_2 *concentration on nett photosynthesis*

The nett photosynthesis of glasshouse grown full nutrient plants was assayed at a range of varying temperatures (10 to 40°C) light intensities (0 to 30 $\times$ 10^4 erg/cm^2/sec) and CO_2 concentrations (0 to 500 ppm).

The results of these experiments are presented in Figs. 1 and 2. It can be seen that this plant has a fairly flat temperature response curve with an optimum in the general region of 20 to 30°C. The response of photosynthesis to changes in light intensity was such that optimum rates were reached at approximately 10 $\times$ 10^4 erg/cm^2/sec and thereafter rates were unaltered to 30 $\times$ 10^4 erg/cm^2/sec. Finally the response of this plant to changes in CO_2 concentration was also examined and was found to be linear up to 300 ppm at a light intensity equivalent to 10 $\times$ 10^4 ergs/cm^2/sec.

(2) *Comparison of gas exchange rates of whole plants and of shoots*

The gas-exchange data presented here for mineral deficient plants were obtained by enclosing the whole plant in the cuvette. This

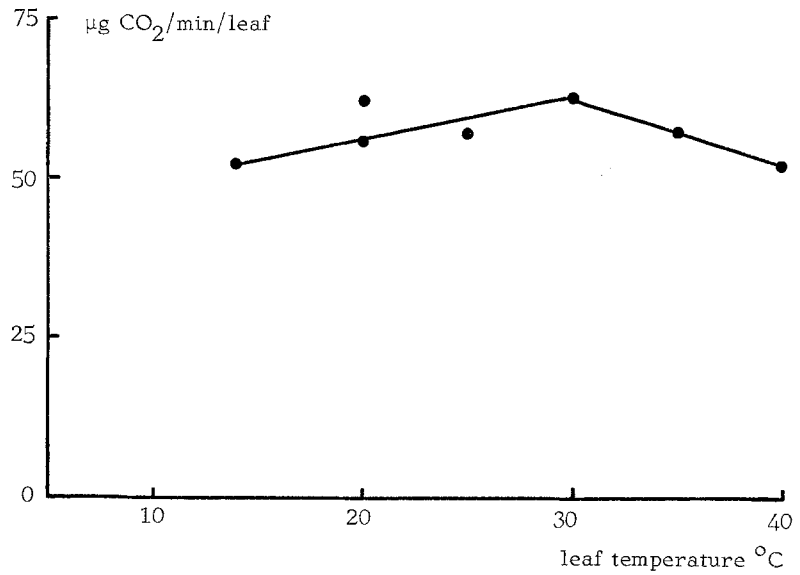

Fig. 1. Effect of temperature on the carbon dioxide uptake of a spinach leaf. Light intensity was maintained at 10×10^4 ergs/cm^2/second.

method of measurement was favoured as American Round Seeded spinach has a short and relatively tender hypocotyl so that it is difficult to enclose only the shoots in the cuvette without damaging the plant. In addition this plant has a rosette habit of growth and is easily damaged when handled.

A limited number of comparisons were made of the gas exchange rates of whole plants and of the shoots of the same plants. The plants used in these experiments were grown in nutrient culture solution but under spring glasshouse conditions. Plants 18 to 21 days old and weighing approximately 15 g fresh weight were transferred to the glass cuvette and measurements were taken of the gas exchange with whole plant and subsequently with only the shoots in circuit.

The results of the experiments are given in Table 1 from which it can be seen that there is a close correspondence between the photosynthetic rates obtained by the two methods of measurement. Also it should be noted that the amounts of CO_2 evolved in the dark by respiration are approximately one fifth of the amount of CO_2 fixed by photosynthesis. The rates of respiration of the roots which were

