Enhancement of Diagnostic Symptoms of Potato Spindle Tuber Virus by Manganese

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ABSTRACT

Although the tomato cultivar Allerfrüheste-Freiland has been used successfully as an indicator host for the mild strain of the potato spindle tuber virus (MPSTV), at times some inconsistency in symptom expression has been encountered. Involvement of manganese nutrition was suspected. Tomato plants were grown in sand cultures containing various levels of Mn, Fe, or Zn. Increasing Mn in the growth medium from 0 to 9 μg/ml dramatically increased the characteristic veinal necrosis symptoms induced by MPSTV and the severe strain. Extensive virus-induced necrosis was obtained whenever Mn contents of plant tissue were 1,500 μg/g or above and Mn/Fe concentration ratios were 12 or more. In sand cultures containing 9 μg/ml of Mn, increasing Fe in the growth medium up to 10 μg/ml decreased tissue Mn/Fe ratios below 12, and resulted in less virus-induced necrosis. Zinc had no effect on virus-induced necrosis. Other tomato cultivars (Bonny Best, Michigan-Ohio, Rutgers, and Sheyenne), reported previously as symptomless carriers of MPSTV, developed virus-induced necrosis when grown in sand cultures receiving 9 μg/ml of Mn.

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Tomato plants (*Lycopersicon esculentum* Mill. 'Allerfrüheste-Freiland') were reported (5) as indicator hosts for the mild strain of potato spindle tuber virus (MPSTV). However, symptom expression has at times been inconsistent. Analysis of the greenhouse potting medium showed variations in pH and soluble Mn. As the pH of the potting medium increased above 5.5, soluble Mn decreased to very low levels. Since Mn has been shown to increase the number of local lesions on *Phaseolus vulgaris* by tobacco mosaic virus (1), the present study was conducted to determine the effect of Mn on the expression of diagnostic symptoms of PSTV in tomato plants.

MATERIALS AND METHODS.—Allerfrüheste-Freiland tomato seeds were germinated in a vermiculite-peat mix in the greenhouse. When cotyledons were fully developed, each seedling was washed thoroughly and transferred to a 13-cm clay pot containing sand. The drainage hole in each pot was covered with a layer of glass wool. Each pot was then lined with a polyethylene bag with a hole cut in the bottom for drainage, and filled with 1.8 kg of coarse silica sand (Ottawa Silica Company). After transfer, tomato seedlings were watered daily with a complete nutrient solution (3) at a pH of 4.6. Two days later, treatments with nutrient solutions containing various levels of either Mn, Fe, or Zn were started at random to groups of seedlings of uniform size. After 1 week of treatment, seedling leaves were rubbed with crude sap from tomato leaves infected with either a mild strain of PSTV (MPSTV) (2) or a severe strain (SPSTV). An 18-hr daylight period of at least 800-900 ft-c of light was provided. Temperatures were maintained between 28 and 31 C. Diagnostic symptoms of veinal necrosis appearing on each leaflet were estimated visually and recorded as the percentage of the total leaflet area. Three weeks

after inoculation, plant tops were harvested, dried, and weighed. Plant tops were wet-ashed with a mixture of concentrated nitric and perchloric acids. Plant digests were analyzed for Mn and Fe by atomic absorption spectrophotometry.

RESULTS AND DISCUSSION.—*Effect of Mn on plant growth and diagnostic symptoms of PSTV.*—Preliminary sand culture experiments indicated that 10 μg/ml of Mn added to the growth medium appeared to be a desirable Mn level for evaluating diagnostic veinal necrosis of SPSTV in tomato plants. A level of 20 μg/ml Mn resulted in severe Mn toxicity symptoms, which made it difficult to obtain an accurate evaluation of virus-induced necrosis. A 4 X 3 factorial experiment, with four Mn levels (0, 3, 6, and 9 μg/ml) as $MnCl_2 \cdot 4H_2O$ and plant inoculations with either MPSTV, SPSTV, or healthy tomato leaf extract, was arranged in a randomized complete block design with six blocks.

