

## SCIENTIFIC OPINION

### Scientific Opinion on pest the categorisation of *Spiroplasma citri*<sup>1</sup>

#### EFSA Panel on Plant Health (PLH)<sup>2,3</sup>

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#### ABSTRACT

The Panel on Plant Health performed a pest categorisation of *Spiroplasma citri*, the causal agent of citrus stubborn disease, horseradish brittle root and carrot purple leaf. *S. citri* is a well-defined species of the genus *Spiroplasma*, a group of helical wall-less bacteria (Mollicutes) mainly associated with arthropods. Routine molecular detection methods are available and are appropriate for identification. *S. citri* is not known to occur in most of the EU Member States. Among citrus-growing countries, only Croatia, Greece, Malta and Portugal do not report *S. citri*. The disease seems to be widespread only in Cyprus. *S. citri* is included in Annex II, Part A, Section II of Directive 2000/29/EC. The insect vectors *Circulifer haematoceps* and *C. tenellus* are included in the same Directive, Annex II, Part A, Section II. Citrus, the main host plant (as a symptomatic host crop), is listed (a) in Annex III, Part A, (b) in Annex IV, Part A, Section II, (c) in the same annex, Part B, and (d) in Annex V, Parts A and B, Section I. *S. citri* is disseminated by plants for planting and by seven species of leafhoppers (Cicadellidae), of which only three species, *C. tenellus*, *C. haematoceps* and *C. opacipennis*, are reported in the EU. Besides the rutaceous hosts, more than 33 other plant species from 12 different families can be hosts, even if only some may show symptoms. Ecological conditions in the risk assessment area are suitable for the establishment and spread of *S. citri*, at least where citrus is currently grown. Yield reductions on citrus crops are reported from Cyprus, with reduction in fruit size, weight and quality. Infected planting material could contribute to the dissemination of *S. citri*, as well as the insect vectors. In areas with a hot, dry climate, the impact on the yield and quality may be high.

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#### KEY WORDS

*Spiroplasma citri*, *Circulifer haematoceps*, *Circulifer tennelus*, citrus, quarantine pest, regulated non-quarantine pest

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## BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The current European Union plant health regime is established by Council Directive 2000/29/EC on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community (OJ L 169, 10.7.2000, p. 1).

The Directive lays down, amongst others, the technical phytosanitary provisions to be met by plants and plant products and the control checks to be carried out at the place of origin on plants and plant products destined for the Union or to be moved within the Union, the list of harmful organisms whose introduction into or spread within the Union is prohibited and the control measures to be carried out at the outer border of the Union on arrival of plants and plant products.

The Commission is currently carrying out a revision of the regulatory status of organisms listed in the Annexes of Directive 2000/29/EC. This revision targets mainly organisms which are already locally present in the EU territory and that in many cases are regulated in the EU since a long time. Therefore it is considered to be appropriate to evaluate whether these organisms still deserve to remain regulated under Council Directive 2000/29/EC, or whether, if appropriate, they should be regulated in the context of the marketing of plant propagation material, or be deregulated. The revision of the regulatory status of these organisms is also in line with the outcome of the recent evaluation of the EU Plant Health Regime, which called for a modernisation of the system through more focus on prevention and better risk targeting (prioritisation).

In order to carry out this evaluation, a recent pest risk analysis is needed which takes into account the latest scientific and technical knowledge on these organisms, including data on their agronomic and environmental impact, as well as their present distribution in the EU territory. In this context, EFSA has already been asked to prepare risk assessments for some organisms listed in Annex IIAII. The current request concerns 23 additional organisms listed in Annex II, Part A, Section II as well as five organisms listed in Annex I, Part A, Section I, one listed in Annex I, Part A, Section II and nine organisms listed in Annex II, Part A, Section I of Council Directive 2000/29/EC. The organisms in question are the following:

### Organisms listed in Annex II, Part A, Section II:

- *Ditylenchus destructor* Thorne
- *Circulifer haematoceps*
- *Circulifer tenellus*
- *Helicoverpa armigera* (Hübner)
- *Radopholus similis* (Cobb) Thome (could be addressed together with the HAI organism *Radopholus citrophilus* Huettel Dickson and Kaplan)
- *Paysandisia archon* (Burmeister)
- *Clavibacter michiganensis* spp. *insidiosus* (McCulloch) Davis *et al.*
- *Erwinia amylovora* (Burr.) Winsl. *et al.* (also listed in Annex IIB)
- *Pseudomonas syringae* pv. *persicae* (Prunier *et al.* ) Young *et al.*
- *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye
- *Xanthomonas campestris* pv. *pruni* (Smith) Dye
- *Xylophilus ampelinus* (Panagopoulos) Willems *et al.*
- *Ceratocystis fimbriata* f. sp. *platani* Walter (also listed in Annex IIB)
- *Cryphonectria parasitica* (Murrill) Barr (also listed in Annex IIB)
- *Phoma tracheiphila* (Petri) Kanchaveli and Gikashvili
- *Verticillium albo-atrum* Reinke and Berthold
- *Verticillium dahliae* Klebahn
- Beet leaf curl virus
- Citrus tristeza virus (European isolates) (also listed in Annex IIB)
- Grapevine flavescence dorée MLO (also listed in Annex IIB)
- Potato stolbur mycoplasma
- *Spiroplasma citri* Saglio *et al.*

- Tomato yellow leaf curl virus

Organisms listed in Annex I, Part A, Section I:

- *Rhagoletis cingulata* (Loew)
- *Rhagoletis ribicola* Doane
- Strawberry vein banding virus
- Strawberry latent C virus
- Elm phloem necrosis mycoplasma

Organisms listed in Annex I, Part A, Section II:

- *Spodoptera littoralis* (Boisd.)

Organisms listed in Annex II, Part A, Section I:

- *Aculops fuchsiae* Keifer
- *Aonidiella citrina* Coquillet
- Prunus necrotic ringspot virus
- Cherry leafroll virus
- *Radopholus citrophilus* Huettel Dickson and Kaplan (could be addressed together with IIAII organism *Radopholus similis* (Cobb) Thome)
- *Scirtothrips dorsalis* Hendel
- *Atropellis* spp.
- *Eotetranychus lewisi* McGregor
- *Diaporthe vaccinii* Shear.

#### TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

EFSA is requested, pursuant to Article 29(1) and Article 22(5) of Regulation (EC) No 178/2002, to provide a pest risk assessment of *Ditylenchus destructor* Thome, *Circulifer haematoceps*, *Circulifer tenellus*, *Helicoverpa armigera* (Hübner), *Radopholus similis* (Cobb) Thome, *Paysandisia archon* (Burmeister), *Clavibacter michiganensis* spp. *insidiosus* (McCulloch) Davis *et al.*, *Erwinia amylovora* (Burr.) Winsl. *et al.*, *Pseudomonas syringae* pv. *persicae* (Prunier *et al.*) Young *et al.*, *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye, *Xanthomonas campestris* pv. *pruni* (Smith) Dye, *Xylophilus ampelinus* (Panagopoulos) Willems *et al.*, *Ceratocystis fimbriata* f. sp. *platani* Walter, *Cryphonectria parasitica* (Murrill) Barr, *Phoma tracheiphila* (Petri) Kanchaveli and Gikashvili, *Verticillium albo-atrum* Reinke and Berthold, *Verticillium dahliae* Klebahn, Beet leaf curl virus, Citrus tristeza virus (European isolates), Grapevine flavescence dorée MLO, Potato stolbur mycoplasma, *Spiroplasma citri* Saglio *et al.*, Tomato yellow leaf curl virus, *Rhagoletis cingulata* (Loew), *Rhagoletis ribicola* Doane, Strawberry vein banding virus, Strawberry latent C virus, Elm phloem necrosis mycoplasma, *Spodoptera littoralis* (Boisd.), *Aculops fuchsiae* Keifer, *Aonidiella citrina* Coquillet, Prunus necrotic ringspot virus, Cherry leafroll virus, *Radopholus citrophilus* Huettel Dickson and Kaplan (to address with the IIAII *Radopholus similis* (Cobb) Thome), *Scirtothrips dorsalis* Hendel, *Atropellis* spp., *Eotetranychus lewisi* McGregor and *Diaporthe vaccinii* Shear., for the EU territory.

