Enhancement of the Infectivity, Nucleoprotein Concentration, and Multiplication Rate of Cowpea Chlorotic Mottle Virus in Manganese-Treated Cowpea

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ABSTRACT

The infectivity of cowpea chlorotic mottle virus was markedly enhanced in systemically infected cowpea leaves treated with toxic concentrations of manganese. The infectivity of sap from plants whose stems were maintained in 0.005 M $MnSO_4$ for 14 days beginning immediately after inoculation increased 2-fold. When treatment began 30 days after inoculation by maintenance of excised stems in 0.01 M $MnSO_4$, the sap infectivity increased 7-fold, the specific infectivity increased 8-fold, the virus nucleoprotein concentration increased 10-70%, and the rate of ^{32}P incorporation into virus particles increased 5- to 7-fold after 14 days of treatment.

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Additional key words: manganese toxicity, specific infectivity.

Cowpea chlorotic mottle virus (CCMV) is unstable in vivo, with the processes of multiplication, inactivation, and particle breakdown occurring simultaneously, although at different rates (1, 6). Little is known about the mechanisms of plant virus multiplication, and almost nothing is known about the mechanisms of inactivation and particle breakdown in vivo. Various chemicals were screened in an attempt to alter these processes with the hope that manipulation would aid in the explanation of their mechanisms. One of the chemicals tried, manganese, applied at toxic concentrations to CCMV-infected cowpea, *Vigna sinensis* (Torner) Savi, enhanced the infectivity of the virus when treatment began at any time during the infection.

This paper describes the effect of manganese on the CCMV infection during two distinct phases: an early infection, beginning immediately after inoculation, and an established infection, beginning 30 days after inoculation. The early infection exhibits rapid virus synthesis with high levels of infectivity and specific infectivity. The established infection has a low level of synthesis with low, stable levels of infectivity and specific infectivity (less than 5% of an early infection) (6).

MATERIALS AND METHODS.–CCMV was cultured in Early Ramshorn cowpea. The techniques for inoculation, purification, and infectivity assays were described previously (2). When treatment began immediately after inoculation, manganese was supplied to inoculated primary leaves by putting 10-day-old cowpea plants, excised at ground level, into Johnson's nutrient solution (5) plus 0.005 M manganese sulfate ($MnSO_4$). Excised control plants were grown in nutrient solution. When treating a 30-day-old infection, the excised stems with all leaves removed except the first two trifoliolate leaves were put into nutrient solution with or without 0.01 M $MnSO_4$. In preliminary experiments and most of the experiments reported here, additional controls consisting of excised plants grown in distilled water, and nonexcised plants grown in pots of soil were included. However, there was little variation among the different controls.

In the radioisotope experiments, infected plants were transferred 14 days before treatment initiation from the greenhouse to a controlled environment chamber at 27 C with a 16-hr photoperiod and an illumination of 850 ft-c. The techniques for application of the radioisotope (carrier-free ^{32}P-orthophosphate), virus isolation, isotope counting, and calculation of ^{32}P incorporation into virus particles were described earlier (2).

RESULTS.–After 7 and 14 days of treatment, the leaves with the manganese-treated early infection exhibited greater infectivity in expressed sap than did control leaves. Representative experiments are shown in Table 1. Extrapolation from the dilution curve of CCMV (2) indicates that the increase in infectivity was 1.5-fold at 7 days and 2-fold after 14 days of treatment (average of five experiments). The infectivity of leaf extracts of an established infection of CCMV treated with manganese was increased more, relative to controls, than that of an early infection (Table 1). The infectivity of sap from the treated established infection was 3 times that of control leaves after 7 days of treatment and 7 times that of control leaves after 14 days (average of seven experiments). In both the early and the established infections, there was a delay of 4-5 days before an increase in infectivity resulting from the manganese treatment was observed.

Although manganese induced large increases in the sap infectivity of established infections of CCMV, this infectivity level was only one-fifth the amount of infectivity of an 8-day-old nontreated infection. The relative infectivity increase was greater in the established infection, but the absolute increase in infectivity was greater in the early infection (Fig. 1).

At equal molarities, manganese chloride was as effective as manganese sulfate, and magnesium sulfate treatment had no effect on the infectivity of CCMV

in cowpea, indicating that the manganese ion was responsible for the increase in infectivity.

