Effect of Manganese and Dimethylsulfoxide on Development of Tobacco Mosaic Virus Local Lesions in Phaseolus vulgaris

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ABSTRACT

Attempts were made to determine the role of manganese, boron, and carbonate, and temp and light on the susceptibility of the bean leaves to tobacco mosaic virus (TMV) infection. Pinto and Scotia bean plants, supplied manganese and grown constantly at 21 C and 40 ft-c light intensity, were approximately 3 times more susceptible than the controls. Scotia under these conditions was more susceptible to TMV than Pinto. Dimethylsulfoxide (DMSO) at 0.05% concn with manganese treatment, 28 C and 200 ft-c light intensity at a day and night cycle of 14 hr light and 10 hr dark, caused approximately 2-fold increase in susceptibility as compared to other treatments. Pinto with DMSO and Scotia without DMSO treatment performed better than controls in hydroponic solutions with manganese. The results were significant at the 1% level. Phytopathology 60:1178-1182.

Bawden & Pirie (1), Kassanis (5), and Yarwood (10) have reviewed the environmental influences on infection by viruses. Samuel et al. (8) observed that the susceptibility of test plants increased after several days of cloudy weather. Mathews (7) reported that the time of inoculation at which most local lesions were produced corresponded approximately to the maximum accumulation of sugars produced by photosynthesis. In contrast, Bawden & Roberts (2) reported that accumulation of photosynthates reduced local lesion production. Yarwood (11) found that lesion counts doubled or trebled in Pinto bean leaves heated for 30-45 sec at 50 C 5-10 hr after inoculation.

The local lesion assay plants, the environmental factors under which they are grown, and the type of nutrients available to the plants affect results with plant viruses. Chessin (3) obtained conflicting results when he assayed tobacco mosaic virus (TMV) purified from normal and from N-deficient plants on both bean and *Nicotiana glutinosa* as assay hosts. Helms & Pound (4) reported that of the three nutrient series used (different concentrations of N and phosphorus, and balanced solution), N produced the most marked effect on host growth, symptom expression, and virus concentration of potato virus X and tobacco ringspot virus.

Little work has been done in exploring the action of nutrients on the susceptibility of *Phaseolus vulgaris* L. to the development of TMV-induced local lesions. The present study was designed to establish the relationship of several inorganic ions and dimethylsulfoxide (DMSO) in nutrient solutions to the number of local lesions on test plants produced by inoculation with TMV.

MATERIALS AND METHODS.—*Phaseolus vulgaris* 'Pinto' and 'Scotia' were used as assay hosts. Plants were grown in 1-liter bottles with complete and different levels of mineral nutrients (Table 1); and with varied light intensities and temp conditions. Bean seeds were germinated in vermiculite at 28 C. When seedlings just emerged, the roots were washed in distilled water and the plants transferred to hydroponic solutions (with and without DMSO), pH 6.0. Air was continuously bubbled through the solutions. Primary leaves were harvested from 10-day-old plants, immersed in water at 45 C for 1 min, and the midribs removed. Then the half-leaves were distributed at random on 1% agar in 28 × 19 × 4-cm aluminum trays. Surface water was removed from the leaves with paper towels, then 350-mesh Carborundum was dusted evenly on the leaves.

Leaves were inoculated with TMV (common strain) purified by the method used by Venekamp & Mosch (9). Dilution was made with 0.1 M phosphate buffer pH 7.0, and the inoculum used on the test plants produced around 50 lesions/half-leaf on Scotia and around 25 lesions/half-leaf on Pinto. A drop of inoculum was put on a leaf (supported by 10 × 5 × 2-cm foam pad) with a 2-ml syringe with a 27 gauge needle. This inoculum was spread evenly on the leaf with the probe of an ultrasonic apparatus (Heat Systems Co., Melville, Long Island, N.Y.), passing the leaf only once under the probe exerting uniform pressure (6). Inoculated leaves were placed between two strips of paper towels, and transferred to an airtight 25 × 17 × 5-cm plastic container (not evacuated) which was then placed in an incubator at 30 C for 18 hr. At the end of postinoculation treatment, leaves were placed on 1% agar in trays and covered with glass plates for 3 days at near 25 C under constant light (40 ft-c). Lesions were counted with binocular microscope using an electronic counter (American Optical Co., Instrument Division, Buffalo, N.Y.). Each experiment was repeated at least 3 times. Statistical comparisons were made using analysis of variance technique.

RESULTS AND DISCUSSION.—*Effect of light, temp, and nutrients.*—The effects of N, phosphorus, potassium, magnesium, iron, manganese, boron, and carbonate were tested in preliminary experiments. Manganese, boron, and carbonate appeared to influence infection, and they were selected for study at the concn listed in Table 1.

