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

Data Sheets on Quarantine Pests

Spiroplasma citri

The vectors of *Spiroplasma citri* are individually included in EU Directive 77/93. Since their importance only arises in relation to *S. citri*, they are covered in this data sheet.

IDENTITY

- ***Spiroplasma citri***
  Name: *Spiroplasma citri* Saglio *et al.*
  Taxonomic position: Bacteria: Tenericutes: Mollicutes
  Common names: Stubborn, little leaf (English)
  Stubborn (French)
  Bayer computer code: SPIRCI
  EU Annex designation: II/A2
- ***Circulifer tenellus***
  Name: *Circulifer tenellus* (Baker)
  Synonyms: *Neoaliturus tenellus* (Baker)
  Eutettix tenellus Baker
  Taxonomic position: Insecta: Hemiptera: Homoptera: Cicadellidae
  Common names: Beet leafhopper (English)
  Cicadelle de la betterave (French)
  Saltahojas de la remolacha (Spanish)
  Notes on taxonomy and nomenclature: Oman (1970) argues for the retention of *Circulifer* and *Neoaliturus* as separate genera; in his concept both *tenellus* and *haematoceps* are placed in *Circulifer*. Nast (1972) treats the two genera as *Neoaliturus* notwithstanding Oman's (1970) arguments for the retention of the two genera. He includes 17 species in the Palaearctic region of which 14 are recorded from the Mediterranean Basin. The two species, *tenellus* and *haematoceps*, are both included in *Circulifer* by della Giustina (1989), who reports that *tenellus* is confirmed in Corsica.
  Bayer computer code: CIRCTE
  EU Annex designation: II/A2
- ***Neoaliturus haematoceps***
  Name: *Neoaliturus haematoceps* (Mulsant & Rey)
  Synonyms: *Circulifer haematoceps* (Mulsant & Rey)
  Jassus haematoceps Mulsant & Rey
  Taxonomic position: Insecta: Hemiptera: Homoptera:
  Cicadellidae
  Bayer computer code: NEOAHA
  EU Annex designation: II/A2 (under the name *Circulifer haematoceps*)

HOSTS

- ***Spiroplasma citri***
  The principal economic hosts of *S. citri* are susceptible *Citrus* spp. including the major commercial species in the Mediterranean area: grapefruits (*C. paradisi*), lemons (*C. limon*),

mandarins (*C. reticulata*), oranges (*C. sinensis*) and sour oranges (*C. aurantium*). Other citrus hosts are *C. grandis*, *C. limettioides*, *C. limonia*, *C. madurensis*, rough lemons (*C. jambhiri*), satsumas (*C. unshiu*), and tangelos (*C. paradisi* x *reticulata*) (Calavan, 1980). Other rutaceous hosts include *Fortunella* spp. and interspecific rootstock hybrids like citranges (*C. sinensis* x *Poncirus trifoliata*). Some forms are symptomlessly infected, including *P. trifoliata*. Many other cultivated or wild plants, particularly Amaranthaceae, Chenopodiaceae, Brassicaceae and Plantaginaceae, have been found to be naturally infected in south-western USA. *S. citri* causes a specific disease (brittle root) of horseradish (*Armoracia rusticana*) in eastern USA. *Catharanthus roseus*, a host for many phytoplasms, has been found naturally infected in Mediterranean countries; there are probably many other hosts there. Yet other plants have been experimentally infected by leafhopper transmission.

- ***Circulifer tenellus* and *Neoaliturus haematoceps***

  Like many other cicadellids, the Mediterranean vectors of stubborn disease feed on a wide range of hosts (field crops, fruit trees, ornamentals, wild plants and weeds). *C. tenellus* has attracted particular attention on sugarbeet (*Beta vulgaris* var. *saccharifera*) in North America because it acts as the vector for beet curly top hybrigeminivirus (EPPO/CABI, 1996); it is not especially associated with this host in the EPPO region. *N. haematoceps* has been found particularly on the ornamental *Matthiola incana* and on the wild plants *M. sinuata* and *Salsola kali* (Fos *et al.*, 1986). Neither insect is particularly associated with citrus, on which feeding is incidental. Oldfield *et al*. (1976) reported that *C. tenellus* could not be reared on citrus as sole host. *C. tenellus* also transmits periwinkle virescence phytoplasma and beet leafhopper-transmitted virescence agent, and *N. haematoceps* sesame phyllody phytoplasma. Klein & Raccah (1991) report on two *N. haematoceps* populations in Israel, one polyphagous and the other host-specific (to *S. kali*).

