

CITRUS VIROIDS

Distinct Disease Symptoms in *Poncirus trifoliata* Induced by Three Citrus Viroids from Three Specific Groups

C. N. Roistacher, J. A. Bash and J. S. Semancik

ABSTRACT. Observations on a Valencia/trifoliata planting inoculated with pure isolates of citrus viroids (CVds) showed the following specific reactions: 1) "Trifoliata deep pit" a symptom caused by citrus viroid Ia which induced deep pits in the wood of the trifoliata trunk with corresponding pointed pegs in the bark. 2) "Trifoliata mild bark cracking" a symptom caused by citrus viroid IIa which induced mild to moderate bark cracking in the trifoliata trunk. 3) "Trifoliata finger imprint" a symptom caused by citrus viroid IIIb which induced grooving in the trifoliata trunk resembling a squeezing or strangling of the trunk leaving indented imprints with horizontal striations. These three distinct symptoms in *Poncirus trifoliata*, associated with representative members of the citrus viroid groups I, II and III are described and illustrated. In addition, viroids Ia and IIIb caused significant reduction of the trunk cross section in Valencia scion and trifoliata rootstock, and the canopy volume of infected trees.

The association of severe bark shelling on trifoliata orange rootstock with the citrus exocortis viroid (CEVd), and the severe epinasty induced by this viroid in citron is well documented. Beginning in the 1960's a collection of isolates, presumed to be mild CEVd variants, was initiated in the Citrus Clonal Protection Program (CCPP) virus bank at the University of California, Riverside. Various mild to moderate symptoms on citron indicator plants were interpreted as the reaction of CEVd isolates. A range of symptoms in citron from a distinct twisting of leaves, petiole wrinkle, petiole browning, leaf tip browning, midrib browning with mild leaf epinasty or bending, cracking or browning of the leaf midrib were described and illustrated (9).

The association of these mild symptoms induced in citron to specific symptoms in trifoliata orange was never tested to verify the presence of the "exocortis" disease. A test of some of these presumptive mild to moderate "exocortis" isolates for reaction to trifoliata orange rootstock was initiated in 1983. At that time, the identification and cataloging of the citrus viroids had not as yet been reported (2, 3, 4).

Subsequently, all the isolates inducing a reaction in citron were analyzed by sequential polyacrylamide gel electrophoresis (sPAGE) and the specific citrus viroid content established. This paper presents the relationship of specific citrus viroids, representative of Groups I, II and III (3) to distinct symptoms induced in the trifoliata orange rootstock and to effects on the Valencia scion. Analysis for the viroid profile in the scion and also in the rootstock was made to confirm the presence of the specific viroids.

MATERIALS AND METHODS

In March, 1983, Campbell Valencia orange as the scion was budded on Rubidoux trifoliata seedlings. The scion source was a virus-tested candidate (V.I. 176) free of all known graft-transmissible pathogens. At the time of scion budding, groups of six trifoliata seedlings were graft-inoculated with two blind buds from each of four sources which induced a mild to moderate reactions in citron.

Trees were grown for one year at the Rubidoux greenhouse in Riverside and in April, 1984, were transferred to

the field at the Lindcove Field Station in Central California. Temperatures at Lindcove are warm during the summer months and are excellent for production of exocortis and cachexia symptoms. Scions and rootstocks were examined and measured in July 1992. Trunks were calipered 15 cm above and 5 cm below the bud union. Height and width measurements were used for estimation of tree volume as a cylinder. Statistic analysis was by Duncan's multiple range test, using the SAS system.

To determine the viroid content of the scion, budwood was collected from the trees in September 1989, and again in August 1991. Buds were graft-inoculated to 861-S1 Etrog citron on rough lemon rootstock. Approximately 3-4 months after inoculation, the young tips of the citron were collected for nucleic acid sPAGE analysis (8, 12). To verify the viroid content of the rootstock as identical to the scions, bark samples were collected in May, 1992 and graft-inoculated to citron/rough lemon for subsequent nucleic acid sPAGE analysis of the citron.

RESULTS

Association of symptom expression in citron with citrus viroids. Symptoms in citron and the viroids found by nucleic acid sPAGE analysis are shown in Table 1. The citrus viroids found in each of the four treatments were uniform for both the Valencia scion and the trifoliate rootstock. The viroids included in treatments were CVd-Ia, CVd-IIa, CVd-IIIb and a mixture of CVds-Ib, -IIa and -IIIb (Fig. 3).

Tree size and growth. Fig. 1 shows the relationship of the presence of various CVds to the trunk section of the trifoliate rootstock and Valencia scion and to the canopy volume of the tree. The presence of CVd-IIIb and mixtures of CVds-Ib, -IIa and -IIIb significantly reduced both the trunk section of the scion and rootstock (Fig. 1a), and reduced the canopy volume (Fig. 1b).

