# Detection of Tobacco Mosaic and Tobacco Ringspot Viruses in Herbaceous and Woody Plants Near Virus-Infected White Ash Trees in Central New York

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**ABSTRACT**

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Leaves, roots, flowers, and seeds from most of the 45 herbaceous and woody plant species located near declining white ash trees infected with tobacco mosaic virus (TMV) and/or tobacco ringspot virus (TbRSV) were indexed by enzyme-linked immunosorbent assay (ELISA) and infectivity bioassay for TMV and TbRSV in 1983. Seven of 45 species indexed positive for TMV, and 17 of 45 indexed positive for TbRSV. Neither virus was detected by infectivity bioassay in 1983; however, both viruses were transmitted from tissues of some plant species collected again in 1984 that had indexed positive for virus by ELISA in 1983. The tissue samples collected in 1984 were clarified, concentrated, and inoculated onto herbaceous indicator hosts. Tissues from many herbaceous and some woody hosts apparently contained compounds that gave high nonspecific absorbance in ELISA for TbRSV detection. High nonspecific absorbance generally was not observed when tissue samples were indexed for TMV by ELISA.

Additional key words: white ash decline

Both tobacco mosaic virus (TMV) and tobacco ringspot virus (TbRSV) have extensive host ranges that encompass species in about 30 and 17 plant families, respectively (16,18). Both viruses also were detected in white ash trees (*Fraxinus americana* L.) with and without symptoms of ash decline (white ash dieback) in central New York (1). Although a positive correlation between virus infection and symptoms characteristic of ash decline has not yet been established, it nevertheless seemed desirable to determine the extent of TMV and TbRSV distribution in plants growing near virus-infected trees. Virus-infected weed and woody hosts may serve as an inoculum source for infection of white ash, other forest tree species, or agricultural crops. In addition, virus distribution studies may furnish insight into the ecology of TMV and TbRSV in central New York. The following plant species (among others) are common coinhabitants with white ash in hedgerows, meadows, and forested areas in central New York and are reported to be hosts of TMV: apple (3,11), grape (4), plantain (9), and black-eyed Susan (9). The following are reported hosts for TbRSV: dandelion (2,13), *Rubus* sp. (15), plantain (15), grape (5), cherry (17), dogwood (14), apple (12), red clover (10), wild carrot (13,15), American elm (2), and wild mustard (13). This paper reports the results of a study to determine the distribution of TMV and TbRSV in known and previously undescribed herbaceous and woody hosts of these viruses growing near virus-infected and declining white ash trees in central New York.

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## MATERIALS AND METHODS

**Sampling procedures and processing.** Three previously established white ash decline observation plots in central New York (1) that contained TMV- and/or TbRSV-infected white ash trees were selected. Leaf and root tissues were collected from 45 plant species representing 26 plant families. Sampling was done monthly from May through September 1983. In addition, flowers and seeds also were collected from most species. No effort was made to collect tissue of a particular age. The presence of viruslike foliar symptoms, if any, was recorded. Tissues from woody plant species were collected from the same individuals (one or two per plot) throughout the growing season, whereas tissues from herbaceous species were collected from three to five individuals per plot and collection date. The tissue samples were placed in separate plastic bags, labeled, brought to the laboratory, rinsed in tap water, and frozen at −20 C. In September 1983, the tissue samples were thawed. Four grams of each tissue sample was placed in a sterile plastic vial, and enzyme-linked immunosorbent assay (ELISA) extraction buffer (phosphate-buffered saline [8 g of NaCl, 0.2 g of $KH_2PO_4$, 1.2 g of $Na_2HPO_4$, and 0.2 g of KCl per liter of distilled water], pH 7.4, + 0.05% Tween 20 + 1% β-mercaptoethanol) was added to give a 1:5 ratio of tissue to buffer. Each sample was triturated in a Janke and Kunkel Tissumizer (Tekmar Co., Cincinnati, OH) and refrozen at −20 C until indexed by ELISA.

