Apple Tip Leaf Antigens That Cause Spurious Reactions With Tomato Ringspot Virus Antisera in Enzyme-Linked Immunosorbent Assay

G. I. Mink, W. E. Howell, and P. R. Fridlund

Plant pathologist, agriculture research technologist III, and plant pathologist, respectively, Irrigated Agriculture Research and Extension Center, Washington State University, Prosser 99350.
Washington State University College of Agriculture and Home Economics, Pullman 99164, Scientific Paper 6747, Project 1719.
Accepted for publication 1 October 1984.

ABSTRACT

Mink, G. I., Howell, W. E., and Fridlund, P. R. 1985. Apple tip leaf antigens that cause spurious reactions with tomato ringspot virus antisera in enzyme-linked immunosorbent assay. Phytopathology 75:325-329.

Extracts from rapidly growing shoot tip leaves collected during the summer from apparently healthy apple rootstock and scion trees produced absorbance values ($A_{405\ nm}$) in enzyme-linked immunosorbent assay that indicated the presence of tomato ringspot virus (TmRSV). However, no virus was detected in these tissues by bioassay or partial purification. Leaves from the tips of rapidly growing apple shoots appear to contain unidentified, possible nonviral, antigens that react with antibodies in several TmRSV antisera. These reactions were indistinguishable from those obtained with TmRSV. The significance of this finding relative to propagating stock certification is discussed.

Additional key words: clonal apple rootstocks.

Tomato ringspot virus (TmRSV) has been associated repeatedly with apple union necrosis and decline (AUND), a graft union disorder that appears several years after some scion cultivars have been grafted onto certain clonal rootstocks, especially Malling-Merton (MM) 106 (3,4,9,11,16–18). In several eastern states in the United States where both TmRSV and its nematode vectors are widely distributed (5,6,8,12–14), orchard trees presumably can be infected with TmRSV from local sources (12). In addition, rootstocks such as MM 106 can be infected with TmRSV in the nursery and remain symptomless until grafted (3,4,16–18). Consequently, this virus is of concern to both nurserymen and growers.

In 1981, AUND was reported in eastern Washington (11). In that study, it was not determined whether TmRSV occurred naturally in the orchards or was introduced with the nursery stock. However, the virus is known in other areas of the state (8). For this reason, stool-beds of all clonal apple rootstocks produced for virus certification in Washington are tested annually for TmRSV.

In 1982, leaf and stem samples were taken at random during June from stool-beds of all clonal rootstock cultivars produced by participating Washington nurseries and tested for TmRSV by enzyme-linked immunosorbent assay (ELISA). No virus was detected in any of more than 5,000 plants tested. To accommodate nursery inspectors and to simplify sample collection, the 1983 tests were made with rapidly growing shoot tips. The initial ELISA results suggested that nearly every plant tested was infected.

The purpose of the studies reported here was to investigate this phenomenon and to determine whether rapidly growing tips of many cultivars of apparently healthy apple trees contain antigens that react either with TmRSV antibodies or with antibodies against some unidentified antigen frequently co-purified with TmRSV.

MATERIALS AND METHODS

Source of apple tissues. The initial series of ELISA tests that precipitated this study were made with rapidly growing tips (growing point plus one to three young leaves) collected at random in July from 100 stool-bed plants of each of four clonal apple rootstock cultivars (MM 106, MM 109, MM 111, and EM VII) grown by a commercial nursery in Washington. During the remainder of the growing season, we tested numerous trees of apple rootstock and scion cultivars grown in containers under screen or in fields near Prosser. Many of these same plants were retested during the spring of 1984.