In line with the experience gained with the previous two batches of pest risk assessments of organisms listed in Annex II, Part A, Section II, requested to EFSA, and in order to further streamline the preparation of risk assessments for regulated pests, the work should be split in two stages, each with a specific output. EFSA is requested to prepare and deliver first a pest categorisation for each of these 38 regulated pests (step 1). Upon receipt and analysis of this output, the Commission will inform EFSA for which organisms it is necessary to complete the pest risk assessment, to identify risk reduction options and to provide an assessment of the effectiveness of current EU phytosanitary requirements (step 2). *Clavibacter michiganensis* spp. *michiganensis* (Smith) Davis *et al.* and *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye, from the second batch of risk assessment requests for Annex IIAII organisms requested to EFSA (ARES(2012)880155), could be used as pilot cases for this approach, given that the working group for the preparation of their pest risk assessments has been constituted and it is currently dealing with the step 1 "pest categorisation". This proposed

modification of previous request would allow a rapid delivery by EFSA by May 2014 of the first two outputs for step 1 "pest categorisation", that could be used as pilot case for this request and obtain a prompt feedback on its fitness for purpose from the risk manager's point of view.

As indicated in previous requests of risk assessments for regulated pests, in order to target its level of detail to the needs of the risk manager, and thereby to rationalise the resources used for their preparation and to speed up their delivery, for the preparation of the pest categorisations EFSA is requested, in order to define the potential for establishment, spread and impact in the risk assessment area, to concentrate in particular on the analysis of the present distribution of the organism in comparison with the distribution of the main hosts and on the analysis of the observed impacts of the organism in the risk assessment area.

## ASSESSMENT

### 1. Introduction

#### 1.1. Purpose

This document presents a pest categorization prepared by the EFSA Scientific Panel on Plant Health (hereinafter referred to as the Panel) for the species *Spiroplasma citri* in response to a request from the European Commission (EC).

#### 1.2. Scope

This pest categorisation is for *Spiroplasma citri*. The risk assessment area is the territory of the European Union (hereinafter referred to as the EU) with 28 Member States (hereinafter referred to as EU MSs), restricted to the area of application of Council Directive 2000/29/EC.

### 2. Methodology and data

#### 2.1. Methodology

The Panel performed the pest categorisation for *Spiroplasma citri* following guiding principles and steps presented in the EFSA guidance on the harmonised framework for pest risk assessment (EFSA PLH Panel, 2010) and as defined in the International Standard for Phytosanitary Measures No 11 (FAO, 2013) and International Standard for Phytosanitary Measures No 21 (FAO, 2004).

In accordance with the harmonised framework for pest risk assessment in the EU (EFSA PLH Panel, 2010), this work was initiated as result of the review or revision of phytosanitary policies and priorities. As explained in the background of the European Commission request, the objective of this mandate is to provide updated scientific advice to European risk managers to take into consideration when evaluating whether those organisms listed in the Annexes of Council Directive 2000/29/EC deserve to remain regulated under Council Directive 2000/29/EC, or whether they should be regulated in the context of the marketing of plant propagation material, or should be deregulated. Therefore, to facilitate the decision-making process, in the conclusions of the pest categorisation, the Panel addresses explicitly each criterion for a quarantine pest in accordance with ISPM 11 (FAO, 2013) but also for a regulated non-quarantine pest (RNQP) in accordance with ISPM 21 (FAO, 2004) and includes additional information required as per the specific terms of reference received by the European Commission. In addition, for each conclusion, the Panel provides a short description of its associated uncertainty.

Table 1 below presents the ISPM 11 (FAO, 2013) and ISPM 21 (FAO, 2004) pest categorisation criteria on which the Panel bases its conclusions. It should be noted that the Panel's conclusions are formulated respecting its remit and particularly with regards to the principle of separation between risk assessment and risk management (EFSA founding regulation<sup>4</sup>); therefore, instead of determining whether the pest is likely to have an unacceptable impact, the Panel will present a summary of the observed pest impacts. Economic impacts are expressed in terms of yield and quality losses and not in monetary terms, in agreement with EFSA guidance on a harmonised framework for pest risk assessment (EFSA PLH Panel, 2010).

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<sup>4</sup> Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31/1, 1.2.2002, p. 1–24.



**Table 1:** International Standards for Phytosanitary Measures ISPM 11 (FAO, 2013) and ISPM 21 (FAO, 2004) pest categorisation criteria under evaluation.

<b>Pest categorisation criteria</b>	<b>ISPM 11 for being a potential quarantine pest</b>	<b>ISPM 21 for being a potential regulated non-quarantine pest</b>
<b>Identity of the pest</b>	The identity of the pest should be clearly defined to ensure that the assessment is being performed on a distinct organism, and that biological and other information used in the assessment is relevant to the organism in question. If this is not possible because the causal agent of particular symptoms has not yet been fully identified, then it should have been shown to produce consistent symptoms and to be transmissible	The identity of the pest is clearly defined
<b>Presence (ISPM 11) or absence (ISPM 21) in the PRA area</b>	The pest should be <b>absent from all or a defined part of the PRA area</b>	The pest is <b>present</b> in the PRA area
<b>Regulatory status</b>	If the pest is present but not widely distributed in the PRA area, it should be under official control or expected to be under official control in the near future	The pest is under official control (or being considered for official control) in the PRA area with respect to the specified plants for planting
<b>Potential for establishment and spread in the PRA area</b>	The PRA area should have ecological/climatic conditions including those in protected conditions suitable for the establishment and spread of the pest and, where relevant, host species (or near relatives), alternate hosts and vectors should be present in the PRA area	–
<b>Association of the pest with the plants for planting and the effect on their intended use</b>	–	Plants for planting are a pathway for introduction and spread of this pest
<b>Potential for consequences (including environmental consequences) in the PRA area</b>	There should be clear indications that the pest is likely to have an unacceptable economic impact (including environmental impact) in the PRA area	–
<b>Indication of impact(s) of the pest on the intended use of the plants for planting</b>	–	The pest may cause severe economic impact on the intended use of the plants for planting
<b>Conclusion</b>	If it has been determined that the pest has the potential to be a quarantine pest, the PRA process should continue. If a pest does not fulfil all of the criteria for a quarantine pest, the PRA process for that pest may stop. In the absence of sufficient information, the uncertainties should be identified and the PRA process should continue	If a pest does not fulfil all the criteria for a regulated non-quarantine pest, the PRA process may stop



In addition, in order to reply to the specific questions listed in the terms of reference, three issues are specifically discussed only for pests already present in the EU: the analysis of the present EU distribution of the organism in comparison with the EU distribution of the main hosts, the analysis of the observed impacts of the organism in the EU, and the pest control and cultural measures currently implemented in the EU.

The Panel will not indicate in its conclusions of the pest categorisation whether to continue the risk assessment as it is clearly stated in the terms of reference that at the end the pest categorisation the European Commission will indicate if further risk assessment work is required following their analysis of the Panel's scientific opinion.

## **2.2. Data**

### **2.2.1. Literature search**

A literature search on *Spiroplasma citri* was conducted at the beginning of the mandate. The search was conducted for the scientific name of the pest on the CAB Abstract and web based search engines such as Google Scholar. Further references and information were obtained from experts, from citations within the references as well as from grey literature.

### **2.2.2. Data collection**

To complement the information concerning the current situation of the pest provided by the literature and online databases on pest distribution, damage and management, the PLH Panel sent a short questionnaire on the current situation at country level based on the information available in the European and Mediterranean Plant Protection Organization (EPPO) Plant Quarantine Retrieval (PQR) to the National Plant Protection Organisation (NPPO) contacts of all the EU Member States and of Iceland and Norway. Iceland and Norway are part of the European Free Trade Association (EFTA) and are contributing to EFSA data collection activities, as part of the agreements EFSA has with these two countries. A summary table on the pest status based on EPPO PQR and MS replies is presented in Table 2.