**ELISA.** A direct, double-antibody sandwich (horseradish peroxidase) ELISA system was developed for each virus and used as described previously (1). Plate wells were coated with purified gamma globulin at 20 or 5 μg/ml for the TMV or TbRSV ELISA, respectively (1). Conjugate was diluted 1/100 and 1/200 for the TMV and TbRSV ELISA, respectively. Positive, negative, and buffer controls were included on each plate. Positive controls consisted of dilutions of purified virus. Buffer controls consisted of wells that contained extraction buffer. Negative controls were of two types; wells that contained healthy tobacco sap diluted 1/5 in extraction buffer and centrifuged plant sap of the target species to guard against the possibility of spurious reactions to nonvirus plant constituents. These controls were prepared as follows: One-milliliter aliquots were removed from each triturated tissue sample and combined according to species tissue type. Each mixture then was centrifuged at 250,000 *g* for 90 min to pellet intact or fragmented virus particles. The supernatant liquids were used as virus-free plant sap (negative) controls. To determine if this procedure would reduce virus concentration to below detectable limits, purified TMV and TbRSV were each diluted to 2,000 ng/ml in healthy tobacco sap prepared in extraction buffer, frozen and thawed, and centrifuged as described. The supernatants were assayed by ELISA and infectivity bioassay for the presence of virus. For ELISA, all samples and controls were replicated in a minimum of two wells per plate.

Standard curves were developed for each virus to determine the sensitivity of

ELISA for virus detection and to establish the linear range of ELISA for assessment of virus concentration in tissue samples. Standard curves were prepared by plotting the absorbance at 450 nm ($A_{450nm}$) of dilutions of purified virus in extraction buffer vs. $\log_{10}$ of virus concentration.

Tissue samples were considered positive for virus if the mean $A_{450nm}$ of the sample wells was greater than twice the mean $A_{450nm}$ of the corresponding centrifuged plant sap control wells and if the mean sample $A_{450nm}$ minus the mean $A_{450nm}$ of the corresponding centrifuged plant sap control wells was greater than the $A_{450nm}$ of purified virus at the limit of virus detection by ELISA as determined from the standard curve.

**Infectivity bioassay.** All tissue samples that indexed positive for virus by ELISA and all centrifuged plant sap controls were indexed by infectivity bioassay by rubbing the extracts onto Celite-dusted leaves of tobacco (*Nicotiana tabacum* L. 'Windsor Shade 117') and cucumber (*Cucumis sativus* L. 'National Pickling'). The leaves were rinsed with tap water immediately after inoculation. The plants were incubated in the greenhouse at 25 C for 3 wk and monitored for symptom development.

To compare the sensitivity of ELISA with that of infectivity bioassay for detection of TMV and TbRSV, dilutions of purified virus were prepared in ELISA extraction buffer, then frozen for 1 wk at −20 C to approximate tissue sample preparation, thawed, and indexed by ELISA and infectivity bioassay as described.

In June 1984, tissue samples were collected again from individuals of five plant species. Several individuals of each of these species had indexed positive for virus by ELISA in 1983. Leaf and/or root tissue from these species was triturated in 0.05 M phosphate buffer (pH 8.0) containing 0.02 M sodium diethyldithiocarbamate and 0.02 M sodium thioglycolate. The homogenate was concentrated through one cycle of differential centrifugation (7) and/or precipitation with polyethylene glycol (PEG) (6). Extracts concentrated about 20- to 40-fold were then inoculated onto tobacco and cowpea (*Vigna unguiculata* (L.) Walp. 'California Blackeye') and incubated in the greenhouse for 3 wk.

## RESULTS

The limit of sensitivity of infectivity bioassay for reliable detection of purified TMV and TbRSV diluted in extraction buffer was about 250 ng of virus per milliliter (Table 1). The lower limits of detection by ELISA of purified TMV and TbRSV were 5 and 50 ng/ml, respectively (Table 1, Fig. 1). Absorbance values of dilutions of purified virus from Table 1, when corrected for the absorbance of extraction buffer and plotted against $\log_{10}$ of virus concentration, displayed a linear relationship between 5 and 100 ng of TMV per milliliter and 50 and 5,000 ng of TbRSV per milliliter (Fig. 1). Correlation coefficients were 0.999 and 0.998, respectively.