The concentrations of manganese used in these experiments were toxic to the cowpea plants. After 3-4 days of treatment, small necrotic flecks developed, and after 3-4 weeks, the plants usually died. Lower concentrations of manganese caused smaller and sporatic increases in infectivity. Spraying a solution of manganese (0.05 M $MnSO_4$) upon the leaves also caused inconsistent increases in infectivity. There appeared to be no correlation between the amount of damage to the host and the amount of increase in infectivity of CCMV. Occasionally, large increases occurred in leaves with little visible damage caused by manganese. However, in order to produce consistent increases in the infectivity, toxic concentrations of manganese were required.

To determine the effect of manganese on the virus, the amount of nucleoprotein and the specific infectivity (infectivity per unit virus) of CCMV from treated and nontreated leaves were compared. There was little measurable effect of manganese treatment on the specific infectivity or the virus nucleoprotein concentration in an infection where treatment began immediately after inoculation. However, in the established infection, as the sap infectivity and specific infectivity of CCMV were still decreasing in control plants, both increased strikingly in treated leaves. Figure 2 shows a representative experiment. Averaging five experiments, the specific infectivity of the virus from manganese-treated leaves after 14 days of treatment was 8 times greater than that from nontreated leaves. The virus nucleoprotein per plant increased 10-70% in treated plants, whereas that in control plants remained stable. There was a delay of about 5-7 days between treatment initiation and an observed increase in specific infectivity or virus nucleoprotein.

Since CCMV particles are simultaneously being synthesized, inactivated, and broken down, there are several possible explanations for the large increases in the infectivity and specific infectivity of CCMV with a relatively small increase in virus nucleoprotein which results from the manganese treatment of the established infection. A stimulation of the multiplication rate adding new, highly infectious virus, an inhibition of the inactivation process, or a reversal of the inactivation process (activation of uninfectious virus) could all produce this effect. To determine which processes were affected by manganese, the multiplication rates of CCMV in treated and nontreated plants were determined by measuring the rate of incorporation of ^{32}P into virus particles. Although the amount taken up by the treated leaves decreased with the length of treatment (Fig. 3-A), the double dilutions of ^{32}P given the groups of plants insured that the total amounts taken into treated and nontreated leaves overlapped. After 10 days of treatment, the manganese treatment induced a 5- to 7-fold increase in the synthesis rate of CCMV (Fig. 3-B). The increased rate of synthesis induced by the manganese treatment is approximately one-fifth the maximum multiplication rate in nontreated plants which occurs

TABLE 1. The effect of manganese on the infectivity of CCMV in expressed sap

Experiment No.	Excised plants grown in:	Infectivity (lesions/half-leaf) [a] 7 days	Infectivity (lesions/half-leaf) [a] 14 days
	Early infection[b]		
1	NS[c] + 0.005 M $MnSO_4$	173	85
	NS	150	40
2	NS + 0.005 M $MnSO_4$	150	148
	NS	78	40
	Established infection[d]		
3	NS + 0.01 M $MnSO_4$	279	331
	NS	163	109
4	NS + 0.01 M $MnSO_4$	33	233
	NS	9	9

[a]Independent assays were made for each test and at each harvest date of 7 and 14 days.

[b]Treatment began immediately after inoculation, and infectivity of sap was assayed after 7 and 14 days of treatment.

[c]NS = nutrient solution.

[d]Treatment began 30 days after inoculation, and infectivity of sap was assayed after 7 and 14 days of treatment.

3-5 days after inoculation of primary leaves of 10-day-old cowpea plants (1). The delay which occurred before the manganese treatment induced an increase in the multiplication rate was similar to the delay observed before the infectivity and specific infectivity increased.

DISCUSSION.—Little work has been done on the effect of manganese upon plant viruses in vivo. Welkie

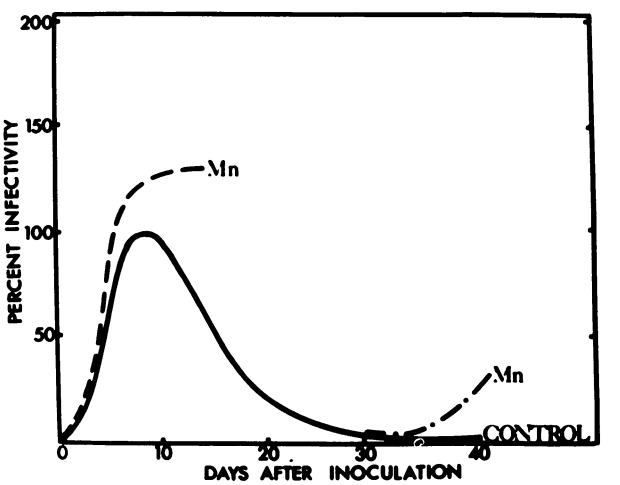

Fig. 1. A summary of several experiments showing the infectivity of cowpea chlorotic mottle virus (CCMV) in manganese-treated leaves in relation to the infectivity of CCMV in nontreated leaves when treatment began at the time of inoculation or 30 days after inoculation. The maximum level of infectivity in nontreated leaves was set equal to 100%.