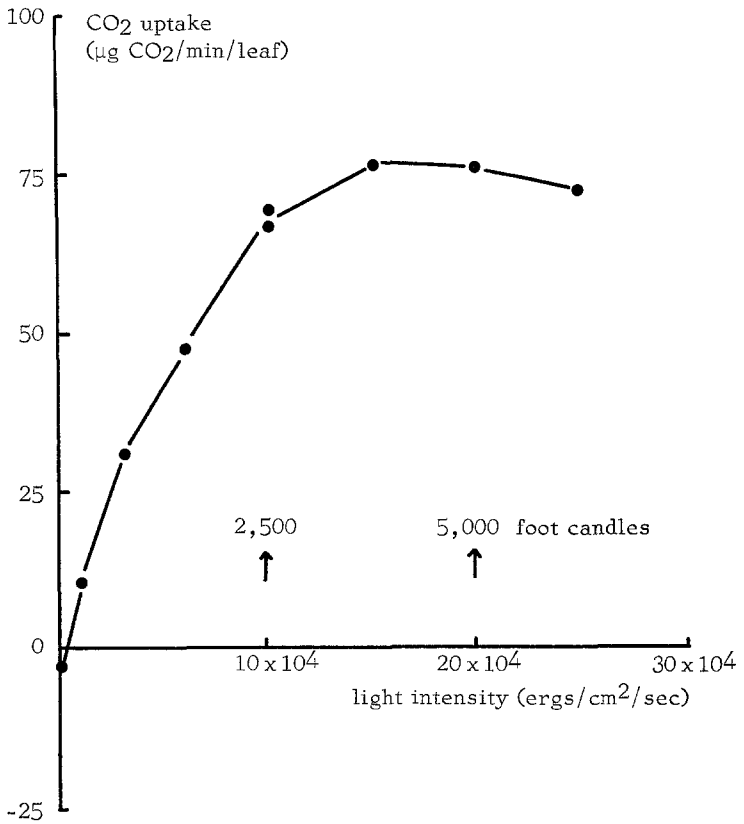

Fig. 2. Effect of light intensity on the carbon dioxide uptake of a spinach leaf. Leaf temperature was maintained at 21°C.

obtained by difference have also been given. Variation in CO_2 assimilation occurred between individual plants but it should be noted that the rates of gas exchange due to roots were less than 5 per cent of the total CO_2 turnover of the plant.

(3) *Effect of macro- and micro-nutrient element deficiencies on plant growth and carbon assimilation*

The photosynthetic activity of mineral-deficient and full-nutrient experiments was compared in a total number of 20 separate experiments. The work extended over a 12-months period and plants grown deficient in each of the essential nutrient elements except

TABLE 1

Plant No.	Photosynthesis *		Respiration *		
	Whole plant in Circuit	Tops only in Circuit	Whole plant in Circuit	Tops only in Circuit	Roots by Difference
1	67.01	74.18	13.17	11.67	1.50
2	77.01	75.18	8.33	7.33	1.00
3	91.68	88.02	16.50	11.00	5.50
4	52.18	55.51	9.17	7.33	1.84
Mean *	72.01	73.18	11.79	9.33	2.46
Mean +	70.16	71.82	7.86	9.23	4.97
Mean **	176.7	181.5			

* μg CO_2 fixed or evolved per plant.
+ μg CO_2 fixed or evolved/min./g. fresh wt.
** μg CO_2 fixed/min./mg. chlorophyll.

chloride and cobalt were examined. All the plants for these experiments were grown in a controlled environment cabinet. At each harvest the rates of gas exchange and other growth characteristics of mineral-deficient plants were compared with those of control plants of the same age. The general experimental design involved a series of harvests of deficient and corresponding full-nutrient plants when the plants were between 14 and 21 days old. The first harvest was made when the first visual signs of deficiency symptoms were present and further harvests were made over the following 7 to 14 days. The data obtained in this series of experiments can be summarized as follows:

(a) Effect of nutrient deficiencies on plant growth

Figures 3 and 4 show the total fresh weight per plant of the plants used in these experiments. In all cases mineral-deficient plants weighed less than control plants of the same age. The supplementary levels of nutrient added to deficient cultures were carefully selected so that mineral-deficient plants were obtained that were in the general size range of control plants. Further, only plants displaying visual symptoms of nutrient deficiency were harvested. These procedures helped to improve the uniformity of the plants that were assayed. Accordingly for statistical analysis standard errors were averaged for the calculation of least significant differences. A series of least significant difference values permitting comparison of con-

