Symptoms Incited by Apple Type II Virus Isolates in Virginia Crabapple Trees

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ABSTRACT

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Isolates of Type II viruses from four apple cultivars were, in earlier work, mechanically transmitted to squash and from squash to apple seedlings. These seedlings have now been used to transmit these isolates by budding to virus indicators Virginia crabapple K-6, R-12740-7A (Russian), and Spy 227 to relate symptoms to the characterized virus. After 3 yr, some Virginia crabapple trees were yellowed and in decline; all had necrosis at the graft union, but some isolates caused more severe necrosis than others. No grooving symptoms appeared after 4 yr on the stocks or scions within 15 cm of the graft union with any of the four virus isolates. These isolates also did not incite symptoms in the Russian or Spy 227 trees or in five pear virus indicator cultivars.

Most viruses that are mechanically transmissible from apple, except apple mosaic virus, can be categorized as Type I or Type II (6). The two types are readily distinguishable in *Chenopodium quinoa* (6,13). In apple trees, Type I isolates cause chlorotic leaf spot in the Russian indicator R-12740-7A and no symptoms in Virginia crabapple. A single virus, chlorotic leaf spot virus, is involved. It is not known whether all isolates resembling Type II are a single virus or, with few exceptions, what symptoms they incite when returned to apple trees after study in herbaceous species.

Type II isolates have been called apple stem grooving (5), E-36 (1), dark green epinasty (10,13), and C-431 (5). All have similar herbaceous host ranges, stability in vitro properties, and virion morphology, but whether all of these and many other isolates are the same virus remains obscure. Type II isolates are widespread in apple and pear cultivars but have seldom been characterized in the laboratory (1,2,7,10). The difficulty of reinfecting fruit trees has greatly hindered attempts to relate characterized or purified viruses to the diseases they incite (4). Two serologically related isolates, C-431 and E-36, were successfully returned to apple trees and incited stem grooving in Virginia crabapple indicators (1,7). Our four Type II isolates were previously characterized, found to be similar to C-431 and E-36 in herbaceous species, and returned to apple seedlings (4); their serological relationship to C-431 and E-36 was not determined.

The purpose of this study was to determine what symptoms these Type II isolates would incite in Virginia crabapple and in other apple and pear indicators.

MATERIALS AND METHODS

The Type II virus isolates, referred to as apple stem grooving virus (ASGV) isolates A, B, C, and D, were originally isolated from four cultivars of orchard trees in New York in 1971 (4). The source trees were Red Gravenstein (A), Mutsu (B), Duke of Clarence (C), and Queen Cox (D).

Virus indicator trees were produced in the field by grafting two virus-free buds into apple seedling stocks about 15 cm above the ground. Two weeks later these trees were inoculated by placing two buds from the infected apple seedlings (4) on the seedling stock immediately below the two indicator buds. A week later the field seedlings were cut off just above the upper indicator bud; growth of the indicator buds began at this time. Five trees with indicator buds of Virginia crabapple K-6 and three each with buds of Russian R-12740-7A and Spy 227 were inoculated with each of the four ASGV isolates (4).

Buds from a seedling mechanically infected with apple chlorotic leaf spot virus (4) were placed on three additional trees of these indicators to serve as controls. Six unbudded trees were also included as negative controls.

Bark, leaf, and fruit of all trees were inspected for symptoms at least once a year for 4 yr. The trees were then cut down, the bark was removed from 15 cm above to 15 cm below the graft union, and the xylem was examined.

In 1977 ASGV isolate A was bud-inoculated in the field to the five pear cultivar virus indicators Beurre Hardy, Long Ashton 62, and Jules d'Airolles and the Japanese indicators HN36 and HN39. The purpose was to determine whether any of these indicators would develop symptoms like those produced by some of the pear viruses in these indicators.

RESULTS AND DISCUSSION

The four ASGV isolates incited symptoms only in the Virginia crabapple indicators. When a strip of bark was removed 3 yr after inoculation, graft union necrosis was apparent in all trees infected with ASGV. Trees inoculated with isolates C and D also were clearly stunted.

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Differences in symptoms caused by the four isolates became more apparent by the end of the fourth year. Trees inoculated with isolate A were about 2 m tall and had sparse foliage. Those inoculated with isolate B were 3 m tall and appeared similar in all respects to uninoculated control trees. In contrast, all trees inoculated with virus isolates C and D were 1 to 2 m tall and 0.5 to 1 m tall, respectively, and all were dead. Thus, after 4 yr the gross effects induced by the four ASGV isolates in Virginia crabapple trees ranged from normal 3-m trees to dead trees that had reached a height of only 60 cm. The graft unions in three Virginia crabapple trees inoculated with virus isolates A and B are shown in Fig. 1. All trees showed union necrosis—the A trees more than the B trees. Swelling of the scion was not prominent and was apparent only in one tree inoculated with isolate A and one with isolate B. An important feature was the absence of grooves in the scion near the graft union. In two earlier studies (1,7) in which ASGV was transmitted from herbaceous plants back to Virginia crabapple, it induced long grooves in the xylem 1 or 2 yr after inoculation; hence, the name "stem grooving virus" has been used.

