

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



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

Santa Olga Cacciola, Assunta Bertaccini,
Antonella Pane and Pio Maria Furneri

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/66481>

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

Species	Host species	Geographic distribution	Authors
<i>Spiroplasma monobiae</i>	Vespid wasp (<i>Monobia quadridens</i>)	USA (MD)	[26]
<i>Spiroplasma montanense</i>	Tabanid fly (<i>Hybomitra opaca</i>)	USA (MN)	[27]
<i>Spiroplasma penaei</i>	Pacific white shrimp (<i>Penaeus vannamei</i>)	Colombia	[28]
<i>Spiroplasma phoeniceum</i>	Periwinkle (<i>Catharanthus roseus</i>)	Syria	[29]
<i>Spiroplasma platyhelix</i>	Dragonfly (<i>Pachydiplax longipennis</i>)	USA (MD)	[30]
<i>Spiroplasma poulsonii</i>	Fruit fly (<i>Drosophila willistonii</i>)	South America	[31]
<i>Spiroplasma sabaudiense</i>	Mosquitoes (<i>Aedes stricticus</i> , <i>Aedes vexans</i>)	France	[32]
<i>Spiroplasma syrphidicola</i>	Syrphid fly (<i>Eristalis arbustorum</i>)	Unknown	[33]
<i>Spiroplasma tabanidicola</i>	Horsefly (<i>Tabanus abactur</i>)	USA (OK)	[7]
<i>Spiroplasma taiwanese</i>	Mosquitoes (<i>Culex tritueniorhynchus</i>)	Taiwan	[34]
<i>Spiroplasma tunicum</i>	Fly (<i>Haematopota</i> sp.)	France	[35]
<i>Spiroplasma velocicrescens</i>	Vespid wasp (<i>Monobia quadridens</i>)	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* house-keeping 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. limettoides*, *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. trifoliata* [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 *Macrostoteles 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 maydis* (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. maydis* 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. kunkelii* appears to be pathogenic also to its insect vectors, in particular it is able to shorten the life of *D. maydis* [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 severe 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 markers [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, piperacillin-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 previously 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 find no structures similar to *Spiroplasma* spp. Humphrey-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 necropsy 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.

Author details

Santa Olga Cacciola¹, Assunta Bertaccini², Antonella Pane¹ and Pio Maria Furneri^{3*}

*Address all correspondence to: furneri@unict.it

1 Department of Agriculture, Food and Environment (Di3A), University of Catania, Catania, Italy

2 Department of Agricultural Sciences (DipSA), Plant Pathology, *Alma Mater Studiorum*–University of Bologna, Bologna, Italy

3 Department of Biomedical and Biotechnological Sciences – BIOMETEC, Section of Microbiology, Torre Biologica F. Latteri, University of Catania, Catania, Italy

References

- [1] Brown DR, Bradbury JM, Whitcomb RF. Entomoplasmatales. *Bergey's Manual of Systematics of Archaea and Bacteria*. 2015; vol. 1–2. Tully, Bové, Laigret and Whitcomb. 381. Published by John Wiley & Sons, Inc., in association with Bergey's Manual Trust
- [2] Saglio P, L'Hospital M, Laflèche D, Dupont G, Bové JM, Tully JG, Freundt EA. *Spiroplasma citri* gen. and sp. n. A mycoplasma-like organism associated with "stubborn" disease of citrus. *International Journal of Systematic and Evolutionary Microbiology*. 1973; **23**: 191–204. doi: 10.1099/00207713-23-3-191
- [3] Adams JR, Whitcomb RF, Tully JG, Clark EA, Rose DL, Carle P, Konai M, Bové JM, Henegar RB, Williamson DL. *Spiroplasma alleghenense* sp. nov., a new species from the scorpion fly *Panorpa helena* (Mecoptera: Panorpidae). *International Journal of Systematic and Evolutionary Microbiology*. 1997; **47**: 759–762. doi: 10.1099/00207713-47-3-759
- [4] Mouches C, Bové JM, Tully JG, Rose DL, McCoy RE, Carle-Junca P, Garnier M, Saillard C. *Spiroplasma apis*, a new species from the honey-bee *Apis mellifera*. *Annals of Microbiology (Institut Pasteur)*. 1983; **134A**: 383–397.
- [5] Koerber T, Gasparich GE, Frana MF, Grogan WL, Jr. *Spiroplasma atrichopogonis* sp. nov., from a ceratopogonid biting midge. *International Journal of Systematic and Evolutionary Microbiology*. 2005; **55**: 289–292. doi: 10.1099/ijss.0.02465-0
- [6] Whitcomb RF, Chastel C, Abalain-Colloc M, Stevens C, Tully JG, Rose DL, Carle P, Bové JM, Henegar RB, Hackett KJ, Clark TB, Konai M, Williamson DL. *Spiroplasma*

