Cucumber Mosaic Virus Infection of Tobacco Transplants and Purslane (*Portulaca oleracea*)

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ABSTRACT

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Plants of a cigar-wrapper tobacco cultivar with the local lesion response to tobacco mosaic virus developed systemic mosaic symptoms in the field and a greenhouse after seedling propagation in trays resting on crushed stone in a hoop-house. In the field, infected plants were sequential within rows and not in groups across rows. A virus with the morphology and antigenicity of cucumber mosaic virus (CMV) isolated from field plants with symptoms induced a mosaic when inoculated into squash and tobacco. The same virus was isolated from plants of purslane (*Portulaca oleracea*) and one plant of plantain (*Plantago major*) showing mosaic symptoms and growing as weeds in the crushed stone floor of the hoop-house. The tobacco plants probably became infected as seedlings, with purslane as a possible source of CMV. This is the first report of CMV in purslane in the United States.

Shade-grown cigar-wrapper tobacco cultivars in the Connecticut Valley carry a local lesion response to tobacco mosaic virus (TMV). When systemic mosaic symptoms appeared in a crop in June 1979, we suspected cucumber mosaic virus (CMV) (5). This report confirms that diagnosis and suggests that one source of CMV may have been purslane, a common weed not previously reported as a host to CMV.

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MATERIALS AND METHODS

Symptom distribution. The physical location of all plants with mosaic symptoms in approximately 0.16 ha of a commercial field of shade-grown cigar-wrapper tobacco was recorded on 28 June 1979. Plants had been in the field about 3 wk and were about 0.6 m tall. They had been propagated in trays (2) resting upon crushed stone in several of nine plastic-covered hoop-houses. Other tobacco plants from this propagation area that had been moved to and maintained in a greenhouse were also observed for mosaic symptoms.

Virus identification. Tobacco plants from the field and plants of two weed species, purslane (*Portulaca oleracea* L.) and plantain (*Plantago major* L.) found growing in the crushed stone floor of the

hoop-houses were examined for CMV by three procedures.

Electron microscopy. Leaf pieces were crushed in 0.01 M potassium phosphate (pH 7.2), and a drop of extract was placed on a Formvar-coated, carbon-fronted grid for 10 min. The grid was washed with 20 drops of 2% phosphotungstic acid (pH 3.0), and the final drop of negative stain was blotted with the edge of a piece of filter paper. Negatively stained grids were examined in a Zeiss EM9 S-2 electron microscope at a magnification of ×60,000.

Indicator hosts. Leaf pieces were ground with a mortar and pestle in 0.02 M potassium phosphate buffer (pH 7.2), 0.3% sodium thioglycollate, 0.1% sodium diethyldithiocarbamate, and 1% Celite and inoculated to squash (*Cucurbita pepo* 'Early Prolific Straightneck') and to tobacco (*Nicotiana tabacum* 'Windsor Shade 117'); this tobacco cultivar reacts hypersensitively to TMV.

Immunodiffusion tests. Buffered extracts from leaves were reacted against CMV antibody in immunodiffusion tests in 0.7% agar. The buffer used was 0.005 M borate (pH 8.5), 0.005 M EDTA, and 0.02% sodium azide (3).

RESULTS

Symptom distribution. Mosaic symptoms were observed in 11.4% of the tobacco plants in the selected area of the field. The plants had been infected for some time, since all leaves showed symptoms. Groups of three to nine infected plants were often in sequence within a row, with no counterpart grouping in adjacent rows (Fig. 1). About one-fourth of the rows were free of symptomatic plants. The pattern suggested that infected transplants had been set and that some transfer of infection occurred during the transplanting procedure.

Mosaic symptoms developed in some of the tobacco seedlings that had been transferred from the seedling houses to the experimental greenhouse. Seedlings from other sources remained symptomless.

Purslane was the dominant weed in the nine hoop-houses where seedlings were propagated. Mosaic symptoms were observed in more than 10% of the purslane plants in five of the houses. The purslane plants in the other four houses were symptomless. A single plantain plant with mosaic symptoms was also observed.

