Thigmomorphogenesis and Predisposition of Hosts to Fusarium Wilt

Omar Shawish and Ralph Baker

Graduate student and professor, respectively, Department of Botany and Plant Pathology, Colorado State University, Fort Collins 80523.
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ABSTRACT

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Tomato, flax, and pea plants were subjected to mechanical stimulation (MS) in a greenhouse environment by gently shaking the stems and leaves for 1 min each day. The incubation period for development of Fusarium wilt, which is induced by various *formae speciales* of *Fusarium oxysporum*, was shorter than in inoculated plants not exposed to MS. Symptom expression also was more severe in MS-treated plants as measured by foliar symptoms and vascular discoloration. This increase in symptom expression was accompanied by reduction in stem length and increase in stem thickness as a result of MS in comparison with untreated controls. Cuttings were taken from tomato plants and inoculated with *F. oxysporum* f. sp. *lycopersici*. These were rooted for 15 days and transplanted. Half of the transplants were subjected to MS and half were not. All combinations of MS-treated, untreated, and inoculated and uninoculated controls also were included in the experiment. Symptom expression, as measured in foliar responses, was significantly ($P = 0.05$) most severe in plants treated with MS both on the mother plants and as transplants followed in descending order by those treated only as transplants, only as cuttings on the mother plant, and untreated inoculated controls. Similar responses were observed when transplants were derived from seedlings rather than cuttings from mother plants in the same experimental design. These phenomena suggest that there was a long-term effect of MS on disease expression after MS was withdrawn because transplants treated while they were on mother plants or as seedlings had more severe foliar symptoms than untreated controls. In the field, MS is furnished predominantly by wind. Thus, wind may be added to the list of predisposing factors in epidemiology which have an effect on disease expression.

Additional key words: resistance.

Jaffe (6–8) and others (3,5,9) reported an interesting phenomenon involving a relatively new discipline in sensory physiology of higher plants. The application of mechanical stimulation induces dramatic morphological alterations; growth is markedly retarded and radial stem diameter is increased in comparison with plants not exposed to mechanical perturbations. Such changes may be induced in greenhouse environments, which are relatively undisturbed by wind or other mechanical insults, by gently shaking or rubbing the plants for less than 1 min each day (6). The phenomenon, which is called thigmomorphogenesis (8), appears to be a basic response of plants to mechanical cues in the natural environment such as bending by the wind and mechanical rubbing by soil particles. Mechanical perturbation also is

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accompanied by changes in metabolic activity (8). Since thigmomorphogenesis has such an impact on plant growth and metabolism, it is opportune to determine how it relates to the onset and development of plant disease.

It is well known that plants can be predisposed to disease by various environmental factors. Calhoun (1) recently reviewed the subject; however, he did not treat thigmomorphogenesis as a predisposing factor. Thus, if mechanical perturbation has an effect on plant disease, a new field of investigation in plant pathology would be initiated.

The objectives of this study were to establish the effects of mechanical stimulation on the susceptibility of three plant species to the Fusarium wilt pathogens and to determine the long-term predisposing effect of thigmomorphogenesis.

MATERIALS AND METHODS

To assess the effects of mechanical stimulation (MS) on a number of plant species, the following hosts were assayed: tomato (*Lycopersicon esculentum* Mill., 'Bonny Best'), flax (*Linum usitatissimum* L., 'Taichung #1') and pea (*Pisum sativum* L., 'Little Marvel'). Tomato seedlings, transplanted when they had two to five expanded leaves, were placed in 15-cm-diameter plastic pots containing a greenhouse soil mixture (peat, Perlite® [Persolite Products Inc., Florence, CA 90001], and soil, 1:1:3, v/v). Pea and flax were grown from seeds in the same type of soil.