In total, about 730 tissue samples from 45 herbaceous and woody plant species were collected from May through September 1983 and indexed for viruses by ELISA. Plants of seven species indexed positive for TMV, and plants of 17 species indexed positive for TbRSV by ELISA (Table 2). In Tables 3 and 4, the absorbance of tissue samples that indexed positive for virus, absorbance of the corresponding centrifuged sap controls, and an estimate of virus concentration (ng/ml) are presented for species that indexed positive for TMV and TbRSV, respectively. Virus concentration was estimated by substituting the mean sample $A_{450nm}$ minus the mean $A_{450nm}$ of the corresponding centrifuged sap control wells (Tables 3 and 4, column A − B) for $Y$ in the equations $\log_{10} X = (Y + 0.238)/0.383$ for TMV and $\log_{10} X = (Y + 0.343)/0.234$ for TbRSV and solving for $\log_{10} X$ (virus concentration). These equations were derived from the general equation for a straight line ($Y = mx + b$) using the absorbance readings of purified virus dilutions minus the absorbance of extraction buffer controls presented in Table 1.

According to the described criteria, plants of the following species did not index positive for either virus by ELISA: *Abies concolor* (Gord. & Glend.) Lindl., *Acer rubrum* L., *A. saccharum* Marsh., *Achillea millifolium* L., *Asclepias syriaca* L., *Chrysanthemum leucanthemium* L., *Daucus carota* L., *Equisetum arvense* L., *Fragaria virginiana* Duch., *F. americana* L., *Galium* sp., *Hieracium aurantiacum* L., *Lonicera japonica* Thunb., *Medicago sativa* L., *Prunus virginiana* L., *Ranunculus acris* L., *Rhamnus catharticus* L., *Rhus typhina* L., *Rosa* sp., *Rudbeckia hirta* L., *Sisyrinchium augustifolium* Mill., *Taraxacum officinale* Weber, *Tilia americana* L., *Trifolium pratense* L., and *Viburnum* sp.

Neither TMV nor TbRSV was detected by ELISA or infectivity bioassay in the supernatants derived from centrifugation of purified virus diluted in healthy tobacco sap. This procedure, therefore, was presumed to be effective in reducing virus concentration in infected plant sap to below the limits detectable by our ELISA and bioassay methods.

Tissues from many plant species apparently contained a compound, or

**Table 1.** Comparison between ELISA and infectivity bioassay for detection of purified tobacco mosaic virus (TMV) and tobacco ringspot virus (TbRSV) diluted in ELISA extraction buffer

| Virus concentration[a] (ng/ml) | TMV | | TbRSV | |
|---|---|---|---|---|
| | $A_{450nm}$[b] | No. of local lesions per half leaf[c] | $A_{450nm}$ | Plants infected (%) |
| 10,000 | 0.796 | 48 | 0.585 | 100 |
| 5,000 | 0.751 | 48 | 0.588 | 100 |
| 2,000 | 0.726 | 11 | 0.499 | 80 |
| 1,000 | 0.665 | 63 | 0.437 | 100 |
| 500 | 0.612 | 11 | 0.384 | 60 |
| 250 | 0.596 | 3 | 0.296 | 30 |
| 100 | 0.551 | 0 | 0.208 | 10 |
| 50 | 0.421 | 0 | 0.124 | 0 |
| 25 | 0.304 | 0 | 0.120 | 0 |
| 10 | 0.168 | 0 | 0.104 | 0 |
| 5 | 0.053 | 0 | 0.112 | 0 |
| Healthy tobacco[d] | 0.027 | 0 | 0.046 | 0 |
| Buffer | 0.023 | 0 | 0.070 | 0 |

[a] Dilutions of purified virus were prepared in ELISA extraction buffer and frozen at −20 C for 1 wk. These dilutions were thawed and used both for ELISA and infectivity bioassay.
[b] The values for absorbance at 450 nm ($A_{450nm}$) represent an average of two wells each.
[c] Each virus suspension was inoculated onto Carborundum-dusted leaves of one *Nicotiana tabacum* 'Windsor Shade 117' plant (TMV) or 10 cucumber plants (TbRSV).
[d] Healthy tobacco sap was diluted 1/5 in extraction buffer.

