Prune Brown Line Disease Occurrence in Declining 'Stanley' Plum orchards in Michigan and Factors Related to Its Incidence

D.C. Ramsdell¹, V.A. Adler²,
and C.R. Kesner³

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Summary. Twenty-one declining 'Stanley' prune (*Prunus domestica* L.) commercial orchards in the southwestern, west-central, and northwestern regions of Michigan's lower peninsula were surveyed for prune brown line disease, associated with tomato ringspot virus (TmRSV). Fifty trees from each orchard were examined for a brown line and pitting-grooving symptoms beneath the bark at the graft union. Inner bark and cambium were taken at the graft union for ELISA testing for TmRSV. Dagger nematodes (*Xiphinema americanum* Cobb 1913) (the vector) were extracted from soil samples and enumerated. Dandelions (a TmRSV weed host) also were tested for TmRSV. Information on orchard cultural practices and orchard histories was compiled. The percentage of trees ELISA-positive for TmRSV ranged from 4% to 82%, with a mean of 27.9%. The percentage of orchards in the northwestern, west-central, and southwestern regions in which TmRSV was detected by ELISA was 18.0%, 32.3%, and 35.1%, respectively. There was a strong positive correlation between the percentage of trees with a brown

line at the graft union and the percentage of trees in which TmRSV was detected at each location. The brown line symptom is a good indicator for the presence of TmRSV, but graft-union pitting and grooving did not correlate strongly with the presence of the virus. TmRSV was detected in dandelion plants in 63% of the orchards tested. Dandelion densities, which ranged from <0.5 to 10/m², did not correlate positively with percentage of ELISA-positive trees. Numbers of dagger nematodes ranged from 0 to 132 per cm³ of soil. Vector nematode populations correlated positively with ELISA-positive trees from southwestern Michigan, but not in the other two regions. Orchard age, which ranged from 6 to 22 years, did not seem to relate to the percentage of trees in which TmRSV was detected, nor did the source of the plant material used to establish the orchards. Both 'Myrobalan' and peach rootstocks were heavily infected. Preplant and at-planting applications of fenamiphos as a strip treatment were ineffective in preventing infection. We believe that TmRSV is endemic in Michigan orchard soils and that the virus is not being introduced to new orchards through the use of infected planting material.

Tomato ringspot virus (TmRSV) is well-documented as the cause of economically serious diseases in woody fruit crops throughout the north-central and northeastern United States and eastern Canada (State-Smith and Ramsdell, 1987). The array of fruit crops infected by this virus is wide, including apple (Rosenberger et al., 1985), blueberry (unpublished data), cherry (Morrissey, 1988; State-Smith and Ramsdell, 1987), grape (Powell et al., 1990; Uyemoto, 1975), peach (Bitterlin et al., 1988), raspberry (Converse et al., 1970), and 'Stanley' plum (Cummins and Gonsalves, 1986a, 1986b). A brownline disease of 'French' prune has been reported in California (Mircetich and Hoy, 1981), and is also caused by TmRSV.

An increase in the number of declining 'Stanley' plum trees in orchards throughout western Michigan has been reported by county agents over the past 10 to 15 years. Symptoms shown by these declining trees fit the description of 'Stanley' constriction and decline disease (SCAD) (Cummins and Gonsalves, 1986a) and prune brown

linedisease (Mircetich and Hoy, 1981).

The purpose of this research was to: 1) determine the incidence of brown line disease in 'Stanley' plum in Michigan's declining plum orchards in relationship to the presence of TmRSV (the disease will be referred to as "brown line disease" hereafter); 2) ascertain the correlation between positive ELISA results for TmRSV and a brown line and/or pitting-grooving at the graft union; 3) determine relationships between brown line disease incidence and the dagger nematode vector/dandelion populations; 4) assess the effect of grower-applied nematicides on brown line incidence; and 5) determine if orchard age, rootstock type, or nursery source influence the incidence of brown line disease in commercial plum orchards.