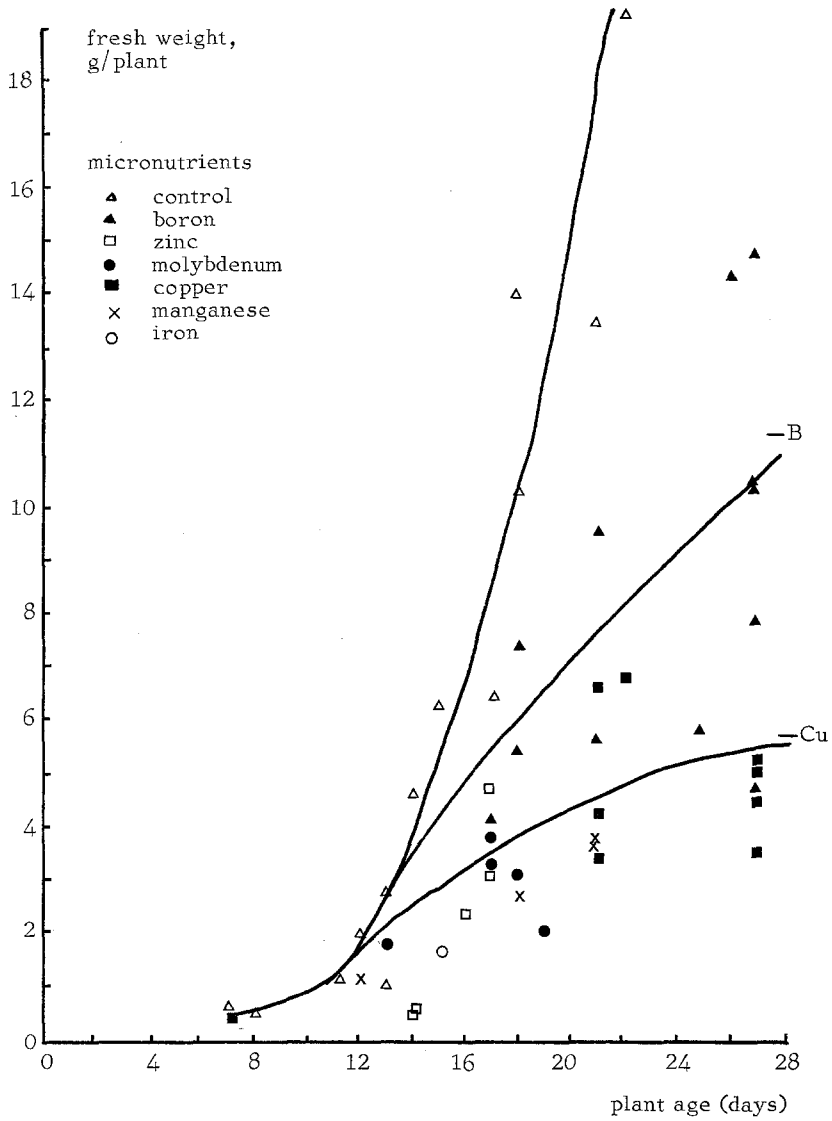

Fig. 3. Comparison of the size of micronutrient-deficient plants at the time of assay with control plants of the same age.

trol means with treatment means assembled from varying numbers of replicates is provided.

