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Data Sheets on Quarantine Pests

Citrus leprosis 'rhabdovirus'

IDENTITY

Name: Citrus leprosis 'rhabdovirus'
Taxonomic position: Viruses: Possible unassigned rhabdovirus
Common names: CiLV (acronym)
Léprose des agrumes (French)
Leprose dos citros (Portuguese)
Leprosis, lepra explosiva de los cítricos (Spanish)
Notes on taxonomy and nomenclature: The bulk of available evidence suggests that a virus, probably a rhabdovirus, is the causal agent of citrus leprosis. To avoid semantic confusion, this possibility is accepted in this data sheet.
EPPO computer code: CSLXXX
EU Annex designation: II/A1 - as Leprosis

HOSTS

Citrus spp., especially grapefruits (*C. paradisi*) and oranges (*Citrus sinensis*) are found naturally infected by CiLV. Lemons (*C. limon*) and mandarins (*C. reticulata*) however, are considered much less susceptible. No other plant species is known to serve as a natural host for the agent causing citrus leprosis. Recently, local lesions have been obtained on several herbaceous hosts (*Chenopodium amaranticolor*, *C. quinoa*, *Gomphrena globosa*) when experimentally inoculated by mechanical means.

GEOGRAPHICAL DISTRIBUTION

- **Citrus leprosis rhabdovirus**
Citrus leprosis has been reported only in the American continent.
EPPO region: Absent.
North America: USA (Florida).
South America: Argentina, Brazil, Paraguay, Uruguay, Venezuela (Fawcett, 1936; Bitancourt, 1937, 1955; Vergani, 1945).
EU: Absent.
- **Vectors**
The vectors of leprosis are much more widely distributed. Since this distribution is relevant to the phytosanitary risk presented by CiLV, it is given as follows.
Brevipalpus californicus
EPPO region: Algeria, Cyprus, Egypt, France, Greece (including Crete), Israel, Italy (including Sicily), Libya, Portugal.
Asia: Cyprus, India (Assam, Karnataka, Kerala, Punjab, Tamil Nadu), Israel, Japan (Ryukyu Islands), Malaysia (Peninsular), Nepal, Sri Lanka, Thailand.
Africa: Algeria, Angola, Congo, Egypt, Libya, Mauritania, Mozambique, Senegal, South Africa, Zimbabwe.

North America: Mexico, USA (Arizona, California, Florida, Hawaii, Kansas, Maryland).
Central America and Caribbean: French Guiana.
South America: Brazil (São Paulo).
Oceania: Australia (New South Wales, Northern Territory, Queensland, South Australia, Victoria, Western Australia), Papua New Guinea.
EU: Present.
Distribution map: See CIE (1975, No. 107).

Brevipalpus obovatus

EPPO region: Algeria, Belgium, Bosnia, Bulgaria, Croatia, Cyprus, Egypt, France, Greece, Israel, Italy, Lebanon, Libya, Portugal (including Azores), Romania, Serbia, Spain (including Canary Islands), Sweden, Syria, Turkey, Ukraine.
Asia: China (Anhui, Fujian, Guangdong, Guangxi, Jiangsu, Jianxi, Shandong), Georgia, India (Assam, Delhi, Himachal Pradesh, Kashmir, Punjab, Tripura, Uttar Pradesh, West Bengal), Iran, Israel, Japan (Honshu), Kazakhstan, Lebanon, Nepal, Pakistan, Philippines, Sri Lanka, Syria, Tadzhikistan, Taiwan, Turkey, Yemen.
Africa: Algeria, Angola, Burundi, Cameroon, Central African Republic, Egypt, Kenya, Libya, Malawi, Mauritius, Mozambique, Rwanda, South Africa, Sudan, Tanzania, Uganda, Zaire, Zimbabwe.
North America: Canada (Ontario), Mexico, USA (Alabama, Arizona, California, Florida, Hawaii, Kansas, Louisiana, Maine, Maryland, Mississippi, Missouri, New Jersey, North Carolina, Ohio, Texas).
Central America and Caribbean: Puerto Rico.
South America: Argentina, Brazil (Bahía, Minas Gerais, São Paulo), Uruguay, Venezuela.
Oceania: Australia (Queensland, South Australia), Fiji, New Zealand, Vanuatu.
EU: Present.
Distribution map: See CIE (1988, No. 128).