GEOGRAPHICAL DISTRIBUTION

- ***Spiroplasma citri***

  Described separately in the 1900s in California (USA) and in the 1930s in Palestine, stubborn disease has not been reported from eastern Asia (where citrus originates) or tropical Africa. *S. citri* has presumably moved to citrus from other hosts, indigenous to the Mediterranean area. It is not clear whether *S. citri* is indigenous or introduced in North America. Its main vector in North America, the leafhopper *C. tenellus*, is of Mediterranean origin.

  EPPO region: Algeria, Cyprus, Egypt, France (Corsica only), Greece, Israel, Italy (few records; at least Sardinia, Sicily), Lebanon, Libya, Morocco, Spain, Syria, Tunisia, Turkey.

  Asia: Cyprus, Iran, Iraq, Israel, Jordan (UNDP/FAO, 1988), Lebanon, Pakistan, Saudi Arabia, Syria, Turkey, Yemen (UNDP/FAO, 1988).

  Africa: Algeria, Egypt, Libya, Morocco, Tunisia. A doubtful record for Madagascar mentioned in the CMI Distribution Map (CMI, 1970) is so old and insubstantial that it should be considered erroneous.

  North America: Mexico, USA (Arizona, California, Illinois, Maryland).

  South America: All the following 'records' from South America are based on suspicious symptoms only and should be considered as unconfirmed: Argentina (Tucumán), Brazil (São Paulo), Peru, Suriname, Venezuela.

  Oceania: New Zealand (isolated reports).

  EU: Present.

  Distribution map: See IMI (1993, No. 375).

- ***Circulifer tenellus***
  EPPO region: Algeria, Egypt, France (Corsica; della Giustina, 1989), Israel, Italy (Sicily), Libya, Morocco, Spain (including Canary Islands), Tunisia, Turkey.
  Asia: India (northwestern states), Israel, Kyrgyzstan, Tajikistan, Turkey, Turkmenistan, Uzbekistan.
  Africa: Algeria, Egypt, Libya, Morocco, Namibia, South Africa, Sudan, Tunisia.
  North America: Mexico, USA (Arizona, California, Colorado, Florida, Hawaii, Idaho, Illinois, Kansas, Montana, Nebraska, Nevada, New Mexico, Oklahoma, Oregon, Texas, Utah, Washington, Wyoming).
  Central America and Caribbean: Jamaica, Puerto Rico.
  EU: Present.
  Distribution map: See CIE (1961, No. 134).
- ***Neoaliturus haematoceps***
  EPPO region: Mediterranean countries generally, France (including Corsica), Israel, Morocco, Spain, Syria, Turkey.
  Asia: Iran, Israel, Syria, Turkey.
  Africa: Morocco.
  EU: Present.

BIOLOGY

S. citri infects the phloem sieve tubes of its hosts. The pathogen persists in affected trees as they decline. It is in practice an obligate parasite, surviving in citrus or in a variety of other host plants, with no saprophytic phase. It is naturally transmitted by leafhoppers: *Circulifer tenellus*, *Scaphytopius nitridus* and *S. acutus delongi* in California (USA) (Oldfield, 1988), *Neoaliturus haematoceps* (Bové, 1986) and *C. tenellus* (Klein *et al*., 1988) in the Mediterranean area. None of these vectors have a particular preference for citrus as a host; they may therefore acquire *S. citri* from other hosts. *S. citri* multiplies in its insect vectors, which become infective some 10-20 days after acquisition feeding (Liu *et al*., 1983a, b). The insects can remain infective throughout their lives (which may be shortened by the infection), but there is no transovarial transmission. Other Homoptera may acquire the spiroplasm, but not transmit it (Rana *et al*., 1975; Bové *et al*., 1979). It remains possible that other species may act as vectors in the Mediterranean area (Klein *et al*., 1982).