## **3. Pest categorisation**

### **3.1. Identity and biology of the pest**

#### **3.1.1. Taxonomy**

Kingdom: bacteria; phylum: Tenericutes; class: Mollicutes; order: Entomoplasmatales; family: Spiroplasmataceae; genus: *Spiroplasma*, subgroup: I-1 (Williamson et al., 1998); species: *Spiroplasma citri* (Saglio et al., 1973).

#### **3.1.2. Biology of *Spiroplasma citri***

*S. citri* belongs to the genus *Spiroplasma*, a group of helical wall-less bacteria (Mollicutes) mainly associated with arthropods (insects, mites, crustaceans). *Spiroplasma* spp. can be commensals or parasites and even be involved in symbiosis. Out of 45 known species, only three have been associated with plant diseases propagated by leafhoppers: *S. citri*, *S. kunkelii* (maize stunt disease), and *S. phoeniceum* (periwinkle yellows) (Gasparich, 2010).

*S. citri* is responsible for stubborn disease of citrus (Saglio *et al.*, 1973). It inhabits the phloem sieve tubes of infected plants to which it is transmitted by polyphagous phloem feeding leafhoppers in a persistent and propagative manner (Liu *et al.*, 1983a; b). *S. citri* can infect a wide range of plant species including crops such as citrus, horse radish, sesame, carrot and safflower (Saglio *et al.*, 1971; Fletcher *et al.*, 1981; Kersting and Segonca, 1992; Lee *et al.*, 2006; Khanchezar *et al.*, 2012) and several wild plants (Calavan and Bové, 1989). The pathway of *S. citri* through one of its natural vectors, *Circulifer tenellus*, is well established (Liu *et al.*, 1983a;b). In order to be transmitted to

plants, two physical barriers within the leafhopper, the gut and salivary gland walls, must be crossed by *S. citri*. When the insects are feeding on infected plants, spiroplasmas move from the food canal through the foregut and accumulate in the midgut and hindgut of the insect. Then, they move across the basal lamina, circulate in the hemocoel where they multiply, enter the salivary glands, multiply again and are released into the salivary canal. In both midgut epithelia and salivary gland cells, spiroplasmas usually occur in membrane-bound cytoplasmic vesicles (Kwon *et al.*, 1999). There is no known transovarial transmission of *S. citri* from infected female insect vectors to their progeny (Liu *et al.*, 1983a; Fletcher and Eastman, 1984; Oldfield *et al.*, 1984). *S. citri* affects the longevity and fecundity of *C. tenellus* (De Almeida *et al.*, 1997). Once present in the salivary canal, *S. citri* can flow with salivary secretions through the saliva duct into the host plant during feeding. *S. citri* colonizes plants by spreading and multiplying in the phloem sap. *S. citri* induces chlorotic leaf mottling and stunting of the plant by consuming the sap fructose produced through sucrose hydrolysis by the phloemian companion cells (Bové *et al.*, 2003). There is no report of transmission through seeds from infected plants (Uygur, 1998; Chang and Zheng, 1999). The optimal temperature promoting multiplication of *S. citri* is 32°C, a temperature at which *S. citri* induces wilting in herbaceous hosts such as the Madagascar periwinkle *Catharanthus roseus* (Saillard *et al.*, 1984). It has also been shown that *S. citri* very poorly multiply at temperatures below 25°C in artificial culture medium (Saglio *et al.*, 1973).

### 3.1.3. Intraspecific diversity

*S. citri* strains differ in chromosome size, which varies from 1.6 to 1.9 Mbp (Ye *et al.*, 1995). Part of the size variation is thought to result from differences in the quantity of prophage sequences (Melcher and Fletcher., 1999), which are particularly abundant in the *S. citri* chromosome (Carle *et al.*, 2010). *S. citri* strains can also carry various plasmids (Ranhand *et al.*, 1980) whose absence or presence has direct impacts on insect transmissibility of the different strains as they encode surface proteins involved in insect transmission (Davis *et al.*, 2005; Berho *et al.*, 2006; Saillard *et al.*, 2008; Breton *et al.*, 2010). *S. citri* strains were initially classified upon sequencing of the gene encoding spiralin, a variable major surface protein involved in insect transmission (Foissac *et al.*, 1996; Killiny *et al.*, 2005; Duret *et al.*, 2003, 2014). Up until now, eight different genotypes have been differentiated (Khanchezar *et al.*, 2014). Random amplified polymorphism DNA polymerase chain reaction (RAPD-PCR) proved to be efficient in differentiating *S. citri* strains occurring in the USA, but no strain classification was described (Mello *et al.*, 2008). Recent surveys found no correlation between symptom severity and particular genotype but, instead, confirmed that severity depends on the spiroplasma titre in the plant (Mello *et al.*, 2010b).

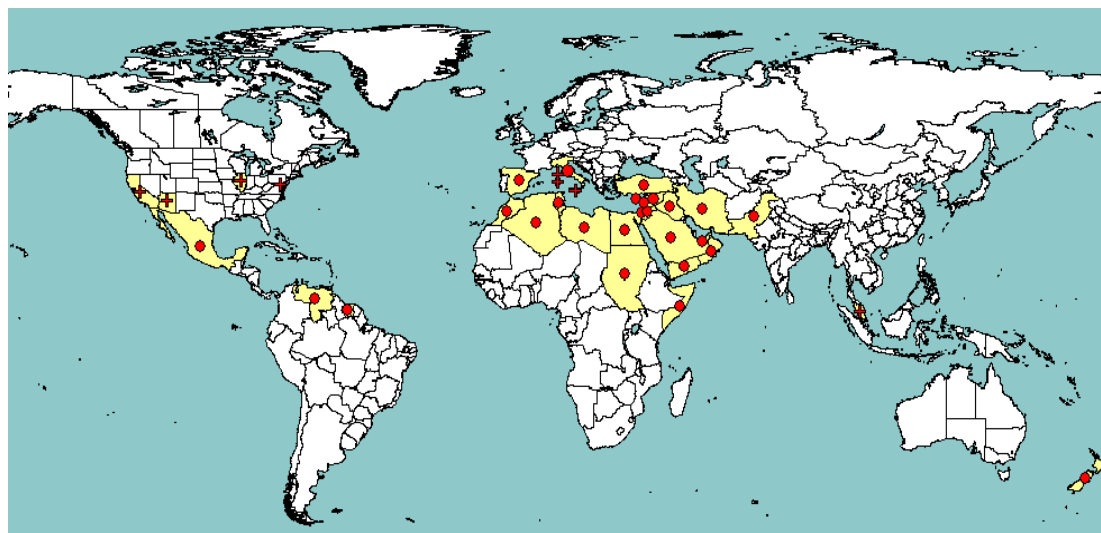
### 3.1.4. Detection and identification of *Spiroplasma citri*

Field diagnosis of *S. citri*-induced diseases is difficult because foliar symptoms can resemble nutritional deficiencies or symptoms caused by other phloem-restricted pathogens such as huanglongbing in citrus (Bové and Garnier, 2000). Routine detection of *S. citri* from both plants and insects is by culturing in cell-free liquid medium containing animal serum followed by examination of the organism for helical morphology and motility by darkfield microscopy (Saglio *et al.*, 1973; Tully *et al.*, 1977; Lee *et al.*, 1983). This method, however, lacks sensitivity in comparison with serological and molecular approaches. Serological detection by enzyme-linked immunosorbent assay (ELISA) using polyclonal antisera has been proven to be efficient and reliable (Clark *et al.*, 1978; Saillard *et al.*, 1984a). It must be noted that anti-*S. citri* polyclonal antisera cross-react with other spiroplasmas of group I (Whitcomb *et al.*, 1987). PCR amplification of the spiralin gene (Foissac *et al.*, 1996) has been recently improved to give better sensitivity by implementing nested PCR (Khanchezar *et al.*, 2012). Proper identification can be achieved by sequencing spiralin-amplified PCR products or 16S PCR amplification and sequencing (Gasparich *et al.*, 2004). PCR and real-time PCR targeting multicopy genes *P89* and *P58* are currently the most sensitive detection systems (Yokomi *et al.*, 2008). Recently, a rapid detection system based on the serological detection of a *S. citri*-secreted protein has been developed and proven to be as efficient as real-time PCR (Shi *et al.*, 2014).

