Persistence of Tomato Mosaic Virus in Tomato Debris and Soil Under Field Conditions

J. M. LANTER, Graduate Assistant, and J. M. McGUIRE and M. J. GOODE, Professors, Department of Plant Pathology, University of Arkansas, Fayetteville 72701

ABSTRACT

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The fruit necrosis strain of tomato mosaic virus (ToMV-FN) was recovered from tomato debris and soil in an experimental field where tomato infected with ToMV-FN had been grown the previous year. Healthy tomato transplanted to that field the following year became infected and developed typical fruit necrosis symptoms. ToMV was also recovered from debris and soil collected from fields in southeast Arkansas where fruit necrosis had occurred in Pinkwrap tomato from 1978 seed; the disease recurred the following year. Cultural practices affected the amount of debris present and disease recurrence, and in general, less tillage resulted in higher disease incidence. The effects of tillage and of a winter wheat cover crop were tested in a field where tomato uniformly infected with ToMV-FN had been grown. ToMV-FN was recovered from some plants in plots that received one tilling before healthy tomato seedlings were transplanted into them. One plant was infected in a plot that had a wheat cover crop. Environmental conditions were severe and atypical that season, and no fruit necrosis symptoms were observed. Seed transmission of ToMV-FN was demonstrated. ToMV-FN was serologically related to but different from tobacco mosaic virus and a commonly occurring "Purple" strain of ToMV (ToMV-P). Serologic tests indicated that mixed infections of ToMV-P and ToMV-FN occurred in plants from southeast Arkansas fields. This was also demonstrated in tomato mechanically inoculated with a mixture of the two strains.

The fruit necrosis strain of tomato mosaic virus (ToMV-FN) was first described in 1978 from tomato (*Lycopersicon esculentum* Mill. 'Pinkwrap') grown in the fresh-market production area of southeast Arkansas (15). Fruit symptoms caused by ToMV-FN are unlike those caused by other strains of ToMV described by Rast (18). Lesions on immature infected fruit appear water-soaked and later become necrotic (Fig. 1). They may resemble lesions reportedly caused by tobacco mosaic virus (TMV) on tomato in Canada (1). Mild mosaic is the usual foliar symptom. Other strains of ToMV causing mild mosaic but no fruit necrosis, and a strain designated "Purple" (ToMV-P) (14) that causes severe leaf curling and purple pigmentation, are commonly found in Arkansas.

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Initial occurrences of fruit necrosis were correlated with use of a particular seed source (16), suggesting that the virus was introduced into Arkansas on the seed. ToMV has been shown to be seed-transmitted (4).

The ease of mechanical transmission of ToMV has made diseases caused by one or more strains of this virus endemic to most fresh-market tomato-growing areas, reducing yield significantly and causing economic loss (2,10). Moreover, infectious virus persists on clothing, tools, and greenhouse structures (5) and in debris-contaminated soil, providing a source of infection for subsequent crops (3,6,7,11,12,14,19).

Broadbent et al (6) recovered infectious ToMV from tomato debris in a field after 22 mo of fallow. Johnson (11) found that tobacco debris contained infectious TMV 2 yr after a tobacco field had been abandoned. Johnson and Ogden (12) suggested that TMV-contaminated debris breaks down faster in moist, well-aerated soils. Lehman (14) found that either removing tobacco debris or disking in fall and spring was an acceptable method of eliminating TMV in sandy loam soil.

Preliminary observations in tomato fields in southeast Arkansas suggested that cultural practices promoting decomposition of tomato debris reduced occurrence of fruit necrosis in the succeeding crop. Therefore, research was initiated to determine how long ToMV-

FN persists in the soil under field conditions in Arkansas, study the effects of cultural manipulations on persistence of the virus, and associate occurrence of the disease with seed source or overwintering of the virus in tomato debris or both. A preliminary report has been published (13).

MATERIALS AND METHODS

Presence of virus in live plant material or plant debris was determined by inoculating *Chenopodium quinoa* Willd. and tobacco (*Nicotiana tabacum* L. 'Xanthi nc' selection), which developed necrotic local lesions in reaction to all isolates of ToMV and TMV (ATCC-221), and Traveler-76 (T-76) tomato, which was a systemic host.

