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Spiroplasma spp.: A Plant, Arthropod, Animal and Human Pathogen

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Additional information is available at the end of the chapter

Abstract

Mollicutes is a class of smallest and free-living bacteria. They have no cell wall and their plasma membrane contains cholesterol; nevertheless, cellular organization does not differ from that of other prokaryotes. They are used as simple model systems for studying general biological problems, such as those concerning membrane structure and functions, symbiosis between arthropods and microrganisms, animal and plant pathogens. Mollicutes includes the family of *Spiroplamataceae*, which contains *Spiroplasma* genus, a group of species associated, in different manner, with arthropods (insects, mites, crustaceans). *Spiroplasma* species can be commensals or parasites and even be involved in more close symbiosis, such as synergism or mutualism. Out of 38 described *Spiroplasma* species, only three have been associated with plant diseases and three with arthropod diseases. Moreover, some species have been related to animal diseases, such as transmissible spongiform encephalopathy (TSE), and their role in human disease has been assessed. The chapter describes the taxonomic situation of the genus and reports the most important diseases due to the presence of *Spiroplasma* in different living organisms with special emphasis on citrus in which it causes one of the most economically damaging infectious diseases in a number of citrus growing areas worldwide.

Keywords: *Spiroplasma*, citrus, disease, crab, transmissible spongiform encephalopathy, insect vector

1. Introduction

Mollicutes is a class of microorganisms composed by the smallest and free-living bacteria, which have no cell wall. Their cellular organization does not differ from that of other prokaryotes: plasma membrane, which, however, contains cholesterol, cytoplasm, and ribosomes;

their metabolic pathways are simpler than those of other eubacteria and their DNA has a low GC content. The cell biology of these organisms is interesting to many researchers who use *Mollicutes* as simple model systems for studying general biological problems, such as those concerning membrane structure and functions, symbiosis between arthropods and microorganisms, animal and plant pathogens. The genus *Spiroplasma* has arthropods as a peculiar host. *Spiroplasma* species (spiroplasmas) have developed different types of symbiosis with insects and mites, and, as recently shown, with crustaceans. Among the 38 described *Spiroplasma* species, only three have been associated with plant diseases. In this chapter, the relevance of the spiroplasma diseases in citrus is described together with some relevant diseases reported as associated with the presence of spiroplasma in other living organisms, such as arthropods, animals, and humans.

2. Methods

Bibliographical sources have been obtained through a PubMed search, integrating the bibliographic citations from PMC. The used keywords to select bibliographic items were *Spiroplasma*, *Spiroplasma* pathogenesis, *Spiroplasma*, and human infections, *Spiroplasma* taxonomy. As of today, there are 709 bibliographic entries, since 1973, in PubMed and 1140 citations in PMC, since 1981. This discrepancy is due to the fact that PMC also includes sources from the conference proceedings, books, etc., etc. A literature search on *Spiroplasma citri*, *Spiroplasma kunkelii*, and *Spiroplasma phoeniceum* was conducted on the CAB Abstract and web-based search engines, such as Google Scholar. Further references and information were obtained from experts, citations within the references, as well as from gray literature.

3. Classification and taxonomy

The spiroplasmas belong to the Class of *Mollicutes*, Order *Entomoplasmatales*, Family *Spiroplasmataceae*, and Genus *Spiroplasma* [1]. Species of officially recognized *Spiroplasma* are shown in **Table 1**. Most species have mandatory relationships with insects, with whom they develop different types of symbiosis [37, 38], while a number species were reported to be pathogenic for animals, arthropods, and plants and a few has been involved in human diseases.

