Effect of Citrus Exocortis Viroid on Flower and Fruit Structure and Development on Etrog Citron

W. P. BITTERS, Horticulturist Emeritus, University of California, Riverside 92521; N. DURAN-VILA, IVIA, Moncada (Valencia), Spain; and J. S. SEMANCIK, Plant Pathologist, University of California, Riverside 92521

ABSTRACT

Bitters, W. P., Duran-Vila, N., and Semancik, J. S. 1987. Effect of citrus exocortis viroid on flower and fruit structure and development on Etrog citron. Plant Disease 71: 397-399.

Rooted cuttings of Etrog citron (*Citrus medica*) were inoculated with the citrus exocortis viroid (CEV). Typical exocortis symptoms were expressed in these plants within 2 mo. Affected plants were retained after the initial foliage symptoms for between 2.5 and 3 yr, which provided an opportunity for the plants to flower and set fruit. Floral morphology was abnormal from that of healthy plants in that the corolla failed to open. Fruits that developed from these flowers were characterized by a ringed constriction resembling an asymmetrical "hourglass" that contained a high percentage of seeds that aborted and lacked viability. S-1 citron cuttings inoculated with the citron variable viroid (CVaV) did not produce these types of symptoms, which therefore might be considered characteristic of severe CEV infection.

Etrog citron (*Citrus medica* L.) is used as an indicator plant for the citrus exocortis viroid (CEV) (1). Propagations of Etrog citron are made by growing seedlings of Arizona 861 (3) and cuttings or young budded plants of the S-1 selection (4), both of which are currently used in the indexing technique. The S-1 cuttings are now generally preferred. Symptoms of citrus exocortis viroid (CEV) on citron Arizona 861 or S-1 include chlorosis and epinasty of the newly formed and developing leaves; corking of the midrib and large lateral veins on the ventral leaf surface; and vertical, striated, corky lesions on the stem (2). However, no floral or fruit symptoms have yet been reported.

In the spring of 1985, cuttings of S-1 citron infected with a severe isolate of CEV were retained in the greenhouse longer than 1 yr. This delay allowed the infected cuttings an opportunity to flower and set fruit, a situation not previously observed. Observations of the flowering and fruiting structures of the infected plants revealed abnormalities in these organs not previously associated with the presence of the CEV-RNA.

The morphology of healthy citrus flowers has been characterized (5,7) and is shown in Figure 1A. In normal, healthy flowers, the corolla opens in basipetal order and the base of the petals remains attached at the calyx cup until after anthesis. Most of the flowers of the CEV-infected plants are characterized by the corolla remaining intact before, during, and after anthesis. The petals do not dehisce normally. Instead, they remain attached at the apex of the corolla, and the entire corolla is pushed off the receptacle as the ovary develops. The petals do separate at the base of the corolla and sometimes flare out in a somewhat star-shaped pattern as the dried uppermost half of the corolla forms a cap over the stylar end of the immature fruit, where it may remain for several months (Fig. 1B).

Although no anatomical studies have been made, abnormalities of the flower, developing ovulary, and immature and mature fruit are apparent. Although the calyx of an infected flower appears to be normal in structure, closer observations suggest that the calyx is much more adherent to the upper portion of the receptacle and the floral disk (Fig. 1C, left) than is found on normal, healthy flowers (Fig. 1C, right). In fact, the adherence is so tight it is restrictive to the normal development of the ovulary and the developing ovary as cell multiplication and enlargement take place in the process of forming a mature hesperidium (Fig. 2). The tightness seems to be concentrated at the apex of the sepals so that the approximate middle of the ovulary or ovary is constricted. This constriction varies from mild to severe (Fig. 3), almost amounting to a severe girdling effect in extreme cases. The ultimate response to this girdling effect is that some of the developing fruit are distorted to varying degrees with an asymmetrical "hourglass" configuration (Fig. 3), the most common symptom. The stylar half of the fruit is usually smaller than the stem half, and in some cases much more so. Although