Changes occurred in the proportion of plant fresh weight present

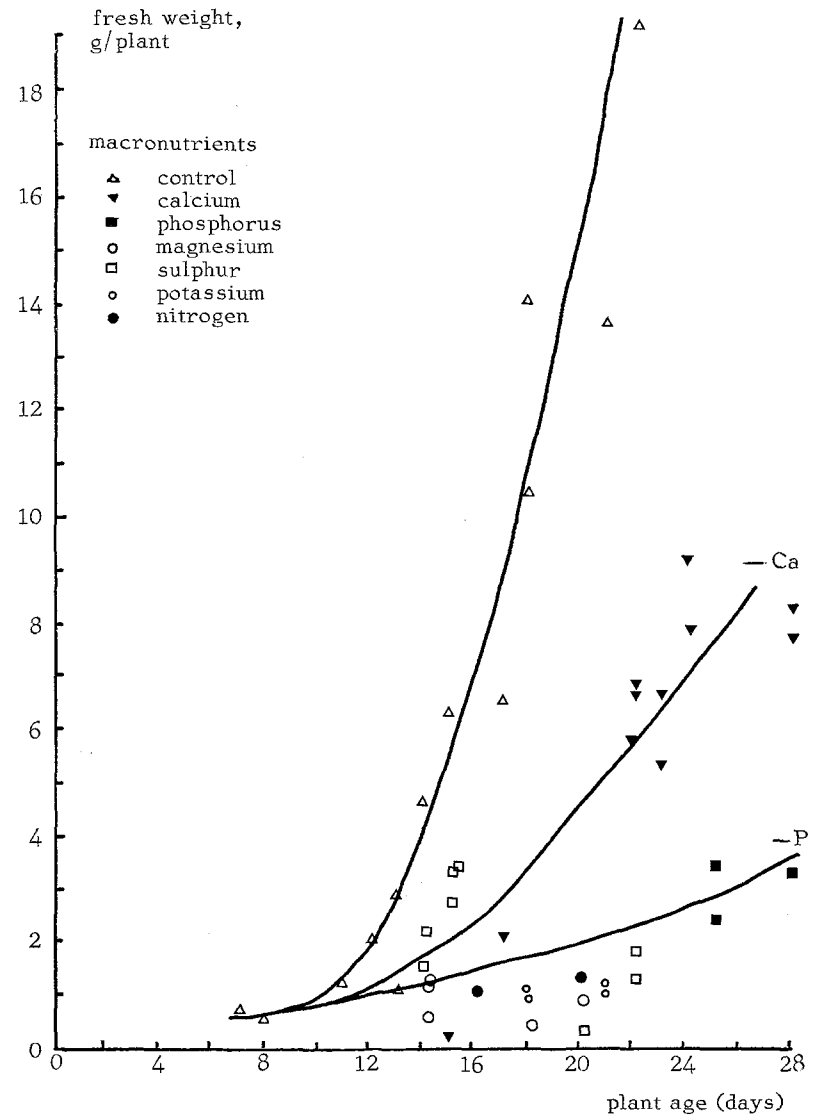

Fig. 4. Comparison of the size of macronutrient-deficient plants at the time of assay with control plants of the same age.

in shoots and roots (Table 2). The shoot-root ratio was increased by deficiencies of manganese and boron and decreased by deficiencies of nitrogen, phosphorus, potassium and molybdenum. Deficiencies

TABLE 2

Effect of nutrient deficiencies on the growth of spinach plants

Deficiency	Shoot f. wt. / Root f. wt.	mg insol. N* / g f. wt.	μg chlorophyll / g f. wt. shoot	Chlorophyll A / Chlorophyll B
Control	2.10 (15)+	2.20 (13)+	546 (19)+	3.25 (15)+
−N	0.80 (4)	1.59 (4)	312 (5)	2.47 (4)
−P	0.72 (5)	2.06 (5)	764 (5)	2.67 (5)
−K	0.84 (4)	5.06 (4)	1353 (4)	2.60 (4)
−Mg	1.64 (5)	2.77 (5)	369 (5)	2.86 (5)
−S	1.79 (8)	1.93 (9)	270 (8)	2.44 (8)
−Ca	1.84 (12)	2.76 (12)	669 (12)	3.08 (12)
−Fe	1.90 (2)	2.49 (3)	253 (3)	3.58 (3)
−Mn	3.76 (6)	2.40 (6)	343 (8)	2.07 (6)
−Cu	2.04 (9)	2.82 (9)	604 (9)	3.35 (9)
−B	3.32 (13)	2.84 (13)	651 (13)	2.77 (13)
−Zn	1.79 (5)	1.91 (5)	417 (5)	2.77 (5)
−Mo	2.93 (5)	1.61 (4)	235 (5)	3.59 (5)
LSD $P = 0.05$ for comparison of control with means				
of 2 values	0.92	—	—	—
3 values	—	0.57	161	0.70
4 values	0.69	0.51	143	0.62
5 values	0.63	0.47	130	0.57
6 values	0.59	0.44	—	0.53
8 values	0.54	0.40	109	0.48
9 values	0.52	0.39	105	0.46
12 values	0.47	0.36	96	0.43
13 values	0.46	0.35	93	0.42

* Nitrogen insoluble in 80% acetone.

\+ Within brackets no. of values used for calculation of mean.

of magnesium, iron, sulphur, calcium, copper and zinc had no effect.

The insoluble nitrogen content of the plant was increased by deficiencies of potassium, magnesium, calcium, copper and boron and reduced by deficiencies of nitrogen and molybdenum. Phosphorus, iron, sulphur, manganese and zinc deficiencies had no effect on nitrogen content.