The growth of plant tops inoculated with either MPSTV or SPSTV were 0.93 and 0.92 g/plant, respectively. These top yields were stunted, as compared to 1.3 g/plant for plants rubbed with healthy leaf extract. The addition of 6 μg/ml or more of Mn decreased plant growth regardless of the inoculation (Table 1). The addition of 3 μg/ml of Mn to the growth medium increased the Mn content of plant tissue to 818 μg/g and the Mn/Fe ratio in plant tissues to 7.3 (Table 1). At this same Mn level, the percentage of symptomless leaflets was decreased and the percentage of necrotic leaflets was increased in

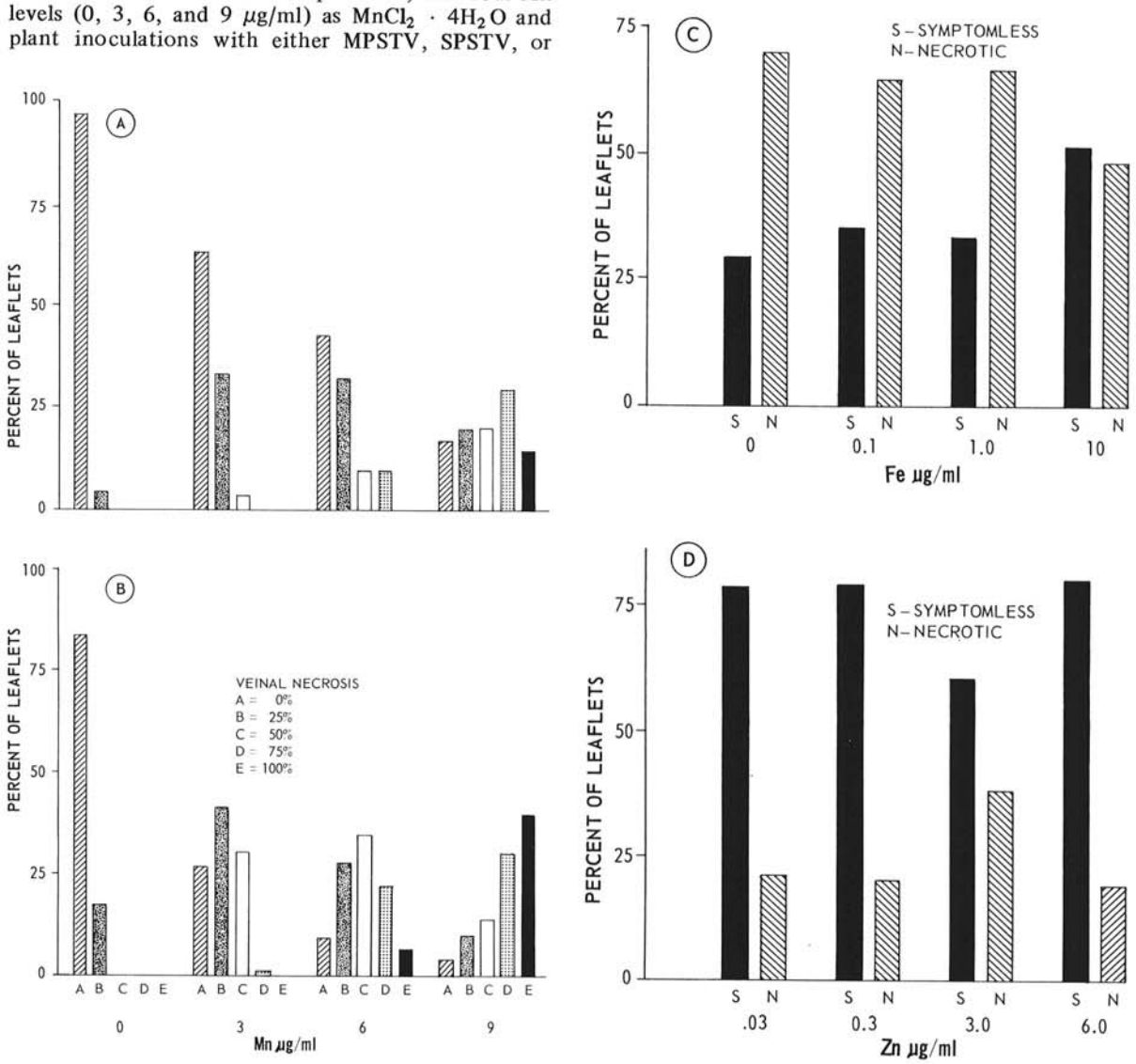

Fig. 1. A, B) Effect of manganese levels on per cent of leaflets exhibiting veinal necrosis due to potato spindle tuber virus. **A)** Mild strain; **B)** severe strain. Least significant difference at $P = .05$ was 8%. **C)** Effect of iron levels on per cent of leaflets exhibiting veinal necrosis of a severe strain of potato spindle tuber virus. Least significant difference at $P = .05$ was 9%. **D)** Effect of zinc levels on per cent of leaflets exhibiting veinal necrosis of a severe strain of potato spindle tuber virus. Least significant difference at $P = .05$ was not significant.