- cantharicola* sp. nov., from cantharid beetles (Coleoptera: Cantharidae). International Journal of Systematic and Evolutionary Microbiology. 1993; **43**: 421–424. doi: 10.1099/00207713-43-3-421
- [7] Guo JH, Chen TE, Whitcomb RF, Rose DL, Tully JG, Williamson DL, Ye XD, Chen YX. *Spiroplasma chinense* sp. nov. from flowers of *Calystegia hederacea* in China. International Journal of Systematic and Evolutionary Microbiology. 1990; **40**: 421–425, doi: 10.1099/00207713-40-4-421
- [8] Whitcomb RF, French FE, Tully JG, Gasparich GE, Rose DL, Carle P, Bové JM, Henegar RB, Konai M, Hackett KJ, Adams JR, Clark TB, Williamson DL. *Spiroplasma chrysopicola* sp. nov., *Spiroplasma gladiatoris* sp. nov., *Spiroplasma helicoides* sp. nov., and *Spiroplasma tabanidicola* sp. nov., from Tabanid (Diptera: Tabanidae) flies. International Journal of Systematic and Evolutionary Microbiology. 1997; **47**: 713–719. doi: 10.1099/00207713-47-3-713
- [9] Whitcomb RF, Vignault J-C, Tully JG, Rose DL, Carle P, Bové JM, Hackett KJ, Henegar RB, Konai M, Williamson DL. *Spiroplasma clarkii* sp. nov. from the green June beetle (Coleoptera: Scarabaeidae). International Journal of Systematic and Evolutionary Microbiology. 1993; **43**: 261–265. doi: 10.1099/00207713-43-2-261
- [10] Hackett KJ, Whitcomb RF, French FE, Tully JG, Gasparich GE, Rose DL, Carle P, Bové JM, Henegar RB, Clark TB, Konai M, Clark EA, Williamson DL. *Spiroplasma corruscae* sp. nov., from a firefly beetle (Coleoptera: Lampyridae) and tabanid flies (Diptera: Tabanidae). International Journal of Systematic and Evolutionary Microbiology. 1996; **46**: 947–950. doi: 10.1099/00207713-46-4-947
- [11] Hung SHY, Chen TA, Whitcomb RF, Tully JG, Chen YX. *Spiroplasma culicicola* sp. nov. from the salt marsh mosquito *Aedes sollicitans*. International Journal of Systematic and Evolutionary Microbiology. 1987; **37**: 365–370. doi: 10.1099/00207713-37-4-365
- [12] Carle P, Whitcomb RF, Hackett KJ, Tully GJ, Rose DL, Bové JM, Henegar RB, Konai M, Williamson DL. *Spiroplasma diabroticae* sp. nov., from the Southern corn root-worm beetle, *Diabrotica undecimpunctata* (Coleoptera: Chrysomelidae). International Journal of Systematic and Evolutionary Microbiology. 1997; **47**: 78–80. doi: 10.1099/00207713-47-1-78
- [13] Williamson DL, Tully JG, Rosen L, Rose DL, Whitcomb RF, Abalain-Colloc M-L, Carle P, Bové JM, Smyth J. *Spiroplasma diminutum* sp. nov., from *Culex annulus* mosquitoes collected in Taiwan. International Journal of Systematic and Evolutionary Microbiology. 1996; **46**: 229–233. doi: 10.1099/00207713-46-1-229
- [14] Wang W, Gu W, Gasparich Ge, Bi K, Ou J, Meng Q, Liang T, Feng Q, Zhang J, Zhang Y. *Spiroplasma eriocheiris* sp. nov., associated with mortality in the Chinese mitten crab, *Eriocheir sinensis*. International Journal of Systematic and Evolutionary Microbiology. 2011; **61**: 703–708. doi: 10.1099/ijls.0.020529-0

