A Study of Tomato Ringspot Virus and Prune Brown Line Resistance in Twenty-five Rootstock-Scion Combinations

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ADDITIONAL INDEX WORDS. *Prunus*, plum, graft union, incompatibility

SUMMARY. In a greenhouse study, fifty 1-year-old 'Stanley'/'Myrobalan 29C' plum (*Prunus* sp.) trees were inoculated with tomato ringspot nepovirus (ToRSV) by either nematode inoculation or slash inoculation to compare how inoculation effects the onset of the prune brown line (PBL) disease. In six tests (over 2 years), slash-inoculated trees had a higher percentage of ToRSV infection than nematode-inoculated trees when root and bark samples were tested by enzyme-linked immunosorbent assay (ELISA). ELISA differences between the two treatments were significant by chi-square analysis. None of the ToRSV positives by ELISA developed a brown line at the graft union. In a second experiment, five rootstock ('Myrobalan 29C', 'Marianna 4001', 'Marianna 2624', 'Marianna GF8-1', and 'St. Julian 655-2') and five scion ('Carolyn Harris', 'New York 58.900.12', 'Stanley', 'Valor', and '70031') combinations (total combination = 25) were established in a field plot in Traverse City, Mich., and infected with ToRSV by slash and nematode inoculation. All five rootstocks were infected, with incidences of 40% to 60% ToRSV infections after 3 years. 'Marianna 2624' had the lowest incidence of PBL (5%) compared to 'Myrobalan 29C', which had the highest incidence (30%). The scion 70031 in combination with either 'Myrobalan 29C' or 'Marianna 4001' rootstocks, produced PBL in

100% of the trees. ToRSV was detected by ELISA and northern hybridization assays. ELISA consistently detected more positives when root or bark tissues were tested, and northern hybridization assay consistently detected more positives when rootstock sucker leaves were used.

Tomato ringspot nepovirus (ToRSV) causes prune brown line (PBL) disease in plum and prune (*Prunus* sp.) trees (Cummins and Gonsalves, 1986b; Mircetich and Hoy, 1981). PBL is diagnosed by the presence of a narrow strip of brown necrotic tissue (a brown line) at the graft union. The brown line is caused by the necrosis of the cambial and phloem tissues (Mircetich and Hoy, 1981) that girdle the tree and leads to tree decline and death (Hoy and Mircetich, 1984). The brown line is a result of a hypersensitive reaction of the scion to ToRSV infection in the rootstock. The trees sometimes develop an inverted shoulder of the scion at the graft union and chlorotic spots on the leaves of the rootstock suckers (Brase and Parker, 1955; Mircetich and Hoy, 1981). The disease is also referred to as 'Stanley' constriction and decline (SCAD) (Cummins and Gonsalves, 1986a).

ToRSV is vectored by the dagger nematode *Xiphinema americanum* Cobb 1913 (Hoy and Mircetich, 1984; Stace-Smith, 1984; Teliz et al., 1966, 1967). In California, *X. californicum* Lamberti and Blevé-Zacheo is also a ToRSV vector found associated with fruit trees (Hoy et al., 1984). When virus-infected rootstocks are used in propagation, they can serve as a source of inoculum and, coupled with the vector, result in spread of PBL. Based on field observations, Mircetich and Hoy (1981) suggested that a clonal rootstock, 'Marianna 2624' (*P. cerasifera* Ehrh. x *P. munsonia* Wight & Hedr.), may be more resistant to PBL than peach or 'Myrobalan' plum rootstocks. 'Marianna 2624' was resistant to ToRSV infection when challenged with root chip inoculum from PBL-affected orchard trees (Hoy and Mircetich 1984). A chi-square analysis of a pool of PBL-affected trees propagated on *P. domestica* L. and *P. cerasifera* rootstocks revealed no differences among rootstocks for incidence of infection or for lateral distribution of infection within an individual tree (Cummins and Gonsalves 1986b).

