Prepared by CABI and EPPO for the EU
under Contract 90/399003

Data Sheets on Quarantine Pests

Citrus greening bacterium

IDENTITY

Name: Citrus greening bacterium
Taxonomic position: Bacteria: Gracilicutes
Common names: Greening (Africa), leaf mottling (Philippines), decline (India), vein phloem degeneration (Indonesia) (English)
Huang long bin (yellow shoot), likubin (decline) (Chinese)
Greening (French)
Enverdecimiento (Spanish)
Notes on taxonomy and nomenclature: A fastidious, phloem-limited Gram-negative bacterium, which has not been cultured. Two forms occur: a heat-tolerant (Asian) form and a heat-sensitive (African) form. Jagoueix *et al.* (1994) have recently proposed the genus *Liberobacter* for the greening pathogen, considered to be a member of the alpha subdivision of the Proteobacteria on the basis of PCR studies. The names *L. asiaticum* and *L. africanum* have been proposed for the two forms, which can be distinguished as separate species on the basis of sequence homology (Planet *et al.*, 1995). However, the names have yet to be accepted as valid.
Bayer computer code: CSGXXX
EPPO A1 list: No. 151
EU Annex designation: II/A1

HOSTS

Citrus greening bacterium infects citrus generally. The bacterium may persist and multiply in most *Citrus* spp. but most severe symptoms are found on oranges (*C. sinensis*), mandarins (*C. reticulata*) and tangelos (*C. reticulata* x *C. paradisi*). Somewhat less severe symptoms are found on lemons (*C. limon*), grapefruits (*C. paradisi*), *C. limonia*, *C. limettioides*, rough lemons (*C. jambhiri*), kumquats (*Fortunella* spp.) and citrons (*C. medica*) (McClean & Schwarz, 1970). Symptoms are even weaker on limes (*C. aurantiifolia*) and pummelos (*C. grandis*). Though other Rutaceae have been infected artificially, there are not apparently any records of their natural infection.

Citrus greening bacterium has been experimentally transmitted, by *Cuscuta campestris*, from citrus to one non-rutaceous host *Catharanthus roseus* (Garnier & Bové, 1983).

GEOGRAPHICAL DISTRIBUTION

The distribution is given separately for the two forms of the disease.

- **Heat-sensitive form ("*Liberobacter africanum*")**

EPPO region: Absent.
Asia: Saudi Arabia, Yemen.

Africa: Burundi, Cameroon, Central African Republic, Comoros, Ethiopia, Kenya, Madagascar, Malawi, Mauritius, Réunion, Rwanda, Somalia, South Africa, St. Helena (unconfirmed), Swaziland, Tanzania, Zimbabwe. Recent records are from Aubert *et al.* (1988). Surveys have failed to find the disease in Gabon, Namibia and Zambia.
EU: Absent.

- **Heat-tolerant form ("*Liberobacter asiaticum*")**

EPPO region: Absent.
Asia: Bangladesh, China (Guangdong, Guangxi), Hong Kong, India (Arunachal Pradesh, Assam, Haryana, Maharashtra, Manipur, Meghalaya, Punjab, Sikkim, West Bengal), Indonesia (Java, Sumatra), Japan (Ryukyu Islands), Malaysia (Peninsular), Nepal, Pakistan, Philippines, Saudi Arabia, Taiwan, Thailand, Viet Nam.
Africa: Mauritius, Réunion.
EU: Absent.
Note that both forms exist in Mauritius, Réunion and Saudi Arabia. The Mediterranean area and most of the Middle East (e.g. Iran) are still free from the disease. The infested area which is closest to the Mediterranean zone extends south of Mecca, along the Red Sea.
Distribution map: See Commonwealth Department of Health (1982).

BIOLOGY

Graca (1991) has recently provided a comprehensive review of the disease. Transmission of the greening agent by graft inoculation was first reported in China (Lin, 1956). The agent was initially thought to be a phytoplasma, but later work showed it to be a bacterium with a peptidoglycan-containing membranous cell wall of the Gram-negative type (Moll & Martin, 1973; Garnier *et al*., 1984). The bacterial nature of the greening organism explains why penicillin treatment of infected plants results in symptom remission (Aubert & Bové, 1980; Bové *et al*., 1980). A report claiming culture of citrus greening bacterium has appeared (Garnett, 1985). There is, however, as yet, no experimental evidence to show that the cultured organism is really the greening bacterium.

