

Manganese Effect on the Local Lesion Symptom of Potato Spindle Tuber 'Virus' in *Scopolia sinensis*

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ABSTRACT

The influence of manganese on the development of local lesions in *Scopolia sinensis* was followed in plants inoculated with the potato spindle tuber 'virus' and maintained in sand culture at levels of Mn which varied from 0 to 18 $\mu\text{g}/\text{ml}$. Optimum expression of symptoms occurred on leaves which contained 1,900 μg Mn/g leaf tissue at a Mn/Fe ratio of 27. Over the shorter periods of lesion development, the effect of Mn at this concn in the leaf tissue was to produce a greater

number of lesions in the upper leaves of *S. sinensis* than in the lower ones. Magnesium was without effect on the enhancement of symptom expression by Mn in this local-lesion indicator plant. These and other considerations suggest that higher-than-normal Mn levels are required for the replication of potato spindle tuber RNA in the tissues of its various hosts.

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Additional key words: host nutrition, sand culture, RNA polymerase, replication.

It has already been demonstrated that manganese has a pronounced effect on the enhancement of the diagnostic symptoms of potato spindle tuber 'virus' (PSTV) in the systemic indicator, tomato (6). For example, it was shown

in these studies (6) that in order to obtain extensive veinal necrosis in PSTV-infected plants, the tomato leaf tissue must contain 1,500 $\mu\text{g}/\text{g}$ or more of Mn and at the same time possess Mn/Fe ratios of 12 or more. Apart from its

TABLE 1. Influence of Mn level on tissue Mn content and Mn/Fe ratio, number of local lesions on *Scopolia sinensis* and percent of half leaves exhibiting local lesions after infection with potato spindle tuber 'virus'

Mn Level ($\mu\text{g/ml}$)	Tissue content ^a ($\mu\text{g/g}$) Mn Mn/Fe		Infectivity (lesions/half-leaf) ^b				Half-leaves exhibiting lesions (%)			
			12 days		19 days		12 days		19 days	
			Leaf position		Leaf position		Leaf position		Leaf position	
			First	Second	First	Second	First	Second	First	Second
0	63	1.0	17 fg	6 g	40 kl	29 l	100	67	100	100
3	1,076	15.2	38 defg	41 defg	85 jkl	169 hij	93	100	100	100
6	1,882	27.0	143 c	40 defg	172 hi	194 h	100	100	100	100
9	2,518	32.0	85 de	39 defg	160 hij	117 hijk	100	93	100	100
12	3,118	44.6	87 d	54 defg	102 ijkl	100 ijkl	100	67	100	67
15	3,216	45.1	70 def	10 g	94 ijkl	38 kl	100	67	100	100
18	5,278	70.9	32 efg	9 g	89 ijkl	67 kl	100	57	100	100

^aEach value is a composite leaf sample from all plants at each Mn level.

^bAt 12 days after inoculation, each value is a mean of 14 half-leaves; at 19 days after inoculation, each value is a mean of 6 half-leaves. Values followed by the same letters are not significantly different ($P=0.05$) according to Duncan's new multiple range test.

value in facilitating the rapid and reliable detection of PSTV, this enhancement of symptom expression by Mn raises the question of whether the primary effect is on host susceptibility, or on the multiplication of PSTV itself. As part of an attempt to answer this and other questions, the effect of Mn on symptom expression of PSTV was examined in the local lesion host plant, *Scopolia sinensis* Hemsl. (9). The present paper describes the results of this attempt.

MATERIALS AND METHODS.—Plants of *Scopolia sinensis* were grown from seed which was produced locally (3) and stored at 4 C for several months. Seeds were germinated on moist filter paper at room temp and then transferred to sand beds where they were kept moist with tap water. After the seedlings had reached about 5 cm in height, they were transferred to individual pots and maintained according to procedures already described (6).

In experiments 1 and 2, a nutrient solution (5) containing no Mn was applied to each seedling for 1 wk. Seedlings were then selected and grouped according to size, and the individual groups received nutrient treatments which contained levels of Mn ranging from 0 to 18 $\mu\text{g/ml}$. Treatments were applied in a completely randomized design with six replicates in experiment 1 and eight replicates in experiment 2.

In a third experiment, a nutrient solution containing neither Mn nor Mg was applied to each seedling for 1 wk. Seedlings were again selected according to size and each group received a nutrient solution containing differing levels of Mn and Mg in combinations which maintained a constant Mg/Mn ratio of 5.0. Individual levels of Mg and Mn ranged from 2.5 - 100 $\mu\text{g/ml}$ and 0.5-20 $\mu\text{g/ml}$, respectively. The statistical design of this experiment was identical to that described above, but with 12 replicates per treatment.

