THE YALE JOURNAL OF BIOLOGY AND MEDICINE 56 (1983), 573-582

Spiroplasmas of Group I: The *Spiroplasma citri* Cluster

J.M. BOVÉ, Ph.D.,[a] C. MOUCHES, Ph.D.,[a] P. CARLE-JUNCA, M.Sc.,[a]
J.R. DEGORCE-DUMAS, Ph.D.,[a] J.G. TULLY, Ph.D.,[b]
AND R.F. WHITCOMB, Ph.D.[c]

[a]*Laboratoire de Biologie Cellulaire et Moléculaire, INRA, Domaine de la Grande Ferrade, Pont de la Maye, France;* [b]*Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, Frederick, Maryland;* [c]*Plant Protection Institute, Insect Pathology Laboratory, U.S. Department of Agriculture, Beltsville, Maryland*

Received January 4, 1983

We propose that Group I spiroplasmas be subdivided into seven, rather than four, subgroups. The seven subgroups showed remarkable homogeneity when several representative strains were compared. Hybridization reactions between DNAs of representative strains within subgroups were generally at least 90 percent, and usually at least 80 percent co-migrating cell proteins were found. In addition, when plasmid DNA was excluded, profiles of restricted DNA among strains within subgroups were very similar. In contrast, comparisons between Group I subgroups showed substantial heterogeneity. This heterogeneity was indicated by DNA-DNA hybridization reactions as low as 10–20 percent and only 10–15 percent co-migrating cell proteins.

Spiroplasma citri (subgroup I-1), the honeybee spiroplasma (subgroup I-2), and the corn stunt spiroplasma (subgroup I-3) are all pathogenic organisms with more or less limited host ranges. Strains of these three subgroups have been repeatedly isolated from affected hosts. Since strains of subgroups I-2 and I-3 can be clearly differentiated from other Group I subgroups and all other spiroplasmas, the DNA-DNA hybridization reactions of the subgroups do not exceed 70 percent, and because they are important pathogens, we propose (subject to completion of standard requirements for species descriptions) that they be recognized as new species of the genus *Spiroplasma*.

DISCOVERY OF SPIROPLASMAS AND NEED FOR CLASSIFICATION SCHEMES

At the first international congress on mycoplasmas, held in Bordeaux, France, in 1974, only three helical mycoplasma-like organisms were known: the citrus stubborn agent, the corn stunt spiroplasma (CSS), and the *Drosophila* sex ratio agent. *Spiroplasma citri,* the stubborn disease agent, was the only organism in the group that had been cultured, characterized, and named at that time [1,2,3,4]. That *S. citri* was a true mollicute was clearly established at the Bordeaux meeting, when data were presented that the organism lacked both cell-wall peptidoglycan and its known precursors [5]. Recognition that the *Drosophila* agent was not a spirochete [6] but a spiroplasma [7] came as a result of the discovery of *S. citri* and CSS and the morphologic similarities among these three helical, wall-less prokaryotes. The CSS was cultured soon thereafter by two independent groups [8,9].

Address reprint requests to: J.M. Bové, Ph.D., Laboratoire de Biologie Cellulaire et Moléculaire, INRA, Domaine de la Grande Ferrade, 33140 Pont de la Maye, France

In the late 1960s, at a time when the agents of citrus stubborn and corn stunt had not yet been identified, there was no reason to suspect a relationship between these two diseases. However, when both agents were found to be spiroplasmas, their morphologic similarity rasied questions about possible serologic relatedness. Such a relationship was indeed found, even before the CSS could be cultured [10], and this interrelationship has since been confirmed in a number of laboratories [11,12,13, 14,15,16] and by a variety of techniques [10,11,13,15,16].

A significant number of spiroplasmas have been identified and cultivated on artificial medium since the initial discovery of *S. citri* and its taxonomic description in 1973 [4]. In certain instances, "new" spiroplasmas were agents that had been grown previously on a variety of substrates (broth medium or chick embryos) but whose microbial nature had been misinterpreted. The 277F spiroplasma from rabbit ticks [17] was first grown on artificial medium and described as a spirochete in 1968 [18]. Two other isolates from rabbit ticks (SMCA and GT-48) were first grown in the chick embryo and described either as virus [19] or mycoplasma [20] before they were recognized as spiroplasmas [21,22]. However, most newly discovered spiroplasmas represent previously unknown agents that have been isolated from insects or from plant surfaces (e.g., flowers). T.B. Clark [23] first found spiroplasmas at high titers in the hemolymph of honeybees. This critical observation led to a search for spiroplasmas on the surfaces of flowers that might have been visited by foraging bees. Indeed, many such "flower" spiroplasmas have since been found, especially in the United States, France, and Morocco. However, as important as the flower habitat has been in the discovery of new spiroplasmas, it seems apparent now that occurrence of spiroplasmas in this habitat is only an occasional, secondary result of the colonization of insects by these organisms [24].