### 3.2. Current distribution of *Spiroplasma citri*

#### 3.2.1. Global distribution of *Spiroplasma citri*

Figure 1 shows the global distribution of *S. citri*. Although “stubborn disease” was reported in 1944 by Fawcett et al., and “little leaf disease” of citrus was described in Palestine by Reichert in 1928 (reviewed by Calavan and Bové, 1989), its causal agent, *S. citri*, was not identified until the 1970s (Saglio et al., 1973).



**Figure 1:** Global distribution of *Spiroplasma citri* (extracted from EPPO PQR, version 5.3.1, accessed July 2014). Red circles represent pest presence as national records and red crosses represent pest presence as sub-national records (note that this figure combines information from different dates, some of which could be out of date).

**Africa:** Algeria, Egypt, Libya, Madagascar, Morocco, Somalia, Sudan and Tunisia. Madagascar: unreliable records according to EPPO PQR.

**America:** Mexico, Suriname, USA and Venezuela.  
Argentina, Brazil, and Peru: unreliable records according to EPPO PQR.

**Asia:** Iran, Iraq, Israel, Jordan, Lebanon, Malaysia, Oman, Pakistan, Turkey, Saudi Arabia, Syria, United Arab Emirates, and Yemen.

**Oceania:** New Zealand.

#### 3.2.2. *Spiroplasma citri* distribution in the EU

No interception from third countries is documented to date in the EUROPHYT database.

**Table 2:** Current distribution of *Spiroplasma citri* in the 28 EU MSs, Iceland and Norway, based on answers received via email from N:PPOs or/and other sources.

Country	NPPO answers	Other sources
Austria	Absent, no pest record	
Belgium	Absent, no pest records	
Bulgaria	Absent	
Croatia	Absent	
Cyprus		Present, widespread (EPPO PQR)

Country	NPPO answers	Other sources
Czech Republic	Absent, no record	
Denmark	Not known to occur	
Estonia	Absent, no pest record	
Finland	Absent, no pest record	
France	Present	Present, only in Corsica with restricted distribution (Fos et al 1986, EPPO PQR, 2014)
Germany	Absent, no pest record	
Greece		Absent, no longer present
Hungary	Absent, no data	
Ireland	Absent, no pest record	
Italy	Absent in Sicily	Present, few occurrences (EPPO PQR)
Latvia	-	-
Lithuania	-	-
Luxembourg	-	-
Malta	Absent, no pest record	
Poland	Absent, no pest records	
Portugal	No records	
Romania	-	-
Slovak Republic	Absent: no pest records	
Slovenia	Absent: no pest records on <i>Citrus</i> L., <i>Fortunella</i> Swingle, <i>Poncirus</i> Raf.	
Spain	Present, few occurrences, no recent reports	In Spain, <i>S. citri</i> symptoms are not easily observable as the temperatures are not as high as in the countries where it normally occurs Cambra M, 2008, unpublished data Cambra M, Curso pasaporte Fitosanitari. Non published. First report of <i>Spiroplasma citri</i> in carrot in Europe (Cebrián, <i>et al.</i> , 2010)
Sweden	Absent Comment: no citrus host plants	
The Netherlands	absent, no pest records	
United Kingdom	Absent	
Iceland	-	-
Norway	-	-
Switzerland <sup>(a)</sup>	-	-

(a): Switzerland was not included in the NPPO consultation.

– no information available; EPPO PQR, European and Mediterranean Plant Protection Organization Plant Quarantine Data Retrieval System; NPPO, National Plant Protection Organisation.

### 3.2.3. Vectors and their distribution in the EU

*S. citri* is known to be transmitted by seven species of leafhoppers (Cicadellidae Deltocephalinae): *C. tenellus* (Baker), *C. haematoceps* (Mulsant & Rey), *C. opacipennis* (Lethierry), *Macrostelus fascifrons* Fieber, *Scaphytopius nitridus* DeLong, *S. acutus* (Say) and *S. californiensis* Hepner (Calavan and Bové, 1989; Kersting and Sengonca, 1992). Of these, only the first three species are reported in Europe (de Jong, 2013) and are believed to be of Old World origin (Calavan and Bové, 1989). The other species are native to, and spread in, the New World.

Within the EU, *C. tenellus* is reported from Southern France, Italy (Sicily) and Spain (including the Canary Islands) while *C. haematoceps* is reported from Austria, Cyprus, the Czech Republic, France (including Corsica), Greece, Hungary, Italy (including Sardinia and Sicily), Poland, Romania and Spain (including the Canary Islands) (de Jong, 2013). According to Calavan and Bové (1989), *C.*

*haematoceps* is also present in Germany. This species is therefore more common than *C. tenellus* in Europe, the Mediterranean area and the Middle East (Calavan and Bové, 1989). *C. opacipennis* has been reported in Cyprus, France, Greece, Hungary, Italy (including Sardinia and Sicily) and Spain (de Jong, 2013). This species has been found to be very abundant in France on lavender (Nusillard and Villevielle, 1998).

*C. tenellus* and *C. haematoceps* are polyphagous and multivoltine and they overwinter as adults on suitable weed hosts, largely in uncultivated areas. Females lay eggs in the leaf veins and petioles of weeds. Populations of *C. haematoceps* and *C. tenellus* can be abundant on *Atriplex sp* or *Salsola pestifer* in California (*Amaranthaceae* and *Chenopodiaceae*) (Severin, 1933), on *S. kali*, *Chenopodium album* (*Chenopodiaceae*), *Mathiola sinuata* and *M. incana* (*Brassicaceae*) or *Alhagi mannifera* (*Fabaceae*) (Fos et al., 1986; Bové et al., 1988; Klein and Raccach, 1991). Most of these plant species are widely distributed around the Mediterranean and the Middle East regions (Bové et al., 1988). However, these plant species have not been described as hosts for *S. citri*. Both insect species are known vectors of the beet curly top viruses (Oldfield et al., 1977b; Taheri et al., 2012); *C. tenellus* also transmits the phytoplasma associated with the beet leafhopper-transmitted virescence (Golino et al., 1987). Similarly, *C. opacipennis* is polyphagous and multivoltine and is a known vector of the beet curly top virus (Kaur et al., 1971). Polyphagous vectors can acquire the spiroplasmas from weed species and transmit them to citrus (Calavan and Bové, 1989; De Almeida et al., 1997). However, citrus to citrus transmission has also been demonstrated (Oldfield et al., 1977a).

Besides vector species (that have been proven to be able to transmit *S. citri* from plant to plant), a number of other insects showed some relationships with this mollicute. After membrane feeding on a concentrated *S. citri* suspension, the leafhoppers *Aceratagallia sp.*, *Acinopterus angulatus*, *Erythroneura variabilis* and *Hordnia circellata* and the membracid *Spissistilus festinus* acquired the pathogen, which was then successfully cultured from the insects' bodies (Rana et al., 1975). The leafhopper *Euscelidius variegatus* has been extensively used as an experimental vector after injection with *S. citri* or feeding through Parafilm™ on *S. citri* suspension (Markham, 1983; Markham and Townsend, 1979; reviewed by Calavan and Bové, 1989). Similarly, the leafhopper *Euscelis incisus* (Kirschabum) (formerly *E. plebejus*) has been used as experimental vector following injection (Townsend et al., 1977). Moreover, *S. citri* was cultured from field-collected *Ollarianus strictus* leafhoppers (Oldfield et al., 1984) and other leafhopper species that acquired *S. citri* from periwinkle (i.e. *Aceratagallia curvata*, *Euscelidius variegatus* and *Graminella sonora*) retained the infection (Oldfield et al., 1984). However, all transmission attempts with these leafhoppers were unsuccessful (Oldfield et al., 1984; reviewed by Calavan and Bové, 1989). Among all these species not known to be vectors, only *E. variegatus* is present (and widespread) in Europe, but the fact that, in addition to the above, it has never been found infected under natural conditions, or after feeding on source plants under experimental conditions, suggests that it is a very unlikely vector.