Inoculum of *Fusarium oxysporum* Schlecht. f. sp. *lycopersici* (Sacc.) Snyd. & Hans. race 1 was grown in potato-dextrose broth in flasks on a rotary shaker for 5 days to produce a bud-cell suspension. The contents of the flasks were filtered through eight layers of cheesecloth to remove most mycelial fragments. *Fusarium oxysporum* Schlecht. f. sp. *lini* (Bolley) Snyd. & Hans., and *F. oxysporum* Schlecht. f. sp. *pisi* (Lindf.) Snyd. & Hans. race 5 were grown on sterilized carnation tissue, which was ground to a dry powder, and used later to infest soil (4,10).

Tomato seedlings were inoculated by dipping roots into bud cell suspensions (2×10^5 per milliliter) for 4 hr before transplanting. When unrooted tomato cuttings were used, they were similarly exposed to the pathogen. Peas and flax were inoculated by germinating seed in a layer of raw soil approximately 2 cm deep overlaying soil infested with the appropriate pathogen. This way, the seeds were placed in soil not infested with the pathogen but, after germination, the roots invaded infested soil (4).

The experiments were done in a greenhouse. Temperatures were adjusted to 18–24 C. Plants were irrigated as necessary with a 1:200 dilution of a stock nutrient solution containing 11.34 kg KNO_3, 5.44 kg $MgSO_4 \cdot 7H_2O$, 2.27 kg NH_4NO_3, and 400 ml H_3PO_4 in 94.6 liters of water.

Mechanical stimulation was accomplished by gently shaking plants by brushing the tops laterally by hand at a rate of 1–2 cycles per sec for 1 min per day (6). No detectable injury was induced on the plants by this method.

Disease severity for tomato was rated according to the leaf grade and vascular discoloration indices of Dimond et al (2). For leaf grade 0 = no disease symptoms, 1 = epinasty and/or distortion of leaf shape, 2 = slight yellowing, 3 = yellowing, 4 = yellowing more intense than grade 3, and 5 = all leaves dead or completely yellow. The disease rating for each plant was calculated as the total of the individual leaf grades divided by the total number of leaves. As suggested by Dimond et al (2), the lower leaves were not recorded because they become prematurely senescent. Symptom expression on flax also was evaluated by leaf grade index. Vascular discoloration of tomato was rated as follows from stems split at the end of the experiments: 0 = no vascular discoloration; 1 = slight discoloration; 2 = brown discoloration, more than grade 1; and 3 = discoloration dark and extensive. Disease severity was assessed for peas by determining the proportion of wilted leaves (4). For measurements reflecting the morphological responses of the hosts to treatments and pathogens, the length and diameter of plant stems and also the lengths of the internodes were recorded.

Data were subjected to analyses of variance and mean separations were performed according to Duncan's multiple range test. Values of standard error, however, are presented in the figures (illustrating the results) only when data points were close enough so that there was some doubt regarding significant differences.

RESULTS

Host response to MS. Tomato seedlings were dipped in bud cell suspensions of *F. oxysporum* f. sp. *lycopersici* and transplanted. Seven days after inoculation, plants were subjected daily to MS until the end of the experiment 21 days later. MS also was applied daily to peas and flax 7 days after seeds were planted in soil infested with the appropriate Fusarium wilt pathogen and continued until the end of the experiments. Other hosts were not exposed to MS treatments and uninoculated plants served as appropriate controls. There were 15 plants in each treatment and four replications.