Virus isolates, purification, and antisera. In 1980, an isolate of TmRSV from a tree affected by AUND and subsequently designated TmRSV-A (11), was obtained from R. M. Stouffer and maintained by serial passage in cucumber or cowpea seedlings. The virus was purified from cucumber or cowpea seedings by the method of Stace-Smith (15). Density gradient zones that contained intact virus were removed from three 10–40% rate sucrose density gradient tubes, combined, concentrated into 2 ml by differential ultracentrifugation, and given a second density gradient ultracentrifugation. The single visible infectious zone was removed and used for antiserum production. A rabbit was given four 1.0-ml intramuscular injections (at 1-wk intervals) with purified virus (0.1 μg/ml) mixed with an equal volume of Freund's complete adjuvant (first injection) or incomplete adjuvant (three subsequent injections). In preliminary tests, this antiserum, when used as described below, produced ELISA absorbance values near zero (0.010) with healthy expanded MM 106 apple leaves and above 1.0 with TmRSV-infected apple leaves provided by R. M. Stouffer. Additional TmRSV antisera used in some tests were provided by C. E. Powell, J. K. Uyemoto (via R. M. Lister), and R. M. Converse.

ELISA conditions. Gamma globulins were prepared from each antiserum by the methods of Clark and Adams (1), diluted to 1–2 μg/ml in pH 9.6 coating buffer, and incubated for 4 hr at 20 C in 50-well Gilford cuvette pacs. Tissue samples (0.2 g) were placed in metal grinding cups with 2 ml of phosphate-buffered saline (PBS), pH 7.4, containing 0.05% Tween-20, 2% polyvinylpyrrolidone, and 0.2% egg albumin and triturated with a rotary file attached to a variable-speed drill (10). Each sample was tested in duplicate wells. Because we had no consistent supply of TmRSV-infected apple leaf tissue, we used expanded cucumber leaves as the standard controls. In each test, expanded leaves from healthy and TmRSV-infected cucumber plants were included in duplicate wells. In many tests, tip leaves from a flowering crab apple cultivar (Jay Darling) that consistently gave low ELISA readings were also included as a negative control. To monitor background color development, all plates contained at least five wells that received grinding buffer only. Samples were incubated overnight at 4 C. Alkaline

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.

©1985 The American Phytopathological Society

phosphatase conjugates were used at dilutions of 1:500 to 1:4,000 and incubated for 3–4 hr at 37 C. Substrate (*p*-nitrophenyl phosphate) at 1 μg/ml was incubated 1–2 hr at 22–24 C. Absorbance at 405 nm ($A_{405\ nm}$) was measured with a Gilford PR-50 automatic reader.

RESULTS

ELISA reactions with apple tissues. Initially, shoot tips from 100 MM 106 stool-bed plants were tested in a total of five plates (20 plants per plate). In each of the five plates the healthy and TmRSV-infected cucumber controls reacted as expected. Absorbance values of buffer-only wells remained at zero for over 2 hr while the $A_{405\ nm}$ values obtained with healthy cucumber leaf tissue ranged between 0.12 and 0.18 (avg, 0.16); values obtained with TmRSV-infected leaves ranged between 1.32 and 2.05 (avg, 1.77). However, the average $A_{405\ nm}$ values obtained with apple shoot tips in these tests ranged from 0.13 to 0.88. The fact that 58 of 100 stool-bed plants produced $A_{405\ nm}$ values in excess of 0.60 (50–75% of the infected cucumber control values) created some concern. Similar results (*unpublished*) were obtained when tip leaves of Jay Darling apple were used as the negative control.

Since the report of Clark and Adams (1) most workers have considered $A_{405\ nm}$ values exceeding 2–3× the healthy control values to be evidence for a positive test. This ratio (sample $A_{405\ nm}$/ negative control $A_{405\ nm}$) was determined for all MM 106 samples (Fig. 1). If 2× the negative control value was considered a positive reaction, 92 of the 100 plants in this test would have been rated as infected with TmRSV. Only two stool-bed plants produced $A_{405\ nm}$ values that were less than those of the healthy cucumber or Jay Darling apple control. This test was repeated twice with young, succulent, light-green leaves (but not growing points) from the same shoots. Although $A_{405\ nm}$ values for individual plants varied substantially among the three tests, the combined results could be interpreted to mean that all 100 plants were infected. Similar results (not reported) were obtained with shoot tips of MM 109, MM 111, and EM VII.