Brase and Parker (1955), in a comparison of three prune cultivars—Fellenberg, Stanley, and Abundance—

propagated on 'Myrobalan' seedling rootstock with chlorotic fleck, found that constriction of the rootstock below the union developed on the 'Stanley' but not the other scion trees. Another symptom observed was alternating long ridges and depressions on the rootstock immediately below the union, resulting in an appearance of angularity in cross-section. The authors suggested that there may be some factor in 'Stanley' that causes the angularity of the stock without serious injury. They also suggested that when this factor combines with the one that causes chlorotic fleck, a constriction is formed at the bud union, roots develop poorly, and some of the trees die.

Twenty-one declining 'Stanley' prune commercial orchards were surveyed for ToRSV and PBL in Michigan (Ramsdell et al., 1993). The percentage of trees testing for positive by enzyme-linked immunosorbent assay (ELISA) ranged from 4% to 82% with a mean of 27.9%. A strong correlation between percentage of trees with PBL and percentage of ToRSV-positive trees was also found. Because of the presence of ToRSV in dandelion populations and the fact that the source of the plant material (nursery stock) did not correlate with percent ToRSV infection, Ramsdell et al. (1993) concluded that ToRSV is endemic in Michigan orchard soils and was not necessarily due to infected nursery stock. A thorough testing of possible resistant plum rootstock-scion combinations for Michigan was needed.

The first objective of this study was to compare the inoculation treatment effects of nematode and slash inoculation on the onset of PBL. The second objective was to test five rootstock and five scions in different combinations for their susceptibility to PBL development after challenge with ToRSV inoculum.

Materials and Methods

Virus purification and RNA extraction

ToRSV was purified from infected 'National Pickling' cucumber seedlings (Stace-Smith, 1984). Leaf and stem tissue was homogenized in cold 0.5 M borate buffer, pH 7.4 (1:2 w/v). The extract was filtered through cheesecloth and clarified with low-speed centrifugation. Ammonium sulfate (15 g/100 mL) was added to the supernatant and the virus precipitated overnight. After a low and high speed centrifugation, the resultant pellet was resuspended in 0.05 M phosphate buffer, pH 7.5. The virus was purified through 5% to 30% (w/v) linear-

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log sucrose density gradients. The purified virus fraction was concentrated by a high speed centrifugation and the pellet was resuspended in 0.05 M phosphate buffer.

The resuspended virus was mixed with sodium dodecyl sulfate (SDS) for a final concentration of 1% SDS, and gently vortexed for 2 min at room temperature. The virus suspension was extracted twice with phenol-chloroform and the viral RNA precipitated with 3 M sodium acetate (0.1 vol), pH 5.0, and chilled ethanol (3 vol). The concentration and purity of the RNA was determined with spectrophotometer readings at 260 and 280 nm.

Plum tree inoculation

NEMATODE INOCULATION FOR THE GREENHOUSE STUDY. Twenty five 1-year-old 'Stanley'/'Myrobalan 29C' plum trees were planted in 5-gal plastic pots in sterilized soil, August 1993. The nematode vector *X. americanum* was added to the soil in the root zone at the rate of 1000 ± 15 per pot and then 'National Pickling' cucumber seedlings were planted five to six per pot and inoculated with ToRSV to serve as an inoculum source for the nematodes. As a control, five 'Stanley' plum trees were treated as above except the cucumbers were mock inoculated with phosphate buffer. The plants were maintained in the greenhouse for the entire duration of the study except when they were kept in cold storage to undergo dormancy for 40 d in the winter.

SLASH INOCULATION FOR THE GREENHOUSE STUDY. In September 1993, another group of 25 'Stanley'/'Myrobalan 29C' plum trees were slash inoculated with purified ToRSV ≈ 1 cm below the graft union. Sterile razor blades were used to make cuts on the bark while a solution of $0.25 \text{ mg}\cdot\text{mL}^{-1}$ ToRSV in sucrose was simultaneously dripped with a pasteur pipette over the area being cut. As a control, 10 plum trees were mock-inoculated as above with unamended sucrose solution. This group of plants was treated in a manner similar to the nematode inoculated trees above.