As a result of MS treatment, inoculated tomato plants had a

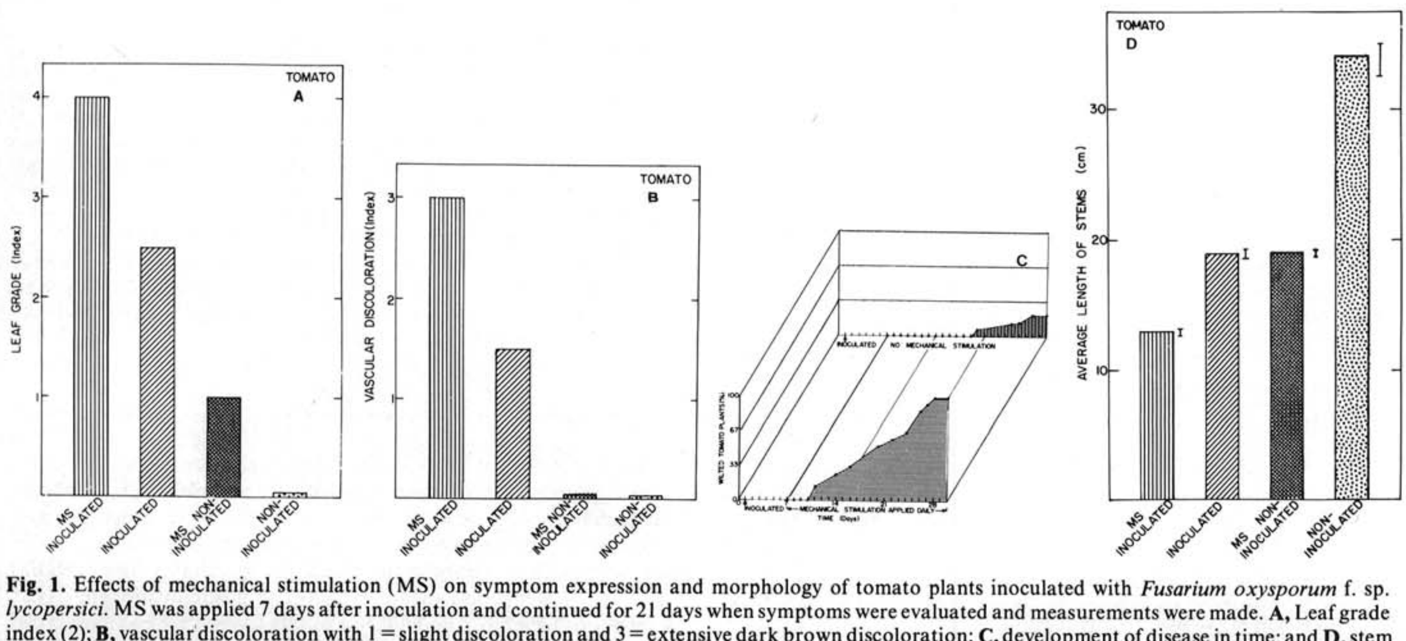

Fig. 1. Effects of mechanical stimulation (MS) on symptom expression and morphology of tomato plants inoculated with *Fusarium oxysporum* f. sp. *lycopersici*. MS was applied 7 days after inoculation and continued for 21 days when symptoms were evaluated and measurements were made. **A,** Leaf grade index (2); **B,** vascular discoloration with 1 = slight discoloration and 3 = extensive dark brown discoloration; **C,** development of disease in time; and **D,** stem lengths. The standard errors shown as vertical brackets were computed from original data. Differences in symptom expression were significant ($P = 0.05$) among all treatments.

significantly higher leaf grade index (Fig. 1A), more vascular discoloration (Fig. 1B) and higher disease incidence with a shorter incubation period (Fig. 1C) than similar inoculated controls not exposed to MS ($P = 0.05$). The application of MS to uninoculated and inoculated plants also significantly reduced the average length of stems ($P = 0.05$) when compared, respectively, with uninoculated and inoculated controls not exposed to MS (Fig. 1D). The same trends were observed when flax was used as a host (Fig. 2).

Disease severity was significantly increased ($P = 0.01$) and the incubation period was shortened by 1 wk when MS was applied to inoculated peas in comparison with those not receiving MS (Fig. 3 A and B) in tests with the same experimental design. Whereas uninoculated plants exposed to MS were significantly shorter ($P = 0.05$) than those not exposed to MS (Fig. 3D), the magnitude of the difference was not as great as for similar treatments of tomato (Fig. 1D) and flax (Fig. 2B). There was no significant difference ($P = 0.05$) in stem length among inoculated plants regardless of treatment (Fig. 3C).

