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Recovery of Tomato Ringspot Virus from Inoculated Apple Trees[1]

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Abstract. **Tomato ringspot virus (TmRSV) was detected by ELISA indexing in leaf tissue from 23 of 59 clones of *Malus domestica* Borkh., 1 to 6 years after inoculation with TmRSV-infected buds. However, TmRSV was repeatedly detected from only 10 of the 23 clones: Malling (M) 26, MM 106, MAC-30, MAC-39, and P-2 and the fruiting cultivars 'Northern Spy', 'Spigold', 'Spijon', 'Stayman', and 'Wayne'. 'Northern Spy' and most of its derivatives appeared to be highly susceptible to TmRSV infection, in that the virus was consistently recovered from inoculated plants.**

The first reported isolation of TmRSV from *Malus* was from the cultivar 'McIntosh' (8). Since that report, TmRSV has been recovered but rarely from naturally infected cultivars of *Malus domestica,* and has not been reported from other *Malus* species. The virus has been recovered from naturally infected Malling-Merton (MM) 106 rootstocks in numerous localities (1, 2, 6, 7; personal communications, R. Cameron, D. Rosenberger).

Tomato ringspot virus has been consistently associated with the "apple union necrosis and decline" (AUND) syndrome. The AUND syndrome has been observed almost exclusively on trees growing on MM 106 roots; the cultivars 'Delicious', 'Jersey-mac', 'Quinte', 'Rhode Island Greening', and 'Tydeman's Early' have been most often affected. AUND occasionally appears on 'Idared', 'Jonathan', 'McIntosh', and 'Spartan'. Trees of 'Empire', 'Golden Delicious', 'Rome Beauty', and 'York Imperial' on MM 106 stocks, growing in the same orchards as symptomatic 'Delicious'/MM 106 trees, have not exhibited AUND (1, 2, 6).

The AUND symdrome is typically expressed in young bearing trees as a marked invagination at the graft union, with or without embedded necrotic tissue, followed by a general decline in growth and productivity of the tree (7). Formation of a necrotic plate at the union is typical for 'Delicious' but has not been observed in 'McIntosh' or 'Tydeman's Early' trees. Symptoms are similar to but distinct from other graft union disorders, such as those expressed by 'Virginia Crab' and other virus indicator clones in response to apple stem pitting and apple stem grooving viruses (7).

The hypothesis has been advanced that AUND results when a "hypersensitive" cultivar, such as 'Delicious', is grown on "tolerant" stock, such as MM 106, which is systematically infected with TmRSV (1). In the study reported here, we examined 59 *Malus* clones for capacity to multiply TmRSV when subjected to long-term exposure to TmRSV-infected MM 106.

Materials and Methods

Twenty-four fruiting cultivars and 35 rootstock clones were evaluated over a period of 4 to 6 years. Inoculation with TmRSV was done by 1 or more of the following procedures:

1) Inoculation of scion shoots of 1-year-old trees on MM 106 rootstocks with 3 TmRSV-infected buds. Shoots were forced from the inoculum buds and allowed to grow to provide a constant source of virus.

2) Bud-inoculation of the rootstock portion of a 1-year-old tree composed of the test cultivar growing on MM 106 rootstocks. Subsequently at least 1 rootstock shoot was maintained.

3) Budding of the clone to be tested directly onto a shoot of MM 106 that had been shown by previous indexing to be systemically infected with TmRSV.

In each of the 500 plants studied, MM 106 tissue infected with TmRSV was united with tissue of the subject. In about one-half of the plants, the infected MM 106 was the distal tissue. Inoculum buds were vegetative propagules of MM 106 stocks naturally infected with either the Amberg or the Chickadee isolate of TmRSV. The MM 106 infected with the Amberg strain was obtained originally from Amberg Bros. Nursery, Stanley, N.Y.; this isolate appears to be serologically identical with a previously described grapevine isolate from New York (9). The MM 106 infected with the Chickadee isolate was originally obtained from Chickadee Nursery, Sherwood, Ore. In ELISA tests, the Chickadee isolate reacts strongly (4) and the Amberg isolate reacts poorly with antisera specific to the grape yellow vein strain of TmRSV (4, 5).

All trees were grown in a peat-vermiculite medium in the greenhouse for 3 years. For an additional 3 years, about one-half of the trees were grown in the greenhouse and one-half were grown in fumigated soil in the nursery.

Leaves from the tips of actively growing shoots were indexed by the ELISA technique (3). Antisera to the grape (9) and the grape yellow vein (4) isolates of TmRSV were used to index for the Amberg and Chickadee isolates, respectively. We indexed the test clones 1 to 4 times each growing season following inoculation.

Results

Many of the test trees began fruiting 1 to 2 years after inoculation. We observed none of the visual symptoms associated with incompatibility or with AUND—to initial rejection of test buds, no union invagination, no necrotic plates, no decline in vigor, and no unusual production of root suckers.

