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Influence of Manganese Deficiency and Toxicity on Isoprenoid Syntheses[1]

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ABSTRACT

Twenty-eight day old wheat (*Triticum aestivum* L. cv Stacy) response to varying Mn concentration (10.1–10,000 micromolar) in nutrient solution was measured. Manganese concentrations in the most recently matured leaves (blade 1) were 0.21 to 19.03 mmol Mn per kilogram dry weight, respectively. Fresh and dry weights increased to a maximum at the 5 micromolar Mn nutritional level (0.37 millimole Mn per kilogram dry weight) and were decreased at Mn above and below this concentration. Blade 1 chloroplast pigment concentrations increased up to the 20 micromolar Mn nutritional level (1.98 millimole Mn per kilogram dry weight) and decreased at higher Mn concentrations. Thylakoid Mn content was above 1 mole Mn/100 mole chloroplast at Mn nutrition levels which resulted in greatly decreased plant growth. Total phytoene biosynthesis was decreased by Mn deficiency and toxicity. *In vitro* ent-kaurene synthesis was greatly influenced by Mn concentration with a maximal biosynthesis at 1 micromolar Mn and decreases at Mn levels above and below this concentration. *In vivo* blade 1 gibberellic acid equivalent concentrations were maximal at 20 parts per million Mn nutrition solution levels (1.98 millimole Mn per kilogram dry weight) and decreased at Mn tissue concentrations above and below this value; additionally, gibberellic acid concentrations were reciprocal to extracted C_{20} alcohol concentrations. Mn influence on gibberellin and chloroplast pigment biosyntheses exactly matched the measured changes in growth.

Manganese is a required microelement for growth and photosynthesis of heterotrophic and autotrophic plants (2, 3, 8, 9, 17–20). Excess concentrations of Mn in plant tissues result in decreased growth (9, 10, 17–20). Manganese deficiency has been shown to limit photosynthetic capacity (26). This limitation of photosynthesis was presumed to be the major influence which results in limited growth during Mn deficiency (9). The requirement for Mn in PSII O_2-evolution has tended to corroborate this hypothesis (22, 32). However, many other metabolic syntheses also require Mn. The possibility exists that the concentrations of Mn required for plant growth and development may be primarily a function of the limitation of metabolic syntheses whose decreased activity ultimately results in a limitation of total photosynthesis and/or growth.

The isoprenoid biosynthetic system (Fig. 1) produces carotenoids, Chl, GA, sterols, and several quinones that are requisite for growth and photosynthesis. Phytoene synthetase (16) (Fig. 2) activity specifically requires Mn while Mg is inhibitory. *Ent*-kaurene synthetase (13) requires Mg but can use Mn at lower concentrations; and, a mixture of Mg:Mn = 2:1 is superior to either cation at any concentration. In both of these biosynthetic systems, maximum activity was attained at 1 to 2 mM Mn with sharp decreases in enzyme activity at lower or greater concentrations of Mn (13, 16). Mevalonic kinase utilizes Mn or Mg with activities of either divalent ion being dependent upon ion concentration (27). Thus, Mn activities are requisite or highly beneficial for enzymes directly involved with the synthesis of GA and/or chloroplast pigments. Consequently, a Mn deficiency would result in decreased chloroplast pigment synthesis. A consequence would be smaller plants and decreased net photosynthesis.

Experiments were designed to evaluate the hypothesis that Mn deficiency and toxicity were explicable as influences on isoprenoid biosynthetic processes rather than PSII limitation.

MATERIALS AND METHODS

Plant Growth. Wheat (*Triticum aestivum* L. 'Stacy') seeds were grown with 10 Mn levels (0.1, 0.4, 1.0, 5.0, 20, 100, 500, 2,000, 4,000, and 10,000 μM Mn as $MnSO_4$) in the nutrient solution (19).

