Maize White Line Mosaic Virus Transmission to Maize Seedlings in Hydroponic Culture

RAYMOND LOUIE, Research Plant Pathologist and Adjunct Professor, J. J. ABT, Agricultural Research Technician, and J. K. KNOKE, Research Entomologist (retired), Corn and Soybean Research Unit, USDA-ARS, and Department of Plant Pathology, The Ohio State University, Ohio Agricultural Research and Development Center, Wooster 44691

ABSTRACT

Louie, R., Abt, J. J., and Knoke, J. K. 1992. Maize white line mosaic virus transmission to maize seedlings in hydroponic culture. Plant Dis. 76:1069-1073.

A hydroponic culture system and the effects of nonchemical and chemical treatments of root inoculum on transmission of maize white line mosaic virus (MWLMV) were evaluated. Root pieces (0.2 g) from 8- to 10-wk-old infected Seneca Chief sweet corn (*Zea mays* var. *saccharata*) plants were used as inoculum to transmit MWLMV to roots of sweet corn test seedlings. MWLMV was transmitted consistently to seedling roots (>90%) as shown by enzyme-linked immunosorbent assays (ELISA). The ELISA values (absorbances at 405 nm) of root inocula and inoculated roots were not significantly correlated. Moreover, none of the nonchemical (amount of inoculum, exposure period, filter or distance barrier, depth of nutrient solution, and temperature of plant growth) or chemical (fungicide, antibiotic, insecticide, sterilant, and solvent) treatments of root inoculum prevented transmission of MWLMV. These tests indicated that in this hydroponic system, a vector need not be involved in the transmission of MWLMV to roots of seedlings with inoculum from infected roots.

Additional keywords: soilborne pathogen

In the early 1980s, maize white line mosaic (MWLM) was widespread in the northeastern United States (8) and also was reported in France and Italy (5,14). Studies on the field distribution of maize plants (*Zea mays* L.) infected with maize white line mosaic virus (MWLMV) indicated that the virus might be soilborne and might involve a soilborne vector (1,8). Tests in the greenhouse with naturally infested soils and infected roots added to autoclaved soils (8) supported this hypothesis, but evidence of a vector was inconclusive. This lack of evidence may have resulted from the use of field and greenhouse soil inocula consisting of a complex of microorganisms and from inability to mechanically transmit the virus. In studies where an isolation of soil microorganisms was first attempted, the approach was usually limited to methods involving the use of nonselective media. Consequently, these methods excluded fastidious microorganisms that might act as vectors. However, when researchers were unable to isolate an obligate parasitic vector in pure culture (e.g., in soilborne wheat mosaic virus or wheat spindle streak mosaic virus), they were able to demonstrate the involvement of an obligately parasitic vector more easily when the virus could be transmitted mechanically (2,11,13).

Recently, there have been few new reports of MWLM. Furthermore, plants with symptoms of MWLM are seldom found in fields in Ohio where the disease was once prevalent. Because the occurrence of MWLM under field conditions was no longer predictable, we stopped experimentation in field plots. In addition, because the percentage of MWLMV transmissions under greenhouse conditions using pots of naturally infested soil also diminished (R. Louie, *unpublished*), we had to increase the number of samples in a treatment to distinguish treatment effects. If studies on disease resistance, virus ecology, and particularly, the involvement of a vector(s) in MWLMV transmission are to continue, a reliable method of transmission is needed.

When this study was nearly completed, Zhang et al reported on an embryo-wound inoculation technique for MWLMV transmission (15). They placed a drop of purified virus or juice from infected leaves on the embryo of an ungerminated seed and then cut the embryo with a scalpel. Although the method was lethal to some inoculated seeds, infected plants that survived the inoculation developed the characteristic white line symptoms. Our method is not injurious, and it relies on the infection of roots of test seedlings that have been exposed to root inoculum in a slant-board (hydroponic culture) system developed by Kendall and Leath (6) and modified by Olah and Schmitthenner (12). This study reports on the usefulness of this method and the effects of various treatments of root inoculum on MWLMV transmission. A preliminary report has been published (7).