In July 1978, T-76 tomato plants were transplanted into an experimental field and inoculated with isolates of ToMV from three fields in which the fruit necrosis disease had first appeared (15). Fruit necrosis symptoms occurred on most of these plants. From February through June 1979, samples of tomato debris were collected from this field at monthly or bimonthly intervals and indexed. Three samples of debris, either stem or root, were taken from each of eight rows in February, March, and April. Two root and stem debris samples were collected from each of four rows in June. Fruit debris was also collected in February through April. Virus isolates recovered from debris were checked serologically in Ouchterlony double-diffusion tests in 0.75% agarose, using intragel absorption with healthy sap.

Soil samples were taken from each site where debris was collected. Healthy T-76 seedlings were transplanted to 250-ml beakers containing portions of each soil sample. For controls, beakers of soil were autoclaved for 30 min at 129 C before receiving the transplant. Presence of ToMV was determined by occurrence of mosaic symptoms in tomato plants or by indexing foliage and roots to tobacco after 6–8 wk of growth.

Half of the 1978 field (four rows) was disked in May 1979, and healthy tomato seedlings were transplanted into it as a test for root acquisition of virus from soil or debris. Because no attempt was made to prevent movement of virus between plants, virus present in any one plant could have been moved to others during pruning and tying procedures.

In 1979, tomato debris and soil samples collected from southeast Arkansas fields where fruit necrosis had appeared in 1978 were indexed. Fruit and foliage samples taken in 1979 and 1980 from plants showing fruit necrosis symptoms in southeast Arkansas fields were also indexed on the three indicator hosts. Virus isolates were tested serologically.

In 1978 and 1979, attempts were made to isolate virus from commonly occurring weeds in fields where fruit necrosis occurred in tomato.

A second experimental field was planted in 1979 with T-76 tomato, and the plants were inoculated with a selected isolate of ToMV-FN that had been propagated in tomato. In fall 1979, the field was divided into 16 plots (4 × 4.5 m) in which four cultural practices were replicated four times each. The treatments were 1) tilling three times in the fall and three times in the spring, 2) tilling three times in the spring, 3) tilling once in the fall before planting winter wheat and once in the spring before transplanting tomato, and 4) tilling once in the spring before transplanting tomato. The Rototiller was scraped free of soil and sprayed with a 20% solution of household bleach (1% sodium hypochlorite) between each treatment. Soil was tilled to a depth of about 15 cm.

In May 1980, 12 T-76 plants were set 60 cm apart in two rows in each plot, giving a total of 48 plants per treatment. Fifty plants were set 60 cm apart in the control row located 4 m from the plots in soil where tomato had not been grown the previous year. Each plant was separately staked, pruned to two stems, and tied. Workers washed their hands in a 20% bleach solution between plants, and stakes were dipped in a 20% bleach solution. Hand-weeding, hoeing, tilling, watering, and picking were performed as needed, with care to avoid mechanical transmission of ToMV from possible virus-infected plants to healthy plants.

Foliage samples were taken from the apexes of these plants 5, 8, 11, 14, and 17 wk after transplanting. A root sample was taken from each plant after 20 wk. Samples were indexed, and recovered virus was transferred to tomato for serologic testing.

T-76 tomato seeds, extracted from fruit with necrotic symptoms and shown to be roughly 100% infested with ToMV-FN (16), were germinated and transplanted at the cotyledonary stage to determine seed transmissibility of ToMV-FN. Twenty seedlings from a noninfested seed source were transplanted and indexed as controls.

ToMV-FN and ToMV-P were purified from tomato by a modified chloroform-butanol procedure (20). Antiserum to each was prepared by injection into rabbits. The two strains were compared serologically and with TMV (ATCC-221) in Ouchterlony double-diffusion tests.

Tomato seedlings were inoculated in the greenhouse with ToMV-FN, ToMV-P, or a combination of both strains to determine whether a mixed infection could occur and how it would react serologically to each antiserum. A purified preparation of ToMV-FN was negatively stained with 1% phosphotungstic acid and examined by electron microscopy.

RESULTS

Serology and electron microscopy. ToMV-FN, ToMV-P, and TMV were serologically related but distinct when reacted with antiserum to each in Ouchterlony double-diffusion reactions. Electron micrographs of purified ToMV-FN showed typical rods.

