Transmission of Tomato Ringspot Virus from Dandelion via Seed and Dagger Nematodes

WILBUR L. MOUNTAIN, Plant Industry Technician, CHARLES A. POWELL, Plant Pathologist, and LYLE B. FORER, Chief Plant Pathologist, Pennsylvania Department of Agriculture, Harrisburg, PA 17110, and RICHARD F. STOUFFER, Professor, Department of Plant Pathology, Pennsylvania State University, Fruit Research Laboratory, Biglerville, PA 17307

ABSTRACT

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Seedlings from five tomato ringspot virus (TmRSV)-infected common dandelion (*Taraxacum officinale*) plants were assayed for the virus. An average of 24% of the seedlings were found infected. Mean percent germination of seeds from TmRSV-infected and uninfected dandelion plants was the same. *Xiphinema rivesi* was able to acquire TmRSV from infected dandelion plants and transmit it to uninfected seedlings. Common dandelion is a major natural reservoir for TmRSV in Pennsylvania, and the potential importance of this weed in the epidemiology of TmRSV-induced orchard diseases is discussed.

Prunus stem pitting (PSP) and apple union necrosis (AUN) are major problems in peach and apple orchards in Pennsylvania. PSP-like symptoms also have been reported from most other peach-growing states of the northeastern United States and Canada (8,10). The PSP disorder was described in detail by Barrat et al (2), Stouffer and Forer (14), and Stouffer and Smith (17), although PSP-like symptoms had been reported previously (1,3,8,9). Reports from Pennsylvania and Virginia of poor growth and decline of certain apple cultivars propagated on size-controlling rootstocks were first received in about 1970–1971 (15). These reports continued with increasing frequency and similar disorders were recognized in apple-growing areas in other parts of the United States and Canada (15).

TmRSV was identified as the causal agent of PSP (13) and has been implicated as the causal agent of AUN (15). TmRSV is transmitted by dagger nematodes, *Xiphinema americanum* (18) and/or *X. rivesi* (7) and natural spread from tree to tree presumably occurs via these nematode species. Infected nursery stock originally accounted for a large percentage of infected trees (16). With TmRSV-free nursery stock now generally available, however, this source of the virus is no longer a significant factor in most Pennsylvania orchards. Yet, even with the use of "virus-free" nursery stock, the incidence of TmRSV-induced diseases remains high. Major attention has been focused, therefore, on the identification of possible weed reservoirs of the virus and on feeding hosts for the nematode vector. Extensive surveys have determined that field infection of dandelion with TmRSV is correlated with TmRSV-induced orchard disease (C. A. Powell et al, *unpublished*). Results presented in this paper establish that TmRSV is transmitted through dandelion seed and that the dagger nematode can acquire TmRSV from dandelion.

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MATERIALS AND METHODS

Nematode transmission. Field soil adjacent to a vineyard and containing *X. rivesi* was bait tested by transplanting two Wisconsin SMR-18 cucumber seedlings into soil samples in 8-oz Styrofoam cups. After a 4-wk bait period, a composite root-leaf sample of each cucumber plant was analyzed for virus by mechanical inoculation to *Chenopodium quinoa*. Root-leaf extracts in 0.05 M potassium phosphate buffer, pH 7.0, were rubbed onto Carborundum-dusted leaves of the indicator plants. After 10 days, these symptomless indicator plants were assayed for TmRSV by enzyme-linked immunosorbent assay (ELISA) (4) and were found negative. Transmission to *C. quinoa* prior to ELISA was performed because of nonspecific ELISA reactions obtained with cucumber sap.

Nematodes were extracted from the bait-tested soil by the wet-sieve/Baerman funnel technique (6) and added to a pasteurized 1:1 sand/vermiculite mix containing TmRSV-infected dandelion. After a 3-wk acquisition period, nematodes were extracted from this donor plant medium and 50 potentially viruliferous *X. rivesi* were added to the sand/vermiculite mix of each of 12 and 10 potted uninfected dandelion and cucumber seedlings, respectively. Controls consisted of 10 dandelion and 10 cucumber seedlings to which 50 of the bait-tested, nonviruliferous *X. rivesi* were added. Root-leaf samples of the dandelion and cucumber plants were analyzed for virus as described previously after a 4 wk transmission period.

Seed transmission of TmRSV in common dandelion. Five dandelions naturally infected with TmRSV were maintained in the greenhouse. Seeds from these five source plants were collected and germinated; the resulting seedlings were indexed for TmRSV by inoculation of a composite root-leaf sample to *C. quinoa* followed by ELISA serodiagnosis as described previously. Seedlings from uninfected dandelion source plants served as controls.