Following a serological analysis of the then-current spiroplasma strains [16], the first extensive classification of these organisms was proposed in 1980 [25]. On the basis of both serological relationships and properties of the spiroplasmal genomes, this classification scheme recognized five major groups and four subgroups. Serological studies [11,12,15,16] showed the honeybee spiroplasma (HBS) of Clark and the 277F spiroplasma to be related to the complex formed by *S. citri* and the CSS. While the serological relatedness between *S. citri*, the CSS, and the HBS was rather close, the 277F spiroplasma seemed to be less closely related. Although these four spiroplasmas were assigned to Group I in the classification scheme, it was apparent that these organisms also exhibited undeniable differences. To accommodate these distinctions, the collaborative group proposing the scheme suggested division of Group I spiroplasmas into four subgroups (Table 1).

The classification scheme proposed by Junca et al. [25] was subsequently revised to include several newly discovered spiroplasmas [26]. Three new serogroups were proposed (based upon serological evidence only): Groups (VI), (VII), and (VIII). In addition, three additional spiroplasmas entered Group I as unclassified serovars, including the green leaf bug spiroplasma (LB-12), the *Cocos* spiroplasma (N525), and the Maryland flower spiroplasma (M55) (Table 1). A further update [27] of the scheme added three additional serogroups: (IX), (X), and (XI). In all of these proposed schemes, spiroplasma groups and subgroups were defined not only on the basis of serological distinctions, but also on recognized characteristics of spiroplasmal DNA—particularly the guanine + cytosine (G + C) content—and the pattern of cell proteins of the organisms—as defined by one- and two-dimensional polyacrylamide gel electrophoresis. How these concepts apply to spiroplasmas in Group I (the *S. citri* cluster) is detailed below.

Intragroup heterogeneity in spiroplasmas is not restricted to Group I. Spiroplasma strains within Group IV (now designated *Spiroplasma apis* [32]) also show substantial serological heterogeneity, although the variation noted is less than that found within Group I. Spiroplasmas assigned to Group IV were first isolated from surfaces of flowers [28,29] but were later recovered from insects [25]. The DNA of all strains examined has G + C values of about 30 moles %, while flower and insect spiroplasmas of Group III have G + C values of about 26 moles %. Further data on the characteristics of Group IV spiroplasmas are presented elsewhere [30,31,32].

SPIROPLASMAS OF GROUP I: ORIGIN, HOST, AND DISEASE RELATIONSHIPS

Table 1 lists the seven proposed subgroups of Group I spiroplasmas, including three new subgroups represented by the LB-12, M55, and N525 strains. The assignment of these new spiroplasmas to subgroups I-5, I-6, and I-7 is based upon data to be presented below.

Group I contains the only two spiroplasmas known to be pathogenic to plants: *S. citri* (subgroup I-1) and the corn stunt spiroplasma (subgroup I-3). Both of these spiroplasmas are confined to the phloem and are therefore, in a sense, intracellular pathogens. The organisms are transmitted by leafhopper vectors; spiroplasma replication occurs within the insect hosts, especially in the hemolymph. Although *S. citri* causes an economically important disease in citrus (stubborn disease), many