Recovery of ToMV from debris, soil, and weeds. ToMV was recovered from tomato root and stem debris collected from the 1978 experimental field in February through June 1979 (Table 1). All root samples contained virus, but the percentage of infectious stem samples decreased with each sampling period. Root and stem debris were collected from the same plants in June; virus was recovered from all roots but from only 25% of the stems. Virus was also present in fruit debris collected in February and March but not in April; no fruit debris could be found in June. March was dry and windy, causing desiccation of surface debris and perhaps contributing to loss of virus. Serologic tests showed that 52% of the ToMV isolates recovered from root

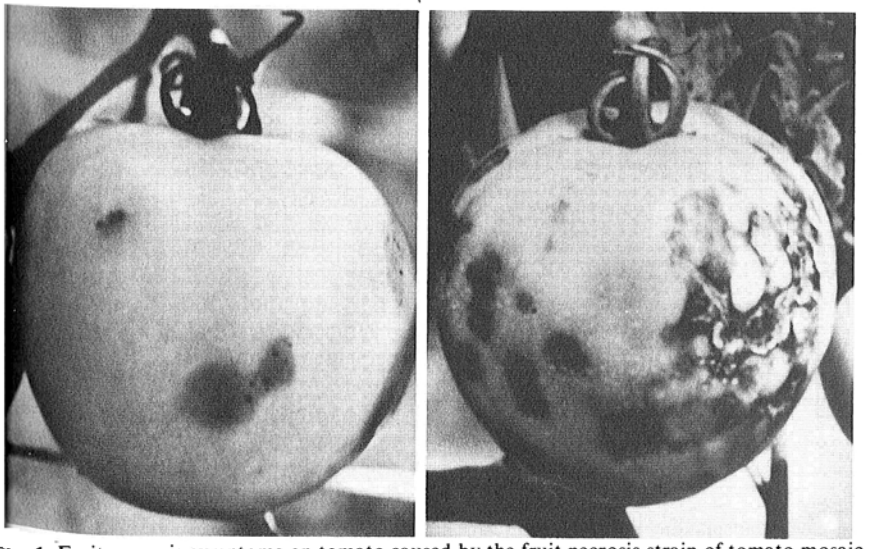

Fig. 1. Fruit necrosis symptoms on tomato caused by the fruit necrosis strain of tomato mosaic virus: water-soaked appearance of lesions on immature fruit (left); necrotic fruit lesions (right).

or stem debris and 13% of those from fruit debris were ToMV-FN.

Healthy T-76 seedlings transplanted to soil collected in February through April 1979 from the 1978 experimental field became infected with ToMV (Table 1). Mosaic symptoms typical of ToMV infection developed in some plants, and ToMV was recovered from foliage or roots of these plants and others not showing symptoms. Serologic tests showed that 56% of the virus isolates were ToMV-FN.

ToMV was recovered from debris collected from two of the four southeast Arkansas fields (fields 1 and 4) sampled in 1979 that had had fruit necrosis in plants grown from Pinkwrap tomato seed the previous year (Table 2). Both fields had been disked once in the spring. A small amount of debris was collected from field 3, which had been disked several times in fall and spring, but no virus was obtained from that debris. No debris could be found in field 2, which had been disked once in the fall and had had a winter wheat cover crop. Virus was also recovered from one soil sample from field 1 but not from soil samples from fields 2, 3, or 4 (Table 2). None of the ToMV recovered from debris or soil from southeast Arkansas was identified as ToMV-FN.

No virus was recovered from any weeds collected from fields where ToMV occurred.

Recurrence of fruit necrosis. A mild foliar mosaic and typical necrotic fruit lesions developed on some of the healthy tomato seedlings transplanted into half of the 1978 experimental field in May 1979. ToMV was recovered from both foliage and fruits, and several isolates were identified serologically as ToMV-FN.

In 1979, fruit necrosis also recurred in southeast Arkansas. Only two of the four fields (3 and 4) in which the disease had originally occurred were planted to tomato in 1979 (Table 2). Of these, field 4 had a severe outbreak after minimum tillage, while field 3 had a very low incidence of fruit necrosis after being disked six times before tomato seedlings were transplanted.

