DETECTION OF TOMATO RINGSPOT VIRUS IN PELARGONIUM IN ONTARIO[1]

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Abstract

A virus transmitted by mechanical inoculation from Pelargonium with ringspot symptoms to cucumber was identified as tomato ringspot virus. Its identity was established primarily by serological comparisons with a known culture of tomato ringspot virus and secondarily by simultaneous-infection tests, symptomatology, host reaction, and properties in vitro. It was transferred by means of Xiphinema americanum from infected geranium roots to cucumber bait plants.

Introduction

In March 1967, a sap-transmissible virus was isolated from geranium (Pelargonium hortorum Bailey cv. Appleblossom) that was distinct from any virus previously described from Pelargonium in Canada. It was recovered from one stock plant that succumbed to bacterial stem rot later in the summer. Besides symptoms suggestive of this bacterial disease, a few large, chlorotic ringspots were observed on the lower, older leaves but not on the youngest ones. The virus failed to react with antiserum against tobacco ringspot virus previously isolated from geranium by the author (4). Mechanical transmission of the virus from geranium leaves to other herbaceous plants was erratic and occurred on only two occasions, both in March, when cucumber seedlings developed symptoms 10 days after inoculation. Results of studies on symptomatology, transmission, host range, and serological identity of the virus are presented in this paper.

Materials and methods

Stock cultures of the unknown geranium virus under investigation, as well as an isolate of tobacco ringspot virus and a tomato ringspot virus culture provided by Dr. R. Stace-Smith, Research Station, Canada Department of Agriculture, Vancouver, B.C., were maintained in cucumber for use in comparative tests.

Infected cucumber leaf ground in 0.25% Na_2SO_3 or 2.5% nicotine solutions was used as inoculum in various tests. Five-hundred mesh carborundum was added to the inoculum.

Virus-indexed geranium seedlings (cv. Nittany Lion) at the 4 leaf stage were preconditioned for 48 hr in complete darkness before mechanical inoculation with the virus and were then reinoculated 10 days later after a second 24-hr dark exposure.

The thermal inactivation point, dilution end point, and longevity of the virus in crude sap were determined by conventional methods.

Nematode transmission tests were done by the sequential planting of virus-infected geranium and cucumber bait plants in nematode-infested soil. Three soil samples collected from a cherry orchard at Fonthill, Ontario, with varying populations of Xiphinema americanum Cobb, 1913 were used. Control tests with cucumber indicated that the nematodes were initially virus-free.

An antiserum prepared by the author (4) against tobacco ringspot virus from geranium and another against tomato ringspot virus supplied by Dr. R. Stace-Smith were used in the identification of the virus in question. Agar-diffusion precipitin tests were performed in plastic plates prepared as described previously (3).

Results

TRANSMISSION TO PELARGONIUM

Of 22 inoculated 'Nittany Lion' seedlings, 21 showed visible symptoms within 3 to 4 weeks. The virus was recovered from each plant within 3 months. Later, when the virus was identified serologically, these isolates were checked and found to be identical to the one used in the original inoculation. Eight control plants consisting of seedlings of the same age were planted and prepared for inoculation in the same way but were not actually inoculated. None developed symptoms.

Most of the infected seedlings were stunted; leaves were small and many of them showed yellow veins and chlorotic spots and rings (Figure 1). Leaves on one plant showed whitish rings 1/8- to 1/4 inch in diameter. Two months after inoculation the newest leaves were symptomless, but the plants remained somewhat smaller than comparable healthy controls. No stem lesions were noted.