Brevipalpus phoenicis

EPPO region: Austria (under glass only), Cyprus, Egypt, Italy (including Sicily), Netherlands (under glass only), Poland (under glass only), Portugal, Spain, Tunisia, Turkey.
Asia: Cambodia, Cyprus, India (northeast; Assam, Maharashtra, West Bengal), Indonesia (Java), Japan (Ryukyu Islands), Lao, Malaysia (peninsular), Pakistan, Philippines, Sri Lanka, Taiwan, Turkey, Viet Nam, Yemen.
Africa: Angola, Burundi, Cameroon, Egypt, Kenya, Malawi, Mauritania, Mauritius, Mozambique, Réunion, Rwanda, South Africa, St. Helena, Sudan, Tanzania, Tunisia, Uganda, Zaire, Zimbabwe.
North America: Mexico, USA (California, District of Columbia (under glass only), Florida, Hawaii).
Central America and Caribbean: Cuba, Jamaica, Puerto Rico, Trinidad and Tobago.
South America: Argentina, Brazil (São Paulo), Guyana, Paraguay, Venezuela.
Oceania: Australia (Queensland, Western Australia), Fiji, Norfolk Island, Solomon Islands.
EU: Present.
Distribution map: See CIE (1970, No. 106).

BIOLOGY

Citrus leprosis is always associated with infestation by a false spider mite of the genus *Brevipalpus* (Tenuipalpidae). Knorr (1950) reported that *B. inornatus* is associated with citrus leprosis in Florida; later he also demonstrated that *B. obovatus* collected from *Bidens pilosa* was able to induce citrus leprosis (Knorr, 1968). The same mite species was found

associated with citrus leprosis in Argentina (Knorr & Ducharme, 1951). Garnsey *et al.* (1990) cite *B. californicus* as a vector in Florida. In Brazil, Rossetti *et al.* (1959) showed that *B. phoenicis* transmitted the disease under experimental conditions and that natural infestation of orchards by this mite was associated with the incidence of citrus leprosis. Larvae are more efficient vectors than adults and nymphs (Musumeci & Rossetti, 1963; Chagas *et al.*, 1984).

The disease is characterized by lesions in leaves, twigs and fruits which do not become systemic. Its etiology has been controversial, since the agent has been considered to be either a toxin produced by the mite, or a virus with localized infection transmitted by the mite. Several pieces of experimental evidence support viral etiology: (a) only mites which have access to lesions cause leprosis (Rossetti *et al.*, 1959); (b) tip grafting of infected shoots results in spread from the graft to the receptor tissue (Knorr, 1968; Chagas & Rossetti, 1980); (c) lesions can be reproduced experimentally by mechanical transmission from citrus to citrus and from citrus to several herbaceous plants (Colariccio *et al.*, 1995); (d) unenveloped or enveloped rhabdovirus-like particles have been consistently found in cells from citrus leprosis lesions of citrus fruit, leaf or stem (Kitajima *et al.*, 1972, 1974; Colariccio *et al.*, 1995). The same type of particle was found in both inoculum tissue and in the lesions produced by mechanical transmission (Colariccio *et al.*, 1995). Indirect evidence comes from the fact that the mite vectors occur in many parts of the world where leprosis has never been recorded.

