

Evidence that Tomato Ringspot Virus Causes Apple Union Necrosis and Decline: Symptom Development in Inoculated Apple Trees

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ABSTRACT

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Rootstocks of cultivar Delicious/ MM.106 apple trees were inoculated with tomato ringspot virus (TmRSV). Inoculum consisted of bark patches from TmRSV-infected MM.106 rootstocks of Delicious apple trees with distinct graft union symptoms of apple union necrosis and decline (AUND). Two of 31 trees inoculated in 1981 developed graft union symptoms after 2 yr and 26 additional trees had symptoms after 3 yr. TmRSV was detected serologically in rootstocks of most trees with symptoms of AUND. Only one of 37 control trees was infected with TmRSV 4 yr after planting. Inoculated trees were subjected to various crop-load, nutritional, and water stress regimes, but none of these factors affected the incidence or severity of AUND symptoms.

Apple union necrosis and decline (AUND) is a graft union disease of apple first reported in 1976 (12). Diagnostic

symptoms of AUND include pitting, invagination, or a brown line in the woody cylinder at the graft union. Severely infected trees may break off at the graft union due to an increase of ray and axial parenchyma cells and a corresponding decrease in vessel and fiber cells at the union (14). AUND has been observed most frequently in cultivar Delicious trees propagated on Malling-Merton (MM.) 106 rootstock, but it also

occurs in numerous other cultivars propagated on MM.106 (4,7,10,12).

Tomato ringspot virus (TmRSV) has been detected in the rootstocks but not in the scions of diseased apple trees (4,7,8,11). However, early attempts to reproduce the disease by inoculating apple trees with TmRSV failed. Trees inoculated with the virus became infected but did not develop symptoms at the graft unions (2,11). The purpose of this study was to determine if AUND could be reproduced by inoculating apple trees with bark from the rootstocks of diseased trees and to determine if symptom development was dependent on physiological stress factors in inoculated trees. Initial results from part of this study were reported earlier (6).

MATERIALS AND METHODS

Eighty Redchief Delicious trees on MM.106 rootstocks were obtained from a commercial nursery and were planted in a double row at the New York Agri-

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cultural Experiment Station, Hudson Valley Laboratory, Highland, NY, in the spring of 1981. Trees were spaced 2.7 m apart within rows and 3.7 m between rows. They were planted with graft unions 12–20 cm above the ground to allow access to the rootstocks. The 80 trees were divided into four replicates, each containing four four-tree plots and two two-tree plots.

On 15 July, half of the four-tree plots (a total of 32 trees) were inoculated using bark patches from the rootstock of a Redchief Delicious/MM.106 tree that was showing AUND symptoms. The tree used for inoculum was from Orchard A (8), had a necrotic plate at the graft union, and had given a serological reaction positive for TmRSV in our 1981 survey. The tree was dug up and brought to the test orchard where bark patches approximately 25 mm long by 7 mm wide were removed from the trunk and roots for use as inoculum. A single inoculum patch was carefully fitted into a rectangle of identical size where bark had been removed from the rootstock of the test tree. Inoculum patches were held in position with rubber budding strips. Thirty-two control trees were similarly inoculated in the rootstocks using bark patches taken from the Delicious scions of the diseased source tree. To assure that all inoculated trees in the four-tree plots were exposed to TmRSV inoculum, the rootstocks on these trees were reinoculated 13 July 1983, using two bark patches 25 mm long by 13 mm wide from two different trees with AUND symptoms from Orchard B (8). On 13 July, we also used the same inoculum sources to inoculate one tree in each of the two-tree plots that had been established but left uninoculated in 1981.

Additional cultural treatments were applied to some of the trees to determine if physiological stress factors affected symptom development. In each of the four replicates, one four-tree plot with TmRSV-inoculated trees and one four-tree plot with control trees received annual applications of fertilizer at the usual commercial rates, whereas the other four-tree plots (one TmRSV-inoculated and one control) were never

fertilized. Within each four-tree plot, one tree was defruited shortly after bloom each year to minimize the normal physiological stresses associated with fruit production. The second tree in each four-tree plot was temporarily girdled 25 May 1982, by making a spiral cut two-and-a-half times around the trunk just above the graft union with the intent of stimulating heavy early fruiting. These trees were allowed to fruit heavily in subsequent years. The third tree in each plot was treated according to normal commercial practices. We attempted to subject the fourth tree in each group to water stress. Efforts to induce water stress were initiated in June 1982 by covering the root zone with a plastic sheet attached to a frame 15–30 cm above the soil. Because the plastic sheets were difficult to maintain, we changed tactics and in May 1983 we installed fiberglass panels to restrict root growth. The fiberglass panels, 152 cm long by 61 cm wide, were buried 45–50 cm deep and approximately 45 cm away from the trunk along two sides of each water-stressed tree. The root restriction caused by the fiberglass panels resulted in increasingly severe water stress as the tree canopies increased in size from year to year.