[1] Contribution No. 176, Research Station, Canada Department of Agriculture, Vineland Station, Ontario

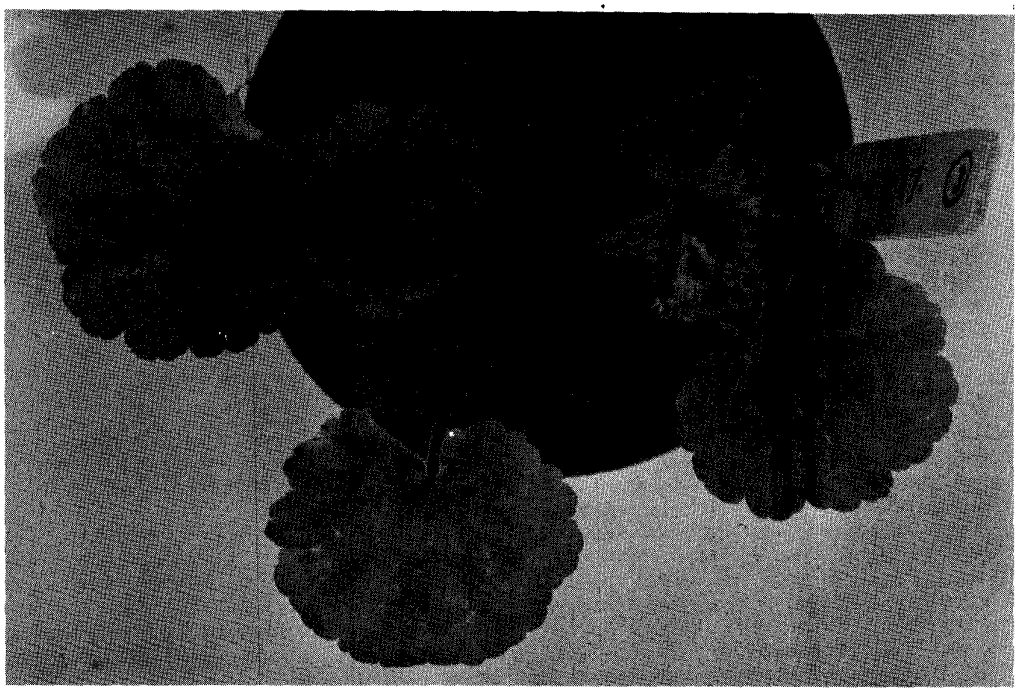

Figure 1. *Pelargonium hortorum* ('Nittany Lion') seedling showing systemic symptoms 5 weeks after mechanical inoculation with a geranium isolate of tomato ringspot virus.

HERBACEOUS HOST REACTIONS

The host range study was restricted to species considered to be of value in identifying viruses of the ringspot type. Symptoms on the various hosts are summarized in Table 1.

Only bean (Phaseolus vulgaris) failed to react to the virus: each of the other plants developed chlorotic or necrotic lesions on the inoculated leaves. Systemic symptoms appeared as chlorotic or necrotic spots or rings, veinal chlorosis, and/or mild mottle. Apical stem necrosis occurred on Chenopodium amaranticolor, C. quinoa, and Vigna sinensis. The reactions of these herbaceous plants suggested that the geranium virus possibly was related to tomato ringspot virus. Subsequently, the virus and an isolate of tomato ringspot virus were inoculated into the same range of test plants under the same conditions. With the exception of certain variations in tobacco and cucumber, the responses of the test plants to the two isolates were similar.

BIOLOGICAL AND PHYSICO-CHEMICAL PROPERTIES

Interference - The geranium virus and the tomato ringspot virus acted as closely related viruses in the presence of each other in two simultaneous-infection tests. No double-virus effect, indicating unrelatedness, was observed (2). Single and double infections with these viruses did not induce any marked differences: all plants were stunted to approximately the same degree and showed no symptoms other than those caused by either of the viruses alone.

Serology - In agar diffusion tests, crude sap from infected cucumber leaves containing the geranium virus reacted specifically with antiserum to tomato ringspot virus but not with antiserum to tobacco ringspot virus. Sap from healthy cucumber failed to react with either antiserum. Furthermore, the precipitin pattern produced when both of these virus isolates were compared in adjacent wells against tomato ringspot virus antiserum was a single, coalescing zone. The fusion of precipitation zones (Figure 2) suggests a close serological relationship between these viruses (1).