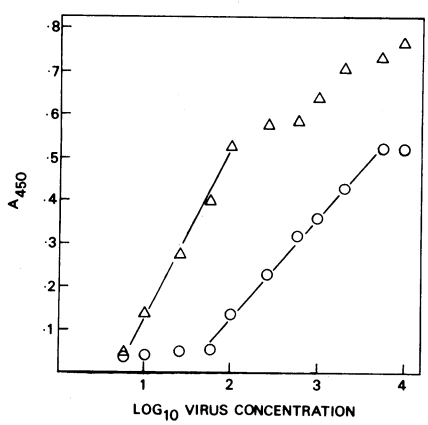


**Fig. 1.** Absorbance at 450 nm ($A_{450nm}$) of dilutions of purified TMV (△) and TbRSV (O) plotted against $\log_{10}$ of virus concentration. Solid lines represent the range in $\log_{10}$ of virus concentrations for which the curves are linear: △—△ = 5–100 ng/ml for TMV and O—O = 50–5000 ng/ml for TbRSV. Correlation coefficients were 0.999 and 0.998 for TMV and TbRSV, respectively. Purified virus was diluted in extraction buffer to 5, 10, 25, 50, 100, 250, 500, 1,000, 2,000, 5,000, and 10,000 ng/ml.

compounds, that gave a high nonspecific background absorbance in ELISA (Tables 3 and 4). The absorbance of centrifuged sap controls, from tissues of plants that contained these substances, were in all cases greater than twice the absorbance of healthy tobacco sap controls included on each plate. (The mean absorbance of 160 healthy tobacco control wells included on about 80 plates each for the TMV and TbRSV ELISA was 0.014 ± 0.006 and 0.034 ± 0.017, respectively.)

The occurrence of high nonspecific absorbance readings (greater than twice the healthy tobacco control values) were more pronounced in the TbRSV-ELISA system (Table 4) than in the TMV-ELISA system (Table 3). Of the 45 species tested, only centrifuged sap controls prepared from some tissues of *Ranunculus, Lonicera* (data not shown), and *Sanguisorba* (Table 3) contained compounds that gave high nonspecific absorbance readings in the TMV-ELISA system. However, centrifuged sap controls prepared from some tissues of 34 of the 45 species tested for TbRSV contained compounds that gave high nonspecific absorbance readings. Centrifuged sap controls prepared from some tissues of species in the genera *Achillea, Hieracium, Taraxacum, Sanguisorba, Medicago, Trifolium, Lonicera, Salix, Galium,* and *Tilia* gave absorbance readings greater than 10 times that of healthy tobacco control wells.

Centrifuged sap controls prepared from tissues of apparently healthy, symptomless white ash did not yield high nonspecific absorbance readings in either the TMV- or TbRSV-ELISA system.

Neither TMV nor TbRSV were transmitted from any of the tissue samples collected, processed, and indexed positive for virus by ELISA in 1983. In addition, neither virus was transmitted from the supernatants of the centrifuged plant sap controls.

Based on virus concentration and detection frequencies (Tables 3 and 4), virus transmission was attempted again in 1984 from leaf tissues of *Solidago, Arctium, Potentilla*, and *Cornus*

**Table 2.** Indexing results of tissues collected from herbaceous and woody plant species that tested positive for tobacco mosaic virus (TMV) and tobacco ringspot virus (TbRSV) by ELISA[a]