Plum tree inoculation in a field plot in Traverse City, Mich.

A field plot was established at the Michigan State Univ. Northwest Horticultural Research station in Traverse City in Summer 1993. The plot was a completely randomized design of 1-year-old plum cultivars of five scions and five rootstocks in combination. The plum rootstocks were 'Myrobalan 29C',

'Marianna 4001', 'Marianna 2624', 'Marianna GF8-1', and 'St. Julian 655-2'. The scion cultivars used were 'Carolyn Harris', 'New York 58.900.12', 'Stanley', 'Valor', and '70031'. As described for the potted trees, the plum trees in the experimental plot were nematode-inoculated with the vector *X. americanum* and slash inoculated with purified ToRSV in the same manner. An adjacent control plot was planted with replications of the same rootstock-scion combinations as in the treatment block and were mock-slash inoculated with unamended sucrose solution as detailed above. Trees in the control plot were used as negative checks in ELISA and northern hybridization assay (NHA).

Sampling of the plum trees

From each greenhouse tree, root samples (0.8 g) and bark samples below the graft union (0.4 g) were tested six times in 3 years by ELISA and northern hybridization. Half of each sample was processed for ELISA and half used for total nucleic acid extraction. Only root samples (2.0 g) from the Traverse City field trees were collected in August 1995 and 1996 and processed as above. Some trees occasionally produced rootstock suckers and the leaves were sampled (0.5 to 1.0 g) and processed.

NHA

The plasmid T22 construct with ToRSV cDNA insert and the SP6 promoter was obtained from Ahmed Hadidi (USDA, Beltsville, Md.). The plasmid was linearized with Eco RI restriction enzyme and digoxigenin labeled according to standard protocols (Boehringer Mannheim Corp. Indianapolis, Ind.).

Total nucleic acids were extracted as described by Hadidi and Hammond (1989), and the preparations were dot-blotted (5 to 10 μL) onto a positively

charged nylon membrane prewetted with $20\times$ SSC (3 M sodium chloride, 300 mM sodium citrate, pH 7.0). The nucleic acids were immobilized on the membrane by baking for 1 h at 80 °C. Prehybridization, hybridization, and chemiluminescent detection was done according to standard protocols accompanying kits (Boehringer Mannheim Corp.).

ELISA

The procedure for direct antibody sandwich ELISA was according to Clark (1981). The p-nitrophenyl phosphate substrate was used at a concentration of $0.5 \text{ mg}\cdot\text{mL}^{-1}$ and absorbance readings taken at 405 nm. The threshold value for positive virus identification was the mean A_{405} nm value plus three standard deviations of healthy control samples.

Detection of PBL in Traverse City plum trees

Each tree in the Traverse City plot was examined in 1995 and 1996 for the presence of PBL by removing a 5-cm² bark piece at the graft union and visually evaluating the bark and underlying woody cylinder for evidence of necrotic tissue and a brown line.

Height and shoot growth measurements of the Traverse City plum trees

Each tree in the Traverse City plot was measured for height and shoot growth. Ten shoot growth measurements were taken for each tree and averaged. A Student's *t* test statistical analysis was done on all growth measurements between diseased and healthy scion-rootstock trees of the same combination.

Results

DETECTION OF TRSV IN THE GREENHOUSE PLUM TREES. The results obtained

Table 1. Number and agreement of TRSV positive in nematode-inoculated (NI) and slash-inoculated (SI) greenhouse plum trees from combined results of six enzyme-linked immunosorbent assay (ELISA) and northern hybridization assay (NHA) tests.

	NI		SI	
	Root	Bark	Root	Bark
ELISA	8/25 ²	12/25	21/25	22/25
NHA	13/25	6/25	12/25	3/25
Total ³ (E+NH)	16/25	15/25	21/25	22/25
Agreement ⁴ (E+NH)	5	3	12	3

²Numerator = total trees positive at least once in six tests denominator = total number of trees in test.

³Number of trees that were positive at least once by either ELISA or NHA.

