Infection of Cultivated Strawberries by Tomato Ringspot Virus

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ABSTRACT

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Tomato ringspot virus (TomRSV) was detected by enzyme-linked immunosorbent assay in commercial strawberry cultivars Lassen, Olympus, Puget Beauty, and Sequoia during 19 mo of growth in a field known to be infested with viruliferous dagger nematodes (*Xiphinema americanum*). Eighteen cultivars were not infected by TomRSV in this test. Leaf symptoms were unreliable for virus detection; some infected plants were symptomless, but many showed reduced vigor and resembled plants infected by the aphidborne strawberry viruses. Field symptoms of TomRSV-infected Olympus often resembled those of Verticillium wilt. In leaflet-grafting tests in the greenhouse, TomRSV from strawberry and raspberry sources infected and caused reduction in vigor in Puget Beauty within 30 days. Parallel grafts to Shasta strawberry from both virus sources resulted in death of infected plants within 30 days, which indicated that differences in strawberry symptomatology were associated with host genotype rather than virus source or mode of inoculation. Tomato ringspot virus is probably a common, but unassessed, cause of crop loss wherever susceptible strawberry cultivars are planted in fields infested with viruliferous dagger nematodes.

Tomato ringspot virus (TomRSV) has been reported to occur in symptomless native populations of the beach strawberry (*Fragaria chiloensis* (L.) Duch.) in California as well as in many other local flora in the area (8). Experimentally, TomRSV has been graft-transmitted from infected raspberry to cultivated strawberry, killing 8 of 11 strawberry cultivars tested (9), and to *Fragaria vesca* (9) and to *F. chiloensis* (7) by graft transmission and by the dagger nematode, *Xiphinema americanum* Cobb (6). Natural infection of strawberry cultivars by TomRSV has not been previously reported and is the subject of this paper. An abstract on this subject has appeared (5). In addition, graft-transmission experiments of TomRSV to strawberry were conducted to relate these natural infection studies to previous Canadian (9) graft studies.

MATERIALS AND METHODS

Twenty-two strawberry cultivars grown on the U.S. Pacific Coast were planted in a field near Corvallis, OR, in soil known from previous studies on TomRSV in red raspberries (3) to contain an average of 285 *X. americanum* per liter of soil as determined by the Baermann funnel technique. In greenhouse trap-crop tests with cucumbers, these nematodes were found to transmit TomRSV. The occurrence of dagger nematodes and of TomRSV had been plotted in the previous crop of red raspberries, and the strawberry plants were subsequently planted in the same rows. Certified-grade strawberry plants, free of TomRSV, were obtained from commercial nurseries in Washington and California and were used for this test. Five replications, five plants per replicate, of each cultivar were planted in May 1978, but herbicide carry-over from the previous crop of red raspberries killed more than half of the stand. Ten-plant blocks of each cultivar were also planted as

healthy control plots in a nearby field that was free from *X. americanum* and had no known history of TomRSV occurrence. Standard strawberry management practices were used in the plantings, but no aphicides were applied.

For TomRSV detection, roots from daughter plants dug in November 1978 were sampled. Subsequently, leaf samples were taken directly from mother plants for TomRSV detection in June 1979 and the test was arbitrarily terminated with final leaf samples in December 1979. The occurrence of TomRSV in these samples was determined by agar gel serology and by ELISA by using alkaline phosphatase and *p*-nitrophenyl phosphate (Sigma Chemical Co., St. Louis, MO 63178) in Microelisa® (Dynatech Corp., Alexandria, VA 22314) plates (2). Antiserum prepared by C. L. Parish (USDA, SEA-AR, Wenatchee, WA 98801) against a Pennsylvania isolate of TomRSV from apple was used in the study. N. W. Frazier, University of California, Davis 95616, supplied *F. vesca* plants known to be infected with TomRSV (7). Cultivars from plots free from TomRSV infection were used as controls. The threshold value for positive readings by ELISA in a given plate was taken as the mean absorbance reading (A_{405nm}) of control wells (healthy strawberry sap in buffer) plus twice its standard deviation. In the threshold range, absorbance values varied as a direct linear function of TomRSV concentration in known samples. Symptoms and vigor ratings were recorded from plants in test and control plantings in November 1978 and in June and December 1979. Detection of TomRSV was also attempted by bioassay. Leaves of strawberry plants, known by ELISA to be infected with TomRSV, were triturated (1:10, w:v) either in 3% nicotine alkaloid in 0.01 M phosphate buffer (pH 9.1) plus 0.01 M cysteine hydrochloride (4), or in 0.05 M phosphate buffer (pH 7.0) with 1% bovine serum albumin (Sigma Chemical Co., St. Louis, MO), 2% polyvinyl pyrrolidone (MW 10,000, Sigma), and 0.01 M potassium metabisulfite plus a little Celite. The latter buffer will be referred to as BSA buffer. The resulting triturates were rubbed onto young leaves of *Chenopodium quinoa* Willd. and cotyledons of *Cucumis sativus* L. 'National Pickling' in the greenhouse, and the indicator plants were observed for symptoms for 2 wk.