TmRSV can be transmitted from weed hosts by the nematodes *Xiphinema americanum* Cobb and *X. rivesi* Dalmasso (3,5,13). TmRSV-infected dandelions were present in the test site, and *Xiphinema* populations in an adjacent orchard ranged from 0 to 45 per 100 cm³ in samples collected in July 1982 (Rosenberger, unpublished). However, the rocky soil conditions and unavailability of appropriate equipment made preplant fumigation unfeasible. To reduce the likelihood of natural transmission of TmRSV to test trees, annual herbicide applications were used to maintain a continuous weed-free strip along the tree rows. The width of the herbicide-treated strip was increased annually from 1.2 m the first year to 1.5 m in 1987. Each year from 1982 through 1986, trees were also treated with a postplant nematicide during May. Fenamiphos (16.8 kg/treated ha) was

used in 1982 and carbofuran (6.73 kg/treated ha) was used in subsequent years. For each tree, the nematicides were applied to an area 1.5 × 1.5 m, with the tree at the center of the square.

All trees in the block were indexed for TmRSV during July in 1983, 1985, and 1986. Samples were collected from bark tissue and indexed using enzyme-linked immunosorbent assay (ELISA) as previously described (8), except that in 1983 and subsequent years we used antiserum from the peach yellow bud strain of TmRSV (1) for ELISA work. ELISA reactions for test samples and for appropriate controls were evaluated visually by two independent observers. Reactions were considered positive only if detected by both observers. In 1983, samples for indexing were collected by scraping inner bark and cambial tissue from the bark patches that were removed in the process of reinoculating the trees. One of these patches was consistently collected from just above or below the original 1981 inoculation site to increase the likelihood of detecting TmRSV. In 1985 and 1986, the samples were collected by removing 15-mm-diameter circular patches from two locations close to the inoculation sites on each tree.

During September or October of each year from 1983 to 1986, graft unions were checked for visual symptoms of AUND by removing bark across 5–10 mm of the graft union circumference on one or two sides of each tree.

RESULTS

Pitting at the graft union was detected on two trees in 1983, 2 years after they were inoculated. By 1984, all but four of the trees inoculated in 1981 had developed graft union symptoms of AUND (Table 1). The most common symptom was pitting (Fig. 1) or a very faint brown line in the trunk at the graft union, but seven trees had a distinct brown line at the union. When bark at the graft union was removed from trees that showed pitting or a faint brown line in the woody cylinder, the graft union symptoms on the exposed phloem tissue in the bark were faint at first but became more distinct after several minutes, probably

Table 1. Incidence of graft union symptoms of apple union necrosis and decline (AUND) and of serologically detectable tomato ringspot virus (TmRSV) in Redchief Delicious/MM.106 apple trees planted in 1981

Year	Uninoculated trees (control)		Trees inoculated with TmRSV			
	With AUND graft union symptoms	With TmRSV, detectable by ELISA ^a	1981		1983	
			With AUND graft union symptoms	With TmRSV, detectable by ELISA	With AUND graft union symptoms	With TmRSV, detectable by ELISA
1983	0/37	0/37	2/31	3/31 ^b	0/7	0/7
1984	0/37	0/37	27/31	...	0/7	...
1985	0/37	1/37	27/31	27/31 ^b	0/6	1/6
1986	1/35	2/35	29/31	29/31	2/6	3/6

^aThe enzyme-linked immunosorbent assay (ELISA) was used to index cambium and inner bark tissue removed from rootstocks of the trees.