### 3.3. Regulatory status

*S. citri* is considered as a harmful organism in the EU. It is listed as such in Council Directive 2000/29/EC. In addition, the Directive also considers some host plants and insect vectors of *S. citri*.

Among the known vectors, only *C. haematoceps* and *C. tenellus* are considered in Council Directive 2000/29/CE, when present on plants of *Citrus spp.*, *Fortunella spp.*, *Poncirus spp.* and their hybrids, other than fruit and seeds.

Among the host plants, only *Citrus spp.* are considered in Council Directive 2000/29/CE.

#### 3.3.1. Legislation addressing *Spiroplasma citri*

*Spiroplasma citri* is considered as a harmful organism in the EU. It is listed as such in Council Directive 2000/29/EC in Tables 3-7.



**Table 3:** *Spiroplasma citri* in Council Directive 2000/29/EC

<b>Annex II, Part A</b>	Harmful organisms whose introduction into, and whose spread within, all Member States shall be banned if they are present on certain plants or plant products,
<b>Section II</b>	Harmful organisms known to occur in the Community and relevant for the entire Community,
<b>(d)</b>	Viruses and virus-like organisms
<b>10</b>	Plants of <i>Citrus L.</i> , <i>Fortunella Swingle</i> , <i>Poncirus Raf.</i> , and their hybrids, other than fruit and seeds

### 3.3.2. Legislation addressing vectors of *Spiroplasma citri*

**Table 4:** Vectors of *Spiroplasma citri* in Council Directive 2000/29/EC

<b>Annex II, Part A</b>	Harmful organisms whose introduction into, and whose spread within, all Member States shall be banned if they are present on certain plants or plant products,
<b>Section II</b>	Harmful organisms known to occur in the Community and relevant for the entire Community,
<b>(a)</b>	Insects, mites and nematodes, at all stages of their development
<b>5. <i>Circulifer haematoceps</i> and 6. <i>Circulifer tenellus</i></b>	Plants of <i>Citrus L.</i> , <i>Fortunella Swingle</i> , <i>Poncirus Raf.</i> , and their hybrids, other than fruit and seeds

### 3.3.3. Legislation addressing plants, plant parts or other objects of species hosts of *Spiroplasma citri*

EU Council Directive 2000/29/CE addresses *Citrus spp.* in its Annexes III, IV and V. *Fortunella* and *Poncirus* are considered to be minor or incidental hosts for *S. citri* (EPPO PQR, 2014). Other plant species are not considered in relation to *S. citri*.

Annex III deals with plants, plant products and other objects the introduction of which shall be prohibited in all MSs or in certain protected zones.

Annex IV deals with special requirements which must be laid down by all MSs for the introduction and movement of plants, plant products and other objects into and within all MSs, or into and within certain protected zones.

Annex V deals with plants, plant products and other objects which must be subject to a plant health inspection (at the place of production if originating in the Community, before being moved within the Community in the country of origin or the consignor country, if originating outside the Community) before being permitted to enter the Community.

**Table 5:** Plants considered to be hosts of *Spiroplasma citri* in Annex III of Council Directive 2000/29/EC

<b>Annex III Part A</b>	Plants and plant products and other objects the introduction of which shall be prohibited in all Member States as it follows
<b>Description</b>	<b>Country of origin</b>
16. Plants of <i>Citrus L.</i> , <i>Fortunella Swingle</i> , <i>Poncirus Raf.</i> , and their hybrids, other than fruit and seeds	Third countries

**Table 6:** Plants or plant parts considered as hosts of *Spiroplasma citri* in Annex IV of Council Directive 2000/29/EC

<b>Annex IV, Part A</b>	Special requirements which must be laid down by all Member States for the introduction and movement of plants, plant products and other objects into and within all Member States
<b>Section II</b>	Plants, plant products and other objects originating in the Community,
<b>Plants, plant products and other objects</b>	<b>Special requirements</b>
10. Plants of <i>Citrus</i> L., <i>Fortunella</i> Swingle, <i>Poncirus</i> Raf., and their hybrids, other than fruit and seeds	<p>Official statement that:</p> <p>(a) the plants originate in areas known to be free from <i>Spiroplasma citri</i> (...); or</p> <p>(b) the plants derive from a certification scheme requiring them to be derived in direct line from material which has been maintained under appropriate conditions (...) and have been growing permanently in an insect proof glasshouse or in an isolated cage on which no symptoms of <i>Spiroplasma citri</i> (...) have been observed ;</p> <p>and</p> <p>have been inspected and no symptoms of <i>Spiroplasma citri</i> (...) have been observed since the beginning of the last complete cycle of vegetation.</p> <p>or</p> <p>(c) the plants: — have been derived from a certification scheme requiring them to be derived in direct line from material which has been maintained under appropriate conditions and has been subjected to official individual testing for, at least Citrus vein enation woody gall and Citrus tristeza virus (European strains), using appropriate indicators or equivalent methods, approved in accordance with the procedure referred to in Article 18(2), and has been found in these tests, free from Citrus tristeza virus (European strains), and certified free from at least Citrus tristeza virus (European strains) in official individuals tests carried out according to the methods mentioned in this indent, and — have been inspected and no symptoms of <i>Spiroplasma citri</i> Saglio et al., <i>Phoma tracheiphila</i> (Pandri) Kanchaveli et Gikashvili, and of Citrus vein enation woody gall and Citrus tristeza virus have been observed since the beginning of the last complete cycle of vegetation.</p>

**Table 7:** Plants or part of plants that are hosts of *Spiroplasma citri* in Annex V of Council Directive 2000/29/EC

<b>Annex V, Part A</b>	Plants, plant products and other objects originating in the Community
<b>Section I</b>	Plants, plant products and other objects which are potential carriers of harmful organisms of relevance for the entire Community and which must be accompanied by a plant passport
<b>1.4</b>	Plants of <i>Fortunella</i> Swingle, <i>Poncirus</i> Raf., and their hybrids and <i>Citrus</i> L., other than fruit and seeds.
<b>1.5</b>	Plants of <i>Citrus</i> L. and their hybrids other than fruit and seeds.

### 3.3.4. Marketing directives

Council Directive 2008/90/EC regulates the marketing of fruit plant propagating material and fruit plants intended for fruit production.



### 3.4. Elements to assess the potential for establishment and spread in the EU

#### 3.4.1. Host range

*S. citri* had been identified as a pathogen of sweet orange (*Citrus sinensis*) but its plant host range is much wider than citrus owing to its transmission by polyphagous leafhoppers. The known host range was reviewed by Calavan and Bové (1989) with few new plant hosts being discovered later on (Table 8). In addition to naturally infected plant hosts, several experimental plant hosts have been infected through forced inoculation with leafhopper vectors (Calavan and Bové; 1989).

**Table 8:** Natural plant hosts of *Spiroplasma citri* as reviewed by Calavan and Bové, 1989 and Kersting and Baspinar, 1997.