To assess the sensitivity of one ELISA procedure, TomRSV-infected strawberry roots were mixed in various ratios with known healthy strawberry roots, homogenized, and ELISA readings were made on the mixtures. Similar tests were performed with leaf mixtures. In one test, A_{405nm} readings for various dilutions of infected root sap were: undiluted, 1.45; 1:4, 0.92; 1:9; 0.62; and 1:19, 0.31; and for healthy sap it was 0.06. Leaf sample dilutions gave similar results. Samples from individual test plants were pooled in batches of 20 or less, usually 10, in preliminary ELISA tests. Thereafter, pooled samples exceeding threshold values were retested as individual plant samples.

In studies of graft infection of strawberry cultivars with TomRSV from an infected red raspberry, Mellor and Stace-Smith (9) found that most susceptible strawberry cultivars (including Shasta) died soon after being grafted. In contrast, in the present field inoculation studies death of strawberry plants naturally infected with TomRSV occurred only in cultivar Olympus. To determine whether the differences between these two tests were related to differences in virus strains, methods of inoculation, or genetic differences among cultivars, grafting experiments were done on greenhouse-grown plants at Corvallis. Healthy strawberry plants of Puget Beauty and other cultivars were planted in flats of field soil that contained viruliferous nematodes from the same field where the natural infection studies of strawberry cultivars had been conducted. Details of these techniques for inoculating greenhouse-grown plants will be reported elsewhere. Cultivated strawberry plants from this test, found by ELISA to be naturally infected by TomRSV and free from other known viruses, were used as sources of TomRSV in leaflet-grafting (1) experiments. These plants are called "greenhouse strawberries with TomRSV" in Table 2. In addition, TomRSV isolates in Willamette red raspberry from Eddyville, OR, and Puyallup red raspberry from the experimental TomRSV field at Corvallis, OR, already described, were used as sources of inoculum. Both indexed free of known viruses other than TomRSV. Willamette-65, Puyallup-65, Puget Beauty, and Shasta were used as sources of healthy leaflets. All virus and control sources were each leaf-grafted to 4–15 healthy Puget Beauty, Shasta, and Alpine plants. After two months of growth and symptom evaluation all successfully leaf-grafted plants were indexed for TomRSV by ELISA.

TABLE 1. Susceptibility of 22 strawberry cultivars to natural infection by tomato ringspot virus after 19 mo in field tests at Corvallis, OR, as determined by ELISA

Cultivars	No. infected/ total tested	Cultivars	No. infected/ total tested
Aiko	0/10	Puget Beauty	8/12
Aliso	0/7	Quinault	0/11
Benton	0/8	Rainier	0/15
Cruz	0/11	Sequoia	2/15
Fresno	0/7	Shasta	0/9
Fort Laramie	0/9	Shuksan	0/17
Hood	0/8	Siletz	0/9
Lassen	1/11	Solano	0/16
Linn	0/7	Tioga	0/12
Northwest	0/7	Totem	0/7
Olympus	4/12	Tufts	0/7

RESULTS

A total of 15 plants of four of the 22 strawberry cultivars tested became infected with TomRSV during the field study (Table 1). These cultivars were Lassen, Olympus, Puget Beauty, and Sequoia. The vigor ratings of the infected Lassen and Sequoia plants were the same as those of comparable healthy plants. Plants of Olympus and Puget Beauty infected with TomRSV were less vigorous than were comparable healthy plants. During the 1978 and 1979 growing seasons some Olympus plants developed a whorl of dead outer leaves and subsequently died. Tomato ringspot virus was recovered from these plants, but not from adjacent symptomless Olympus plants. Infected Puget Beauty plants were dwarfed and produced fewer runners than those not infected by TomRSV. New leaves developing on infected plants in the spring were sometimes abnormally erect and exhibited broad yellow vein-clearing (Fig. 1). These symptoms have also developed in other plantings of Puget Beauty in the greenhouse and the field and are associated only with TomRSV infection. The number of infected Puget Beauty plants increased from two to four to eight when they were ELISA-indexed 6, 13, and 19 mo, respectively, after planting. Lassen, Olympus, and Sequoia were symptomless at 6 mo, and the number of plants infected at 13 mo (Table 1) remained unchanged 19 mo after planting.