| | No. of tissue samples that indexed positive for virus/no. of samples indexed | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Leaves | | Roots | | Flowers | | Seeds | |
| Host plant | TMV | TbRSV | TMV | TbRSV | TMV | TbRSV | TMV | TbRSV |
| Compositae | | | | | | | | |
| *Arctium lappa* L. | 0/8 | 1/8 | 0/2 | 0/2 | 0/1 | 0/1 | 0/2 | 0/2 |
| *Solidago canadensis* L. | 8/20 | 0/20 | 0/16 | 0/16 | 0/8 | 0/8 | 0/2 | 0/2 |
| Cornaceae | | | | | | | | |
| *Cornus racemosa* Lam. | 0/11 | 1/11 | 1/9 | 2/9 | 0/3 | 0/3 | 0/7 | 3/7 |
| *C. stolonifera* Michx. | 0/11 | 1/11 | 2/6 | 6/6 | 0/2 | 0/2 | 0/3 | 0/3 |
| Cruciferae | | | | | | | | |
| *Brassica* sp. | 0/4 | 2/4 | 0/3 | 0/3 | 0/3 | 0/3 | –[b] | – |
| Fabaceae | | | | | | | | |
| *Vicia* sp. | 0/2 | 1/2 | 0/2 | 0/2 | – | – | – | – |
| Gramineae | | | | | | | | |
| *Avena* sp. | 2/4 | 0/4 | 0/4 | 0/4 | 0/1 | 0/1 | 0/1 | 0/1 |
| Liliaceae | | | | | | | | |
| *Erythronium americanum* Ker. | 0/1 | 0/1 | 0/1 | 1/1 | – | – | – | – |
| Osmundaceae | | | | | | | | |
| *Osmunda cinnamomea* L. | 0/2 | 1/2 | 0/2 | 0/2 | – | – | – | – |
| Papaveraceae | | | | | | | | |
| *Plantago lanceolata* L. | 3/7 | 1/7 | 0/7 | 0/7 | 0/4 | 0/4 | – | – |
| Primulaceae | | | | | | | | |
| *Lycimachia nummularia* L. | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 | 1/1 | – | – |
| Rosaceae | | | | | | | | |
| *Crataegus* sp. | 0/11 | 1/11 | 0/3 | 0/3 | 0/1 | 0/1 | 0/1 | 0/1 |
| *Potentilla* sp. | 2/10 | 1/10 | 4/9 | 6/9 | 0/2 | 0/2 | 0/2 | 1/2 |
| *Pyrus malus* L. | 0/5 | 1/5 | – | – | 0/1 | 0/1 | 0/3 | 0/3 |
| *Rubus occidentalis* L. | 0/19 | 0/19 | 0/14 | 6/14 | 0/8 | 0/8 | 0/5 | 0/5 |
| *Sanguisorba minor* Scop. | 1/5 | 0/5 | 0/5 | 0/5 | – | -- | – | – |
| Salicaceae | | | | | | | | |
| *Populus tremuloides* Michx. | 0/11 | 1/11 | 0/1 | 0/1 | – | – | – | – |
| *Salix nigra* March. | 0/7 | 1/7 | – | – | 0/1 | 0/1 | 0/1 | 0/1 |
| Ulmaceae | | | | | | | | |
| *Ulmus americana* L. | 0/10 | 2/10 | – | – | – | – | – | – |
| Vitaceae | | | | | | | | |
| *Vitis aestivalis* Michx. | 0/5 | 1/5 | 0/4 | 0/4 | – | – | 0/4 | 0/4 |

[a] A sample was considered positive for virus if the $A_{450nm}$ of the sample well was greater than twice the $A_{450nm}$ of the corresponding centrifuged sap control, and the $A_{450nm}$ of the sample well was greater than the $A_{450nm}$ of purified virus at the limit of detection by ELISA (from Fig. 1).

[b] – = Not tested.

*stolonifera* and root tissues of *Potentilla, C. stolonifera*, and *C. racemosa*. Local lesions characteristic of TMV developed on tobacco inoculated with leaf sap of *Solidago* sp. concentrated about 40-fold by differential centrifugation. Leaf tissue of *Solidago* sp. frequently indexed positive for TMV by ELISA in 1983 (Table 3). In addition, TMV was transmitted to tobacco in 1984 from burdock (*Arctium* sp.) leaf sap concentrated 18-fold by the PEG method. Burdock did not index positive for TMV by ELISA in 1983 but did index positive for TbRSV. TbRSV was transmitted to cowpea from leaf sap of *C. stolonifera* concentrated 23-fold by both methods and from root sap of *C. racemosa* also concentrated 23-fold by both methods.

## DISCUSSION

High-speed centrifugation was an effective means to reduce virus concentration to below the limits detectable by our ELISA and infectivity bioassay systems. However, centrifuged sap prepared from tissues of many plant species and used as negative controls in ELISA still gave high absorbance interpreted to be due to nonspecific reactions.

The following are three possible explanations for the high nonspecific absorbance in ELISA:

1. Some plant species may contain compound(s), perhaps peroxidases, that cause high nonspecific absorbance and therefore effectively reduce the sensitivity of ELISA for virus detection. Nevertheless, the use of centrifuged plant sap controls permitted the detection of both TMV and TbRSV in plant tissues by ELISA.