In North America, the distribution of *S. citri* follows rather closely that of *C. tenellus* (primarily a sugarbeet insect). In the Mediterranean area, one or other vector is present practically wherever citrus is grown, so that availability of vectors does not appear to be a limiting factor in the spread of stubborn disease to new areas. For further information on vectors, see also Golino & Oldfield (1990).

The spiroplasm develops best in citrus under hot conditions (28-32°C) and may not give rise to conspicuous symptoms at lower temperatures. Bové (1986) explains in this way the greater perceived damage due to stubborn in Syria, by comparison with Corsica (France). Annual plants experimentally infected are rapidly killed at temperatures over 30°C, but may again not show symptoms at lower temperatures (Oldfield, 1988).

S. citri from areas where citrus does not occur can be experimentally vector-transmitted to citrus (Gumpf, 1988), while the pathogen from citrus can be experimentally transmitted to horseradish (Sullivan *et al.*, 1987). Thus, there is no indication at present of special races or strains attacking citrus.

DETECTION AND IDENTIFICATION

Symptoms

The name 'stubborn' arises from the persistence of the original characters of a tree when it is 'top-worked' with healthy budwood. Affected trees are more or less stunted. Leaves are shorter and broader ('little leaf'), cupped, abnormally upright, sometimes mottled or chlorotic. Under very hot conditions, leaves on some shoots may have misshapen, blunted or heart-shaped yellow tips (a highly diagnostic character). Shoots may be abnormally bunched and development of multiple axillary buds may give rise to witches' brooms. Fruiting tends to be suppressed in infected plants. Fruits may be stunted, lopsided or acorn-shaped (i.e. with thick rind at the base and thin rind at the tip), and may show colour inversion (peduncular end discolours while stylar end remains green). For more details, see Bové (1984, 1988). The vectors, as such, cause no particular symptoms.

Morphology

- ***Spiroplasma citri***

  S. citri forms wall-less pleomorphic cells with a characteristic spiral morphology. The minimum viable length of a helix is 2.0 x 0.1-0.2 µm. The helices are motile by flexing or rotation. Some strains are non-motile and non-helical. *S. citri* is one of the very few plant pathogenic mollicutes to have been cultured. For more details on morphology, biochemical tests and behaviour in culture, see Saglio *et al*. (1973), Bradbury (1991).

- ***Circulifer tenellus* and *Neoaliturus haematoceps***

  Species in the genera *Neoaliturus* and *Circulifer* may be characterized as being small leafhoppers, males 2.5-3.6 mm and females 2.7-3.8 mm. The head is slightly wider than the pronotum with the anterior margin rounded. The overall colour is brown or stramineous with variable darker markings. The male aedeagus is symmetrical with a stout tapered shaft at the apex of which are a pair of slender curved processes which lie in a horizontal plane and together form a nearly complete circle.

  The species in the two genera are very difficult to separate and identification requires use of characters in the male genitalia (Ribaut, 1936, 1952). Positive identification will require specialist help. The differences between *haematoceps* and *tenellus* lie chiefly in the subgenital plates of the male. In *haematoceps* the subgenital plates are pointed apically (Ribaut, 1952), while in *tenellus* they are truncate (Ribaut, 1952; della Giustina, 1989).

Detection and inspection methods

S. citri can be detected by graft inoculation of indicator plants, of which the most suitable is orange cv. Madame Vinous, kept at 32°C in the day and 27°C at night (Bové, 1988). Other indicators are grapefruit cv. Marsh and tangelo cv. Sexton. The best inoculum is young leaf patches including midrib.