& Pound (7) reported that manganese-deficient tobacco plants produced more tobacco mosaic virus (TMV) with less symptom expression that did control plants. However, a 10-fold excess (1×10^{-4} M) over the optimum level of manganese had no effect.

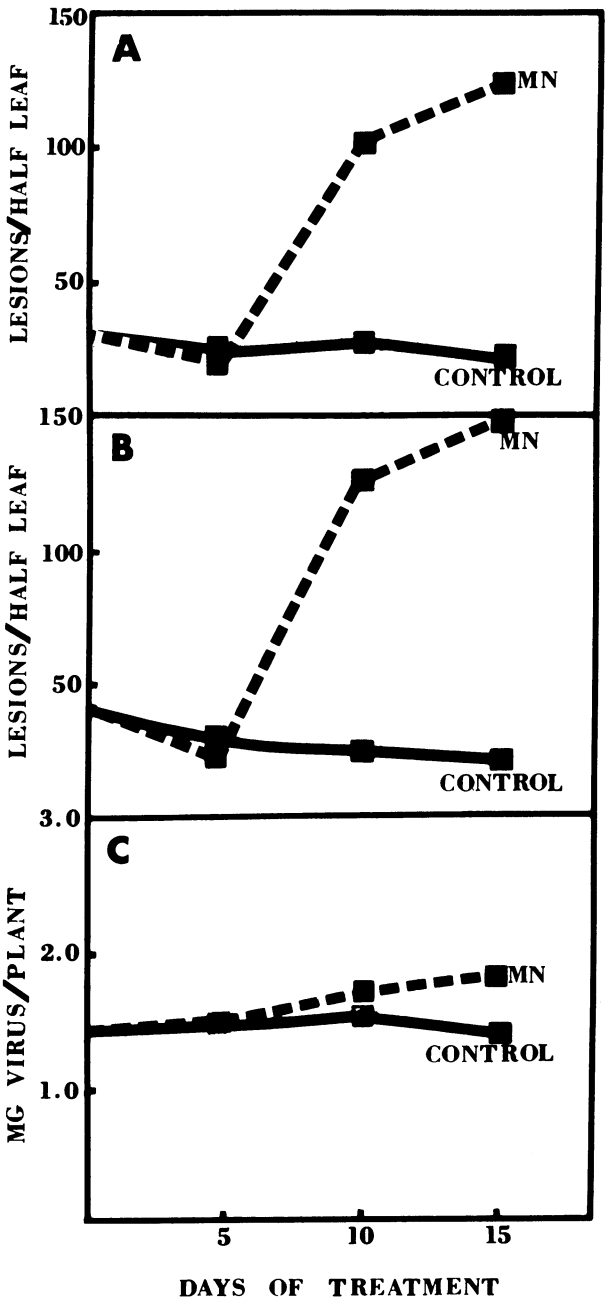

Fig. 2. The effect of manganese on the infectivity of expressed sap (A), specific infectivity (purified virus assayed at 1 μg/ml) (B), and virus nucleoprotein (C) of an established infection of cowpea chlorotic mottle virus. Excised plants with all leaves removed except the first two trifoliolate leaves were grown in nutrient solution with or without 0.01 M $MnSO_4$.

