Reservoirs of Tomato Ringspot Virus in Fruit Orchards

CHARLES A. POWELL, Plant Pathologist, and LYLE B. FORER, Chief Plant Pathologist, Pennsylvania Department of Agriculture, Harrisburg 17110, and RICHARD F. STOUFFER, Professor, The Pennsylvania State University, Fruit Research Laboratory, Biglerville 17307

ABSTRACT

Powell, C. A., Forer, L. B., and Stouffer, R. F. 1982. Reservoirs of tomato ringspot virus in fruit orchards. Plant Disease 66:583-584.

Xiphinema spp. overwintered in tubs containing soil collected in late summer from root zones of four nectarine trees with symptoms of Prunus stem pitting disease. Weeds that grew in these tubs were indexed for tomato ringspot virus in May and July, and positive transmissions were confirmed serologically. At least one plant of each of the following species of dicotyledonous weed found in the tubs was infected with the virus: common chickweed (*Stellaria media*), henbit (*Laminum amplexicaule*), dandelion (*Taraxacum officinale*), creeping woodsorrel (*Oxalis corniculata*), common plantain (*Plantago major*), strawberry (*Fragaria virginiana*), sorrel (*Rumex acetosella*), and red clover (*Trifolium pratense*).

Prunus stem pitting disease was identified as a serious problem in peach and nectarine orchards in Pennsylvania in the late 1960s. The disease also was observed in most other peach-growing states in the northeastern United States and in Canada (5,8,13,15). The suspected viral etiology (3) was confirmed by Smith et al (11) in 1973 when they reproduced the stem pitting symptoms in peach seedlings by mechanical inoculation with tomato ringspot virus (TmRSV).

TmRSV is also the probable causal agent of apple union necrosis, a disorder characterized by necrotic plate formation at the graft union, subsequent tree decline, and occasional breakage at the graft union. TmRSV can be isolated from rootstock suckers of trees with symptoms of union necrosis (14); buds from such rootstock suckers incite typical Prunus stem pitting symptoms when grafted into peach seedlings (Stouffer and Powell, *unpublished*).

Prunus stem pitting was considered primarily a nursery problem because large numbers of diseased trees could be traced to infected nursery stock. Even though the disease has been eliminated from Pennsylvania nursery stock by fumigating the nursery soil, the disease is still prevalent in Pennsylvania orchards; disease-free trees frequently become infected after they are planted in the orchard.

TmRSV is transmitted by the dagger nematodes *Xiphinema americanum* Cobb (16) and *X. rivesi* Dalmasso (Forer and Powell, *unpublished*), which are widespread in the fruit production areas of Pennsylvania (1) (Forer, *unpublished*). In addition, TmRSV is seed-transmitted in soybean (2) and strawberry (7) and both seed- and pollen-transmitted in geraniums (10). The natural host range of TmRSV is extensive and includes both herbaceous and woody plant species.

Results from experiments with other nepoviruses indicate that weeds and weed seed are important factors in disease epidemiology (9). Our investigations were performed to determine whether weeds serve as virus reservoirs for TmRSV-induced diseases of deciduous fruit trees.

Accepted for publication 5 October 1981.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

0191-2917/82/07058302/$03.00/0
©1982 American Phytopathological Society

MATERIALS AND METHODS

Soil samples and nematode analysis. In late August, soil was removed from the root zones of four nectarine trees with symptoms of Prunus stem pitting disease. Each sample was placed in a 57-L tub and set outdoors in a shaded area. Controls were four soil samples collected from an orchard that had received a preplant fumigation with Dowfume MC-33 (67% methyl bromide, 33% chloropicrin; 308.3 kg/ha). The control samples were placed in similar tubs and were located adjacent to the tubs with nonfumigated soil. Two 100-cc soil samples were removed from each tub the following spring, and *Xiphinema* spp. were extracted (12) and counted.

Indexing. Individual weeds that grew in the tubs were removed, and a composite sample from each weed consisting of approximately 1 g of leaf and 1 g of root tissue was triturated with a mortar and pestle in approximately 5 ml of 0.05 M sodium phosphate buffer, pH 7.1. The sap extract from each weed was rubbed on the cotyledons of six to eight cucumber (*Cucumis sativus* L. 'National Pickling') plants. The cucumber plants were observed for local lesions 5–10 days after inoculation. Questionable lesions were transferred to additional cucumber for verification. Weeds were indexed in May and July, 9 and 11 mo, respectively, after the soil was collected.

Serology. Presence of TmRSV in cucumber plants that showed symptoms was determined by the Ouchterlony double-diffusion technique in 0.75% ionagar. Crude sap from the cucumber plant in this buffer was reacted against a 1:10 dilution of TmRSV antiserum (American Type Culture Collection, PV AS #25). A cucumber plant known to be infected with TmRSV and one known to be healthy were used as positive and negative controls, respectively.

RESULTS AND DISCUSSION

The four tubs of orchard soil collected from the root zones of four nectarine

Table 1. Summary of indexing for tomato ringspot virus in weeds in orchard soil

Weed	Positive/Indexed[a]	
	May	July
Common chickweed	3/8	3/8
Common plantain	2/4	1/2
Dandelion	6/8	1/4
Henbit	1/6	1/2
Oxalis	2/6	1/4
Red clover	1/2	1/2
Sorrel	1/4	1/2
Strawberry	1/2	0/2

[a] Number of weeds with serologically confirmed tomato ringspot virus infection/total number indexed.

trees with Prunus stem pitting disease contained overwintering *Xiphinema* spp. (*X. americanum, X. rivesi,* or both) populations of 21, 26, 45, and 65 per 100 cc of soil. No *Xiphinema* spp. were detected in the four control tubs of soil from a fumigated orchard.