S. citri can fairly reliably be cultured from trees showing symptoms, the best material to use being seeds with various degrees of abortion, the peduncular end of the fruit axis, or mottled summer leaves collected in October (Bové *et al.*, 1984). Since the organism can be cultured, antisera are relatively easy to obtain, and ELISA can be used for detection and/or identification of *S. citri* in extracts from infected plants and insects (Saillard & Bové, 1983; Clark *et al.*, 1989). The latex agglutination technique is also suitable for rapid detection (Fletcher & Slack, 1986). cDNA probes are under development, and are potentially much more sensitive than ELISA (Bové, 1986). Dale (1988) has described a rapid DAPI staining technique applied to crushed leaf midribs, which detects *S. citri* and distinguishes it from other phytoplasms.

MEANS OF MOVEMENT AND DISPERSAL

Natural transmission by leafhoppers will carry *S. citri* over local distances. International spread would be more likely to occur in infected budwood (although this does not transmit the pathogen very reliably). Although the theoretical possibility exists that infective vectors may be carried on citrus plants, the insects concerned are actively mobile and do not preferentially feed on citrus, so the risk seems minor.

PEST SIGNIFICANCE

Economic impact

Stubborn is a serious disease of citrus, which under hot dry conditions can much reduce the quality and quantity of yield. The fact that it is vector-transmitted, unlike most other graft-transmissible pathogens of citrus, makes it more difficult to control by use of healthy planting material. In California (USA), the main economic hosts are oranges, grapefruits and tangelos, of which 5-10% of trees are estimated to be affected. In the Mediterranean area, stubborn is very serious in some countries, especially in Syria where the vector *Neoaliturus haematoceps* is common and introduced healthy budwood was rapidly reinfected (Bové, 1986). The Syrian practice of rebudding imported healthy stock has also favoured reinfection (UNDP/FAO, 1988). Iraq and Turkey are two other countries where the disease is reported to be widespread and important. In other Mediterranean countries (Cyprus, Egypt, Jordan, Morocco) the disease is present, but only rather rarely on certain cultivars. In others (Algeria, Libya, Tunisia), stubborn was reported to be common in the past but the survey of UNDP/FAO (1988) found few or no trees with characteristic symptoms. Importance no doubt depends largely on the presence or abundance of vectors. *N. haematoceps* occurs throughout the Mediterranean area, while *Circulifer tenellus* occurs mainly in its southern and eastern parts. Newly planted citrus orchards are readily invaded by leafhoppers from surrounding crops, and can suffer serious attacks, which then decline as the trees become older and less attractive to the insects (Bové, 1986). Stubborn also tends to occur only in certain years, when the vectors are abundant.

Although *S. citri* naturally infects many other hosts, it is not reported to have any economic impact on these. Their main significance would be as reservoirs of *S. citri* for infection of citrus. Horseradish brittle root is of purely anecdotal interest.

Control

Production of healthy budwood is the only practical means of control, but it must be accompanied by suitable siting of orchards to avoid reinfection as far as possible during the first years of development. In practice, this means that *S. citri* has to be covered by virus-free certification schemes for citrus, such as the one now under development by EPPO.

Trees showing symptoms should be rogued and replaced, not so much because they constitute a risk to neighbouring trees, but because they will never bear fruit satisfactorily. Insecticide treatments against the vectors are not effective, because *S. citri* can be transmitted very rapidly after arrival of infective vectors in an orchard. It has been suggested (Gumpf, 1988) that trap plants (attractive to the vector, but not hosts of *S. citri*, e.g. sugarbeet) should be planted in the vicinity of orchards. Gumpf (1988) summarizes the main elements recommended for stubborn control in North America.

Phytosanitary risk

S. citri was assessed as a quarantine pest by EPPO and rejected, on the grounds that it is already widely distributed, though not necessarily common, throughout the citrus-growing areas of the region. Its vectors in the EPPO region are widely distributed and undoubtedly

important in facilitating reinfection of newly planted healthy stocks. However, it is difficult to conceive what phytosanitary measures against the vectors could be taken internationally.