At harvest on D28 following transplanting, the plants were in the vegetative stage. Blade 1 (most recently matured leaf with a developed ligule) samples were taken from 3 plants per replicate from developed tillers in each Mn level. A sample of these leaves (blade 1) was weighed and analyzed for pigments and precursors (see below). The remainder of the tops was washed, blotted dry, weighed, dried in a 70°C forced draft oven, and weighed again. The total top dry weight per pot consisted of 3 plants. A second

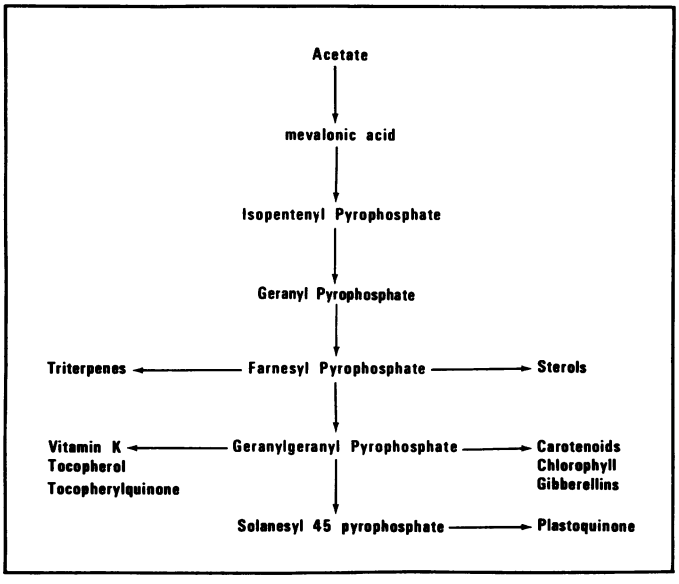

FIG. 1. Isoprenoid biosynthetic pathway.

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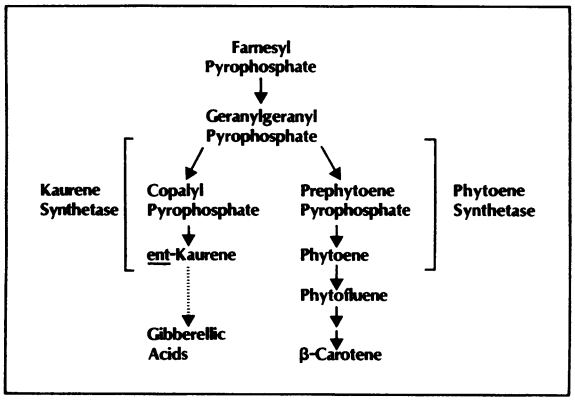

FIG. 2. Conversion of GGPP to β-carotene or gibberellins.

blade 1 sample from the remaining 12 plants was washed, freeze-dried, and analyzed for Mn content. The dried plant material was ground in a Wiley mill to pass a 40-mesh stainless steel screen. Elemental analyses were conducted on nitric-perchloric acid wet ashings of the dried and ground plant samples. Mn was determined by atomic absorption spectrophotometry.

Pigment Analyses. Blade 1 samples from each pot were extracted with acetone (85%) and an aliquot was analyzed spectrophotometrically for Chl α, Chl β, and carotenoids (24). After return of the aliquot to the total pigments, total volume was reduced to about 30 ml using a rotary evaporator (20°C, dark). The acetone extract was washed thrice with *n*-pentane. After TLC plate activation and cooling in a desiccator, carotenoid components in the *n*-pentane extract were separated by TLC using 0.25 mm layers of $CaCO_3$, MgO, and $Ca(OH)_2$ made up with 30% KOH developed with *n*-octane:acetone:chloroform (60:50:40, v/v/v) (11). The top 2 cm of the chromatogram were scraped into *n*-pentane. Carotene, phytoene, and phytofluene were quantitated spectrophotometrically using $E_1^{1\%}$ for carotene (2505) (451 nm), phytoene (1250) (285 nm), and phytofluene (1350) (348 nm) (5).