In 1980, field 2 was replanted with tomato following a winter wheat cover crop and corn in 1979; no fruit necrosis occurred. Tomato was grown in field 3 again in 1980, without any recurrence of the disease. Tomato was not grown in fields 1 and 4 in 1980.

Plants in a fifth field reportedly had fruit necrosis in 1978 and 1979. Recurrence of the disease was documented in 1980 when virus was recovered from 10 samples, and all were identified serologically as ToMV-FN or a mixture of ToMV-FN and ToMV-P. Pinkwrap seed of the 1978 seed lot had been used in the field in 1978.

Effects of cultural practices. Tomato plants in the second experimental field developed foliage and fruit symptoms after being inoculated with ToMV-FN in 1979. Foliage samples taken from two plants in each of the 16 plots contained ToMV-FN.

ToMV was recovered from nine of 189 tomato transplants in the 16 plots in 1980. In the 11th wk after planting, virus was recovered in low titer from eight foliage samples—four from each of two replicates of the once-tilled treatment. Foliage samples collected during the 14th and 17th wk and foliage and roots collected during the 20th wk from these same eight plants did not yield virus when indexed. Virus was recovered from roots of one plant in the winter wheat cover crop treatment. Serologic tests showed that seven of the nine isolates were ToMV-FN.

Seed transmission and new occurrences. In the seed transmission study conducted in the greenhouse, two of 77 tomato plants were infected with a virus identified serologically as ToMV-FN.

All four southeast Arkansas fields in which fruit necrosis first appeared in 1978 had been planted with Pinkwrap tomato. In 1979, fruit necrosis occurred in eight additional fields, six of which had been planted with tomato from 1978 Pinkwrap seed. Pinkwrap had been grown in one of the other two fields in 1978. The eighth field had been pruned by laborers who also worked in one of the original four fields and who could have moved the virus on their hands, clothing, or machinery. New infections in 1980 also coincided with the use of 1978 Pinkwrap seed.

Serologic identification of mixed infections. Sap from greenhouse-grown tomato inoculated with ToMV-FN or ToMV-P reacted as FN and P, respectively, against both antisera. Sap from plants inoculated with a mixture of the two viruses reacted homologously with FN

Table 1. Recovery of tomato mosaic virus (ToMV) in 1979 from tomato debris and soil from an experimental tomato field infected with the fruit necrosis strain of ToMV (ToMV-FN) in 1978

Collection date Virus strain[a]	Tomato debris[b] Total samples	Root samples	Stem samples	Fruit debris[b]	Soil samples[b]
February 1979					
ToMV	23/24	16/16	7/8	1/2	5/24
ToMV-FN	12/23	10/16	2/7	0/1	4/5
March 1979					
ToMV	21/24	16/16	5/8	7/8	2/24
ToMV-FN	12/21	10/16	2/5	1/7	1/2
April 1979					
ToMV	18/24	16/16	2/8	0/8	11/24
ToMV-FN	11/18	10/16	1/2	0/0	5/11
June 1979					
ToMV	8/8	8/8	2/8[c]	NS[d]	0/8
ToMV-FN	2/8	2/8	1/8	NS	0/0

[a] ToMV-FN was identified serologically.
[b] Debris was ground in phosphate buffer, pH 7.2, and inoculated onto tobacco, *Chenopodium quinoa*, and tomato. A healthy tomato was transplanted into each soil sample, and leaves and/or roots were indexed 8 wk later. Results are expressed as number of samples from which ToMV was recovered/total number of samples.
[c] Root or stem debris was taken from 24 plants in February through April; in June, root and stem samples were taken from each of eight plants and were indexed separately. Therefore, June stem samples were not added into the total because they were from the same plants as root samples.
[d] NS = no sample.