⁴Number of trees that were positive at least once in both ELISA and NHA.

Table 2. Detection of TRSV in rootstock sucker leaf samples of the greenhouse plum trees with enzyme-linked immunosorbent assay (ELISA) and northern hybridization assay (NHA), 1995.

Leaf sample type	February		July	
	ELISA	NHA	ELISA	NHA
Nematode inoculated	1/2 ²	2/2	4/5	5/5
Slash inoculated	0/3	1/3	5/6	6/6

²Number of positive trees with suckers/number of trees with suckers; (not all trees had rootstock sucker leaves available for sampling).

by testing root and bark samples of the nematode- and slash-inoculated plum trees in the greenhouse with ELISA and NHA are presented in Table 1. Data in this table represent the combined positives detected in six tests between November 1993 and July 1995. In comparison, ELISA detected ToRSV infections in a higher number of samples than NHA among extracts prepared from nematode-inoculated bark, slash-inoculated root, and slash-inoculated bark. With nematode-inoculated root extracts, however, NHA detected more samples with ToRSV.

Table 1 also shows the agreement between ELISA and NHA. With well-infected material, as in the case of the slash-inoculated trees, ELISA and NHA were in high agreement, although ELISA detected more positives. That is, all of the NHA positive samples of slash-inoculated root or bark were also detected by ELISA, although ELISA was able to detect more positive samples. With material that may not have been as well infected, as in the case of the nematode-inoculated trees, there was less agreement between the two assays. That is, out of 16 nematode-inoculated root and 15 nematode-inoculated bark samples that tested ToRSV positive by either ELISA or NH, only 5 of the 16 (31%) and 3 of the 15 (20%) were detected positive by both assay methods.

The results obtained by testing the

rootstock sucker leaf samples are presented in Table 2. NHA detected one more ToRSV positive samples than ELISA.

DETECTION OF TRSV IN TRAVERSE CITY PLUM TREES. The plum trees in the field plot at Traverse City were tested twice in 1995 and repeated in 1996. Roots were tested for ToRSV by ELISA and NHA. Positive results were combined and are shown in Table 3. None of the rootstocks were immune to infection by ToRSV. 'St. Julian 655-2' had the least number of ToRSV positive trees (8 out of 20), although this is a high infection rate (40%).

The Traverse City plum trees were also examined each year for symptoms of a ToRSV-induced graft union incompatibility and PBL. Table 4 shows individual scion-rootstock combinations and totals with respect to PBL formation. 'Marianna 2624' was the least affected (1/20), which was reported to be immune to PBL in California (Hoy and Mircetich, 1984). As expected, 'Myrobalan 29C' had the highest PBL formation (6/20). 'Marianna GF8-1' and 'St. Julian 655-2' showed intermediate responses (3/20 and 2/20, respectively). Of the scions, noteworthy is '70031', which triggered formation of PBL on infected rootstocks, especially in combination with 'Myrobalan 29C' and 'Marianna 4001' rootstocks (4/4 in each case.).

GROWTH MEASUREMENTS OF THE TRAVERSE CITY PLUM TREES. There were no significant differences in tree height and shoot growth between any of the rootstock-scion combinations when statistically tested by Student's *t* test (*p* = 0.05).

Discussion

COMPARISON OF ELISA AND NHA. When choosing a detection assay for viruses, the decision is balanced between sensitivity and ease of processing samples. In the case of ToRSV and plum trees, ELISA overall detected more ToRSV-positive trees than NHA (Table 1). This was surprising, as NHA, a more time-consuming assay than ELISA, is generally considered to be more sensitive.