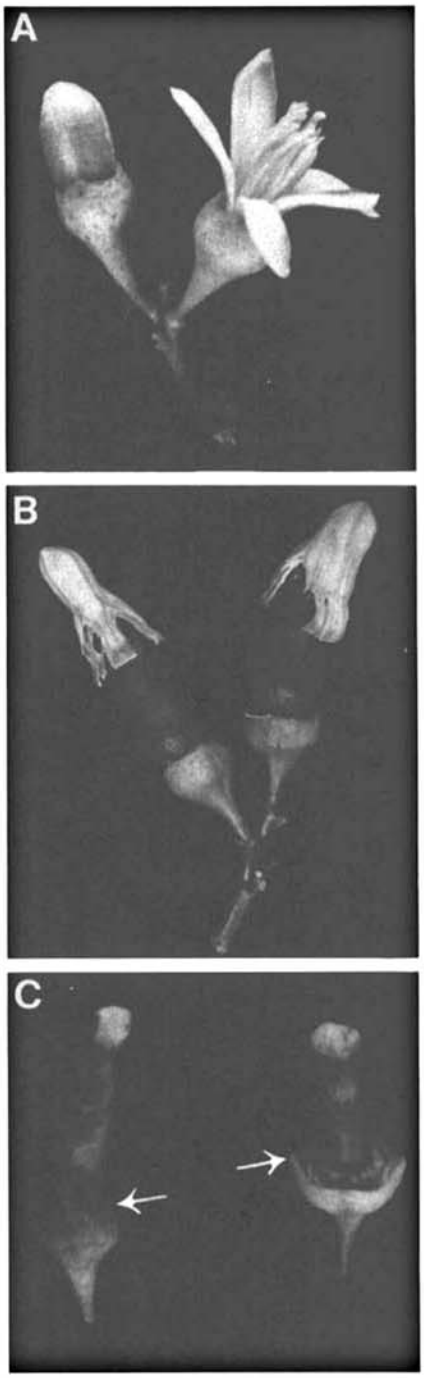

Fig. 1. (A) Normal, healthy citron flower. **(B)** Abnormal citron flower from a plant infected with citrus exocortis viroid (CEV). **(C)** (Left) Pistil, constricted calyx, and receptacle from an abnormal CEV-infected citron flower; (right) same structure from a healthy flower. Arrows indicate lip of calyx.

Accepted for publication 28 October 1986.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

some of the fruits are vertically oriented about the placental axis, other fruits will deviate from this with the stylar half of the fruit bent away from the perpendicular. Infected fruits are generally smaller than healthy fruits and tend to turn yellow prematurely. Severely affected fruits may be only one-fourth the size of normal fruits.

If longitudinal or cross sections are made of the infected fruits, it is quite evident that the ovules have been affected (Fig. 4). However, severely affected, small, distorted fruits (Fig. 4A, right) may contain over a hundred small seed primordia that never develop into normal plump seeds. In contrast, mildly affected fruits (Fig. 4A, left), when they reach maturity and attain a size of about 10 cm in length, have about 20% normal plump seeds that will germinate readily and produce symptomless healthy plants. The remaining 80% of the seeds never grow longer than 2 mm, and these seed primordia never develop into normal seeds. All laboratory attempts to force those undeveloped seeds (100–150)