Grooves in the xylem of the Virginia crabapple K-6 selections that were bud-inoculated with various sources of ASGV have been observed under the same climatic conditions (12). Therefore, our failure to observe grooving in the xylem with the four isolates used in this study is probably not due to unique environmental factors such as high summer temperatures (11) or use of the wrong selection of Virginia crabapple indicator. Rather, we believe that not all Type II isolates cause grooving. Welsh and Uyemoto (15) described in detail the various combina-

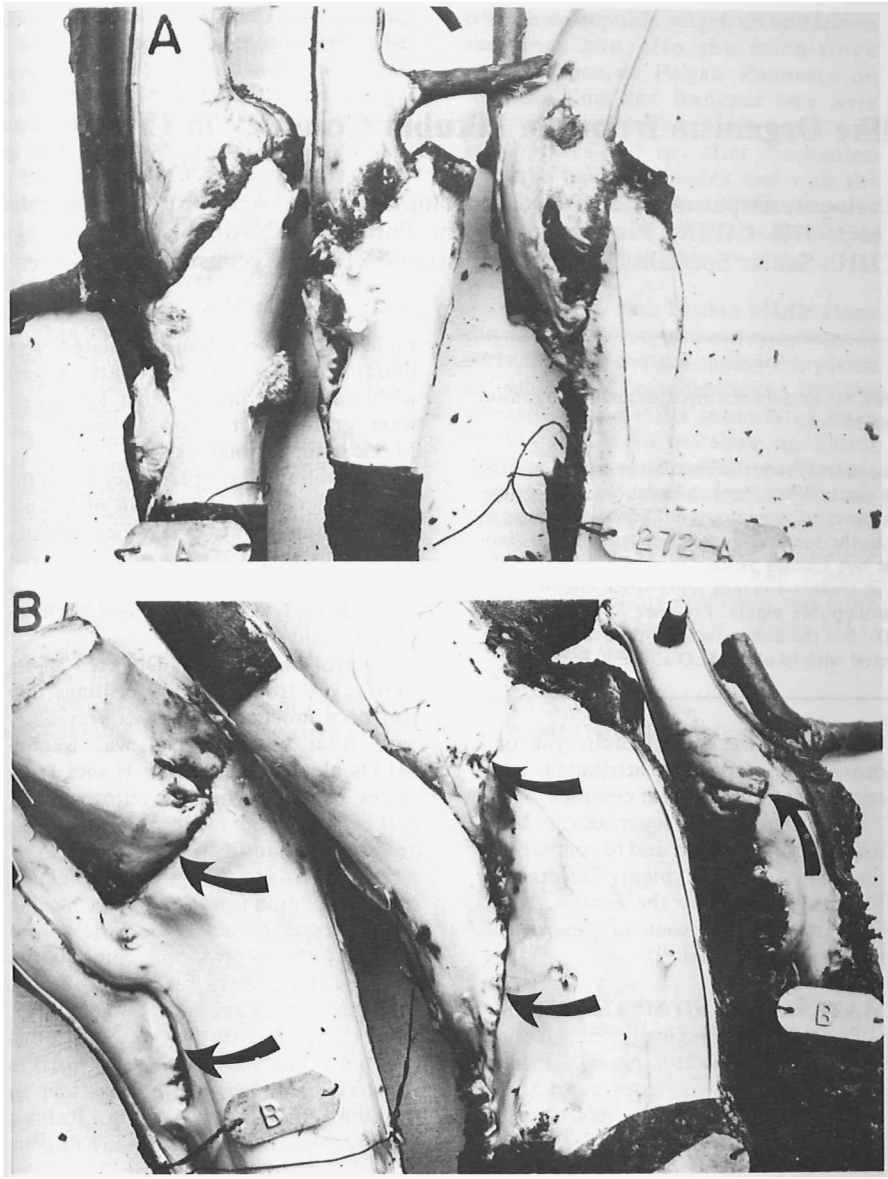

Fig. 1. Graft union region of 4-yr-old Virginia crabapple trees on apple seedlings rootstocks. The bark was removed to expose the xylem and the union between stock and scion. All trees were inoculated by placing two buds infected with apple stem grooving virus onto the stocks when the scions were 0.5–1 cm long. Trees inoculated with **(A)** the more severe source (originally from Red Gravenstein) and **(B)** a milder source (from Mutsu). Note brown line of union necrosis in B (arrows), the near vertical growth of the scions in A, and the absence of grooves in both sets of scions.

tions of xylem aberrations in virus-infected crabapple trees.

The wide angle at which the scion grows in relation to the stock is also a symptom of ASGV-infected Virginia crabapple trees (1,5). Although this occurred in some previous experiments here, it was not a dependable symptom. In fact, the scion growth of most trees in this study was nearly vertical (Fig. 1).

With few exceptions (3,7), the most characteristic symptom incited by ASGV isolates probably is necrosis at the graft union (1,3,8,14). But this necrosis should not be confused with the union necrosis caused by tomato ringspot virus in specific stock-scion combinations (9). The two apparently unrelated diseases are similar in some respects and could be confused when grooving is absent. In 1965 Welsh and Nyland (14) described a union necrosis disorder incited by an agent that was not eliminated by heat treatments; they too saw no grooves in some of the Virginia crabapple trees. The causal agent, not yet named, probably was ASGV, for it is especially difficult to eliminate by heat therapy (P. R. Fridlund, *unpublished*).

None of the four ASGV isolates incited symptoms in the Spy 227 or Russian R-12740-7A indicators or in the five pear indicators. The chlorotic leaf spot virus control incited the expected chlorosis in the R-12740-7A and Spy 227 indicators but incited no symptoms in Virginia crabapple trees.

In conclusion, Type II isolates may "look alike" in *Chenopodium quinoa*, squash, and other herbaceous species, but they do not all incite the same combination of symptoms in Virginia crabapple trees. Some Type II-like isolates may in fact be different viruses or at least different pathotypes if a single virus is involved.

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