Tomato Ringspot Virus Associated with Apple Union Necrosis and Decline in Western United States

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ABSTRACT

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Union necrosis and decline were observed in Delicious apples on Malling-Merton 106 and 111 rootstocks in five commercial orchards in central Washington. Virus cultures, which were isolated from Malling-Merton 106 rootstock suckers by sap transmission to tobacco and cucumber, contained tomato ringspot virus, shown by agar gel serology and enzyme-linked immunosorbent assay.

In the northeastern United States, graft union failure followed by progressive decline of certain apple stock-scion combinations has been associated with tomato ringspot virus (TomRSV) (3,4). This disease, named union necrosis and decline (4), has not previously been reported in the western United States, although TomRSV has been found in New York and Pennsylvania in unbudded Malling-Merton (MM) 106 apple rootstocks that originated in Oregon (5). This paper reports union necrosis and decline and the association of TomRSV with the disease in commercial fruiting apple orchards in Washington.

MATERIALS AND METHODS

In an apple orchard near Basin City, WA, spring foliage from rootstock suckers of 3-yr-old cv. Delicious/MM 106 trees with weak, necrotic graft unions and poor scion growth was used as inoculum for sap transmissions. Apple leaves were triturated in cold cysteine-hydrochloride-phosphate buffer plus 3% nicotine (7) and were used to inoculate *Nicotiana tabacum* L. 'Turkish' and *Cucumis sativus* L. 'Ohio MR17.' Three standard virus cultures were included for reference: TomRSV-P from apple in Pennsylvania, supplied by R. F. Stouffer; TomRSV-O from red raspberry in Oregon; and apple mosaic virus (ApMV) from apple in Washington, supplied by P. R. Fridlund.

MM 106 trees used as healthy controls in some tests were obtained from the Washington State Department of Agriculture nursery foundation planting, which originated from the IR-2 program directed by P. R. Fridlund.

TomRSV inoculum for serologic tests was harvested from cucumbers inoculated 7–10 days previously. All Washington isolates of TomRSV used in the tests reported in this paper came from one orchard near Basin City, WA.

For antiserum production in two

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Table 1. Enzyme-linked immunosorbent assay absorbance values at 405 nm,[a] with antiserum to the Pennsylvania isolate of tomato ringspot virus (TomRSV)

Source of virus isolate	Test host	A_{405nm}
Washington apple tree[b]		
1	Cucumber	0.30
2	Cucumber	0.19
3	Cucumber	0.18
4	Cucumber	0.25
Pennsylvania apple TomRSV	Cucumber	5.54
Oregon red raspberry TomRSV	Cucumber	0.84
Washington apple with apple mosaic virus	Apple	0.00
Healthy cucumber	Cucumber	0.02 ± 0.004
Buffer		0.01

[a] Mean of four replicates.
[b] Delicious/MM 106 trees with union necrosis and decline.

Fig. 1. Delicious apple scion broken off at the graft union with Malling-Merton 106 rootstock from an orchard in central Washington. Note lack of tissue continuity between stock and scion and also necrosis and swelling of the scion above the graft union.

Table 2. Enzyme-linked immunosorbent assay absorbance values at 405 nm,[a] with antiserum to the Pennsylvania isolate of tomato ringspot virus (TomRSV-P)

Source of extract	Test host	A_{405nm}
Tree with union decline and decline necrosis		
1	MM 106	0.382
2	MM 106	0.426
3	MM 106	0.404
Normal-appearing tree		
1	MM 106	0.082
2	MM 106	0.107
3	MM 106	0.088
4	MM 106	0.105
5	MM 106	0.106
6	MM 106	0.092
7	MM 106	0.077
8	MM 106	0.095
Healthy MM106 rootstock (nursery)	MM 106	0.104 s = 0.030
Healthy cucumber	Cucumber	0.130 s = 0.039
TomRSV-P infected cucumber	Cucumber	0.510
Buffer		0.094

[a]Mean of four replicates of leaf extracts.

rabbits, TomRSV-P was increased in tobacco and purified using Uyemoto's method (6), modified as follows: Leaf tissue infected systemically (approximately 80 g fresh weight) was homogenized (1:2,w/v) in 0.02 M potassium phosphate buffer, pH 7, containing 0.1% mercaptoacetic acid, filtered through cheesecloth, and frozen overnight. After thawing, the buffered extract was subjected to two cycles of low-speed (10,000 *g*, 20 min) and high-speed (150,000 *g*, 90 min) centrifugation. The final high-speed pellets, resuspended in 0.01 M phosphate buffer, pH 7, were mixed with equal volumes of Freund's complete adjuvant and used for antiserum production.