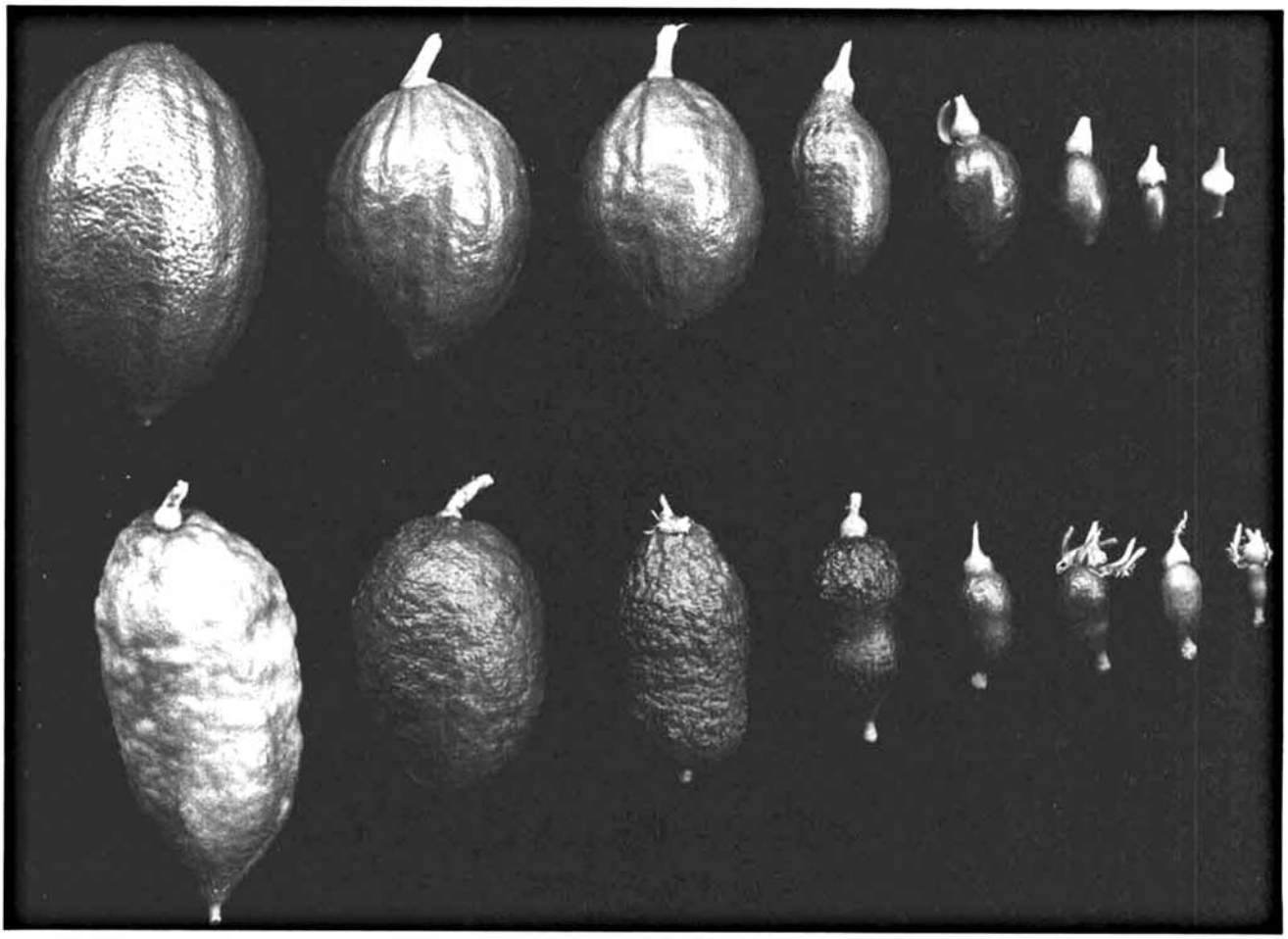

Fig. 2. (Top) Progressive developmental stages from right to left in healthy citron fruits. **(Bottom)** Similar developmental stages in citrus exocortis viroid-infected fruits.

Fig. 3. Examples of the range in types of citron fruit malformation expressed as a result of the presence of the citrus exocortis viroid.

Fig. 4. (A) Longitudinal sections of citrus fruit infected with citrus exocortis viroid showing different degrees of malformation and a decrease in seed count and viable seeds with the severity of the malformation increases. **(B)** Longitudinal sections of healthy fruit.

obtained from either CEV severely or mildly affected fruits to produce plants or any kind of tissue proliferation such as callus, either in vivo or in vitro, failed. Healthy S-1 fruits have many viable plump seeds that germinate at virtually 100%.

Thus, seed abortion in S-1 citron seems to be an important aspect of the disorder and may at least in part contribute to the absence or extremely low incidence of seed transmission of CEV. The floral and fruit symptoms described here have not been reported for any commercial cultivar of citrus infected with CEV. In fact, tree vigor as well as fruit size and quality do not appear to be affected when CEV is present in a tolerant rootstock-scion combination.

None of the above symptoms have been observed on healthy S-1 citron cuttings, nor have they been observed on S-1 citron cuttings inoculated with the citron variable viroid (6), which is characterized by foliage symptoms that are milder but nevertheless similar to that induced by CEV. Therefore, the described symptom expression in the flowers and fruit is believed to be specifically due to the presence of CEV.

ACKNOWLEDGMENTS

We wish to thank J. Juarez of IVIA, Moncada, for in vitro culture of the seeds and Kathy Harper and R. Rivera-Bustamonte for illustration preparation. Supported in part by the US-Spain Joint Committee for Scientific and Technological Cooperation.

LITERATURE CITED

1. Calavan, E. C., Frolich, E. F., Carpenter, J. B., Roistacher, C. N., and Christiansen, D. W. 1964. Rapid indexing for exocortis of citrus. Phytopathology 54:1359-1362.
2. Childs, J. F. L. 1968. Exocortis. Pages 28–34 in: Indexing Procedures for 15 Virus Diseases of Citrus Trees. J. F. L. Childs, ed. U.S. Dep. Agric. Agric. Handb. 333. 96 pp.
3. Garnsey, F. M., and Whidden, R. 1973. Severe, uniform response by monoembryonic seedlings of the Arizona 861 selection of 'Etrog' citron (*Citrus medica*) to the citrus exocortis virus (CEV). Plant Dis. Rep. 57:1010-1012.
4. Roistacher, C. N., Calavan, E. C., Blue, R. L., Navarro, L., and Gonzales, R. 1977. A new more sensitive citron indicator for mild isolates of citrus exocortis viroid (CEV). Plant Dis. Rep. 61:135-139.
5. Schneider, H. 1968. The anatomy of citrus. Pages 1–85 in: The Citrus Industry. Vol. 2. W. Reuther, L. D. Batchelor, and H. J. Webber, eds. University of California, Division of Agricultural Sciences, Berkeley. 398 pp.
6. Schlemmer, A., Roistacher, C. N., and Semancik, J. S. 1985. A unique infectious RNA associated with citron showing symptoms typical of citrus exocortis disease. Phytopathology 75:946-949.
7. Tillson, A. H., and Bamford, R. 1938. The floral anatomy of the *Aurantioideae*. Am. J. Bot. 25:780-793.