Irregular Distribution of Tomato Ringspot Virus at the Graft Unions of Naturally Infected Stanley Plum Trees

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ABSTRACT

Cummins, J. N., and Gonsalves, D. 1986. Irregular distribution of tomato ringspot virus at the graft unions of naturally infected Stanley plum trees. Plant Disease 70:257-258.

Graft unions of 20 14-yr-old Stanley plum trees (*Prunus domestica*) naturally infected with tomato ringspot virus (TmRSV) were examined visually for brownline symptoms, and rootstocks were indexed by enzyme-linked immunosorbent assay (ELISA). Samples for visual and ELISA detection of TmRSV were taken around the trunk circumference at each graft union. Static sampling revealed that both lateral distribution of TmRSV, as indicated by ELISA, and lateral development of brownline symptoms at the graft union were nonuniform. Visual samplings from all four quadrants were required to identify 90% of the infected trees, whereas ELISA samples from three quadrants were required for this degree of accuracy.

Additional key words: prune brownline, *Prunus cerasifera*

In Stanley plum trees, the prune brownline (PBL) syndrome, including constriction and subsequent decline, has consistently been associated with tomato ringspot virus (TmRSV) (3). Recently, PBL was produced after bud-inoculating Stanley plum trees with TmRSV (D. Gonsalves and J. N. Cummins, *unpublished*), and TmRSV is therefore considered the incitant of PBL.

TmRSV is irregularly distributed throughout trees of *Malus domestica* Borkh. (MM106 rootstock) (1); other viruses also have been reported to be irregularly distributed in apple (4), in sweet cherries (*P. avium* L.), and in tart cherries (*P. cerasus* L.) (5). However, in surveys in which we routinely indexed apple and plum orchards for TmRSV, we assumed that a large proportion, perhaps 90%, of TmRSV-infected trees were detected. The actual number of samples required to detect at least 90% of infections was never carefully investigated. This study was designed to determine appropriate sampling methods for field surveys of TmRSV infection in plums.

MATERIALS AND METHODS

Visual examination of graft unions and enzyme-linked immunosorbent assay (ELISA) (2) indexing for TmRSV were used to study virus distribution in 20 14-yr-old Stanley plum trees growing on a site with large populations of *Xiphinema americanum* Cobb, a major vector of TmRSV (3,8,9). Rootstocks studied included *P. domestica* cvs. Brompton, Yellow Egg, and St. Julien A; *P. cerasifera* Ehr. cv. Myrobalan B; and five seedling myrobalan lines. In 1982 and 1983, ELISA indices of the rootstock portions of these trees were negative. We took as many as eight samples from each tree by removing strips 1 cm wide across the union, observing each sample for brownline (BL) symptoms at the union, and securing samples of inner bark 1 cm below and 1 cm above each union for ELISA indexing. The number of samples varied with the surface topography of the trunk. Circumferences of the Stanley trees 20 cm above the union ranged from 18 to 25 cm.

These bark samples were indexed individually for TmRSV with antisera to peach yellow bud mosaic virus (9) and grapevine (6) and elderberry (10) isolates of TmRSV. All three antisera react strongly to the TmRSV isolate found in this plum planting (M. W. Bitterlin and D. Gonsalves, *unpublished*). Microtiter plates were coated with gamma globulin at 2–5 μg/ml, and alkaline phosphatase-labeled gamma globulins were used at 1/300–1/800 (v/v) dilutions. Tissues were collected and processed for ELISA as described by Rosenberger et al (7), except bark samples were put into cold buffer in the field and hand-ground with mortar and pestle in the laboratory. Reactions in microtiter plates were read at 405 nm with a MR580 Microelisa Autoreader (Dynatech Laboratories, Inc., Torrance, CA). A reading was considered TmRSV-positive if it was at least double that of the healthy control and had a minimum $A_{405\,nm}$ value of 0.20.