Plant family	Latin name	Common name
<i>Amaryllidaceae</i>	<i>Allium cepa</i>	Onion
<i>Apiaceae</i>	<i>Daucus carota</i>	Carrot
<i>Apocynaceae</i>	<i>Catharanthus roseus</i>	Madagascar periwinkle
<i>Asteraceae</i>	<i>Callistephus chinensis</i>	China aster
	<i>Lactuca sp.</i>	Lettuce
	<i>Tagetes erecta</i>	American marigold
	<i>Zinnia elegans</i>	Zinnia
<b>Brassicaceae</b>	<i>Arabidopsis thaliana</i>	Arabidopsis
	<i>Armoracia rusticana</i>	Horse radish
	<i>Brassica chinensis</i> ,	Chinese mustard
	<i>Brassica geniculata</i>	Short pod mustard
	<i>Brassica nigra</i>	Black mustard
	<i>Brassica napobrassica</i>	Rutabaga
	<i>Brassica oleracea botrytis</i>	Broccoli
	<i>Brassica oleracea capitata</i>	Cabbage
	<i>Brassica oleracea gemmifera</i>	Brussels sprout
	<i>Brassica pekinensis</i>	Chinese cabbage
	<i>Brassica rapa</i>	Turnip
	<i>Brassica tournefortii</i>	Wild turnip
	<i>Hirshfeldia incana</i>	Hoary Mustard
	<i>Sisymbrium irio</i> , <i>S. orientale</i>	Rockets
	<i>Raphanus sativus</i>	Radish
<i>Raphanus raphanistrus</i>	Wild radish	
<i>Cactaceae</i>	<i>Opuntia tuna monstrosa</i>	Tree cactus
<i>Crassulaceae</i>	<i>Sedum prealtum</i>	<i>Sedum prealtum</i>
<i>Cucurbitaceae</i>	<i>Citrullus vulgaris</i>	Watermelon
	<i>Cucurbita pepo melopepo</i>	Squash
<i>Plantaginaceae</i>	<i>Plantago ovata</i>	Blond Psyllium

Plant family	Latin name	Common name
<i>Rosaceae</i>	<i>Prunus avium</i>	Cherry
	<i>Prunus persica</i>	Peach
	<i>Pyrus communis</i>	Pear
<i>Rutaceae</i>	<i>Citrus sinensis</i>	Sweet orange
	<i>Citrus grandis</i>	Pomelo
	<i>Citrus paradisi</i>	Grapefruit
	<i>Citrus reticulata</i>	Tangerin
<i>Scrophulariaceae</i>	<i>Digitalis purpurea</i>	Foxglove
<i>Violaceae</i>	<i>Viola cornuta</i>	Violet

### 3.4.2. EU distribution of main host plants

The citrus species commonly cultivated in Europe belong to three rutaceous genera: *Citrus*, *Fortunella* and *Poncirus*. All three genera are considered together in the EU Regulation. *Citrus* is known as the main host of *S. citri* but *Fortunella* and *Poncirus* are considered to be minor or incidental host (EPPO PQR, 2014). Twelve citrus species are cultivated worldwide.

Citrus is grown commercially for fruit production in all the countries of the southern EU with a Mediterranean climate: Croatia, Cyprus, France, Greece, Italy, Malta, Portugal and Spain.

The cultivated area of orange, lemon and small fruit citrus varieties in the EU by country and NUTS2 (Nomenclature of Territorial Units for Statistics) region is given in Table 9. Citrus are widely available with a production area in the EU-28 estimated at 494 913 ha in 2007 and located in eight countries: (1) Spain (314 908 ha); (2) Italy (112 417 ha); (3) Greece (44 252 ha); (4) Portugal (16 145 ha); (5) Cyprus (3 985 ha); (6) France (1 705 ha); (7) Croatia (1 500 ha); and (8) Malta (193 ha). Figure 2 is a map showing the EU NUTS3 citrus-growing regions, based on total area cultivated with citrus species in the EU NUTS3 regions as extracted from the national statistical databases of the EU citrus-growing countries (Portugal, Spain, France, Italy, Croatia, Greece, Malta, Cyprus).

**Table 9:** Area of production (× hectares) of citrus in the EU in 2007. Citrus-producing EU MSs are sorted alphabetically in the first column. Data extracted from EUROSTAT on 21 February 2013.

Country /region	Orange varieties	Lemon varieties	Small-fruited citrus varieties	All citrus varieties (*)
European Union (27 countries)	279 048.00	62 854.80	151 510.00	493 413.00
<b>Cyprus</b>	1 554.00	665.00	1 766.00	3 985.00
<b>France</b> (Corsica)	28.55	22.70	1 654.21	1 705.46
<b>Greece</b>	32 439.9	5 180.49	6 631.71	44 252.1
<b>Italy</b>	73 785.90	16 633.60	21 997.90	112 417.40
<b>Portugal</b>	12 416.00	494.04	3 235.21	16 145.00
<b>Spain</b>	158 824.00	39 859.00	116 225.00	314 908.00

(\*) = calculated as the sum of orange varieties, lemon varieties and small fruited citrus varieties.

### 3.4.3. Analysis of the potential distribution of *Spiroplasma citri* in the EU

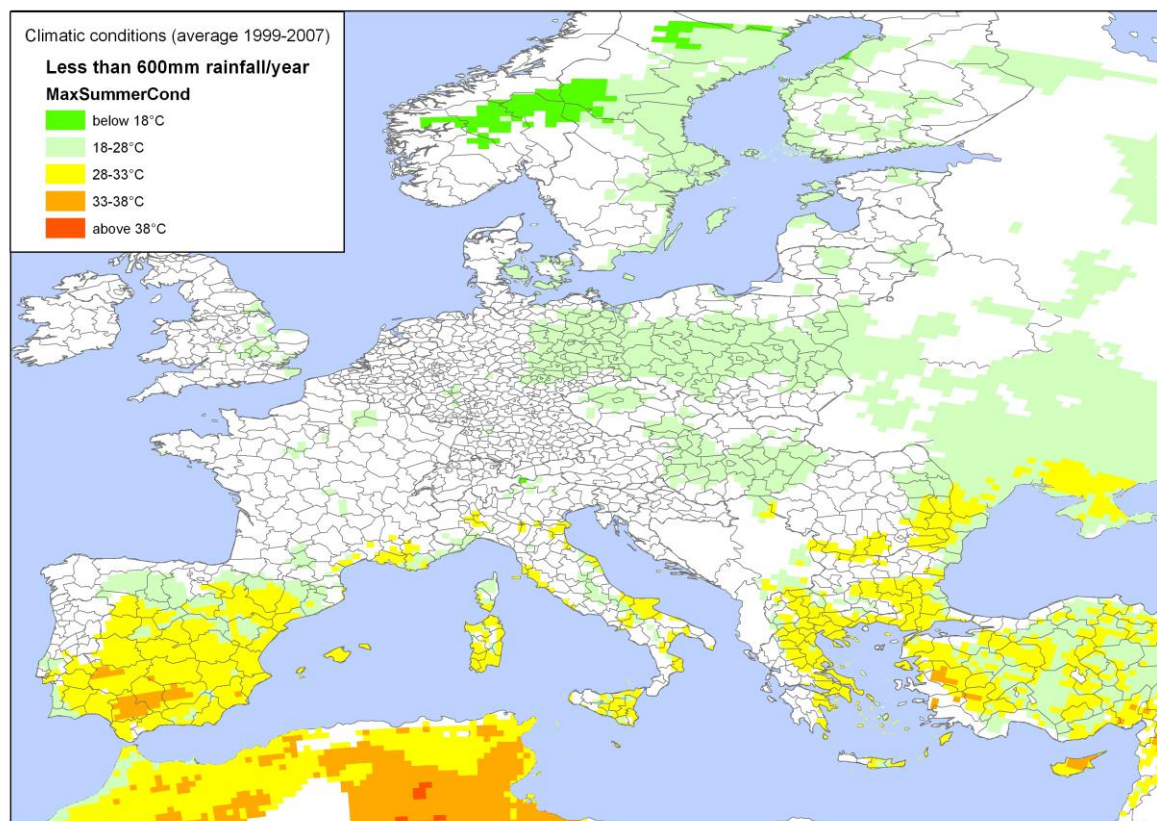
*S. citri* is currently present in Cyprus where it is widespread. It has scarcely been reported in Italy and Spain. In France (Corsica), *S. citri* has been detected only in the insect vectors (Brun et al., 1987). It is present in North Africa, the Near East and Middle East, mostly in warm and semi-arid areas (precipitation 250-500 mm/year) and in deserts (precipitation below 250 mm/year) where both suitable host plants and vectors do occur (Bové et al., 1988).

