

A New Graft-transmissible Disease of Bergamot in Greece

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ABSTRACT. Bergamot trees growing in Chania and Peloponnesse in Greece have been commonly observed showing symptoms of leaf vein yellowing. The disease was graft-transmitted to healthy bergamot seedlings, with more severe symptoms developing in plants kept at 32°C compared to those at 25°C. Inoculated citron plants of three selections all developed severe epinasty and yellow blotching, and one of them, Roxane, also developed mild yellow vein. Samples were tested for *Citrus exocortis viroid* and *Hop stunt viroid* by sPAGE and by tissue printing and hybridization and were positive, but the roles of these viroids in the etiology of the disease remain to be determined. Biological indexing showed that *Citrus tristeza virus*, *Citrus psorosis virus* and Citrus vein enation virus were not present.

Surveys of bergamot trees in Greece have revealed the presence of many trees in Chania and Peloponnesse with marked leaf vein yellowing symptoms. These symptoms resemble those reported in California on limequat in the 1950s (7).

To determine whether this disease was graft-transmissible to bergamot or other citrus species, five seedlings each of bergamot, sour orange, Volkamer lemon, Washington navel sweet orange, Marisol Clementine, Mexican lime, Eureka and Vakalou lemons, and Etrog 60-13, Etrog Arizona 861 and Roxane citrons were wedge-graft inoculated with two buds from a 15-yr-old bergamot tree showing leaf vein yellowing.

The inoculated plants and the uninoculated controls were maintained in a greenhouse at 21-35°C. Bright yellowing of the mid and lateral veins, typical of the disease observed in the field, appeared in the bergamot seedlings 14 mo after inoculation (Table 1). The symptoms were more severe at 32°C than below 25°C.

A set of five healthy seedlings was graft-inoculated periodically during 1999 and 2000 to study the effect of seasonal temperature changes on the appearance of symptoms.

No symptoms of *Citrus tristeza virus*, *Citrus psorosis virus* or Citrus vein enation virus were observed on the other indicator species (Table 1). The absence of yellow vein symptoms

TABLE 1
INFECTIVITY ASSAY AND OBSERVED SYMPTOMS OF BERGAMOT VEIN YELLOWING

Species	Symptoms			
	Yellow vein	Epinasty	Yellow blotch	Drooping leaves
Bergamot	severe	none	none	none
Sour orange	none	none	none	none
Volkamer lemon	none	none	none	none
Sweet orange	none	none	none	none
Clementine	none	none	none	none
Lemon				
-Eureka	none	none	faint	none
-Vakalou	none	none	faint	none
Mexican lime	none	none	faint	none
Citron				
-Etrog 60-13	none	severe	severe	random
-Arizona 861	none	severe	severe	random
-Roxane	mild	severe	severe	severe

in the Mexican lime, and the fact that symptoms on bergamot took 14 mo to appear, suggests that this disease is different from the one reported in limequats in California, where symptoms appeared 3-5 mo after inoculation (7); the limes and lemons did, however, develop faint yellow blotching. The citrons all reacted with severe epinasty, indicating the presence of *Citrus exocortis viroid* (CEVd). Roxane citron also showed mild yellow vein, and “drooping leaves”, a symptom associated with *Citrus viroid III* and *Citrus viroid IV* (1, 2).

Because of the reactions observed in the inoculated citrons, additional tests were conducted to determine the presence of other citrus viroids. Young leaves from inoculated citrons, Mexican lime, Washington navel sweet orange, Marisol Clementine, and Volkamer lemon were phenol extracted and partitioned in 2M LiCl, essentially as described by Semancik et al. (5). The nucleic acids were then separated by sPAGE, electroblotted onto membranes (2) and hybridized against CEVd and *Hop stunt viroid* (HSVd) specific probes. In addition, hybridization of tissue imprints of leaves was conducted as described by Palacio et al. (3). [α - 32 P]-labeled probes were synthesized by a transcription reaction using as templates

the plasmids pCEV6 and PH121 (kindly donated by Dr. H. Puchta) containing a monomeric cDNA of CEVd ligated into the pGEMTeasy and a head to tail dimeric cDNA of HSVd ligated into the pBluescript cloning vectors, respectively. Hybridization was performed for 16 h at 60°C and 65°C for CEVd and HSVd probes respectively. Positive reactions were visualized by autoradiography against X-ray films. The results of these tests indicated that both CEVd and HSVd were present.

These preliminary studies have shown that this previously unreported disease of bergamot is graft-transmissible. Based on incubation times and host reactions, it appears to be different from citrus yellow vein (7). Although we demonstrated the presence of two viroids in the affected trees, other citrus viroids have not been tested. Further investigations are required to determine what role, if any, viroids are playing. Both exocortis caused by CEVd and cachexia caused by specific HSVd variants (4, 6) have been reported in Greece, and are believed to be widespread. CEVd also causes a problem called ‘condylitis’ in commercial citrons in Chania. We propose the term “bergamot vein yellowing” for this graft-transmissible disease.

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