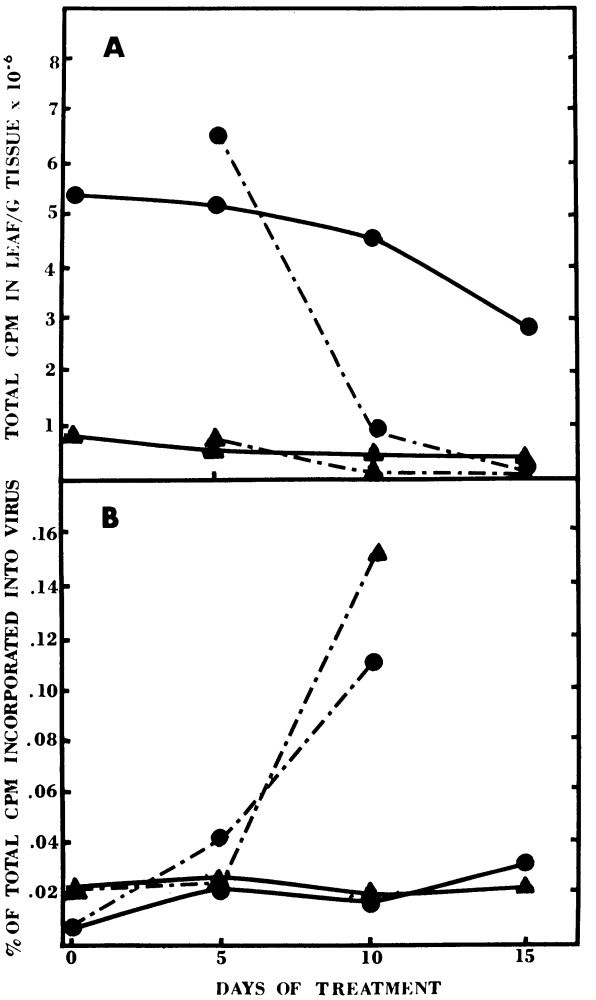

Fig. 3. The effect of manganese on the multiplication rate of cowpea chlorotic mottle virus in cowpea when treatment began 30 days after inoculation by growing excised plants in 0.01 M $MnSO_4$ in nutrient solution. Manganese-treated leaves (dashed lines) were labeled with 0.40 mc/ml (circles) and 0.04 mc/ml (triangles) ^{32}P. Nontreated leaves (solid lines) were labeled with 0.20 mc/ml (circles) and 0.02 mc/ml (triangles) ^{32}P. A) The total cpm taken up into the leaves. B) Per cent of total cpm taken up into the leaves incorporated into virus particles.

Manganese (0.001 M $MnCl_2$) has also been shown to increase by 3-fold the susceptibility (number of local lesions formed) of the local lesion host bean to TMV (3).

There are few clues as to the effect of manganese on the CCMV infection. Manganese is the cofactor for several host enzymes, and, in in vitro enzyme studies, manganese is able to replace magnesium as the cofactor of many enzymes, usually with reduced activity. Haruna & Spiegelman (4) reported that when manganese replaces the normal cofactor magnesium of the RNA replicase of the bacteriophage QB, the enzyme in vitro loses its specificity for homologous template RNA and produces defective viral RNA. If

manganese could be added to plants at concentrations high enough to replace magnesium as the cofactor of the viral RNA replicase, the manganese should inhibit virus synthesis. However, toxic concentrations of manganese increased CCMV synthesis instead of decreasing it.

The effect of manganese upon the infectivity of CCMV in vivo is similar to the effect of 2-thiouracil upon CCMV, which was reported earlier (2). Both manganese and thiouracil (i) enhanced the sap infectivity of CCMV when treatment was begun at any time during the infection; (ii) were toxic to the host plant; (iii) required a lag of 4-5 days after initiation of treatment before enhancement occurred; and (iv) markedly increased the specific infectivity and synthesis of CCMV in an established infection. The major difference between the two treatments is that manganese induced an increase in the amount of virus nucleoprotein, whereas thiouracil caused a decrease.

Treatment of an established infection of CCMV with manganese induced a 5- to 7-fold increase in the multiplication rate, which normally is very low in an established infection (1). Although the manganese-induced multiplication rate is only about one-fifth the maximum multiplication rate which occurs in inoculated primary leaves of 10-day-old plants 3-5 days after inoculation, it may be similar to the maximum multiplication rate which occurs after infection of leaves which are more than 30 days old, where multiplication is slower than in younger leaves (W. O. Dawson, *unpublished data*). Since the infectivity and specific infectivity levels of the established infection of CCMV were very low, the addition of a small amount of new, highly infectious virus infectivity in treated plants while only slightly increasing the total amount of virus nucleoprotein.

The toxic manganese treatment of senescent leaves which have a low rate of anabolism produced an unexpected increase in virus synthesis. Also, large increases in total host RNA and large and nonspecific increases in host protein were observed in CCMV-infected and noninfected cowpea plants treated with toxic levels of manganese (W. O. Dawson, *unpublished data*). Toxic levels of manganese may drastically alter the regulation processes in cowpea leaves.

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