TABLE 1
Spiroplasmas of Group I

Binomial or Common Name	Subgroup	Strains	Hosts		Disease Incited	References
			Plant	Arthropod		
Spiroplasma citri	I-1	Maroc (R8A2)	Citrus	Leafhoppers	Stubborn	[3,4,5]
		C189, etc.	Citrus	Leafhoppers	Stubborn	
		M4, etc.	*Vinca*	Leafhoppers	Yellows[a]	
			Dicots	Leafhoppers	Yellows[a]	[33]
Honeybee spiroplasma	I-2	BC-3	–	Honeybees	Spiroplasmosis	[23]
		AS 576	–	Honeybees	Spiroplasmosis	
		B63	–	Honeybees	Spiroplasmosis	
		G1	Flowers	–	–	
		G2	Flowers	–	–	
		BW	Flowers	–	–	
Corn stunt spiroplasma	I-3	I-747	Maize	Leafhoppers	Corn stunt	[8,9]
		E275	Maize	Leafhoppers	Corn stunt	
		B655	Maize	Leafhoppers	Corn stunt	[47]
277F spiroplasma	I-4	277F	–	Rabbit tick	None known	[17,18]
Green leaf bug spiroplasma	I-5	LB-12	–	Green leaf bug	None known	[35]
Maryland flower spiroplasma	I-6	M52	Flowers	–	None known	[36]
		M55	Flowers	–	None known	
		M58	Flowers	–	None known	
Cocos spiroplasma	I-7	N525	*Cocos* palm	–	None known	[37]
		N628	*Cocos* palm	–	None known	

[a] Yellows disease without virescence of flowers

other plants in nature have been found to be infected with the organism, and a number of plant species are experimentally susceptible [33]. In these induced infections, the disease is frequently more severe than in citrus infections, but *S. citri* has never been found to cause floral virescence or phyllody (characteristic symptoms of "yellows" diseases). In the few instances where *S. citri* has been isolated from plants showing virescence, mixed infections have been found to be present. A mycoplasma-like organism (MLO) present in such mixed infections is responsible for virescence symptoms, and the dual infections of these two organisms observed in nature can be reproduced experimentally [34].

The first spiroplasmas identified in honeybees [23] were those assigned to subgroup I-2. While these organisms are pathogenic to the bee, similar or identical isolates were later obtained from flower surfaces [12]. However, in France, a well-known disease of honeybees (May disease) was found to be associated with Group IV spiroplasmas (*S. apis*) [31,32]. Thus, spiroplasmas from two distinct groups are now clearly documented as bee pathogens.

Subgroup I-4 is represented, at this point, by a single spiroplasma (strain 277F) [17]. Although this organism was apparently recovered from rabbit ticks, additional isolations of members of this subgroup will be required to clearly establish the true host origin. Subgroup I-5 spiroplasmas also contain a single representative, recovered from the green leaf bug (*Trigonotylus ruficornis*) in Taiwan [35]. Spiroplasmas placed in subgroup I-6 represent three strains recovered from the surface of flowers [36], while subgroup I-7 contains two isolates from coconut palms [37]. In contrast to the intracellular location of *S. citri* and CSS, the spiroplasmas of subgroups I-6 and I-7 have been found only on plant surfaces. At this time, there is no evidence that these new subgroup members are associated with any plant disease.

SEROLOGICAL RELATEDNESS OF GROUP I SPIROPLASMAS

The spiroplasmas of the first three subgroups show significant heterologous crossing in all serological tests with which they have been examined, including growth inhibition (GI), deformation (DF), and metabolism inhibition (MI) [26], and by enzyme-linked immunosorbent assay (ELISA) [Saillard C: unpublished]. Spiroplasma 277F (subgroup I-4) clearly cross-reacts in a number of serological procedures with the corn stunt spiroplasma E275 (subgroup I-3) and with spiroplasmas of the new subgroups I-5, I-6, and I-7 [26]. Little or no serologic crossing was observed between 277F and strains of either *S. citri* (subgroups I-1) or honeybee spiroplasma (subgroup I-2).

DNA CHARACTERISTICS OF GROUP I SPIROPLASMAS

Genome Size and Guanine + Cytosine Content

S. citri has been found to have a genome size of 10^9 daltons [4], although Lee and Davis reported a value of 1.2×10^9 daltons for the AS 576 strain of subgroup I-2 [38].

The guanine plus cytosine (G + C) content of the DNA of strains within the Group I complex has been examined rather extensively [25,38,39]. The DNAs of all Group I spiroplasmas, including those of subgroups I-5, I-6, and I-7 [Carle-Junca P: unpublished] have been found to be 26 ± 1 mole % G + C.

