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Thermochimica Acta 349 (2000) 141–146

thermochimica acta

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Methylcyclopentadienyl manganese tricarbonyl (MMT), plant uptake and effects on metabolism

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Received 30 July 1999; received in revised form 9 September 1999; accepted 9 September 1999

Abstract

In the USA and Canada, methylcyclopentadienyl manganese (MMT) is currently added to gasoline to replace tetraethyl lead as an antiknock fuel additive. Manganese concentrations in roadside soil and plants are increasing and correlated with distance from the roadway, traffic volume, plant type, and microhabitat. Radish (*Raphanus sativus* L.) seedlings were treated for either five or thirty-five days with different levels of manganous chloride (0–1000 ppm). Metabolic heat rates (q) and respiration rates (R_{CO_2}), measured calorimetrically, indicated severe stress at Mn concentrations between 10 and 100 ppm and at temperatures above 20°C. Predicted growth rates (R_{SG}) also decreased in these circumstances.

Keywords: Calorimetry; Manganese; Radish; Respiration; Temperature

1. Introduction

Methylcyclopentadienyl manganese tricarbonyl (MMT: $C_9H_7MnO_3$) is an organic derivative of manganese used in gasoline as an antiknock agent and to improve octane ratings [1]. Combustion leads to the formation of oxides of manganese which are deposited along roadsides in North America in amounts proportional to traffic density [2]. Plants absorb the manganous ion and show the highest concentrations of Mn when the root and soil environment is depleted in oxygen, such as in submerged or emergent plants [3]. At moderate concentrations of Mn, visible symptoms of toxicity may not be similar in different species for a variety of reasons [4]. Lead and cadmium inhibited transpiration and photosynthesis [5] while stimulating synthesis of respiration and abscisic acid [6,7].

There is a growing consensus that manganese and other forms of environmental stress result in formation of free radicals (superoxide, superhydroxide, hydrogen peroxide, etc.), which can oxidize the fatty acids in membrane lipids, which leads to membrane leakage and eventual death [8,9]. Mechanisms for free-radical formation may differ in different compartments of the cell [10]. Plant lipoxigenases stimulate the synthesis of jasmonic acid, polyamines, glutathione, polychetalin, and ABA in response to environmental stress [11]. Included in the defense mechanisms are enzymes, such as superoxide dismutase, catalase,

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0040-6031/00/$ – see front matter
PII: S0040-6031(99)00507-9

peroxidase, ascorbic acid oxidase, and polyphenol oxidase [12,13].

The purpose of this study was to use respiration rate and metabolic heat rate to define the relationship between temperature and manganese toxicity. Metabolic heat rate (q) and respiration rate (R_{CO_2}) can be used to determine temperatures and Mn concentrations responsible for plant stress [14]. Growth rate and efficiency in response to environmental stress can be predicted [15].

2. Materials and methods

Radish (*Raphanus sativus* L.) seedlings have good germination rates and rapid growth in the laboratory. They were sprouted in distilled water with manganous chloride (0, 0.25, 0.5, 1.0, 5.0, 10.0, 50.0, 100.0, 500.0, 750.0, and 1000.0 ppm Mn). While these levels were within the range of Mn concentrations found near highways [2], sublethal concentrations were necessary for metabolic measurements. Seedlings were sprouted in a climate-controlled growth chamber with a 16 h/8 h light/dark cycle, 24°C/14°C temperature cycle and 70% humidity. When the seedlings were 5 days old, calorimetry was used to measure the metabolic heat rate (q) and respiratory rate of CO_2 (R_{CO_2}) at nine temperatures between 5 and 45°C at 5°C intervals. Metabolism was measured at several temperatures in order to determine the optimal temperature for Mn effects. Cotyledon tissue (80–100 mg fresh weight) from radish seedlings germinated in each Mn concentration was placed in three ampules. Measurements were made in a Hart Scientific model 7707 differential scanning calorimeter operated in the isothermal mode. Respiratory CO_2 rate was determined following the procedure used by Criddle et al. [14]. A small vial filled with 40 μl of 0.40 M NaOH is placed into the calorimeter ampule with the tissue. As the CO_2 and NaOH react in solution, heat is produced. The heat of reaction for carbonate formation (-108.5 kJ mol^{-1}) was used to convert the heat rate into the rate of CO_2 evolution by the plant.

