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Translocation of Tobacco Ringspot Virus in Cucumber and Zinnia[1]

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Tobacco ringspot virus (TRSV) is effectively transmitted to the roots of several species of plants by the American dagger nematode, *Xiphinema americanum* Cobb (2, 4). Since demonstrated effect of TRSV on host plants has been on above ground parts of the plant, translocation of the virus from points of inoculation by the nematode vector to other parts of the plant is important. Fulton (3) found that TRSV, as well as several other viruses, moved downward from points of inoculation of tobacco and tomato roots much more rapidly than they moved upward. Bergeson, et. al (1) reported limited movement of TRSV from inoculated roots to tops of soybean plants.

In initial tests of nematode transmission of TRSV, differences in translocation of the virus from roots to tops of cucumber and zinnia became evident (J. P. Fulton, personal communication). Studies were thus initiated to determine patterns of translocation of TRSV from roots to tops of cucumber and zinnia and subsequently to determine whether restricted translocation to tops of zinnia could be accounted for by lack of translocation in the roots following transmission by *X. americanum*.

MATERIALS AND METHODS

The TRSV used in these tests was a yellow strain used by Fulton (2) and McGuire (4). Nematodes were obtained from a soilbed in the greenhouse cropped with Sudan grass [*Sorghum vulgare* Pers. var. *sudanense* (Piper) Hitchc.]. Cucumber (*Cucumis sativus* L. 'Model') and zinnia (*zinnia elegans* Jacq. 'Will Rogers') were used as test plants for nematode transmission and study of virus translocation.

Nematodes were handled essentially as described by McGuire (4). Virus-free nematodes were screened from soil, handpicked, and washed into sterilized fine river sand in which TRSV-infected cucumber was growing. The plants were kept at 28°C under fluorescent lights. After an acquisition access period of at least 7 days, nematodes were removed from the sand by screening and active nematodes were handpicked and added to root zones of healthy cucumber or zinna test plants growing in sterilized fine river sand in pots or

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cups without holes. All test plants were held at 28°-30°C soil temperature in a heated sandbed in the greenhouse.

Blackeye cowpea (*Vigna sinensis* L. 'Monarch') and cucumber were used as indicator plants to detect virus infection. Portions of test plants were ground in 0.05 M phosphate buffer, pH 7.0, and rubbed on primary leaves of cowpea and cotyledons of cucumber seedlings after dusting with 600 mesh Carborundum powder. Presence of symptoms of TRSV on indicator plants (local necrotic lesions on cowpea and a systemic chlorotic mottle on cucumber) was considered as evidence for presence of TRSV in the portion of the plant that was indexed.

Three types of tests were conducted to study translocation of TRSV in roots. In the first test, single viruliferous nematodes were washed into sand in pots, each containing a single healthy cucumber or zinnia seedling in the cotyledonary stage. The test plants were divided ito four groups and, at weekly intervals for four weeks, roots of a group of zinnia and cucumber were indexed for presence of TRSV. Each plant was washed from the sand and its root system was floated on a pan of water, separated into four sections and each section was labeled. Each section of the root system was removed and indexed.

Since the points of virus inoculation by the nematode could not be determined in the first test, alterations were made in the second test. Test plants were started in sand in 100 ml plastic beakers with holes in the bottoms of the beakers. As the roots began to grow through the holes, the beakers were placed in 3 inch pots containing sterilized sand. A single viruliferous nematode was washed into each lower pot near the root that had grown into it. Groups of these plants were indexed 2, 3, and 4 weeks after nematodes were added. Roots of each plant from the pot and the beaker were indexed separately.

In the third test, a split root system was used. Zinnia, used as test plants, were grown for approximately 2 weeks in sterilized soil, then the root system of each plant was divided by splitting the tap root to the stem with a razor blade. The split root system was divided in the same pot by a plastic barrier, or in separate plastic cups held together by staples. Ten viruliferous nematodes were added to one side of each root system by washing them into the sand, or one side of the root system was mechanically inoculated by scratching the root and injecting purified virus into and on the scratched area. Plants were removed at various intervals and roots from each side of the split system and tops were indexed.