Long-term effect of MS. Tomatoes grown from seedlings were subjected daily to MS beginning 25 days after transplanting. Others were not exposed to MS to serve as controls. Five days after the beginning of MS treatments, 20 cuttings were taken from plants in the MS treatment and a like number from the control. In each case, half of the cuttings were inoculated with a bud-cell suspension of *F. oxysporium* f. sp. *lycopersici*. Cuttings were placed in flats, separated by treatment, for propagation. After 15 days, roots had formed and each cutting was transplanted into a pot. MS was applied daily to half of the rooted cuttings in each treatment for 21 wk. Thus, 20 plants were inoculated and 20 were not inoculated. In each of these treatments, five plants received MS on the mother plant and after transplanting, five were exposed to MS only during growth on the mother plant, five received MS treatment only after rooting, and five received no treatment at any time during the experiment. In this case, each of the five plants within each treatment was considered a replication for statistical purposes and blocks were randomized.

Disease ratings were recorded 35 days after transplanting. When symptom expression was measured by leaf grade index, disease severity was significantly higher ($P = 0.05$) among (inoculated) plants derived as cuttings from mother plants exposed to MS than among those that had not received MS (Fig. 4A). Additionally, there was a significant increase ($P = 0.05$) in disease severity observed in inoculated plants receiving MS after transplanting whether mother plants had received treatment or not. The same trends were observed when ratings were made of vascular

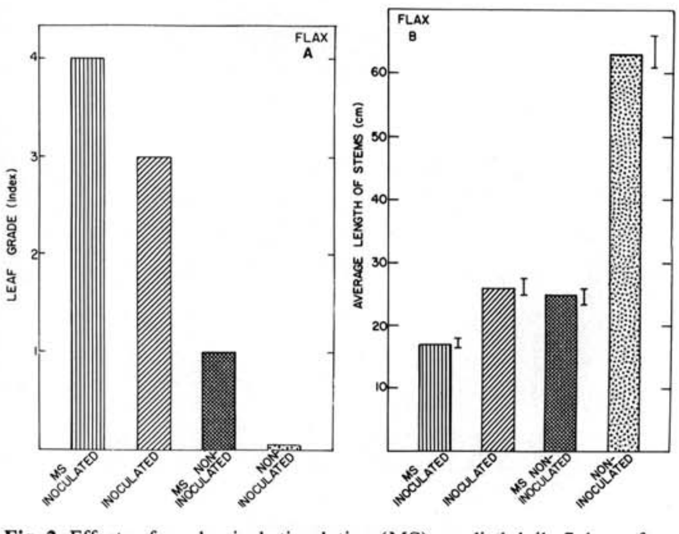

Fig. 2. Effects of mechanical stimulation (MS), applied daily 7 days after seeding, on symptom expression and morphology of flax inoculated with *Fusarium oxysporum* f. sp. *lini*. **A,** Leaf grade index (2); and **B,** stem lengths 5 wk after the beginning of MS. The standard errors shown as vertical brackets were computed from original data. Differences in symptom expression were significant ($P = 0.05$) among all treatments.