4. Pathogenetic mechanisms and interactions with insect vectors

Spiroplasma species are mainly transmitted to plants by specific insect vectors; in order to achieve the transmission, they must cross the salivary gland barrier [39]. *S. citri* and *S. kunkelii* invade the hemocoel through the gut epithelium of the insect host by a process of receptor-mediated cell endocytosis [40]. Receptors on leafhopper gut epithelial cells likely recognize specific spiroplasma membrane proteins. Several candidate *S. citri* attachment protein genes have been studied, including spiralin (immunodominant membrane protein) [41], P58 [42], SARP1 [43], P32 of pSci6 plasmid [44–46], and phosphoglycerate kinase (PGK) [47]. It has

Species	Host species	Geographic distribution	Authors
Spiroplasma alleghenense	Common scorpion fly (*Panorpa helena*)	USA (WV)	[3]
Spiroplasma apis	Honey-bee (*Apis mellifera*)	France	[4]
Spiroplasma atrichopogonis	Biting midge (*Atrichopogon* spp.)	USA (MD)	[5]
Spiroplasma cantharicola	Soldier beetle (*Cantharis carulinus*)	USA (MD)	[6]
Spiroplasma chinense	False bindweed (*Calystegia hederacea*)	China (Jiangsu)	[7]
Spiroplasma chrysopicola	Deerfly (*Chrysops* sp.)	USA (MD)	[8]
Spiroplasma citri	*Citrus* spp.	USA	[2]
Spiroplasma clarkii	Green June beetle (*Cotinus nitida*)	USA (MD)	[9]
Spiroplasma corruscae	Lampyrid beetle (*Ellychnia corrusca*)	USA (MD)	[10]
Spiroplasma culicicola	Salt marsh mosquito (*Aedes sollicitans*)	Worldwide	[11]
Spiroplasma diabroticae	Corn rootworm (*Diabrotica undecimpunctata*)	USA (MD)	[12]
Spiroplasma diminutum	Mosquito (*Culex annulus*)	Taiwan	[13]
Spiroplasma eriocheiris	Chinese mitten crab (*Eriocheir sinesis*)	China	[14]
Spiroplasma floricola	Tulip tree (*Liriodendron tulipifera*)	USA	[15]
Spiroplasma gladiatoris	Maryland horsefly (*Tabanus gladiator*)	USA (MD)	[7]
Spiroplasma helicoids	Horseflies (*Tabanus abdominalis-limbatinevris*)	USA (MD)	[7]
Spiroplasma insolitum	Fall flower (*Bidens* sp.)	USA (MD)	[16]
Spiroplasma ixodetis	Black-legged ticks (*Ixodes pacificus*)	USA (OR)	[17]
Spiroplasma kunkelii	Corn (*Zea mays*)	America	[18]
Spiroplasma lampyridicola	Firefly beetle (*Photuris pennsylvanicus*)	USA (MD)	[19]
Spiroplasma leptinotarsae	Colorado potato beetle (*Leptinotarsa decemlineata*)	USA (MD)	[20]
Spiroplasma leucomae	Satin moth larvae, (*Leucoma salicis*)	Poland	[21]
Spiroplasma lineolae	Striped horsefly (*Tabanus lineola*)	USA (GE)	[22]
Spiroplasma litorale	Horsefly (*Tabanus nigrovittatus*)	USA (NC)	[23]
Spiroplasma melliferum	Honey bee (*Apis mellifera*)	worldwide	[24]
Spiroplasma mirum	Rabbit ticks (*Haemaphysalis leporispalustris*)	USA (GE, MD)	[25]

Species	Host species	Geographic distribution	Authors
Spiroplasma monobiae	Vespid wasp (*Monobia quadridens*)	USA (MD)	[26]
Spiroplasma montanense	Tabanid fly (*Hybomitra opaca*)	USA (MN)	[27]
Spiroplasma penaei	Pacific white shrimp (*Penaeus vannamei*)	Colombia	[28]
Spiroplasma phoeniceum	Periwinkle (*Catharanthus roseus*)	Syria	[29]
Spiroplasma platyhelix	Dragonfly (*Pachydiplax longipennis*)	USA (MD)	[30]
Spiroplasma poulsonii	Fruit fly (*Drosophila willistonii*)	South America	[31]
Spiroplasma sabaudiense	Mosquitoes (*Aedes stricticus, Aedes vexans*)	France	[32]
Spiroplasma syrphidicola	Syrphid fly (*Eristalis arbustorum*)	Unknown	[33]
Spiroplasma tabanidicola	Horsefly (*Tabanus abactur*)	USA (OK)	[7]
Spiroplasma taiwanese	Mosquitoes (*Culex tritueniorhynchus*)	Taiwan	[34]
Spiroplasma turonicum	Fly (*Haematopota* sp.)	France	[35]
Spiroplasma velocicrescens	Vespid wasp (*Monobia quadridens*)	USA (MD)	[36]