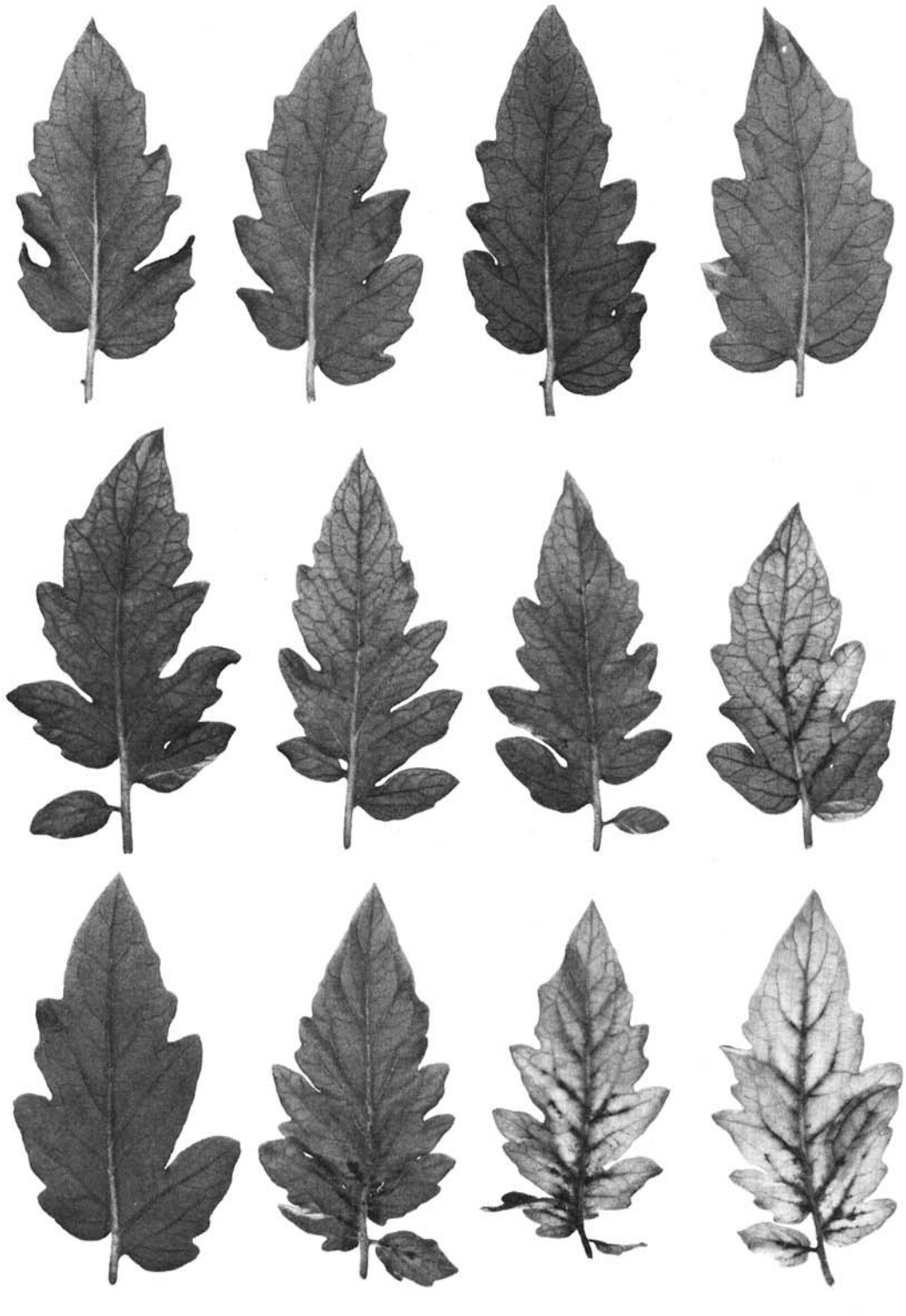

Fig. 2. Effect of manganese on symptom expression of tomato plants rubbed with 1) healthy tomato sap; 2) mild strain of potato spindle tuber virus; 3) severe strain of potato spindle tuber virus. Manganese levels (left to right) 0, 3, 6, and 9 μg/ml.

TABLE 1. Influence of Mn and Fe treatments on yield of tomato plant tops, plant tissue contents of Mn and Fe, and Mn/Fe ratios in tomato tissues[a,b]

Treatments (μg/ml)		Plant top yields	Plant tissue content (μg/g)		
Mn	Fe	(g)	Mn	Fe	Mn/Fe[b]
Mn Experiment					
0	0.1	1.20 a	159 a	142 a	1.1 a
3	0.1	1.09 ab	818 b	112 a	7.3 b
6	0.1	0.99 bc	1,595 c	135 a	11.8 c
9	0.1	0.92 c	2,313 d	139 a	16.6 d
Fe Experiment					
9	0	0.97 c	1,946 e	124 b	15.7 e
9	0.1	1.14 c	1,952 e	133 bc	14.7 f
9	1.0	1.37 c	1,840 e	139 c	13.2 g
9	10	1.16 c	1,819 e	315 d	4.8 h

[a]Values under each heading within each experiment followed by different letters are significantly different at $P = .05$.

[b]Each value is a mean of six replicates in the Mn experiment and eight replicates in the Fe experiment.

plants inoculated with MPSTV or SPSTV (Fig. 1-A, B, respectively). While Mn additions increased leaf veinal necrosis resulting from both strains, SPSTV showed a greater response to Mn than MPSTV (Fig. 1-A, B, 2). The addition of 9 μg/ml of Mn to the growth medium resulted in an 82% decrease in the number of symptomless leaflets for MPSTV (Fig. 1-A). Severe PSTV required only 6 μg/ml of Mn