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We detected TmRSV in 11 of the 24 fruiting cultivars and in 12 of the 35 clonal rootstocks (Table 1). The virus was detected repeatedly in M 26, MM 106, MAC-30, MAC-39, P-2, 'Northern Spy', 'Spigold', 'Spijon', 'Stayman', and 'Wayne'. Although we recovered TmRSV from 'Burgundy', 'Empire', Budagovsky 9, M 27, 'Mark' (MAC-9), and MM 111, recovery was not consistent from a particular tree or even from a given leaf.

The 59 clones that we inoculated may be grouped according to consistency of detection of TmRSV (Table 1):

I. Consistent recovery from leaf tissue at almost every indexing during the growing season; typified by MM 106.

II. Inconsistent: sometimes detection very early in the growing season but not after leaves have matured; typified by MM 111.

III. No recovery of TmRSV from leaf tissue; typified by 'Delicious'.

Recovery of TmRSV was almost always during the first 2 or 3 weeks of growth following winter dormancy. For example, we recovered TmRSV from shoot tips of 3/6 Bud.9 and 2/4 M 27 plants 10 days after budbreak the second season following inoculation, but we did not identify TmRSV from these plants in the 3 seasons following. We did not recover TmRSV from every MM 106 inoculated with TmRSV. In almost 10% of infected MM 106 plants, we were able to recover TmRSV only once.

Discussion

Earlier interpretation of orchard observations gave rise to the "hypersensitive" hypothesis cited above (1). Our consistent failure to find union necroses or other union aberrations between TmRSV-infected MM 106 and supposedly hypersensitive scion cultivars such as 'Delicious' and 'Quinte' does not support a rigorous application of this hypothesis. It now appears that other factors, e.g., fruiting and environmental stresses, may play significant roles in inducing symptom expression.

Of the 11 fruiting cultivars in which we have been able to detect TmRSV, only 'McIntosh' and 'Spartan' have exhibited AUND in commercial orchards; these 2 cultivars develop symptoms of AUND much more slowly than does 'Delicious'. We did not detect TmRSV in 4 cultivars which normally exhibit severe symptoms at the graft union: 'Delicious', 'Jerseymac', 'Quinte', or 'Tydeman's Early'; this finding supports the hypothesis cited earlier (1), i.e., that TmRSV is not able to invade and multiply in a "hypersensitive" clone. By contrast, we expected to, but did not, recover TmRSV from leaf tissue of the "tolerant" cultivars 'Golden Delicious', 'Rome Beauty', and 'York Imperial'.

Table 1. Grouping of apple clones according to consistency of detection of tomato ringspot virus (TmRSV) from leaves of inoculated plants.

Class	Detection of TmRSV	Type	Clones
I	Consistent	MM 106	Northern Spy, Spigold, Spijon, Stayman, Wayne, M 26, MM 106, MAC-30, MAC-39, P-2
II	Inconsistent	MM 111	Antonovka Kamenichka, Burgundy, Empire, Idared, McIntosh, Spartan, M 27, MM 111, Budagovsky 9, MAC-1, Mark (MAC-9), Ottawa 3, P-18
III	None	Delicious	Delicious, Golden Delicious, Jerseymac, Jonagold, Jonamac, Julyred, Liberty, MacSpur, Mutsu, Paulared, Rome Beauty, Tydeman's Early, York Imperial, C6, M 2, M 4, M 7, M 9, M 13, Robusta 5, Bud. 54-118, Bud. 54-146, Bud. 57-490, Bud. 57-491, CG.10, CG.24, Kansas-14, MAC-24, MM 102, OAR-1, Ottawa 7, Ottawa 11, P-1, P-13, P-16, P-22

Cultivars of class I appear to be fully hospitable to TmRSV, supporting multiplication, distribution, and maintenance of the virus. Cultivars of class III, on the other hand, appear to be immune, supporting neither multiplication nor distribution. Class II cultivars appear to be able to support only very low levels of TmRSV multiplication and/or translocation. Subsequently, virus titer in the first leaves apparently becomes too low to detect by ELISA, and after 1 to 4 weeks not enough is present in the extending shoot tip to detect.

The inconsistency of detection of TmRSV in the Class II clones by the ELISA technique suggests that the distribution of the virus within tissue of these hosts is not uniform, and that virus in most tissues of these hosts is usually below detectable levels. Preliminary results indicate that titer in inner bark may be more uniformly detectable than in leaves (A. Gottlieb, personal communication). We are also examining the low titer concept by budding "amplifier" buds of virus-free MM 106 onto suspected hosts (unpublished).

