EPPO Datasheet: *Apple fruit crinkle viroid*

Last updated: 2021-07-28

IDENTITY

Preferred name: *Apple fruit crinkle viroid*
Taxonomic position: Viruses and viroids: Viroids: Pospiviroidae
Other scientific names: *AFCVd, Apple fruit crinkle apscaviroid*
Common names: apple blister bark
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EU Categorization: Quarantine pest ((EU) 2019/2072 Annex II A)
EPPO Code: AFCVD0



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Notes on taxonomy and nomenclature

AFCVd is an unclassified viroid in the genus *Apscaviroid*. Indeed, based on structural features [a central conserved region (CCR) identical to that of the other members of the genus *Apscaviroid*, with which it also shares the terminal conserved region (TCR)], AFCVd should be classified in the genus *Apscaviroid*. However, its classification at species level is still tentative. The current criteria, established by the International Committee on Taxonomy of Viruses, to create a novel viroid species, are i) less than 90% sequence identity (over the entire genome) between the viroid to be classified and the other viroids and ii) at least one divergent biological feature with respect to the members of the closest viroid species (Di Serio *et al.*, 2020). AFCVd shares the highest sequence identity (89.4%%) with Australian grapevine viroid (AGVd, species *Australian grapevine viroid*, genus *Apscaviroid*), but no evidence has been provided so far that AFCVd diverges from AGVd from a biological point of view, explaining its still partially unresolved taxonomic status.

HOSTS

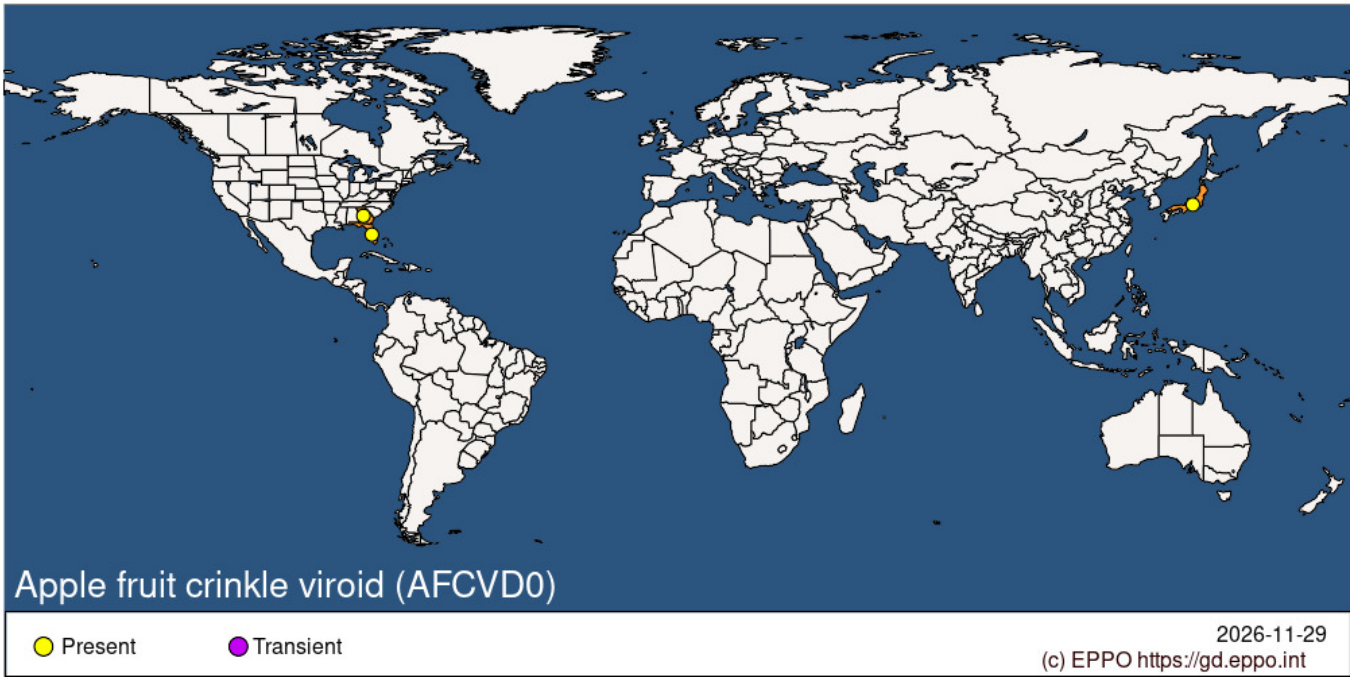
AFCVd was initially identified from apple trees (*Malus domestica*) in Japan (Ito *et al.*, 1993). It was later identified, again in Japan, in natural infections of hop (*Humulus lupulus*) (Sano *et al.*, 2004) and of oriental persimmon (*Diospyros kaki*) (Nakaune and Nakano, 2008). Research involving germplasm material in the USA (Lin *et al.*, 2011) indicates that pear (*Pyrus communis*) is an experimental host and suggests that it is also a natural host. However, it is unclear whether the detection of natural infection in pear, performed by molecular hybridization, was confirmed by another technique. The status of pear as a natural host should therefore be considered as still doubtful and not unambiguously established.

By mechanical inoculation, cucumber (*Cucumis sativus*) and tomato (*Solanum lycopersicum*) have been shown to be experimental hosts of AFCVd (Suzuki *et al.*, 2017).