In the first series of experiments, plants were grown at 21 C under constant light intensity (40 ft-c) for 5

TABLE 1. Nutrient solution used for the culture of *Phaseolus vulgaris* 'Pinto' and 'Scotia'. The figures indicate ml of each stock solution per liter of nutrient solution

Nutrient solution	$Ca(NO_3)_2 \cdot 4H_2O$ 1 M	KNO_3 1 M	$MgSO_4 \cdot 7H_2O$ 1 M	KH_2PO_4 1 M	Minor[a] elements	$MnCl_2 \cdot 4H_2O$ 1 M	H_3BO_4 0.5 M	$NaHCO_3$ 0.5 M	H_2O	pH
Complete	5	5	2	1	1	0	0	0	986	6
Complete + Mn	5	5	2	1	1	1	0	0	985	6
Complete + HCO_3	5	5	2	1	1	0	0	30	956	6
Complete + B	5	5	2	1	1	0	2	0	984	6

[a] Fe, 5·6 ppm; Cu, 0·064 ppm; Zn, 0·065 ppm; Mo, 0·019 ppm; Co, 0·006 ppm.

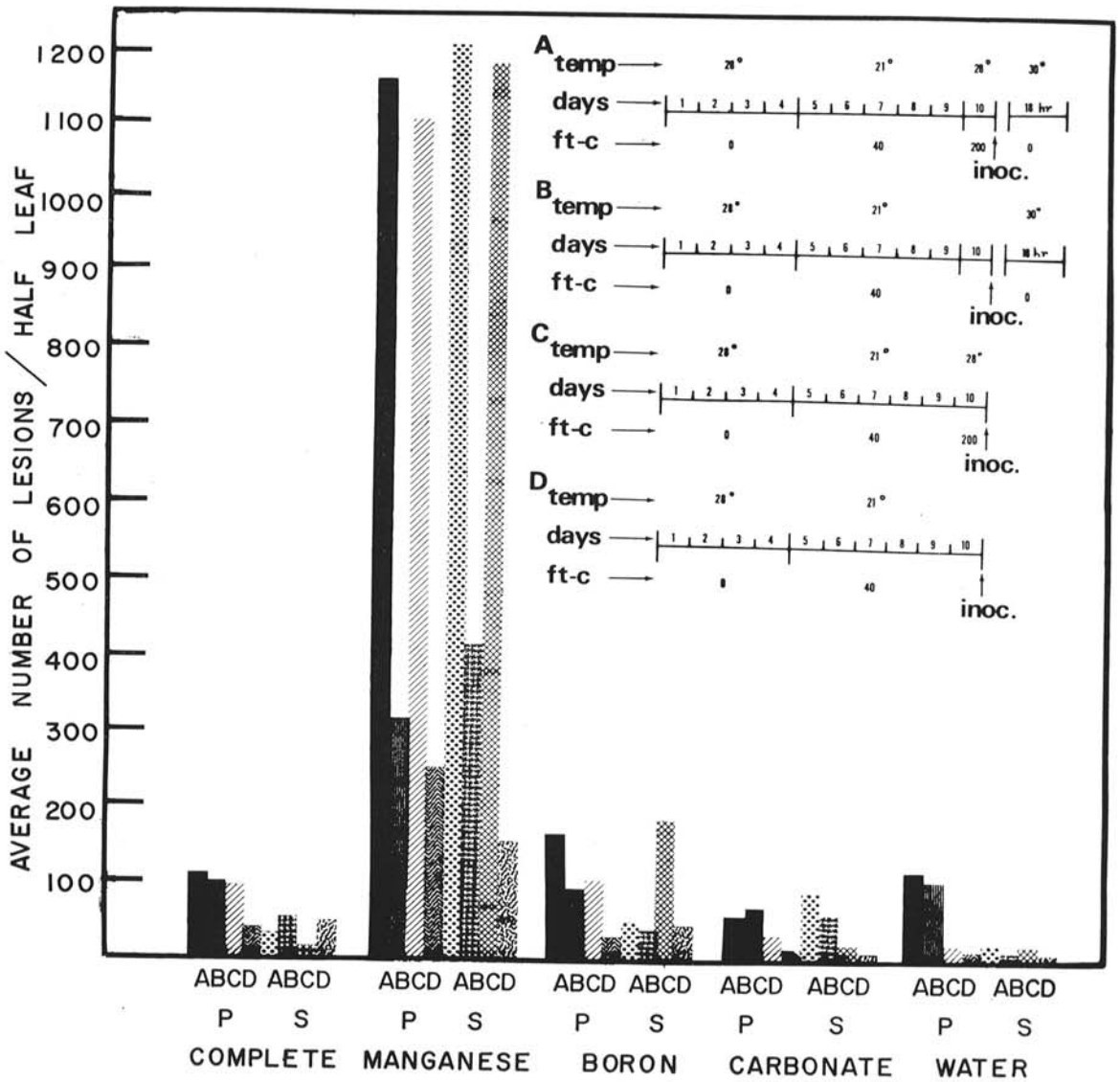

Fig. 1. Effect of manganese, boron, and carbonate on tobacco mosaic virus local lesion development in *Phaseolus vulgaris* 'Pinto' (P) and 'Scotia' (S). Treatments A-D represent various pre- and postinoculation temp and light regimes. **A)** Received 21 C and 40 ft-c light for 5 days, and 28 C and 200 ft-c for 1 day before inoculation and 18 hr of 30-C postinoculation treatment. **B)** Similar to (A) but did not receive 28 C and 200 ft-c for 1 day before inoculation. **C)** Similar to (A) but did not receive 30-C postinoculation treatment. **D)** Similar to (B) but did not receive 30-C postinoculation treatment. Statistical comparison on A, B, C, and D in Scotia and Pinto with different treatments shows a significant increase in local lesions with manganese treatment at the 1% level of significance. The comparisons on A, B, C, and D in both the varieties show a nonsignificant increase in local lesion with boron and carbonate over controls, except (C) in Scotia, which is significant. Other differences among the treatments not mentioned here are nonsignificant.