Geranylgeraniol contents were released from the blade 1 leaf residues by acid hydrolysis (0.1 M HCl, 50–60°C, 30 min) and an excess of 10 M KOH (to pH 8) was added to the tube. Since acid hydrolysis induced partial rearrangement of GGPP[2] to other C_{20} alcohols (21), data are presented as GG equivalents as quantitated from geranylgeraniol. Isoprenoid alcohols were extracted into benzene:acetone (3:1) thrice (21). Separation and quantitation of the isoprenoid alcohols utilized 100 μg 1-docosanol as an internal standard, BSTFA silylation (23), and GLC quantitation (183 × 0.3 cm diameter steel columns filled with 80/100 Anakrom ABS coated with 5% OV-1 at 210°C isothermal oven temperature) (31). All constituents were converted to μg/gm FW. Analyses of variance were conducted on a randomized complete block design.

Chloroplast Analyses. Plants were grown as described above. Blade 1 leaves were sliced into 3 mm strips, and mesophyll cells were isolated by digestion with 2% maceroenzyme (500 mM sorbitol, 1mM $CaCl_2$, 5 mM MES [pH 5.5]) (25). After removal of debris by filtration through polyethylene (508 μm openings), the cells were centrifuged (10,000*g* for 20 min at 4°C), resuspended in medium B (500 μM sucrose, 1 mM $CaCl_2$, and 5 mM MS [pH 6.0]) and centrifuged again under the same conditions. The cells were resuspended in medium B and sampled for Chl and Mn contents. The cells were disrupted by sheering them through a hypodermic needle (22 mesh), and the broken cell mixture was centrifuged (as above). Resuspended chloroplasts were sampled for Chl and Mn contents. The chloroplasts were centrifuged (as above) and the chloroplast pellet was resuspended in medium C (10 mM phosphate buffer [pH 7.4] and 4 mM $MgCl_2$). Thylakoids from these osmotically disrupted chloroplasts were pelleted by centrifugation (20,000*g*, 30 min, 4°C), and thylakoids were sampled for Chl and Mn contents.

2, 6-Dichlorophenol-Indophenol Reduction. Plants were grown under a supplementary light photon flux density (400–700 nm) of 420 μmol m^{-2} s^{-1} at plant tops from 0600 to 0900 and 1700 to 2000 h during February and March (7). Blade 1 leaves (10 g) were segmented to 1 cm sections, and chloroplasts were extracted (mannitol [0.3 M], EDTA [1 mM], DTE [6 mM], BSA [1 g/L], KH_2PO_4 [50 mM], and Hepes [0.1 M] pH 7.2) at o°C by four 1/2 s bursts in a Waring Blendor. After filtering through four layers of cheesecloth and centrifuging at 5000*g* for 10 min, the chloroplasts were resuspended in 4 ml extraction solution. Chl was quantitated (1) and diluted to 0.5 mg/ml. All extractions were retained at 0 to 4°C. DCPIP reduction was conducted with a recording spectrophotometer (Beckman DBG) in 3 ml containing phosphate buffer (0.037 M) pH 7.2, sorbitol (0.22 M), KCl (7.3 mM), Chl (16.7 μg), and DCPIP (400 μM) and was measured at 660 nm.

Mn content of the chloroplast suspension was determined by placing a 1 ml aliquot into a 3 ml plastic vial and lyophilizing the suspension. The Mn content of the vials was quantitated by neutron activation (limit 5 pg). Chl contents were determined spectrophotometrically (24).

Each assay was repeated three times and four replications were utilized. Data were subjected to analysis of variance on a randomized plot design.

Carbon Dioxide Fixation. Carbon dioxide reduction was conducted by previously published methods (20). Four replications were utilized and data were analyzed for significance by linear regression.

