

Maize White Line Mosaic Virus in Ohio

RAYMOND LOUIE, Research Plant Pathologist, ARS/USDA; D. T. GORDON, Professor; J. K. KNOKE, Research Entomologist, ARS/USDA; R. E. GINGERY, Research Chemist, ARS/USDA; O. E. BRADFUTE, Professor; and P. E. LIPPS, Assistant Professor, Department of Plant Pathology and (third author) Department of Entomology, Ohio Agricultural Research and Development Center, Wooster 44691 and Ohio State University, Columbus 43210

ABSTRACT

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Maize white line mosaic virus (MWLMV) was detected in corn (*Zea mays*) and soil samples from Lake, Lorain, and Wayne counties in Ohio in 1979-1980. A spherical particle with a diameter of about 34-36 nm, buoyant density of 1.333 g/ml in cesium chloride, and a protein subunit molecular mass of $35,300 \pm 1,600$ daltons was associated with plants with disease symptoms. Serial transmission of MWLMV was successful with soil fractions and root inocula placed on greenhouse soil below seeds or seedlings. Transmission of MWLMV was reduced by benomyl applied as a drench. MWLMV was detected in pollen, anthers, silks, and seeds of infected plants, but seed transmission was not demonstrated in 10,288 seeds of diseased sweet and dent corn. MWLMV was detected in 15 of 22 symptomless plants. MWLMV was not transmitted by *Graminella nigrifrons*, *Macrostes fasciifrons*, *Empoasca fabae*, *Rhopalosiphum maidis*, or *Schizaphis graminum*. Yield loss and height reduction in MWLMV-infected plants averaged 44 and 21%, respectively. *Digitaria sanguinalis*, *Panicum dichotomiflorum*, and *Setaria faberi* were identified as MWLMV hosts.

Additional key words: corn virus disease, soilborne virus, soil transmission

Maize white line mosaic (MWLM) has been found in New York (1,2), Michigan (Gordon and B. P. Singh, *unpublished*), Wisconsin (5), and Vermont (6). In July 1979, J. Abt (*personal communication*) collected a corn (*Zea mays* L.) plant with unusual viruslike symptoms from Lake County, Ohio. Abundant isometric, viruslike particles were found in this plant by electron microscopy of leaf dip preparations. Plants with similar symptoms and isometric particles were also found in Lorain County in August 1979 by L. R. Nault (*personal communication*). Serologic tests (Gordon, *unpublished*) for maize chlorotic dwarf, maize chlorotic mottle, and maize rayado fino viruses (all isometric particles) were negative. These results, symptomatology, and the viruslike particles suggested that

this disease was new to Ohio and similar to MWLM described by Boothroyd and Israel (1,2) from New York. Serologic tests with antiserum to the Ohio isolate indicated a relationship to isolates from New York, Vermont (Gordon, *unpublished*), and Wisconsin (5). Based on this information, we assume that the isometric particle was the etiologic agent of MWLM and will refer to it as maize white line mosaic virus (MWLMV).

We report here the occurrence of MWLMV in Ohio, several of its characteristics, and our attempts to isolate and transmit it.

MATERIALS AND METHODS

Field surveys. In 1979, one field in Lake County (in July) and one field in Lorain County (in late August) were surveyed for MWLM. In 1980, eight fields of sweet corn (in May) in Lorain County, two fields of sweet corn (in May) in Erie County, and 14 fields including both sweet and dent corn (in July-August) in Wayne County were surveyed.

In fields measuring 1-1.5 ha, 200 plants along each edge-row at both ends of a field and 200 plants at each end of a center row bisecting the field were examined for MWLM symptoms. In larger fields, additional groups of 200 plants from rows dividing the field into three or four equal parts were examined. At survey times, plant heights ranged from 15-20 cm to 150-215 cm. Incidence of MWLM in corn was based on diagnostic symptoms (1). Identity of the pathogen in corn and weed hosts with viruslike symptoms was determined by serologic tests for some plants.

