Pathogenicity of Tobacco Ringspot Virus in Cherry

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ABSTRACT

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During indexing trials, tobacco ringspot virus (TRSV) was sap-transmitted to herbaceous indicator plants: once from leaf or dormant bud extracts of two mature symptomless sweet cherry trees (*Prunus avium*) and repeatedly from a flowering cherry tree (*P. serrulata*). Attempts to repeat the isolation of TRSV in two successive yearly indexing seasons from these source trees failed. However, two virus isolates were introduced to healthy Mazzard seedlings (*P. avium*) by mechanical transmission. Inoculated leaves developed large chlorotic lesions, and systemic symptoms consisted of chlorotic lesions or sectors of chlorosis bordered with necrotic tissues. Collectively, the virus isolates were transmitted to 17 of 24 seedlings. In the second and third ensuing dormancy periods, the surviving seedlings yielded 6/23 and 9/22 TRSV transmissions, respectively. Recurrent leaf symptoms were observed on only four trees. Healthy Mazzard tree controls indexed negative in all assays.

Stace-Smith and Hansen (5) provided substantial evidence that tobacco ringspot virus (TRSV) is the causal agent of a disease of sweet cherry (*Prunus avium* L.) although they were unable to complete the proof by mechanically introducing the TRSV isolates to test trees of two species of *Prunus*.

In this paper we record isolations of the virus from two *Prunus* species, provide evidence for nonsystemic distribution of the virus in tree tissues, and describe mechanical inoculation trials, using cherry seedlings as test trees.

MATERIALS, METHODS, AND RESULTS

Virus isolation from sweet cherry.—The sources were two adjacent trees of *P. avium* L. growing in an orchard at Geneva, New York; the cultivars were Early Rivers and an unnamed New York selection, No. 1169. In 1972, dormant buds, or forced immature leaves on detached 1-yr shoots, were triturated in nicotine-phosphate buffer (6) and rubbed on corundum-dusted leaves of *Chenopodium quinoa* Willd., cowpea (*Vigna unguiculata* L. 'Early Ramshorn') and cucumber (*Cucumis sativus* L. 'Marketmore'). Several inocula incited primary lesions and systemic mottle on *C. quinoa* and cucumber, symptoms typical of *Prunus* necrotic ringspot virus (NRSV) infection. In addition, two inocula, one from each source tree, incited necrotic primary lesions and ringspots on cowpea.

Antisera specific for NRSV (provided by R. W. Fulton, Wisconsin) and TRSV (2) were tested in agar gel plates against freshly expressed sap of systemically infected host range plants (6). All suspected NRSV extracts reacted with homologous antiserum. The two isolates that had infected cowpea reacted with TRSV antiserum.

During the 1972 spring season, and in two subsequent spring seasons, additional buffered extracts were made of buds, leaves, or flower petals in attempts to again recover TRSV. In each trial, only NRSV was transmitted to the herbaceous host plants.

The TRSV cultures were single-lesioned and maintained for pathogenicity studies.

Virus isolation from oriental flowering cherry.—In November 1973, budsticks of five flowering cherry trees (cultivars of *P. serrulata* Lindl., *P. incisa* Thunb., and *P. serrula* Franch.) were received from the U. S. Department of Agriculture Plant Introduction Station, Glenn Dale, Maryland. Dormant bud inocula were prepared (tissue extracted in nicotine-phosphate buffer or in 0.05 M phosphate buffer, pH 7.0, containing 2.5% PEG, M. W. 6,000) and rubbed onto *C. quinoa*, cucumber, tobacco (*Nicotiana tabacum* L. 'Havana 423'), and snapdragon (*Antirrhinum majus* L. 'Crimson Giant'). Three inocula from one tree (*P. serrulata* Lindl. 'Asagi') incited systemic TRSV symptoms on all hosts. Serological tests were positive for TRSV (Fig. 1).