The pest is considered to be absent in Europe except in the Mediterranean area, despite the fact that some alternative host plants (see section 3.4.1., Table 7) are largely distributed in Europe. According to Calavan and Bové (1989), spiroplasmas are directly affected by temperature, with a clear relationship between temperature, in vivo growth of *S. citri* and severity of disease symptoms. Mello et al. (2010b) have also shown a clear relationship between the symptom severity and *S. citri* titer. The disease is far more severe under hot and arid climates. The heat tolerances of *S. citri* and citrus are considered as similar based on the work of Olson and Rogers (1969). Only mild symptoms, if any, are recorded in areas where the field temperature does not exceed 28°C (Calavan and Gumpf, 1974; Vogel and Bové, 1974).

Known hosts of *S. citri* (see table 8) are mostly annual herbaceous plants. Some are herbaceous perennials and a few, such as *Citrus* spp. or *Prunus* spp. are woody perennials. Notably, the host survival varies according to the plant species, but *Citrus* spp. are rarely killed.

*S. citri* can overwinter in herbaceous biennials or perennials, or even in winter annuals such as plantain or wild turnip (Calavan et al., 1979, Oldfield and Kaloostian, 1979, Kloepper and Garrott, 1981). *S. citri* is also known to overwinter in the adult stage of the vectors (Calavan and Bové, 1989).

Stubborn disease of citrus has been studied for over more than half a century in the Mediterranean area, where it is thought to have occurred for a long time (Calavan and Bové, 1989). According to Figure 2 and the temperature and precipitation conditions found in areas where the disease is reported, it appears that the extension of the disease North of the Mediterranean rim in Europe would probably be limited. Nevertheless horseradish brittle root is an example of the possible movement of *S. citri* outside the citrus growing area (Fletcher, 1983). Based on the climatic conditions that favour the disease (Calavan and Gumpf, 1974, Vogel and Bové, 1974) (see Figure 2), extension in unaffected citrus areas is possible.



**Figure 2:** Map of Europe showing areas where the annual average rainfall (average for years 1999–2007) is less than 600 mm, together with the maximum temperature in June, July and August. (Climatic data from the JRC-MARS (Joint Research Centre Monitoring Agricultural Resources) meteorological database interpolated to a grid of 25 km resolution, average of years 1999–2007) (Biavetti et al, 2014).

#### 3.4.4. Spread capacity

Many elements above support the idea that *S. citri* has a large capacity to spread:

#### 3.5. Elements to assess the potential for consequences in the EU

- It is widely distributed worldwide, including islands, e.g. Corsica (France), Sicily and Sardinia (Italy) (see sections 3.2.1, Global distribution of *S. citri*, and 3.2.2, *S. citri* distribution in the EU).
- It has a large number of host plants (see sections 3.4.1, Host range, and 3.4.2, EU distribution of main hosts), including weeds as well as cultivated plants, allowing it a choice of host in space and time.
- Three known vectors are present in the EU (among which two species, *C. haematoceps* and *C. opacipennis*, are widely distributed) and a large number of host plants of these vectors is also present (see section 3.2.3, Vectors and their distribution in the EU).
- Some of its main vectors considerable fecundity and dispersal capacity. For example, DeLong (1971) mentions a maximum record of 675 eggs for a female of *C. tenellus* and describes mass migrations, presumably wind-borne with “piling up” at weather fronts and usually associated with large populations. To illustrate this, Glick (1957) reports catching a *C. tenellus* individual at an altitude of 2 000 feet (610 m) from a plane in Texas.

### 3.5.1. Pest effects of *Spiroplasma citri*

Stubborn disease was named based on the fact that bud-grafted trees grew slowly (Calavan and Bové, 1989). The disease affects both the quality and the yield of fruit (Mello et al., 2010a). It is correlated with the occurrence of warm and dry periods of weather.

The disease is characterized by stunted trees, with short internodes and small, abnormally upright leaves sometimes mottled or chlorotic (Shi *et al.*, 2014). Shoots may be abnormally bunched and like a witches' broom, premature leaf drop and twig dieback are also found. Flowering sometimes occurs off-season. Fruits are, misshapen or abnormally coloured. Fruit production may be reduced in affected trees (Bové et al, 1988; Gumpf, 1988).

Yield losses are variable. In California, USA, losses of Valencia oranges of 44 to 74 % and of navel oranges of up to 100 % have been reported (Calavan, 1979). Mello et al. (2010a) have studied the impact of citrus stubborn disease on navel orange. They showed that a significant reduction in fruit number occurred only in severely symptomatic trees in which *S. citri* was largely distributed within the tree.

The disease was at first believed to spread only by budding. However, the detection of several thousands of stubborn-diseased trees in southern California provided evidence for wider natural spread. Calavan (1969) estimated that about 1 000 000 trees were affected by stubborn disease. Visual surveys indicated that from under 1 % to over 50 % of trees in affected orchards appeared to be infected with stubborn disease in California and Morocco (Calavan and Carpenter, 1965). Surveys conducted by Chapot (1959) stressed the wide distribution of stubborn disease around the Mediterranean Sea but found differences with regard to distribution and impact on production (Anonymous, 1970).

Both horseradish brittle root (Fletcher *et al.*, 1981) and carrot purple leaf (Lee *et al.*, 2006) are considered anecdotal, although carrot purple leaf was recently reported in Spain and Israel (Gera *et al.*, 2011).

### 3.5.2. Observed pest impact of *Spiroplasma citri* in the EU

Although stubborn disease of citrus has been reported in several Mediterranean countries, including Spain (Hernandez Gimenez, 1975) and other EU territories such as the islands of Sardinia, Sicily and Corsica (Gumpf, 1988), almost no data on the impact of this disease in the EU are available. In Cyprus, Kyriakou et al. (1996) reported yield reductions of 19 % to 34%, with a reduction in fruit size, weight and quality for both orange cultivars Frost Washington Navel and Frost Valencia.

*S. citri* is commonly thought to be present at low levels in areas where the disease is known to occur (Bové et al., 1988), and damage depends largely on the abundance of the vector and on the occurrence of warm and dry periods of weather. Although *S. citri* affects several host plants other than citrus, most often it does not cause them any economic damage.

Only a limited number of published papers on the pest impact of *S. citri* in the EU are available.

### 3.6. Currently applied control methods

As previously indicated, *S. citri* is not widespread in the EU so information regarding field control methods in use is scarce and relate to the North American situation.

As for most phytoplasmas, it is in practice not possible to cure a plant in the field that is infected by *S. citri*. Thus, it is of primary importance to use healthy planting material. Nevertheless, as the disease is insect transmitted and as other host plants, including weeds, may be present in the environment, removal of already infected plants is of primary importance before planting a new orchard. Wallace (1978), cited by Gumpf (1988), suggested that good control of host weeds in the orchard and its vicinity is good practice, but this is not supported by experimental evidence.



Gumpf (1988) suggested that a trap plants strategy could be used. This consists in planting in the areas surrounding orchards plants that are very attractive to the insect vectors but that are not hosts of *S. citri*. Nevertheless, the practical efficiency of such a strategy has not been confirmed by other authors.

According to Gumpf (1988), it is important to keep young orchards (up to six years old) as clean as possible to limit the impact of *S. citri*. During that period, orchards should be monitored carefully and symptomatic plants rogued as soon as possible.