Pathogen purification and characterization. *Purification.* MWLMV-infected corn leaf tissue was ground in a blender with a phosphate-citrate buffer, pH 6.0, and 0.5% 2-mercaptoethanol (3 ml of buffer per gram of tissue). The buffer was made by adjusting 0.2 M K_2HPO_4 to pH 6.0 with 0.1 M citric acid (phosphate-citrate). Leaf homogenates were clarified by emulsifying with one-third volume chloroform. The aqueous phase was recovered after low-speed centrifugation (8,000 or 10,000 rpm for 15 min, GSA rotor), and the virus was sedimented by high-speed centrifugation (40,000 rpm for 90 min at 10 C in the Beckman Type 42.1 rotor) and resuspended in phosphate-citrate.

Concentrated virus was layered onto a 10-40% linear sucrose gradient in phosphate-citrate and centrifuged for 2.5 hr at 40,000 rpm in the Beckman SW 41 rotor. The virus zone was collected using an ISCO model 640 density gradient fractionator (Instrumentation Specialites Co., Lincoln, NE) and diluted threefold with phosphate-citrate. Solid cesium chloride (CsCl) was added slowly at 0 C until the density was 1.33 g/ml, and the resulting suspension was centrifuged at 36,000 rpm for 18-24 hr in the Beckman SW 50.1 rotor at 10 C. The virus zone was collected, and the virus was separated from the CsCl by high-speed centrifugation and suspended in phosphate-citrate. Virus at this stage is referred to as purified.

Buoyant density determination. Purified virus was adjusted to a density of 1.333 g/ml with solid CsCl and centrifuged at 35,000 rpm for 18-24 hr in the Beckman SW 50.1 rotor at 20 C. Densities of 0.4-ml gradient fractions were determined by weighing 50- μ l aliquots in a micropipette previously calibrated with water. Gradient density at the peak of virus-band absorbance was considered the virus density.

Polyacrylamide gel electrophoresis. Protein was released from purified virus by boiling for 1 min in 0.01 M sodium phosphate, 1% sodium dodecyl sulfate (SDS), and 1% 2-mercaptoethanol, pH 8.0. Reference proteins (bovine serum albumin: mol wt = 68,000; ovalbumin: mol wt = 43,000; pancreatic ribonuclease: mol wt = 13,700) were treated similarly. Electrophoresis was by the procedure of Weber and Osborn (11), except that 7.5% polyacrylamide gels were used. Gel tops

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were formed by trimming gels to 6 cm with a scalpel. Stained gels were scanned in an ISCO model 1310 gel scanner.

Electron microscopy. Expressed leaf sap or virus fractions from centrifuged sucrose gradients were negatively stained on Formvar-coated grids with 2% phosphotungstic acid neutralized to pH 7.0 with potassium hydroxide or with 2% ammonium molybdate.

Assays. Serology. The microprecipitin assay (MPA) was used to detect MWLMV in leaf and root tissues as described previously (7). Extracts for MPA were prepared by grinding tissue in physiologically buffered saline (PBS: 0.15 M sodium chloride + 0.02 M sodium phosphate + 0.02% sodium azide, pH 7.4; 1 g of tissue per 2–4 ml of PBS) in a mortar with a pestle. When roots were used, they were rinsed to remove soil and blotted dry before extraction. Extracts were filtered through two layers of fine-mesh cheesecloth, and the filtrate was centrifuged at 3,000 or 10,000 rpm for 10 min in the JA 20 rotor of the Beckman J21 centrifuge. The supernatant fraction (clarified extract) and two dilutions (1:2 and 1:4 in PBS) were tested. Antigen controls included extracts of leaves from MWLMV-infected corn plants (a New York or an Ohio isolate) and extracts from healthy corn plants. In all MPAs, MWLMV-infected corn extracts reacted positively and healthy corn extracts negatively.