Additional budsticks of Asagi cherry were obtained immediately, and dormant buds assayed individually on plants of cucumber and snapdragon. The budsticks were divided into two classes, based on lengths of terminal shoots. Class 1 shoots comprised seven or eight buds borne on 5-cm terminals (bud intervals less than 6 mm) and class 2 shoots were over 35 cm with bud intervals 12-50 mm. Transmissions of TRSV were afforded by 11/15 and 8/28 inocula, respectively, for classes 1 and 2. Flower

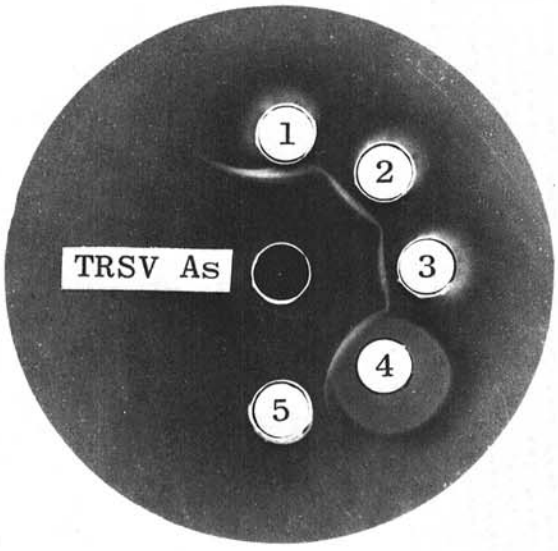

Fig. 1. Agar gel diffusion serology plate. Center well charged with tobacco ringspot virus antiserum (TRSV As); peripheral wells 1 to 4 containing TRSV isolates of grape, Early Rivers cherry, New York No. 1169 cherry, and Asagi flowering cherry, respectively. Test antigens as expressed cucumber sap. Well 5 with healthy sap control.

buds borne on 2-yr wood of class 1 budsticks failed to yield TRSV.

In the early spring months of 1974, 1975, and 1976, additional and extensive assays were conducted on dormant buds of Asagi cherry. In each assay, none of the inocula transmitted TRSV. However, NRSV was routinely transmitted in 1975 and 1976.

Mechanical transmission of tobacco ringspot virus to cherry seedlings.—One-yr-old Mazzard seedlings were potted in a sterilized soil mix and allowed to break dormancy in the greenhouse. When the leaves were at least 50% expanded, the seedling trees were predarkened 24 hr prior to inoculation. Each of the two sweet cherry TRSV isolates was increased in cucumber and concentrated by partial purification (2). For each isolate, 12 corundum-dusted Mazzard seedlings were rubbed with virus inoculum; eight seedlings were rubbed with buffer solution to serve as controls. Cowpea indicator plants were used to test inoculum titer.

Within 10-14 days, each TRSV inoculum, but not buffer control, incited numerous necrotic lesions and ringspots on cowpea leaves. On the cherry seedlings, some inoculated leaves reacted with occasional chlorotic lesions (10-mm diameter) within 14 days, and systemic symptoms appeared after 22-32 days (Fig. 2, 3). These systemic symptoms comprised chlorotic lesions or sectors delimited by necrotic margins. Three seedlings displayed apical symptoms initially, and these recurred during the two successive growth periods (Fig. 4). One seedling produced chlorotic leaf symptoms only in the third season of infection.