The presence of CVd-IIIb alone caused a 47% reduction of scion cross sectional area and a 50% reduction in tree volume. The mixed infection of CVds-Ib, -IIa and -IIIb induced a similar reduction of tree volume and scion

TABLE 1
SYMPTOMS OBSERVED ON 861-S1 ETROG CITRON AND IN THE TRUNKS OF THE TRIFOLIATE ROOTSTOCKS IN ASSOCIATION WITH CITRUS VIROIDS.

Citrus Viroids	Symptoms on Citron	Symptoms on Trifoliate Rootstock
Ia	Foliage: Mild and variable bent-leaf induced by point necrosis. Size: Little to no reduction.	Deep pits with corresponding pegs in the bark. (Fig. 2a).
IIa	Foliage: Leaf twist, very mild tip browning, petiole wrinkle and petiole browning. Size: No reduction.	Mild bark cracks. (Fig. 2b).
IIIb	Foliage: Tip browning, petiole wrinkle and browning plus leaf twist. Leaf droop and bent leaf induced by point necroses of the midvein. Size: Little to no reduction.	Finger imprint and bark striations. (Fig. 2c)
Ib IIa IIIb	Foliage: Moderate to strong leaf epinasty, petiole browning and stem lesions. Size: Significant reduction.	Finger imprint and bark striations plus mild bark cracks (Fig. 2d).

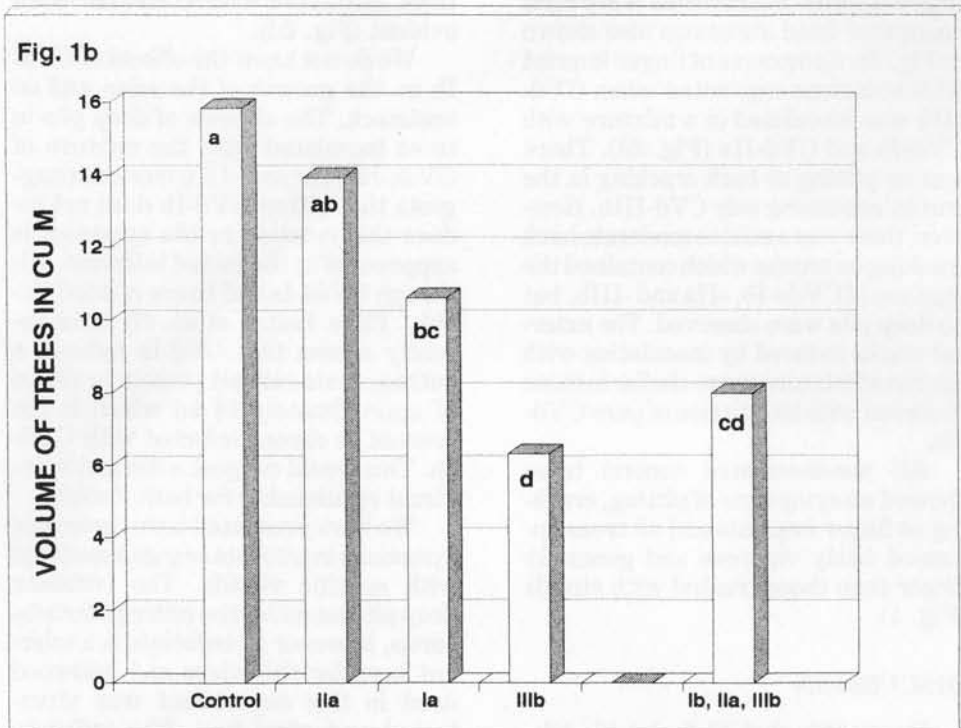
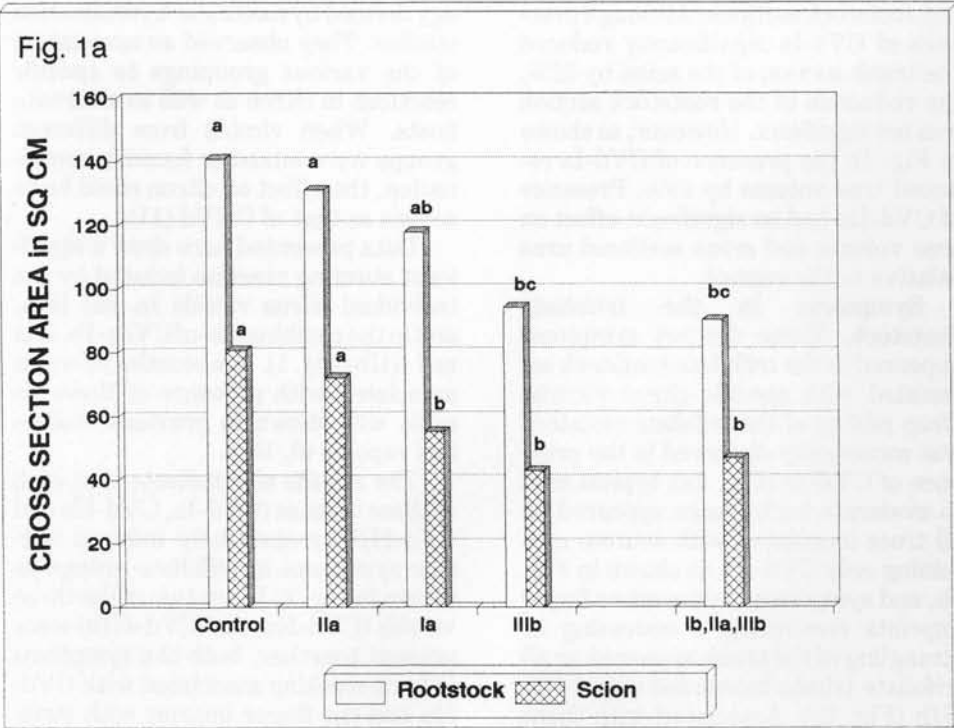


