Symptomless Infection and Incidence of Maize White Line Mosaic

RAYMOND LOUIE, Research Plant Pathologist, ARS, USDA, D. T. GORDON, Professor, and L. V. MADDEN, Systems Specialist, Department of Plant Pathology, and J. K. KNOKE, Research Entomologist, ARS, USDA, Department of Entomology, Ohio Agricultural Research and Development Center and The Ohio State University, Wooster 44691

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Symptomless infections in sweet corn (Zea mays) from three field plantings with a history of maize white line mosaic (MWLM) were detected by enzyme-linked immunosorbent assays (ELISA) for the virus. Symptomless infections were detected similarly in two of three fields where MWLM was not previously found. The numbers of plants with symptomless infections in a sample of 15 consecutive plants within a row were 13/15, 11/15, and 7/15, and 4/15, 1/15, and 0/15 for three fields with and without a history of MWLM, respectively. Two plants with characteristic symptoms of MWLM were included in samples from each field with a known history of MWLM and were positive for maize white line mosaic virus (MWLMV) infection in ELISA. In experimental plantings, symptomless infections were detected by ELISA in root samples as early as 10 days after planting (DAP) and 100% were assayed positive by 55 DAP in two of four plantings. Symptomless shoot infections ($\sim 13\%$), based on ELISA of leaf samples, were detected by 40 DAP in the same two plantings. Disease incidences based on symptoms were low (<10%) for all planting dates except the third planting, where it reached ~20% level. Infections appeared to be more dependent on the time of season at which the plants were growing than on plant age. Detection of symptomless infection in plants and observations of plants with characteristic symptoms indicate that symptom development depends on some factor in addition to MWLMV infection.

The distribution of maize white line mosaic virus (MWLMV), which is presumably soilborne (2,5), includes Michigan (D. T. Gordon and B. P. Singh, unpublished), New York (1), Ohio (5), Vermont (4), and Wisconsin (2). Symptoms of maize white line mosaic (MWLM), often confirmed by serological assays, were the basis for disease identifications. Published accounts of symptomless infection in corn (Zea mays L.) have thus far only been reported from

Symptomless infections can significantly alter our perception of disease incidence. Determinations of the extent of symptomless infection in corn grown in areas with or without a history of MWLM and any associations between

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symptom expression, time of infection, and date of planting are therefore important considerations for diseaseincidence assessements. In this paper, we report our findings related to those studies. A short report has been published previously (6).

MATERIALS AND METHODS

Virus detection. Characteristic mosaic and white line symptoms on leaves (1) were used to determine disease incidence in field plants. Enzyme-linked immunosorbent assays (ELISA) for MWLMV were used on leaf and root tissue samples as described previously (5).

Tissue sampling from field and experimental plantings. On 10 and 17 August, 15 consecutive plants within a row of sweet corn hybrid (Candy Corn, Golden Sweet, Guardian, or Spring Gold) in the milk stage were dug from each of three fields with a known history of MWLM (Lorain County, two locations; Wayne County, one location). Two plants with obvious MWLM symptoms were included in each 15-plant sample. An additional 15 consecutive plants without MWLM symptoms were dug from fields (within 1 km of the above fields) that did not have a history of

For experimental planting, seeds of Seneca Chief sweet corn were planted in Wayne County at 20-cm intervals (3 seeds per hill) in plots 5 m long with four replicates on 21 and 31 May and 11 and 21 June. Distance between plot rows was 76 cm. Disease incidence in plots was

determined on 1, 16, and 29 July and on 6 and 19 August. On 14, 21, and 28 July and on 4 and 18 August, the first two plants in each plot were dug. All plants (both field and experimental plantings) were returned to the laboratory, where roots were washed free of soil and a top leaf and root sample taken for ELISA.

Data analysis. The percentage of plants infected based on symptoms or on ELISA of roots or shoots (leaves) was analyzed as a repeated-measures experimental design with four replicates. This design is analogous to a split-plot design. Planting date was the main effect (whole plot), and survey time was the repeated measure (split plot). Analysis of variance (ANOVA) was used to assess the effects of planting date and survey time and their interactions on MWLM incidence. Duncan's modified least significant difference test (7) was used to separate means when ANOVA indicated that a factor or its interaction was significant.

The association among MWLM incidences based on field symptoms and root or shoot infections based on ELISA was tested at each planting and survey date, using a Kendall-Tau correlation coefficient (3).

RESULTS

MWLMV infections in field plantings. Detection of MWLMV by ELISA in both roots and shoots (leaves) was independent of MWLM symptoms (Table 1). MWLMV was detected in roots more often than in shoots. MWLMV was detected in roots or shoots or in both roots and shoots. Symptomless infections were also detected in roots of 4/15 and 1/15 plants (number infected over total tested) collected from two of three fields without a history of MWLM. Both fields were located in Lorain County.

MWLMV infection in experimental planting. ANOVA indicated a significant (P = 0.05) effect of planting date and survey time as well as the survey X planting date interaction for disease incidence based on symptoms, ELISA of roots, and ELISA of shoots. Incidence based on symptoms generally increased over time for each planting date (Table 2). The significant interaction indicated that the increase was not equal for each planting date. The highest level of MWLM in the field was observed during the last two surveys of the third planting.