to give an 89% decrease in the number of symptomless leaflets (Fig. 1-B). There were more leaflets exhibiting 100% veinal necrosis at either 6 or 9 μg/ml of Mn treatment for SPSTV than for MPSTV. These results show that the addition of Mn to the growth medium, which induces higher tissue Mn contents and higher Mn/Fe ratios in host plant tissues, enhances the diagnostic symptoms of PSTV.

In another test, SPSTV-infected tomato plants receiving the 0 μg/ml Mn treatment failed to develop veinal necrosis in 5 weeks. However, when 9 μg/ml of Mn were added to the sand culture for only 3 days, virus-induced necrosis developed in all plants. These results indicate that the virus was present in the plants, but veinal necrosis was not observed until the Mn level in the growth medium was increased.

Mechanism of enhancement of PSTV symptoms in tomato plants by Mn.—High levels of Mn adversely affect plant metabolism of Fe (8). Therefore, the enhancement of diagnostic symptoms of PSTV by Mn may be due to two factors. One factor could be subnormal plant Fe metabolism giving rise to unhealthy plant tissues induced by high Mn levels in tissue. These unhealthy tissues might then be more susceptible to virus infection, as suggested by Dhaliwal & Rudd (1). The other factor contributing to the enhancement of PSTV symptoms by Mn could be the presence of high Mn levels in the host plant. Subsequently, additional experiments were conducted to test these hypotheses.