Fig. 1. The relationship of presence of various citrus viroids to growth of Valencia orange trees grafted on *Poncirus trifoliata*. 1a) Effect of viroids on the cross sectional area of the trifoliolate rootstock and the Valencia scion. 1b) Effect of the viroids on canopy volume. Different letters at the top of bars with the same marking indicate significant differences at $P \leq 0.05$ (Fig. 1b) or $P \leq 0.01$ (Fig. 1a), based on Duncan's multiple range test.

and rootstock sections. Although presence of CVd-Ia significantly reduced the trunk section of the scion by 32%, the reduction of the rootstock section was not significant. However, as shown in Fig. 1b the presence of CVd-Ia reduced tree volume by 33%. Presence of CVd-IIa had no significant effect on tree volume and cross sectional area relative to the control.

Symptoms in the trifoliolate rootstock. Three distinct symptoms appeared in the trifoliolate rootstock associated with specific citrus viroids. Deep pitting of the trifoliolate rootstock was consistently observed in the presence of CVd-Ia (Fig. 2a); typical mild to moderate bark cracks appeared on all trees inoculated with sources containing only CVd-IIa as shown in Fig. 3b, and symptoms of grooves or finger imprints resembling a squeezing or strangling of the trunk appeared on all trifoliolate trunks inoculated with CVd-IIIb (Fig. 2c). Associated with these finger-imprint impressions were light horizontal lined striations also shown in Fig. 2c. Symptoms of finger imprint with striations was noted when CVd-IIIb was inoculated in a mixture with CVd-Ib and CVd-IIa (Fig. 2d). There was no pitting or bark cracking in the trunks containing only CVd-IIIb. However, there was a mild to moderate bark cracking on trunks which contained the mixture of CVds-Ib, -IIa and -IIIb, but no deep pits were observed. The external cracks induced by inoculation with the viroid mixtures were similar to those observed with inoculation of pure CVd-IIa.

All non-inoculated control trees showed no symptoms of pitting, cracking or finger imprints and all trees appeared fairly vigorous and generally larger than those treated with viroids (Fig. 1).

DISCUSSION

Duran-Vila et al. (2, 3) classified the citrus viroids into five groups (CEVd, CVd-I, CVd-II, CVd-III and CVd-IV) based on electrophoretic mobility of their nucleic acids and sequence homol-

ogy defined by molecular hybridization studies. They observed an association of the various groupings to specific reactions in citron as well as alternate hosts. When viroids from different groups were mixed or found in combination, the effect on citron could be as severe as that of CEVd (11).

Data presented here show a significant stunting reaction induced by the individual citrus viroids Ia and IIIb, and in the combination of CVds-Ib, -IIa and -IIIb (Fig. 1). The stunting of scions associated with presence of these viroids was shown in previous studies and reports (6, 10).

The results also indicate that each of these isolates (CVd-Ia, CVd-IIa and CVd-IIIb) respectively induced specific symptoms in trifoliolate orange as shown in Fig. 2. When two of the three viroids (CVd-IIa, and CVd-IIIb) were present together, both the symptoms of bark cracking associated with CVd-IIa and the finger imprint with striations associated with CVd-IIIb were evident (Fig. 2d).