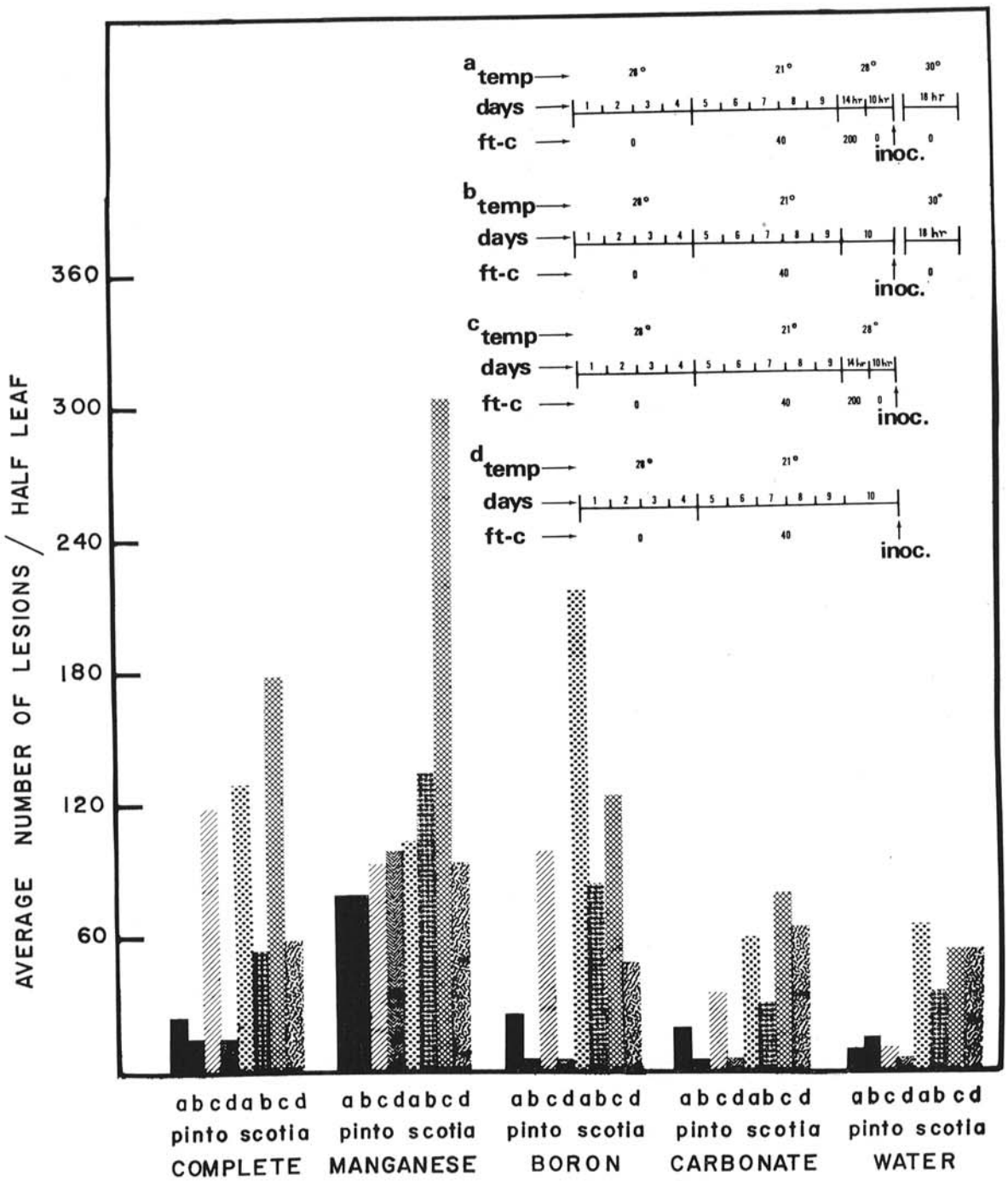

Fig. 2. Effect of manganese, boron, and carbonate, 21 C and 40 ft-c light, 28 C and 200 ft-c light, and 30 C postinoculation treatment for 18 hr on tobacco mosaic local lesion development in Pinto and Scotia beans. **a)** Received 21 C and 40 ft-c for 5 days and 28 C and 200 ft-c for 14 hr and 10 hr of complete darkness before inoculation and 30-C postinoculation treatment for 18 hr. **b)** Similar to (a), but did not receive 28 C and 200 ft-c for 14 hr and 10 hr of complete darkness before inoculation. **c)** Similar to (a) but did not receive 30-C postinoculation treatment. **d)** Same as (b) but did not receive 30 C postinoculation treatment. Increase in lesion counts in a, b, and c with manganese and boron in Scotia is significant at the 5% level over the counts with water as control. In Pinto, a and b give significant increases with manganese over all other treatments. But (c) in manganese gives significant increases over all other treatments including controls. Other differences among the treatments not mentioned here are nonsignificant.

days. At 6 days (10th day after seeding), half the plants were placed at 28 C with 200 ft-c light before inoculation. Treatment with manganese produced approximately 2 to 10 times more lesions than controls (complete nutrients and H_2O) (Fig. 1). The treatments with boron or carbonate failed to produce any significant results. Plants transferred to 28 C and 200 ft-c with 18-hr postinoculation treatment (Fig. 1-A) were similar in susceptibility to plants without postinoculation treatment (Fig. 1-C) in both Pinto and Scotia. Furthermore, 28 C and 200 ft-c treatment increased the number of local lesions 2- to 5-fold (Fig. 1-D). In general, Scotia (Fig. 1-S) was more susceptible to TMV infection than Pinto (Fig. 1-P). The increase of local lesions resulting from the manganese treatment over those of other treatments was significant at the 1% level.