Two forms of greening disease are known (Bové *et al*., 1974). One, in the southern part of Africa, is heat-sensitive, as symptoms do not develop in hot climates where temperatures above 30°C are reached several hours a day. The other form is heat-tolerant and withstands high temperatures, and is predominantly Asian in distribution. The Asian heat-tolerant form of the disease has been discovered in Saudi Arabia, and the African heat-sensitive form in Yemen. When the African and the Asian forms of the greening bacterium were transmitted from citrus to *Catharanthus roseus* by *Cuscuta campestris* (Garnier & Bové, 1983), the African form remained heat-sensitive and the Asian form heat-tolerant in both *C. roseus* and citrus.

Monoclonal antibodies to citrus greening bacterium and more specifically to an isolate from India, have been obtained (Garnier *et al.*, 1987). Using immunofluorescence on sections, the monoclonal antibodies reacted not only with the homologous (Indian) isolate of citrus greening bacterium, but also with isolates from the Philippines, Réunion and elsewhere in Africa. There are therefore serological relationships between African and Asian forms of citrus greening bacterium, but it is possible that further work will discover strain differences. Under natural conditions greening is transmitted in Africa and Yemen by *Trioza erytreae* (McClean & Oberholzer, 1965; EPPO/CABI, 1996b) and in Asia (including Saudi Arabia) by *Diaphorina citri* (Capoor *et al*., 1967; EPPO/CABI, 1996a). However, it was shown experimentally that *T. erytreae* can transmit the Indian form of greening (Massonie *et al*., 1976) and that *D. citri* is able to transmit the African form (Lallemand *et al*., 1986).

DETECTION AND IDENTIFICATION

Symptoms

The general aspect of affected trees is open growth, stunting, twig dieback, sparse yellow foliage, severe fruit drop; on certain trees and in certain countries (China), symptoms are seen initially on one limb of an affected tree (yellow branch aspect); severe decline is mainly seen with Asian greening.

On fruits

Some fruits are under-developed, lopsided, and poorly coloured. When pressure is exerted with a finger, a greyish-white waxy mark appears sometimes on the rind surface. Seeds are often aborted. The greening symptom, mainly occurring in Africa, is seen on fruits which mature only on the side exposed to the sun, the unexposed side remaining dull olive-green (Commonwealth Department of Health, l982).

On leaves

Mottling and zinc deficiency-like symptoms are the most common and characteristic. Mature leaves often show irregular patches between the main veins. The veins are often prominent and yellow.

On trunk, limbs and shoots

No symptoms apparent.

Histological symptoms

Localized zones of necrotic phloem are scattered through the vascular system of the leaf. Massive accumulation of starch in the plastids is seen together with aberrations in cambial activity and excessive phloem formation.

Morphology

Elongated sinuous rod-like structures, 0.15-0.25 µm in diameter and µm long. These can be seen by electron microscopy in the sieve tubes of infected trees. Similar structures have been seen in both vectors.

Detection and inspection methods

Suspect material may be grafted onto sensitive indicator plants. Preferred indicator plants are Orlando tangelo and sweet orange seedlings. Inoculation should preferably be with pieces of mottled leaves. Because of the variable results in graft transmission, at least ten seedlings should be used for each tree to be indexed. After inoculation, the indicator seedling should be kept at 24°C (South African form) or 32°C (Asian form). Symptoms usually show after 4-5 months. The presence of a specific fluorescent marker, gentisoyl glucoside (Feldman & Hanks, 1969), in greening-infected tissue has been used for detection (Schwarz, 1968a; 1968b). The reliability of this method was further confirmed by Hooker *et al*. (1993). Suspect trees may be analysed by electron microscopy to confirm the presence of the characteristic bacteria in the sieve tubes. Serological identification using monoclonal antibodies against the Indian form of citrus greening bacterium has been successfully used to detect the bacterium in greenhouse-grown citrus and *Catharanthus roseus* by immunofluorescence and ELISA (Garnier *et al*., 1987). DNA probes have recently been obtained which detect the pathogen and differentiate between its two forms (Villechanoux *et al*., 1992).

MEANS OF MOVEMENT AND DISPERSAL

Citrus greening bacterium can be moved by its vectors or in citrus plants for planting. Seed transmission does not occur. Since the two known psyllid vectors of the disease are not present in the Mediterranean area, parts of the Middle East and America, the disease, if introduced into these areas, should in theory only spread by propagation of infected plant material. However, it is conceivable that hitherto unrecognized vectors of greening exist.

PEST SIGNIFICANCE

Economic impact

Citrus greening, transmitted by *D. citri* and *T. erytreae*, is an extremely severe disease. In South Africa, in 1965, fruit losses from the disease were 30-100% in individual orchards; many of these had subsequently to be abandoned or removed. Earlier outbreaks occurred in l932-l936 and 1939-1946. Annual losses in 1991 are estimated at ZAR 35 million. In Réunion, large areas of citrus cultivation had to be abandoned (Catling, 1973), and also in Thailand (Schwarz & Knorr, 1973). In the Philippines, mandarin production fell from 11700 t in 1960 to 100 t in 1968. In south-western Saudi Arabia, sweet orange and mandarin practically disappeared during the 1970s. In Asia, an FAO-UNDP programme has recently been established to try to control the disease.