The immunization schedule consisted of three weekly subcutaneous and intramuscular injections, 1 ml each. The rabbits were bled by cardiac puncture 14 days after the last injection. The more active of the two resulting sera had reactive endpoints of 1:8192 and 1:4 against TomRSV-P and healthy cucumber sap, respectively. Serum titers were determined in agar gel double diffusion plates containing 0.7% purified agar (Oxoid Limited, London); wells were 3 mm in diameter and spaced 2 mm apart.

Enzyme-linked immunosorbent assay (ELISA) tests were performed as described by Clark and Adams (1) using Microelisa reaction plates (Dynatech Co., Alexandria, VA) and alkaline phosphatase, type VII (Sigma Chemical Co., St. Louis, MO). Coating globulin was used at a concentration of 1 μg/ml and conjugated globulin at a dilution of 1:800. Test samples derived from cotyledons of 14-day-old cucumbers inoculated 7 days before harvest were homogenized 1:10 in phosphate-buffered saline, pH 7.4, plus 0.05% Tween 20, 2% polyvinyl pyrrolidone (10,000 mol wt), and 0.2% egg albumin (1). Absorbance readings at 405 nm (Beckman Model 25 spectrophotometer; Beckman Instruments, Palo Alto, CA) equipped with an 80-μl flow cell with a 1-cm light path) were made after 1 hr of incubation with *p*-nitrophenyl phosphate. The linearity of the TomRSV ELISA test in the range of absorbances read was established ($r^2 = 0.90$) (2).

RESULTS

Union necrosis and decline were observed in 3- to 5-yr-old trees in two apple orchards near Basin City, two near Omak, and one near Riverside, WA. Delicious was grown on MM 106 in the two Basin City orchards and on MM 111 at the other three locations. Detailed examination of symptomatic trees indicated that the Delicious scion had formed a good vascular union with the under-stock only during the first year of growth. In subsequent years there appeared to be little continuity between the xylem of stock and scion, giving rise to a smooth break at the graft union when the tree was bent. On such trees the scions were swollen at the graft union as if girdled. Rings of darkened summer wood often occurred internally across the xylem (Fig. 1). Production of rootstock suckers, dwarfing of tops, and early purpling of foliage in the autumn were common on infected trees.

Virus isolates were recovered from rootstock suckers of four apple trees showing union necrosis and decline at Basin City. These isolates produced mild ringspotting and mottling on tobacco and cucumber, respectively. Infectivity endpoints of extracts from diseased herbaceous tissue ranged from 1:1 to 1:8, as determined by inoculating cucumber. In contrast, isolates TomRSV-O and TomRSV-P produced severe symptoms in the herbaceous indicator hosts and higher infective titers, 1:256 for TomRSV-O and 1:30 for TomRSV-P.

In agar gel double diffusion tests, three of the four Washington apple isolates (trees 1, 3, and 4; Table 1) reacted positively with TomRSV-P antiserum. However, in simultaneous infection studies, all four Washington apple isolates tested positive for TomRSV by ELISA (Table 1) by using healthy cucumber sap, TomRSV-P and TomRSV-O in cucumber sap, and ApMV in apple leaf sap as controls. The results (Table 1) are average values derived from four replicate wells per sample. As a threshold positive value, we chose to use the mean reading of healthy cucumber sap plus two standard deviations (= 0.03 A_{405}). TomRSV was also detected by ELISA directly in leaf extracts of apple rootstocks from both Basin City orchards.

In another series of experiments, leaf extracts from the orchard near Basin City were tested by using apple rootstock suckers from trees appearing normal and trees expressing symptoms typical of union necrosis and decline. All three extracts from trees showing union necrosis and decline reacted positively in TomRSV-P ELISA tests; none of the extracts from eight normal-appearing trees reacted positively with TomRSV-P antiserum in ELISA tests. Leaf extracts of normal apple rootstock, healthy cucumber, cucumber infected with TomRSV-P, and extracting buffer were used as controls. The results (Table 2) are average values of four replicated wells per sample. As a positive threshold value, we again used the extract of healthy leaves plus two standard deviations.

DISCUSSION

This is the first report of apple union necrosis and decline disease in and isolation of TomRSV from commercial fruiting apple orchards in the western United States. The incidence and distribution of this disease in Washington are unknown. We believe, however, that some of the abnormal autumn purple coloration of apple trees in Washington, previously attributed to collar rot caused by *Phytophthora cactorum* (Leb. & Cohn) Schroet., may be connected with union necrosis and decline.

The lack of serologic reactions for one of the four Washington apple isolates against antiserum to TomRSV-P in agar gel and the low ELISA absorbance readings for these same heterologous combinations, compared with the high homologous reading, suggest to us that the Washington apple isolates of TomRSV

that we tested are serologically distinct from the Pennsylvania isolate. Stouffer and Uyemoto (5) also found that TomRSV isolates from MM 106 from Oregon were serologically distinct from New York and Pennsylvania isolates.

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