- [15] Davis RE, Lee I-M, Worley JF. *Spiroplasma floricola*, a new species isolated from surfaces of flowers of the tulip tree, *Liriodendron tulipifera* L. International Journal of Systematic and Evolutionary Microbiology. 1981; **31**: 456–464. doi: 10.1099/00207713-31-4-456
- [16] Hackett KJ, Whitcomb RF, Tully JG, Rose DL, Carle P, Bové JM, Henegar RB, Clark TB, Clark EA, Konai M, Adams JR, Williamson DL. *Spiroplasma insolitum* sp. nov., a new species of group I spiroplasma with an unusual DNA base composition. International Journal of Systematic and Evolutionary Microbiology. 1993; **43**: 272–277. doi:10.1099/00207713-43-2-272
- [17] Tully JG, Rose DL, Yunker CE, Carle P, Bové JM, Williamson DL, Whitcomb RF. *Spiroplasma ixodetis* sp. nov., a new species from *Ixodes pacificus* ticks collected in Oregon. International Journal of Systematic and Evolutionary Microbiology. 1995; **45**: 23–28. doi: 10.1099/00207713-45-1-23
- [18] Whitcomb RF, Chen TA, Williamson DL, Liao C, Tully JG, Bové JM, Mouches C, Rose DL, Coan ME, Clark TB. *Spiroplasma kunkelii* sp. nov.: characterization of the etiological agent of corn stunt disease. International Journal of Systematic and Evolutionary Microbiology. 1986; **36**: 170–178. doi: 10.1099/00207713-36-2-170
- [19] Stevens C, Tang AY, Jenkins E, Goins RL, Tully JG, Rose DL, Konai M, Williamson DL, Carle P, Bové JM, Hackett KJ, French FE, Wedincamp J, Henegar RB, Whitcomb RF. *Spiroplasma lampyridicola* sp. nov., from the firefly beetle *Photuris pennsylvanicus*. International Journal of Systematic and Evolutionary Microbiology. 1997; **47**: 709–712. doi: 10.1099/00207713-47-3-709
- [20] Hackett KJ, Whitcomb RF, Clark TB, Henegar RB, Lynn DE, Wagner AG, Tully JG, Gasparich GE, Rose DL, Carle P, Bové JM, Konai M, Clark EA, Adams JR, Williamson DL. *Spiroplasma leptinotarsae* sp. nov., a mollicute uniquely adapted to its host, the Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). International Journal of Systematic and Evolutionary Microbiology. 1996; **46**: 906–911. doi: 10.1099/00207713-46-4-906
- [21] Oduori MA, Lipa JJ, Gasparich GE. *Spiroplasma leucomae* sp. nov., isolated in Poland from white satin moth (*Leucoma salicis* L.) larvae. International Journal of Systematic and Evolutionary Microbiology. 2005; **55**: 2447–2450. doi: 10.1099/ijs.0.63685-0
- [22] French FE, Whitcomb RF, Tully JG, Carle P, Bové JM, Henegar RB, Adams JR, Gasparich GE, Williamson DL. *Spiroplasma lineolae* sp. nov., from the horsefly *Tabanus lineola* (Diptera: Tabanidae). International Journal of Systematic and Evolutionary Microbiology. 1997; **47**: 1078–1081. doi: 10.1099/00207713-47-4-1078
- [23] Konai M, Whitcomb RF, French FE, Tully JG, Rose DL, Carle P, Bové JM, Hackett KJ, Henegar RB, Clark TB, Williamson DL. *Spiroplasma litorale* sp. nov., from tabanid flies (Tabanidae: Diptera) in the Southeastern United States. International Journal of Systematic and Evolutionary Microbiology. 1997; **47**: 359–362. doi: 10.1099/00207713-47-2-359

- [24] Clark TB, Whitcomb RF, Tully JG, Mouches C, Saillard C, Bové JM, Wróblewski H, Carle P, Rose DL, Henegar RB, Williamson DL. *Spiroplasma melliferum*, a new species from the honeybee (*Apis mellifera*). International Journal of Systematic and Evolutionary Microbiology. 1985; **35**: 296–308. doi: 10.1099/00207713-35-3-296
- [25] Tully JG, Whitcomb RF, Rose DL, Bové JM. *Spiroplasma mirum*, a new species from the rabbit tick (*Haemaphysalis leporispalustris*). International Journal of Systematic and Evolutionary Microbiology. 1982; **32**: 92–100. doi: 10.1099/00207713-32-1-92
- [26] Whitcomb RF, Tully JG, Rose DL, Carle P, Bové JM, Henegar RB, Hackett KJ, Clark TB, Konai M, Adams J, Williamson DL. *Spiroplasma monobiae* sp. nov. from the vespid wasp *Monobia quadridens* (Hymenoptera: Vespidae). International Journal of Systematic and Evolutionary Microbiology. 1993; **43**: 256–260. doi: 10.1099/00207713-43-2-256
- [27] Whitcomb RF, French FE, Tully JG, Rose DL, Carle P, Bové JM, Clark EA, Henegar RB, Konai M, Hackett KJ, Adams JR, Williamson DL. *Spiroplasma montanense* sp. nov., from hybomitra horseflies at Northern latitudes in North America. International Journal of Systematic and Evolutionary Microbiology. 1997; **47**: 720–723. doi: 10.1099/00207713-47-3-720
- [28] Nunan LM, Lightner DV, Oduori MA, Gasparich GE. *Spiroplasma penaei* sp. nov., associated with mortalities in *Penaeus vannamei*, Pacific white shrimp. International Journal of Systematic and Evolutionary Microbiology. 2005; **55**: 2317–2322. doi: 10.1099/ijs.0.63555-0
- [29] Saillard C, Vignault JC, Bové JM, Raie A, Tully JG, Williamson DL, Fos A, Garnier M, Gadeau A, Carle P, Whitcomb RF. *Spiroplasma phoeniceum* sp. nov., a new plant-pathogenic species from Syria. International Journal of Systematic and Evolutionary Microbiology. 1987; **37**: 106–115. doi: 10.1099/00207713-37-2-106
- [30] Williamson DL, Adams JR, Whitcomb RF, Tully JG, Carle P, Konai M, Bové JM, Henegar RB. *Spiroplasma platyhelix* sp. nov., a new mollicute with unusual morphology and genome size from the dragonfly *Pachydiplax longipennis*. International Journal of Systematic and Evolutionary Microbiology. 1997; **47**: 763–766. doi: 10.1099/00207713-47-3-763
- [31] Williamson DL, Sakaguchi B, Hackett KJ, Whitcomb RF, Tully JG, Carle P, Bové JM, Adams JR, Konai M, Henegar RB. *Spiroplasma poulsonii* sp. nov., a new species associated with male-lethality in *Drosophila willistoni*, a neotropical species of fruit fly. International Journal of Systematic and Evolutionary Microbiology. 1999; **49**: 611–618. doi: 10.1099/00207713-49-2-611
- [32] Abalain-Colloc ML, Chastel C, Tully JG, Bové JM, Whitcomb RF, Gilot B, Williamson DL. *Spiroplasma sabaudiense* sp. nov. from mosquitoes collected in France International Journal of Systematic and Evolutionary Microbiology. 1987; **37**: 260–265. doi: 10.1099/00207713-37-3-260
- [33] Whitcomb RF, Gasparich GE, French FE, Tully JG, Rose DL, Carle P, Bové JM, Henegar RB, Konai M, Hackett KJ, Adams JR, Clark TB, Williamson DL. *Spiroplasma syrphidicola* sp. nov., from a syrphid fly (Diptera: Syrphidae). International Journal of Systematic and Evolutionary Microbiology. 1996; **46**: 797–801. doi: 10.1099/00207713-46-3-797