Gibberellin Precursor Studies. Previously published procedures were utilized for gibberellin precursor studies (29). Briefly, wheat seeds were germinated and grown in the dark at 28°C. After 96 h, the etiolated coleoptiles were harvested and ground in a mortar and pestle under liquid N_2. After centrifugation at 25,000*g*, the supernatant was utilized as the enzyme source. GA precursors were synthesized using [^{14}C]MVA (0.1 μCi/tube) (53 mCi/mmol), 10 μg protein/assay, 10 mM $MgCl_2$, and varying concentrations of Mn (0, 0.01, 0.1, 1, 2, 4, 7, or 10 μM). Protein was determined by the Lowry method (29). Ancymidol was added to prevent kaurene oxidation (4, 29). After 2 h, the reaction was stopped with acetone and *ent*-kaurene was extracted with benzene:acetone (3:1) three times. *Ent*-kaurene was separated by TLC on silica gel G using *n*-pentane as a developing solvent utilizing a paper-lined TLC chamber (29). In an unlined chamber, *ent*-kaurene and squalene chromatograph together $R_f \sim 0.95$. In a lined chamber, *ent*-kaurene $R_f \sim 0.95$ but squalene $R_f \sim 0.80$ (29). Silica gel containing *ent*-kaurene was scraped into vials, and ^{14}C was assayed by liquid scintillation spectrometry (29).

All samples were converted to percent of total ^{14}C incorporated. Data were subjected to analysis of variance on a randomized complete block. All analyses were conducted in quadruplicate.

GA Extraction and Quantitation. Previously described procedures were utilized for GA extraction and quantitation. (6, 30). Briefly, the sample was macerated in 80% acetone (4°C) and

[2] Abbreviations: GGPP, geranylgeranylpyrophosphate; GG, geranylgeraniol; ancymidol, α-cyclopropyl-α-(*p*-methoxyphenyl)-5-pyrimidine methyl alcohol; kaurene, *ent*-kaurene; PPFD, photosynthetic photon flux density; MVA, mevalonic acid; BSTFA, N, O, bis-(trimethylsilyl)-trifluoroacetamide; DCPIP, 2, 6-dichloro-phenol-indophenol; ppmw, parts per million; MK, mevalonic kinase; EtAc, ethyl acetate; FW, fresh weight; DW, dry weight.

stored for 24 h. After filtering (glass wool) the acetone was removed by rotary evaporation. The aqueous residue was acidified (pH 2) and extracted twice with EtAc. The combined EtAc extracts were washed with 100 ml 5% $NaHCO_3$; the water was acidified (pH 2) and extracted with EtAc three times. The EtAc was evaporated under vacuum in a dry Teflon-capped, screw-topped test tube and stored under N_2 at −20°C.

Samples were warmed to room temperature, 1 ml BSTFA was inserted, and the sample was heated at 65°C for 1 h. Quantitation was on a dual-flame-ionization GLC equipped with a digital integrator and 183-cm stainless steel columns, 3 mm o.d., packed with 10% OV-1 on Chromosorb W(AW) (DMCS) (100/120 mesh). Temperatures were programmed from 100 to 300° C at 10°C/min. Acid hydrolysis of gibberellic acid yields rearrangement products. Only those products eluting at the same R_f as authentic GA_3 were utilized for presentation. Quantities were determined by the area under the curve relative to 100 μg *n*-heptadecanoic acid added to the original acetone extract. Each sample was analyzed three times, and the experiment was replicated four times. Analytical values were subjected to analysis of variance on a randomized block design.

RESULTS

Plant Growth. Total FW/plant increased as Mn nutrition concentrations increased to a level of 5 μM Mn in the nutrient solution (Table I) and decreased as Mn nutrition concentrations were increased above 5 μM Mn. This is a typical demonstration of Mn deficiency and toxicity of higher plants and corroborates previous data (9, 17–20). The exact form of the response curve depends upon species and varieties (9, 12, 19, 20).

Blade 1 leaves showed similar trends in FW and DW indicating that blade 1 was a good physiological indicator of the Mn status of the plants. The Mn concentration in blade 1 leaves remained at approximately 0.2 mmol Mn kg^{-1} DW (Table I) for the 0.1 to 1.0 mmol Mn nutrient levels. At 5 μM Mn nutrition, the Mn concentration was increased to 0.37 mmol/kg DW. Above 5 μM Mn nutrition, the blade 1 Mn concentration increased rapidly (Table I). Using Liebig's "Law of the Minimum" (15), growth at these Mn concentrations showed that 0.2 mmol Mn kg^{-1} DW is an approximate minimum to support growth. This corroborates previous data (18–20).