MATERIALS AND METHODS

Inoculum and test plant preparations. Roots from infected Seneca Chief sweet corn (*Zea mays* L. var. *saccharata* (Sturtev.) L. H. Bailey) plants (8- to 10-wk old) grown in 10-cm pots of infested field soil (bulked from six collections) were used as inoculum. The soil was collected from the bases of sweet corn naturally infected with MWLMV. Occasionally, inoculum was prepared from roots of infected plants grown in greenhouse soil infested with root inoculum. Roots of infected plants grown from seeds inoculated by embryo wound (15) also were used in three tests. The roots from each plant were shaken to remove loose soil, washed with flowing tap water while rubbing the roots to remove the residual soil (1–3 min), and blotted dry between layers of paper towels. They were then cut into pieces (2–3 mm long) and combined. Because infected plants were usually symptomless, two or three root samples (0.3 g [20–30 pieces]/sample) from each plant were tested by enzyme-linked immunosorbent assay (ELISA) (8). If the ELISA value (absorbance at 405 nm) of any root sample was greater than 0.5, the remaining root pieces were combined with root pieces from other infected plants and stored at 4 C in a beaker sealed with Parafilm. The inoculum was used within 15 days.

Crude juice inoculum was produced from leaves or roots of symptomatic, MWLMV-infected plants that were homogenized in a blender in 0.01 M potassium phosphate buffer, pH 7.0 (1:10, w/v).

Test plants were Seneca Chief sweet corn seedlings grown in vermiculite in a 15-cm pot for 6–8 days. Before their use, the seedlings were washed with

Cooperative investigation of USDA-ARS and OSU-OARDC. Salaries and research support provided by state and federal funds appropriated to the USDA-ARS and OSU-OARDC. Submitted as OSU-OARDC Journal Article 246-90.

Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

Accepted for publication 14 June 1992.

flowing tap water to remove the vermiculite from the roots.

Root inoculum treatments. Nonchemical (Table 1) and chemical (Table 2) treatments of root inoculum were used to try to modify virus transmission. Nonchemical treatments included use of 0.1, 0.2, or 0.3 g of root inoculum per test seedling; inoculum exposure periods of 1, 2, or 3 wk; placement of root inoculum at 0, 1, or 2 cm distance from the test seedling; separation of root inoculum from test seedlings with Whatman no. 1 filter paper, 3.0, or 0.2-μm Nucleopore filters, or confinement of root inoculum in bags made from 0.2- or 0.05-μm Nucleopore filters sealed with silicone or heat; placement of inoculated test seedlings in a plant nutrient solution (Hoagland's solution) that was 5 or 20 cm deep; and growth of test seedlings in growth chambers with 21/15 C or 27/21 C day/night temperatures and a 14-hr day length (250 $\mu E \cdot s^{-1} \cdot m^{-2}$).

Chemical treatments included fungicides (metalaxyl [active ingredients, 10 ppm], benomyl [60 ppm], and propiconazole [97 ppm]); antibiotics (vancomycin [250 ppm], gentamicin [100 ppm], and mixtures of streptomycin-chloramphenicol [250 and 250 ppm] and lincomycin-spectinomycin [100 and 100 ppm]); an insecticide (diazinon [61.5 ppm]); sterilants (sodium azide [200 ppm] and sodium hypochlorite [5,250 ppm]); solvents (xylene, chloroform, carbon tetrachloride, and petroleum ether); and a detergent (3% sodium dodecyl sulfate [SDS]).

At the beginning of an experiment, the fungicides, antibiotics, and insecticide were dissolved in Hoagland's solution and applied simultaneously to the root inoculum (0.2 g/plant) and test seedlings. The root inoculum in the solvent treatment was submerged first in a solvent for 18–24 hr, air-dried, and then placed on roots of test seedlings. Similarly, root inoculum in the sterilant treatments was treated with either sodium hypochlorite or sodium azide, blotted, and air-dried before use.