Materials and methods

Survey of plum orchards. To determine the impact of TmRSV-induced disease on 'Stanley' plum in Michigan, a total of 21 commercial orchards were surveyed. Seven orchards from each of three geographical areas of Michigan's lower peninsula were sampled. These areas included the northwestern region of the state (Grand Traverse and Leelanau Counties), the west-central region (Benzie, Manistee, and Oceana Counties), and the southwestern region of Michigan (Van Buren County). Each of these areas have about 1820 ha (4500 acres) of trees.

After consultation with county agents in each of the areas, only horticulturally well-managed orchards were selected for the survey to minimize the interference of factors other than TmRSV [e.g., peach tree borer (*Synanthedon pictipes*), black knot disease (*Dibotryon morbosum*), Armillaria root rot (*Armillaria melea*), and Cytospora canker (*Valsa leucostoma*).

Sampling for ELISA, examination of the graft union for symptoms of brown line disease, and sampling for dagger nematodes and dandelions. Fifty trees in each orchard were selected for ELISA tests and bark examination. In all cases, there were patchy areas of trees throughout surveyed orchards that showed sparse growth and leaf yellowing (Fig. 1). Trees in and around these areas were sampled. In many cases, the growers pushed the trees over, which allowed us to examine thoroughly the graft unions for the presence of a brown

Michigan State University, East Lansing, MI 48824.

¹Professor of Plant Pathology, Dept. of Botany and Plant Pathology.

²Former Veterinary Medicine student, Dept. of Veterinary.

³Professor of Horticulture (retired), Dept. of Horticulture.

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Fig. 1. Appearance of a 'Stanley' prune tree with advanced prune brownline disease caused by tomato ringspot virus. Note chlorosis of leaves and sparse growth.

line and pitting-grooving symptoms. We also were able to examine the trunk more thoroughly for the presence of lesser peach tree borer damage and/or other disorders, e.g., sunburn. A special tool (Fig. 2) was used to remove an ellipsoidal piece of bark $\approx 2 \times 7.5$ cm (0.8×3.0 inches), centered on the graft union. The tool, when struck with a hammer, cut through the bark. The cut bark was removed from the tree with a knife. This was done in three places spread evenly around the rootstock circumference near ground level because it has been shown that bark sampling from three places on the trunk gives a 90% rate in detecting TmRSV in 'Stanley' plum (Cummins and Gonsalves, 1986). The bark pieces were placed in a plastic bag and kept on ice until processed for ELISA testing 5 to 7 h later. Each tree was examined for the presence of a brown line and/or stem pitting-grooving at the graft union (Fig. 3). One to nine dandelion plants (depending on availability) were collected from beneath trees showing a graft union brown line. Known TmRSV-free dandelions were used as controls in ELISA tests. Powell et al. (1982) and Mountain et al. (1984) have implicated dandelions as important in the spread of TmRSV in fruit crops. Dandelion plant density (plants/ m^2) was determined at each location in the immediate area sampled.

Fifteen soil samples were taken throughout the area sampled in each orchard. These were taken with a shovel at a depth of 15.2 to 19.3 cm (6 to 8 inches) and subsampled to make a single aggregated soil sample of 2 liters. The samples from each site were processed by sieving, screening, and the Jenkins sugar flotation technique (Jenkins, 1964) to extract the dagger nematode (Bloom et al., 1972), which is the vector of TmRSV. Dagger nematodes were counted and expressed as numbers per 100 cm^3 of soil.

ELISA testing. Usually, two orchards were sampled on a given day. On the evening of the sampling day, bark samples were processed for ELISA. One gram of cambial tissue was scraped from the inner face of the bark samples taken from each tree and ground in 10 ml of PBS-Tween-PVP ELISA extraction buffer, pH 7.4, using a high-speed homogenizer (Tekmar Co. Inc., Cincinnati). In addition, 1 g of dandelion leaves was ground in 10 ml of the same buffer. F(ab')_2 -ELISA (Barbara

Fig. 2. Metal punch tool used to take ellipsoidally shaped bark samples at the graft union. Note brown line formation at graft union.