Control

In the Transvaal (South Africa), citrus is treated against greening by injection of tetracycline (up to 20 g per adult tree) with high-capacity compressors working at 10 kg/cm^2. Such methods have been tried, but not widely used, in Asia. Treatment with rolitetracycline also reduces symptom expression. However, the main emphasis of control in South Africa is based on the use of healthy nursery trees and effective systemic insecticides (against the vector *T. erytreae*) as trunk treatments (Buitendag, 1991). Santokh Singh *et al.* (1994) have obtained promising results using cross protection with mild strains of greening, but this approach has not been used in practice.

Phytosanitary risk

Citrus greening bacterium is listed as an A1 quarantine pest by EPPO (OEPP/EPPO, 1988) and is also of quarantine significance for COSAVE, CPPC and IAPSC. In the citrus-growing areas of the EPPO region, it could only present a problem if introduced with one or other of its vectors. There is no suggestion that native Mediterranean vectors could exist. In view of its severity it is essential to keep the disease and its vectors out of the Mediterranean zone and to prevent their spread in the Middle East. *D. citri* and *T. erytreae* are also considered as A1 quarantine pests by EPPO (OEPP/EPPO, 1988; EPPO/CABI, 1996a, b), in their own right as well as because of their vector status.

PHYTOSANITARY MEASURES

EPPO recommends (OEPP/EPPO, 1990) that importation of plants for planting and cut branches of citrus from countries where citrus greening bacterium or either of its vectors occur should be prohibited. Healthy budwood can be obtained from material under quarantine by shoot-tip grafting, or alternatively by heat treatment (e.g. water-saturated hot air at 49°C for 50 min) of budwood in tetracycline solution (1000 p.p.m.), which results in a high percentage of greening-free buds. Various other heat-treatment regimes are also possible. Such greening-free material should be kept and propagated under insect-proof screenhouse conditions, and its health status checked by grafting onto sweet orange.

BIBLIOGRAPHY

Aubert, B.; Bové, J.M. (1980) Effect of penicillin or tetracycline injections of citrus trees affected by greening disease under field conditions in Réunion Island. In: *Proceedings of the 8th Conference of the International Organization of Citrus Virologists* (Ed. by Calavan, E.C.; Garnsey, S.M.; Timmer, L.W.), pp. 103-108. University of California, Riverside, USA.

Aubert, B.; Garnier, M.; Cassim, J.C.; Bertin, Y. (1988) Citrus greening disease in east and west African countries south of the Sahara. In: *Proceedings of the 10th Conference of the International Organization of Citrus Virologists*, pp. 226-230.

Bové, J.M.; Calavan, E.D.; Capoor, S.P.; Cortez, R.E.; Schwarz, R.E. (1974) Influence of temperature on symptom of Californian stubborn, South African greening, Indian citrus decline and Philippines leaf mottling disease. In: *Proceedings of the 6th Conference of the International Organization of Citrus Virologists* (Ed. by Weathers, L.G.; Cohen, M.), pp. 12-15. University of California, Berkeley, USA.

Bové, J.M.; Bonnet, P.; Garnier, M.; Aubert, B. (1980) Penicillin and tetracycline treatments of greening disease affected citrus plants in the glasshouse and the bacterial nature of the prokaryote associated with greening. In: *Proceedings of the 8th Conference of the International Organization of Citrus Virologists* (Ed. by Calavan, E.C.; Garnsey, S.M.; Timmer, L.W.), pp. 91-97. University of California, Riverside, USA.

Buitendag, C.H. (1991) The current status and the control of greening disease of citrus in the Republic of South Africa. *Citrus Journal* **1**, 35-40.

Capoor, S.P.; Rao, D.B.; Viswanath, S.M. (1967) *Diaphorina citri*, a vector of the greening disease of citrus in India. *Indian Journal of Agricultural Science* **37**, 572-576.

Catling, H.D. (1973) Notes on the biology of the South African citrus psylla *Trioza erytreae*. *Journal of the Entomological Society of South Africa* **36**, 299-306.

Commonwealth Department of Health (1982) Citrus dieback and greening. *Plant Quarantine Leaflet* No. 26. Australian Government Publishing Service, Canberra, Australia.

EPPO/CABI (1996a) *Diaphorina 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.