^bThe total number of trees with symptoms and/or with TmRSV-positive ELISA was five in 1983 and 28 in 1985.

due to rapid oxidation of phenolics in affected phloem cells. Neither the nutritional nor the physiological stress factors applied to trees within the four-tree plots had any effect on the time symptoms first appeared or on the severity of symptoms observed. All trees, except those defruited each year, carried heavy crops in 1986 and 1987, but none of the trees broke at the graft union. One tree inoculated in 1981 and one inoculated in 1983 died of apparent low temperature injury incurred during the winter of 1985–1986. The tree inoculated in 1981 had given a positive ELISA for TmRSV in 1985 but did not have graft union symptoms. The tree inoculated in 1983 had neither symptoms nor a positive ELISA reaction in 1985. Several other trees were also removed during the course of the experiment. By September of 1987, two inoculated trees and one infected control tree had light-colored foliage typical of trees declining from AUND. The remainder of the trees showed no foliar symptoms of AUND.

Although 87% of the trees inoculated in 1981 developed graft union symptoms within 3 yr, only two of the six surviving TmRSV-inoculated trees in the two-tree plots developed symptoms within 3 yr after their 1983 inoculation. TmRSV was detected by ELISA in one control tree in 1985 and in a second in 1986. The former had distinct graft union symptoms in 1986. A third control developed graft union symptoms in 1987.

DISCUSSION

The experiment reported here is the first in which artificially inoculated apple trees developed classical graft union

symptoms of AUND. A strong association between TmRSV and AUND has been reported by numerous researchers (4,8,12), but previous attempts to reproduce AUND symptoms by artificial inoculation were unsuccessful (2). A discussion among researchers involved in the Northeast Regional Project on Virus and Virus-like Diseases of Woody Deciduous Fruit Crops (NE-14) revealed that earlier unsuccessful attempts to reproduce AUND symptoms in inoculated trees in New York (2) and Pennsylvania (11) differed from our experiment in two significant ways. First, inoculum in the earlier New York and Pennsylvania experiments came from trees without AUND symptoms (i.e., the inoculum consisted of buds or bark patches from either ungrafted TmRSV-infected stock or from rootstocks of grafted trees with detectable TmRSV but no disease symptoms). Second, as far as the NE-14 group could determine, all of the earlier unsuccessful inoculation experiments involved either the Chickadee strain of TmRSV or unidentified strains that might have been the Chickadee strain. The Chickadee strain is now known to be serologically distinct from most other strains of TmRSV and appears unable to cause disease in apples (1).

Our experiment demonstrating that AUND symptoms can be reliably reproduced in artificially inoculated Delicious/MM.106 trees is important because it allows us to proceed with evaluation of disease development in other apple rootstock/scion combinations for which the effects of TmRSV infection are unknown. Inoculated Delicious/MM.106 trees can now be

used as positive controls in future experiments.

Our experiment provides additional support for the association between TmRSV and AUND, but it does not completely fulfill Koch's postulates because we did not use purified TmRSV in our inoculations. The trees we used as inoculum may have contained any of several apple latent viruses. However, except for TmRSV, none of the known viruses in apple are restricted to the rootstock in Delicious/MM.106 trees. By inoculating our controls with bark patches from the scions of our inoculum trees, we ensured that any of the known latent viruses were present in both TmRSV-inoculated and control trees. From our results we cannot rule out the possibilities that 1) TmRSV requires a latent virus "helper" to cause AUND, or 2) that some new virus or viruslike entity that is also root-restricted in Delicious/MM.106 trees could have caused the AUND symptoms we observed. Both of these are unlikely possibilities because no consistent association has been found between AUND and the common latent apple viruses (11), and no unusual virus or viruslike entity was detected in previous studies on the TmRSV/AUND association (4,11).

The lower percentage of successful inoculations in trees inoculated for the first time in 1983 as compared with 1981 may reflect differences in the effectiveness of the inoculum, environmental conditions during and after inoculation, or susceptibility of trees at different ages. Development of AUND symptoms in two of the trees first inoculated when they were 3 yr old in 1983 provides evidence