Table 1. *Spiroplasma* species reported in literature after the genus description by Saglio et al. [2] (in bold strains recognized as pathogenic).

been reported that a spiralin mutant is less effectively transmitted [41]; moreover, the spiralin binds to glycoproteins of its insect vector [48] and it is, therefore, a candidate molecule for insect vector specificity. It was also shown that *S. citri* phosphoglycerate kinase (PGK), a glycolytic enzyme, could bind to actin of its leafhopper vector for the internalization of *S. citri* into leafhopper cells [47]. In addition, the PGK protein or partial PGK peptides were shown to inhibit spiroplasma transmission by leafhoppers [49].

A relationship between plasmids of *S. citri* and insect-transmissibility has been demonstrated for spiroplasmas [44–51]. The plasmid pSci6 confers insect transmissibility to a nontransmissible strain of *S. citri* [44] encoding protein *P32*; however, when only the p32 gene was introduced into the nontransmissible strain of *S. citri*, its insect transmissibility was not restored; therefore, pSci6-encoded determinants other than P32 might be essential for insect transmission [46]. There is no report of transmission through seeds from infected plants [52].

The mechanisms by which spiroplasmas cause disease in plants are poorly understood, and the genetic determinants involved, are unknown. For *S. citri* toxins and lactic acid production seems to play a role in the disease development; spiroplasmas cause also a shortage of auxins, most probably, due to the utilization of sterols [53]. In particular, it was shown that the pathogenicity of *S. citri*, i.e., the ability to induce diseases, correlates with its ability to use fructose [54], indicating that the sugar metabolism is certainly an important factor in the relationships

of *S. citri* with its two hosts, the plant and the leafhopper vector. Indeed, carbohydrate partitioning was shown to be impaired in infected plants [55].

5. Diagnosis and epidemiology

Citrus stubborn disease (CSD) caused by *S. citri*, is a relevant threat to the citrus industry in several citrus-growing countries, such as California and Cyprus; however, the knowledge of its epidemiology is mostly anecdotal and in most cases diagnosis was only based on symptoms; in particular on the acorn-shaped fruits. The pathogen is graft-transmissible and vectored by leafhoppers in a persistent and propagative manner [56]. Field diagnosis of CSD is difficult because foliar symptoms can resemble nutritional deficiencies or symptoms induced by other phloem-restricted pathogens, such as, the huanglongbing agents ('*Candidatus* Liberibacter' species). Moreover, symptoms of CSD can vary with season, citrus cultivar, and disease severity. The isolation and *in vitro* culture of *S. citri* are time consuming and technically demanding since it is typically low in concentration and unevenly distributed in citrus tissues, making its reliable detection challenging. Currently, the preferred detection methods are based on polymerase chain reaction (PCR) assays with primers developed from sequences of *S. citri* housekeeping genes [57]. Recent genome sequencing revealed that the bacterium harbors multiple copies of prophage genes; therefore, it was hypothesized that targeting these genes could improve the sensitivity of PCR detection. Several different pathogen detection protocols have been optimized to evaluate the pathogen presence in commercial groves and assess its impact on fruit quality and yield. PCR and quantitative PCR targeting multicopy genes *P89* and *P58* are currently the most sensitive detection systems [54]. Recently, a rapid serological method based on the detection of a protein secreted by *S. citri* has been developed and proven to be as efficient as quantitative PCR [58].

Genetic diversity among *S. citri* strains has been observed; however, in recent surveys, no correlation has been observed between symptom severity and genotypes. Findings suggest that CSD incidence in commercial groves in California could be as high as more than 80% and its impact on yield and fruit quality is significant. The principal economic hosts of *S. citri* are susceptible *Citrus* species, including the major commercial species in the Mediterranean area: grapefruits (*C. paradisi*), lemons (*C. limon*), mandarins (*C. reticulata*), orange (*C. sinensis*), and sour orange (*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 × reticulata*) [59]. Other rutaceous hosts include *Fortunella* species and interspecific hybrid rootstock, such as citrange (*C. sinensis × Poncirus trifoliata*); however, they are considered minor or incidental hosts. Some forms are symptomlessly infected including *P. trifoliat*a [60]. Many other cultivated or wild plants 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.