As mentioned above, in 1982 we had indexed over 5,000 stool-bed plants of these same cultivars; most produced by the same nursery, and found no unusual absorbance or other evidence suggesting the presence of TmRSV. In those tests we tested partially expanded leaves (but not tips) and in some cases stem tissue from plants selected at intervals of 30.5 m (100 ft) in each nursery row. In 1983 growing tips were tested from plants selected at random from each row. Individual plants were not marked either year. As a result we could not retest specific plants either serologically or biologically. Consequently, we cannot state unequivocally that every plant tested either year was free of TmRSV. However, it seemed unlikely to us that all plants tested in one season would be free of TmRSV and all plants selected the following season would be infected. Rather, these results suggested that the use of tip leaves for testing by ELISA introduced an artifact of considerable significance to our virus indexing program. Because of the potential importance of these results to virus certification programs of this and other states, we examined possible causes of this phenomenon.

Attempts to transmit TmRSV from selected rootstocks. To determine if all apple rootstock cultivars produced high absorbance values under our conditions, we tested shoot tips from 17 clones of 12 cultivars maintained at Prosser. Eight of these clones had been produced in thermotherapy. One propagant of each clone had been grown continuously under screen in containers which were tested in 1983 and found to be free of dagger nematodes. A second propagant of each clone had grown for 1–4 yr in the field. Both propagants had been indexed at least once on a range of woody indicator plants that included peach (*Prunus persicae*) and *P. tomentosa*, both sensitive to TmRSV strains; some had been indexed by rub-inoculation on cucumber. All were presumed to be free of TmRSV. When tests were made in July absorbance values for 20 of the 34 propagants exceeded 3× the healthy cucumber (or Jay Darling apple) control values. For nine propagants, the $A_{405\ nm}$ values exceeded those of the TmRSV-infected controls (Table 1). The highest absorbance values were associated with rapid shoot growth. Shoot tips which had ceased growth invariably produced $A_{405\ nm}$ values in the range of either the healthy cucumber controls (Table 1) or the controls which consisted of Jay Darling tip leaves (*unpublished*). Tip leaves from all plants were rub-inoculated on three to five cucumber seedlings. All were negative.

In April 1984, the above experiment was repeated. For each of the 34 propagants listed in Table 1, the $A_{405\ nm}$ values obtained with rapidly growing shoot tips was equal to or less than values obtained with healthy cucumber leaves. At least two rapidly growing tips from each propagant were indexed by rub-inoculation on three to five plants each of *Chenopodium quinoa*, cowpea (cultivar Early Ramshorn), and cucumber. All were negative. Parallel inoculations from tip leaves of known TmRSV-infected plants

TABLE 1. Absorbance values ($A_{405\ nm}$) obtained in enzyme-linked immunosorbent assay with tomato ringspot virus antiserum using shoot tips collected in July 1983 from two propagants of 17 apple rootstock clones grown in the field or in a screenhouse

Cultivar	Clone no.	Location[a]	
		Field	Screenhouse[c]
EM II	168	0.36	0.20
	89	0.01	0.39
EM IV	187	0.04	0.23
EM VIIA	188	0.02	0.03[d]
EM VIII	136	0.01[d]	0.05[d]
EM IX	146	0.69	0.15
	100	0.35	0.12
	90	0.01[d]	0.46
EM 25	166	0.38	0.03[d]
EM 26	35	0.50	0.18
MM 102	39	0.00[d]	0.00[d]
MM 104	40	0.32	0.23
MM 106	41	0.21	0.08
MM 109	167	0.76	0.21
	224	0.00[d]	0.05[d]
MM 111	32	0.48	0.13
	42	0.26	0.14
Buffer control		0.00	0.00
Healthy cucumber		0.01	0.05
Infected cucumber		0.33	0.38

[a] All propagants from a given location were tested in a single ELISA plate incubated for 1 hr at 20 C.
[b] Clone numbers above 100 were produced in thermotherapy.
[c] All screenhouse trees were grown in containers which were found to be free of dagger nematodes in 1983.
[d] Terminal growth had ceased before samples were taken.