We think it likely that under orchard conditions, fruiting cultivars grouped in Class III (e.g., 'Delicious') will develop AUND on the rootstocks grouped in Class I (e.g., MM 106). Also, fruiting cultivars of Class II on TmRSV-infected rootstocks of Class I would probably develop symptoms more slowly. Based on these hypotheses, we postulate that 'Jonamac' on TmRSV-infected MM 106 will usually develop AUND, that 'Empire', 'Idared', and 'Spijon' may develop symptoms relatively late in the life of the tree, and that 'Burgundy', 'Northern Spy', 'Spigold', 'Stayman', and 'Wayne' would not develop symptoms (Table 1). Symptoms of AUND have not been observed in the orchard on trees on M 7, M 9, or MM 111 stocks. This is consistent with our failure to recover TmRSV from leaves of inoculated M 7 andM 9 and to the infrequent recovery from MM 111.

The rootstocks M 26, MAC-30, MAC-39, Bud.9, and P-2, from which we recovered TmRSV regularly, will probably behave much like MM 106; 'Delicious' trees on these stocks should develop severe AUND. 'Delicious' on the rootstocks Robusta 5, Ottawa 7, and Ottawa 11 should not develop AUND symptoms. However, on these stocks, TmRSV-infected 'Stayman' or 'York Imperial' would probably develop AUND.

'Northern Spy' and 6 of its derivatives ('Spigold', 'Spijon', 'Wayne', MM 102, MM 106, and MM 111) were included in our tests. We recorded at least 1 positive index for all but MM 102. It is possible that 'Northern Spy' transmits unusual susceptibility to TmRSV infection. We observed no long-term difference in virus content related to method of inocuation. For example, of 16 'Delicious', 17 'McIntosh', 10 'Rome Beauty', and 10 'Tydeman's Early', about one-half of the trees of each cultivar were inoculated by method 1 and one-half by method 2. Of these 53 trees, only 2 'McIntosh', 1 inoculated by each tecnique, yielded TmRSV. TmRSV was recovered regularly from MM 106 trees the season following inoculation, regardless of method.

Transmission, maintenance, reproduction, and recovery of TmRSV appear probably to be influenced by a number of factors.

There may be—no doubt there are —variations in the establishment of new vascular tissues between stock and scion. There may be differences among cultivars in capacity to support reproduction of TmRSV. There may be differences in capacity to destroy TmRSV particles or components. There may be variation related to differences in physical status of the host plants within a given clone.

One further possibility cannot be ruled out by our data or by others' reports we have seen: the possibility exists that at least some plants may respond to relatively slow virus influx with some sort of defense mechanism that either temporarily or permanently inhibits access of the virus.

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Ethylene, Fungi, and Summer Fruit Drop of Navel Orange[1]

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Abstract. **Species of *Alternaria* and *Gloeosporium* were most often isolated from fruit with blossom-end yellowing (BEY), a disorder associated with summer fruit drop of navel orange [*Citrus sinensis* (L.) Osbeck]. Fruit inoculated with pure cultures of these fungi did not develop BEY; however, wounded fruit which were inoculated with fungi produced higher levels of ethylene and more extensive BEY than wounded, noninoculated fruit. Fruit with BEY produced higher amounts of ethylene than symptomless fruit. The methoxy analog of rhizobitoxine (methoxyvinylglycine) did not reduce ethylene levels, and silver nitrate increased ethylene production from fruit with BEY. Ethylene and fungi are associated with BEY of navel orange but do not appear to be causal factors.**

The yield of navel orange is generally lower than that of other citrus cultivars (10, 17, 26, 27). Several periods of heavy fruit drop contribute to this reduced yield, including a recently characterized summer fruit drop (19). Fruit that drop during this period usually first develop blossom-end yellowing (BEY), which is then followed by abscission.

BEY begins in the secondary fruit (navel), advances to surrounding tissue and intensifies within the navel area prior to abscission. Insects also invade the navel and have been associated with fruit severely affected by BEY (10, 19). *Alternaria citri* Ellis & Pierce has been associated with "June drop" of 'Washington' navel orange in California (10) and BEY of navel oranges in Florida (13). However, BEY symptoms observed in the field are unlike those symptoms characteristic of *Alternaria* black rot of orange caused by *Alternaria citri*. Ethylene is known to cause yellowing of diseased tissue (7, 28), but has not previously been measured in BEY fruit. Objectives of this research were to investigate the roles of fungi and ethylene in navel orange BEY and summer fruit drop. Species of *Alternaria* and *Gloeosporium* were selected for use in this study because these fungi have been frequently isolated from citrus fruit (25). Moreover, they are known to cause disease symptoms similar to BEY.

Materials and Methods

Plant material. Fruit were collected from 15-year-old 'Washington'-type navel orange trees on sour orange (*Citrus aurantium* L.) rootstock near Eustis, Fla.

Field pretreatments and inoculations. Fruit at stage II were selected from the periphery of trees about 1.0 to 2.0 m above soil surface on May 30, 1980. Two ethylene-producing materials were applied to fruit as treatments prior to fungal inoculation: (2-chloroethyl)phosphonic acid (ethephon), an ethylene-releas-

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