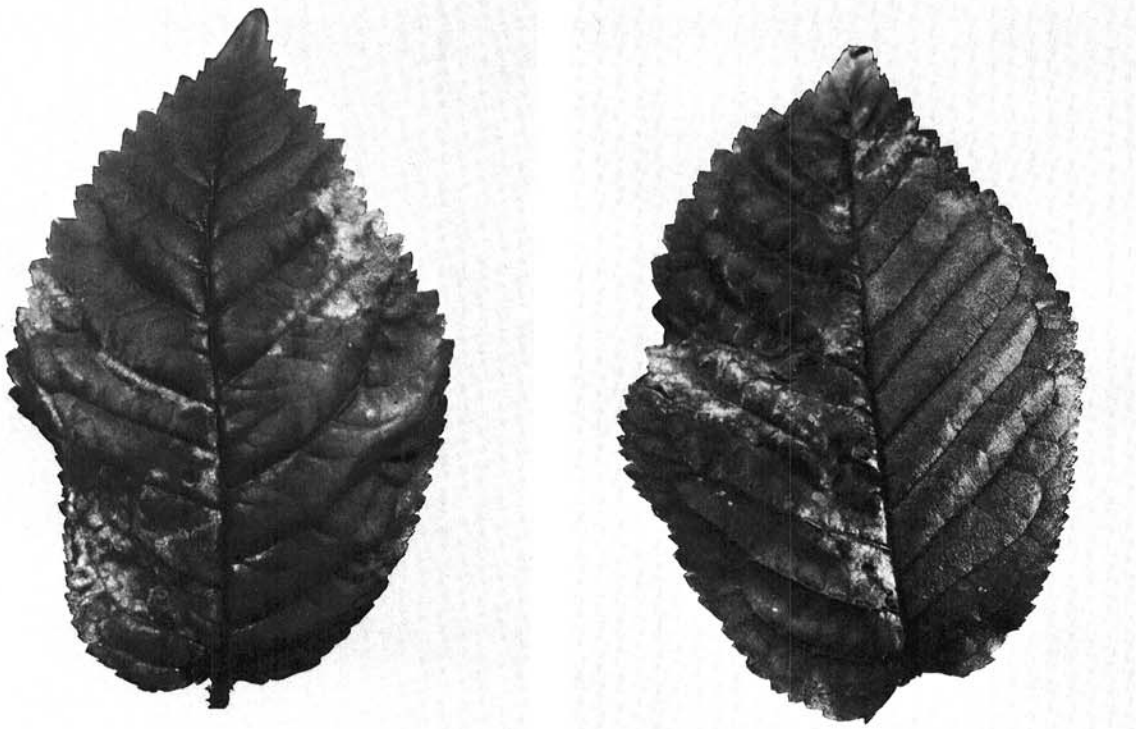

Fig. 2 and 3. Mazzard leaves showing systemic tobacco ringspot virus symptoms at **2)** 22- and **3)** 32-days after inoculation of lower leaves.

The first virus recovery from cherry seedlings was attempted 4 wk after inoculation. Inoculated leaves were triturated in nicotine-phosphate buffer and rubbed on *C. quinoa* and cucumber. The second (1973) and third (1974) recovery attempts used immature leaf or dormant bud tissues. Virus transmission was achieved in each trial. The TRSV-Early Rivers isolate was recovered from 9/12, 4/12, and 6/11 Mazzard trees, respectively, during 1972, 1973, and 1974. The N. Y. 1169 isolate of TRSV was recovered from 8/12, 2/11, and 3/11 trees in the same successive years. All infected trees detected in 1973 yielded TRSV again in 1974. None of the extracts prepared from control trees proved infectious. Serological tests confirmed TRSV identification in all recoveries.

It was evident that TRSV was not uniformly and persistently distributed in the infected Mazzard trees. For example, seven trees yielded TRSV only once (first assay), five trees in two trials, and the remainder in all trials. In the final trial, a comparison was made of inocula from dormant buds and from immature leaf tissues of each tree, by inoculation of *C. quinoa*. Five trees yielded TRSV in both leaf and bud inocula; four additional trees in bud inocula only.

Fig. 4. Mazzard seedling tree with recurrent systemic virus infection symptoms 1 yr following inoculation with tobacco ringspot virus.

DISCUSSION

Tobacco ringspot virus (TRSV) has been identified or isolated from trees of several *Prunus* spp. by Liu and Allen (3), Smith et al. (4), Stace-Smith and Hansen (5), and Wilkinson (7). Most of these reported lack of consistency in isolation results in repeated attempts; also, none have successfully sap-transmitted TRSV to healthy cherry trees. However, we have established the pathogenicity of TRSV in sweet cherry trees by isolating the virus from mature trees, by partially purifying and serologically identifying the isolates, by inducing symptoms in mechanically-inoculated cherry seedlings, and by isolating TRSV from these test trees.

Stace-Smith and Hansen (5) repeatedly isolated TRSV from symptomatic leaf tissues of a naturally infected mature sweet cherry through a 3-mo period. Our results suggest that frequently TRSV infections in cherry trees are not as reliably systemic; in fact, that the invasions can occur as flushes of very short duration. Despite the use of various soft and hard tree tissues as inocula, the virus could be isolated from each of the sweet and flowering cherry trees only during a brief period in one season. This is in striking contrast to NRSV which is easily and consistently transmitted in repeated assays.

In the tests reported by Stace-Smith and Hansen, bud-inoculated cherry seedlings yielded TRSV from symptom-displaying parts only. The naturally infected trees used in our tests lacked obvious leaf symptoms; however, our higher yields of virus from stunted shoots of flowering cherry suggest that such stunting is a symptom of TRSV infection.

In our assays, a higher percentage of TRSV isolates was yielded by extracts of cherry buds than by leaf or blossom extracts. This corresponds to the experience of Davidson and Rundans (1) in isolation of prune dwarf and necrotic ringspot viruses, and confirms that dormant bud-herbaceous host assay of sap-transmitted viruses affecting cherry is superior and more convenient than use of immature leaf or flower petal tissue.

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