Virus identification. *Electron microscopy.* Small spherical particles with diameters of 30 nm were detected in sap from tobacco and purslane plants with mosaic symptoms (three samples of each were tested). The cores of the particles were penetrated by negative stain, the expected result for CMV (6). Rod-shaped particles were not observed in these samples. Particles were not detected in sap from symptomless plants (three samples of each were tested).

Indicator hosts. Sap from symptomatic tobacco and purslane plants was infectious and induced mosaic symptoms in squash (10 samples of each were tested) and a mosaic in Windsor Shade 117 tobacco but no local lesions (two samples of each were tested). The symptoms on squash and tobacco were typical of those induced by local isolates of CMV. Mosaic symptoms in squash were also induced by the extract from plantain. Sap from symptomless tobacco and purslane induced no symptoms in squash (10 samples of each were tested).

Immunodiffusion tests. Sap from field-collected tobacco and purslane plants

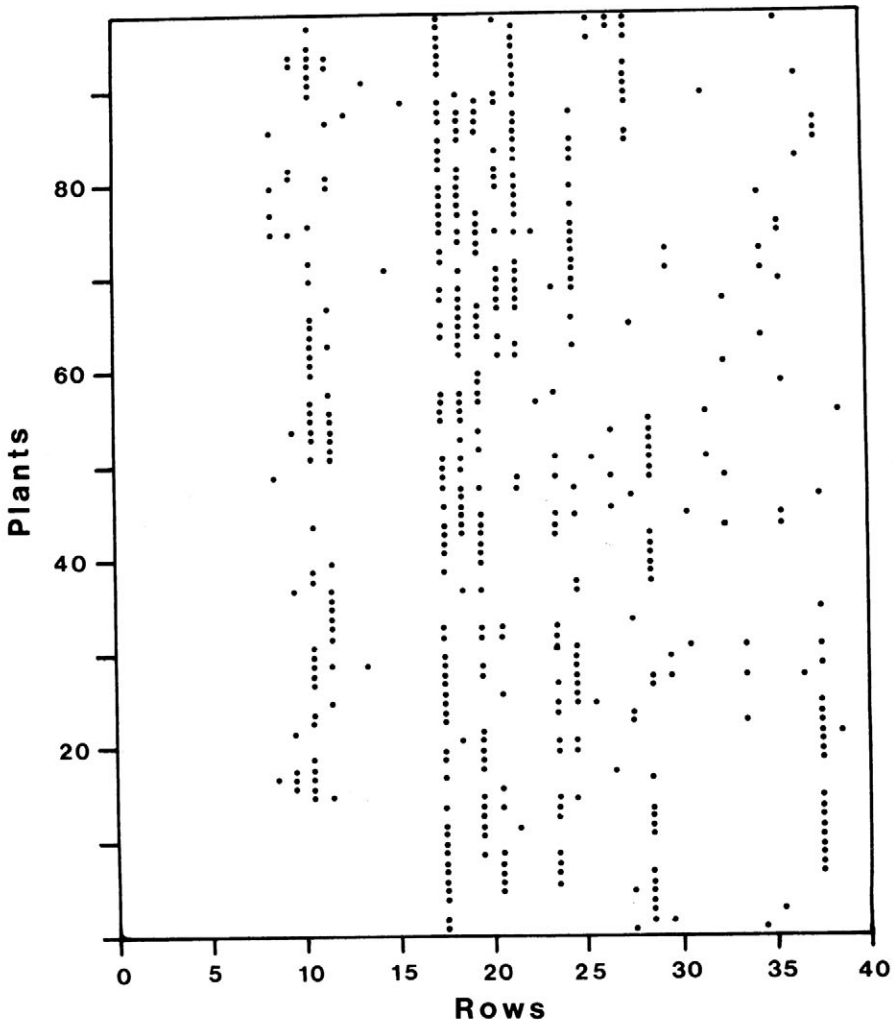

Fig. 1. Distribution of plants with mosaic symptoms in part of a field of shade-grown tobacco.