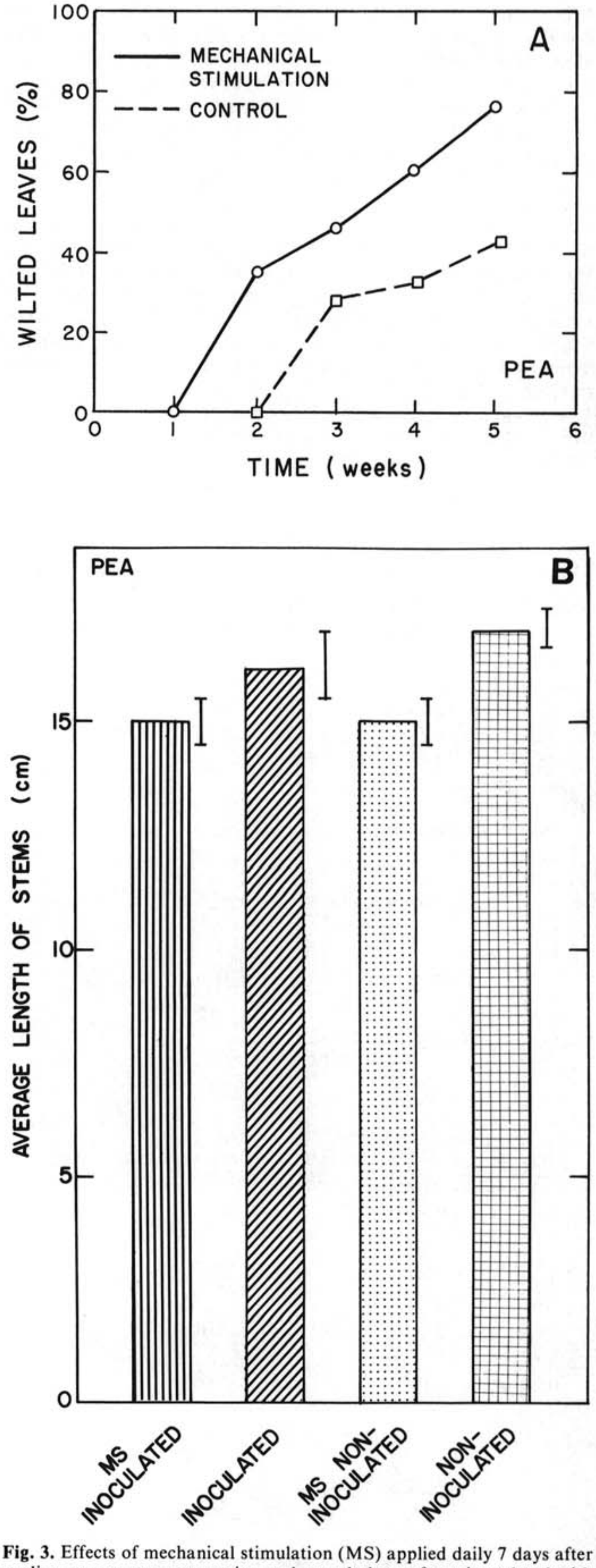

Fig. 3. Effects of mechanical stimulation (MS) applied daily 7 days after seeding on symptom expression and morphology of pea inoculated with *Fusarium oxysporum* f. sp. *pisi*. **A,** Proportions of wilted leaves (4) over time after inoculation in host plants exposed to MS. There were no wilted leaves on uninoculated controls whether plants were or were not exposed to MS. **B,** Stem lengths 5 wk after beginning of MS. The standard errors shown as vertical brackets were computed from original data.

discoloration measured at the end of the experiment (Fig. 4B) except that there was no significant ($P = 0.05$) difference in ratings of this symptom between plants receiving MS while on the mother plant as cuttings and those not receiving any treatment. Increases in leaf grade index were observed among uninoculated plants receiving MS after rooting compared with those subjected to the treatment before rooting or not at all (Fig. 4A). No differences were observed among uninoculated controls in ratings for vascular discoloration (Fig. 4B).

Morphological changes were apparent on all plants that had received MS. Significant ($P = 0.05$) decreases in stem (Fig. 4C–F) and internode length (Fig. 4G) and increases in stem diameter (Fig. 4H) 21 wk after transplanting were evident when MS was administered on mother plants and/or transplants, except for stem length measurements which compared uninoculated plants derived from mother plants subjected to MS (Fig. 4D) with those that received no MS (Fig. 4F).

A similar experiment was performed at the same time except that tomato seedlings were subjected to MS for 7 days, uprooted, inoculated, and again exposed to MS for 12 wk. Appropriate uninoculated controls and combinations of MS treatments (or not treated) were applied to seedlings or transplants as in the experiment described immediately above. The same trends in symptom expression and morphological responses as in the experiment involving rooted cuttings also were apparent in this case (Fig. 5).

DISCUSSION

The various *formae speciales* of *F. oxysporum* pathogenic to tomato, flax and peas used in this research induced greater disease severity as evidenced by reduction in incubation periods (Figs. 1C and 3A,B) and increase in symptom expression (Figs. 1–5) when MS was applied to host plants. In addition, the effects of MS on development of symptoms of Fusarium wilt of tomato continued after the treatment was withdrawn (Figs. 4A and 5A,B). This suggests that MS had a profound effect on the disease reaction of hosts of the Fusarium wilt pathogens and that the effect persisted as did the effects on plant morphology (Figs. 4C–H and 5C–H).