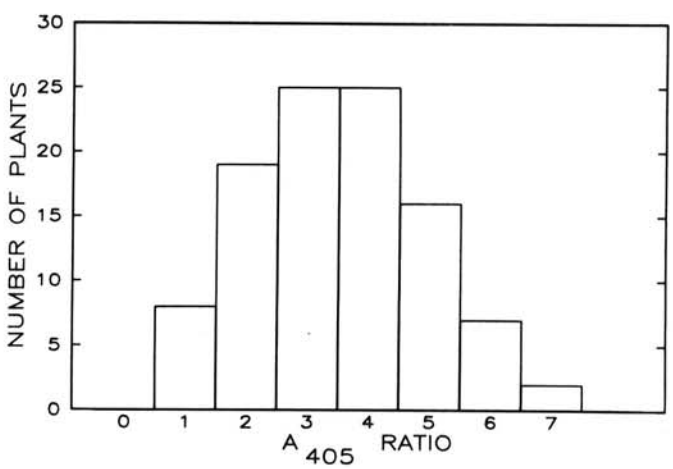

Fig. 1. Histogram of the absorbance ratios (sample $A_{405\ nm}$/ negative control $A_{405\ nm}$) obtained when shoot tips of 100 MM 106 stool-bed plants were tested against a tomato ringspot virus antiserum in five enzyme-linked immunosorbent assay plates. Average $A_{405\ nm}$ values for negative and positive controls were 0.16 and 1.77, respectively.

(peach and apple) all produced typical symptoms on these hosts.

The high absorbance obtained with rapidly growing shoot tips during the summer was not limited to rootstock cultivars. We tested shoot tips from five scion cultivars (Starking Delicious, Golden Delicious, Holly, Smokehouse, and Fireside) and obtained $A_{405\ nm}$ ratios (sample/negative control) of 2.1, 2.6, 4, 11.4, and 13, respectively. Subsequently, in August we assayed the growing tip and 19 leaves (in downward sequence) of two rapidly growing shoots from each of the five cultivars. All 20 samples from a given shoot were tested in duplicate in a single plate and the $A_{405\ nm}$ values from the two shoots were averaged. All tips from cultivars Fireside, Smokehouse, and Holly produced $A_{405\ nm}$ values greater than 0.75 which was 5× that of the healthy cucumber controls (Fig. 2). From the results in Fig. 2, we concluded that the antigens responsible for high $A_{405\ nm}$ values were concentrated in the shoot tip and to a lesser extent in one to four of the youngest leaves. These leaves were less than one-half expanded, light-green, and succulent. The $A_{405\ nm}$ values obtained with newly expanded leaves which were medium-green in color or mature leaves which were dark green were comparable to those obtained with fully expanded leaves of cucumber plants or tip leaves of Jay Darling apple. These results suggest an explanation for why we obtained no unusual absorbance values in the 1982 rootstock tests which were made with nearly fully expanded leaves.

To determine if TmRSV could be transmitted from apple tip leaves that produced high $A_{405\ nm}$ values, we divided the tip of three shoots from Smokehouse in half longitudinally; one-half of each shoot was tested by ELISA, the other was triturated in 0.1 M neutral phosphate buffer containing 2% nicotine and rub-inoculated onto three to five plants of *C. quinoa*, *C. amaranticolor*, cowpea, bean, cucumber, and tobacco. Although each shoot tip produced $A_{405\ nm}$ values above 1.0 in ELISA, none of the inoculated herbaceous plants expressed symptoms. Furthermore, none of the inoculated herbaceous plants subsequently reacted in ELISA at levels above those obtained with either uninoculated plants of the same species or healthy expanded cucumber leaves.