In another experiment, radish plants were grown as before, but for 35 days. Plants were watered with Hoagland's solution with varying amounts of manganese added (0, 0.5, 5.0, 10.0, 50.0, 100.0, and 500.0 ppm Mn). These levels were in the range likely to be encountered in the environment [2], but low enough so that the plants do not die. At harvest, wet and dry weights, shoot length, and number of leaves were measured.

Physical signs of toxicity were also noted. Calorimetry was used to measure metabolic heat rate and respiratory CO_2 rate of the leaf tissue at temperatures from 5 to 45°C at 5°C intervals.

Concentrations of manganese in both seedlings and leaf tissue of radish plants were determined. Plants were dried in a vacuum oven at 70°C, ground to pass a 40-mesh screen, and 0.25 g samples were wet-ash digested with concentrated HNO_3 and 70% $HClO_4$ [16]. Supernatant Mn concentrations were determined by direct aspiration with a Perkin–Elmer Model 5000 atomic absorption spectrophotometer. Blanks and standards were run with the same procedures [2].

3. Results

The linear relationship between manganese concentration in solution and higher Mn concentrations in seedling tissue after 5 days can be seen in Table 1. Metabolic heat rate (q) (Table 2, Fig. 1) increased with temperature as expected, but showed severe stress when environmental Mn reached 100 ppm.

The response of respiration rate (R_{CO_2}) (Fig. 2) was very similar, but showed a stronger Mn vs. temperature interaction, including a peak of respiration just

Table 1
Manganese uptake for 5-day-old radish seedlings

Mn in sprouting solution (ppm)	Mn in tissue (ppm)
0	29.5
0.25	53.4
0.75	98.6
1.0	78.5
2.5	118.7
5.0	50.0
7.5	81.1
10.0	151.4
25.0	148.3
50.0	572.3
100.0	814.4
250.0	1748.6
500.0	2393.7
750.0	4460.5
1000.0	4971.9

Table 2
Metabolic heat rate (q) (±S.D.) for five-day-old radish seedlings (μW mg DW^{-1})[a]

Mn in sprouting solution (ppm)	Temperature (°C)								
	5	10	15	20	25	30	35	40	45
0	2.9±0.5	5.3±0.8	8.1±1.6	12.6±1.6	14.4±1.6	16.8±1.9	27.1±3.4	25.4±4.3	25.1±3.5
0.25	4.4±0.6	6.7±1.0	10.3±2.0	14.4±3.3	14.5±2.3	19.3±2.5	29.7±3.6	39.3±5.1	
0.5	3.7±0.4	5.6±0.9	10.5±0.3	16.4±1.0	22.5±3.1	35.9±2.9	25.0±2.1	34.6±1.0	
1.0	3.9±0.5	6.6±0.7	8.2±1.1	13.2±1.7	21.6±1.7	19.7±1.7	18.6±0.9	18.5±1.9	
5.0	3.3±0.3	6.5±0.5	11.7±1.0	21.1±1.7	28.9±3.1	27.1±3.5	29.8±1.4	30.1±2.6	25.9±1.8
10.0	3.4±0.7	6.1±1.5	9.5±2.2	16.6±3.9	27.3±1.2	26.5±1.6	22.5±4.3		
50.0	3.5±1.7	8.0±1.3	12.1±2.1	19.5±2.5	24.3±6.4	23.8±7.0	19.7±3.9	33.0±8.4	
100.0	3.1±0.9	6.0±1.0	10.3±0.6	13.3±0.8	19.8±2.6	19.3±3.0	18.5±1.8	16.3±2.2	25.9±1.6
500.0	3.5±0.6	3.5±0.9	5.5±0.9	8.5±1.2	12.9±1.4	12.1±1.5	11.0±2.0	18.5±3.0	20.5±1.6
750.0	2.9±2.6	4.5±1.0	7.2±1.1	16.0±4.2	17.1±4.2	17.2±2.6	22.1±1.5	15.2±2.5	12.6±5.5
1000.0	3.3±1.2	5.6±0.7	8.9±1.7	12.0±1.8	14.5±1.7	16.6±1.3	25.7±2.8	25.0±1.4	24.4±2.2
2500.0	2.3±0.9	3.6±0.2	5.7±0.9	10.6±0.9	13.1±0.9	14.8±1.5	19.4±3.9	20.3±3.2	21.0±1.8
5000.0	2.1±1.6	3.8±0.7	6.2±0.7	9.6±0.9	13.9±1.8	13.4±1.3	19.0±2.4	20.1±4.2	20.2±4.0

[a] Measurements were replicated three times. Three seedlings were used for each measurement. The average S.D. is ±2.0 μW mg DW^{-1}. Fig. 1 was plotted from this data.

before exhibiting severe stress. Changes in the graph surface at high Mn concentration followed the onset of Mn-related stress and, may be, oxidative events associated with membrane leakage [10,12,13].