Insecticide treatments may be used to control vector populations. However, insects already present in an orchard when the insecticide is applied may have enough time to transmit the disease before dying (Gumpf, 1988). Further information on the control of insect vectors will be given in the EFSA opinion on *C. haematoceps* and *C. tenellus*.<sup>5</sup>

### 3.7. Uncertainty

Some uncertainties are linked to the actual distribution of the disease, as symptoms on citrus might be confused with nutritional deficiencies or are simply not easily spotted at low temperatures. On other hosts, the disease might also be asymptomatic. Purple leaf symptoms on carrots may also be caused by phytoplasmas (Cebrián et al., 2010).

In general, knowledge of the epidemiology of *S. citri* is limited (Mello et al., 2010a). The role of hosts other than citrus in the ecology of *S. citri* is still not fully understood. The presence of those host plants throughout the risk assessment area and their epidemiological roles is not fully documented.

The distribution of the insect vectors of *S. citri* in Europe is not completely known.

The literature available on the impact of the disease in Europe where it has been reported so far is very limited. Knowing that the disease impact is correlated with the abundance of the insect vectors, there is some uncertainty in the appraisal of the disease.

## CONCLUSIONS

**Table 10:** The Panel's conclusions on the pest categorisation criteria defined in the International standards for Phytosanitary measures No 11 and No 21 and on the additional questions formulated in the terms of reference (ToR).

Criterion of pest categorisation	Panel's conclusions against ISPM11 criterion	Panel's conclusions against ISPM21 criterion	Uncertainties
<b>Identity of the pest</b>	<p><i>Is the identity of the pest clearly defined?</i>  <i>S. citri</i> is clearly defined as one of the 45 known species of bacteria belonging to the Spiroplasmataceae family, genus <i>Spiroplasma</i>. Different strains are known, but no infra-specific classification is available to date.</p> <p><i>Do clearly discriminative detection methods exist for the pest?</i>            Various detection methods are available at the moment and are appropriate for a discriminative identification of <i>S. citri</i>.</p>		Uncertainty is low.

<sup>5</sup> The EFSA Plant Health Panel scientific opinion on the pest categorisation of *C. haematoceps* and *C. tenellus* is currently under preparation and is expected to be published in January 2015 at <http://www.efsa.europa.eu/en/publications/efsajournal.htm>

Criterion of pest categorisation	Panel's conclusions against ISPM11 criterion	Panel's conclusions against ISPM21 criterion	Uncertainties
<p><b>Absence/presence of the pest in the risk assessment area</b></p>	<p><i>Is the pest absent from all or a defined part of the risk assessment area?</i> According to questionnaires sent back by NPPOs in the risk assessment area, to the EPPO PQR database and to the literature, <i>S. citri</i> is reported to be absent from the risk assessment area except from Cyprus (where it is widespread), France, Italy and Spain (where it is locally reported).</p>	<p><i>Is the pest present in the risk assessment area?</i> <i>S. citri</i> is present in Cyprus, France (Corsica detected on infectious insects), Italy and Spain only. Nevertheless, the host range is large and in most cases, no symptoms are shown.</p>	<p>Uncertainty is medium as information does not result from extensive surveys in most Member-states, as the host range is large and as symptom expression requires high temperature. <i>S. citri</i> may be present asymptotically on many host plants grown throughout the risk assessment area.</p>
<p><b>Regulatory status</b></p>	<p><i>In consideration that the pest under scrutiny is already regulated just mention in which annexes of 2000/29/EC and the marketing directives the pest and associated hosts are listed without further analysis. (the risk manager will have to consider the relevance of the regulation against official control)</i> <i>S. citri</i> is included in Annex II, Part A, Section II of Directive 2000/29/EC. The insect vectors <i>C. haematoceps</i> and <i>C. tenellus</i> are included into the same Directive; Annex II, Part A, Section II. <i>Citrus</i>, the main host plant (as a symptomatic crop host) is listed (a) in Annex III, Part A; (b) in Annex IV, Part A, Section II; (c) in the same Annex, Part B and (d) in Annex V, parts A and B, Section I.</p>		
<p><b>Potential establishment and spread</b></p>	<p><i>Does the risk assessment area have ecological conditions (including climate and those in protected conditions) suitable for the establishment and spread of the pest?</i> <i>And, where relevant, are host species (or near relatives), alternate hosts and vectors present in the risk assessment area?</i> <i>S. citri</i> is disseminated by plants for planting and by seven species of leafhoppers, only three being reported in the risk assessment area. Various plants can be hosts even if only some may show symptoms. The risk assessment area therefore has ecological conditions suitable for the establishment and spread of <i>S. citri</i> at least where citrus is currently grown.</p>	<p><i>Are plants for planting a pathway for introduction and spread of the pest?</i> Plants for planting are a pathway for both introduction and spread of <i>S. citri</i>.</p>	<p>The uncertainties are low.</p>



Criterion of pest categorisation	Panel's conclusions against ISPM11 criterion	Panel's conclusions against ISPM21 criterion	Uncertainties
<b>Potential for consequences in the risk assessment area</b>	<p><i>What are the potential for consequences in the risk assessment area?</i> Provide a summary of impact in terms of yield and quality losses and environmental consequences</p> <p>Although present or reported in the past in Member States around the Mediterranean sea, poor information is available on the impact of the disease. Data from Cyprus indicate yield reductions from 19 to 34%, with reduction in fruit size, weight and quality for two cultivars of navel oranges.</p>	<p><i>If applicable is there indication of impact(s) of the pest as a result of the intended use of the plants for planting?</i></p> <p>There are clear indications of the impact of <i>S. citri</i> as a result of the use of plant for planting as the disease is spread by plant used as planting material.</p>	<p>Uncertainty is considered as medium as the susceptibility of the Citrus species is not fully known.</p>
<b>Conclusion on pest categorisation</b>	<p><i>S. citri</i> is not known to occur from most of the Member States. Nevertheless among citrus growing countries only Croatia, Greece, Malta and Portugal do not report <i>S. citri</i>. The disease seems to be widespread only in Cyprus. Symptom expression is dependent on high temperature. Three leafhopper vector species are reported and widely distributed in the risk assessment area. Yield losses are reported in Cyprus.</p>	<p>Infected planting material could contribute to the dissemination of <i>S. citri</i> as well as the insect vectors. In areas with a hot and dry climate, the impact on the yield and quality may be high.</p>	<p>Overall uncertainty is low to medium.</p>
<b>Conclusion on specific ToR questions</b>	<p><i>If the pest is already present in the EU, provide a brief summary of</i></p> <ul style="list-style-type: none"> <li>- <i>the analysis of the present distribution of the organism in comparison with the distribution of the main hosts, and the distribution of hardiness/climate zones, indicating in particular if in the risk assessment area, the pest is absent from areas where host plants are present and where the ecological conditions (including climate and those in protected conditions) are suitable for its establishment,</i></li> </ul> <p>The main Member states that grow citrus crops are Croatia, Cyprus, France, Greece, Italy, Malta, Portugal and Spain, all countries where the ecological conditions are suitable for the establishment of <i>S. citri</i>. The disease is considered as widespread only in Cyprus, and to be restricted in Italy, Spain and France (Corsica and only on vectors in the early 1980s). <i>S. citri</i> is not reported in the other Member States.</p> <ul style="list-style-type: none"> <li>- <i>the analysis of the observed impacts of the organism in the risk assessment area</i></li> </ul> <p>Although present or reported in the past in Member states around the Mediterranean sea, poor information is available on the impact of the disease. Data from Cyprus indicate yield reductions from 19 to 34%, with reduction in fruit size, weight and quality for two cultivars of navel oranges.</p>		

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## ABBREVIATIONS

EFSA	European Food Safety Authority
EPPO	European and Mediterranean Plant Protection Organization
EPPO-PQR	European and Mediterranean Plant Protection Organization Plant Quarantine Retrieval System
ISPM	International Standard for Phytosanitary Measures
MS	Member State(s)
NPPO	National Plant Protection Organisation
PLH Panel	Plant Health Panel
PCR	polymerase chain reaction
PRA	pest risk analysis
RNQP	Regulated Non Quarantine Pest