

Susceptibility of Some Strawberry Cultivars to Tomato Ringspot Virus as Determined by ELISA

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ABSTRACT

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The susceptibility of 52 strawberry cultivars to graft infection by an isolate of tomato ringspot virus (TmRSV) from strawberry was determined by leaflet-graft inoculations and subsequent detection by enzyme-linked immunosorbent assay (ELISA). In 18 cultivars, TmRSV was readily detected by ELISA, whereas in 21 cultivars, it was infrequently detected and then usually at low levels. In 13 cultivars, TmRSV was not detected. In preliminary inoculation tests of three susceptible cultivars, leaflet grafts were found by ELISA to transmit virus only 42% of the time. This level of reliability requires that 10 of 10 grafted plants of a given cultivar must test negative for TmRSV by ELISA to state with a confidence level of $P = 0.996$ that the cultivar did not contain ELISA-detectable TmRSV. Therefore, in this study, at least 10 separate graft inoculations of a given cultivar were tested for TmRSV before determining that no ELISA-detectable virus was present.

Tomato ringspot virus (TmRSV) and its American dagger nematode vectors (*Xiphinema americanum* (Cobb) and/or closely related species) are known to be widespread in temperate regions of North America (9). Although severe outbreaks of TmRSV infection in strawberry fields have not been reported and the rate of infection in strawberry in the field appears to be low, naturally infected plants are weakened and occasionally killed (2). The objective of this study was to screen many of the major U.S. strawberry cultivars by enzyme-linked immunosorbent assay (ELISA) for the presence of TmRSV after graft inoculation.

An isolate of TmRSV from red raspberry infected 11 of 13 commercial strawberry cultivars in leaf-grafting studies conducted in Canada (6). Most of the infected cultivars were killed; however,

Catskill survived in weakened condition, and British Sovereign and Sparta were found to be immune to infection by leaf grafting followed by sap-inoculation assay to *Cucumis sativus* L. (cucumber) or *Nicotiana tabacum* L. (tobacco). In a replicated study of the susceptibility of 22 strawberry cultivars grown on the U.S. Pacific Coast, four (Lassen, Olympus, Puget Beauty, and Sequoia) were found to be infected during 19 mo of growth in a field naturally infested with American dagger nematodes known to be carrying TmRSV (2). In subsequent unpublished studies, two more of those 22 cultivars (Rainier and Totem) were found to be susceptible to TmRSV by dagger nematode transmission in the field or greenhouse.

The identification of modern U.S. strawberry cultivars in which no TmRSV can be detected by ELISA after graft inoculation may provide a pool of germ plasm from which plant breeders can develop cultivars that can be safely planted on unfumigated land without the resulting disease loss from this virus. Therefore, many of the major strawberry cultivars grown in the United States were collected and inoculated with TmRSV in the greenhouse by leaf grafts to expand the list of strawberry cultivars whose response to graft inoculation by TmRSV is known. An abstract of some of these results has appeared (3).

MATERIALS AND METHODS

Plants of 52 strawberry cultivars currently grown commercially in the United States were obtained as certified stock from several commercial nurseries

and grown in the greenhouse at Oregon State University in Corvallis. Randomly sampled plants from 21 of these cultivars were found by ELISA to test free of TmRSV. Stock of Olympus, Puget Beauty, Rainier, OR-US 4681, and OR-US 4682 that indexed negative for known viruses was grown in the greenhouse in flats of soil from a previously tested strawberry planting (2) known to be infested with dagger nematodes carrying TmRSV. Clones of these cultivars were subsequently identified by ELISA as positive for TmRSV and negative for tobacco ringspot virus and were pooled for leaf tissues to inoculate the collection of 52 strawberry cultivars. Leaflets from infected plants were grafted into two petioles each of at least five plants of each cultivar. All other expanded leaves on the inoculated plants were removed at the time of grafting, as described by Frazier (5). Plants were held in intermittent mist for 10 days, and those with one or (generally) two surviving grafted leaflets were then grown on the greenhouse bench for an additional 5 wk.