CO_2 and DCPIP Reduction. Neither CO_2 reduction (μmol CO_2/mg Chl-min) nor DCPIP reduction (μM DCPIP/mg Chl-min) were correlated with Mn content in the Chl or nutrient solution, respectively (data not shown). There was a decreased CO_2 reduction (μmol CO_2/mg Chl-min) at deficient and toxic Mn concentrations. Carbon dioxide fixation (μmol CO_2/g FW) (Fig. 3) was linearly correlated with Chl content (mg Chl/g FW) (significance 0.01% for slope and intercept, slope = $y = 4.703 + 6.612\,x$).

Cell Fraction Mn Contents. Thylakoid Mn content (ng Mn/g FW) increased as Mn nutrition increased (Table II). But, Mn content/unit Chl did not (Table II). Mn is known to be required for PSII at concentrations about 1 mol Mn/100 mol Chl (22, 26, 32). Yet at thylakoid concentrations equal to this value (Table II), the plant growth and FW productions were greatly limited (Table I). And this decrease in growth was not due to a limitation by Mn deficiency on CO_2 reduction or PSII electron transport.

Pigment Analyses. Total Chl *a* per plant and total carotenoids (μg/plant) concentrations followed the same trends as were shown for total FW and blade 1 FW (Tables I and III). Blade 1 Chl *a* content (μg/gm FW) and carotenoid contents (μg/gm FW) (Table III) were increased at the 20 μM nutrition level. Since stroma Mn content (mol Mn/100 mol Chl [in chloroplasts]) increased >70% with increase in Mn nutrition from 0.1 to 0.4 μM, the level of Mn availability in the stroma for phytoene synthetase activity would appear to be a major factor in carotenoid synthesis. Therefore, pigment synthesis was, apparently, saturated with Mn at approximately 0.2 mmol Mn kg^{-1} DW so that increased pigment synthesis and DW production occurred simultaneously at increased Mn nutrition availability levels. Additionally, *a/b*, *a/c*, and *b/c* ratios did not vary greatly. Thus, relative ratios of chloroplast pigment synthesis were uniform throughout the entire Mn nutrition series. This uniformity of pigment synthesis may be due to equivalent synthetic activities. An alternative hypothesis for roughly equivalent Chl and carotenoid synthesis would involve photooxidation of Chl when carotene contents were deficient (14). Blade 1 leaves demonstrated

Table I. *Influence of Mn in Nutrient Solution on the Growth and Dry Weight Production of Wheat*

Culture	Total Fresh Weight per Plant[a]	Blade 1 Leaf[a]		
		FW	DW	Mn Content
μM	gm	gm		mmol/kg DW
0.1	28.5e	4.52f	0.42d	0.21 ± 0.04
0.4	50.1d	8.99d	0.95c	0.18 ± 0.02
1.0	75.8c	12.06c	1.33c	0.21 ± 0.02
5.0	112.4a	18.93a	2.55a	0.37 ± 0.04
20.0	94.4b	16.31b	2.11b	1.98 ± 0.19
100.0	85.7bc	14.73b	1.97b	4.40 ± 0.19
500.0	86.9bc	14.68b	1.97b	6.94 ± 0.27
2,000.0	58.1d	9.49d	1.31c	12.48 ± 0.75
4,000.0	47.7d	7.34de	1.27c	17.23 ± 0.61
10,000.0	27.6e	5.13ef	1.14c	19.03 ± 0.62

[a] Values in a column followed by the same letter are not significantly different at the 5% level as separated by the Fisher's Least Significant Difference Test.