Absorbance values similar to those produced by healthy expanded cucumber leaves were obtained consistently with the following: mature tip leaves of all apple trees collected after terminal growth ceased; dormant terminal or lateral buds; bark tissue from shoots, trunks, or roots; and fruit tissue or seeds.

Reaction with other antisera. The high $A_{405\ nm}$ values produced by apple shoot tips was not a phenomenon associated with a single gamma globulin preparation. Gamma globulins from five different TmRSV antisera produced against four TmRSV isolates gave similar results (Table 2).

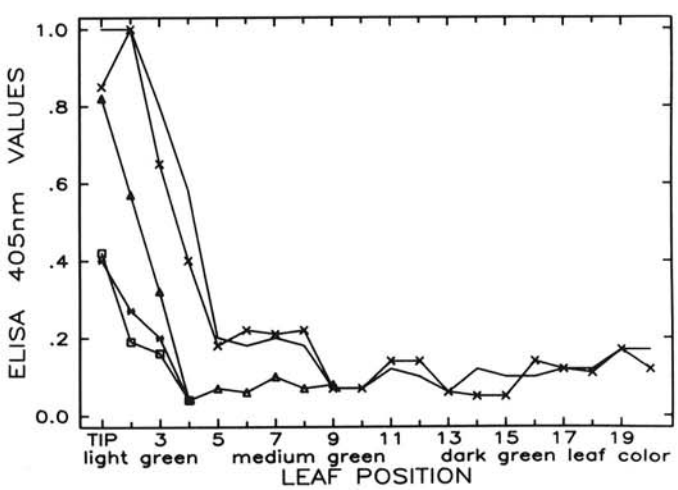

Fig. 2. Absorbance values ($A_{405\ nm}$) obtained with the tip and 19 leaves from rapidly growing shoots from five apple scion cultivars (—— = Fireside, x——x = Smokehouse, △——△ = Holly, *——* = Starking Delicious, and □——□ = Golden Delicious). Each point represents the average of values from two shoots.

Attempts to isolate TmRSV from apple leaves. The field-grown propagant of MM 109 clone 224 (Table 1) exhibited relatively weak growth whereas clone 167 grew vigorously. Shoot tips from clone 224 consistently produced $A_{405\ nm}$ values near zero in contrast to those of clone 167 which produced $A_{405\ nm}$ values that ranged from 24 to 224% of the TmRSV-infected controls (Tables 1 and 2). To determine if TmRSV could be isolated from clone 167, we processed approximately 35 g of tip leaf tissue from each clone according to the TmRSV-purification procedure of Stace-Smith (15). Aliquots were removed from each low- or high-speed supernatant and from each resuspended pellet, treated as described in Table 3, and assayed by ELISA. No ELISA-reactive material was detected at any step from clone 224. With clone 167, high $A_{405\ nm}$ values were obtained with samples taken at every step except the second high-speed supernatant (Table 3).

Density gradient tubes layered with preparations from both clones contained light-scattering materials throughout the lower two-thirds of the tube. Neither tube contained a discrete visible zone in the region where TmRSV was normally found (approximately 26–29 mm below the meniscus under our

TABLE 2. Absorbance values ($A_{405\ nm}$) obtained with healthy and tomato ringspot-infected cucumber leaves and shoot tips from apple rootstock cultivar MM 109 clone 167 using gamma globulins prepared from five different tomato ringspot virus antisera

Antiserum prepared against:	Prepared by:	Buffer	Healthy cucumber	Infected cucumber	Apple tip leaves
Grape isolate (7)	Uyemoto	0.03	0.04	3.18	0.80
Raspberry isolate	Converse	0.03	0.07	3.18	0.97
AUND[a] isolate	Powell	0.00	0.02	2.53	0.99
AUND isolate	Powell	0.05	0.08	3.18	1.06
AUND isolate	Mink	0.00	0.04	3.17	1.04

[a] Apple union necrosis and decline.