- [34] Abalain-Colloc ML, Rosen L, Tully JG, Bové JM, Chastel C, Williamson DL. *Spiroplasma taiwanense* sp. nov. from *Culex tritaeniorhynchus* mosquitoes collected in Taiwan. International Journal of Systematic and Evolutionary Microbiology. 1988; **38**: 103–107. doi: 10.1099/00207713-38-1-103
- [35] Hélias C, Vazeille-Falcoz M, Le Goff F, Abalain-Colloc M-L, Rodhain F, Carle P, Whitcomb RF, Williamson DL, Tully JG, Bové JM, Chastel C. *Spiroplasma turonicum* sp. nov. from *Haematopota* horse flies (Diptera: *Tabanidae*) in France. International Journal of Systematic and Evolutionary Microbiology. 1998; **48**: 457–461. doi: 10.1099/00207713-48-2-457
- [36] Konai K, Whitcomb RF, Tully Jg, Rose DL, Carle P, Bové JM, Henegar RB, Hackett KJ, Clark TB, Williamson DL. *Spiroplasma velocicrescens* sp. nov., from the vespid wasp *Monobia quadridens*. International Journal of Systematic and Evolutionary Microbiology. 1995; **45**: 203–206. doi: 10.1099/00207713-45-2-203
- [37] Gasparich GE. Spiroplasmas: evolution, adaptation and diversity. Frontiers in Biosciences. 2002; **7**: 19–40. doi: 10.2741/gaspari
- [38] Regassa LB, Gasparich GE. Spiroplasmas: evolutionary relationships and biodiversity. Frontiers in Biosciences. 2006; **11**: 2983–3002. doi: 10.2741/2027
- [39] Markham PJ, Townsend R. Experimental vectors of spiroplasmas. In: Leafhopper Vectors and Plant Disease Agents (Ed. by Maramorosch K, Harris KF). 1979; Academic Press, New York, USA, pp. 413–445.
- [40] Kwon M, Wayadande A, Fletcher J. *Spiroplasma citri* movement into the intestines and salivary glands of its leafhopper vector, *Circulifer tenellus*. Phytopathology. 1999; **89**: 1144–1151. doi: 10.1094/PHYTO.1999.89.12.1144.
- [41] Duret S, Berho N, Danet JL, Garnier M, Renaudin J. Spiralin is not essential for helicity, motility, or pathogenicity but is required for efficient transmission of *Spiroplasma citri* by its leafhopper vector *Circulifer haematocephus*. Applied and Environmental Microbiology. 2003; **69**: 6225–6234. doi: 10.1128/AEM.69.10.6225-6234.2003
- [42] Ye F, Melcher U, Fletcher J. Molecular characterization of a gene encoding a membrane protein of *Spiroplasma citri*. Gene. 1997; **189**: 95–100. doi: 10.1016/S0378-1119(96)00840-2
- [43] Berg M, Melcher U, Fletcher J. Characterization of *Spiroplasma citri* adhesion related protein SARP1, which contains a domain of a novel family designated sarpin. Gene. 2001; **275**: 57–64. doi: 10.1016/S0378-1119(01)00655-2
- [44] Berho N, Duret S, Danet JL, Renaudin J. Plasmid pSci6 from *Spiroplasma citri* GII-3 confers insect transmissibility to the non-transmissible strain *S. citri* 44. Microbiology. 2006; **152**: 2703–2716. doi: 10.1099/mic.0.29085-0
- [45] Berho N, Duret S, Renaudin J. Absence of plasmids encoding adhesion-related proteins in non-insect-transmissible strains of *Spiroplasma citri*. Microbiology. 2006; **152**: 873–886. doi: 10.1099/mic.0.28541-0

