Data Sheets on Quarantine Pests

"Xanthomonas axonopodis pv. citrumelo"

IDENTITY

Name: "Xanthomonas axonopodis pv. citrumelo" (Vauterin et al. 1995) Synonyms: "Xanthomonas campestris pv. citrumelo" Gabriel et al.

Xanthomonas campestris pv. citri strain E

Taxonomic position: Bacteria: Gracilicutes

Common names: Citrus bacterial spot, citrus canker (nursery strain), canker E (English) Notes on taxonomy and nomenclature: Certain strains of xanthomonads on citrus in Florida (USA) have been known since 1984 as E strains or nursery strains of *X. campestris* pv. *citri*. Gabriel *et al.* (1989) reclassified them as *X. campestris* pv. *citrumelo*. However, objections have been made to this (Vauterin *et al.*, 1990; Young *et al.*, 1991) as the reclassification is based mainly on differences in restriction fragment length polymorphism (RFLP), which cannot readily be reproduced in other laboratories. Evidence is nevertheless accumulating (Brlansky *et al.*, 1990; Stall & Civerolo, 1991) that these strains are genuinely distinct from what is now *X. axonopodis* pv. *citri* (EPPO/CABI, 1996). More recently, Vauterin *et al.* (1995), on the additional basis of data on DNA-DNA hybridization and the use of BIOLOG microplates, have proposed new species delineations within the genus *Xanthomonas*. The new name *X. axonopodis* pv. *citrumelo* has been proposed for the E strains. However, no substantially new data was presented for this revived pathovar name and it has been rejected (Young *et al.*, 1996). There is therefore, currently, no satisfactory valid name for the pathogen causing citrus bacterial spot.

Bayer computer code: XANTCM

EU Annex designation: II/A1 - included among "all *Xanthomonas campestris* strains pathogenic to citrus"

HOSTS

In citrus nurseries, "X. axonopodis pv. citrumelo" most commonly infects the rootstocks citrumelo (Citrus paradisi x Poncirus trifoliata), especially cv. Swingle, and also citrange (C. sinensis x P. trifoliata) and P. trifoliata itself. In artificial inoculation experiments, leaves of oranges, grapefruits (C. paradisi) and other Citrus spp. were less affected (Gottwald et al., 1993). Fruits of citrumelo were more susceptible to infection by an aggressive strain of "X. axonopodis pv. citrumelo" than were grapefruits, while other citrus fruits were even less infected (Graham et al., 1992).

GEOGRAPHICAL DISTRIBUTION

The pathogen was discovered in Florida (USA), after an outbreak in 1984, then believed to be of a form of citrus canker, caused by *X. campestris* pv. *citri* (Graham & Gottwald, 1991). It has not been reported, as a disease or as a pathovar of *X. campestris*, from any other citrus-growing countries.

EPPO region: Absent.

North America: USA (Florida).

EU: Absent.

BIOLOGY

The pathogen was first recognized as distinct in 1987, after analysis of a first outbreak of citrus bacterial spot at a citrus nursery in central Florida (USA) (Schoulties *et al.*, 1987). The disease particularly affects young citrus plants (in nurseries), rather than established trees (in citrus groves). It causes lesions on leaves, fruits and stems of citrus, like *X. axonopodis* pv. *citri*, but these are sunken and not raised (see Symptoms).

Some strains of "X. axonopodis pv. citrumelo" are less aggressive (Lawson et al., 1989; Graham & Gottwald, 1990), not causing any water-soaking of tissues. These lesions also expand more slowly. On artificial infection with fixed numbers of bacterial cells (Graham et al., 1990a), only the most aggressive strains maintained the bacterial population in the leaf, as with X. axonopodis pv. citri; numbers of the less aggressive strains declined with time. It has accordingly been suggested that, though isolated from citrus, these strains may not be true pathogens of citrus. Fewer bacteria are also released from bacterial spot lesions than from canker lesions, and even fewer from lesions caused by the less aggressive strains (Timmer et al., 1991). Graham & Gottwald (1990) suggest that the less aggressive strains do not spread naturally, and are only propagated by mechanical pruning operations. In a comparison of simulated epidemics of pvs citri and "citrumelo", Gottwald et al. (1988, 1989) found that the latter did not spread at all on orange, and only a few metres on grapefruit, whether in a simulated grove or a simulated nursery. There was slightly more spread, in terms of appearance of disease, in the simulated nursery. Despite the relative absence of disease, the bacterium itself spread extensively, as an epiphytic population, in the simulated grapefruit nursery.