The specific growth rate ($R_{SG}\Delta H_B$) can be calculated from measurements of q and R_{CO_2}. Fig. 3 predicts an increase in growth with increasing temperature, but only at low Mn concentrations. At higher external Mn concentrations, radish seedlings are stressed.

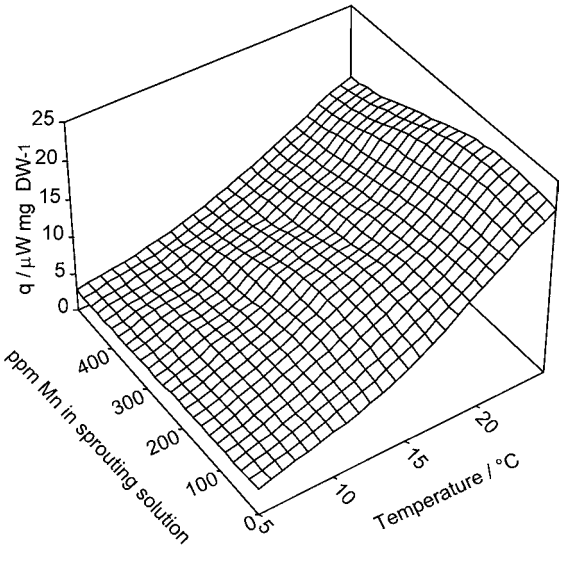

Fig. 1. Surface plot of the specific metabolic heat rate (q) for five-day-old radish seedlings is presented for a range of temperatures (5–25°C) and manganese concentrations (0–500 ppm). Each measurement was replicated three times and three seedlings were used for each measurement. The average S.D. ±2.0 μW mg DW^{-1}.

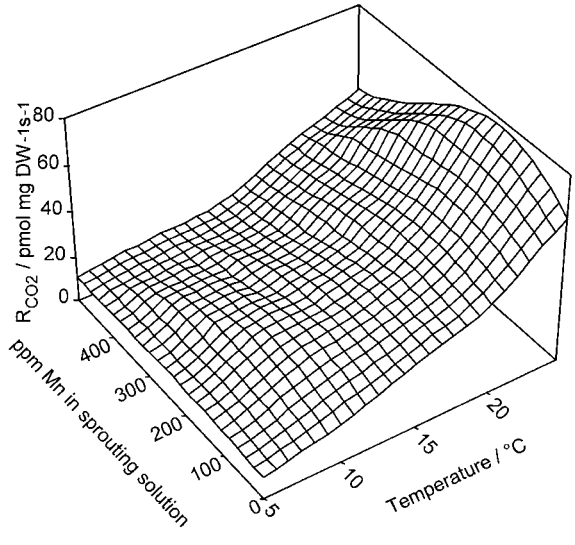

Fig. 2. Surface plot of the carbon dioxide evolution rate (R_{CO_2}) for five-day-old radish seedlings over a range of temperatures and manganese concentrations, as in Fig. 1. Average S.D. is ±22 pmol s^{-1} mg DW^{-1}.

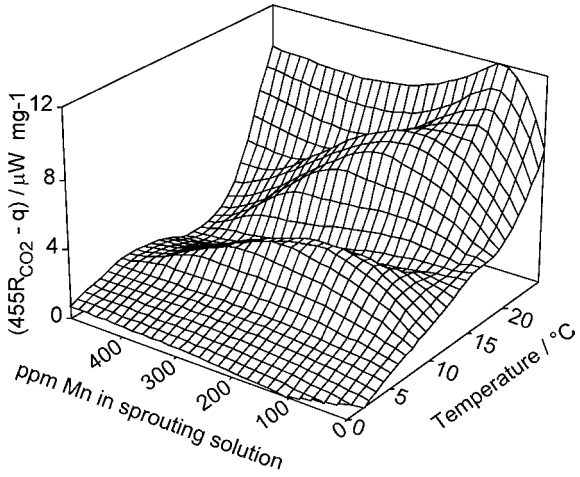

Fig. 3. Surface plot of the relative specific growth rate ($R_{SG}\Delta H_B$)=($455R_{CO_2}-q$) for five-day-old radish seedlings. Calculated from data in Figs. 1 and 2.