Virus replication in roots. To test for residual virus on root surfaces of test seedlings, inoculated primary roots were washed in a water bath with or without sonication or in SDS. For inoculation, the roots were first submerged in fresh crude juice from infected roots for 60 min or were exposed to root inoculum for 3 wk as described below. After inoculation, roots were either rinsed in a 4-L water bath, not rinsed, sonicated in a water bath for 10–15 sec, or treated for 30 min with 3% SDS heated to 65 C before being tested for virus by ELISA. In a separate test, secondary roots that developed during the 3-wk inoculation period also were tested to determine the extent of systemic or secondary infection.

Experimental design. Three test seedlings were placed on a nylon cloth (22 × 60 cm), which overlaid a polyester wicking material (22 × 30 cm), and all were supported by a plastic tray (27 × 33 cm) (12) (Fig. 1). Except when modified by a treatment, 0.2 g of root inoculum (F in Fig. 1) was distributed

Table 1. Effects of nonchemical treatments on maize white line mosaic virus transmission[t]

Test Treatment	Inoculum (root pieces) ELISA value[u]	Inoculum (root pieces) Pos[v] (%)	Apical root[w] ELISA value	Apical root[w] Pos (%)	Basal root[w] ELISA value	Basal root[w] Pos (%)	Transmission to seedling[x] (%)
Amount of inoculum							
0.1 g	1.69	100	0.55 b	96	0.87 c	96	96
0.2 g	2.66	100	0.99 a	89	1.23 b	100	100
0.3 g	2.67	100	1.13 a	89	1.64 a	100	100
Healthy control[y]	0.04	...	0.03	...	0.02	...	...
Inoculum exposure period							
1 wk	2.64	100	0.43	100	0.56	100	100
2 wk	2.47	100	0.34	96	0.65	100	100
3 wk	2.70	100	0.32	85	1.14	100	100
Healthy control	0.01	...	0.00	...	0.00	...	...
Barriers							
Whatman filter no. 1	2.83	100	0.14 b	56	0.23	79	85
0 cm	2.69	100	0.47 a	81	0.87	100	96
1 cm	2.63	100	0.05 b	15	0.39	85	70
2 cm	2.61	100	0.05 b	41	0.43	70	70
Healthy control	0.03	...	0.00	...	0.00	...	...
Hoagland's solution depth							
5 cm	1.44	100	0.52	60	0.73	81	85
20 cm	1.48	92	0.08	18	0.39	66	69
Healthy control	0.04	...	0.03	...	0.03	...	...
Filters (Nucleopore)							
No filter	2.18	100	1.16 ab	94 a	1.42 a	98 a	98
3.0-μm not enclosed[z]	2.89	100	1.63 a	100 a	1.39 a	100 a	100
0.2-μm not enclosed[z]	1.85	100	0.83 bc	100 a	0.87 ab	100 a	100
0.2-μm silicone-sealed bag	2.67	100	0.52 bcd	93 a	0.41 bc	100 a	100
0.2-μm heat-sealed bag	2.42	100	0.40 cd	78 ab	0.61 bc	78 ab	85
0.05-μm silicone-sealed bag	1.83	96	0.13 d	63 b	0.03 c	37 c	63
0.05-μm heat-sealed bag	2.39	100	0.18 d	63 b	0.23 bc	56 bc	74
Nontreated healthy control	0.03	...	0.01	...	0.01	...	...
Treated healthy control	0.02	...	0.00	...	0.00	...	...
Temperature							
21/15 C day/night	1.88	100	0.52	92	0.82	97	97
27/21 C day/night	1.69	100	0.86	94	0.93	100	100
Healthy control	0.02	...	0.01	...	0.01	...	...

[t] Values are based on three replications of nine plants. The values in a column within a test (except controls) followed by the same letter or no letter are not significantly different at $P \leq 0.05$ according to Duncan's new multiple range test.
[u] Average absorbance at 405 nm.
[v] The percentage of samples judged positive for the presence of virus (i.e., ELISA values greater than the mean of the healthy controls plus three times its standard deviation).
[w] Response of apical or basal portion of root.
[x] Percent transmission, based on at least one positive assay in samples of apical and/or basal roots.
[y] A 0.2-g sample of nontreated roots from healthy plants was distributed over the entire length of each seedling root.
[z] A sheet of Nucleopore filter (20.5 × 25.5 cm) was placed between the test seedlings and root inoculum.