An antiserum to one isolate of "X. axonopodis pv. citrumelo" (Brlansky et al., 1990) did not cross-react at all with strain A of X. axonopodis pv. citri. However, it reacted with only about half the bacterial spot isolates tested, and did cross-react with several other pathovars of X. campestris. Permar & Gottwald (1989) obtained a monoclonal antibody which gave no cross reactions, but still did not react with all bacterial spot strains. Alvarez et al. (1991) found bacterial spot isolates to be antigenically heterogeneous. Hartung & Civerolo (1987, 1989) distinguished pvs "citrumelo" and citri by DNA fingerprining and RFLP analysis, and showed the former to be heterogeneous. Finally, Gottwald et al. (1991) found that the classification of "X. axonopodis py. citrumelo" isolates by serology and RFLP profiles coincided with the variations in aggressiveness. In this connection, it is interesting to note that Graham et al. (1990b) were able to obtain bacterial spot-like symptoms on leaves of citrumelo by artificial inoculation with isolates of several non-citrus pathovars of X. campestris. These isolates were indistinguishable from weakly aggressive strains of "X. axonopodis pv. citrumelo" in serological and RFLP profiles. The authors therefore speculated that some strains of so-called "X. axonopodis pv. citrumelo" may in fact be X. campestris from other hosts.

DETECTION AND IDENTIFICATION

Symptoms

Bacterial spot differs from classic citrus canker in that lesions on leaves and fruits are usually flat or sunken rather than raised and corky (Stall & Civerolo, 1991). Water-soaking

of the leaf tissues surrounding necrotic areas is prominent in young lesions (but absent in canker). Lesions on stems resemble those of canker.

Detection and inspection methods

Though serological and DNA-based tests can be used for *X. axonopodis* pv. *citri* and other *X. campestris* from citrus, the heterogeneity of "*X. axonopodis* pv. *citrumelo*" (see Biology) means that, while isolates may probably be identified with reasonable certainty as not being pv. *citri*, it is more difficult to achieve a positive identification of pv. "*citrumelo*".

MEANS OF MOVEMENT AND DISPERSAL

"X. axonopodis pv. citrumelo" spreads more slowly than pv. citri in citrus nursery conditions, and the less aggressive strains may only be spread by mechanical means. In international trade, "X. axonopodis pv. citrumelo" could be carried on planting material of citrus, and particularly on the hybrids which are especially susceptible (citrumelo, citrange). It may be noted that it can be carried epiphytically, without symptoms.

PEST SIGNIFICANCE

Economic impact

"X. a. pv. citrumelo" caused an epiphytotic disease on young citrus in nurseries in Florida (USA) in the mid-1980s. In the course of an attempted eradication, over 20 million trees were destroyed, at a coast of over 94 million USD. However, the severity of the disease was considerably less than that of citrus canker caused by pv. citri, and it only affected young plants. In the late 1980s, outbreaks of X. axonopodis pv. citri itself were also found in Florida, and subjected to an official eradication programme. The campaign against "X. axonopodis pv. citrumelo" was officially terminated in September 1990, on the grounds that "none of the various forms of Florida nursery disease causes a disease dangerous to citrus or other plants or fruit". It may be noted, in addition, that the less aggressive strains of "X. axonopodis pv. citrumelo" were more common than the moderately or most aggressive strains (Graham & Gottwald, 1990). Indeed, the most aggressive strains associated with the initial outbreaks were never found on mature commercial citrus, and have not been found again in nature since 1987 (Stall & Civerolo, 1991). So, the strains which can now be isolated as "X. axonopodis pv. citrumelo" are relatively harmless.

Control

It is thought that the aggressive strains of "X. axonopodis pv. citrumelo" which caused the original bacterial spot outbreaks in Florida were eliminated by eradicative action mainly targeting pv. citri. In any case, the disease can be controlled by sanitation in nurseries, since its spread is relatively slow. The less aggressive strains which now occur do not require any particular control measures. In the USA, it has been decided to make no further attempt to eradicate "X. axonopodis pv. citrumelo". Graham & Gottwald (1991) reviewed the status of canker and bacterial spot in Florida (USA) and the possibilities for their eradication.