Our low detection of ToRSV by NHA may be due to the efficiency in the total nucleic acid (TNA) extraction procedure. Using an extraction procedure different from the one used in this study, Powell et al. (1991) found that hybridization was more efficient than ELISA in detecting ToRSV infection of nectarine trees when bark extracts were analyzed, but ELISA was more efficient when root extracts were analyzed. The authors did not state a reason for these differences. The buffer used by Powell et al. (1991) was also tried in the total nucleic acid extraction from plum root and bark tissue of our study but did not result in any improved detection of ToRSV RNA. Woody plants are known for their interfering compounds (e.g., phenols and quinones), which present problems in the extraction of proteins and nucleic acids. Other factors that may have contributed to the low detection of the virus were the uneven distribution of the virus in the plant, the low titers of the virus in the woody plant tissue, and the small sample size used in this study (0.4 and 0.8 g in case of bark and root, respectively).

Table 3. Number of tomato ringspot positive² plum trees from four enzyme-linked immunosorbent assay (ELISA) and northern hybridization assay (NHA) tests during the 1995 and 1996 growing seasons at Traverse City, Mich., field plot.

Scion	Rootstock					Total
	M29C	M2624	GF8-1	SJ655-2	M4001	
Stanley	4/4 ²	2/4	0/4	3/4	1/4	10/20
70031	3/4	1/4	4/4	2/4	3/4	13/20
Valor	1/4	3/4	3/4	0/4	1/4	8/20
Carolyn Harris	2/4	2/4	1/4	1/4	4/4	10/20
New York	2/4	2/4	4/4	2/4	2/4	12/20
Total	12/20	12/20	12/20	8/20	11/20	

²Positive by ELISA, NHA, or both; samples were sucker leaf, root, or both.

²Numerator = number of trees positive in at least one assay. denominator = total number of trees of that scion-rootstock combination.

Table 4. Number of plum trees exhibiting brown line symptoms determined by a boat-shaped knife cut at the graft union. Examinations done in 1995 and 1996 at Traverse City, Mich., field plot.

Scion	Rootstock					Total
	M29C	M2624	GF8-1	SJ655-2	M4001	
Stanley	0/4 ²	0/4	1/4	1/4	0/4	2/20
70031	4/4	0/4	1/4	0/4	4/4	9/20
Valor	0/4	0/4	1/4	1/4	0/4	2/20
Carolyn Harris	1/4	1/4	0/4	0/4	0/4	2/20
New York	1/4	0/4	0/4	0/4	0/4	1/20
Total	6/20	1/20	3/20	2/20	4/20	

²Numerator = number of trees exhibiting brown line, denominator = total number of trees of that scion-rootstock combination.

When agreement between ELISA and NHA was examined, some differences were found (Table 1). As a group, the slash-inoculated greenhouse trees were infected at a higher percentage rate and so, presumably, contained a higher titer of virus. Not only did NHA detect fewer positives than ELISA, it did not detect any additional positives over ELISA. In the case of the nematode-inoculated trees, where the infection rate and virus concentration was not as high as in the slash-inoculated trees, there were fewer detected positives and less agreement between ELISA and NHA. This points out the limits of each assay and the need for repeated testing, especially with woody plants, to arrive at a true assessment of percent infection.

While NHA was generally less sensitive than ELISA in detecting ToRSV in root and bark tissue, it was more sensitive than ELISA in the rootstock sucker leaf samples from the greenhouse study (Table 2) and the field plot. Extraction of total nucleic acids from the plum sucker leaf is not apparently inhibited by interfering compounds or perhaps the amount of nucleic acid is much higher in leaf than in bark or root tissue. Additionally, the low detection of ToRSV by ELISA in rootstock sucker leaves may be due to the presence of interfering antibodies with epitopes to plant proteins. ToRSV was propagated in cucumber leaf material for antiserum production and there may have been some plant proteins carried along in the purification process. ToRSV detection by ELISA may work better in bark and roots because those samples do not contain as many of the plant proteins present in leaf material, resulting in a lower ELISA background than when leaf material is tested. Lister et al. (1980) suggested that bark extracts may be more reliable than extracts of rootstock sprout leaves in apple trees for a uniform and efficient detection of ToRSV by

ELISA due to erratic distribution of the virus in the rootstock sprouts. Bitterlin et al. (1984), suggested that the time of year is an important factor for virus detection in different parts of woody plants. ToRSV was consistently detected in the leaves of orchard peach trees in May and in the roots and bark in July (Bitterlin et al., 1984).