DNA-DNA Hybridization

Table 2 lists the percentages of hybridization of spiroplasmal DNAs, as determined by the hydroxyapatite technique [40], which yields both the hybridization per-

TABLE 2
Percentages of Hybridization of Spiroplasmal DNAs

Source of Unlabeled DNA	Group or Subgroup	Hydroxyapatite Technique Source of ^{3}H DNA			S1 Nuclease Technique Source of ^{3}H DNA			
		R8A2	BC-3	277F	R8A2	BC-3	E275	277F
S. citri (R8A2)	I-1	100[a] (81.4)[b]	51.5 (78.0)	20.4 (76.2)	100	51	48	23
BC-3	I-2	63.7 (79.8)	100 (81.0)	19.4 (76.0)	65	100	48	23
E275	I-3	49.2 (78.3)	48.5 (76.0)	23.8 (77.0)	49	49	100	28
277F	I-4	19.8 (76.7)	21.1 (74.0)	100 (81.0)	18	17	25	100
M55	I-6	ND	ND	ND	17	ND	ND	32
S. floricola (OBMG)	III	9.3	10.9	13.4	9	8	8	17
S. floricola (BNR1)	III	8.8	ND	ND	ND	ND	ND	ND
PPS1	IV	7.8	8.9	10.2	9	6	9	17
F1	IV	7.2	ND	ND	ND	ND	ND	ND
S. mirum	V	7.8	8.7	11.9	9	6	ND	17
E. coli		7.4	8.6	9.0	7	4	7	12
No DNA		7.6	9.0	10.4	7	6	7	13

[a]The actual hybridization in the homologous reactions was between 70 and 88 percent, which is here normalized to 100 percent.
[b]Melting temperature (°C)
ND, Not done

centage and the melting temperature of the hybrid DNA, and by the S1 nuclease technique [38]. The two methods gave essentially identical results that confirm earlier findings [39]. There is considerable hybridization between *S. citri* (^{3}H) DNA and unlabeled DNA from strains of the honeybee spiroplasma (subgroup I-2); the percentage of hybridization varied between 62 and 67 percent. With *S. citri* (^{3}H) DNA and unlabeled DNA from the corn stunt spiroplasma (subgroup I-3), the percentage varied from 47 to 52 percent. The DNA hybrids formed in the heterologous reactions have melting temperatures close to those of the homologous hybrids. From the differences in melting temperatures, it can be calculated that the hybrid between *S. citri* DNA and the BC-3 strain (subgroup I-2) DNA has only 1 to 2 percent mismatched bases, while the *S. citri* DNA-277F DNA hybrid has only 2 to 4 percent mismatched bases.

Hybridization percentages considerably less than 50 percent are obtained when spiroplasma 277F (subgroup I-4) is involved in heterologous hybridization reactions [39] (Table 2). With *S. citri* or subgroup I-2 DNA, the figures are about 20 percent; they seem to be somewhat higher with DNA from the corn stunt spiroplasma (about 25 percent). Results of hybridization involving DNA from spiroplasmas of subgroups I-5 and I-7 are not yet available. However, S1 nuclease experiments with unlabeled M55 (subgroup I-6) DNA compared against probes of *S. citri* and 277F organisms gave values of 17 percent and 32 percent, respectively (Table 2). Hybridization reactions between the DNAs of strain pairs of the same subgroup (intrasubgroup hybridization) have given values about 90 percent for subgroups I-1, I-2, and I-3 [39].

Finally, the percentages of hybridization between Group I spiroplasma DNA and DNA from spiroplasmas of Groups III, IV, or V were not significantly higher than the values obtained when *Escherichia coli* DNA was used as the unlabeled DNA, or when no unlabeled DNA was added (Table 2).

Influence of Plasmid DNA on Hybridization

It is known that many spiroplasmas contain extrachromosomal DNA molecules, particularly plasmid DNA [11,41,42,43]. Several Group I spiroplasmas have plasmid DNA, as witnessed by the DNA bands obtained in agarose gel electrophoresis. A radioactive probe of plasmid pM41 from *S. citri* strain M4 hybridizes not only with plasmid DNA of other *S. citri* strains (Iran and Arizona), but also with DNA of corn stunt spiroplasma E275 (subgroup I-3) and spiroplasma 277F (subgroup I-4); the plasmid DNA does not hybridize with DNA from *S. citri* strains R8A2, Algeria, MH, R7A10, Israel, or CES 3033, with honeybee spiroplasma strains B88 or BC-3 (subgroup I-2), or with a flower spiroplasma strain, F-1 (Group IV) [44].