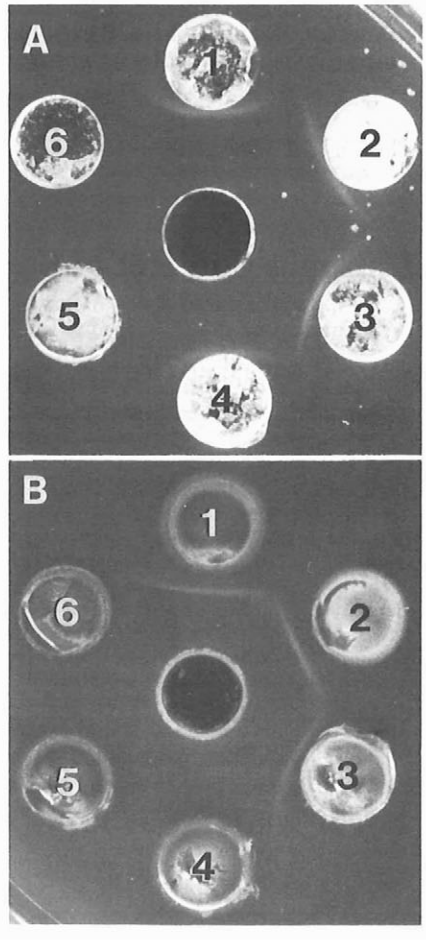

Fig. 2. Reaction of cucumber mosaic virus antiserum (center wells) with **(A)** buffered sap from purslane leaves showing mosaic symptoms (1–4) or from symptomless leaves (5 and 6) and with **(B)** buffer (5) or buffered sap from squash either noninoculated (4 and 6) or inoculated with sap from tobacco (1), purslane (2), and plantain (3) with mosaic symptoms.

with mosaic symptoms reacted positively with CMV-Ab (eight samples of each were tested). Sap from symptomless plants failed to react with CMV-Ab (two samples of each were tested). Sap from squash plants showing symptoms after inoculation with sap from field-collected tobacco, purslane, and plantain reacted positively with CMV-Ab (Fig. 2).

DISCUSSION

Mosaics in shaded tobacco have not been common in Connecticut since the introduction of hypersensitivity to TMV into commercial cultivars. Primary infection by aphid transmission in the field is an unlikely explanation for the linear and disjunct distribution of CMV-infected plants in our study. The distribution is consistent with secondary plant-to-plant manual transmission before or at transplanting time. Plants appear to have been infected at that time because some developed symptoms in an experimental greenhouse in which new infections to other plants did not occur.

The spread of CMV into or between the annuals purslane and tobacco is not, however, resolved by our study. Infected annual weeds or escapes could not have survived the winter in the gravel beds that remained exposed until March, when the hoops were covered with plastic and the houses were heated. When infected plants were brought to our attention in June, it was too late to test whether CMV entered the houses from an external source or from perennial weeds, such as plantain, growing in the gravel beds. Another possibility, now being tested, is that CMV is seed-transmitted in purslane. The discontinuous distribution of both the infected purslane plants among the hoop-houses and the infected tobacco plants in the field suggests that a buildup in the number of infected plants occurred in only some houses. Purslane probably played a role in this buildup, since it was the dominant weed and was susceptible to CMV. The absence of such a buildup in other adjacent houses, even though purslane was present and exposure to external sources was the same, suggests that the important reservoirs of CMV may have been internal ones.

The use of hoop-houses for the propagation of tobacco seedlings is a recent development in Connecticut (2). Our observations indicate that without effective weed and insect control this practice could lead to increased incidence of CMV in the field crop.

The presence of CMV in purslane has been reported from Japan (4). Bruckart and Lorbeer reviewed reports of CMV in weeds in North America (1), but purslane and plantain were not mentioned as hosts, even though purslane was tested. The incidence of purslane with mosaic symptoms was sufficiently high in our study to indicate that this common weed may be an important overlooked, or under-reported host of CMV.

ACKNOWLEDGMENT

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