An experiment was designed to determine if subnormal plant Fe metabolism was, in fact, the reason for the enhancement of PSTV veinal necrosis in tomato by Mn. A 4 X 2 factorial experiment, with four Fe levels (0, 0.1, 1.0, and 10.0 μg/ml) and plant inoculations with either SPSTV or healthy tomato leaf extract, was arranged in a randomized complete block design with eight blocks. The source of Fe was Fe EDDHA [sodium ferric ethylene diamine (*O*-hydroxyphenylacetate), Geigy Chemical Corporation]. All nutrient solutions contained 9 μg/ml of Mn. The addition of Fe to the growth medium tended to increase the growth of plant tops regardless of inoculation (Table 1). This increase at 1.0 μg/ml Fe was significant at 10% level of significance. Although the addition of 1.0 μg/ml or more of Fe increased the Fe content of tissue and decreased the Mn/Fe ratio in plant tissue, symptom expression was not affected until 10 μg/ml of Fe had been added and the Mn/Fe ratio in plant tissues had been decreased below 13.2 (Table 1, Fig. 1-C). These results suggest that diagnostic symptoms of PSTV are increased with high Mn/Fe tissue ratios inducing subnormal Fe metabolism of the host plant. Elimination of subnormal Fe metabolism by reducing Mn/Fe concentration ratios in host plant tissues to below 13.2 with Fe additions can decrease virus-induced necrosis.

Another experiment was designed to create subnormal Fe metabolism in host plants by the addition of Zn to the growth medium. Higher than normal Zn levels in a growth medium induce subnormal plant Fe metabolism (4). A 4 X 2 factorial experiment, with four Zn levels (0.03, 0.3, 3.0, and 6.0 μg/ml) as $ZnSO_4 \cdot 7H_2O$ and plant inoculations with either SPSTV or healthy tomato leaf extract, was arranged in a randomized complete block design with eight blocks. Regardless of the inoculation, the addition of 3.0 or 6.0 μg/ml of Zn to the growth medium reduced the growth of plant tops to 1.24 and 0.70 g/plant, respectively, as compared to 1.63 g/plant for plants receiving a normal Zn level of 0.03 μg/ml. Plant tops exhibited typical Fe deficiency symptoms at both the 3.0 and 6.0 μg/ml Zn levels. However, Zn additions did not affect the expression of veinal necrosis of SPSTV (Fig. 1-D). These results indicate that subnormal plant Fe metabolism is not the major factor determining PSTV symptoms in tomato plants. The reason for the enhancement of PSTV symptoms by Mn is the presence of high tissue Mn contents of 1,500 μg/g or more and Mn/Fe concentration ratios of 12 or more in host tomato plants.

Effect of Mn on diagnostic symptom expression by both strains of PSTV in other tomato cultivars.—Since the foregoing experiments showed that symptoms of PSTV in Allerfrüheste-Freiland tomato were enhanced by the addition of 6 or 9 μg/ml of Mn to the sand cultures, an experiment was designed to determine if other tomato cultivars would be affected in the same way. A 5 X 3 factorial experiment, with five tomato cultivars (Bonny Best, Michigan-Ohio, Rutgers, Sheyenne, and

Allerfrüheste-Freiland) and plant inoculations with MPSTV, SPSTV, or healthy tomato leaf extract, was arranged in a randomized complete block design with five blocks. All nutrient solutions contained 9 μg/ml of Mn. The cultivars Bonny Best and Michigan-Ohio were reported resistant to PSTV when grown in soil (7), whereas cultivars Rutgers (2) and Sheyenne (6) were reported to be symptomless carriers of MPSTV. Generally, all cultivars exhibited diagnostic veinal necrosis when grown in sand cultures containing 9 μg/ml of Mn. At least three or four of the five plants inoculated with MPSTV or SPSTV, respectively, exhibited veinal necrosis within each variety. These results further confirm the previous experiments, and show that Mn enhances veinal necrosis caused by PSTV in tomato plants. Other reported symptomless hosts for PSTV may not remain symptomless when additional soluble Mn is supplied to the host plant's growth medium.

These experiments emphasize that more carefully controlled nutrition of host tomato plants will give more accurate and consistent assays for PSTV. Extensive diagnostic symptoms of PSTV require higher than normal Mn contents and Mn/Fe concentration ratios in host plant tissues. Sufficient soluble Mn must be available in the potting medium to give higher than normal Mn contents in tissues. Tomato tissues containing 1,500 μg/g or more of Mn and having Mn/Fe concentration ratios of 12 or more are required to consistently give extensive PSTV-induced necrosis.

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