over the entire primary root (standard nontreated root inoculum control). The nylon cloth (D in Fig. 1) was folded to contain the seedlings and root inocula and then dampened with Hoagland's solution (and the designated chemical additive). For each treatment, there were three seedlings per tray, three trays per replication, and three replications per experiment. For an experiment, each treatment was replicated in three complete blocks; blocks were separated by time. The three trays (C in Fig. 1) in each replication were stacked and wrapped tightly with a 4-mil, clear polyethylene sheet (B in Fig. 1) that formed a three-sided envelope from which the trays extended 2–3 cm. After the sides of the envelope were taped, Hoagland's solution (with its corresponding chemical additive) was poured into the envelope until it reached 5 cm above the lower edge of the tray. The plastic-wrapped trays were placed in a narrow box (A in Fig. 1) to keep them in an upright position. Controls were nontreated MWLMV root inoculum and virus-free root inoculum that was either treated or not treated (healthy control). Metalaxyl and benomyl (10 and 60 ppm) were included in the Hoagland's solution used for the MWLMV control, virus-free root inoculum control, and all nonchemical treatments. All experiments, except the one with temperature, were maintained in the greenhouse. Hoagland's solution (and the designated chemical additive) was added to maintain a 5 cm depth (20 cm for depth of Hoagland's solution treatment). After a 3-wk test period, the primary roots were assayed for MWLMV.

Virus assays and data analyses. Root samples of test seedlings were sonicated in a water bath for 10–15 sec and then ground in a mortar with a pestle in phosphate buffered saline (PBS)-Tween (0.2 g/0.8 ml of PBST buffer). The resultant brei was assayed by ELISA (8). An automated microplate reader equipped with a 405-nm filter (model EL 309, Bio-Tek Instruments, Winooski, Vermont) was used to evaluate ELISA reactions at 2 hr. Preliminary tests (R. Louie, *unpublished*) showed that infections in roots were often localized. Consequently, when the seedling root weight was greater than 0.4 g, the apical portion of the inoculated root (0.2 g) and the basal portion (0.2 g, the portion closest to the seed) were tested separately. When the seedling root weight was less than 0.4 g, only one assay was done. The root inoculum for each test seedling was also recovered and assayed by ELISA.

In each ELISA, a buffer control, healthy leaf control, and juice from MWLMV-infected leaves diluted in PBST buffer (1:10, w/v) were included in each microtitration plate. The test was considered valid if the ELISA values of the PBST buffer and juice from healthy leaves were less than 0.075 and those of MWLMV-infected leaves were greater than 1.0. A sample was judged positive for the presence of virus (i.e., virus was transmitted) if its average ELISA value was greater than the mean of the healthy control or, where available, the healthy, treated control plus three times its standard deviation. The ELISA values and percentage of samples with detectable virus were analyzed for significance by an analysis of variance (ANOVA), and their means were separated by Duncan's new multiple range test (3) at $P = 0.05$. Significant differences between results from assays of apical and basal root portions were determined by the sign test (3). Correlation coefficients between ELISA values of inoculum and those of root assays also were determined (10).

RESULTS

The average ELISA values of root inocula for the various nonchemical treatments exceeded 1.4 (Table 1). The values within a treatment for the inoculum were not consistent, and some values were lower than those of the healthy controls, suggesting that despite combining root samples and pretesting them for virus before use for inoculum, virus was not uniformly present in all root pieces. For example, the values of some samples in the 20-cm deep nutrient solution (four with an average ELISA value of 0.05) and in the 0.05-μm silicone-

Table 2. Effects of chemical treatments on maize white line mosaic virus transmission[t]