Physical Properties - Virus-containing sap maintained at room temperature (ca. 20C) was infective after 24 hr but not after 48 hr. The dilution end point was between 10^{-4} and 10^{-5}, and the virus was thermally inactivated after 10 minutes at 55C. These properties were similar to thoseof the tomato ringspot virus isolate when both were tested in a comparative study.

Table 1. Symptoms induced in test plants by a ringspot virus from Pelargonium

Host plant	Inoculated leaves	Uninoculated leaves and stems
Chenopodium amaranticolor Coste & Reyn.	chlorotic lesions	chlorotic spots, mottle, leaf distortion, stem necrosis
Chenopodium quinoa Willd.	chlorotic lesions	veinal chlorosis, stem necrosis
Cucumis sativus L.	chlorotic lesions	veinal chlorosis, mottle
Gomphrena globosa L.	buff-centered lesions	mottle, irregular buff-centered lesions
Lycopersicon esculentum Mill.	necrotic lesions	veinal necrosis
Nicotiana glutinosa L.	chlorotic, necrotic spots	chlorotic mottle
Nicotiana tabacum L.	necrotic spots, broken concentric rings	few broken buff rings
Petunia hybrida Vilm.	necrotic spots	veinal chlorosis, mild mottle
Phaseolus vulgaris L.	none	none
Vigna sinensis Endl.	red spots, rings	reddish-brown veins, stem necrosis

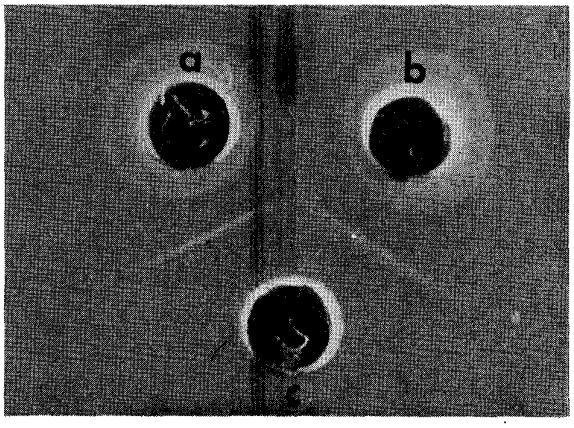

Figure 2. Photograph of precipitation pattern in agar. The meeting of the two precipitin lines indicates that the geranium ringspot virus (a) and the tomato ringspot virus (b) are identical or closely related; well (c) contained antiserum of tomato ringspot virus.

NEMATODE TRANSMISSION

Two of three populations of Xiphinema americanum transmitted the geranium virus. Populations containing 64 and 42 virus-free nematodes per 100 gm of soil acquired the virus frominfected geranium roots within 3 weeks and transmitted it to cucumber bait plants within 1 month of their planting in these soils. No transmission to cucumber occurred in soil originally planted with infected geraniums and containing 8 nematodes per 100 gm of soil.

Discussion

The results substantiate an earlier report (5) of the isolation of a probable strain of tomato ringspot virus from geranium. The identification of tomato ringspot virus in geranium in Ontario has been made primarily on a serological comparison with an isolate of tomato ringspot virus. Cursory serological tests indicated that the isolates tested here are

closely related, but the reactions induced by them in tobacco and cucumber under the same conditions suggest that they may be distinct strains. Tomato ringspot virus is the second NEPO-virus detected in geraniums in Ontario; the first, tobacco ringspot virus, was found by the author in the cultivar Mme. Salleron in 1964(4). Obviously field soils in Ontario contain nematodes that are vectors of this virus. At present, the prevalence and economic importance of tomato ringspot virus in geraniums under greenhouse conditions is uncertain.

Literature cited

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