In hybridization experiments, the presence of the same (or very similar) plasmid sequences in each of two spiroplasmal DNAs to be compared might give positive hybridizable sequences. For instance, in the experiments shown in Table 2, hybridization between corn stunt E275 spiroplasmal DNA and spiroplasma 277F DNA must involve plasmid pM41, since this plasmid is present in both spiroplasma strains. This might explain (Table 2) the somewhat higher hybridization percentages between 277F DNA and E275 DNA than between 277F DNA and the DNA of *S. citri* R8A2 or honeybee spiroplasma BC-3, two spiroplasmas that do not contain the pM41 plasmid sequence. On the other hand, the significant hybridization observed between *S. citri* R8A2 DNA and the DNA of tick spiroplasma 277F is not due to, or affected by, hybridization between plasmid DNA, since *S. citri* strain R8A2 does not contain significant amounts of plasmid DNA.

Restriction Enzyme Patterns

S. citri strains without significant amounts of plasmid DNA, such as strains R8A2, C189, and CES 3033, yield very similar and specific EcoRI restriction enzyme profiles when their restricted DNA is submitted to electrophoresis on polyacrylamide gels [11,39]. These results show that *S. citri* strains represent a cluster of very homogeneous strains when plasmid DNA is not a significant factor. However, *S. citri* strains containing significant amounts of plasmid DNA that differs between strains do not yield the typical *S. citri* EcoRI profile [39].

Three honeybee spiroplasma strains of subgroup I-2 (B88, BC-3, and B1701) have been analyzed by the EcoRI technique [39]. These strains do contain significant amounts of plasmid DNA, as can be seen from the presence of two distinct DNA bands in polyacrylamide gel electrophoresis. These two bands, from each of the spiroplasma strains examined, have the same electrophoretic mobility. The EcoRI profiles of these strains are very similar, providing further evidence that this subgroup (I-2) of spiroplasma strains is very homogeneous.

COMPARISON OF SPIROPLASMA PROTEIN MAPS

We have previously shown that spiroplasmas with each of the first four subgroups (I-1 to I-4) of the *S. citri* complex have characteristic protein maps when subjected to two-dimensional polyacrylamide gel analysis [45]. The extent of relatedness between two spiroplasma strains can be estimated from the percentage of their co-migrating and homologous proteins. Co-migrating proteins are proteins with indistinguishable electrophoretic mobilities on two-dimensional gels; homologous proteins, on the other hand, have slightly different electrophoretic mobilities [45,46].

We have now extended the comparisons of two-dimensional spiroplasma protein maps to include the three new subgroups of Group I: the green leaf bug spiroplasma LB-12 (I-5), the Maryland (M55) flower spiroplasma (subgroup I-6), and the *Cocos* (N525) spiroplasma (I-7) (Table 3).

In previous work [45], the percentage of co-migrating protein between two *S. citri* (subgroup I-1) strains was found to be 91 percent; two honeybee spiroplasma strains (subgroup I-2) shared 82 percent co-migrating proteins. When two pairs of Group

TABLE 3
Percentages of Co-Migrating (C) and Homologous (H) Proteins Shared by Various Pairs of Group I Spiroplasmas as Determined by Two-Dimensional Electrophoretic Analyses

		Percentages of Proteins Shared with													
		I-1 R8A2		I-2 BC-3		I-3 E275		I-4 277F		I-5 LB-12		I-6 M55		I-7 N525	
Group	Strain	C	H	C	H	C	H	C	H	C	H	C	H	C	H
I-1	R8A2	100	0	45	31	28	32	15	31	27	21	26	22	19	30
I-2	BC-3	45	31	100	0	31	20	21	21	28	17	23	28	22	30
I-3	E275	28	32	31	20	100	0	28	30	18	33	27	29	20	32
I-4	277F	15	31	21	21	28	30	100	0	15	28	38	23	34	18
I-5	LB-12	27	21	28	17	18	33	15	28	100	0	22	34	23	15
I-6	M55	26	22	23	28	27	29	38	23	22	34	100	0	32	17
I-7	N525	19	30	22	30	20	32	34	18	23	15	32	17	100	0
III	OBMG	7	20	9	20	8	14	6	13	9	20	9	20		
IV	B31	7	14												