Both CPPC and IAPSC consider *S. citri* to be a quarantine pest. For the EPPO region, it can best be considered as a quality pest, to be controlled by official virus-free certification, following for example the EPPO-recommended scheme for citrus (OEPP/EPPO, 1995).

PHYTOSANITARY MEASURES

No particular specific measures for imported citrus material are recommended. All planting material of citrus and its hybrids, imported or nationally produced, should meet virus-free certification standards.

BIBLIOGRAPHY

Bové, J.M. (1984) Wall-less prokaryotes of plants. *Annual Review of Phytopathology* **22**, 361-396.

Bové, J.M. (1986) Stubborn and its natural transmission in the Mediterranean area and in the Near East. *FAO Plant Protection Bulletin* **34**, 15-23.

Bové, J.M. (1988) *Spiroplasma citri*. In: *European handbook of plant diseases* (Ed. by Smith, I.M.; Dunez, J.; Lelliott, R.A.; Phillips, D.H.; Archer, S.A.), pp. 129-131. Blackwell Scientific Publications, Oxford, UK.

Bové, J.M.; Moutous, G.; Saillard, C.; Fos, A.; Bonfils, J.; Vignault, J.-C.; Nhami, A.; Abassi, M.; Kabbage, K.; Hafidi, B.; Mouches, C.; Viennot-Bourgin, G. (1979) Mise en évidence de *Spiroplasma citri*, l'agent causal de la maladie du 'stubborn' des agrumes dans 7 cicadelles du Maroc. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences D* No. 288, 335-338.

Bové, J.M.; Vignault, J.C.; Fos, A. (1984) Citrus stubborn disease in Iraq and Syria: correlation between symptom expression and detection of *Spiroplasma citri* by culture and ELISA. In: *Proceedings of the 9th Conference of the International Organization of Citrus Virologists*. IOCV, Riverside, USA.

Bradbury, J.F. (1991) *Spiroplasma citri*. *CMI Descriptions of Fungi and Bacteria* No. 1046. CAB International, Wallingford, UK.

Calavan, E.C. (1980) Stubborn. In: *Description and illustration of virus and virus-like diseases of citrus* (Ed. by Bové, J.; Vogel, R.). SETCO-IRFA, Paris, France.

CIE (1961) *Distribution Maps of Insect Pests* No. 134. CAB International, Wallingford, UK.

Clark, M.F.; Davies, D.L.; Buss, S.L.; Morton, A. (1989) Serological discrimination among mycoplasma-like organisms using polyclonal and monoclonal antibodies. *Acta Horticulturae* No. 235, pp. 107-113.

Dale, J.L. (1988) Rapid compression technique for detecting mycoplasma-like organisms in leaf midrib sieve tubes by fluorescence microscopy. *Phytopathology* **78**, 118-120.

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

Fletcher, J.; Slack, S.A. (1986) Latex agglutination as a rapid detection assay for *Spiroplasma citri*. *Plant Disease* **70**, 754-756.

Fos, A.; Bové, J.M.; Lallemand, J.; Saillard, C.; Vignault, J.C.; Ali, Y.; Brun, P.; Vogel, R. (1986) The leafhopper *Neoaliturus haematoceps* is a vector of *Spiroplasma citri* in the Mediterranean area. *Annales de l'Institut Pasteur Microbiologie* **137A**, 97-101.

Giustina, W. della (1989) Homoptéres Cicadellidae. *Faune de France* **73**, 350 pp.

Golino, D.A.; Oldfield, G.N. (1990) Plant pathogenic spiroplasmas and their leafhopper vectors. *Advances in Disease Vector Research* No. 6, pp. 267-299.

Gumpf, D.J. (1988) Stubborn diseases of citrus caused by *Spiroplasma citri*. In: *Mycoplasma diseases of crops. Basic and applied aspects* (Ed. by Maramorosch, K.; Raychaudhuri, S.P.), pp. 327-342. Springer-Verlag, New York, USA.

IMI (1993) *Distribution Maps of Plant Diseases* No. 375 (edition 3). CAB International, Wallingford, UK.