TABLE 3. Absorbance values ($A_{405\ nm}$) obtained with samples removed at various steps when tip leaves of two apple rootstock cultivar MM 109 clones were processed according to the tomato ringspot virus purification procedure of Stace Smith (15)

		Clone no.	
Step	Treatment	224	167
Freeze	LS supernatant[a]	0.00	0.42
	LS pellet[b]	0.00	0.42
Ammonium sulfate	LS supernatant[a]	0.00	0.33
	LS pellet[b]	0.00	0.43
1st High-speed	Supernatant[a]	0.00	0.22
	Pellet[c]	0.00	0.83
2nd High-speed	Supernatant[a]	0.00	0.01
	Pellet	NT	NT
Density gradient	Fractions 1–4	0.01	0.01
	Fractions 5–7	0.06	0.58
	Fractions 8, 9	0.00	0.47
	Fractions 10, 11	0.00	0.42
	Fractions 12, 13	0.00	0.51
	Fractions 14–16	0.02	0.47
	Fractions 17–20	0.03	0.34
Control	Healthy cucumber	0.03	0.03
	Infected cucumber	0.52	0.53

[a] One-milliliter samples were removed after low-speed (2,000–10,000 *g*) or high-speed (90,000 *g*) centrifugation, dialyzed overnight against half-strength PBS and assayed in ELISA against TmRSV antiserum.

[b] Pellets were suspended to initial volume in half-strength PBS, dialyzed overnight against the same buffer; a 1-ml sample was removed and subjected to ELISA. NT = not tested.

[c] Pellets suspended overnight in 2 ml of 0.01 M EDTA, 0.5 ml was removed, dialyzed against half-strength PBS, and assayed in ELISA.

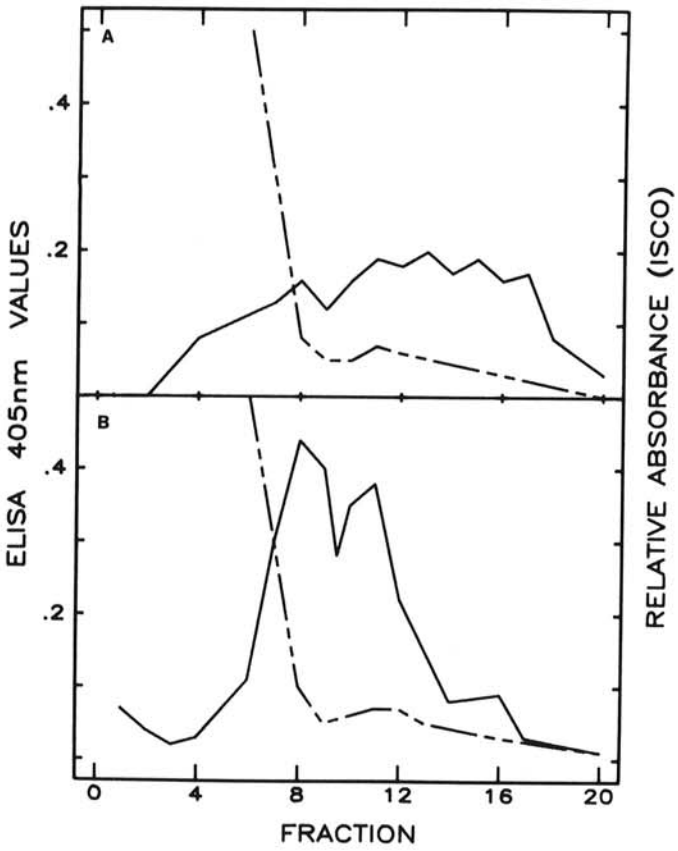

Fig. 3. Absorbance profiles of density-gradient tubes layered with preparations of apple tip leaves (cultivar MM 109 clone 167) processed as described in the text. Absorbance at 254 nm monitored by an ISCO franctionator (broken line). Absorbance at 405 nm of density gradient fractions tested by enzyme-linked immunosorbent assay (solid line). **A,** Initial low-speed pellet. **B,** Resuspended LS pellet.

conditions). However, the tube layered with clone 167 contained ELISA-reactive material scattered throughout the lower four-fifths of the tube (Table 3).