The enzyme-linked immunosorbent assay (ELISA) for MWLMV was used for anthers, pollen, roots, seeds, silks, and leaves of corn and for leaves of other grasses. Plant tissues were ground in PBS-Tween (PBS containing 0.05% polyethylene sorbitan monolaurate [Tween 20]; 1 g of tissue per 1.6–7 ml of PBS-Tween) in a mortar with a pestle. The juice was transferred to duplicate wells (0.1 ml/well) of polystyrene microtiter plates (Dynatech Laboratories Inc., Alexandria, VA). Soil fractions were prepared and diluted with PBS-Tween (1:4) before ELISA.

Antiserum to MWLMV (Ohio isolate) was prepared by injecting a rabbit in the lymph nodes of hind legs with a total of 1.3 mg of purified virus at five intervals over a period of 32 days. The virus was suspended in 0.15 M sodium chloride and 0.01 M potassium phosphate, pH 7.0, and emulsified in an equal volume of Freund's complete adjuvant. About 1.0 ml of the emulsion was injected per lymph node. Antiserum used in MPA was collected 6 days after a total of 0.392 mg of virus had been injected (third injection), and γ -globulin for ELISA was purified from antiserum collected 2 mo after the fifth injection.

Purification of γ -globulin, conjugation of alkaline phosphatase with γ -globulin, and the ELISA procedure were performed according to the methods of Clark and Adams (4), except as

previously described (8). For coating microtiter plate wells, the γ -globulin concentration was 1 μ g/ml and the γ -globulin-enzyme dilution was 1:800. After addition of sodium hydroxide to terminate the reaction, the absorbancies of well contents were measured at 405 nm with a Gilford Stasar II spectrophotometer (Gilford Instrument Laboratories Inc., Oberlin, OH) equipped with a rapid sampling system for withdrawing well contents automatically into a microcuvette. Samples were judged positive for virus if the absorbance was greater than twice the mean value of uninfected control preparations.

Pathogen-vector isolation and transmission. Soil transmission. Soil samples (usually three sites from each of six fields) collected from Lake, Lorain, Scioto, and Wayne counties and classified as Red Hook, Haskins-Jimtown-Oshtemo, Fox-Genesse-Ockley, and Wooster-Canfield, respectively, were used in soil transmission studies. Samples were collected at a depth of 5–8 cm from areas adjacent to diseased plants or from plantfree areas where diseased plants had previously grown. In plantfree areas, samples were also collected at depths of 30–38 cm and 51–58 cm. The samples, totaling 35–105 L from each field, were combined according to collection depth, mixed in a rotary mixer, passed through 1-cm² hardware cloth to remove large debris and organic matter, placed in 10- or 15-cm pots, and planted with five seeds of captan-treated Seneca Chief sweet corn or, occasionally, inbred dent corn Oh28. Results were recorded after 10–12 wk.

Soil samples were serially passed through U.S. Standard Sieve Series numbers 8, 12, 25, 40, 120, and 400. A 0.25-g (dry weight) portion of each soil fraction was tested for MWLMV by ELISA, and the rest was mixed with sterile greenhouse soil and tested for the vector by planting seeds or transplanting 7- to 10-day-old seedlings of Seneca Chief sweet corn.

Soil samples were fractionated by low-speed centrifugation. About 900 cc of soil, including roots, was removed from 15-cm pots. The soil was placed into a pail with 1 L of water, shaken for 1 hr, and then loosened from roots by hand. The resulting mixture was passed through two layers of cheesecloth and centrifuged at 3,500 rpm for 60 sec in a Sorvall GSA rotor with a Sorvall RC2-B centrifuge. The supernatant was recentrifuged at 10,000 rpm for 10 min. Roots of Seneca Chief seedlings grown in 10-cm pots were rubbed gently with cotton swabs and soaked in the last supernatant fraction. Also, the pastelike mixture from the resuspended pellet was spread onto the root surface with a wooden pot label.

Plant tissue inocula. Juice from diseased leaves and roots, diluted 1:10 (w/v) with 0.01 M potassium phosphate buffer (pH 6.8), was poured into pots

containing corn seeds or 7- to 10-day-old seedlings. In another treatment, the juice and 600-mesh silicon carbide (0.25%) were sprayed onto roots of corn seedlings with an artist's airbrush at 6.3 kg/cm² of pressure. Surface-sterilized and unsterilized roots from diseased, greenhouse-grown corn plants were cut into 1–2 cm pieces and placed 1–2 cm below corn seeds or seedlings in 10-cm pots. Similarly, diseased leaf pieces (ca. 1 × 2 cm) were used as inocula. Isolation of vector and virus from roots was attempted using the "pie pan" extraction method for nematodes (9).