Seed germination. Fifty seeds from each of the five TmRSV-infected dandelion plants were sown in "kord" fiber market paks (5 × 7 in.) in a pasteurized soil mix, using a vacuum planter head to count and evenly space the seeds. This procedure was duplicated using seeds from five uninfected dandelion plants. The number of seeds from each plant that had germinated was determined after 1 and 3.5 mo.

RESULTS

Nematode acquisition and transmission of TmRSV. In this study, *X. rivesi* fed on

Table 1. Seed transmission of TmRSV by dandelion

Source plant[a]	No. infected/[b] no. assayed	Transmission (%)
Infected		
A	14/49	28.6
B	3/42	7.1
C	12/42	28.6
D	9/26	34.6
E	7/30	23.3
Total	45/189	23.8
Uninfected		
F	0/42	0
G	0/46	0
H	0/49	0
I	0/34	0
J	0/21	0
Total	0/192	0

[a] Source plants B, D, F, G, and I were first-generation progeny of plant A. Fifty seeds were sown per source plant.

[b] Represents number of seedlings with serologically confirmed TmRSV infection.

Table 2. Germination of seeds from TmRSV-infected and uninfected dandelion

Source plant[a]	Percent germination[b] After 1 mo	After 3.5 mo
Infected		
A	62	98
B	64	84
C	72	84
D	42	52
E	46	60
Avg.	57	76
Uninfected		
F	84	84
G	92	92
H	98	98
I	68	68
J	42	42
Avg.	77	77

[a] After germination counts, seedlings were used for seed transmission tests (Table 1).
[b] Based on 50 seeds per plant.

dandelion and was able to acquire TmRSV and transmit the virus to dandelion and cucumber. Seedlings of seven of 12 dandelions and eight of 10 cucumbers became infected with TmRSV as a result of nematode transmission. None of 10 dandelion or 10 cucumber seedlings exposed to nonviruliferous nematodes became infected.

Seed transmission of TmRSV. TmRSV was seed transmitted in dandelion. Of 189 seeds which germinated from five TmRSV infected plants, 45 (23.8%) were infected (Table 1). Except for plant B, there was limited variation in percent seed transmission among individual plants.

Dandelion seed germination. TmRSV did not significantly affect the number of seeds that germinated; percent germination from infected and uninfected source plants was 76 and 77, respectively (Table 2). Germination time, however, was affected. All seed lots from TmRSV-infected plants had some seeds that germinated late (Table 2).

DISCUSSION

Because of their relative immobility, nematode vectors are unable to efficiently disseminate a virus over long distances as is the case with aerial vectors. Thus, any alternate means of dissemination becomes extremely important in the epidemiology of such diseases as PSP and AUN. Infected dandelion seed may serve as a mechanism for intraorchard as well as interorchard dissemination of TmRSV.

Many nematodeborne viruses are transmitted through seeds of some of their hosts, and the proportion of infected seeds is often high. The seed transmission of TmRSV by *Fragaria* × *ananassa* (cultivated strawberry) and *Glycine max* (soybean) was 55 and 78%, respectively (11). An interesting feature of seed-transmitted nepoviruses is that infected seedlings are usually free from obvious symptoms. This also was the case with TmRSV-infected dandelion seedlings. Reduced vigor, however, is indicated by delayed germination of some seeds from infected source plants (Table 2). Whether TmRSV affects dandelion reproduction or vigor under field conditions is not yet known.

Traditional measures to control PSP and AUN have centered around planting "virus-free" nursery stock to eliminate the possible source of virus and soil fumigation to suppress the nematode vector. Another important factor in an integrated disease management program is the elimination or suppression of weed reservoirs of TmRSV (5,12). Establishing a dense aggressive orchard floor cover and eliminating broad-leaf weeds in the tree row and in the orchard floor cover are practical and inexpensive procedures that can contribute to a total disease management program.

Dandelion has many attributes for complicating control of TmRSV-induced diseases: 1) it is a host for the virus, 2) *X. rivesi* can acquire TmRSV from it, 3) TmRSV is transmitted through it's seed, and 4) it is perennial, adaptable, and very prolific in seed production and dissemination. Thus, the maximum benefit from using clean nursery stock, fumigants, and nonhost cover crops can be obtained only by employing good weed control measures, especially where dandelion is prevalent.

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