Fig. 3. Close-up view of brown line and pitting-grooving of trunk at graft union of 'Stanley' plum on 'Myrobalan' rootstock infected with tomato ringspot virus, the cause of prune brownline disease ('Stanley' constriction and decline disease).

and Clark, 1982) was used for all tests. This variation of ELISA was chosen over double antibody sandwich ELISA because it has been shown to be two to three times more sensitive for detection of TmRSV in cherry bark samples (Morrissey, 1988). Wells of Immulon-1 microtiter plates (Dynatech, Alexandria, Va.) were precoated with 200 μ l of a dilution of 1 μ g anti-TmRSV-IgG-F(ab')₂ fragments per ml of coating buffer. The rabbit antiserum had been made in our laboratory to a Michigan raspberry isolate of TmRSV. Following incubation for 4 h at 37C, plates were washed three times with PBS-Tween. Next, 200 μ l of each sample extract was plated in duplicate wells in separate plates and incubated at 4C overnight. The next day, wells were rinsed three times with PBS-Tween and 200 μ l of a solution containing 1.5 μ g·ml⁻¹ of anti-TmRSV-IgG in extraction buffer was placed in each well and incubated at 37C for 3 h. After rinsing as before, 200 μ l of a 1:1000 (v/v) dilution of protein-A

alkaline phosphatase (Sigma, St. Louis) was added to each well. After incubation for 3 h at 37C, wells were washed as before. Para-nitrophenyl-phosphate substrate (Sigma) (1 mg·ml⁻¹ in diethanolamine buffer, pH 9.8) was added at a volume of 200 μ l per well. Reactions were read after 30 and 60 min with an ELISA reader (Model No. EL307, Biotech Co., Winooska, Vt.) at 405. Absorbance values exceeding twice the mean absorbance of healthy prune cambial samples were considered as TmRSV-positive.

Growers cultural practices in surveyed orchards. Growers were interviewed at the site, or later by telephone or questionnaire, regarding the history of the surveyed plum planting. Information requested consisted of the nursery stock source, planting age, rootstock type, and preplant fumigation practices.

Results and discussion

Data from all aspects of this survey are summarized in Table 1.

ELISA-positive trees. ELISA-positive trees within the orchard blocks ranged from 4% to 82%, with a mean of 27.9%. The northwestern, west-central, and southwestern Michigan regions had mean percentages of 18.0%, 32.3%, and 35.1% trees ELISA-positive, respectively.

Correlation between trees with an ELISA-positive reaction for TmRSV and presence of a brown line or pitting at graft union. In general, the presence of a brown line and/or pitting-grooving at the graft union correlated positively (highly significant) with an ELISA-positive reaction ($r = 0.77$; $P \geq 0.01$). The correlation of graft-union pitting-grooving by itself with ELISA-positive reactions was nonsignificant ($r = 0.27$; $PI 0.27$).

Nematode levels relative to TmRSV incidence in orchards. Overall, the level of detectable dagger nematodes varied from 0 to 132 per 100 cm³ of soil, with a mean of 20.6 per 100 cm³ of soil. Throughout the three geographical areas, correlation of dagger nematode populations with ELISA-positive trees was low ($r = 0.300$; $PI 0.18$). Analysis by region gives a somewhat different picture. The northwestern orchards had a mean population of 19.1 per 100 cm³ of soil, while the west-central and southwestern orchards had mean populations of 0.7 and 42 per 100 cm³ of soil, respectively. Correlations between dagger nematode populations and ELISA-positive trees by geographic area were low, except for the southwestern region, which had a significantly high positive correlation ($r = 0.71$; $P \geq 0.07$).

The southwestern orchards were on the sandiest soils. It is known that dagger nematodes prefer sandy soils. The west-central orchards were on heavier clay loam-type soils. Northwestern soils were of an intermediate loamy sand type. Differences in nematode populations probably are related to these different soil types.