Insect transmission. Field collections of about 100 individuals each of *Graminella nigrifrons* (Forbes) and *Macrostelus fascifrons* (Stål) and 100–200 individuals of *Empoasca fabae* (Harris), *Rhopalosiphum maidis* (Fitch), and *Schizaphis graminum* (Rondani) (a laboratory biotype) were tested en masse as vectors of MWLMV. A detached MWLMV-infected leaf was maintained in a vial of water so that about 20 cm extended above the water surface. Test insects were placed in an insect cage measuring 38 × 38 × 19 cm with the inoculum source and 15 pots of 14-day-old Oh28 corn plants per pot and allowed an acquisition-inoculation access period of 10 days. The test was repeated three times for each insect species, and plants were checked for symptoms 31 days after inoculation.

G. nigrifrons was tested as a vector in long-term acquisition access periods (AAP). Three groups, one group at a time, of Oh28 corn seedlings (two 14-day-old plants per pot, 20–40 pots per test) were exposed to 400 *G. nigrifrons* and one MWLMV-infected plant for successive 1-wk periods.

Groups (five insects per plant) of *S. graminum* and *R. padi* (L.) were tested for nonpersistent transmission of MWLMV. Apterous aphids were starved for 1 hr in a petri dish, allowed a 5-min AAP in the whorl of an infected plant, and transferred individually with an aspirator to the whorl of an Oh28 test plant for at least an 18-hr inoculation access period.

Seed transmission. Ears from naturally infected sweet and dent corn were harvested at maturity, dried, and shelled, and the seeds were planted in steam-sterilized or methyl bromide-treated soil. Seedlings were observed for 8–12 wk for MWLMV symptoms. Seeds from symptomless plants collected from the field and seeds of Seneca Chief presumed to be virus-free were used as controls. Leaf tissues from seedlings suspected of virus infection were tested for MWLMV by ELISA.

Plant growth environment. Greenhouse temperatures were usually 27–31 C and 20–24 C during day and night, respectively. However, temperature extremes of 10 and 40 C occasionally occurred. During

July and early August, plants suffered water stress. In two growth chambers, temperature regimes of 32/21 C and 27/15.5 C were used with a 12-hr day/night cycle.

Yield loss and height reduction. Yield loss and height reduction were estimated from naturally infected field plants. Plant height was the distance from soil level to the base of the top leaf; yield was the weight of grain dried to about 10% moisture. Control values were those obtained from the first adjacent and second adjacent pairs of symptomless plants on both sides of an MWLMV-infected plant.

Control of soilborne vector. Soil was steam-treated in an autoclave at 1.1–1.2 kg/cm² for 6 hr. Chemical drenches (application rates per 15-cm pot) included benomyl (71 mg a.i./237 ml of water), fenaminosulf (57 mg a.i./118 ml of water), captafol (39 mg a.i./20 ml of water), mancozeb (40 mg a.i./10 ml of water), pentachloronitrobenzene (375 mg a.i./10 ml of water), and metalaxyl (19.5 mg a.i. placed on the soil surface and watered in). Carbofuran as 10% granules (13 mg a.i./pot) was mixed with soil that was used to fill the upper 2–3 cm of each pot. Except for carbofuran, chemical applications were repeated 1 and 2 wk later.

RESULTS

Disease incidence. In 1979, two sweet corn plants from Lake County (J. Abt, *personal communication*) and <0.1% of a sweet corn planting in Lorain County (L. R. Nault, *personal communication*) were identified by symptoms and serology as MWLMV infected. In 1980, MWLM was observed in 0.3 and 0.7% of the sweet corn plants surveyed in two of eight fields in Lorain County but not in two fields in Erie County. In Wayne County, MWLM was found in 10 of 14 fields in which disease incidence ranged from <0.01 to 6.7%. Except for two fields within 2 km of each other in which MWLM incidence was 2.4 and 6.7%, MWLM incidence was usually about 1–5/1,000–10,000 plants per field.