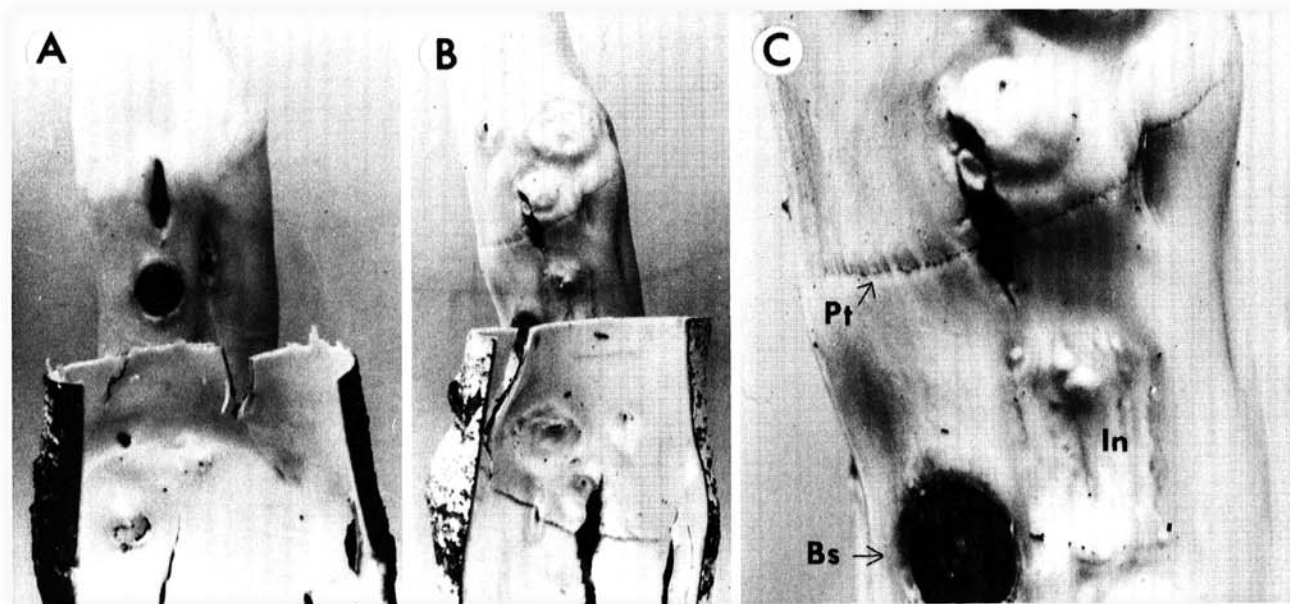


Fig. 1. Appearance of the woody cylinder and inner bark at the graft unions of healthy and tomato ringspot virus (TmRSV) infected Delicious/MM.106 apple trees. (A) Healthy control tree inoculated with bark patches from TmRSV-free scion of infected source tree. (B) Diseased tree inoculated with TmRSV-infected bark patches from the rootstock of infected source tree. (C) Close-up of the graft union showing the line of pitting (Pt), one of the inoculation sites (In), and one of the enzyme-linked immunosorbent assay (ELISA) bark-sampling sites (Bs).

that susceptibility to TmRSV is not limited to very young or newly planted trees.

Because preplant fumigation of our test site was not feasible, we used a large number of uninoculated control trees to ensure that we would detect even low levels of natural transmission of TmRSV by nematodes. None of 37 control trees developed AUND symptoms or had ELISA-detectable TmRSV before 1985. We therefore conclude that natural transmission of TmRSV by nematodes did not contribute to infection of the 1981-inoculated trees that developed symptoms in 1983 and 1984. Natural transmission by nematodes cannot be ruled out as the source of some of the infections in the 3-yr-old trees first inoculated in 1983 because two control trees developed symptoms at the same time as the 1983-inoculated trees. Natural transmission by nematodes was not unexpected because the nematicides we used do not completely eliminate the *Xiphinema* vectors (9). As the trees increased in size, some of the tree roots may have extended into the sodded row middles where TmRSV-infected weeds were present and nematicides were not applied.

Field observations on the incidence of AUND in commercial plantings and difficulties in reproducing disease symptoms in inoculated trees have led to the suggestion that stress factors might

be important in inducing symptoms in infected trees (2,11). This hypothesis was not supported by our results. However, we cannot rule out the possibility that stress factors might affect nematode transmission of TmRSV to trees or the early establishment of the virus in the trees. The bark patch inoculations we used probably introduced more virus into trees than a single nematode would introduce, and the introduction site was on the trunk, whereas nematodes would introduce the virus into fine roots. We will continue observing our inoculated trees to determine if stress factors will affect the survival of trees after graft union symptoms appear.

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