COMPARISON OF THE EFFECTS OF TWO INOCULATION METHODS ON INFECTION. The objective of the greenhouse study, in which two groups of 'Stanley'/'Myrobalan 29C' plum trees were either slash or nematode inoculated, was to compare the inoculation treatment effects on the onset of the PBL. Nematode inoculation with *X. americanum*, using ToRSV-infected cucumber as a source of inoculum, is comparable to the natural transmission mode of the virus. Slash inoculation, a mechanical transmission method, is comparable to the situation in which the virus is introduced into the plant through injuries or through infected rootstock planting material. None of the trees in either greenhouse study group developed PBL. Greenhouse trees did not grow as vigorously as the Traverse City trees, presumably because field conditions favored better growth than greenhouse conditions. This could have delayed the onset of any PBL response in the greenhouse trees to some point beyond the time limits of the study. However, more greenhouse slash-inoculated trees tested ToRSV positive than greenhouse nematode-inoculated trees (84% to 88% vs. 32% to 48%, respectively). A chi-square analysis of the ELISA results obtained at the end of six tests indicates a significant difference between the two treatment effects on 'Stanley'/'Myrobalan 29C' plum. In a study comparing different methods of mechanical transmission of ToRSV to peach, Bitterlin et al. (1987), found the knife-slash technique to give the highest and most consistent trans-

mission rate. In the present study, slash inoculation was superior to nematode inoculation.

ROLE OF ROOTSTOCK AND SCION IN PBL DISEASE. Since the greenhouse study demonstrated that slash inoculation results in higher infection rate than nematode inoculation, the choice was made to infect the field-plot trees in Traverse City via the slash inoculation method because of the inherent problems of slow infection with woody plants. The questions to be answered were at what rate relative to each other would the rootstocks become infected and would the fatal PBL disease develop? The results showed that, under high-pressure inoculum rates, all of the five rootstocks were susceptible to ToRSV, even 'Marianna 2624'. However, when occurrence of PBL was examined, several trends were observed. The known susceptible rootstock, 'Myrobalan 29C', had the highest occurrence of PBL (6/20 or 30%), followed by 'Marianna 4001', 'GF8-1', 'St. Julian 655-2', and 'Marianna 2624' (20%, 15%, 10%, and 5%, respectively, Table 4). 'Marianna 2624', which was reported to be immune to PBL in California (Hoy and Mircetich, 1984), may be immune under natural conditions in Michigan also. Only one out of twenty 'Marianna 2624' trees developed a PBL. This is quite low, especially given the high inoculation pressure of slash inoculation.

The effect of the scion on PBL development should be studied further. The scion '70031' combined with 'Marianna 29C' and 'Marianna 4001' rootstocks produced PBL symptoms in all trees. This suggests that the scion also plays an important role in PBL expression.

Literature cited

Brase, K.D. and K.G. Parker. 1955. Decline of Stanley prune trees. Plant Dis. Rpt. 39(5):358-362.

Bitterlin, M.W., D. Gonsalves, and J.N. Cummins. 1984. Irregular distribution of tomato ringspot virus in apple trees. *Plant Dis.* 68(7):567-71.

Bitterlin, M.W., D. Gonsalves, and R. Scorza. 1987. Improved mechanical transmission of tomato ringspot virus to *Prunus* seedlings. *Phytopathology* 77(4):560-63.

Clark, M.F. 1981. Immunosorbent assays in plant pathology. *Annu. Rev. Phytopathol.* 19:83-106.

Cummins, J.N. and D. Gonsalves. 1986a. Constriction and decline of 'Stanley' prune associated with tomato ringspot virus. *J. Amer. Soc. Hort. Sci.* 111(2):315-318.

Cummins, J.N. and D. Gonsalves. 1986b. Irregular distribution of tomato ringspot virus at the graft unions of naturally infected Stanley plum trees. *Plant Dis.* 70(3):257-258.