Symptoms and disease detection. Symptoms of MWLM in the field were similar to those described by Boothroyd (1). In the greenhouse, typical mosaic interspersed by characteristic white lines was not always present in all plants. In some plants, dark green spots (1 mm wide and 2–4 mm long) on a lighter green background were observed during the acute phase of disease. Later, however, the diagnostic white line symptoms appeared. MWLMV was detected serologically in plants with dark green spots.

Symptomless infections in the field were detected by ELISA. In three tests, MWLMV was detected in 15 of 27 mature, symptomless plants tested. Symptoms were still not observed in any

of these 27 plants when they were reexamined 6 and 13 days later. In these tests, MWLMV was detected in 18 of 18 plants with typical MWLM symptoms. In three tests involving greenhouse-grown plants, none of the 41 symptomless plants tested positive for MWLMV by serology, whereas six of six MWLMV-infected plants tested positive.

Alternate hosts. *Setaria faberi* Herrm. and *Panicum dichotomiflorum* Michx. were found naturally infected in the field. A volunteer plant of *Digitaria sanguinalis* (L.) Scop. growing in field soil placed in the greenhouse was infected. These weed hosts showed dark green mottle, and MWLMV infection was confirmed by ELISA.

Pathogen identification and characterization. *Physical properties.* The yield of MWLMV was 400–600 mg of virus per kilogram of fresh tissue, based on $E_{1\text{ cm}}^{0.1\%} = 3.9$ at 260 nm (5). Degradation of MWLMV resulted in a single protein species of mol wt = 35,300 ± 1,600. The buoyant density of MWLMV in CsCl in phosphate-citrate buffer was 1.333 g/ml.

Electron microscopy. Abundant isometric, viruslike particles were found in negatively stained preparations from leaves of 7 of 7 and 1 of 1 corn plants with MWLM symptoms from Ohio field collections in 1979 and 1980, respectively; from leaves of 4 of 4 corn plants with MWLM symptoms from experimental transmission; and from fractions from centrifuged gradients containing preparations from MWLM-diseased leaves collected from Ohio fields. The viruslike particles were 26–29 nm in diameter and could not be distinguished from viruslike particles found in negatively stained preparations of field-collected leaves with MWLM symptoms in 1979 from New York (15/15) and Vermont (5/5).

MWLMV transmission. *Soil transmission.* MWLMV was found in soil samples from 12 of 17 fields randomly selected in Ohio. MWLMV was found in 12 of 17 samples collected at the 5–8 cm depth, in 3 of 17 samples collected at the 30–38 cm depth, but in none of the 10 soil samples collected at 50–58 cm. Percentage of infection in test plants was erratic within a soil test unit (one pot) and among all units. In the 12 samples where MWLMV was found, percentage of infected plants among the survivors on a pot basis (14–50 pots per sample) ranged from 4.8 to 78.6%. In 17 soil samples, percentage of infected plants among the survivors on a soil sample basis ranged from 0 to 71.2%.

In one test with suspensions from a resuspended pellet or supernatant from high-speed centrifugation of soil obtained from 900 cc of soil and diseased roots, MWLMV was transmitted to 4 of 39 plants. MWLMV was not transmitted using the soil fraction obtained by centrifugation of the material that passed through the no. 400 sieve (0/16; one test);

it was also not detected by ELISA in the other soil fractions that were retained by nos. 8, 12, 25, 40, 120, and 400 sieves. MWLMV was not transmitted with the extract (0/107; one test) or pulp (0/118; one test) of diseased roots blended with water or with the leachate from field soil containing diseased plants (0/52; one test).

In two of six tests, MWLMV was transmitted when either surface-sterilized or untreated root inoculum was placed beneath seedlings or seeds (19/227 = number infected/total inoculated in positive tests). MWLMV was serially transmitted three times using diseased roots from a previous test as inocula for the next successive transfer. MWLMV was also maintained in the same infested field soil in the greenhouse through eight successive sweet corn plantings.

