

VIROIDS

A Contribution to the Natural History of Viroids

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ABSTRACT. Presently recognized viroid diseases were not known to exist prior to the 20th century and, as such, it has been suggested that viroid diseases of cultivated plants are of recent origin. This paper presents descriptions of archaeological articles that depict Etrog citron fruit with citrus viroid disease (CVds) symptoms from an ancient synagogue from the early 6th century C.E. in Israel. Because CVds are not known to be seed-borne and that citron was introduced to the Mediterranean Basin by seed around 300 B.C., early citron plants in Israel were likely CVd-free. It is postulated that CVds could have resulted from contamination from endemic grapevines which can harbor low concentrations of several CVds species by pruning and other cultural practices. CVds were then perpetuated by graft propagation and became a problem when certain sensitive rootstocks were used with CVd-infected "old line" budwood.

Viroids are the smallest known agents of infectious diseases. They are small single stranded circular RNA molecules of 246 to 375 nucleotides that lack a capsid protein and detectable mRNA activity. At present, there are about 20 known viroid species which are grouped in two families (14). All viroids have a host range restricted to the plant kingdom. The origin of viroids is unknown, however, Diener, in his discussion of the nature of viroids, has stated that none of the presently recognized viroid diseases was known to exist before the 20th century and postulated that viroid diseases of cultivated plants are of recent origin (4). Accordingly, he suggested that viroid diseases of cultivated plants are caused by man and his agricultural activities and that viroid diseases usually originate by accidental introduction of viroids from wild plants into susceptible cultivated plants.

This paper indicates that viroids were present in cultivated plants for at least 1.5 to 2 millennia and postulates that viroid diseases occurred ever since new viroid-sensitive plant genotypes such as Etrog Citron (*Citrus medica*) were introduced

to areas cultivated with symptomless viroid carrier plants. Etrog is one of the oldest cultivated citrus and is a host of a wide range of citrus viroids (CVds) (5).

Viroid-infected Etrog plants often bear malformed fruit with typical symptoms (Fig. 1B) during the summer crop (3, 9). A floor mosaic from an ancient synagogue dating to the early 6th century C.E. at Maon in the Northern Negev, Israel (Fig. 1A) shows decorations with a variety of Jewish sanctual relics, including the Menorah (the large candle holder), Shofar (ram horn trumpet) and of Etrog citron fruits (11). The latter were used for the ceremonial rites of the Sucot (Tabernacles) Feast. The Etrog fruits in Fig. 1A (two on each side of the Menorah base) clearly show malformation symptoms similar to those caused by viroid infection (Fig. 1B). Similar symptoms were also present on coins dating to 130 to 133 C.E. (not shown). Other coins and mosaics, such as the one from a 4th century C.E. synagogue at Tiberya (near the Sea of Galilee) (11), depicts a fruit (Fig. 1C, on the left near the Menorah tripod) similar to one from a viroid-free Etrog plant (Fig. 1D). These archeological findings indi-