We do not know the effects of CVd-Ib on the growth of the scion and on rootstock. The absence of deep pits in trees inoculated with the mixture of CVds-Ia, -IIa and -IIIb inoculum suggests that either CVd-Ib does not induce this reaction or the symptom is suppressed in the mixed infection. Although CVds-Ia and Ib are related viroids, Pires Leitao *et al.* (7) have recently shown that CVd-Ia induces a pathogenesis-related protein in citron of approximately 14 kd which is not present in citrons infected with CVd-Ib. This would suggest a distinct host-viroid relationship for both viroids.

We have presented here three new symptoms in trifoliolate orange associated with specific viroids. The trifoliolate deep pit resembles the pitting of *Crataegus*, however *P. trifoliata* is a tolerant host for this virus and budwood used in this experiment was virus-tested and virus-free. The trifoliolate finger imprint resembles somewhat the 'finger mark' symptoms on limbs of sweet orange, lemon, grapefruit and mandarin reported from Italy, Peru,



Fig. 2. Symptoms on trifoliate rootstock associated with specific citrus viroids. A) Deep pits in the wood and pegs in bark associated with CVd-Ia. B) Mild cracking in the bark associated with CVd-IIa. C) Finger imprint with striations in the bark associated with CVd-IIIb. D) Finger imprint and mild bark cracks associated with a mixture of CVds-Ib, CVd-IIa and CVd-IIIb.

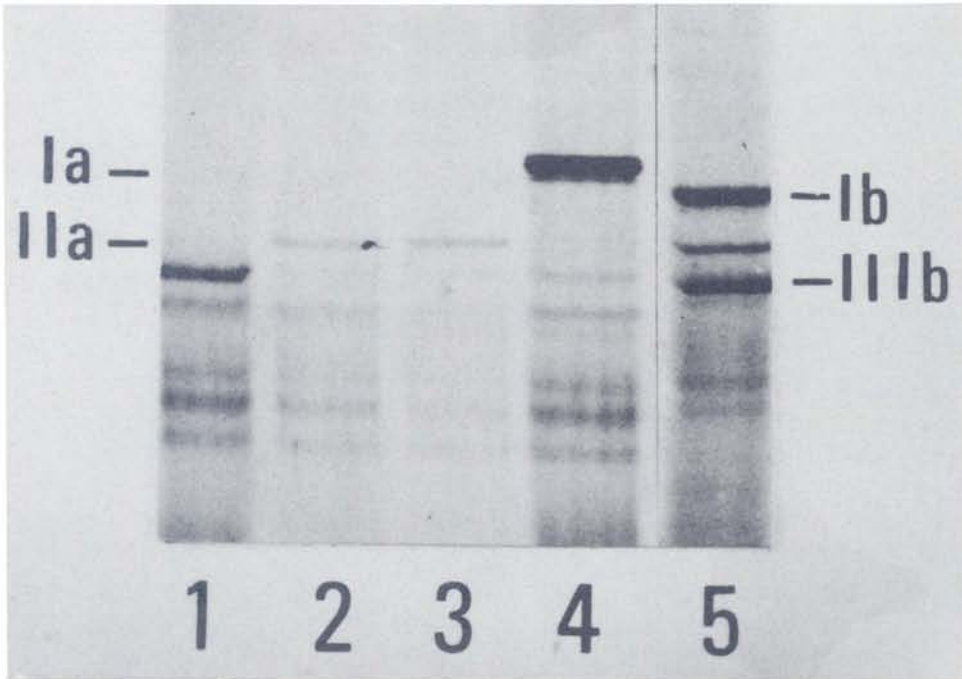


Fig. 3. sPAGE of nucleic acid extracts obtained from citron plants following inoculation from Valencia scion (lanes 1, 2, 4, and 5) or trifoliolate rootstock (lane 3) containing CVd-IIIb (lane 1), CVd-IIa (lanes 2 and 3), CVd-Ia (lane 4) or a mixture of CVds-Ib, -IIa, and -IIIb (lane 5). Gels were stained with silver nitrate.

and Argentina (5), which some authors have related to the concave gum virus. This 'finger mark' has never been transmitted and no specific pathogen implicated. The symptoms do resemble the effect of a tight wrap on young trees. However, wraps were removed very early in the experiment and there was no similar symptom present on any other tree in the experiment except those inoculated with CVd-IIIb, thus indicating that this finger imprint reaction was a response to the presence of

the viroid. Bark cracks induced by CVd-IIa resemble symptoms reported on trifoliolate rootstocks grafted with the Atwood navel orange scion (1, 9). The Atwood navel was subsequently confirmed to contain CVd-IIa (3, 4).

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