RESULTS AND DISCUSSION

BL symptoms were observed in 19/20 trees. No positive ELISA readings were obtained from Stanley tissue. In three trees on which we observed BL symptoms on only 1/10 to one-third of the circumference, we obtained positive ELISA readings 4–10 mm circumferentially beyond the BL. On tree 310 (Table 1) only one of six ELISA samples was positive, and no BL was detected at any of six sample sites.

Chi-square tests revealed no differences among rootstocks for incidence of infection or for lateral distribution of infection within an individual tree. We therefore pooled data for all rootstocks. Of the samples from the rootstock portions of these infected trees assayed by ELISA, 54% were positive; 44% of the union examinations revealed BL symptoms (Table 1).

We calculated probabilities of obtaining negative ELISA readings or of failing to detect BL symptoms visually in TmRSV-infected trees by using the probability equation $Q = (1 - P)^n$, where Q = probability of failure to detect infection, P = probability of successful detection, and n = number of samples taken (Table 2). Because 54% of the ELISA samples in

Table 1. Record of ELISA readings and visual observations of unions of individual Stanley plum trees in which tomato ringspot virus infection was first detected in 1984

Rootstock Tree no.	No. of positive observations/no. of observations: ELISA	Visual brownline symptoms
St. Julien A		
101	1/4	1/5
208	4/4	4/5
211	2/3	3/7
215	1/3	2/5
310	1/6	0/6
705	2/4	2/4
714	1/3	3/5
Brompton		
105	1/4	1/4
208	4/4	4/5
307	2/4	2/4
308	5/5	4/5
313	1/2	1/4
Myrobalan		
212 (Myro. B clonal)	1/4	1/4
730 (Myro. B)	5/5	4/8
1208 (G-13 seedling)	1/1	3/5
1120 (G-20 seedling)	2/5	2/5
1121 (G-20 seedling)	0/1	1/5
210 (G-25 seedling)	1/4	1/6
312 (G-25 seedling)	1/2	1/3
420 (G-29 seedling)	1/1	3/3

Accepted for publication 26 September 1985.

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Table 2. Probability of detecting a tomato ringspot virus-infected rootstock under Stanley plum

No. of graft union samples per tree	Detection by ELISA	Detection by visual examination of union
1	0.54	0.44
2	0.78	0.68
3	0.90	0.82
4	0.95	0.90
5	0.98	0.94

this experiment were positive, the probability for failure (Q) to detect TmRSV by ELISA with two samples is $(1.000 - 0.54)^2 = 0.21$ and probability of successful detection is $1.00 - Q = 0.79$.

In the block of Stanley plum trees considered in this experiment, samples from three quadrants per tree were required to achieve a 90% rate of successful detection by ELISA. Using two samples taken from opposite sides of the tree as we had originally planned would have resulted in failure to identify 20% of the infected trees. Visual examination for BL symptoms was less accurate than ELISA indexing; a sample from each quadrant of the tree was required for a 90% detection rate, and our conventional two samples per tree would have missed one-third of the infected trees. Because four visual examinations of unions per tree can be done very rapidly and inexpensively, we will be using this as our basic orchard survey method, and we will use ELISA only for confirmation.

The work reported in this paper was done in June, July, and August, when bark was slipping freely. Visual examination is much more difficult to conduct during other seasons, and results may therefore be less accurate.

In Delicious/MM106 apple trees with TmRSV-infected root systems, lateral development of the BL symptom is similar to that reported here for Stanley plum. In infected MM106 rootstocks, detectability of TmRSV by ELISA also varies around the trunk. We therefore recommend that multiple sampling around the circumference at the graft union should be done on apples as well as plums.

ACKNOWLEDGMENTS

We thank Eunice Williams, Mary Jean Welser, David Gill, and David Hummer for technical support. This research was supported in part through USDA General Cooperative Agreement 58-32U4-0-151 (Appalachian Fruit Research Station) and by a grant from International Dwarf Fruit Tree Association, which we gratefully acknowledge.

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