Mechanical transmission. In one test, MWLMV was not transmitted by rubbing leaves with leaf inocula buffered with phosphate (0/12 = number infected/number inoculated) or citrate-phosphate (0/12); with MWLMV-infected, crushed leaves (0/12); or with cut edges of infected leaf pieces (0/12). Airbrush inoculation of roots with root inocula was also not successful (0/52 in two tests).

Insect transmission. Mass inoculation by groups of 100 adult *G. nigrifrons* and *M. fascifrons* and groups of 100–200 adults and nymphs of *E. fabae*, *R. maidis*, and *S. graminum* resulted in 0/30 (number infected/number inoculated), 0/23, 0/26, 0/6, and 0/18 transmission, respectively. *G. nigrifrons* given long-term AAP's resulted in 0/16, 0/37, and 0/19 transmission when tested at 1, 2, and 3 wk, respectively. *S. graminum* and *R. padi* given a 1-hr starvation period and then a 5-min AAP resulted in 0/6 and 0/9 transmissions, respectively.

Seed transmission. MWLMV was detected by ELISA in pollen (4/7 = number positive/number tested) and silks (7/7) of diseased sweet corn grown in the greenhouse and in kernels (49/64 from diseased and 3/16 from symptomless plants) from field-grown sweet and dent corn. None of 10,228 seedlings from seed from 16 ears of infected sweet corn (2 of Silver Queen, 11 of Calico, and 3 of Bellringer) or 36 ears of infected dent hybrids (all from a mixed planting of Columbiana H-2420 and Funk's G-4224) showed symptoms of MWLM. In a control planting, none of 300 seedlings from presumably virus-free Seneca Chief nor the 454 seedlings from symptomless dent corn hybrid (field collected) showed MWLM symptoms during an 8- to 12-wk test period.

Environmental effects. MWLMV transmission in two tests involving two lots of the same soil at growth chamber temperatures of 32 C day/21 C night and 27 C day/15.5 C night were 14.1 and 28.6% (first test) and 56.2 and 41.2% (second test), respectively. The trans-

Table 1. Effect of chemical soil treatments on transmission of maize white line mosaic virus

Treatment	Transmission (%)		
	Soil 1 ^a		Soil 2 ^b
	Test 1	Test 2	
Control	56.3 a ^c	88.9 a	34.6 a
Metalaxyl		93.3 a	8.8 bc
Fenaminosulf	74.6 a	88.9 a	
Pentachloro-nitrobenzene			1.3 bc
Captafol			5.0 bc
Mancozeb			20.8 ab
Benomyl	0.0 c	22.2 b	0.0 bc
Carbofuran	23.5 b	55.6 ab	
Autoclaved field soil	0.0 c		
Autoclaved soil mix	0.0 c		

^aHaskins-Jimtown-Oshtemo soil; percentage based on an average of 83 and 15 plants per treatment for tests 1 and 2, respectively.

^bWooster-Canfield soil; percentage based on an average of 66 plants per treatment.

^cColumn values followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's new multiple range test (10).

mission rates were significantly different for the two temperature regimes in the first but not in the second test ($P = 0.05$).

Yield loss and height reduction. MWLMV infection in a field planting of mixed dent hybrids (Columbiana H-2420 and Funk's G-4224) reduced the yield by 44.6%. Mean weights (two groups of 36 sets) of the first adjacent pair and the second pair of symptomless plants in a row on both sides of the infected plants were 128.3 and 145.0 g for the first plants and 125.6 and 123.4 g for the second plants as compared with 72.4 g for the infected plants. The differences between diseased and healthy plants but not among the healthy plants were significant ($P = 0.05$).

In this same field, height reduction in MWLMV-infected plants averaged 21.3%. Means of 20 sets of first adjacent pairs of plants preceding and following an MWLMV-infected plant were 175.4 and 170.7 cm; for the second pair, means were 179.3 and 176.4 cm compared with

138.0 cm for the infected plant. The differences between diseased and healthy plants but not among the healthy plants were significant ($P = 0.05$).