S. citri is known to be transmitted by seven species of leafhoppers (Cicadellidae Deltocephalinae). In California (USA), its main natural vectors are the leafhoppers *Circulifer tenellus*, *Scaphytopius nitridus*, and *Scaphytopius acutus* [61]. In the Mediterranean area, it is reported to be transmitted by *Neoaliturus haematoceps* [62] and *C. tenellus* [63]. Other

Homoptera may acquire the spiroplasma, but not transmit it [64, 65]. In North America, the distribution of *S. citri* follows rather closely that of *C. tenellus* (primarily a sugarbeet insect). In the Mediterranean area, insects known as vectors are present practically wherever citrus is grown, so that their presence does not appear to be a limiting factor in the spread of CSD to new areas. Spiroplasma develops best in citrus under hot conditions (28–32°C) and may not induce conspicuous symptoms at lower temperatures. Annual plants experimentally infected are rapidly killed at temperatures over 30°C, but may recover at lower temperatures [62].

C. tenellus and *N. haematoceps* are the Mediterranean vectors of stubborn disease and they feed on a wide range of host plants, such as weeds, ornamental plants, and crops. *N. haematoceps* has been found particularly on the ornamental *Matthiola incana* and on wild plants, *M. sinuata* and *Salsola kali* [66]. Neither insect is particularly associated with citrus, on which feeding is incidental. Oldfield et al. [67] reported that *C. tenellus* could not be reared on citrus as sole host. Klein and Raccah [68] reported the presence of two *N. haematoceps* populations in Israel, one polyphagous and the other host-specific. Although *S. citri* naturally infects many other hosts, it is not reported to have any economic impact on those. Their main significance would be as reservoirs of *S. citri* for infection of citrus. Horseradish brittle root is of purely anecdotal interest.

S. kunkelii is transmitted by leafhoppers in the field, mainly by *Dalbulus maidis* (Homoptera: Cicadellidae) that is a subtropical species which occurs throughout the Americas and causes severe production losses in corn cultivation. *S. phoenicium* was only experimentally transmitted from cultures by *Macrosteles fascifrons* on aster and periwinkle plants.

6. Plant diseases

Only three *Spiroplasma* species are reported as agents of plant diseases: *S. citri*, *S. kunkelii*, and *S. phoeniceum*.

Citrus stubborn disease (CSD) was first detected in California, and it is a widespread bacterial disease caused by *S. citri*, mostly found in arid areas of the United States and the Mediterranean Region, where citrus is mainly produced. It causes quite relevant loss in both fruit production and quality. Affected trees are stunted and quite often flat topped, because the stems have shortened internodes and undersized leaves showing mottle and chlorosis. Most fruits drop while very small. The few fruits that could reach maturity are misshapen and abnormally matured with aborted seed. The pathogen induces symptoms by consuming the sap fructose produced through sucrose hydrolysis by the companion cells of phloem [69]. *S. citri* had been identified as a pathogen of several citrus species as above-mentioned and it has a wide plant host range than that of citrus, since it is transmitted by polyphagous leafhoppers. In addition to naturally infected plant hosts, several experimental plant hosts have been infected through the forced inoculation with leafhopper vectors. The disease is considered to be absent in Europe except in the Mediterranean area and it is quite severe in areas where the climate is hot and arid. Only mild symptoms, if any, are recorded where the field temperature does not exceed 28°C. Presently, *S. citri* is a quality pathogen in the European and Mediterranean

Regions, however, it is listed as an harmful organism in Council Directive 2000/29/EC; in addition, the Directive also considers some of its host plants and insect vectors [70].