After 3 wk of treatment with Mn or Mg/Mn, when seedlings were 12 to 15 cm high with four to five leaves, the first and second uppermost fully expanded leaves on each plant were inoculated. Inoculation consisted of rubbing each half-leaf with 0.05 ml of a suspension containing 50 mg/ml of Celite and 50 $\mu\text{g/ml}$

(Experiments 1 and 2) or 100 $\mu\text{g/ml}$ (Experiment 3) of a partially purified preparation of infectious, low-molecular-weight RNA (11). This RNA was prepared from tomato plants infected with a severe strain of potato spindle tuber 'virus'.

Environmental conditions in the greenhouse were maintained as described earlier (10) for all experiments except number three, where greenhouse temp were kept between 24 and 29 C.

Local lesions were counted on each half-leaf at 7, 12, and 19 days after inoculation. Following these counts, both inoculated leaves on each plant were used for tissue analysis of both Mn and Fe, according to procedures previously described.

RESULTS.—*Experiments 1 and 2, Mn levels.* — Increasing the level of Mn added to the sand medium, increased the tissue content of Mn and Mn/Fe concn ratios (Table 1).

Tissue Mn content and Mn/Fe ratios influenced the number of local lesions observed in *S. sinensis* (Table 1). There was a definite interaction between the number of local lesions observed on the first or second uppermost leaf of a plant at each Mn level, and the number of days after inoculation. At 12 days after inoculation, a tissue Mn content of approximately 1,900 $\mu\text{g/g}$ and a Mn/Fe ratio of 27 gave the highest number of local lesions on the first uppermost mature leaf of each plant. In contrast, there were no differences in the number of local lesions observed on the second uppermost leaf over the range of Mn at this first count. However, at 19 days after inoculation (second count), both uppermost leaves showed similar counts at each Mn level.

At 12 days after inoculation, nearly all half-leaves of the first uppermost leaf showed lesions (Table 1). However, only those half-leaves on the second uppermost leaf containing 1,076 to 1,882 $\mu\text{g/g}$ Mn showed consistent lesion development on all half-leaves. At 19 days after inoculation, all half-leaves on the first uppermost leaf showed lesions. By this time, nearly all the half-leaves on the second uppermost leaf exhibited lesions.

The number of local lesions increased sharply up to a tissue Mn content of approximately 1,900 $\mu\text{g/g}$ and a