- [46] Killiny N, Batailler B, Foissac X, Saillard C. Identification of a *Spiroplasma citri* hydrophilic protein associated with insect transmissibility. *Microbiology*. 2006; **152**: 1221–1230. doi: 10.1099/mic.0.28602-0
- [47] Labroussaa F, Arricau-Bouvery N, Dubrana MP, Saillard C. Entry of *Spiroplasma citri* into *Circulifer haematoceps* cells involves interaction between spiroplasma phosphoglycerate kinase and leafhopper actin. *Applied Environmental and Microbiology*. 2010; **76**: 1879–1886. doi: 10.1128/AEM.02384-09
- [48] Killiny N, Castroviejo M, Saillard C. *Spiroplasma citri* spiralin acts in vitro as a lectin binding to glycoproteins from its insect vector *Circulifer haematoceps*. *Phytopathology*. 2005; **95**: 541–548. doi:10.1094/PHYTO-95-0541
- [49] Labroussaa F, Dubrana MP, Arricau-Bouvery N, Beven L, Saillard C. Involvement of a minimal actin-binding region of *Spiroplasma citri* phosphoglycerate kinase in spiroplasma transmission by its leafhopper vector. *PLoS One*. 2011; **6**: e17357. doi: 10.1371/journal.pone.0017357
- [50] Breton M, Duret S, Beven L, Dubrana MP, Renaudin J. I-SceI-mediated plasmid deletion and intra-molecular recombination in *Spiroplasma citri*. *Journal of Microbiological Methods*. 2010; **84**: 216–222. doi: 10.1016/j.mimet.2010.11.020
- [51] Breton M, Duret S, Danet JL, Dubrana MP, Renaudin J. Sequences essential for transmission of *Spiroplasma citri* by its leafhopper vector, *Circulifer haematoceps*, revealed by plasmid curing and replacement based on incompatibility. *Applied and Environmental Microbiology*. 2010; **76**: 3198–3205. doi: 10.1128/AEM.00181-10
- [52] EFSA PLH Panel (EFSA on Plant Health). Scientific Opinion on pest categorisation of *Spiroplasma citri*. *EFSA Journal*. 2014; **12**(12): 3925. 29 pp. doi: 10.2903/j.efsa.2014.3925
- [53] Chang C-J. Pathogenicity of aster yellows phytoplasma and *Spiroplasma citri* on periwinkle. *Phytopathology*. 1998; **88**: 1347–1350. doi: 10.1094/PHYTO.1998.88.12.1347
- [54] Gaurivaud P, Danet JL, Laigret F, Garnier M, Bové JM. Fructose utilization and phytopathogenicity of *Spiroplasma citri*. *Molecular Plant-Microbe Interactions*. 2000; **13**: 1145–1155. doi:10.1094/MPMI.2000.13.10.1145
- [55] André A, Maucourt M, Moing A, Rolin D, Renaudin J. Sugar import and phytopathogenicity of *Spiroplasma citri*: glucose and fructose play distinct roles. *Molecular Plant-Microbe Interactions*. 2005; **18**: 33–42. doi: 10.1094/MPMI-18-0033
- [56] Liu HY, Gumpf DJ, Oldfield GN, Calavan EC. Transmission of *Spiroplasma citri* by *Circulifer tenellus*. *Phytopathology*. 1983; **73**: 582–585. doi: 10.1094/Phyto-73-582
- [57] Carle P, Saillard C, Carrere N, Carrere S, Duret S, Eveillard S, Gaurivaud P, Gourgues G, Gouzy J, Salar P, Verdin E, Breton M, Blanchard A, Laigret F, Bové JM, Renaudin J, Foissac X. Partial chromosome sequence of *Spiroplasma citri* reveals extensive viral invasion and important gene decay. *Applied and Environmental Microbiology*. 2010; **76**: 3420–3426. doi: 10.1128/AEM.02954-09