2. Recently, G. I. Mink reported (*personal communication*) that expanding leaves from virus-free apple trees produced high absorbance values when tested for tomato ringspot virus (TmRSV) by ELISA. He postulated the existence of a widespread immunogenic plant compound that was copurified from cucumber

**Table 3.** Mean absorbance values and standard deviations of tissue samples from species that indexed positive for tobacco mosaic virus (TMV), the corresponding centrifuged plant sap controls, and conversion of sample absorbance values minus control absorbance values to virus concentration

| Plant host | Tissue | No. of samples that indexed positive | Mean $A_{450nm}$ and SD of positive samples[a] (A) | Mean $A_{450nm}$ and SD of centrifuged plant sap controls[b] (B) | (A − B) | Estimated virus concentration[c] (ng/ml) |
|---|---|---|---|---|---|---|
| *Potentilla* sp. | Leaves | 2 | 0.068 ± 0.013 | 0.026 ± 0.001 | 0.042 ± 0.013 | 5.4 |
| | Roots | 4 | 0.070 ± 0.039 | 0.017 ± 0.003 | 0.053 ± 0.039 | 5.8 |
| *Sanguisorba minor* | Leaves | 1 | 0.135 ± 0.005 | 0.056 ± 0.002 | 0.079 ± 0.005 | 6.7 |
| *Cornus racemosa* | Roots | 1 | 0.058 ± 0.003 | 0.016 ± 0.001 | 0.042 ± 0.003 | 5.4 |
| *C. stolonifera* | Roots | 2 | 0.052 ± 0.011 | 0.018 ± 0.001 | 0.034 ± 0.011 | 5.1 |
| *Solidago canadensis* | Leaves | 8 | 0.077 ± 0.037 | 0.016 ± 0.037 | 0.061 ± 0.037 | 6.0 |
| *Plantago lanceolata* | Leaves | 3 | 0.063 ± 0.019 | 0.029 ± 0.001 | 0.034 ± 0.019 | 5.1 |
| *Avena* sp. | Leaves | 2 | 0.117 ± 0.060 | 0.030 ± 0.003 | 0.087 ± 0.060 | 7.0 |

[a] Mean absorbance at 450 nm ($A_{450nm}$) and standard deviations are based on two wells per sample. A sample was considered positive for virus if the mean $A_{450nm}$ of the sample wells were greater than twice the mean $A_{450nm}$ of the corresponding centrifuged plant sap control wells. In addition, the mean $A_{450nm}$ of the sample wells must be >0.030 ($A_{450nm}$ of purified TMV at 5 ng/ml minus the $A_{450nm}$ of extraction buffer, from Table 1).
[b] Mean $A_{450nm}$ and standard deviations are based on a minimum of two wells per sample.
[c] Virus concentration estimated by the equation $\log_{10} X = (Y + 0.238)/0.383$.

**Table 4.** Mean absorbance values and standard deviations of tissue samples from species that indexed positive for tobacco ringspot virus (TbRSV), the corresponding centrifuged plant sap controls, and conversion of sample absorbance values minus control absorbance values to virus concentration