RESULTS AND DISCUSSION

Tobacco ringspot virus was recovered from the tops of approximately 25% of zinnia plants and 85% of cucumber plants to which

transmission occurred. This is a composite of all tests on transmission of TRSV by *X. americanum* over the past 5 years when plants were indexed about 4 weeks after nematodes were added. In several tests, virus was recovered only from roots of zinnia, whereas virus was always recovered from tops of a high percentage of cucumber plants. Symptoms were usually evident on leaves of a majority of the cucumber plants within 2-3 weeks after addition of nematodes. These results indicate a difference in translocation of TRSV from roots to tops of these plants; or a difference in virus replication in leaves. The former seems more likely, since both plants are easily infected with TRSV by mechanical inoculation of cotyledons.

Tests were conducted to determine whether the difference in translocation to tops of zinnia and cucumber could be accounted for by a difference in time of translocation from the point of root inoculation. When single nematodes were used to inoculate cucumber and zinnia root systems, and roots were divided into four sections which were indexed separately, there was no difference in translocation in roots of the two plants (Table 1). Virus was recovered from roots of 2 cucumber and 1 zinnia within a week after nematodes were added, and in each case it was recovered from more

TABLE 1. Recovery of tobacco ringspot virus from sections of cucumber and zinnia roots in the same pot following transmission by single *X. americanum*

Time after addition of nematode	Number of virus-infected plants		Number of plants with virus in 2 or more root sections[1]	
	Cucumber	Zinnia	Cucumber	Zinnia
1 wk.	2	1	2	1
2 wk.	8	5	8	4
3 wk.	2	6	2	6
4 wk.	8	8	8	8

[1]Each root system was divided into 4 sections, and each section was indexed separately for presence of TRSV.

than one root section on each plant. Indexing at 2, 3 and 4 weeks showed that virus was systemic in root systems of both cucumber and zinnia.

In a second type of test, portions of the root systems of plants grew through holes in the bottoms of plastic cups into pots, and single nematodes were added to each pot. Roots of each plant from the cups and the pots were indexed separately. Most of the plants were

indexed approximately 4 weeks after nematodes were added. Virus was systemic in roots of both cucumber and zinnia (Table 2).

Split root systems were also used to study virus translocation in zinnia. Virus was recovered from roots from the side where nematodes were added and from roots from the side without nematodes of 70% of infected plants. These were indexed 3-12 weeks after nematodes were added. No nematodes were ever found when sand from the side where no nematodes were added was screened. Virus was recovered from the tops of 20% of the infected plants (Table 3).

TABLE 2. Recovery of tobacco ringspot virus from roots of cucumber and zinnia in separate pots following transmission by *X. americanum* in one pot

Time after addition of nematode	Number of virus-infected plants		Number of plants with virus systemic in roots[1]	
	Cucumber	Zinnia	Cucumber	Zinnia
3 wk.	1	0	1	0
4+ wk.[2]	10	18	10	16

[1]Roots from pot where nematode was introduced and from cup without nematode were indexed separately for presence of TRSV.

[2]Plants were indexed over a period of 4 days.

TABLE. 3. Translocation of tobacco ringspot virus in split root systems of zinnia following transmission by *X. americanum*

Time after nematodes added	No. of infected plants	No. of plants with virus systemic in roots[1]	No. of plants with virus in tops[1]
3 wk.	4	4	—
4 wk.	11	8	2
5 wk.	18	14	3
6 wk.	14	6	4
12 wk.	2	2	1

[1]Each side of the split root system and leaves from each plant indexed separately for presence of TRSV.

Roots on one side of the split systems of a few plants were mechanically inoculated with TRSV. Two plants which were indexed after 4 weeks were infected and virus was recovered from both sides of the split root systems. Virus was also recovered from the top of one of these plants.

The results of these studies show TRSV is translocated throughout the root systems of both cucumber and zinnia following transmission to the roots. There was no evident difference in the time of translocation from the points of inoculation to other parts of roots of cucumber and zinnia. Thus, the difference in translocation of TRSV to tops of these plants cannot be accounted for by a difference in behavior of the virus in the roots. Fulton (3) reported that TRSV moved very slowly from point of inoculation of tobacco and tomato roots, but seemed to move more slowly upward than downward in the roots. Up to eleven days after mechanical inoculation TRSV was not recovered above the point of inoculation, and infrequently below this point. He postulated that the virus moved slowly through root parenchyma and was slow reaching the phloem. If this is true in cucumber and zinnia, then the virus must move rapidly throughout the root system once it reaches the phloem. Systemic invasion of roots of both plants occurred in 2 to 3 weeks.

In the plants with split root systems, it was necessary for the virus to move into the base of the stem in order to get to the other side. Yet virus was recovered from tops of only a small part of these plants. It therefore seems that the barrier to movement to the top of the plant must be in the lower stem area.

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