In the next set of experiments, all conditions were kept similar to the varied conditions above except for the last day before inoculation. At 28 C, 14 hr of 200 ft-c and 10 hr of darkness were provided. Under these conditions the action of manganese is pronounced (Fig. 2). Scotia plants exposed to 28 C and 200 ft-c for 14 hr and 10 hr dark without 30 C postinoculation treatment for 18 hr (Fig. 2-C) produced twice as many lesions with manganese. Plants receiving boron and 28 C and 200 ft-c for 14 hr, and darkness for 10 hr, just before inoculation produced 2- to 6-fold more lesions than controls and other treatments grown under similar conditions. These increases are significant at the 1% level. Treatments with carbonate and water produced lower numbers of lesions than complete nutrient solution. Scotia with boron treatment is twice as susceptible to TMV infection as is Pinto (Fig. 2). Scotia with manganese, 28 C and 200 ft-c for 14 hr and 10 hr dark (Fig. 2-C), produced more lesions than other treatments significant at the 5% level.

Comparing the above series of experiments in Fig. 2, it appears that Scotia with boron, carbonate, and complete nutrient solution produces about 2-fold more lesions if the last day before inoculation is divided into 14 hr light (200 ft-c) and 10 hr darkness at 28 C than plants receiving constant light (200 ft-c). Postinoculation treatment has little or no effect on the susceptibility of the leaves, but it does influence the early appearance and size of the local lesions.