EPPO/CABI (1996b) *Trioza erytreae*. In: *Quarantine pests for Europe*. 2nd edition (Ed. by Smith, I.M.; McNamara, D.G.; Scott, P.R.; Holderness, M.). CAB INTERNATIONAL, Wallingford, UK.

Feldman, A.W.; Hanks, R.W. (1969) The occurrence of a gentisic glucoside in the bark and albedo of virus-infected citrus trees. *Phytopathology* **59**, 603-606.

Garnett, H.M. (1985) Isolation of the greening organism. *Citrus and Subtropical Fruit Journal* No. 611, pp. 4-6.

Garnier, M.; Bové, J.M. (1983) Transmission of the organism associated with citrus greening disease from sweet orange to periwinkle by dodder. *Phytopathology* **73**, 1358-1363.

Garnier, M.; Danel, N.; Bové, J.M. (1984) Etiology of citrus greening disease. *Annales de Microbiologie (Institut Pasteur)* **153A**, 169-179.

Garnier, M.; Martin-Gros, G.; Bové, J.M. (1987) Monoclonal antibodies against the bacterial-like organism associated with citrus greening disease. *Annales de Microbiologie (Institut Pasteur)* **138**, 639-650.

Graca, J.V. da (1991) Citrus greening disease. *Annual Review of Phytopathology* **29**, 109-136.

Hooker, M.E.; Lee, R.F.; Civerolo, E.L.; Wang, S.Y. (1993) Reliability of gentisic acid, a fluorescent marker, for diagnosis of citrus greening disease. *Plant Disease* **77**, 174-180.

Jagoueix, S.; Bové, J.M.; Garnier, M. (1994) The phloem-limited bacterium of greening disease of citrus is a member of the alpha subdivision of the Proteobacteria. *International Journal of Systematic Bacteriology* **44**, 379-386.

Lallemand, J.; Fos, A.; Bové, J.M. (1986) Transmission de la bactérie associée à la forme africaine de la maladie du 'greening' par le psylle asiatique *Diaphorina citri*. *Fruits* **41**, 341-343.

Lin, K.H. (1956) Observations on yellow shoot on citrus. Etiological studies of yellow shoot on citrus. *Acta Phytopathologica Sinica* **2**, 1-42.

Massonie, G.; Garnier, M.; Bové, J.M. (1976) Transmission of Indian citrus decline by *Trioza erytreae*, the vector of South African greening. In: *Proceedings of the 7th Conference of the*

International Organization of Citrus Virologists (Ed. by Calavan, E.C.), pp. 18-20. University of California, Riverside, USA.

McClean, A.P.D.; Oberholzer, P.C.J. (1965) Citrus psylla, a vector of the greening disease of sweet orange. *South African Journal of Agricultural Science* **8**, 297-298.

McClean, A.P.D.; Schwarz, R.E. (1970) Greening or blotchy-mottle disease of citrus. *Phytophylactica* **2**, 177-194.

Moll, J.N.; Martin, M.N. (1973) Electron microscope evidence that citrus psylla (*Trioza erytreae*) is a vector of citrus greening in South Africa. *Phytophylactica* **5**, 41-44.

OEPP/EPPO (1988) Data sheets on quarantine organisms No. 151, Citrus greening bacterium and its vectors *Diaphorina citri* & *Trioza erytreae*. *Bulletin OEPP/EPPO Bulletin* **18**, 497-507.

OEPP/EPPO (1990) Specific quarantine requirements. *EPPO Technical Documents* No. 1008.

Planet, P.; Jagoueix, S.; Bové, J.M.; Garnier, M. (1995) Detection and characterization of the African citrus greening liberobacter by amplification, cloning, and sequencing of the rplKAJL-rpoBC operon. *Current Microbiology* **30**, 137-141.

Santokh Singh; Kapur, S.P.; Cheema, S.S. (1994) Management of greening disease through cross-protection. *Indian Journal of Virology* **10**, 113-121.

Schwarz, R.E. (1968a) Indexing of greening and exocortis through fluorescent marker substances. In: *Proceedings of the 5th Conference of the International Organization of Citrus Virologists* (Ed. by Childs, J.F.L.), pp. 118-124. University of Florida Press, Gainesville, USA.

Schwarz, R.E. (1968b) Thin layer chromatographical studies on phenolic markers of the greening virus in various citrus species. *South African Journal of Agricultural Science* **11**, 797-801.

Schwarz, R.E.; Knorr, L.C. (1973) Presence of citrus greening and its psylla vector in Thailand. *FAO Plant Protection Bulletin* **21**, 132-138.

Villechanoux, S.; Garnier, M.; Renaudin, J.; Bové, M. (1992) Detection of several strains of the bacterium-like organism of citrus greening disease by DNA probes. *Current Microbiology* **24**, 89-95.