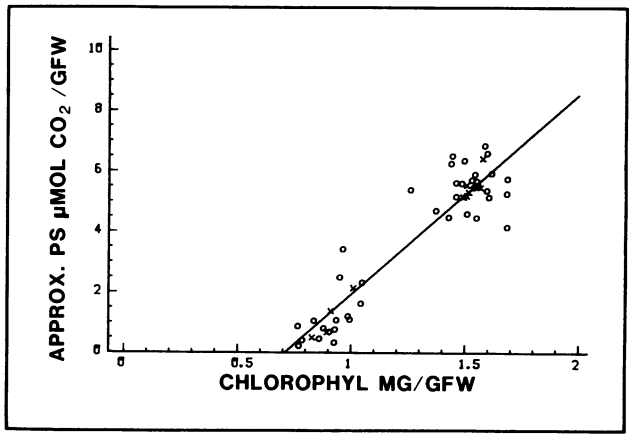

FIG. 3. Correlation of photosynthetic CO_2 fixation (μmol CO_2/g FW) and Chl content (mg Chl/g FW).

Table II. *Manganese Contents of Cell Fractions in Blade 1*

Manganese Nutrition	Cells	Chloroplast	Stroma	Thylakoid
		ng Mn/gm FW		
0.1	1115	664	262	401
0.4	1193	999	229	769
1.0	1264	1005	111	894
5.0	1632	1311	139	1172
		mol Mn/100 mol Chl		
0.1	2.98	2.06	0.76	1.30
0.4	2.70	2.52	1.32	1.20
1.0	1.02	2.28	1.38	0.90
5.0	1.76	2.06	1.26	0.80

Table III. *Influence of Mn in Nutrient Solution on the Chloroplast Pigment Content of blade 1 (most recently matured) of Wheat*

Culture Mn	Blade 1 Leaves[a]			mol Mn per 100 mol Chl *a*
	Chl		Carotenoid	
	a	*b*	*c*	
μM	*μg/gm FW*			
0.1	91.6b	25.3bc	24.6b	19.2
0.4	108.6b	28.9b	30.4ab	15.6
1.0	94.8b	28.9b	27.8ab	21.9
5.0	106.0b	27.3bc	24.6b	42.2
20.0	140.3a	35.5a	33.2a	113.9
100.0	112.5b	24.3bc	24.5b	467.3
500.0	112.7b	25.7b	25.0b	746.2
2,000.0	108.1b	27.8b	25.8b	1428.6
4,000.0	93.2b	21.1c	23.8b	2857.2
10,000.0	50.8c	11.7d	13.8c	7692.3

[a] Values in a column followed by the same letter are not significantly different at the 5% level as separated by the Fisher's Least Significant Difference Test. Extractions were from fresh leaves in 85% acetone.

Table IV. *Influence of Mn in the Nutrient Solution on Carotene, Phytoene, and Phytofluene in blade 1 Leaves*

Mn Nutrition	Carotene	Phytoene	Phytofluene
μM		*μg/gm FW*	
0.1	132.3ab[a]	28.4b	8.9b
0.4	145.7ab	35.9a	13.7a
1.0	163.4ab	34.2a	13.1a
5.0	179.6ab	32.0ab	12.9a
20.0	196.7a	31.6ab	12.8a
100.0	185.3ab	30.8ab	10.8ab
500.0	163.4ab	30.1ab	10.0ab
2,000.0	157.6ab	29.8b	11.3ab
4,000.0	120.9b	29.3b	9.7b
10,000.0	112.8b	27.5b	8.5b

[a] Values in a column followed by the same letter are not significantly different at the 5% level as separated by the Fisher's Least Significant Difference Test. Extractions were from lyophyllized leaves in *n*-pentane.

an integrated response between increase in size, increase in dry weight, and increase in photosynthetic capacity (*i.e.* Chl content) that was based on the concentration of Mn present in the leaves (Table I).

In the isoprenoid biosynthetic pathway (Figs. 1 and 2) there are several enzymic reactions that require or can use Mn as a cofactor. Phytoene synthetase requires Mn (16), and the activity curve is maximal at 1 to 2 mmol Mn per mg protein with sharply decreased activity at Mn concentrations above and below this Mn concentration.