Corn stunt (CSS) [71] is one of the major diseases of maize (*Zea mays*) in the Americas (CABI Crop Protection Compendium). It was reported that the disease caused in Tucuman province in Argentina severe damages that resulted in reduction of the yield ranging from 50% to 90% (with an average of 70%) with infected plants producing grains with weight less than 3 times. In the USA, the disease was considered sporadic, although it was observed in the California central valley every year, until recently. In particular, in 2001, there was an outbreak with losses of more than 5 million USD. Although CSS is due to a complex of pathogens, *Spiroplasma kunkelii* (**Table 1**) seems to play an important role in the etiology and epidemiology of the disease. *S. kunkelii* is transmitted by leafhoppers mainly *Dalbulus maydi*s (Homoptera: Cicadellidae), a subtropical species which occurs throughout the Americas in all the maize growing areas [72]. The same leafhopper transmits maize bushy stunt phytoplasma (MBSP) and *Maize rayado fino virus* (MRFV). *S. kunkelii* has also been detected in teosintes (e.g., *Euchlaena amexicana, Z. perennis*) [73]. The insect vector, *D. maydis,* feeds on cultivated or wild species of the genus *Zea* and occasionally on species of the closely related genus *Tripsacum*. CSS has been detected in maize crop in Brazil, mainly in the second maize growing season at the warm areas [69]. The mollicute-infected leafhoppers migrate from diseased maize crop fields and infect maize seedlings in other places. CSS symptoms appear after the maize flowering, and are usually leaf reddening, shortness of the internodes, and small ears, with small or a few grains, depending on the cultivar [74]. Based only on the foliar symptoms, the CSS can be identified by the presence in the leaves of chlorotic streaks, almost of white color, that extend from the leaf base to the leaf apex. However, these typical and diagnostic streaks in the leaves are not frequently present, and so, it is difficult or impossible to distinguish the CSS from the MBSP symptoms, at field. The spiroplasma and the phytoplasma can be detected and distinguished mainly by PCR tests. The management efficiency of the CSS through the insecticide control of *D. maydis* leafhopper is limited, since this insect vector can infect the maize seedling before its death. The disease management can be achieved by the use of resistant maize cultivars, associated to the insecticide seed treatment for *D. maydi*s control, together with the choice of best month for the maize sowing. There is little information about the genetic control of the maize resistance to corn stunt *spiroplasma*, although some studies carried out in field indicate that additive and nonadditive effects are present in the maize resistance inheritance to this disease [75]. *S. kunkeli*i appears to be pathogenic also to its insect vectors, in particular it is able to shorten the life of *D. maidis* [76, 77]. Moreover, *S. kunkelii* appears not to be seed transmitted, while the aster yellows phytoplasmas associated with the disease resulted recently to be seed transmitted [78].

Spiroplasma phoeniceum was found to be the causal agent of a periwinkle yellows disease (PYD) and described as a new species of *Spiroplasma* in Syria, in the vicinity of orchards with high incidence of CSD, during a survey aimed at demonstrating the natural spread of the latter disease [29]. Infected periwinkle plants showed yellowing symptoms that were indistinguishable from those associated to infections that were associated with phytoplasma presence or due to *S. citri*. The report of this disease has remained anecdotic.

7. Arthropod diseases

Severe mortalities in the marine shrimp *Penaeus vannamei* were reported in early 2002 in one pond at a shrimp farm in the Colombian Caribbean coast. During May-June 2002, two other shrimp ponds at the same farm also experienced very high mortalities. *S. panaei* was then identified as the causative agent of the outbreak and spread during the next grow-out cycle to a neighboring farm, which suffered mortalities ranging from 10 to 90% [27]. Earlier reports on Chinese mitten crab (*Eriocheir sinensis*) showing a tremor disease (TD) resulted to be infected by *S. eriocheiris* by molecular testing, supporting sever epizootic outbreak mainly in aquaculture [79]. A strain isolated from the hemolymph of a Chinese mitten crab with tremor exhibited a predilection for muscle, nerve, and connective tissues and was found to be transported to various tissues and organs by haemocytes. The pathologic features seen in experimentally infected crabs were similar to those observed in naturally occurring infected crabs [14].