DETECTION AND IDENTIFICATION

Symptoms

Round to elliptical local lesions are seen on fruits, leaves and twigs, the severity of which varies with the type of citrus and the region of origin. Leaf symptoms are usually roundish with a dark-brown central spot about 2-3 mm in diameter, surrounded by a chlorotic halo, in which 1 to 3 brownish rings frequently appear surrounding the central spot; the overall lesion size varies from 10 to 20 mm, though larger lesions may form by the fusion of 2 or more adjacent lesions. On fruits, lesions are necrotic spots 10-20 mm wide, with a necrotic centre. Gum exudation is occasionally observed on the lesion. On green fruits, the lesions are initially yellowish, becoming more brown- or blackish, sometimes depressed, and reducing the market value of the fruits. On stems, lesions are protuberant, cortical, grey or brownish or sometimes dark-reddish. Lesions may coalesce when present in large numbers, leading to the death of the twig. In extreme cases, as in "lepra explosiva" in Argentina, severe defoliation and fruit fall are observed (Frezzi, 1940; Bitancourt, 1955; Rossetti *et al.*, 1969).

Citrus leprosis lesions are usually very characteristic, but may sometimes be mistaken for lesions of citrus canker, caused by the bacterium *Xanthomonas axonopodis* pv. *citri* (EPPO/CABI, 1996) or zonate chlorosis (Rossetti, 1980). Zonate chlorosis, which is associated with infestation by the same mites, does not become necrotic; symptoms are essentially concentric green and chlorotic rings (Bitancourt, 1934).

Morphology

Presumed virus particles occur mostly in parenchyma cells of the lesion in affected orange leaves, fruits or stems. Particles are short, bacilliform, 120-130 nm long (occasionally up to 300 nm) and 50-55 nm wide. They occur within the lumen of the endoplasmic reticulum (Kitajima *et al.*, 1974; Colariccio *et al.*, 1995). There is a report of similar but unenveloped particles in the nucleoplasm (Kitajima *et al.*, 1972).

Besides the presence of the rhabdovirus-like particles within endoplasmic reticulum, in tissues from the lesion, dense viroplasm-like material is commonly found in the cytoplasm, near the particles. Small vesicle-containing fibrillar materials are frequently present in the

vacuole, associated with the tonoplast, next to the dense material (Kitajima *et al.*, 1972; Colariccio *et al.*, 1995). Chloroplasts are usually affected with disorganized hypertrophied lamellar system (Kitajima *et al.*, 1972). There is a report in which rod-like particles, considered as naked rhabdovirus, accumulate in the nucleoplasm, associated with the nuclear envelope (Kitajima *et al.*, 1972).

Detection and inspection methods

Citrus leprosis is principally detected by the observation of local lesions with characteristic symptoms. It can be mechanically transmitted in extract from infected tissue, frozen in liquid nitrogen, with tris or phosphate buffer containing several reducing agents, to give lesions on orange and some herbaceous hosts (*Chenopodium amaranticolor*, *C. quinoa* and *Gomphrena globosa*). Electron microscopy of lesion tissues reveals virus-like particles in parenchyma cells (Colariccio *et al.*, 1995). However, these methods have more significance in relation to research on the etiology of leprosis than as practical means of detection. No serological tests or tests on woody indicators have been described.

MEANS OF MOVEMENT AND DISPERSAL

CiLV apparently only infects plants locally, each lesion being associated with infestation by a vector mite. The virus does not apparently move systemically in the host plant, or at most short distances from a grafted shoot tip to the adjacent scion tissue. Accordingly, movement in latently infected planting material (as very commonly occurs for most plant viruses) is not likely to be a major pathway for CiLV. In practice, the main means of movement and dispersal is with the vector mites of the genus *Brevipalpus*. These colonize most *Citrus* spp. and many other plant species; according to Oliveira (1986), *Brevipalpus* mites have been found infesting more than 200 different plant species. Chiavegato & Kharfan (1993) report that fruits with scab lesions (*Elsinoë fawcettii*) were preferred for colonization by mites.

In international trade, CiLV is unlikely, as mentioned above, to be carried latently on citrus budwood. Normal nursery management procedures should ensure that budwood material showing symptoms does not enter trade. CiLV is possibly more likely to be spread on rooted plants, since these are more likely to carry vector mites and may be harder to inspect for symptoms. Infected plants would be most likely to come from nurseries which have not been treated against mites. Since little is known about alternative hosts for the virus, some of which may be asymptomatic carriers, there may be some risk of introducing citrus leprosis via other plant species. However, this is considered rather unlikely. Other plants could possibly also carry viruliferous mites, since the mites concerned are polyphagous and could move from citrus to other hosts.