A Puget Beauty plant naturally infected with TomRSV in the field was transplanted to the greenhouse and was used as a source of inoculum for mechanical transmission attempts to herbaceous hosts. At the same time an isolate of TomRSV from red raspberry maintained in the greenhouse in cucumber was used for parallel inoculation tests. Use of a standard trituration buffer containing 3% nicotine alkaloid (4), failed to result in transmission of the TomRSV from Puget Beauty, but did facilitate transmission of the TomRSV from cucumber. Use of the BSA buffer resulted in transmission of the Puget Beauty isolate to cucumbers, where typical TomRSV symptomatology (yellow cotyledonary lesions followed by systemic mottle and necrosis) developed and the infected cucumber plants tested positive for TomRSV by agar gel serology and ELISA.

An effort was made to determine how well previous TomRSV infections of red raspberries in a field plot known to have a high level of dagger nematodes carrying TomRSV would predict the successful subsequent infection of strawberry plants in the same planting sites. Strawberries were planted in the same rows where many red raspberries had previously been found (3) to be infected with TomRSV. The planting sites of the resulting 15 naturally infected strawberries were found to include locations where both *X. americanum* and TomRSV had been previously found together, as well as areas in which one or both were absent. There seemed to be

little predictive value in assaying the individual planting sites for dagger nematode and TomRSV incidence prior to planting except to locate the area in general as containing viruliferous nematodes.

In greenhouse tests, TomRSV was detected by ELISA in several strawberry plants that had been inoculated by viruliferous dagger nematodes in greenhouse flats. TomRSV was successfully transmitted by leaflet graft from these strawberries to healthy Puget Beauty, Shasta, and Alpine in 24/39, 7/12, and 18/39 instances, respectively, in which grafted leaflets survived 10 or more days (Table 2). Shock and chronic symptoms in Puget Beauty ranged from none to epinasty, chlorosis, mottling, leaf distortion, leaf necrosis, and plant dwarfing; but no infected plants died during the 60 days of the test. Parallel leaflet grafts to Shasta resulted in all seven of the infected plants dying within 1 mo of inoculation. Symptoms on grafted Alpine were as described (9). Grafts to Puget Beauty, Shasta, and Alpine from Willamette and Puyallup red raspberry plants from the field infected with TomRSV resulted in a wide range of shock and chronic symptoms and death of infected Shasta, but not of Puget Beauty plants (Table 2). All healthy control grafts remained symptomless and free from TomRSV.

DISCUSSION

The main purpose of this study was to demonstrate that under natural field conditions TomRSV can cause a severe and hitherto unrecognized disease in several commonly grown strawberry cultivars, and that a range of symptoms result, many resembling those caused by aphidborne viruses. However, after 19 mo in the field in Oregon an average of only 22% (range 9–67%) of the plants of 4 of 22 strawberry cultivars tested became infected. Natural field transmission of TomRSV by dagger nematodes is slow and erratic. The inadequacy of dagger nematode counts from soil samples in predicting subsequent infection by TomRSV is amply demonstrated by the present data. Therefore escapes probably account for the discrepancies in susceptibility data when the results of the present field study, in which Siletz and Shasta remained uninfected, are contrasted with those of both the British Columbia study (in which plants of these and most of the other cultivars tested died when they were infected by leaflet grafting [9]) and the Oregon leaflet grafting test (in which Shasta plants were infected and killed). Differences between Oregon and British Columbia isolates of the virus and the limits of its detection by the ELISA method also may be involved in these discrepancies.

Inoculation of strawberry cultivars with TomRSV by raspberry leaflet grafting resulted in 63% infection in British Columbia as determined by bioassay (9). In Oregon, grafting with TomRSV-infected raspberry leaflets resulted in 78% infection of strawberry cultivars, while grafting with TomRSV-infected strawberry leaflets to plants of strawberry cultivars resulted in 61% infection. Thus, in both locations, about one fourth to one third of the strawberry plants failed to become infected after successful leaflet grafting with TomRSV-infected raspberry or strawberry leaflets. In Oregon, leaflets from the same raspberry and strawberry leaves used for grafting were tested for the presence of TomRSV by ELISA and were found to be positive. It appears that TomRSV is transmitted inefficiently from infected raspberry and strawberry leaflets through leaflet grafts to cultivated strawberry hosts, even when the source leaflet survives.