TmRSV was not detected in strawberry (*Fragaria virginiana*) in July (Table 1), but in May and July, the virus was isolated from at least one plant of the following species of dicotyledonous weed: common chickweed (*Stellaria media* (L. Cyr.), henbit (*Lamium amplexicaule* L.), dandelion (*Taraxacum officinale* Weber), creeping woodsorrel (*Oxalis corniculata*), common plantain (*Plantago major* L.), sorrel (*Rumex acetosella* L.), and red clover (*Trifolium pratense* L.). The virus was detected more frequently in May (42%) than in July (34%). Dandelion was the most frequently infected weed, followed by common chickweed. None of the weeds showed symptoms. No virus was detected in a similar number of identical weed species from the control tubs with fumigated orchard soil.

These results demonstrate that TmRSV overwintered in *Xiphinema*-infested orchard soil and also that several species of common orchard weeds may become infected and serve as reservoirs of the virus. This persistence may be explained by at least four mechanisms: TmRSV may be present as "free" virus in the soil, the virus may be present in infected perennial weeds, overwintering stages of the dagger nematode may carry the virus, and TmRSV may be present in seeds from infected weeds.

Persistence of TmRSV in the form of free virus in the soil seems rather unlikely. Cucumber and *Chenopodium quinoa* seedlings planted in nematode-free soil to which purified TmRSV had been added at 100 μg/cc failed to become infected (Forer and Powell, *unpublished*). Some of the infected perennial weeds could have arisen from previously infected plant parts such as taproots and rhizomes, but it seems highly unlikely that all infected plants in our tests originated in this way. Certainly this could not explain the occurrence of infected annual weeds. Overwintering stages of the dagger nematode have been reported to harbor tobacco ringspot virus (6); it is likely that TmRSV can be retained in a similar manner. Seed transmission of TmRSV has been reported in several commercial crops (2,4,7,10), but its occurrence in common orchard weeds has not been investigated extensively. Our data cannot distinguish between infections that occurred as a result of viruliferous nematodes or virus-infected seed that may have been originally present in the nonfumigated orchard soil.

The hypothesis that the TmRSV in infected weeds originated from viruliferous *Xiphinema* or weed seeds in the nonfumigated orchard soil and not elsewhere (pollen or seeds from weeds growing near the tubs) is supported by the observation that TmRSV-infected weeds were found in tubs of nonfumigated orchard soil; weeds in adjacent tubs of fumigated soil were free of the virus. The conclusion that TmRSV originated from weed tissue and not from viruliferous nematode tissue, which may have been triturated along with the weed roots, is based on experiments in which virus was not detected when *Xiphinema* from a TmRSV-transmitting population were triturated and inoculated to cucumber (Powell and Forer, *unpublished*).

LITERATURE CITED

1. Forer, L. B., and Longenecker, J. L. 1978. Distribution of *Xiphinema americanum* in cereal and corn fields in Pennsylvania. (Abstr.) Phytopathol. News 12:228.
2. Kahn, R. P. 1956. Seed transmission of the tomato-ringspot virus in the Lincoln variety of soybeans. Phytopathology 46:295.
3. Lewis, F. H., Stouffer, R. F., and Hewetson, F. N. 1968. A serious new disorder of peach trees. Plant Dis. Rep. 52:292-294.
4. Lister, R. M., and Murant, A. F. 1967. Seed-transmission of nematode-borne viruses. Ann. Appl. Biol. 59:49-62.
5. Lott, T. B., Keane, F. W. L., and May, J. 1962. Gumming, distortion and pitting in cherry and apricot. Can. Plant Dis. Surv. 42:229-232.
6. McGuire, J. M. 1973. Retention of tobacco ringspot virus by *Xiphinema americanum*. Phytopathology 63:324-326.
7. Mellor, F. C., and Stace-Smith, R. 1963. Reaction of strawberry to a ringspot virus from raspberry. Can. J. Bot. 41:865-870.
8. Mircetich, S. M., and Fogle, H. W. 1969. Stem pitting in *Prunus* spp. other than peach. Plant Dis. Rep. 53:7-11.
9. Murant, A. F., and Lister, R. M. 1967. Seed-transmission in the ecology of nematode-borne viruses. Ann. Appl. Biol. 59:63-76.
10. Scarborough, B. A., and Smith, S. H. 1977. Effects of tobacco- and tomato-ringspot viruses on the reproductive tissues of *Pelargonium* × *hortorum*. Phytopathology 67:292-297.
11. Smith, S. H., Stouffer, R. F., and Soulen, D. M. 1973. Induction of stem pitting in peaches by mechanical inoculation with tomato ringspot virus. Phytopathology 63:1404-1406.
12. Southey, J. F. 1970. Laboratory methods for work with plant and soil nematodes. Min. Agric. Fish Food Tech. Bull. 2, London. 148 pp.
13. Stouffer, R. F., and Forer, L. B. 1969. Prunus stem pitting—How to identify the disorder. PA Dep. Agric. Spec. Bull. 4 pp.
14. Stouffer, R. F., Hickey, K. D., and Welsh, M. F. 1977. Apple union necrosis and decline. Plant Dis. Rep. 61:20-24.
15. Stouffer, R. F., Lewis, F. H., and Soulen, D. M. 1969. Stem pitting in commercial cherry and plum orchards in Pennsylvania. Plant Dis. Rep. 53:434-438.
16. Teliz, D., Lownsberry, B. F., and Grogan, R. G. 1966. Transmission of tomato ringspot virus by *Xiphinema americanum*. (Abstr.) Phytopathology 56:151.