Phytosanitary risk

X. axonopodis pv. citri is an A1 quarantine pest for EPPO (EPPO/CABI, 1996), but the concept of this pathovar used by EPPO excludes X. campestris pv. citrumelo ("E strain"), while including the B, C and D strains, together with the classic A strain. "X. axonopodis pv. citrumelo" certainly presents a much lesser risk for the EPPO region than X. axonopodis pv. citri, because of its more limited host range, its slower spread, its restriction

to young plants and the relative rarity (indeed possible disappearance) of the most aggressive strains. It has been argued that the less aggressive strains are not truly pathogenic to citrus, but only incidental on this host. It would be dangerous, however, to accept such a negative definition: these strains do infect citrus to a certain extent (whatever else they may do), and they cannot clearly be demarcated from the other strains in the taxon under consideration. Only if they can clearly be attributed to other taxa could their minor pathogenicity to citrus be considered irrelevant. "X. axonopodis pv. citrumelo" continues, in any case, to pose an indirect risk: if an outbreak were found in an EPPO country (even causing little damage), it could create problems for export of citrus planting material and fruits, because of the disputed relationship with pv. citri.

PHYTOSANITARY MEASURES

The importation of citrus planting material from countries where *X. axonopodis* pv. *citri* occurs is generally prohibited by citrus-growing EPPO countries. This prohibition also targets a number of other EPPO A1 quarantine pests of citrus, all much more important than "*X. axonopodis* pv. *citrumelo*". Accordingly, no particular measures need to be taken for planting material. For fruits, it is debatable whether any specific measures would be needed for consignments coming from a country where "*X. axonopodis* pv. *citrumelo*" has been reported but *X. axonopodis* pv. *citri* is definitely absent.

BIBLIOGRAPHY

- Alvarez, A.M.; Benedict, A.A.; Mizumoto, C.Y.; Pollard, L.W.; Civerolo, E.L. (1991) Analysis of *Xanthomonas campestris* pv. *citri* and *X. c. citrumelo* with monoclonal antibodies. *Phytopathology* **81**, 857-865.
- Brlansky, R.H.; Lee, R.F.; Civerolo, E.L. (1990) Detecting *Xanthomonas campestris* pv. *citrumelo* and *X. citri* from citrus using membrane entrapment immunofluorescence. *Plant Disease* **74**, 863-868.
- 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.
- Gabriel, D.W.; Kingsley, M.T.; Hunter, J.E.; Gottwald, T. (1989) Reinstatement of *Xanthomonas citri* (ex Hasse) and *X. phaseoli* (ex Smith) to species and reclassification of all *X. campestris* pv. *citri* strains. *International Journal of Systematic Bacteriology* **39**, 14-22.
- Gottwald, T.R.; Civerolo, E.L.; Garnsey, S.M.; Brlansky, R.H.; Graham, J.H.; Gabriel, D.W. (1988) Dynamics and spatial distribution of *Xanthomonas campestris* pv. *citri* group E strains in simulated nursery and new grove situations. *Plant Disease* **72**, 781-787.
- Gottwald, T.R.; Timmer, L.W.; McGuire, R.G. (1989) Analysis of disease progress of citrus canker in nurseries in Argentina. *Phytopathology* **79**, 1276-1283.
- Gottwald, T.R.; Alvarez, A.M.; Hartung, J.S.; Benedict, A.A. (1991) Diversity of *Xanthomonas campestris* pv. *citrumelo* strains associated with epidemics of citrus bacterial spot in Florida citrus nurseries: correlation of detached leaf, monoclonal antibody, and restriction fragment length polymorphism assays. *Phytopathology* 81, 749-753.
- Gottwald, T.R.; Graham, J.H.; Civerolo, E.L.; Barrett, H.C.; Hearn, C.J. (1993) Differential host range reaction of citrus and citrus relatives to citrus canker and citrus bacterial spot determined by leaf mesophyll susceptibility. *Plant Disease* 77, 1004-1009.
- Graham, J.H.; Gottwald, T.R. (1990) Variation in aggressiveness of Xanthomonas campestris pv. citrumelo associated with citrus bacterial spot in Florida citrus nurseries. Phytopathology 80, 190-196
- Graham, J.H.; Gottwald, T.R. (1991) Research perspectives on eradication of citrus bacterial diseases in Florida. *Plant Disease* 75, 1193-1200.