- [58] Shi J, Pagliaccia D, Morgan R, Qiao Y, Pan S, Vidalakis G, Ma W, 2014. Novel diagnosis for citrus stubborn disease by detection of a *Spiroplasma citri*-secreted protein. *Phytopathology*. 2014; **104**: 188–195. doi: 10.1094/PHYTO-06-13-0176-R
- [59] Calavan EC. Stubborn. In: Description and illustration of virus and virus-like diseases of citrus (Ed. by Bové J, Vogel R). 1980; SETCO-IRFA, Paris, France.
- [60] Bové JM. *Spiroplasma citri*. In: European handbook of plant diseases (Ed. by Smith IM, Dunez J, Lelliott RA, Phillips DH, Archer SA). 1988; Blackwell Scientific Publications, Oxford, UK, pp. 129–131.
- [61] Oldfield GN. Ecological associations of *Spiroplasma citri* with insects, plants and other plant mycoplasmas in the western United States. In: Mycoplasma diseases of crops. Basic and applied aspects (Ed. by Maramorosch K, Raychaudhuri SP). 1988; Springer-Verlag, New York, USA, pp. 175–191.
- [62] Bové JM. Stubborn and its natural transmission in the Mediterranean area and the Near East. *FAO Plant Protection Bulletin*. 1986; **34**: 15–23. <http://prodinra.inra.fr/record/100438>.
- [63] Klein M, Rasooly P, Raccach B. New findings on the transmission of *Spiroplasma citri*, the citrus stubborn disease agent in Israel, by a beet leafhopper from the Jordan valley. *Hassadeh*. 1988; **68**: 1736–1737.
- [64] Rana GL, Kaloostian GH, Oldfield GN, Granett AL, Calavan EC, Pierce HD, Lee I-M, Gumpf DJ. Acquisition of *Spiroplasma citri* through membranes by Homopterous insects. *Phytopathology*. 1995; **65**: 1143–1145. doi: 10.1094/Phyto-65-1143
- [65] Bové JM, Moutous G, Saillard C, Fos A, Bonfils J, Vignault J-C, Nhami A, Abassi M, Kabbage K, Hafidi B, Mouches C, Viennot-Bourgin G. Mise en évidence de *Spiroplasma citri*, l'agent causal de la maladie du 'stubborn' des agrumes dans 7 cicadelles du Maroc. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences*. 1979; **288**: 335–338.
- [66] Fos A, Bové JM, Lallemand J, Saillard C, Vignault JC, Ali Y, Brun P, Vogel R. The leafhopper *Neoliturus haematoceps* is a vector of *Spiroplasma citri* in the Mediterranean area. *Annales de l'Institut Pasteur Microbiologie*. 1986; **137A**: 97–101.
- [67] Oldfield GN, Kaloostian GH, Pierce HD, Calavan EC, Granett AL, Blue RL. Beet leafhopper transmits citrus stubborn disease. *California Agriculture*. 1976; **30**: 6–15.
- [68] Klein M, Raccach B. Separation of two leafhopper populations of the *Circulifer haematoceps* complex on different host plants in Israel. *Phytoparasitica*. 1991; **19**: 153–155. doi: 10.1007/BF02980361
- [69] Bové JM, Renaudin J, Saillard C, Foissac X, Garnier M. *Spiroplasma citri*, a plant pathogenic mollicute: relationships with its two hosts, the plant and the leafhopper vector. *Annual Review of Phytopathology*. 2003; **41**: 483–500. doi: 10.1146/annurev.phyto.41.052102.104034

- [70] CABI/EPPO. *Spiroplasma citri*. Distribution Maps of Plant diseases. 2013; n. October, Wallingford, UK: CABI, Map 375 (Edition 4) <http://www.cabi.org/isc/abstract/20133421491>.
- [71] CABI Crop Protection Compendium (Datasheets on *Spiroplasma kunkelii* (corn stunt spiroplasma) and *Dalbulus maidis*). 2016. <http://www.cabicompendium.org/cpc/home.asp>.
- [72] Moya-Raygoza G, Palomera-Avalos V, Galaviz-Mejia C. Field overwintering biology of *Spiroplasma kunkelii* (Mycoplasmatales: *Spiroplasmataceae*) and its vector *Dalbulus maidis* (Hemiptera: Cicadellidae). *Annals of Applied Biology*. 2007;**151**: 373–379.
- [73] Nault LR. Maize bushy stunt and corn stunt: a comparison of disease symptoms, pathogen host ranges, and vectors. *Phytopathology*. 1980; **70**:659–662.
- [74] Bradbury, JF. *Spiroplasma kunkelii*. IMI Descriptions of Fungi and Bacteria (No. 105). 1991; Sheet 1047: CABI Bioscience, Surrey, UK.
- [75] de Oliveira E, Gomes e Gama EE, Teixeira FF, de Oliveira AC, da Silva AR. Genetic control of maize resistance to corn stunt spiroplasma. *Phytopathogenic Mollicutes*. 2013; **3**(2): 67–71. doi: 10.5958/j.2249-4677.3.2.016
- [76] Bourtzis K, Miller TA. *Insect Symbiosis*. 2006; vol. 2. CRC Press, Boca Raton, p 304.
- [77] Ammar E-D, Hogenhout SA Mollicutes associated with arthropods and plants. In: *Symbiosis Insect* (Ed. by Kostas B, Miller T). 2006; vol II. CRC Press, Boca Raton, pp 97–118. ISBN 9780849341946
- [78] Calari A, Paltrinieri S, Contaldo N, Sakalieva D, Mori N, Duduk B, Bertaccini A. Molecular evidence of phytoplasmas in winter oilseed rape, tomato and corn seedlings. *Bulletin of Insectology*. 2011; **64**(Supplement): S157–S158. ISSN: 1721–8861
- [79] Wang W, Wen B, Gasparich GE, Zhu N, Rong L, Chen J, Xu Z. A spiroplasma associated with tremor disease in the Chinese mitten crab (*Eriocheir sinensis*). *Microbiology*. 2004; **150**: 3035–3040. doi: 10.1099/mic.0.26664-0
- [80] Poulson DF, Sakaguchi B. Nature of “sex-ratio” agent in *Drosophila*. *Science*. 1961; **133**: 1489–1490. doi: 10.1126/science.133.3463.1489
- [81] Lorenz B, Schroeder J, Reischl U. First evidence of an endogenous *Spiroplasma* sp. infection in humans manifesting as unilateral cataract associated with anterior uveitis in a premature baby. *Graefe's Archive for Clinical and Experimental Ophthalmology*. 2002; **240**(5): 348–353. doi: 10.1007/s00417-002-0453-3
- [82] Aquilino A, Masiá M, López P, Galiana AJ, Tovar J, Andrés M, Gutiérrez F. First human systemic infection caused by *Spiroplasma*. *Journal of Clinical Microbiology*. 2015; **53**(2): 719–721. doi: 10.1128/JCM.02841-14
- [83] Shepard MC, Lunceford CD. Differential agar medium (A7) for identification of *Ureaplasma urealyticum* (human T mycoplasmas) in primary cultures of clinical material. *Journal of Clinical Microbiology*. 1976; **3**(6): 613–625.