Control of soilborne vector. Seven chemicals were tested for effect on MWLMV transmission in two soil types (Table 1). Percentage of transmission within and among pots and between tests was highly variable. In the first test with Haskins-Jimtown-Oshtemo soil, autoclaving or applying benomyl as a soil drench prevented transmission. Fenaminosulf or carbofuran did not prevent transmission, but carbofuran significantly reduced it. In the second test with the same soil (but previously untreated), metalaxyl, fenaminosulf, and carbofuran did not reduce transmission compared with the control. Benomyl reduced transmission but did not prevent it. In the trial with the Wooster-Canfield soil, benomyl prevented transmission; however, because of the variation between pots, percentage of transmission was not significantly different than that in soil treated with metalaxyl, pentachloro-nitrobenzene, captafol, or mancozeb.

DISCUSSION

Incidence of MWLM in Ohio was low in 1979 and 1980. The low incidence and first observations of MWLM at four locations where we had had test plots for 2-11 yr make it highly probable that MWLM is of recent origin. Recent introduction of MWLMV may have been caused by seed transmission (C. W. Boothroyd, *personal communication*). However, the possibility that the disease may have been present but unrecognized because of symptomless infection or coinfection with maize dwarf mosaic virus has not been excluded. Our discovery of symptomless, field-grown plants supports this possibility. In the past, confirmation of virus infection in plants with mosaic symptoms that relied on bioassays by mechanical transmission and use of maize dwarf mosaic virus antiserum would not have detected MWLMV.

Our results of characterization and biological studies of MWLMV generally

agree with previous reports (1,5). Transmission studies support the hypothesis of a vector/virus associated with soil and roots rather than with a leafhopper or aphid vector. Control of MWLMV transmission from soil with benomyl suggests a fungal vector (3). However, because of wide variations among treatments, replications, and experiments, the data are inclusive.

Our yield loss data show that MWLMV can cause significant economic losses in corn. Significant yield losses may have already occurred but were unrecognized because of symptomless infection.

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LITERATURE CITED

- Boothroyd, C. W., and Israel, H. W. 1980. A new mosaic disease of corn. *Plant Dis.* 64:218-219.
- Boothroyd, C. W., and Israel, H. W. 1980. A new mosaic of corn. (Abstr.) *Phytopathology* 70:459.
- Campbell, R. N. 1980. Effects of benomyl and ribavirin on the lettuce big vein agent and its transmission. *Phytopathology* 70:1190-1192.
- Clark, M. F., and Adams, A. M. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol.* 34:475-483.
- de Zoeten, G. A., Arny, D. C., Grau, C. R., Saad, S. M., and Gaard, G. 1980. Properties of the nucleoprotein associated with maize white line mosaic in Wisconsin. *Phytopathology* 70:1019-1022.
- Gottlieb, A. R., and Liese, A. L. 1980. White line mosaic and stunt of field and sweet corn in Vermont associated with polyhedral virus infection. (Abstr.) *Phytopathology* 70:462.
- Knoke, J. K., Louie, R., Anderson, R. J., and Gordon, D. T. 1974. Distribution of maize dwarf mosaic and aphid vectors in Ohio. *Phytopathology* 64:639-645.
- Nault, L. R., Gordon, D. T., Gingery, R. E., Bradfute, O. E., and Castillo, J. 1979. Identification of maize viruses and mollicutes and their potential insect vectors in Peru. *Phytopathology* 69:824-828.
- Thistlewayte, B. 1969. Hatch of eggs and reproduction in *Pratylenchus penetrans* (Nematoda: Tylenchida). Ph.D. thesis, Cornell University, Ithaca, NY. 166 pp.
- Waller, R. A., and Duncan, D. B. 1969. A Bayco rule for the symmetric multiple comparisons problem. *J. Am. Stat. Assoc.* 64:1484-1503.
- Weber, K., and Osborn, M. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* 244:4406-4412.