Table 2. Cultural practices and recurrence of fruit necrosis strain of tomato mosaic virus (ToMV-FN) in tomato in southeast Arkansas fields

Year	Field 1	Field 2	Field 3	Field 4
1978[a]	ToMV-FN	ToMV-FN	ToMV-FN	ToMV-FN
	disked once (spring)	disked once (fall); planted winter; wheat cover crop	disked six times (spring and fall)	disked once (spring)
1979	debris and soil with ToMV[b]	no debris	little debris and no ToMV[b]	debris with ToMV[b]
	no tomato grown	no tomato grown	tomato grown and little ToMV-FN	tomato grown and severe ToMV-FN
1980	no tomato grown	tomato grown and no ToMV-FN	tomato grown and no ToMV-FN	no tomato grown

[a] Plants were grown from 1978 Pinkwrap tomato seed.
[b] Virus was recovered from debris by indexing and from soil by transplanting a healthy tomato and indexing it after 8 wk. ToMV-FN was distinguished from other strains of tomato mosaic virus (ToMV) serologically.

but spurred with P against ToMV-FN antiserum and gave the reciprocal against ToMV-P antiserum.

In 1980, all 29 samples of plants showing fruit necrosis symptoms collected from three southeast Arkansas fields contained ToMV. In serologic tests, 10 reacted as mixed infections of ToMV-FN and ToMV-P, and 19 reacted as ToMV-FN.

DISCUSSION

Recovery of ToMV from debris and soil in both the 1978 experimental field and the southeast Arkansas fields through the beginning of the next growing season indicates that virus was available as inoculum for healthy transplants. Infection of healthy tomato grown in debris-contaminated soil was demonstrated in the greenhouse and an experimental field and also apparently occurred in some southeast Arkansas fields. Other workers have reported similar findings (3,6,12,14). Inoculation might occur at the time of transplanting, through root wounds incurred during normal root growth, or perhaps by lower leaves contacting infected debris on the soil surface. Gooding (8) demonstrated that infection of tobacco with TMV occurred at transplanting. The failure to isolate ToMV from weeds indicates that infected tomato debris is the source of recurring virus infections in these Arkansas fields. Horsenettle has been shown to be an important host of TMV in North Carolina (8) but was not a common weed in tomato fields with fruit necrosis.

If even one plant in a commercial tomato field becomes infected from contaminated soil or seed, the handling required during fresh-market tomato production can rapidly transmit ToMV to adjacent plants and throughout the field. With a disease as severe as fruit necrosis, even a low rate of infection from contaminated soil or infested seed must be avoided. The 2.6% rate of transmission from roughly 100% infested seed would no doubt be lower if less than 100% of the seed were infested; nevertheless, secondary spread by mechanical transmission is so easy that no risks taken with infested seed can be justified. Effective seed treatments have been investigated by Gooding (9) and Broadbent (4) and were found to eliminate virus from the seed source mentioned above (16).

The experiences of the four southeast Arkansas growers who reported fruit necrosis in 1978 suggested several cultural practices for eliminating contaminated debris from soil. Tillage and/or use of a cover crop hastened decomposition of debris and resulted in reduced fruit necrosis in the following tomato crop. The cultural practices chosen for the experimental plot study resembled those used by growers and were aimed at eliminating debris. Results of this experiment followed the trends we had predicted. With the exception of one plant in the winter wheat plots, virus was recovered only from plants in the plots that had been tilled the least. All plants in the control row and in plots that had been tilled three or more times remained healthy.

Extreme environmental conditions during the summer of 1980 most likely affected viral infection. Temperatures averaged 5 C above normal, and rainfall was significantly below average during July, August, and September. Fruit set occurred only in the first two clusters during the first 6 wk of growth. This is a marginal amount of time for root infection and translocation of virus (3). We do not know what effect the lack of fruit set may have had on viral infection and disease development.

Another unknown is the effect consistently high temperatures have on persistence of ToMV-FN in debris and acquisition of virus through tomato roots. Heat cure (17) of some plants that became infected could also have occurred. Heat cure or an undetectably low virus titer seem to be the most likely explanations for failure to recover virus from the eight plants that had yielded a low titer of virus in the 11-wk foliage sample. Although debris and soil sampling of the 1978 experimental field indicated that ToMV persisted through June in all root debris samples and could infect healthy transplants, it is improbable that root debris remained infected as long under the extreme environmental conditions of 1980.

Serologic reactions of 1980 southeast Arkansas virus isolates were difficult to interpret, because several isolates gave homologous reactions with both ToMV-FN and ToMV-P against their respective antisera. Comparing these reactions with those of sap from tomato inoculated in the greenhouse with both strains simultaneously suggests that mixed infections of ToMV-FN and ToMV-P occurred in southeast Arkansas fields.

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