Although some differences in symptom expression and frequency of transmission were observed among the different TomRSV inoculum sources and inoculation methods used in the Oregon studies, it appears that TomRSV, naturally transmitted by dagger nematodes in the field, produces a disease in cultivated strawberry that can be duplicated in the greenhouse by leaflet grafting from strawberry or raspberry plants infected with TomRSV. The differences in symptomatology in the strawberry cultivars seem to be related to the genotype of that cultivar rather than to the route of infection or the strain of TomRSV. Physiological stress in the field also may contribute to overall

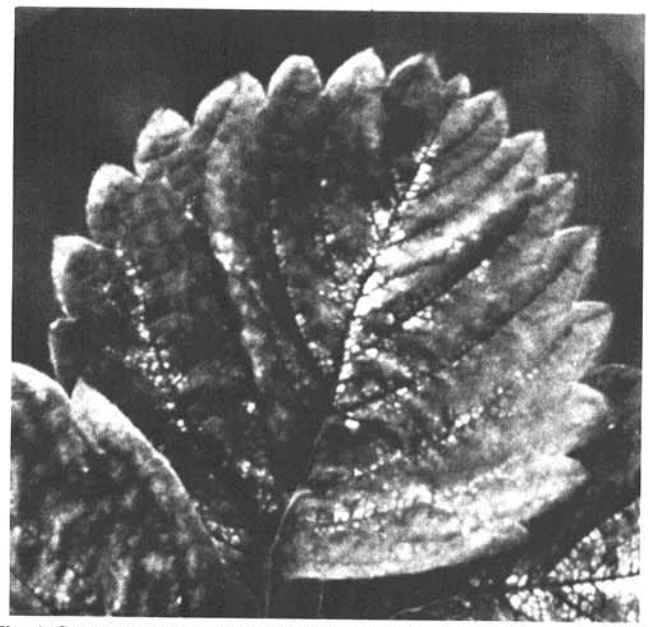

Fig. 1. Symptoms on a leaf of Puget Beauty strawberry after natural field infection with tomato ringspot virus.

TABLE 2. Transmission of tomato ringspot virus (TomRSV) by leaflet graft from naturally infected red raspberry and strawberry plants to strawberry plants

Source of grafted leaflets	Strawberry test plant cultivars								
	Puget Beauty			Shasta			Alpine		
	Trans.[a]	Shock symptoms[b]	Chronic symptoms[b]	Trans.[a]	Shock symptoms[b]	Chronic symptoms[b]	Trans.[a]	Shock symptoms[b]	Chronic symptoms[b]
TomRSV-infected									
Willamette red raspberry	3/5	Y0	W0	3/4	EN0	X0	4/5	RW	WX0
Puyallup red raspberry	4/5	0	W0	4/4	NW0	X0	4/6	DwMN0	MX0
Greenhouse strawberries	24/39	CDEM0	CDwNY0	7/12	N	X	18/39	CDwEMN0	DwNRY0
Healthy									
Willamette-65	0/4	0	0	0/5	0	0	0/4	0	0
Puyallup-65	0/5	0	0	0/5	0	0	0/5	0	0
Puget Beauty	0/5	0	0	0/5	0	0	0/5	0	0
Shasta	...	...	...	0/6	0	0	...	...	...

[a] Numerators indicate the number of plants to which TomRSV was transmitted as determined by ELISA; denominators indicate the number of plants on which grafted source leaflets survived for 10 or more days.

[b] Explanation of symbols: C = leaf chlorosis, D = leaf distortion, Dw = plant dwarfing, E = epinasty, M = leaf mottle, N = leaf necrosis, 0 = no symptoms, R = reddened leaflets or petioles, W = weak plants, X = dead plants, Y = yellow spots on leaflets. In some cases, certain infected plants were symptomless while others expressed the symptoms indicated.

weakness of TomRSV-infected plants. It is likely that high levels of resistance or even immunity are common in many strawberry cultivars, but the demonstration of this genetic resistance will require additional study. Preliminary studies (*unpublished*) suggest that greenhouse screening for TomRSV susceptibility is possible in flats of soil infested with viruliferous *X. americanum*.

Olympus strawberry suffers from a widespread and unexplained sudden-death syndrome in the Pacific Northwest. The syndrome resembles Verticillium wilt disease (10), but isolations from infected plants often fail to detect the presence of *Verticillium* species. The collapse of Olympus plants in the TomRSV test plots resembles that of the sudden-death syndrome. The intriguing possibility that TomRSV is associated with this sudden-death syndrome invites additional study.

TomRSV can be detected in strawberry with reasonable reliability by leaflet grafting to suitable strawberry indicators, and with difficulty (as noted in our results) by sap inoculation to herbaceous test plants (6–9). ELISA has proved to be a fast, sensitive alternative method of detecting TomRSV in roots and leaves of naturally infected strawberry cultivars. ELISA techniques can be applied in their present state to evaluate strawberry breeding lines for susceptibility, resistance, and immunity to infection following exposure to viruliferous nematodes, and to screen commercial nursery stocks for the presence of TomRSV.

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