Group Treatment	Inoculum (root pieces) ELISA value[u]	Inoculum (root pieces) Pos[v] (%)	Apical root[w] ELISA value	Apical root[w] Pos (%)	Basal root[w] ELISA value	Basal root[w] Pos (%)	Transmission to seedling[x] (%)
Group 1							
Nontreated[y]	1.20	84	0.29	60	0.52	70	87
Metalaxyl	1.27	82	0.22	50	0.42	60	76
Benomyl	1.32	89	0.30	57	0.43	70	82
Metalaxyl and benomyl	1.45	87	0.28	47	0.35	73	84
Nontreated healthy control	0.02	...	0.00	...	0.00	...	...
Treated healthy controls[z]	0.02	...	0.00	...	0.00	...	...
Group 2							
Nontreated	1.59	100	0.47	81	0.75	96	96
Propiconazole	2.10	96	1.20	100	0.98	100	100
Vancomycin	1.99	89	0.47	98	0.78	100	100
Nontreated healthy control	0.01	...	0.00	...	0.01	...	...
Treated healthy controls	0.02	...	0.00	...	0.00	...	...
Group 3							
Nontreated	2.01	100	0.51	96 a	0.93	93	100
Diazinon	1.79	100	0.40	89 a	0.37	93	100
Sodium hypochlorite	2.05	93	0.26	56 b	0.46	96	93
Streptomycin and chloramphenicol	2.89	100	1.03	93 a	1.09	96	89
Nontreated healthy control	0.03	...	0.01	...	0.01	...	...
Treated healthy controls	0.02	...	0.01	...	0.02	...	...
Group 4							
Nontreated	2.15	100	1.31	100	1.62	100	100
Gentamicin	2.17	100	1.23	100	1.42	100	100
Lincomycin and spectinomycin	2.18	100	0.97	100	1.35	100	100
Nontreated healthy control	0.03	...	0.01	...	0.01	...	...
Treated healthy controls	0.02	...	0.01	...	0.01	...	...
Group 5							
Nontreated	1.80 a	100	0.74	100	1.06	100	100
Petroleum ether	1.71 ab	100	0.52	100	0.62	100	100
Carbon tetrachloride	1.52 bc	100	0.48	96	0.49	96	100
Sodium azide	1.89 a	100	0.72	96	0.70	100	100
Xylene	1.48 c	100	0.51	100	0.54	100	100
Nontreated healthy control	0.01	...	0.00	...	0.00	...	...
Treated healthy controls	0.00	...	0.00	...	0.00	...	...

[t] Values are based on three replications of nine plants. The values in a column within a test (except controls) followed by the same letter or no letter are not significantly different at $P \leq 0.05$ according to Duncan's new multiple range test.

[u] Average absorbance at 405 nm.

[v] The percentage of samples judged positive for the presence of virus (i.e., ELISA values greater than the mean of the healthy controls plus three times its standard deviation).

[w] Response of apical or basal portion of root.

[x] Percent transmission, based on at least one positive assay in samples of apical and/or basal roots.

[y] A 0.2-g sample of nontreated root inoculum was distributed over the entire length of each seedling root.

[z] A 0.2-g sample of healthy root was distributed over the entire length of each seedling root and treated with the corresponding chemical. ELISA values are a combined average of all treatments.