Ratios of purified β-carotene, phytoene, and phytofluene contents did not vary greatly in the blade 1 leaves from the Mn nutrition concentration series (Table IV). Thus, there were not any major inhibitions of reactivity within the series of conversions phytoene → phytofluene →→ β-carotene; and, β-carotene accumulated while phytoene and phytofluene concentrations remained relatively constant as would be expected from biosynthetic steady state intermediates without inhibitions in that portion of the system (Fig. 2). Additionally, MK utilizes Mn, and MK produces the precursors utilized in the various chloroplast pigments. When the quantities of these pigments (*i.e.* Chl, carotene, phytoene, and phytofluene) present in the tissues were converted to GG equivalents, the total GG equivalents present in blade 1 leaves grown in Mn nutrition levels from 0.1 to 5.0 μM ranged from 361.4 to 408.2 nmol/g FW (Table V). This was

Table V. *Total Geranylgeranyl-Equivalents Contained in Chl, Carotene, Phytoene, and Phytofluene in Blade 1 Leaves from Plants Grown at Various Mn Nutrition Levels*

Mn Nutrition	GG-equiv.
μM	*n mol/q FW*
0.1	361.4
0.4	451.9
1.0	418.2
5.0	408.2
20.0	486.0
100.0	399.3
500.0	397.5
2,000.0	401.3
4,000.0	361.9
10,000.0	255.5

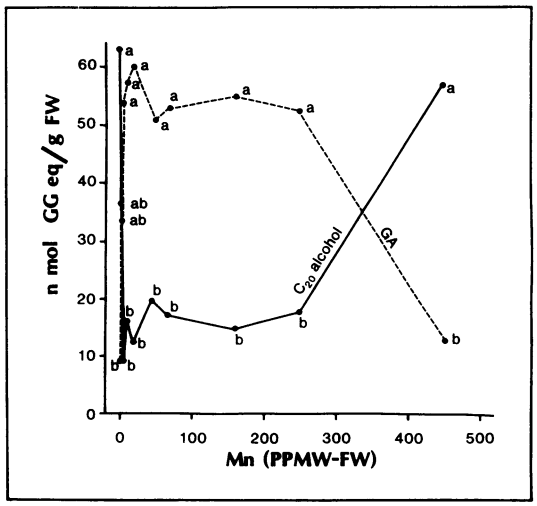

FIG. 4. GG and GA equivalent contents of wheat blade 1 leaves after 28 d growth under differing Mn nutrition levels. Manganese concentrations were determined from whole blade 1 leaves. Points on a line followed by the same letter are not significantly different at the 5% level.

a 12.9% increase in GG-equivalents (nmol/g FW) in leaves whose FW/plant increased 419% (Table I). On a total GG-equivalents/plant basis there was a massive increase in total GG-equivalents (nmol/plant). Thus, major Mn nutrition influence on MK activity was demonstrated.

In the phytoene biosynthetic system (Fig. 2), the C_{20}-alcohol (*i.e.* geranylgeranyl pyrophosphate) accumulated approximately 5 times at the 0.1 μM Mn nutrition level (Fig. 4). As Mn nutrition increased, the C_{20}-alcohol accumulation decreased (Fig. 4) to a minimum at the 10 to 250 μM Mn nutrition level. The C_{20}-alcohol accumulation and constant quantities of carotene, phytoene, and phytofluene are explicable as a steady state synthesis of precursor but an inhibition of utilization of that precursor for phytoene synthesis by the Mn-requiring enzyme phytoene synthetase (16). Since Mn deficiency inhibited one type of utilization of C_{20}-alcohol that is a common intermediate to pigment and GA synthesis (Fig. 2), an influence of Mn on GA biosynthesis could explain growth reductions by Mn deficiency or toxicity. A second biosynthetic reaction which can use Mg and/or Mn is *ent*-kaurene synthetase (Fig. 2) (13).