Citrus fruits are selected and processed in packing houses before export and this eliminates the mites. Thus, it is not likely that CiLV would be introduced with fruits.

PEST SIGNIFICANCE

Economic impact

If proper mite control is not undertaken when the disease appears, severe losses in yield may occur, both in quantity and quality. Fruits with lesions have low commercial value, especially for direct consumption. In severe cases, twigs may die, jeopardizing succeeding production. Furthermore, untreated orchards may serve as a source for the mite and citrus leprosis may spread to other plantations in the area. Usually citrus leprosis occurs in cycles: when citrus prices are high, growers do control the mites but when prices fall, chemical treatments for mites and other pests and pathogens are applied to a lesser extent and

incidence of citrus leprosis increases. The disease is reported to be particulary important in Brazil and Argentina. It has no current importance in the USA.

Control

Citrus leprosis is basically controlled by control of the mites. Most of the currently available chemicals used to control mites are effective. Some of the products presently recommended in Brazil are azocyclotin, cyhexatin, hexythiazox, fenbutatin oxide, propargite and quinomethionate (C. A. Oliveira, pers. comm.).

Phytosanitary risk

CiLV has not been considered to be a quarantine pest by any regional plant protection organization, but is under evaluation for the A1 list of EPPO. Doubts about the etiology of leprosis have probably contributed to this situation. In fact, feeding by the vector mites alone does cause certain symptoms, and this has probably complicated the recognition that, in some countries, a virus is also present and causes distinct symptoms.

The vector mites are present in some EPPO countries but appear to be of no practical importance as pests of citrus. They are pests which are apparently more favoured by a warm humid climate. To a certain extent, control of more important mites (*Panonychus citri*) may also be eliminating them. However, it is clear that citrus leprosis, because of its non-systemic infection, can only be important where attacks by its vector mites are significant. For this reason, the risk to citriculture in the EPPO region from CiLV seems rather low.

PHYTOSANITARY MEASURES

If it is judged useful to take measures against CiLV, then it should be sufficient to require that any imported plants for planting of citrus should be free from leprosis lesions and come from nurseries found free from, and/or treated against, *Brevipalpus* mites during the growing season. The risk from normal commercial consignments of fruits seems insignificant. In practice, citrus from countries where CiLV occurs is already subject to much stricter requirements on account of more serious pests.

BIBLIOGRAPHY

Bitancourt, A.A. (1934) [Zonate chlorosis. A new citrus disease]. *Arquivos do Instituto Biológico de São Paulo* **5**, 245-250.

Bitancourt, A.A. (1937) [Leprosis and the next orange harvest]. *O Biológico* **3**, 37-40.

Bitancourt, A.A. (1940) [Citrus leprosis]. *O Biológico* **6**, 39-45.

Bitancourt, A.A. (1955) [Studies on citrus leprosis]. *Arquivos do Instituto Biológico de São Paulo* **22**, 161-231.

Chagas, C.M.; Rossetti, V. (1980) [Experimental transmission of citrus leprosis by leaf implant into the stem]. *Fitopatologia Brasileira* **6**, 211-214.

Chagas, C.M.; Rossetti, V.; Chiavegato, L.G. (1984) Effectiveness of the different life cycle stages of *Brevipalpus phoenicis* Geijskes in leprosis transmission. In: *Proceedings of the 9th Conference of the International Organization of Citrus Virologists* (Ed. by Garnsey, S.M.; Timmer, L.W.; Dodds, J.A.), pp. 211-214. University of California Press, Riverside, USA.

Chiavegato, L.G.; Kharfan, P.R. (1993). [Behaviour of the mite *Brevipalpus phoenicis* in citrus]. *Anais da Sociedade Entomológica do Brasil* **22**, 355-359.

CIE (1970) *Distribution Maps of Pests, Series A* No. 106. CAB International, Wallingford, UK.