- Graham, J.H.; Gottwald, T.R.; Fardelmann, D. (1990a) Cultivar-specific interactions for strains of *Xanthomonas campestris* from Florida that cause citrus canker and citrus bacterial spot. *Plant Disease* **74**, 753-756.
- Graham, J.H.; Hartung, J.S.; Stall, R.E.; Chase, A.R. (1990b) Pathological, restriction-fragment length polymorphism, and fatty acid profile relationships between *Xanthomonas campestris* from citrus and noncitrus hosts. *Phytopathology* **80**, 829-836.
- Graham, J.H.; Gottwald, T.R.; Riley, T.D.; Bruce, M.A. (1992) Susceptibility of citrus fruit to bacterial spot and citrus canker. *Phytopathology* **82**, 452-457.
- Hartung, J.S.; Civerolo, E.L. (1987) Genomic fingerprints of Xanthomonas campestris pv. citri strains from Asia, South America and Florida. Phytopathology 77, 282-285.
- Hartung, J.S.; Civerolo, E.L. (1989) Restriction fragment length polymorphisms distinguish *Xanthomonas campestris* strains isolated from Florida citrus nurseries from *X. c.* pv. *citri*. *Phytopathology* **79**, 793-799.
- Lawson, R.H.; Dienelt, M.M.; Civerolo, E.L. (1989) Histopathology of Xanthomonas campestris pv. citri from Florida and Mexico in wound-inoculated detached leaves of Citrus aurantiifolia: light and scanning electron microscopy. Phytopathology 79, 329-335.
- Permar, T.A.; Gottwald, T.R. (1989) Specific recognition of a *Xanthomonas campestris* Florida citrus nursery strain by a monoclonal antibody probe in a microfiltration enzyme immunoassay. *Phytopathology* **79**, 780-783.
- Schoulties, C.L.; Civerolo, E.L.; Miller, J.W.; Stall, R.E.; Krass, C.J. (1987) Citrus canker in Florida. *Plant Disease* **71**, 388-395.
- Stall, R.E.; Civerolo, E.L. (1991) Research relating to the recent outbreak of citrus canker in Florida. *Annual Review of Phytopathology* **29**, 399-420.
- Timmer, L.W.; Gottwald, T.R.; Zitko, S.E. (1991) Bacterial exudation from lesions of Asiatic citrus canker and citrus bacterial spot. *Plant Disease* **75**, 192-195.
- Vauterin, L.; Swings, J.; Kersters, K.; Gillis, M.; Mew, T.W.; Schroth, M.N.; Palleroni, N.J.; Hildebrand, D.C.; Stead, D.E.; Civerolo, E.L.; Hayward, A.C.; Maraite, H.; Stall, R.E.; Vidaver, A.K.; Bradbury, J.F. (1990) Towards an improved taxonomy of *Xanthomonas*. *International Journal of Systematic Bacteriology* 40, 312-316.
- Vauterin, L.; Hoste, B.; Kersters, K.; Swings, J. (1995) Reclassification of Xanthomonas. International Journal of Systematic Bacteriology 45, 472-489.
- Young, J.M.; Bradbury, J.F.; Gardan, L.; Grozdyak, R.I.; Stead, D.E.; Takikawa, Y.; Vidaver, A.K. (1991) Comment on the reinstatement of *Xanthomonas citri* (ex Hasse 1915) Gabriel *et al.* 1989 and *Xanthomonas phaseoli* (ex Smith 1897) Gabriel *et al.* 1989: indication of the need for minimal standards for the genus *Xanthomonas*. *International Journal of Systematic Bacteriology* 41, 172-177
- Young, J.M.; Saddler, G.S.; Takikawa, Y.; De Boer, S.H.; Vauterin, L.; Gardan, L.; Gvozdyak, R.I.; Stead, D.E. (1996) Names of plant pathogenic bacteria 1864-1995. *Review of Plant Pathology* 75, 721-763.