The effects of MS applied under greenhouse conditions are believed to be duplicated in field situations (8). In this case, wind,

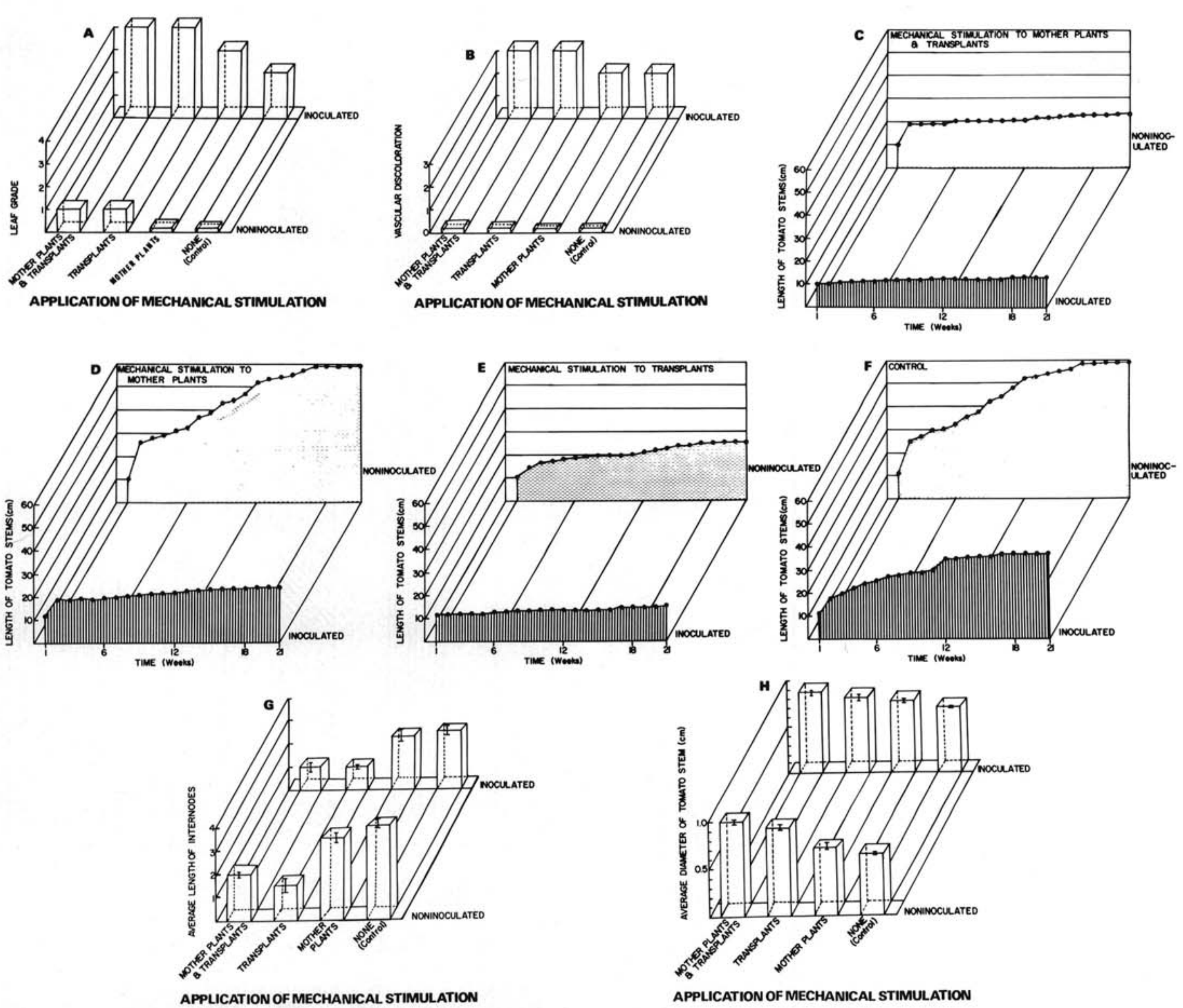

Fig. 4. Effects of mechanical stimulation applied to tomato mother plants for 7 days and/or transplants (rooted cuttings from mother plants) for 21 wk on symptom expression and morphology of tomato inoculated with *Fusarium oxysporum* f. sp. *lycopersici*. **A,** Leaf grade index (2) 35 days after transplanting; **B,** vascular discoloration with 1 = slight discoloration and 3 = extensive dark brown discoloration 21 days after transplanting; **C–F,** length of tomato stems of rooted cuttings over time after transplanting; **G,** average length of internodes after 21 wk; **H,** average diameter of stems after 21 wk. The standard errors shown as vertical brackets were computed from original data.

which exerts a mechanical stress on the plant organs, is the chief agent responsible for MS. Plant pathologists have always been aware of the importance of wind in the study of epidemiology, especially as it is involved in dissemination of pathogens. Thus, to the study of epidemiology and to the list of predisposing factors of plant disease recently reviewed by Calhoun (1), the effects of wind on disease reaction should be added.

Some plant breeders have observed that certain cultivars of plants grown in the greenhouse are resistant; when tested in the field, they are susceptible. Predisposition by MS provides a candidate explanation for this phenomenon. Again, some cultivars of barley and/or wheat respond to MS by a reduction in shoot length while others do not (9). The growth response due to MS is associated with resistance to lodging. This suggests a hypothesis that cultivars insensitive to MS might not be predisposed to disease by this treatment since, in our experiments, alterations in host