Each of the 14 density gradient samples from clone 167 listed in Table 3 was inoculated onto three to six cucumber plants. None were infectious.

In a second purification attempt, we processed 25 g of tip leaves from clone 167 as follows: the low-speed supernatant obtained after the freeze-thaw step was processed as above. The low-speed pellet, which contained considerable ELISA-reactive material (Table 3), was dispersed with gentle stirring for 1 hr in the initial volume of fresh borate buffer, given a second low-speed centrifugation, and that supernatant (designated resuspended LS pellet) was processed separately. Density gradients layered with both preparations appeared similar. The upper portions of both gradients (fractions 1–8 in Fig. 3) contained considerable ultraviolet (UV) absorbance at 254 mm (Fig. 3) but no visible zones. Both tubes contained a single, slightly diffuse, visible zone located approximately 25–30 mm below the meniscus (fractions 10–13 in Fig. 3). However, the material in these zones had little, if any, absorbance at 254 nm. In addition, material from these zones had no characteristic UV absorption spectrum at wavelengths between 240 and 320 nm.

Both density gradient tubes contained ELISA-reactive material which appeared to be unrelated to any materials detected visually or by UV absorbance (Fig. 3). The tube layered with the original low-speed supernatant contained ELISA-reactive material scattered throughout the lower four-fifths of the tube. In the tube layered with the resuspended low-speed pellet, nearly all of the ELISA-reactive material occurred in two bands; a relatively sharp band in fractions 6–9 and a broader band in fractions 10–13 (Fig. 3).

DISCUSSION

Tomato ringspot virus can be transmitted readily to herbaceous plants from succulent terminal leaves of MM 106 rootstock sprouts growing from the base of AUND trees but has rarely been transmitted from the scion portion (9,11,17,18). However, Cummins and Gonsalves (2) reported that TmRSV could be detected consistently by ELISA in leaves of at least five scion cultivars and inconsistently in at least six others.

Our results clearly demonstrate that rapidly growing shoot tips collected during the summer from many apparently healthy apple trees contain antigens which react, in ELISA, with antibodies present in several different TmRSV antisera. So far as we could determine, these antigens are noninfectious and appear to be neither nucleoprotein nor protein. If this is true, these antigens would appear to be unrelated to TmRSV. The nature of these antigens is being investigated.

When apple shoot tips were processed according to the TmRSV purification procedure of Stace-Smith, the ELISA-reactive material was distributed throughout rate sucrose density-gradient tubes. This material was not detectable by visual and UV absorbance methods commonly used to monitor the purity of virus preparations. Studies in progress indicate that similar antigens occur in young tip leaves of cucumber but that these are either in low concentrations or are absent in expanded leaves and cotyledons. Thus far, we can detect these antigens only by serological methods. It appears likely that they are frequently copurified and injected with TmRSV.

Many of the TmRSV antisera currently being used appear to contain antibodies against the unidentified antigens reported here. In ELISA, they produce reactions that are indistinguishable from TmRSV reactions. Very preliminary unpublished evidence suggests that these antigens and their antibodies also react to form visible precipitates in agar gel double diffusion. We have no explanation for why these antigens were not detected in apple tip leaves collected in April, but were readily detected in tip leaves collected between July and September.