| Plant host | Tissue | No. of samples that indexed positive | Mean $A_{450nm}$ and SD of positive samples[a] (A) | Mean $A_{450nm}$ and SD of centrifuged plant sap controls[b] (B) | (A − B) | Estimated virus concentration[c] (ng/ml) |
|---|---|---|---|---|---|---|
| *Crataegus* sp. | Leaves | 1 | 0.297 ± 0.068 | 0.120 ± 0.007 | 0.177 ± 0.068 | 167 |
| *Potentilla* sp. | Leaves | 1 | 0.434 ± 0.020 | 0.190 ± 0.024 | 0.244 ± 0.020 | 322 |
| | Roots | 6 | 0.287 ± 0.111 | 0.064 ± 0.008 | 0.223 ± 0.111 | 262 |
| | Seeds | 1 | 0.157 ± 0.044 | 0.041 ± 0.001 | 0.116 ± 0.044 | 92 |
| *Rubus occidentalis* | Roots | 6 | 0.069 ± 0.025 | 0.021 ± 0.001 | 0.048 ± 0.025 | 47 |
| *Cornus racemosa* | Leaves | 1 | 0.173 ± 0.015 | 0.069 ± 0.004 | 0.104 ± 0.015 | 81 |
| | Roots | 2 | 0.248 ± 0.040 | 0.049 ± 0.002 | 0.199 ± 0.040 | 207 |
| | Seeds | 3 | 0.258 ± 0.037 | 0.114 ± 0.002 | 0.144 ± 0.037 | 120 |
| *C. stolonifera* | Leaves | 1 | 0.311 ± 0.001 | 0.155 ± 0.006 | 0.196 ± 0.001 | 201 |
| | Roots | 6 | 0.257 ± 0.029 | 0.106 ± 0.018 | 0.151 ± 0.029 | 129 |
| *Populus tremuloides* | Leaves | 1 | 0.217 ± 0.059 | 0.084 ± 0.010 | 0.133 ± 0.059 | 108 |
| *Salix nigra* | Leaves | 1 | 0.137 ± 0.006 | 0.059 ± 0.004 | 0.078 ± 0.006 | 63 |
| *Pyrus malus* | Leaves | 1 | 0.153 ± 0.000 | 0.076 ± 0.006 | 0.077 ± 0.000 | 63 |
| *Arctium lappa* | Leaves | 1 | 0.475 ± 0.030 | 0.208 ± 0.001 | 0.267 ± 0.030 | 404 |
| *Vicia* sp. | Leaves | 1 | 0.139 ± 0.008 | 0.056 ± 0.009 | 0.083 ± 0.008 | 66 |
| *Brassica* sp. | Leaves | 2 | 0.161 ± 0.037 | 0.061 ± 0.004 | 0.100 ± 0.037 | 78 |
| *Plantago lanceolata* | Leaves | 1 | 0.284 ± 0.001 | 0.137 ± 0.006 | 0.147 ± 0.001 | 124 |
| *Lycimachia nummularia* | Flowers | 1 | 0.163 ± 0.005 | 0.035 ± 0.001 | 0.128 ± 0.005 | 103 |
| *Ulmus americana* | Leaves | 2 | 0.184 ± 0.002 | 0.062 ± 0.003 | 0.122 ± 0.002 | 97 |
| *Vitis aestivalis* | Leaves | 1 | 0.145 ± 0.016 | 0.057 ± 0.004 | 0.088 ± 0.016 | 69 |
| *Osmunda cinnamomea* | Fronds | 1 | 0.188 ± 0.024 | 0.047 ± 0.005 | 0.141 ± 0.024 | 117 |
| *Erythronium americanum* | Roots | 1 | 0.169 ± 0.020 | 0.063 ± 0.001 | 0.106 ± 0.020 | 83 |

[a] Mean absorbance at 450 nm ($A_{450nm}$) and standard deviations are based on two wells per sample. A sample was considered positive for virus if the mean $A_{450nm}$ of the sample wells were greater than twice the mean $A_{450nm}$ of the corresponding centrifuged plant sap control wells. In addition, the mean $A_{450nm}$ of the sample wells must be >0.054 ($A_{450nm}$ of purified TbRSV at 50 ng/ml minus the $A_{450nm}$ of extraction buffer, from Table 1).
[b] Mean $A_{450nm}$ and standard deviations are based on a minimum of two wells per sample.
[c] Virus concentration estimated by the equation $\log_{10} X = (Y + 0.343)/0.234$.

along with TmRSV during virus purification that induced nonvirus antibodies in subsequent antiserum production. Antibodies to this compound(s), if present in our antisera, may react to closely related or identical compounds in other plant species and cause high nonspecific absorbance. In our study, TbRSV but not TMV was increased in cucumber and purified according to a similar procedure used to purify TmRSV. If the existence of such a compound is verified, it may account for the high, apparently nonspecific absorbance experienced with our TbRSV-ELISA system but not the TMV-ELISA system.

3. Nonspecific absorbance also may be attributed to the presence of antibodies to virus coat protein subunits. It is unlikely that virus coat protein subunits would be removed from plant sap controls by high-speed centrifugation. Therefore, high $A_{405nm}$ values in centrifuged sap (supernatants) controls from some plant species may indicate the presence of virus. However, recent experiments in our laboratory (*unpublished*) involving ELISA for detection of TMV and TbRSV coat protein subunits indicated that our antiserum preparations did not contain antibodies to purified coat protein subunits of either virus.

TMV was detected in seven plant species (Tables 2 and 3), and TbRSV was detected in 17 species (Tables 2 and 4). Both viruses were detected in *C. stolonifera, C. racemosa, Potentilla* sp., and *Plantago lanceolata*, which are common clonally propagated inhabitants of meadows and hedgerows in central New York.