Leaf samples were harvested from five plants of each cultivar 42 days after grafting, then pooled and evaluated for TmRSV by standard ELISA procedures (1). The pooled sample for each cultivar was composed of one or more young, fully expanded leaves from each of the five graft-inoculated plants of that cultivar. For each cultivar where the first ELISA reading was negative, a second set and, if necessary, a third set of leaf grafts were made to the original group of plants of the cultivar and brought up to a total of five plants for each graft round by adding new plants as needed. Leaves showing viruslike symptoms were often harvested when first detected (after the first pooled sample was negative) and were used to index each plant individually by ELISA until an infected plant was found or until a total of 15 leaf-graft inoculations had been made to these five test plants and all had indexed negative up until 42 days after grafting.

For serological detection of TmRSV, a rabbit antiserum was used that had been prepared earlier against an Oregon red raspberry isolate of TmRSV increased in cucumber and purified according to the method of Stace-Smith (9). The double-

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antibody sandwich ELISA method was used with triplicate wells per sample, alkaline phosphatase conjugate, *p*-nitrophenyl phosphate substrate, and Immulon-2 ELISA plates (Dynatech Labs., Alexandria, VA). Absorbance determinations at 405 nm ($A_{405\text{nm}}$) were made on an EL 307 through-the-plate reader (Bio-Tek Instruments, Burlington, VT). Known TmRSV-infected and healthy strawberry leaf samples and buffer controls were used in each ELISA plate. The threshold positive value for a given plate was taken as the mean absorbance ($A_{405\text{nm}}$) for five wells of healthy strawberry sap on that plate plus three times its standard deviation. *Fragaria virginiana* Duch. 'UC-11' plants, known to be very susceptible indicators (4), were included in the graft-inoculation tests. Nine of 102 graft-inoculated UC-11 plants (9%) were negative in subsequent ELISA for TmRSV even though the virus donor leaflets survived equally well in all instances.

RESULTS

Because false-positive ELISA readings have been reported from tests of TmRSV from very young, healthy apple shoot tips (7), very young, young, mature, and old leaves from healthy Totem strawberry plants were tested for TmRSV by ELISA. The average ELISA $A_{405\text{nm}}$ value for all four ages of healthy Totem leaves was 0.023 (range 0.018–0.028), compared with 0.415 for mature leaves of TmRSV-infected Rainier strawberry.

The reliability of ELISA detection of TmRSV was determined for three cultivars (Fletcher, Fresno, and Tioga) known from preliminary studies to be difficult sources for frequent positive detections by ELISA after leaf-graft inoculation. Individual ELISA for TmRSV on grafted plants of each of these cultivars yielded a total of 11 TmRSV positives in 26 evaluations, or 42% detection. If these data are used to estimate the reliability of detecting TmRSV in similar cultivars by these methods, negative ELISA readings on 10 plants of a known susceptible cultivar grafted with TmRSV have a probability of occurrence of $[P(1-0.42)^{10}] = 0.004$.

On the basis of ELISA readings of 52 TmRSV-inoculated strawberry cultivars, the virus was readily detected in 18, infrequently detected in 21, and not detected in 13. Representative ungrafted control plants of the cultivars tested negative for TmRSV by ELISA.

Cultivars with ELISA readings consistently positive for TmRSV. ELISA reactions were positive in all five plants tested of each of the 18 cultivars: Aiko, Allstar, Aptos, Benton, Catskill, Darrow, Fairfax, Florida Belle, Guardian, Hood, Midway, Olympus, Ozark Beauty, Puget Beauty, Sequoia, Sunrise, Totem, and Tufts.

Cultivars with infrequent detection of TmRSV. In the second group of 21

cultivars, TmRSV was detected infrequently, usually at low absorbance levels, after five to 35 graft inoculations (mean of 12) of five grafted plants per cultivar. These 21 cultivars, with the numbers of positive ELISA readings and total number of tests in parentheses, were: Aliso (2/35), Apollo (1/15), Brighton (2/12), Cruz (5/10), Douglas (2/25), Earliglow (1/35), Fletcher (2/21), Fort Laramie (1/25), Fresno (5/16), Hecker (1/40), Jerseybelle (1/30), Rainier (3/13), Redchief (3/23), Redglow (4/25), Scott (1/12), Shasta (5/10), Shuksan (5/10), Surecrop (1/25), Tioga (2/25), Tribute (3/29), and Vesper (4/31).