CIE (1975) *Distribution Maps of Pests, Series A* No. 107. CAB International, Wallingford, UK.

CIE (1988) *Distribution Maps of Pests, Series A* No. 128 (revised). CAB International, Wallingford, UK.

Colariccio, A.; Lovisolo, O.; Chagas, C.M.; Galleti, S.R.; Rossetti, V.; Kitajima, E.W. (1995) Mechanical transmission and ultrastructural aspects of citrus leprosis disease. *Fitopatologia Brasileira* **20** (in press).

EPPO/CABI (1996) *Xanthomonas axonopodis* pv. *citri*. In: *Quarantine pests for Europe*. 2nd edition (Ed. by Smith, I.M.; McNamara, D.G.; Scott, P.R.; Holderness, M.). CAB INTERNATIONAL, Wallingford, UK. (in press).

Fawcett, H.S. (1936) *Citrus diseases and their control* (2nd edition). McGraw Hill Book Co. Inc., New York, USA.

Frezzi, M.J. (1940) ["Lepra explosiva" of orange. Studies in the pathology laboratory of Bella Vista (Corrientes)]. *Boletin Frutas y Hortalizas* **5**, 1-15.

Garnsey, S.M.; Chagas, C.M.; Chiavegato, L.G. (1990) Leprosis and zonate chlorosis. In: *Compendium of citrus diseases*. (Ed. by Whiteside, J.O.; Garnsey, S.M.; Timmer, L.W.), pp. 43-44. American Phytopathological Society, St. Paul, USA.

Kitajima, E.W.; Müller, G.W.; Costa, A.S.; Yuki, V. (1972) Short rod-like particles associated with citrus leprosis. *Virology* **50**, 254-258.

Kitajima, E.W.; Rosillo, M.A.; Portillo, M.M.; Müller, G.W.; Costa, A.S. (1974) [Electron microscopy of leaf tissues of orange trees infected by "lepra explosiva" in Argentina]. *Fitopatologia (Lima)* **9**, 55-56.

Knorr, L.C. (1950) Etiological association of a *Brevipalpus* mite with Florida scaly bark of citrus. *Phytopathology* **40**, 15.

Knorr, L.C. (1968) Studies on the etiology of leprosis in citrus. In: *Proceedings of the 4th Conference of the International Organization of Citrus Virologists* (Ed. by Childs, J.F.L.), pp. 332-340. University of Florida Press, Gainesville, USA.

Knorr, L.C.; Ducharme, E.P. (1951) [Notes on "lepra explosiva"]. *Idia* **42**, 32-38.

Müller, G.W.; Costa, A.S. (1993) *Doencças causadas por virus, viroídes e similares em citrus*, pp. 66-68. Fundação Cargill, Campinas, Brazil.

Musumeci, M.R.; Rossetti, V. (1963) [Transmission of the symptoms of citrus leprosis by the mite *Brevipalpus phoenicis*]. *Ciência e Cultura* **15**, 228.

Oliveira, C.A. (1986) [Population fluctuation and measures for control of the leprosis mite *Brevipalpus phoenicis* on citrus]. *Revista Técnica e Científica da Citricultura* (Novembro) 1-31.

Rossetti, V. (1980) [Differentiation between citrus canker and other diseases]. *Citrus* **1**, 23-26.

Rossetti, V.; Fassa, T.G.; Musumecci, R.M. (1959) [A new mite in the orange groves of São Paulo]. *O Biológico* **25**, 273-275.

Rossetti, V.; Lasca, C.; Negretti, S. (1969) New developments regarding leprosis and zonate chlorosis in citrus. In: *Proceedings of the International Citrus Symposium* Vol. 3 (Ed. by Chapman, H.D.), pp. 1453-1456. University of California Press, Riverside, USA.

Vergani, A.R. (1945) [Transmission and nature of "lepra explosiva" on orange]. *Instituto de Sanidad Vegetal, Ministerio de Agricultura de la Nación, Série A* **5**, 3-11.