Host list: *Diospyros kaki*, *Diospyros virginiana*, *Humulus lupulus*, *Malus domestica*

GEOGRAPHICAL DISTRIBUTION

Almost all reports of AFCVd are from Japan. The presence of AFCVd in oriental persimmon in Georgia (United States of America) has been documented by Gregory *et al.* (2018). There also exists a report of the presence of AFCVd in apple in the Xinjiang province of China (Zhao & Niu, 2009). However, the Genbank AFCVd sequence accessions associated with this report do not appear in Genbank, while the detection appears to rely on a single PCR technique. Taken together, the presence of AFCVd in China should be considered as doubtful.



Asia: Japan (Honshu)
North America: United States of America (Florida, Georgia)

BIOLOGY

AFCVd has been transmitted to apple seedlings by grafting, budding or razor-slashing inoculation using purified RNA preparations (Ito *et al.*, 1993). Similar to the other members of the family *Pospiviroidae*, it is assumed that AFCVd replicates in the nucleus of host plants, moves locally through plasmodesmata and systemically invades the plants through their phloem, leading to generalized infection (Flores *et al.*, 2009). As for most viroids, the natural mode of spread from plant to plant, if any, is still unclear. In apple, natural spread to neighbouring trees has not been documented. AFCVd is however efficiently transmitted by vegetative propagation techniques such as budding or grafting (Koganezawa & Ito, 2011). In hop, AFCVd transmission is also possible through cuttings and mechanical injury during cultural operations (Di Serio *et al.*, 2017).

DETECTION AND IDENTIFICATION

Symptoms

In apple, characteristic symptoms on sensitive varieties consist of crinkled and roughened fruits (Ito *et al.*, 1993; Ito & Yoshida, 1998). In severe cases, fruit crinkling is associated with internal fruit flesh browning, pitting and necrosis. Dappling in the form of discoloured, sometimes slightly depressed, spots is also observed on the fruits of red-skinned cultivars (Koganezawa & Ito, 2011). In the most affected varieties, fruits also tend to drop prematurely. In some varieties, fruits may be smaller, in the absence of other obvious symptoms. Blistering of the bark is also observed in some varieties. Infection is however symptomless in some varieties and no leaf symptoms are observed in any variety (Koganezawa & Ito, 2011).

In hop, an association of AFCVd infection with dwarfing of plants and leaf curling has been suggested, together with a reduction in alpha acid content of hop cones (Sano *et al.*, 2004). Whether AFCVd induces symptoms in infected oriental persimmon remains doubtful (Nakaune & Nakano, 2008; Gregory *et al.*, 2018).

Morphology

AFCVd is a small, circular, naked single-stranded RNA molecule of ~370 nucleotides. As for other members of the

family *Pospiviroidae*, AFCVd genomic molecule lacks ribozymes, adopts a rod-shape secondary structure and contains a central conserved region (CCR) and a terminal conserved region (TCR) (Flores *et al.*, 2009).

Detection and inspection methods

In apple orchards, visual inspections should be carried out on fruit-bearing trees. The visual examination of apples after harvest can also allow the detection of symptoms. However, the practicality of the use of visual examination is dependent on the circumstances (e.g. cultivar and environmental conditions). AFCVd can be detected by indexing on fruit-bearing trees of susceptible varieties (Koganezawa & Ito, 2011), but the test can take up to two years to yield results. Detection can more readily be achieved using either molecular hybridization with molecular probes (Lin *et al.* , 2011) or reverse-transcription polymerase chain reaction (RT-PCR) (Gregory *et al.*, 2018).

PATHWAYS FOR MOVEMENT

In the apparent absence of vectors or of other modes of transmission, movement and trade of contaminated propagation materials is seen as the most significant, if not unique, mode of long-distance movement. Vegetative propagation techniques and cultural operations are the mean of short distance dispersal. Apple fruits are not considered a pathway.

PEST SIGNIFICANCE

Economic impact

While the symptoms induced by AFCVd on sensitive apple cultivars and on hop plants can be severe, the geographic distribution at world scale appears to be very limited. Distribution in Japan, the main country where AFCVd is reported, appears also to be quite limited. Overall, the current economic impact of AFCVd appears very limited.

Control

Given the inefficiency of any known natural spread mechanism, the most efficient control strategy appears to be the development and use of AFCVd-free propagation materials (Koganezawa & Ito, 2011). No known control measures are known in the field, besides the destruction of infected plants, which has proven its efficacy in Japan (Koganezawa & Ito, 2011).

Phytosanitary risk

The phytosanitary risk is essentially linked to infected propagation material and seen as relatively limited given the limited geographical distribution of AFCVd and the apparently inefficient (apple, persimmon) or relatively inefficient (hop) spread in the field. EFSA (2019) considered that climatic conditions in the EPPO region would not impair establishment. However, symptom expression and severity may be affected by climatic conditions (e.g. temperature and light) and by the varieties used.

PHYTOSANITARY MEASURES

Appropriate phytosanitary measures to import apple, oriental persimmon or hop plants for planting into the EPPO region could require that these plants are produced in a pest free area, in a pest free place/site of production, or shown to be free from AFCVd by appropriate molecular diagnostic methods. A number of EPPO countries (e.g. EU countries: Annex VI, points 8 & 9 of Regulation 2019/2072 (EU, 2019)) already ban the import of apple plants for planting (other than seeds) from listed countries including Japan. Host plants for planting could also be imported through post-entry quarantine (in the framework of a bilateral agreement).

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Datasheet history

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