- [84] Mueller NJ, Tini GM, Weber A, Gaspert A, Husmann L, Bloemberg G, Boehler A, Benden C. Hepatitis from *Spiroplasma* sp. in an immunocompromised patient. American Journal of Transplantation. 2015; **15**: 2511–2516. doi: 10.1111/ajt.13254
- [85] Bastian FO. Spiroplasma-like inclusions in Creutzfeldt-Jakob disease. Archives of Pathology & Laboratory Medicine 1979; **103**(13): 665–669.
- [86] Gray A, Francis RJ, Scholtz CL. Spiroplasma and Creutzfeldt-Jakob disease. Lancet. 1980; **316**(8186): 152. doi: 10.1016/S0140-6736(80)90041-0
- [87] Reyes JM, Hoenig EM. Intracellular spiral inclusions in cerebral cell processes in Creutzfeldt-Jakob disease. Journal of Neuropathology and Experimental Neurology. 1981; **40**(1):1–8. doi: 10.1097/00005072-198140010-00001
- [88] Bastian FO, Hart MN, Cancilla PA. Additional evidence of spiroplasma in Creutzfeldt-Jakob disease. Lancet. 1981; **317**(8221): 660. doi: 10.1016/S0140-6736(81)91571-3
- [89] Kirchhoff H, Heitmann J, Trautwein G. Pathogenicity of *Spiroplasma* sp. strain SMCA in rabbits: clinical, microbiological, and histological aspects. Infection and Immunity. 1981; **33**(1): 292–296. doi: 0019-9567/81/070292-05\$02.00/0
- [90] Kirchhoff H, Kuwabara T, Barile MF. Pathogenicity of *Spiroplasma* sp. strain SMCA in Syrian hamsters: clinical, microbiological, and histological aspects. Infection and Immunity. 1981; **31**(1): 445–452. doi: 0019-9567/81/010445-08\$02.00/0
- [91] Leach RH, Matthews WB, Will R. Creutzfeldt-Jakob disease. Failure to detect spiroplasmas by cultivation and serological tests. Journal of Neurological Sciences. 1983; **59**(3): 349–353. doi: 10.1016/0022-510X(83)90020-5
- [92] Tully JG, Bastian FO, Rose DL. Localization and persistence of spiroplasmas in an experimental brain infection in suckling rats. Annales de Microbiologie. 1984;**135A**(1): 111–117.
- [93] Bastian FO, Purnell DM, Tully JG. Neuropathology of spiroplasma infection in the rat brain. American Journal of Pathology. 1984; **114**(3): 496–514. doi: 10.1097/00005072-198305000-00136
- [94] Bastian FO, Jennings RA, Hoff CJ. Neurotropic response of *Spiroplasma mirum* following peripheral inoculation in the rat. Annales Institute Pasteur Microbiologie. 1987; **138**(6): 651–655.
- [95] Bastian FO, Jennings RA, Gardner WA. Antiserum to scrapie-associated fibril protein cross-reacts with *Spiroplasma mirum* fibril proteins. Journal of Clinical Microbiology. 1987; **25**(12): 2430–2431.
- [96] Connolly JH, Allen IV, Dermott E. Transmissible agent in the amyotrophic form of Creutzfeldt-Jakob disease. Journal of Neurology and Neurosurgery Psychiatry. 1988; **51**(11): 1459–1460. doi: 10.1136/jnnp.51.11.1459-a
- [97] Humphrey-Smith I, Chastel C. Creutzfeldt-Jakob disease, spiroplasmas, and crystalline artifacts. Lancet. 1988; **332**(8621): 1199. doi: 10.1016/S0140-6736(88)90271-1