Gibberellin Precursors. *In vitro* incorporation of [^{14}C]MVA into *ent*-kaurene (kaurene synthesis) was greatly influenced by Mn content (Fig. 5). Maximal activity was attained at 1 μmol Mn with decreased activity above and below this Mn concentration. This corroborates previous reports (13). Thus, GA precursor biosynthesis could be highly susceptible to control by Mn con-

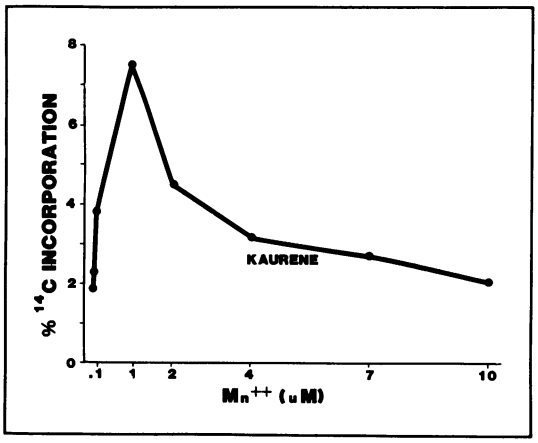

FIG. 5. Percent [^{14}C]MVA incorporation into *ent*-kaurene by a cell-free enzyme system from etiolated wheat coleoptiles.

centration, and growth in FW (Table I) would fit such a hypothesis. Since Mg was present in the reaction medium, these data illustrate a major influence of Mn nutrition on GA production. Extraction of GA from blade 1 showed decreased quantities of GA present in the leaves at low Mn nutrition levels with a maximum at the 10 to 250 μM nutrition level and decreases at higher Mn nutrition levels (Fig. 4). This curve is almost an exact mirror image of the C_{20}-alcohol GG equivalents extracted from the same tissue. The two components combined equalled about 20 nmol/g FW at every Mn nutrition level.

DISCUSSION

Total C_{20}-alcohol GG equivalents accumulated at very low Mn nutrition levels (Fig. 4). The control of phytoene synthetase by Mn (16) resulted in an accumulation of precursor at deficient Mn concentrations (Fig. 4). GA biosynthesis was greatly influenced by Mn concentration (Figs. 4 and 5), and an inhibition of kaurene synthesis also resulted in GG equivalents accumulation (Figs. 4 and 5) (29, 30).

The PSII Mn requirement was saturated first and at a concentration of Mn much lower than the critical level (Table I). In the integrated growth program, Mn deficiency caused decreased capacity to produce pigments and GA. This was equivalent to decreased growth in length. (*i.e.* GA deficiency) and a decreased total Chl and carotene contents. Decreased pigment contents result in lowered net photosynthesis and DW. As Mn concentration increased, the activities of chloroplast pigment and GA precursor biosyntheses ultimately resulted in increased FW and DW to the extent that growth would continue to the limit of the Mn concentration. At optimum Mn concentrations (about 1 mmol Mn), the maximum chloroplast pigment and GA syntheses result. At higher Mn concentrations a progressively increased inhibition of chloroplast pigment and GA syntheses induced decreased growth in FW and DW as well as pigment concentrations.

Massively excessive Mn concentrations would be accompanied by deposition of crystalline Mn (12, 28) or storage in vacuoles as with any other toxic substance (10). Axiomatically, Mn requirements and tolerances for excess Mn would vary between species, varieties, and enzymes (12). Each biosynthetic system would be influenced differently by environmental conditions (10). Additionally, β-carotene deficiencies would limit Chl concentrations through photooxidation (14).

Finally, Mn is utilized in many enzymic e^- transfer reactions which would also be influenced by Mn concentration. For example, 1 mol Mn/100 mol Chl are requisite for PSII O_2-evolution centers (22, 32); but, these Mn ions do not appear to be an integral portion of the PSII reaction centers and can be removed by EDTA (22). Thus, the Mn would appear to function as e^- transfer agent, and a gross excess of Mn could inhibit e^- transfer by competition. The data presented herein suggest a logical scenario for Mn activity in plant growth for deficiency and toxicity of Mn as well as competition for other divalent cations (*i.e.* Mg). Because Mn is utilized in many diverse plant biosynthetic processes, other activities for Mn in plants should be integrated into this proposed hypothesis. The influence of Mn on isoprenoid synthesis is only one of several biosynthetic processes which greatly influence plant growth. Addition of other systems to this integrated approach will require further research.

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