The ratio of q to R_{CO_2} gives a measure of substrate carbon conversion efficiency (ε). As the ratio of q/R_{CO_2} decreases from 455, the plant becomes more efficient. When the ratio is >455, the substrate is more reduced than carbohydrate. Fig. 4 shows a decrease in efficiency at warmer temperatures and higher external manganese concentrations. Radish appears to do best at temperatures below 20°C.

Table 3
Manganese uptake for 35-day-old radish plants

Mn in watering solution (ppm)	Mn in leaf tissue[a] (ppm)
0	60±9
0.5	44±3
5.0	128±43
10.0	96±3
50.0	206±84
100.0	305±144
500.0	4776±1609

[a] Values are Mn±S.D. (n=3).

Radish plants were grown for 35 days, while being watered with solutions containing concentrations of manganous chloride, ranging from 0 to 500 ppm. Table 3 lists the treatments and the final concentrations of Mn in the plant tissue. Time of exposure to Mn may be important. There were no significant differences in wet and dry weights, shoot length, and number of leaves among the different manganese treatments. Only those plants treated with 500 ppm Mn developed small necrotic lesions on older leaves, but showed no signs of chlorosis or leaf crinkling [4].

At temperatures below 20°C, radish plants watered with concentrations of Mn <10 ppm had little effect on metabolic heat (Fig. 5) and respiration (Fig. 6). The

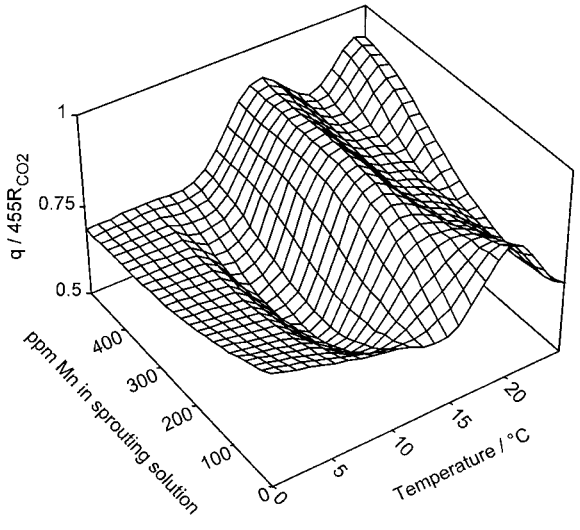

Fig. 4. Surface plot of $q/R_{CO_2}=455-[\varepsilon/(1-\varepsilon)]\Delta H_B$, where ε is the carbon conversion efficiency, for five-day-old radish seedlings. Calculated from data in Figs. 1 and 2. Efficiency increases when q/R_{CO_2} decreases.

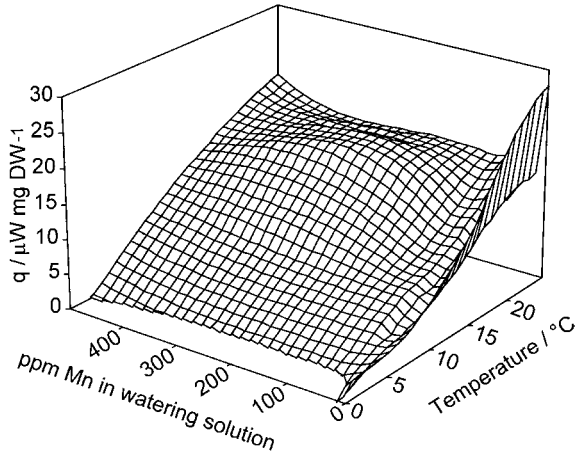

Fig. 5. Surface plot of the specific metabolic heat rate (q) for thirty-five-day old radish plants over a range of temperatures (5–25°C) and manganese concentrations (0–500 ppm).