Klein, M.; Raccah, B. (1991) Separation of two leafhopper populations of the *Circulifer haematoceps* complex on different host plants in Israel. *Phytoparasitica* **19**, 153- 155.

Klein, M.; Raccah, B.; Oman, P.W. (1982) The occurrence of a member of the *Circulifer tenellus* species complex (Homoptera: Cicadellidae: Euscelini) in Israel. *Phytoparasitica* **10**, 237-240.

Klein, M.; Rasooly, P.; Raccah, B. (1988) [New findings on the transmission of *Spiroplasma citri*, the citrus stubborn disease agent in Israel, by a beet leafhopper from the Jordan valley]. *Hassadeh* **68**, 1736-1737.

Liu, H.Y.; Gumpf, D.J.; Oldfield, G.N.; Calavan, E.C. (1983a) Transmission of *Spiroplasma citri* by *Circulifer tenellus*. *Phytopathology* **73**, 582-585.

Liu, H.Y.; Gumpf, D.J.; Oldfield, G.N.; Calavan, E.C. (1983b) The relationship of *Spiroplasma citri* and *Circulifer tenellus*. *Phytopathology* **73**, 585-590.

Nast, J. (1972) *Palaearctic Auchenorrhyncha (Homoptera) - an annotated checklist*, 550 pp. Polish Scientific Publishers, Warsaw, Poland.

OEPP/EPPO (1995) Certification schemes No. 12. Pathogen-tested citrus trees and rootstocks. *Bulletin OEPP/EPPO Bulletin* **25**.

Oldfield, G.N.; Kaloostian, G.H.; Pierce, H.D.; Calavan, E.C.; Granett, A.L.; Blue, R.L. (1976) Beet leafhopper transmits Citrus stubborn disease. *California Agriculture* **30**, 6, 15.

Oldfield, G.N. (1988) Ecological associations of *Spiroplasma citri* with insects, plants and other plant mycoplasmas in the western United States. In: *Mycoplasma diseases of crops. Basic and applied aspects* (Ed. by Maramorosch, K.; Raychaudhuri, S.P.), pp. 175-191. Springer-Verlag, New York, USA.

Oman, P. (1970) Taxonomy and nomenclature of the beet leafhopper, *Circulifer tenellus*. *Annals of the Entomological Society of America* **63**, 507-512.

Rana, G.L.; Kaloostian, G.H.; Oldfield, G.N.; Granett, A.L.; Calavan, E.C.; Pierce, H.D.; Lee, I.M.; Gumpf, D.J. (1975) Acquisition of *Spiroplasma citri* through membranes by Homopterous insects. *Phytopathology* **65**, 1143-1145.

Ribaut, H. (1936) Homoptéres Auchenorrhynques. I (Typhlocybidae). *Faune de France* **31**, 231 pp.

Ribaut, H. (1952) Homoptéres Auchenorrhynques. II (Jassidae). *Faune de France* **57**, 474 pp.

Saglio, P.; L'Hospital, M.; Lafléche, D.; Dupont, G.; Bové, J.M.; Tully, J.G.; Freundt, E.A. (1973) *Spiroplasma citri* gen. and sp. n.: a mycoplasma-like organism associated with stubborn disease of citrus. *International Journal of Systematic Bacteriology* **23**, 191-204.

Saillard, C.; Bové, J.M. (1983) Application of ELISA to spiroplasma detection and classification. In: *Methods in mycoplasmology* (Ed. by Razin, S.; Tully, J.G.), Vol. 1, pp. 471-476. Academic Press, New York, USA.

Sullivan, D.A.; Oldfield, G.N.; Eastman, C.E.; Fletcher, J.; Gumpf, D.J. (1987) Transmission of a citrus-infecting strain of *Spiroplasma citri* to horseradish. *Plant Disease* **71**, 469.

UNDP/FAO (1988) *Fruit crop sanitation in the Mediterranean and Near East region: status and requirements* (Ed. by Taher, M.). RAB/86/018. FAO, Rome, Italy.