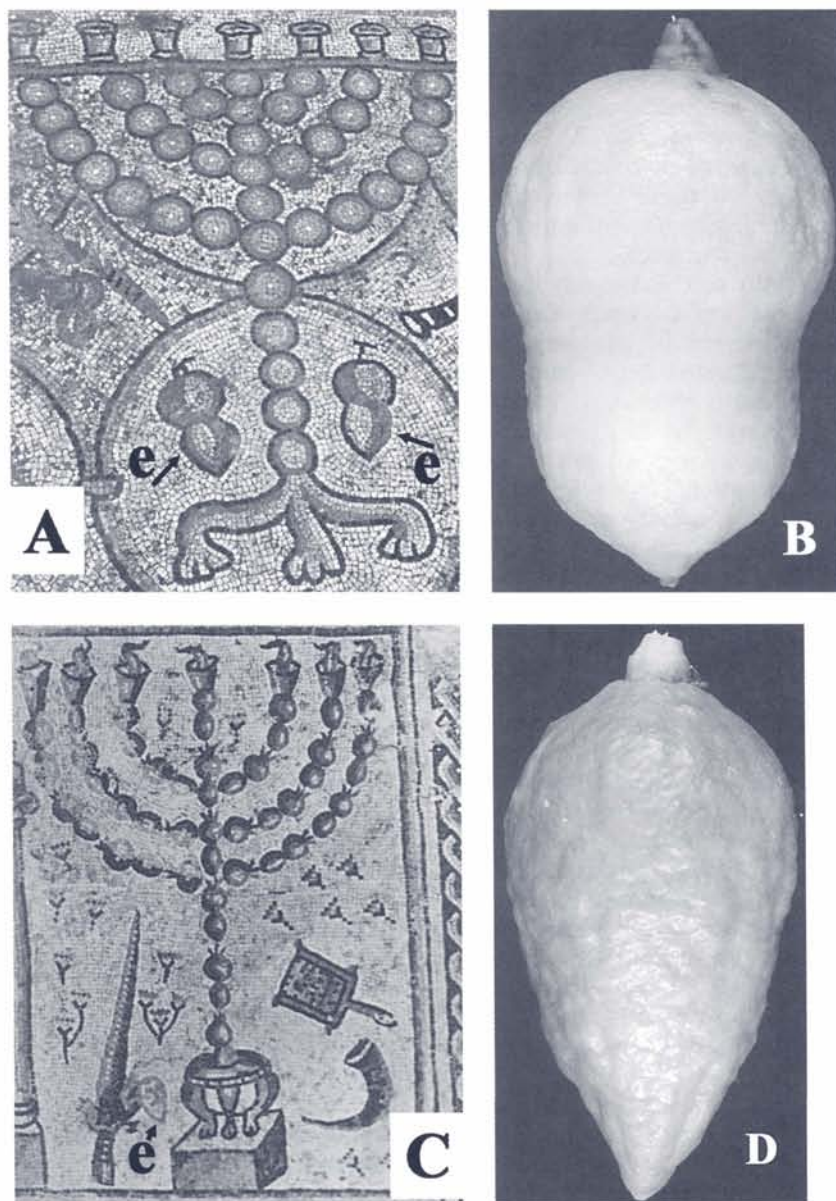


Fig. 1. (A) A fragment from a mosaic recovered from the floor of a 6th century C.E. synagogue at Maon, in the Northern Negev, Israel (11), depicting a variety of sanctual relics including Etrog citron fruits (e) with malformation symptoms. (B) An Etrog citron fruit showing malformation symptoms from a plant inoculated with a combination of citrus viroids. (C) A fragment from the floor of 4th century synagogue from Tiberya (11), depicting an apparently non infected Etrog fruit (e). (D) An Etrog citron fruit from a viroid free Etrog citron plant.

cate, with a reasonable conviction, cases of CVd infections in citrus trees growing in the area 1.5 to 2 millennia ago.

Etrog citron, native to the foothills of the Himalayas in India (10), was the first citrus to reach the Mediterranean basin in 300 B.C., most

probably from Near East Asia (15). Early Etrog introduction was most probably from seeds and none of the known CVds are seed-borne in Etrog (Bar-Joseph, unpublished). It is, therefore, reasonable to assume that the Etrogs became infected by viroids carried in an endemic host plant such as grapevine.

Grapevine is vegetatively propagated mainly by cuttings and was widely cultivated in the area long before the introduction of the Etrog citron and many important varieties have originated in Near-East Asia (16). Grapevines are known to host low concentrations of several CVd-related species, mostly as symptomless carriers (7). Tools and close proximity of the newly introduced Etrog to traditionally cultivated grapevines might have eventually caused the mechanical transmission of some of these viroids to Etrog citron. It is interesting to note that, while viroids have long been associated with vegetatively cultivated fruit trees in the region, generations of horticulturists practicing the "Art of the Plant Breeder" (16) and phytosanitation had eventually eliminated major disease problems caused by viroids. One or a combination of the following four factors probably helped traditional horticulturists to produce acceptable yields of fruit crops in the presence of viroids:

- 1) Selection of varieties and rootstocks tolerant to viroid infection.
- 2) Elimination of severe strains by vegetative propagation from plants with acceptable horticultural performance.
- 3) Recombination between viroid genomes generating new chimeric viroids mostly less pathogenic than either of the parent viroid species (Bar-Joseph, in preparation)
- 4) Interference in symptom expression between different viroids present in some sensitive hosts (12). This phenomena might

have been one of the causes for the omnipresence of several CVd species in many of the old clone citrus varieties.

The continuous preservation of some CVd-sensitive Etrog genotypes in the area may be an exception to the above rules which may have resulted from a variety of reasons including the unusual form of the CVd-infected Etrog fruits (presently named to have a "gartle" which means praying belt in Yiddish) and a greater tolerance to root and vascular diseases (1, 13).

In conclusion, it might be assumed that viroids were associated from a very early period with some major fruits crops in Near-East Asia, but have only caused noticeable disease problems after the transfer of new viroid sensitive genotypes into the area. Thus, the suggestion that viroids have emerged in the last century (4) probably reflects the significant horticultural changes that occurred during the last 150 to 200 years. These included improved access to remote geographical areas and the development of means that enabled the rapid transport of large volumes of vegetatively propagated plant specimens to great distances.

Apparently, new horticultural technology had not only enabled major horticultural improvements, but has also caused the sporadic emergence of "new" viroid and virus disease problems at the meeting places between the introduction of sensitive genotypes with infected tolerant cultivars. Such a situation was experienced several decades ago following the outbreak of citrus tristeza virus (CTV), a man-made disease brought about by the transfer of symptomless CTV carrier varieties from the Far East and South Africa to the South and North America and the Mediterranean basin (2). The stionic combinations of old clone citrus varieties on the sour orange rootstock were CVd-tolerant and

performed well in CTV-free areas. The sour orange sensitivity to CTV had forced horticulturists to experiment with new rootstocks. Rangpur lime was found to be CTV-tolerant; whereas trifoliate orange and its hybrids were resistant to CTV. However, these new rootstocks have eventually displayed severe bark scaling symptoms when grafted with CVd-infected, "old clone" budwood.

It is interesting to note that the "rules of conduct" practiced by farmers in the area since ancient times prohibit the close cultivation of non-related plant species in vineyards and the grafting of non-related plant species (6). It is tempting to assume that these rules represent phytosanitary measures aimed to prevent the outbreak of plant diseases spread by cultivation tools (8), contact (4) and by heterologous graftings (9).

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