The southwestern orchards had the highest percentage of TmRSV-infected trees and the highest dagger nematode populations. However, the west-central orchards had nearly as high a percentage of trees infected, even though we found this area to have the lowest dagger nematode population. The reason for this disparity is not known. Nematode populations may fluctuate seasonally and with environmental conditions.

Table 1. Plum brown line survey—western Michigan (declining 'Stanley' prune orchards).

Region and grower description	Area	Nursery source	No Xiph/100 cm ³	No. plum trees/50 ELISA positive for TmRSV	No. with brown line per 50 trees	Orchard age (years)	Rootstock	Preplant nematocide ^a
Northwestern Michigan								
A	Grand Traverse	Hawley, Mich.	20	2	3	12	Myro	No
B	Grand Traverse	Stark, Mo.	4	12	36	12–14	----	No
C	Grand Traverse	May, Wash.	104	10	5	9	Halford peach	No
D	Grand Traverse	Newark Nursery, Mich.	6	7	0	12–14	---	No
E	Grand Traverse	Emlong, Mich.	0	4	5	15	Myro	No
F	Grand Traverse	Newark Nursery, Mich.	0	11	4	12	Myro	Yes (strip)
G	Grand Traverse	Newark Nursery, Mich.	0	17	17	12	Myro	Yes (strip)
West-central Michigan								
H	Oceana	Hawley, Mich.	0	5	0	14	Myro	Yes (strip)
I	Oceana	C & O, Wash.	1	41	37	6	Myro	No
J*	Oceana	---	0	4	11	---	---	---
K	Ludington	Newark Nursery, Mich.	1	25	26	10	Myro	No
L	Ludington	C & O, Wash.	3	14	19	12–15	Myro	No
M	Ludington	Stark, Mo.	0	18	12	19+	Myro	No
N	Ludington	Stark, Mo. and C & O, Wash.	0	6	6	22	Myro & peach	No
Southwestern Michigan								
O	Van Buren	Newark Nursery, Mich.	23	10	21	9	Myro	Yes (strip)
P	Van Buren	Newark Nursery, Mich.	132	36	45	10	Myro	Yes (strip)
Q	Van Buren	Newark Nursery, Mich.	82	14	34	10	Myro	Yes (strip)
R	Van Buren	Sierra Gold, Calif. or May, Wash.	5	6	18	12	---	No
S	Van Buren	Bountiful Ridge, Md., and Newark Nursery, Mich.	7	6	13	10–12	Myro	No
T	Van Buren	Newark Nursery, Mich.	0	23	28	10	Myro	No
U	Van Buren	Newark Nursery, Mich.	45	22	25	>20	Myro	No

^aFenamiphos strip application 2 to 3 m wide, applied at the rate of 3.36 to 10.1 kg·ha⁻¹ (3 to 9 lb/acre) a.i.

*Dashed = no information.

^aSevere peach borer damage.

Dandelion density relative to TmRSV incidence in orchards.

Dandelion plants were present in all of the orchards surveyed. In 63% of the orchards, 33% to 100% of the dandelion plants sampled were ELISA-positive for TmRSV. In 37% of the orchards, none of the dandelions sampled were ELISA-positive. Even if strip weed control were used, dandelions grew in the treated strip and in the mowed areas. Because none of the orchards had clean cultivation, the effect of nearly total dandelion control could not be measured. The density of dandelions ranged from <0.5 (low) and 0.5 (medium) to 10 per m² (high). Dandelion density had a weak positive correlation

(highly significant) with percentage of TmRSV-infected trees ($r = 0.507$; $P \leq 0.02$).