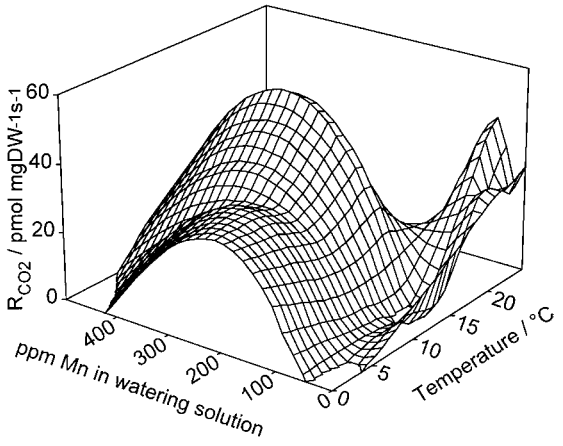

Fig. 6. Surface plot of the carbon dioxide evolution rate (R_{CO_2}) for thirty-five-day old radish plants as in Fig. 5.

plants were stressed at temperatures above 20°C and at ambient Mn concentrations much above 10 ppm. Predicted growth (Fig. 7) and efficiency (Fig. 8) are negatively affected by temperatures >20°C and ambient Mn concentrations much above 10 ppm.

4. Discussion

A complex relationship exists between temperature, tissue manganese concentration and respiration. Tissue content of manganese depends on both concentration of the metal and time of exposure (Tables 1 and 3).

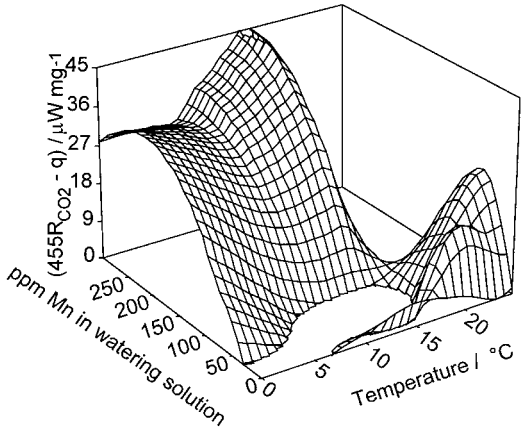

Fig. 7. Surface plot of the relative specific growth rate ($R_{SG}\Delta H_B$)=($455R_{CO_2}-q$) for thirty-five-day old radish plants. Calculated from data in Figs. 5 and 6.

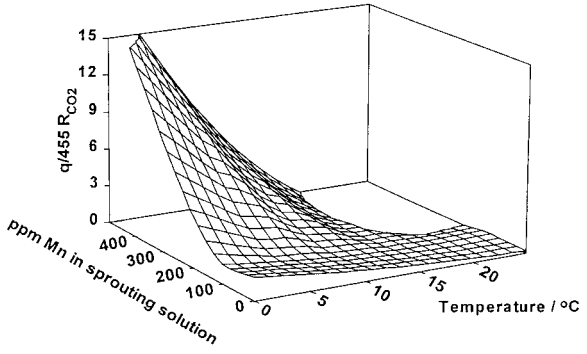

Fig. 8. Surface plot of $q/R_{CO_2}=455-[\varepsilon/(1-\varepsilon)]\Delta H_B$ for thirty-five-day old radish seedlings. Efficiency (ε) increases when q/R_{CO_2} decreases.

After 70 years of research on the effects of air pollutants on plants, prelethal and reversible indexes of damage include the increase of metabolism known as wound respiration, which probably indicates synthesis of plant defense mechanisms [17]. Manganese and other pollutants do not target a key enzyme or metabolite. The first indication of damage is destruction of membranes by free-radical peroxidation of fatty acids in the diacylglycerides which rapidly leads to death of cells [10,12,13].

The effect of manganese on radish metabolism, respiration, efficiency of substrate utilization, and growth is temperature-dependent and, at least somewhat, time-dependent. Some of the effects, seen at temperatures much above 20°C and ambient Mn concentrations from 10 to 100 ppm, may be due to oxidation of metabolites leaking from damaged membranes.

Decrease in dry-matter production has been reported at leaf Mn levels of 160 to 7000 ppm [4,18,19]. Our results, while complex, indicate possible Mn effects at even lower concentrations. Since Mn is mobile only in the 2+ valence, total leaf concentration of Mn may not be a true indicator of activity. Our results indicate that plant stress induced by Mn may profitably be examined using microcalorimetry.

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