In natural populations of some neotropical species of *Drosophila*, single females were found whose progenies consisted of only daughters [80]. After demonstration that this sex-ratio trait was maternally (vertically) inherited and infectious, with the electron microscopic examination of fixed and negatively stained cells, microorganisms similar to spiroplasmas, were observed. The microorganisms were subsequently grown in culture characterized at the serological level and named *S. poulsoni* [31].

8. Human diseases

In 2002, Lorenz et al. reported the first case of human infection due to *Spiroplasma* in a female child, born prematurely, who was diagnosed with a unilateral cataract associated with anterior uveitis [81]. The microscopic examination, cultures for fungi, aerobic and anaerobic bacteria were negative, as well as molecular tests for the *Toxoplasma gondii*, *Herpes simplex virus* (HSV), and *Varicella-Zoster virus* (VZV). Similarly, the serological tests for HSV-1 and HSV-2, CMV, and VZV were negative. By using PCR and TEM, the presence of *Spiroplasma* spp. was confirmed; however, its speciation was not done.

Two additional cases of human infections due to *Spiroplasma* were reported in 2015 [81, 82]. Both patients had a deficiency of the immune system due to hypogammaglobulinaemia or to pharmacologic immunosuppression, after transplantation. In the first case, a patient with rheumatoid arthritis and hypogammaglobulinaemia presented on admission, clinical signs of infection, no signs of autoimmune disease, and negative tumor makers [82]. The only sign of bacterial infection was a positive blood culture, but the bacteriological examination after Gram staining was negative. The presence of beta hemolysis in blood agar subculture, in the absence of visible bacterial growth, was suggestive for the presence of *Mollicutes*. Out of all the tested media, only the subcultures on Agar A7 [83], a specific medium for *Mollicutes*, showed growth. PCR amplification of the 16S rDNA and sequencing, have demonstrated the presence of a bacterium with high homology to *Spiroplasma turonicum*. The infection has been

resolved with doxycycline and levofloxacin therapy. The second case was represented by another transplanted patient hospitalized with signs of liver disease, a diagnosis confirmed by positron emission tomography (PET) [84]. Serological tests for classical agents responsible for liver diseases were negative. Similarly, the parasitological examination of stools was negative as well. The initial therapy, pipera-tazobactam, was replaced by meropenem because of no result. Molecular tests for the detection of 16S rDNA and sequencing, both from blood and liver samples, showed the presence of *Spiroplasma ixodetes*. The therapy with azithromycin and doxycycline led to healing.

9. *Spiroplasma* and transmissible spongiform encephalopathy (TSE)

By entering the query "Spiroplasma and encephalopathy" or "Spiroplasma ad TSE", you get more than 30 citations, in PubMed [85–117]. It is surprising that the first paper dates back to 1979. In fact, in the late 1979, Bastian described membranous structure very similar to *Spiroplasma* spp. cells by the electron microscopy observation, in a brain biopsy from a man with Creutzfeldt-Jakob disease (CJD). Author's conclusion suggested a concurrence of the microorganism with such a disease [85]. One year later, another paper was published, in which, the previous described membranous structures with a spiral shape were found by using the electron microscopy, in axoplasm of brain cells, obtained from a biopsy in a patient with suspected CJD [86]. Two other cases of patients suffering from CJD, in which, brain biopsies showed membranous spiral inclusions resembling spiroplasma cells were reported by Reyes and Hoenig in 1981 [87]. In the same year, Bastian et al. reported other histological evidence of the presence of spiroplasma cells in the brain tissue biopsy from another patient suffering from CJD [88].

An experimental study done on newborn rabbits infected with the *Spiroplasma* spp. responsible for the suckling mouse cataract (SMCA) showed that intracerebral inoculation provoked hemorrhages in the brain, conversely subcutaneous inoculation did not induce any diseases [87]. The intracerebral inoculation of *Spiroplasma* SMCA in newborn Syrian hamster induced severe cerebral damages and death, although adult animals did not develop the disease [90]. The attempt of Leach et al. to isolate either spiroplasmas or mycoplasmas from the brain tissue of 18 patients with CJD was negative; likewise, the detection of antibodies in 15 patients with CJD did not show any positivity [91].