Hadidi, A. and R.W. Hammond. 1989. Construction of molecular clones for identification and detection of tomato ringspot and arabis mosaic viruses. *Acta Hort.* 235:223-230.

Hoy, J.W. and S.M. Mircetich. 1984. Prune brownline disease: Susceptibility of prune rootstocks and tomato ringspot virus detection. *Phytopathology* 74(3):272-276.

Hoy, J.W. S.M. Mircetich, and B.F. Lownsbery. 1984. Differential transmission of *Prunus* tomato ringspot virus strains by *Xiphinema californicum*. *Phytopathology* 74(3):332-335.

Lister, R.M., W.R. Allen, D. Gonsalves, A.R. Gotlieb, C.A. Powell, and R.F. Stouffer. 1980. Detection of tomato ringspot virus in apple and peach by ELISA. *Acta Phytopathol. Acad. Sci. Hungaricae* 15(1-4):47-55.

Little, T.M. and F.J. Hills. 1975. Analysis of

counts, p. 193-198. In: *Statistical methods in agricultural research*. Univ. of California Press. Davis.

Mircetich, S.M. and J.W. Hoy. 1981. Brownline of prune trees, a disease associated with tomato ringspot virus infection of Myrobalan and peach rootstocks. *Phytopathology* 71(1):30-35.

Powell, C.A., A. Hadidi, and J.M. Halbrecht. 1991. Detection of tomato ringspot virus in nectarine trees using ELISA and transcribed RNA probes. *HortScience* 26(10):1290-1292.

Rezaian, M.A. and L.K. Krake. 1987. Nucleic acid extraction and virus detection in grapevine. *J. Virol. Methods* 17:277-285.

Ramsdell, D.C., V.A. Adler, and C.R. Kesner. 1993. Prune brown line disease occurrence in declining 'Stanley' plum orchards in Michigan and factors related to its incidence. *HortTechnology* 3(3): 325-329.

Stace-Smith, R. 1984. Tomato ringspot virus. Description of plant viruses. No 290. Commonwealth Mycol. Inst.-Assn. Applied Biol. Kew, Surrey, England.

Stace-Smith, R. and D.C. Ramsdell. 1987. Nepoviruses of the Americas, p. 131-165. In: K.F. Harris (ed.). *Current topics in vector research*. vol 3. Springer-Verlag, New York.

Teliz, D., R.G. Grogan, and B.F. Lownsbery. 1966. Transmission of tomato ringspot, peach yellow bud mosaic, and grape yellow vein viruses by *Xiphinema americanum*. *Phytopathology* 56:658-663.

Teliz, D., B.F. Lownsbery, R.G. Grogan, and X.X. Kimble. 1967. Transmission of peach yellow bud mosaic virus to peach, apricot, and plum by *Xiphinema americanum*. *Plant Dis. Rpt.* 51(10):841-843.

Chemical Treatments to Control *Erwinia* Soft Rot of Calla Rhizomes

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ADDITIONAL INDEX WORDS. *Zantedeschia*, calla lily, *Erwinia*, soft rot

SUMMARY. Dimethyl ammonium chloride (DAC, 'Triathlon'), sodium hypochlorite, formaldehyde, and streptomycin ('Agri-mycin 17') were used as dips to treat *Zantedeschia rehmannii* *superba* Engl., *Zantedeschia elliotiana* *maculata* (Hook.) Engl., and *Zantedeschia albomaculata* (W. Wats.) Baill. rhizomes to control *Erwinia* soft rot. A 30 min 200 ppm (mg·L⁻¹) streptomycin dip provided the best control of *Erwinia* soft rot for all three *Zantedeschia* species and a 1-hour 10% formaldehyde dip provided the second best control of inoculated rhizomes. Rhizomes inoculated with *Erwinia* required more days to emerge. Chemical treatments did not affect days to emergence or final plant growth.

Bacterial soft rot, caused by *Erwinia carotovora* subsp. *carotovora* (Jones) Bergey et al. (*Ecc*) is a major limiting factor in calla forcing and causes *Zantedeschia*

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