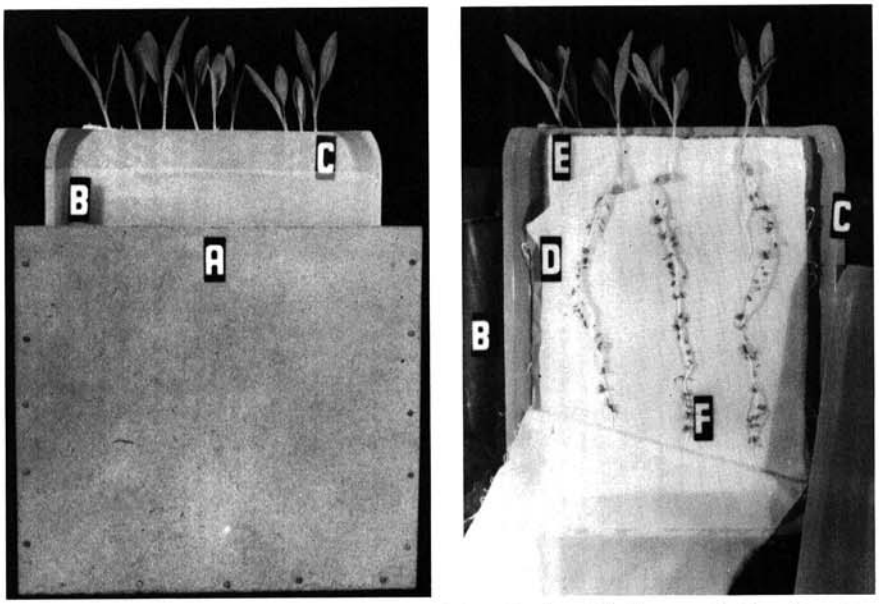

Fig. 1. The hydroponic culture system for transmission of maize white line mosaic virus. Complete system shown at left; internal components of the system shown at right. The various components are the container to hold the trays with test seedlings (three seedlings per tray, three trays per replication) in an upright position **(A)**; the polyethylene envelope to contain the trays and Hoagland's solution in each treatment-replication **(B)**; the tray **(C)** to support the wicking material **(E)**; nylon cloth **(D)**, which is folded to contain the seedlings; and root inoculum **(F)**.

Table 3. Correlation coefficients between ELISA values (absorbances at 405 nm) for root inoculum and values for the root samples

Test Treatment	Correlation coefficient: Apical root	Basal root	Apical and basal roots[v]	Entire root[w]
Nonchemical				
Inoculum exposure period				
1 wk	0.20	0.20	0.30	...
2 wk	0.34	0.32	0.46[x]	...
3 wk	−0.29	0.27	0.13	...
Barriers				
Whatman filter no. 1	0.24	0.28	0.23	0.45
0 cm	0.24	0.22	0.29	−0.18
1 cm	0.05	0.12	0.21	0.47
2 cm	0.15	0.15	0.13	0.08
Hoagland's solution depth				
5 cm	0.07	0.25	0.20	...
20 cm	0.10	0.23	0.25	...
Temperature				
21/15 C day/night	0.14	0.10	0.14	...
27/21 C day/night	0.28	0.29	0.41*	...
21/15 C day/night & metalaxyl	0.24	−0.05	0.09	...
27/21 C day/night & metalaxyl	0.30	0.47*	0.53*	...
Chemicals[y]				
Group 1				
Nontreated root inoculum[z]	0.45*	0.56*	0.66*	0.57*
Metalaxyl and benomyl	0.48*	0.29	0.48*	0.36
Metalaxyl	0.33	0.57*	0.57*	0.37
Benomyl	0.40*	0.44*	0.48*	0.40
Group 2				
Nontreated root inoculum	0.12	0.65*	0.58*	...
Propiconazole	0.58*	0.47*	0.64*	...
Vancomycin	0.35*	0.20	0.38*	...
Group 3				
Nontreated root inoculum	−0.10	0.52*	0.35	0.39
Diazinon	−0.14	0.66*	0.26	0.16
Sodium hypochlorite	0.22	0.36	0.47*	−0.17
Streptomycin and chloramphenicol	0.35	0.25	0.36	0.41

[v] Based on the sum of ELISA values for apical and basal roots.
[w] Assays of an entire root when the root was <0.4 g.
[x] Significant correlation coefficients are indicated by an asterisk.
[y] Groups as in Table 2.
[z] A 0.2-g sample of nontreated root inoculum was distributed over the entire length of each seedling root.

sealed bag (one with an ELISA value of 0.08) were not significantly different from the healthy controls. Hence, only 92 and 96%, respectively, of the root inocula used in these treatments tested positive. Virus was detected in all root inocula used in the other treatments.

In assays of either the apical or the basal portions of the primary root involving amount of inoculum, barriers, or filters, the ELISA values were significantly different ($P \leq 0.05$), but the percentage of positive samples was significantly different only in the filter experiment. On the basis of at least one positive assay per plant, the rate of transmission was greater than 90% in 13 of 21 treatments. The exceptions occurred in treatments where the root inoculum was separated from the test seedlings by distance (1 and 2 cm) or by filters (Whatman filter; heat-sealed 0.2-μm bags; and heat- and silicone-sealed 0.05-μm bags), and where the depth of Hoagland's solution was varied.