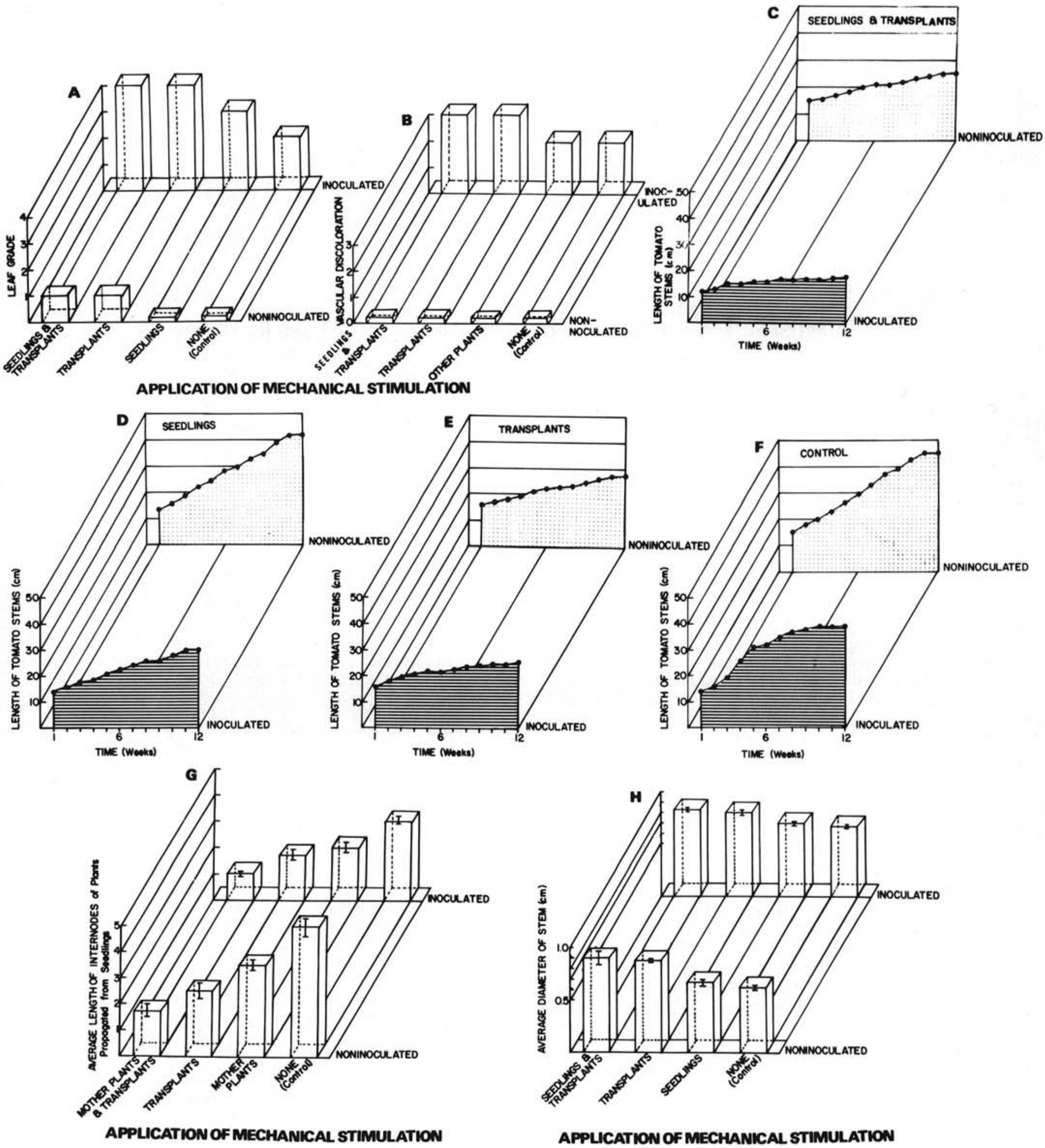

Fig. 5. Effects of mechanical stimulation (MS) applied for 7 days to tomato seedlings which were uprooted, inoculated with *Fusarium oxysporum* f. sp. *lycopersici*, transplanted, and then exposed or not exposed to MS for 12 wk. **A,** Leaf grade index (2) 35 days after transplanting and inoculation; **B,** vascular discoloration with 1 = slight discoloration and 3 = extensive dark brown discoloration; **F,** length of tomato stems of rooted cuttings at 12 wk after transplanting; **G,** length of internodes after 12 wk; **H,** diameter of stems after 12 wk. The standard errors shown as vertical brackets were computed from original data.

morphology were associated in all cases with increased susceptibility.

For the first time, wind may be considered as a predisposing factor in plant disease incidence and severity. Further studies should elucidate more fully the importance of this factor in the epidemiology of plant diseases.

LITERATURE CITED

1. Calhoun, J. 1979. Predisposition in the environment. Pages 75–96 in: J. G. Horsfall and E. B. Cowling, eds. Plant Disease, An Advanced Treatise, Vol. 4. Academic Press, New York, San Francisco and London. 466 pp.