The chlorophyll concentration or amount per unit fresh weight present in the leaves was increased by deficiencies of phosphorus, potassium, calcium and boron. Potassium deficiency elicited large changes in chlorophyll content. Nitrogen, magnesium, iron, sulphur, manganese, zinc and molybdenum deficiencies decreased the chlorophyll content of leaves while copper deficiency had no effect. Sig-

TABLE 3

Effect of nutrient deficiencies on the photosynthesis and respiration of spinach plants

Deficiency	Photosynthesis				Respiration			
Control	46.0*	(15)++	135.7**	(20)++	8.3*	(15)++	4.01+	(16)++
−N	9.9	(4)	75.2	(5)	4.8	(4)	3.37	(4)
−P	17.3	(5)	55.1	(6)	4.5	(5)	2.23	(5)
−K	32.7	(4)	56.1	(4)	11.1	(4)	2.23	(4)
−Mg	21.6	(5)	94.7	(5)	6.4	(5)	2.62	(5)
−S	13.7	(8)	73.2	(8)	4.9	(8)	2.62	(8)
−Ca	43.0	(12)	100.8	(12)	7.1	(12)	2.66	(12)
−Fe	26.6	(3)	124.5	(4)	6.6	(3)	2.77	(3)
−Mn	11.5	(6)	50.4	(10)	3.5	(6)	1.49	(7)
−Cu	24.2	(9)	61.8	(9)	6.8	(9)	2.50	(9)
−B	36.8	(13)	78.1	(13)	7.0	(13)	2.41	(13)
−Zn	20.2	(5)	79.3	(5)	5.1	(5)	2.85	(5)
−Mo	23.2	(5)	134.4	(5)	5.8	(5)	3.84	(4)
LSD $P = 0.05$ for comparison of control with a mean								
of 3 values	11.3		—		2.5		1.27	
4 values	10.1		29.8		2.2		1.13	
5 values	9.3		27.2		2.0		1.03	
6 values	8.7		25.3		1.9		—	
7 values	—		—		—		0.91	
8 values	7.9		22.8		1.7		0.87	
9 values	7.6		21.8		1.6		0.84	
10 values	—		21.1		—		—	
12 values	7.0		19.9		1.5		0.77	
13 values	6.8		19.4		1.5		0.75	

* μg CO_2 fixed or evolved/min./g f. wt.
** μg fixed/min./mg chlorophyll.
\+ μg CO_2 evolved/min./mg insoluble N.
++ number of values used for calculation of means.

nificant changes in the ratio of chlorophyll A to B occurred in plants deficient in nitrogen, phosphorus, potassium, sulphur, manganese and boron. In each case there was a decrease in the ratio.

(b) Effect of nutrient deficiencies on photosynthesis and respiration. All mineral-nutrient deficiencies except calcium reduced the rate of photosynthesis of spinach plants when carbon dioxide fixation rates were expressed per unit of fresh weight. On this basis plants deficient in nitrogen, sulphur, manganese, and phosphorus markedly reduced photosynthesis. However when CO_2 fixation rates were expressed on a per unit chlorophyll basis all

deficiencies except iron and molybdenum reduced photosynthesis. On this basis the effect of calcium deficiency in depressing photosynthesis was small while deficiencies of manganese, copper, phosphorus and potassium had large effects.

Respiration on a fresh-weight basis was markedly increased in potassium-deficient plants and was decreased in nitrogen-, phosphorus-, sulphur-, manganese-, zinc-, and molybdenum-deficient plants. Deficiencies of magnesium, iron, calcium, copper and boron had no effect on respiration rate when calculated on a fresh weight basis. By contrast only deficiencies of iron, molybdenum, and nitrogen had no effect on respiration when expressed per unit of insoluble nitrogen while all other deficiencies resulted in reduced rates of respiration on this basis. The respiration data presented here are average values for whole plants. The method of measurement used in these experiments did not provide separate values for roots and shoots.

DISCUSSION

It is of interest to compare the photosynthetic and respiratory characteristics of spinach leaves recorded here with that of other plants. Spinach generally has a high assimilation rate compared with citrus and deciduous tree species [10]. The assimilation number (mg CO_2 fixed/mg chlorophyll/hour), of between 8 and 11 measured in these experiments compares with 11.6 for glasshouse grown corn, 14 for sunflower [10] and 10.8 previously reported for spinach [9].