Effect of granular nematocide treatments on disease incidence

In all preplant or at-planting time nematocide treatments, fenamiphos was applied as a strip treatment 2 or 3 m (6 to 9 ft) wide, centered on the tree row as a granular treatment. The rate of chemical applied varied from 3.36 to 10.1 kg·ha⁻¹ (3 to 9 lb/acre) (active ingredient basis). These rates would be inadequate even in the banded area for complete control of the dagger nematode at a depth of 0.5 m (1.5 ft). In addition, as the tree roots grew out of the zone of treatment, they would be

exposed to an untreated vector population. Strip nematocide treatments did not prevent infection of prune trees with TmRSV. The six orchards receiving strip treatments of fenamiphos had a mean nematode population of 47.4 per 100 cm³ of soil, while the 14 orchards not receiving treatment had a mean population of 14.0 per 100 cm³ of soil.

Orchard age relative to TmRSV infection.

The age of the orchard had a nonsignificant negative correlation with ELISA-positive trees ($r = -0.36$; $P \leq 0.12$). In the youngest orchard tested (F), which was 6 years old, 82% of the trees were infected—the highest in the survey. The rootstock used in

orchard F was 'Myrobalan', which is known to be susceptible to TmRSV (Cummins and Gonsalves, 1986a). In the one planting of 'Stanley' on 'Halford' peach rootstock, orchard B (Grand Traverse area), TmRSV was detected in only 20% of the trees.

Nursery sources of trees relative to TmRSV incidence in orchards. The source of planting stock did not appear to influence the incidence of TmRSV-infected plum trees in the orchards surveyed. We do not believe that nurseries are a major source of infected trees in Michigan's commercial plum orchards. Rather, we believe that TmRSV infection is endemic in the resident dandelion/dagger nematode populations. Our opinion is based on two observations. First, in 1986, we ELISA-tested 720 sour cherry nursery trees adjacent to the nursery's plum trees at the Emlong, Newark, and Hawley nurseries and found none to be infected with TmRSV (Morrissey, 1988). Second, on several occasions, we have tested dandelion plants near, but not in, orchards, and have found many of them to be infected with TmRSV (D.C.R., unpublished data). The same situation was reported in Pennsylvania (Mountain et al., 1983; Powell et al., 1982).

Rootstock considerations. In California, the rootstock Marianna 2624 (M 2624) has been reported to be immune to TmRSV infection (Hoy and Mircetich, 1984). However, growers might reject its use due its tendency to sucker considerably at the base of the tree. A second drawback to this rootstock is that it is extremely susceptible to damage by the lesser peach tree borer.

'Stanley' plum is difficult to propagate on its own roots. However, brown line-diseased 'Stanley'/'Myrobalan' trees can be forced to make their own roots by mounding soil around the 'Myrobalan' graft union. They will survive and bear a light to medium crop over at least 15 years (J. Cummins, personal communication). This is a "stop-gap" measure that may not solve the problem where the disease already is established in an orchard.

Because of the extent of TmRSV infection in Michigan and the extreme damage the virus does to 'Stanley' plum on 'Myrobalan' rootstock, we have begun a study of several new rootstock and scion combinations (including 'M 2624') that may be resis-

tant or immune to TmRSV infection.

Some recommendations for growers. Plum is a viable fruit crop in Michigan and New York. Because brown line disease associated with infection by TmRSV is so prevalent in these states, the search for resistant or immune rootstock and scion cultivars will be coupled with an increased effort to educate growers in methods of preventing the disease in new plantings and in replant situations. Growers who are contemplating planting a new orchard should sample the soil for the presence of dagger nematode and test for the presence of TmRSV on dandelion plants selected at random from the area to be planted with commercially available ELISA test kits. Dandelions are a good indicator of the presence of TmRSV infection in ground that will be planted to fruit crops (Mountain et al., 1983; Powell et al., 1982, 1984). Positive test results, along with a positive soil test for the dagger nematode, would dictate the need for a thorough preplant soil fumigation with a liquid fumigant (1,3-D) applied using a shank-injector-type applicator (Ramsdell et al., 1983). Nematode kill is most effective on sandier soils. On more coarse-textured soils, there could be some nematode survival, especially at depths of 91.4 cm (3 ft) or more. Planting virus-tested clean stock is equally important.

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