- [98] Chastel C, Le Goff F, Goas JY, Goaguen J, Kerdraon G, Simitzis-Le Flohic AM. Serosurvey on the possible role of mosquito spiroplasma in central nervous system disorders in man. *Zentralblatt für Bakteriologie*. 1990; **20**(No Suppl): 910–911.
- [99] Bastian FO, Foster JW. *Spiroplasma* sp. 16S rDNA in Creutzfeldt-Jakob disease and scrapie as shown by PCR and DNA sequence analysis. *Journal of Neuropathology and Experimental Neurology*. 2001; **60**(6): 613–620. doi: 10.1093/jnen/60.6.613
- [100] Bastian FO, Dash S, Garry RF. Linking chronic wasting disease to scrapie by comparison of *Spiroplasma mirum* ribosomal DNA sequences. *Experimental and Molecular Pathology*. 2004; **77**(1):49–56. doi: 10.1016/j.yexmp.2004.02.002
- [101] Bastian FO, Boudreaux CM, Hagius SD, Bulgin MS, Sorensen-Melson SJ, Enright FM, Elzer PH. Spiroplasma found in the eyes of scrapie affected sheep. *Veterinary Ophthalmology*. 2011; **14**(1):10–17. doi: 10.1111/j.1463-5224.2010.00833.x
- [102] Alexeeva I, Elliott EJ, Rollins S, Gasparich GE, Lazar J, Rohwer RG. Absence of spiroplasma or other bacterial 16s rRNA genes in brain tissue of hamsters with scrapie. *Journal of Clinical Microbiology*. 2006; **44**(1): 91–97. doi: 10.1128/JCM.44.1.91-97.2006
- [103] Hamir AN, Greenlee JJ, Stanton TB, Smith JD, Doucette S, Kunkle RA, Stasko JA, Richt JA, Kehrli ME Jr. Experimental inoculation of raccoons (*Procyon lotor*) with *Spiroplasma mirum* and transmissible mink encephalopathy (TME). *Canadian Journal of Veterinary Research*. 2011; **75**(1):18–24.
- [104] Bastian FO, Elzer PH, Wu X. *Spiroplasma* spp. biofilm formation is instrumental for their role in the pathogenesis of plant, insect and animal diseases. *Experimental and Molecular Pathology* 2012; **93**(1):116–128. doi: 10.1016/j.yexmp.2012.04.017
- [105] Brenner SR, Brown PW. Transmissible spongiform encephalopathy in the 21st Century: neuroscience for the clinical neurologist. *Neurology*. 2009; **72**(3): 292. doi: 10.1212/01.wnl.0000344245.13952.2d
- [106] Bastian FO, Sanders DE, Forbes WA, Hagius SD, Walker JV, Henk WG, Enright FM, Elzer PH. *Spiroplasma* spp. from transmissible spongiform encephalopathy brains or ticks induce spongiform encephalopathy in ruminants. *Journal of Medical Microbiology*. 2007; **56**(Pt 9): 1235–1242. doi: 10.1099/jmm.0.47159-0
- [107] Sikorska B, Hainfellner JA, Mori S, Bratosiewicz J, Liberski PP, Budka H. Echigo-1: a panencephalopathic strain of Creutzfeldt-Jakob disease. II. Ultrastructural studies in hamsters. *Folia Neuropathologica*. 2004; **42**(Suppl B): 167–175.
- [108] Bastian FO, Fermin CD. Slow virus disease: deciphering conflicting data on the transmissible spongiform encephalopathies (TSE) also called prion diseases. *Microscopy Research and Technique*. 2005; **68**(3-4): 239–246. doi: 10.1002/jemt.20223
- [109] Bastian FO. Spiroplasma as a candidate agent for the transmissible spongiform encephalopathies. *Journal of Neuropathology and Experimental Neurology*. 2005; **64**(10): 833–838. doi: 10.1097/01.jnen.0000183553.01458.87

- [110] Bové JM. Spiroplasmas: infectious agents of plants, arthropods and vertebrates. *Wiener Klinische Wochenschrift*. 1997; **109**(14-15): 604–612.
- [111] Liberski PP, Mori S. The Echigo-1: a panencephalopathic strain of Creutzfeldt-Jakob disease: a passage to hamsters and ultrastructural studies. *Folia Neuropathologica*. 1997; **35**(4): 250–254.
- [112] Humphery-Smith I, Chastel C, Le Goff F. Spiroplasmas and spongiform encephalopathies. *The Medical Journal of Australia*. 1992; **156**(2): 142.
- [113] Chastel C, Le Goff F, Humphery-Smith I. Multiplication and persistence of *Spiroplasma melliferum* strain A56 in experimentally infected suckling mice. *Research in Microbiology*. 1991; **142**(4): 411–417. doi: 10.1016/0923-2508(91)90111-M
- [114] Bastian FO, Jennings RA, Hoff CJ. Effect of trimethoprim/sulphamethoxazole and hyperbaric oxygen on experimental *Spiroplasma mirum* encephalitis. *Research in Microbiology*. 1989; **140**(2): 151–158. doi: 10.1016/0923-2508(89)90049-1
- [115] Gray EG. Spongiform encephalopathy: a neurocytologist's viewpoint with a note on Alzheimer's disease. *Neuropathology and Applied Neurobiology*. 1986; **12**(2): 149–172. doi: 10.1111/j.1365-2990.1986.tb00047
- [116] Chastel C, Abalain-Colloc ML, Gilot B, Le Goff F, Gruffaz R, Simitzis AM. [Spiroplasmas and hematophagous arthropods; prospects in tropical pathology]. *Bulletin de la Societe de pathologie exotique et de ses filiales*. 1985; **78**(5 Pt 2): 769–779.
- [117] Bastian FO, Jennings RA. Creutzfeldt-Jakob disease: procedures for handling diagnostic and research materials. *Infection Control: IC*. 1984; **5**(1): 48–50.