Of the species that indexed positive for TMV in this study, to our knowledge, *Cornus* spp., *Sanguisorba minor, Potentilla* sp., *Solidago canadensis*, and *Avena* sp. have not been reported previously as hosts of TMV.

Of the species that indexed positive for TbRSV in this study, to our knowledge, *Crataegus* sp., *Potentilla* sp., *Populus tremuloides, Salix nigra, Arctium lappa, Vicia* sp., *Lycimachia nummularia, Osmunda cinnamomea*, and *Erythronium americanum* have not been reported previously as hosts of TbRSV.

Sap from species that indexed positive for virus by ELISA did not index positive by infectivity bioassay in 1983. The limit of sensitivity of infectivity bioassay for reliable detection of purified TMV and TbRSV, diluted and prepared in a manner similar to tissue preparation for ELISA, was about 250 ng/ml (Table 1). Therefore, with the exception of leaf and root tissues of *Potentilla* sp. and leaf tissue of *A. lappa*, plant tissues did not appear to contain a high enough concentration of either virus to be detected by infectivity bioassay in 1983 (Table 4). Based only on virus concentration (Table 4), it should have been possible to transmit TbRSV from leaf and root tissues of *Potentilla* and leaf tissue of *A. lappa* in 1983. Perhaps a greater number of indicator plants or a more suitable inoculation buffer were needed to achieve virus transmission from these hosts in 1983. The ELISA was more sensitive than infectivity bioassay for detection of TMV and TbRSV.

Both TMV and TbRSV were transmitted in 1984 from tissues of some plant species that indexed positive for virus by ELISA in 1983. The concentration procedures used in 1984 apparently increased the concentration of virus in plant sap of some species to levels now detectable by bioassay or removed virus inhibitors. Although we attempted in 1984 to collect tissue samples from individual plants that had indexed positive for virus in 1983, it was not always possible to determine with certainty which individual plants were sampled in 1983. This may help to explain why virus was not detected by infectivity bioassay in 1984 from all of the plant species that indexed positive by ELISA in 1983 or vice versa. For example, TMV was not detected in *A. lappa* by ELISA in 1983, yet the virus was transmitted from this species in 1984; whereas TbRSV was detected in this species by ELISA in 1983, yet it was not detected by infectivity bioassay in 1984.

Foliar viruslike symptoms were not observed frequently on any of the plant species indexed, although occasionally mosaic, mottle, or other viruslike symptoms were observed. Many virus-infected herbaceous weed hosts often do not display foliar symptoms (15).

Neither virus was detected frequently in flowers or seeds (Tables 3 and 4). TMV was not detected in flowers or seeds of any of the species indexed. TbRSV was detected in seeds of *Potentilla* sp. and *C. racemosa* and in flowers of *Lycimachia nummularia*. Although seed transmission was not investigated in this study, the presence of TbRSV in seeds and flowers of several species indicates that seed transmission may be possible. Furthermore, TbRSV is seed-transmitted in several other plant species (16). TMV generally is not considered to be seed transmitted (18), although transmission has been reported in some hosts including cultivated apple and grape (4,11).

Many of the species in which TMV or TbRSV was detected are clonally propagated in nature, including *C. stolonifera, C. racemosa, Potentilla* sp., *Solidago canadensis, Rubus* sp., *Populus tremuloides, Salix nigra*, and *Osmunda cinnamomea*. The presence of virus in these species provides a means for virus establishment in meadows, hedgerows, and forests in central New York, and from these plants, spread may occur to other plant species. The nematode vector of TbRSV (*Xiphinema americanum* Cobb) is present in New York soils (8) and may aid local spread of TbRSV. In addition, TMV on soil particles may be introduced into the roots of susceptible species through wounding (18).

In central and southeastern New York State, declining white ash almost always grow near dogwood (*C. racemosa* or *C. stolonifera*) and are frequently intermixed with goldenrod and wild grape. In this study, both *Cornus* species, goldenrod, and grape were infected with either or both viruses (Tables 2–4). Virus-infected individuals of these and other plant species are located near virus-infected and declining white ash trees. Therefore, it is possible that virus is transmitted among white ash and these plant species, some of which had not been reported previously as hosts of TMV or TbRSV.

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