The range of average ELISA values of root inocula from chemical treatments was similar to those from nonchemical treatments (Table 2). In the experiment with diazinon, sodium hypochlorite, and streptomycin-chloramphenicol, only the sodium hypochlorite treatment significantly reduced infection of the apical portion of the root. In other assays of the apical or basal root portions, no significant differences were noted in either the ELISA values or the percentages of positive samples. On the basis of at least one positive assay per plant, the differences in average percent transmission within the five experiments were not significant. The percentage of transmission was generally lowest in the test with metalaxyl and benomyl and highest in the tests with gentamicin, lincomycin, and spectinomycin and in the test with solvents.

Washing seedling roots in a water bath with or without sonication or washing them in 3% SDS did not significantly remove virus from roots that were inoculated with pieces of root inoculum. The average ELISA values (four replications of nine plants) for no washing, washing without sonication, washing with sonication, washing with 3% SDS, and the nontreated healthy root control were 0.95, 0.89, 0.82, 0.60, and 0.01, respectively. However, washing with sonication and washing in 3% SDS significantly reduced ELISA values in seedling roots that were inoculated with crude-juice root inoculum. The average ELISA values for the same five treatments were 0.18, 0.05, 0.03, 0.02, and 0.00, respectively.

In both nonchemical and chemical treatments listed in Tables 1 and 2, the ELISA values and percent positives for assays of basal root portions were higher with the sign test (4) ($P \leq 0.012$ and $P \leq 0.022$, respectively) than for assays of the apical root portions. Regardless of

the basis for comparison (assays of apical and/or basal roots versus control), ELISA detected virus in all treatments.

In five of seven plants in one test, ELISA detected MWLMV in the secondary roots that developed after inoculation. Virus also was detected in the primary roots of all seven plants.

The correlation coefficients between the ELISA values of the inoculum and the values for the apical, basal, or entire root were not consistently positive or negative across all treatments (Table 3). This suggests that the amount of inoculum used in these experiments exceeded the minimum threshold amount, or perhaps that transmission with root inoculum (0.2 g) is independent of virus titer, or both. The greatest number of significant positive correlations occurred in the treatments with benomyl, propiconazole, and one with nontreated root inoculum.

DISCUSSION

The main advantages of the hydroponic culture system are consistently high rates of transmission of MWLMV and isolation of the test system from the soil environment. These factors should allow us to evaluate disease resistance, determine the overwintering inoculum, and clarify the mechanism of transmission.

These data may be used to inquire whether a vector is involved in virus transmission, but it is imprudent to do so, because these experiments were not designed to answer that question, and at times the results were contradictory. For example, transmission efficiencies using root inoculum were reduced with sodium hypochlorite but not with any of the other sterilants. This general lack of effect with the biocides may be interpreted as consistent with a mechanical transmission hypothesis. On the other hand, the occurrence of root infections when the inoculum was placed at a distance from the root or when inoculum was confined with Nucleopore filters suggests that the inoculation process may not be entirely mechanical.

Occurrences of symptomless infections (9), variability of ELISA values within treatments, and difficulties in inoculum production were limiting factors in this method of inoculation. In spite of these limitations, the method consistently resulted in transmission and at a greater percentage than those reported by Zhang et al (15). In contrast to the development of symptoms in seedlings when the seeds were wound-inoculated, the test seedlings inoculated with root inoculum, although infected, remained symptomless. Hence, a major concern was that virus was merely adsorbed to the root surfaces and no infection occurred. The removal of virus from seedling roots submerged in crude-juice root inoculum, but not from seedling roots exposed to pieces of root inoculum, suggests otherwise. The occurrence of both virus-positive and virus-negative test plants on the same tray when the root inoculum tested positive for virus, the detection of virus in secondary roots, and the occurrence of statistically higher ELISA values from test seedling roots also indicated that virus infections occurred in the seedling roots. These observations were comparable to those observed for wheat spindle streak mosaic virus (WSSMV) transmission, which also suggested that WSSMV transmission was independent of vector-inoculum dosage (11).