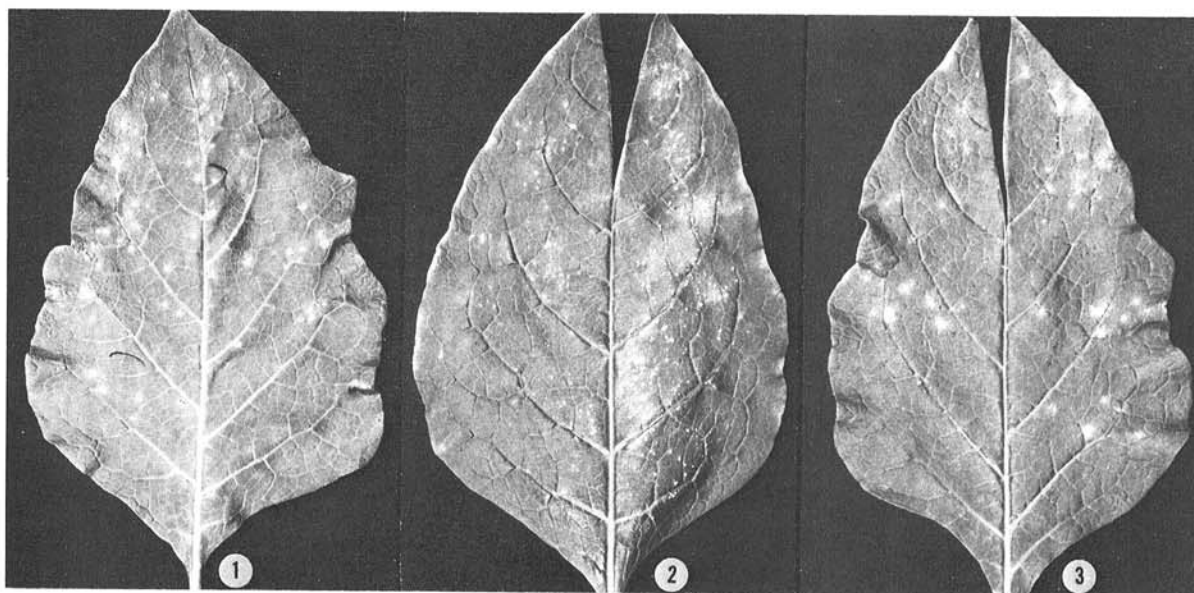


Fig. 1-3. Influence of Mn on local lesion number in *Scopolia sinensis* infected with potato spindle tuber 'virus'. 1) Leaf with Mn content of 63 µg/g, 2) leaf with a Mn content of 1,882 µg/g, and 3) leaf with Mn content of 5,278 µg/g.

Mn/Fe ratio of 27 (Fig. 1, 2, Table 1). Higher tissue Mn contents and Mn/Fe ratios resulted in a decrease in the number of local lesions observed (Fig. 3, Table 1).

Experiment 3, Mg and Mn levels.—The results of the effect of varying levels of Mn and Mg at fixed ratios of these ions are summarized in Table 2 and demonstrate a progressive enhancement of the number of lesions with increasing levels of Mn. At this ratio of Mg to Mn, the effect of Mn on symptom expression in *S. sinensis* appears to occur independently of the levels of Mg. The shortened incubation period (7 days) in this Table can be attributed to the relatively higher temp regime in which the plants were maintained during this experiment.

DISCUSSION.—Symptom expression of PSTV in *S. sinensis* is also enhanced by Mn treatment in much the same way as in the tomato plant. The tissue content of Mn and the Mn/Fe concn ratio that is required for the enhancement of local lesion symptoms in *S. sinensis* may be slightly higher than those in tomato plants. However, the extent to which Mn influences symptom development in both hosts is obvious.

The interaction between leaf position and the time period after inoculation for observing local lesion development implies that in the shorter periods of 7 and 12 days, the uppermost mature leaf of *S. sinensis* is the better choice for local lesion expression. A relatively larger percentage of half-leaves on the uppermost leaf will exhibit lesions at 12 days. In tests conducted over 19 days or longer, either of the two uppermost leaves show consistent lesion expression.

The increased number of local lesions in *S. sinensis* produced by treatment with Mn indicates that either PSTV requires higher than normal levels of Mn in the host tissues for its own multiplication or host susceptibility is somehow altered by Mn. The mechanism by which Mn affects either virus multiplication or host

TABLE 2. Effect of Mn and Mg levels on the number of local lesions on *Scopolia sinensis* after infection with potato spindle tuber 'virus'

Levels (µg/ml)		Infectivity (lesions/half-leaf)*			
		7 days		12 days	
Mg	Mn	Leaf position		Leaf position	
		First	Second	First	Second
2.5	0.5	4 e	0 e	9 g	0 g
25	5	15 de	1 e	29 g	2 g
50	10	38 c	4 e	94 f	28 g
100	20	27 cd	7 c	85 f	27 g

*Values are means of 12 half-leaves. Values followed by similar letters are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.

susceptibility remains to be clearly established. Nevertheless, it is known that both manganese and magnesium are essential co-factors in the activation of both DNA-dependent and RNA-dependent polymerases (7, 8, 12, 13). Manganese, for example, has been shown to stimulate the synthesis of fragmented Q β RNA or foreign RNA in the presence of the Q β replicase (4). Because of its low molecular weight (2, 11), the potato spindle tuber RNA lacks the information in its nucleotide sequence to code for a synthetase or replicase necessary for its replication. Consequently, it may depend on the action of host RNA polymerases to an extent greater than those viruses with a larger genome. Therefore, stimulation of host RNA polymerases by Mn could enhance the synthesis of potato spindle tuber 'virus' in much the same way as has been reported for Q β RNA.

As noted above, the differential and selective activation

of both DNA- and RNA-dependent polymerases by magnesium and manganese is well known (7, 8, 12, 13). However, attempts to observe a differential effect of these ions on the expression of symptoms in *S. sinensis* in the present work (Table 2) indicated that the presence of magnesium had relatively little effect.

Although it would be premature to conclude that Mn exerts its effect primarily on the activity of endogenous polymerases, the similarity of response in the two types of indicator hosts, tomato and *S. sinensis*, is suggestive. A recent report by Dawson (1) that Mn enhances the infectivity, nucleoprotein concn, and multiplication rate of Cowpea chlorotic mottle virus lends some support to the notion that PSTV may require higher-than-normal levels of Mn in the host tissues in order to multiply.

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