Table 1. Occurrence of maize white line mosaic virus (MWLMV) in sweet corn hybrids

	Corn	MWLM ^a	No. of plants ^b		No. of infected/total (ELISA)c			
Field	hybrid	history	MWLM	Healthy	Root	Shoot	Both	Total
1A	Candy Corn	+ +		13	13/13 2/2	13/13 2/2	13/13 2/2	13/13 2/2
1B	Guardian	-	-	15	4/15	0/15	0/15	4/15
2A	Golden Sweet	+ +		13	8/13 2/2	9/13 2/2	7/13 2/2	11/13 2/2
2B	Guardian	_		15	1/15	0/15	0/15	1/15
3A	Candy Corn (mixed seeds)	++	_ 2	13	7/13 2/2	2/13 2/2	2/13 2/2	7/13 2/2
3B	Spring Gold	_	_	15	0/15	0/15	0/15	0/15

^aMWLM history: + = corn fields in which MWLM was previously observed; - = MWLM not previously observed.

Table 2. Disease incidence and serological assay of maize white line mosaic virus (MWLMV)

Planting	Survey ^x	Plant age ^y	Percent MWLMV	Percent MWLMV ELISA assay ²	
date			(symptoms)	Root	Shoot
1	1	40	0.5 de	65.5 bc	12.5 d
	2	55	1.8 de	100.0 a	50.0 c
	3	63	1.4 de	100.0 a	100.0 a
	4	71	2.0 de	100.0 a	100.0 a
	5	84	3.6 cde	100.0 a	81.5 ab
Mean			1.9	92.5	72.5
2	1	30	0.0 de	100.0 a	12.5 d
	2	45	1.7 de	100.0 a	50.0 c
	3	53	6.7 bcd	100.0 a	65.5 bc
	4	61	5.7 bcde	100.0 a	81.5 ab
	5	74	8.8 bc	87.5 ab	75.0 abc
Mean			4.5	97.5	57.5
3	1	20	0.5 de	87.5 ab	0.0 d
	2	35	0.3 de	87.5 ab	0.0 d
	3	43	10.9 b	37.5 cd	0.0 d
	4	51	19.9 a	87.5 ab	87.5 ab
	5	64	18.6 a	100.0 a	75.0 abc
Mean			10.1	80.0	32.5
4	1	10	0.0 de	12.5 de	0.0 d
	2 3	25	0.0 de	0.0 e	0.0 d
	3	33	0.4 de	12.5 de	0.0 d
	4	41	2.2 de	87.5 ab	12.5 d
	5	54	3.6 cde	87.5 ab	0.0 d
Mean			1.2	40.0	2.5

^{*}Planting date: 1 = 21 May, 2 = 31 May, 3 = 11 June, 4 = 21 June.

This was largely attributed to the influence of one replicate where incidence reached 44.0%.

The percentage of MWLMV-infected roots was high for many survey dates, especially for the first three plantings. Detection of diseased plants by ELISA or by symptom expression both increased significantly with time. The lowest level

of root infection was generally associated with the fourth planting date.

The percentage of shoots infected by MWLMV in plants of the first and second plantings increased significantly during the first four surveys and then leveled off. Shoot infections in the third planting were detected in the last two surveys, and in the fourth planting, they

were detected during the fourth survey.

All correlations between disease incidence based on symptoms, ELISA of roots, and ELISA of shoot infections were insignificant (P>0.05). This indicated independence of the three variables.

DISCUSSION

Assessment of the prevalence and incidence of most plant diseases is dependent upon detection of characteristic symptoms. Our results indicate that detection of all MWLMV infections requires other techniques, eg, ELISA, in addition to symptom observations. In this study, MWLMV was detected in plants by ELISA from two of three randomly selected disease-free fields. These results indicate that fields in which MWLM was previously unknown are not necessarily free of diseased plants.

Plant infections appeared to be more dependent on time of season at which the plants were growing than on plant age. Most infections, as determined by ELISA, took place in early July and did not appear to be related to the date of planting.

Symptom development appeared to be dependent on other factors in addition to MWLMV infection. Localized occurrence of diseased plants in poorly drained field areas (1,2) and along edge rows indicated some factor, eg, environment, that might predispose infected plants to develop symptoms. Also, higher proportions of infection were detected earlier by ELISA than by symptoms. For example, 100% root infections were detected in plants by ELISA from the first and second plantings on 16 July, but it was not until 6 August that symptoms developed in $\sim 20\%$ of the plants from the third planting.

Present evidence of symptomless infections of MWLMV is documented by samples from a relatively limited geographical area and does not imply that symptomless infection is a common phenomenon in all areas where MWLMV

^bMWLM = plants with symptoms; healthy = plants without MWLM symptoms.

^cELISA = assays of root and shoot (leaf) tissues by enzyme-linked immunosorbent method.

^x Survey: 1 = 1 July, 2 = 16 July, 3 = 29 July, 4 = 6 August, 5 = 19 August.

y Plant age from date of planting.

² Assays by enzyme-linked immunosorbent method (ELISA). Column values here and under percent MWLMV survey followed by the same letter are not significantly different at $P \approx 0.05$, according to Duncan's modified least significant difference test (7).

occurs or in regions where MWLM symptoms have not yet been observed. Further studies on factors that might relate to symptom development after infection and the geographical distribution of MWLM in Ohio are under way.

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