Although the occurrence of symptomless infections was the major hindrance in these experiments, it may provide an opportunity to determine the mechanism(s) for systemic invasion and symptom expression. In one test (R. Louie, *unpublished*) where root inoculum was applied to a 1–2 cm area of a test seedling root, recovery of virus was restricted to that inoculated area. Because virus must invade other tissues for systemic infection to occur, this suggested that the root infections were mostly localized, possibly limited to cortical tissues. Also suggestive of virus localization (perhaps also of differential rates of multiplication, or of more sites of infection) were the significantly higher ELISA values in assays of the basal, as compared to the apical, root portions of test seedlings. The development of systemic infections following embryo-wound inoculation supports the notion that the site of inoculation is important for systemic invasion and symptom development.

Even though the rates of transmission of MWLMV by embryo-wound inoculation (15) were low, inconsistent, and of limited use for bioassays, and plants inoculated with the hydroponic culture method do not produce disease symptoms, the two methods have specific advantages. Both methods should allow us to determine if systemic movement of MWLMV is dependent upon the inoculation site. Most promising of all, both methods should permit studies on the transmission mechanism of MWLMV in nature.

ACKNOWLEDGMENTS

We thank Ky Tan Lu, Mary Lou Hardesty, Jean Degen, and Cynthia Hardies for technical assistance and Bert L. Bishop for assistance in the statistical analyses.

LITERATURE CITED

1. Boothroyd, C. W., and Israel, H. W. 1980. A new mosaic disease of corn. Plant Dis. 64:218-219.
2. Brakke, M. K., Estes, A. P., and Schuster, M. L. 1965. Transmission of soil-borne wheat mosaic virus. Phytopathology 55:79-86.
3. Carmer, S. F., and Swanson, M. R. 1973. An evaluation of ten pairwised multiple comparison procedures by Monte Carlo methods. J. Am. Stat. Assoc. 68:66-74.
4. Conover, W. J. 1980. Practical Nonparametric Statistics. John Wiley & Sons, New York.
5. Conti, M., Luisoni, E., Caciagli. P., and Milne, R. G. 1980. A new isometric maize virus in northeastern Italy. Pages 9-13 in: Proc. Conf. Virus Dis. Gramineae in Europe, 3rd. Harpenden, U.K.
6. Kendall, W. A., and Leath, K. T. 1974. Slant-board culture methods for root observations of red clover. Crop Sci. 14:317-320.
7. Louie, R., Abt, J. J., and Knoke, J. K. 1989. Transmission studies of maize white line mosaic virus. (Abstr.) Phytopathology 79:1188.
8. Louie, R., Gordon, D. T., Knoke, J. K., Gingery, R. E., Bradfute, O. E., and Lipps, P. E. 1982. Maize white line mosaic virus in Ohio. Plant Dis. 66:167-170.
9. Louie, R., Gordon, D. T., Madden, L. V., and Knoke, J. K. 1983. Symptomless infection and incidence of maize white line mosaic. Plant Dis. 67:371-373.
10. Morrison, D. F. 1976. Multivariate Statistical Methods. 2nd ed. McGraw-Hill, New York. 415 pp.
11. Nolt, B. L., Romaine, C. P., Smith, S. H., and Cole, H., Jr. 1981. Further evidence for the association of *Polymyxa graminis* with the transmission of wheat spindle streak mosaic virus. Phytopathology 71:1269-1272.
12. Olah, A. F., and Schmitthenner, A. F. 1985. A growth chamber test for measuring Phytophthora root rot tolerance in soybean seedlings. Phytopathology 75:546-548.
13. Rao, A. S., and Brakke, M. K. 1969. Relation of soil-borne wheat mosaic virus and its fungal vector, *Polymyxa graminis*. Phytopathology 59:581-587.
14. Signoret, P. A. 1983. Maize virus disease in France. Pages 113-116 in: Proc. Int. Maize Virus Dis. Coll. and Workshop. D. T. Gordon, J. K. Knoke, L. R. Nault, and R. M. Ritter, eds. Ohio State Univ., Ohio Agric. Res. Dev. Cent. Wooster. 261 pp.
15. Zhang, L., Zitter, T. A., and Lulkin, E. J. 1991. Artificial inoculation of maize white line mosaic virus into corn and wheat. Phytopathology 81:397-400.