Because of the presence of what we assume to be nonviral antibodies in many TmRSV antisera, we feel that ELISA data that indicate the presence of TmRSV in apple shoot tips should be interpreted with extreme caution, particularly if the trees appear to be healthy and exhibit vigorous growth during the summer months. For virus certification or other regulatory purposes we recommend that if the ELISA technique must be used without confirmatory biological tests, apple shoot tips should either not be tested or tested only in the early spring. Studies in progress indicate that a situation similar to that reported here for apples also exists with grapes and pears.

LITERATURE CITED

1. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen Virol. 34:475-483.
2. Cummins, J. N., and Gonsalves, D. 1982. Recovery of tomato ringspot virus from inoculated apple trees. J. Am. Soc. Hortic. Sci. 107:798-800.
3. Cummins, J. N., Gonsalves, D., and Rosenberger, D. 1979. Union necrosis of apple trees on MM 106 rootstocks in the Hudson Valley. Proc. NY State Hortic. Soc. 124:61-67.
4. Cummins, J. N., Uyemoto, J. K., and Stouffer, R. 1978. Union necrosis and decline, a newly recognized disease of apple trees associated with tomato ringspot virus. Proc. NY State Hortic. Soc. 123:125-128.
5. Forer, L. B., Hill, N., and Powell, C. A. 1981. *Xiphinema rivesii*, a new tomato ringspot virus vector. (Abstr.) Phytopathology 71:874.
6. Forer, L. B., and Stouffer, R. F. 1982. *Xiphinema* spp. associated with tomato ringspot virus infection of Pennsylvania fruit crops. Plant Dis. 66:735-736.
7. Gilmer, R. M., and Uyemoto, J. K. 1972. Tomato ringspot virus in Baco Noir grapevines in New York. Plant Dis. Rep. 56:133-135.
8. Johnson, F. 1972. Tomato ringspot virus of blueberries in Washington. (Abstr.) Phytopathology 62:1104.
9. Lister, R. M., Allen, W. R., Gonsalves, D., Gotlieb, A. R., Powell, C. A., and Stouffer, R. F. 1980. Detection of tomato ringspot virus in apple and peach by ELISA. Acta Phytopathol. Acad. Sci. Hung. 15:47-55.

10. Mink, G. I., and Aichele, M. D. 1984. Detection of Prunus necrotic ringspot and prune dwarf viruses in *Prunus* seed and seedlings using enzyme-linked immunosorbent assay. Plant Dis. 68:378-381.
11. Parish, C. L., and Converse, R. H. 1981. Tomato ringspot virus associated with apple union necrosis and decline in western USA. Plant Dis. 65:261-263.
12. Powell, C. A., Forer, L. B., and Stouffer, R. F. 1982. Reservoirs of tomato ringspot virus in fruit orchards. Plant Dis. 66:583-584.
13. Rosenberger, D. A., Gonsalves, D., Cummins, J. N., and Harrison, M. B. 1983. Survey for tomato ringspot virus in apple orchards in eastern New York. Acta Hortic. 130:199-206.
14. Rosenberger, D. A., Harrison, M. B., and Gonsalves, D. 1983. Incidence of apple union necrosis, tomato ringspot virus and *Xiphinema* vector species in Hudson Valley orchards. Plant Dis. 67:356-360.
15. Stace-Smith, R. 1970. Tomato ringspot virus. Description of plant viruses No. 18. Commonw. Mycol. Inst., Assoc. Applied Biologists, Kew, Surrey, England. 4 pp.
16. Stouffer, R. F. 1977. Association of tomato ringspot virus with graft union necrosis in apple. Compact Fruit Tree 10:35-37.
17. Stouffer, R. F., Hickey, K. D., and Welsh, M. F. 1977. Apple union necrosis and decline. Plant Dis. Rep. 61:20-24.
18. Stouffer, R. F., and Uyemoto, J. K. 1976. Association of TmRSV with apple union necrosis and decline. Acta Hortic. 67:203-208.