The temperature response of spinach photosynthesis was similar to that of many plants having an optimum in the range 20 to 30°C. Also the response of the plant to changes in light intensity was similar to that of a number of plants as it reached saturation at approximately 2500 foot candles.

Photosynthetic rates in these experiments have not been presented on a leaf area basis as spinach plants have a rosette habit of growth and relatively thick succulent leaves, which make accurate leaf area measurement almost impossible. Also it was felt that more meaningful comparisons of photosynthesis could be obtained by presenting the gas-exchange rates on a per unit chlorophyll basis as this pigment is of course the principal receptor of the light energy used in photosynthesis and is known to vary in mineral-deficient plants [20].

Mention must be made of the measuring arrangement whereby the whole plant (shoots and roots) was placed in the assimilation chamber. The data of Table 1 indicate that this method of measurement gave essentially the same values for photosynthesis as those obtained when the plant top was isolated from the roots. Initially it was thought that the metabolism of micro-organisms present on root surfaces and variations in root size and respiratory capacity might introduce wide variations. However this did not occur mainly because the roots provide less than 5 per cent of the total CO_2 turnover of the plants used in these experiments. In older plants root respiration would have been a larger component. It is considered that the differences in nett photosynthetic rate expressed on a per unit chlorophyll basis which occurred between the two measuring assemblies (Table 1) were largely attributable to differences in the flow rates. The higher values obtained (Table 1) are similar to the maximum rates previously reported [9]. The lower rates measured in comparisons of the nutrient deficient plants (Table 3) would minimize the treatment differences.

The basic design of the present experiments was one in which mineral-deficient plant displaying visual symptoms were compared with control plants of the same age. Earlier studies with isolated tomato chloroplasts indicated that there can be a rapidly changing pattern with time in the degree of inhibition of photochemical reactions in mineral-deficient plants [17]. Similarly with intact plants there almost certainly is a changing pattern of inhibition of both photosynthesis and respiration as deficient plants age. Bouma [5] has shown that the nett photosynthesis of sulphur deficient clover plants was depressed relative to controls but was constant for a period of over a week. By contrast he found marked changes with time in the photosynthetic rates of phosphorus-deficient plants. In unpublished experiments with manganese-deficient plants we found that there was a relatively rapid change in photosynthetic rate as deficient plants deviate from the control. Thereafter the relative difference between control and deficient plants was maintained for a period of over a week.

In the present experiments we have averaged the data for both control and nutrient deficient plants and suggest that these averages provide a general indication of those deficiencies which have a major inhibitory effect on photosynthesis or respiration. It is stressed that

the average values we have provided apply to plants displaying clear visual symptoms of mineral deficiency. We have not attempted to provide information on the initial stages of the deficiency syndrome before visual symptoms of abnormality are established. Also all of the comparisons were made well before the terminal stages of nutrient-deficiency restraint which precedes death.

The results we obtained for the effect of nutrient deficiencies on carbon assimilation in spinach were similar to those separate effects that have been obtained for a range of other species [4 6 7 8 11 15 16]. The effects of the various deficiencies on the photosynthesis of intact spinach plants when expressed on a per unit chlorophyll basis bear a close correspondence to the inhibitory effects previously recorded for nutrient deficiencies on the Hill activity of isolated tomato chloroplasts. For example relative inhibitions due to deficiencies of manganese, phosphorus and sulphur were similar in all cases as was the lack of inhibitory effects with iron and molybdenum deficiencies. By contrast the respiratory data assembled in the present experiment are quite different to the values of the oxygen uptake of homogenates of mineral-deficient tomato leaf tissue [13].

Although the present investigation was intended to document the broad effects of mineral-nutrient deficiencies the specific effects of three elements deserve attention. In the case of iron deficiency it appears that overall photosynthesis was apparently limited by chlorophyll contents because on this basis the CO_2 fixation of iron deficient plants equalled that of controls. A similar result was obtained in the case of molybdenum-deficient plants. The results obtained with manganese-deficient plants where the rates of CO_2 fixation on a chlorophyll basis are low support the now well-documented claim that this element is required for specific reactions of photosynthesis [8 17 18].

It is suggested that a majority of the effects of nutrient deficiencies on photosynthesis and respiration are due to the influence specific nutrient ions exert on particular biochemical reactions. We also consider that at least some of the inhibitory effects we have observed may have been due to mineral-deficient plants having chloroplasts that are structurally abnormal [19].

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