Effect of light, temp, nutrients, and DMSO.—Preliminary experiments assessed the optimum concentration of DMSO. Concentrations above 0.15% in balanced nutrient solution were toxic. DMSO at 0.05% enhanced the production of local lesions without injury. As the concn of DMSO increased, marginal burning developed on the leaves. Similar experiments were conducted using two DMSO concentrations (0.05 and 0.1%) in complete nutrient solution. Plants were grown in DMSO-containing nutrient solutions for 5 days. Inoculations were made at 24-hr intervals starting with 7-day-old leaves. Plants receiving 192-hr DMSO treatment produced more lesions than all other treatments (Fig. 3-a). This increase in lesion number is significant at the 1% level. DMSO at 0.1% increased lesions more than 0.05% DMSO, but the injury on the margins of the leaves with 0.1% DMSO was pronounced; hence, this concn was not desirable. The length of time for which plants were grown in DMSO slightly increased the susceptibility of the leaves to TMV infection. Effectiveness of DMSO was reduced at the 5th day (11-day-old plants). This reduction in lesion number may be due to the age of the plants or to the interaction of age with longer root exposure to DMSO.

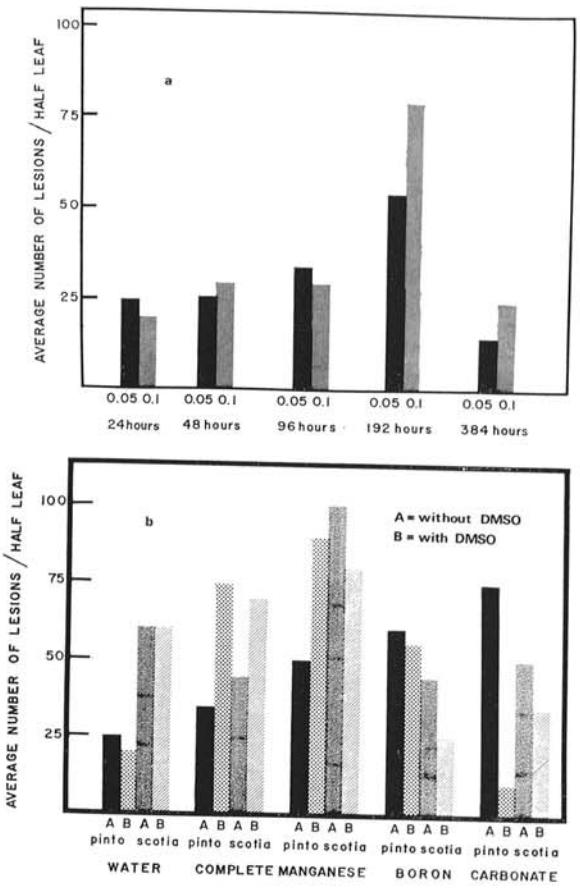

Fig. 3. a) Effect of the length of time (24, 48, 96, 192, and 384 hr) plants are grown in 0.05% and 0.1% dimethylsulfoxide (DMSO) on tobacco mosaic virus local lesion development in Scotia bean. Statistical analysis shows that DMSO at 0.05% and 0.1% concn produced significantly (1% level) increased lesions when roots were exposed for 192 hr. Other differences among treatments are nonsignificant. **b)** Effect of manganese, boron, and carbonate in 0.05% DMSO on tobacco mosaic virus local lesion development in Pinto and Scotia beans grown at 28 C and 200 ft-c light at a day and night cycle of 14 hr light and 10 hr darkness. Statistical analysis shows that Pinto with manganese plus DMSO treatment produces significantly (1% level) more lesions than water plus DMSO and carbonate plus DMSO. In Scotia, manganese, complete, and water with DMSO produce significant increases in lesion counts over the counts with boron and carbonate plus DMSO. Other differences among treatments are nonsignificant except that carbonate, boron, and manganese without DMSO produce significant increases over water and complete without DMSO.

An additional experiment was conducted with Pinto and Scotia grown in 0.05% DMSO containing man-

ganese, boron, and carbonate at the concn levels given in Table 1, with complete nutrient solution and water as controls. Plants were grown at 28 C with 14 hr of light (200 ft-c) and 10 hr of dark for 10 days. Pinto with manganese and DMSO produced significantly (1% level) more lesions, and Scotia with manganese and without DMSO produced significantly higher numbers than controls (complete and H_2O) (Fig. 3-b). Pinto and Scotia differed in their susceptibility to TMV in carbonate with DMSO. In this system, Pinto developed fewer lesions (2- to 4-fold) than Scotia. But in the presence of DMSO, Pinto produced more lesions in manganese solutions but fewer lesions in other nutrient solutions. When plants were grown in complete solution, DMSO treatments in both Pinto and Scotia produced about 2-fold more lesions than without DMSO. These results are significant at the 1% level. We suggest that the nutrient solution for the production of Pinto leaves with maximum susceptibility to TMV should contain 1 ml/1,000 ml of 1 M $MnCl_2 \cdot 4H_2O$ and 0.05%, v/v, DMSO, while for Scotia leaves of max susceptibility, the nutrient solution should contain only manganese at the above concn level.

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