Cultivars with ELISA readings consistently negative for TmRSV. ELISA results were consistently negative for 13 cultivars through three repeated graft-inoculations on each of five plants per cultivar. These cultivars (number of ELISA tests in parentheses) were: British Sovereign (25), Delite (20), Earlidawn (30), Gem (20), Marlate (20), Northwest (25), Ourown (25), Pocahontas (25), Quinault (30), Raritan (20), Redstar (21), Sparkle (25), and Tristar (25).

Twenty-three graft-inoculated cultivars in which TmRSV was either not detected by ELISA or was detected in only a few instances after repeated tests, were sap-inoculated to *Chenopodium quinoa* Willd. with 2% aqueous nicotine alkaloid, but no symptoms developed. Rainier, similarly graft-inoculated, caused necrotic local lesions and systemic necrosis in six of six *C. quinoa* sap-inoculated at the same time.

DISCUSSION

A relatively low level (42%) of successful ELISA detection of TmRSV was observed in this study in certain susceptible strawberry cultivars after leaflet-graft inoculation despite the demonstrated sensitivity of the ELISA technique (1,2). We thus confirm earlier published data from other cultivars of 63 and 61% detection (2,6). Similar data from known-susceptible *F. virginiana* indicator plants (93/102 = 91% detection) in this study compare favorably with 73 and 52% in previously published graft inoculations of known-susceptible *F. vesca* indicators (2,6).

TmRSV was readily detected by ELISA in pooled samples from 18 cultivars. In contrast, 21 cultivars indexed ELISA-positive only from a second or third set of graft inoculations to five plants. After leaflet grafting, the first leaves showing symptoms appear most likely to be positive for TmRSV by ELISA. To increase the chances of detecting TmRSV from these cultivars, several leaves from an inoculated plant were collected and tested together when they first developed symptoms. Even so, several inoculated plants of these cultivars often had to be sampled and tested in this way to obtain positive

ELISA results from at least one plant. With some cultivars, up to eight separate tests were necessary before TmRSV was detected in at least 1 of the 5–35 instances in which grafted plants were individually assayed. In one graft-inoculated Fresno plant, leaves from a subsequently produced side crown had an $A_{405\text{nm}}$ reading of 0.256 compared with a reading of 0.020 obtained from young leaves from the main crown sampled at the same time. The threshold healthy value in this case was 0.016. These results point to the dangers inherent in using negative ELISA values to evaluate genetic resistance of strawberries graft-inoculated with TmRSV, where hypersensitive reactions are likely to occur, although positive ELISA results should be credible, as claimed earlier (2). Problems were also encountered in selecting suitable tissues for detecting TmRSV in apple trees infected with union necrosis and decline disease (10) and in *Prunus* spp. infected with stem-pitting disease (8).

The major objective of this study was to categorize many modern U.S. strawberry cultivars as susceptible or resistant to TmRSV as determined by ELISA. For 13 cultivars, TmRSV was not detected in five plants, each graft-inoculated three times, including the cultivar Sparkle, found susceptible in an earlier Canadian study (6). British Sovereign was found not susceptible to TmRSV in both this and the Canadian study.

Of the 16 strawberry cultivars found in natural infection studies (2; R. H. Converse, *unpublished*) not to be infected by TmRSV, 13 were included in the present leaf-graft inoculation study, and TmRSV was subsequently detected by ELISA in all but two (Northwest and Quinault). TmRSV inoculation of strawberry roots by dagger nematodes probably introduces much less inoculum into the shoot system than leaflet grafting. Whether any cultivars that are readily infected by graft inoculation are resistant to TmRSV infection by dagger nematode must be determined experimentally. We can presume, however, that cultivars that consistently test ELISA-negative after leaflet-graft inoculation by TmRSV should be highly resistant to infection in the field by the same strain of the virus via nematode vectors.

The preliminary testing of graft-inoculated strawberry cultivars for the presence of TmRSV in this study only measured the presence or absence of ELISA-detectable TmRSV coat protein, not necessarily infection. Additional pathological and genetic studies should be undertaken with strawberry cultivars identified in this and in previous studies (2,6) to determine their potential value as sources of high resistance to TmRSV in the development of new strawberry cultivars.

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