2. Dimond, A. E., Davis, D., Chapman, R. A., and Stoddard, E. M. 1952. Plant chemotherapy as evaluated by the Fusarium wilt assay on tomato. Conn. Agric. Exp. Stn. Bull. 557. 82 pp.
3. Grace, J. 1977. Plant response to wind. Academic Press, New York. 200 pp.
4. Guy, S. O., and Baker, R. 1977. Inoculum potential in relation to biological control of Fusarium wilt of peas. Phytopathology 67:72-78.
5. Jacobs, M. R. 1954. The effect of wind sway on the form and development of *Pinus radiata* D. Don. Aust. J. Bot. 2:35-51.
6. Jaffe, M. J. 1973. Thigmomorphogenesis: the response of plant growth and development to mechanical stimulation, with special reference to *Bryonia dioica*. Planta 114:143-157.
7. Jaffe, M. J. 1976. Thigmomorphogenesis: a detailed characterization of the response of beans (*Phaseolus vulgaris* L.) to mechanical stimulation. Z. Pflanzenphysiol. 77:437-453.
8. Jaffe, M. J. 1980. Morphogenetic responses of plants to mechanical stimuli or stress. BioScience 30:239-243.
9. James, D. M., and Guy, L. S. 1974. Response of barley (*Hordeum vulgare*) seedlings to mechanical stress. Penn. Acad. Sci. 40:114-116.
10. Scher, F. M., and Baker, R. 1980. Mechanism of biological control in a Fusarium-suppressive soil. Phytopathology 70:412-417.