Spiroplasma mirum GT-48 strain has been the subject of two studies on experimental animals conducted by Tully et al. [92] and by Bastian et al. [93], respectively. In the first study, it has been shown that the organism spread and multiplied rapidly in the brain; in the second study, spiraling and membranous inclusions in brain cells similar to those described by the electron microscopy in patients with CJD were observed [85–88]. At the end of 1980s, two experimental studies have investigated the pathogenesis of *S. mirum*. In the first, *S. mirum* has been shown to cause a persistent infection of the brain in the suckling rat. Furthermore, the tropism of this microorganism for the brain tissues was correlated to the presence of sterols, which are necessary for its growth [94]. In the second study, it was observed, by western blot, that antibodies

to the fibrils associated with Scrapie interacted with either the brain tissue of patients with CJD either with fibrillar proteins resistant to proteases obtained from *S. mirum* [95].

As previously suggested by Leach et al. [91], even Connolly et al. [96] did not consider that there is a correlation between *Spiroplasma* spp. and CJD, since in their electron microscopy observations they did not found no structures similar to *Spiroplasma* spp. Humphery-Smith and Chastel, whereas have claimed that the results of Gray et al [86] were due to artifacts, reported the finding of *S. sabaudiense* antibodies in a patient with amyotrophic lateral sclerosis and in a patient of the control group [97, 98]. Bastian and Foster by using PCR specific primer for *Spiroplasma* genus and sequencing have investigated the brain tissue from necroscopy of 13 patients suffering from CJD, as well as, 9 scrapie cases, and 50 controls. *Spiroplasma* DNA was detected in 13/13 CJD patients, in 5 out of 9 scrapie brains, but in none of 50 control cases [99]. Successively, 16S rDNA with a homology greater than 99% with *S. mirum* was recovered in most of (>80%) the experimental animals investigated; moreover, 16S rDNA was recovered in two human brains with CJD but not in the control [100]. Moreover, Bastian et al. have shown that there is an association between *Spiroplasma* spp. and scrapie by using an experimental model of sheep suffering from scrapie [101]. Alexeeva et al. in an experimental study on hamster have ruled that either *S. mirum* or other bacteria cannot be considered as causative agents of TSE [102]. Experimental studies have demonstrated that *S. mirum* did not induce TSE-like disease in raccoons [103] and recently suggested that the ability to form biofilms in *Spiroplasma* spp. is the basis of the spread and of the pathogenesis of diseases caused by this *Spiroplasma* [104].

We are far from having clarified the role of *Spiroplasma* in TSE. To date, despite Bastian et al. studies and studies of other authors, there are still many doubts about the role these microorganisms can play in TSE. Probably, in the years to come, more extensive studies on molecular biology and serology may give rise to some answers.

10. Conclusions

CSD and CSS are the only two diseases caused by spiroplasmas of relevant economic importance on a world scale. Although, CSD cause serious damages to the citrus industry in North America and seems widespread in most citrus-producing areas, including North Africa, the Mediterranean basin, and the Middle-East, information on its distribution and incidence, as well as, its impact in citrus orchards are still limited. Routine molecular detection methods have been developed for the identification of *S. citri*; however, robust diagnostic methods remain challenging and most of the reports about this pathogen are not reliable because based only on symptoms. Recently, the European Food Safety Authority (EFSA) has reconsidered the literature on *S. citri* and confirmed that it should be categorized as an harmful organism for the citrus industry in the Mediterranean Region.

In 2008, as the importance of CSS was increasing in several American countries, S. *kunkelii* was added to the EPPO Alert List and it has been included in this list for more than 3 years. During this period, no particular International action was requested by the EPPO member countries and in 2012, it was therefore considered that sufficient alert has been given and the pest was deleted from the list.

Human infections due to *Spiroplasma* spp. are, probably, a new clinical reality: their role as opportunistic pathogens should not be undervalued. Moreover, despite the many evidences on the possible involvement of spiroplasmas in TSE, more studies are needed to definitively associate these bacteria with such a disease.

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