IV spiroplasmas were compared, values of 79 percent to 84 percent co-migrating proteins were recorded. More recently, values of 88 percent, and 91 percent were obtained when we compared two other pairs of Group IV spiroplasmas [30]. On the basis of these studies and other unpublished work, we suggest that spiroplasmas of Group I can be considered to belong to the same subgroup (intrasubgroup spiroplasmas) if they share no less than 80 percent co-migrating proteins. Table 3 gives the results of a number of intersubgroup comparisons. Compared to *S. citri* (subgroup I-1), the honeybee spiroplasma BC-3 (subgroup I-2) has the highest percentage of co-migrating proteins (45 percent), followed by the corn stunt spiroplasma E275 (subgroup I-3) (28 percent), the green leaf bug spiroplasma LB-12 (subgroup I-5) (27 percent), and the Maryland flower spiroplasma M55 (subgroup I-6) (26 percent). The *Cocos* spiroplasma N525 (subgroup I-7) and the 277F organism (subgroup I-4) complete the current comparisons, with values of 19 percent and 15 percent co-migrating proteins, respectively. From these results, it appears that candidates for inclusion in Group I spiroplasmas should share a minimum of 15 percent co-migrating proteins with at least one of the subgroup members. These findings also confirm the substantial heterogeneity among Group I spiroplasmas.

In reciprocal comparisons between specific Group I subgroups, spiroplasma 277F shared more co-migrating proteins with either the corn stunt spiroplasma E275 (28 percent), the M55 spiroplasma (38 percent), or the N525 spiroplasma (34 percent), than with *S. citri* (R8A2) or with LB-12 spiroplasma (15 percent co-migrating proteins each). The three new spiroplasmas of Group I (LB-12, M55, and N525) share only 22 to 32 percent co-migrating proteins, confirming other indications of relatively significant heterogeneity among these three spiroplasmas. These results justify the designation of specific subgroup designations for the green leaf bug (I-5), Maryland flower (I-6), and *Cocos* (I-7) spiroplasmas.

As part of this study, we have also examined the protein maps of *Spiroplasma apis* (Group IV) strains. The lowest percentage of co-migrating proteins found among a group of selected strains was approximately 36 percent, indicating that *S. apis* strains are less heterogeneous than Group I subgroup representatives.

PATHOGENICITY AND CLASSIFICATION: CONCLUSIONS

S. citri (subgroup I-1), the honeybee spiroplasma (subgroup I-2), and the corn stunt spiroplasma (subgroup I-3) are all clearly pathogenic organisms (Table 1). Since a number of susceptible and diseased hosts are readily available within each of the three groups, a respectable collection of representative spiroplasma strains from each of these subgroups have become available for intragroup comparisons. Such comparisons have shown remarkable homogeneity within representatives of subgroups I-1, I-2, and I-3. Thus, spiroplasma strains within any of the subgroups can be identified by a variety of techniques outlined here. This is possible despite the fact that these organisms show intergroup relatedness, as measured by serology, DNA hybridization, and cell protein analysis. Subgroup I-1 spiroplasma strains have received a specific taxonomic designation (*Spiroplasma citri*). Because it is of considerable practical importance to distinguish between pathogenic spiroplasmas, and because differences in DNA-DNA homology are within acceptable levels for prokaryote species designations, Latin binomials for the honeybee spiroplasmas (subgroup I-2) and corn stunt spiroplasmas (subgroup I-3) appear to be appropriate.

REFERENCES

1. Cole RM, Tully JG, Popkin TJ, et al: Morphology, ultrastructure and bacteriophage infection of the helical mycoplasma-like organism (*Spiroplasma citri* gen. and sp. nov.) cultured from "stubborn" disease of citrus. J Bacteriol 115:367-386, 1973
2. Fudl-Allah AE-SA, Calavan EC, Igwegbe ECK: Culture of a mycoplasma-like organism associated with stubborn disease of citrus. Phytopathology 62:729-731, 1972
3. Saglio P, Laflèche D, Bonissol C, et al: Isolement, culture et observation au microscope électronique des structures de type mycoplasme associées à la maladie du stubborn des agrumes et leur comparaison avec les structures observées dans le cas de la maladie du greening des agrumes. Physiol Veg 9:569-582, 1971
4. Saglio P, Lhospital M, Laflèche D, et al: *Spiroplasma citri* gen. and sp. n.: a mycoplasma-like organism associated with "stubborn" disease of citrus. Int J Syst Bacteriol 23:191-204, 1974
5. Bebear C, Latrille J, Fleck J: *Spiroplasma citri:* un mollicute. Colloq Inst Natl Sante Rech Med 33: 35-41, 1974
6. Poulson DF, Sakaguchi B: Nature of sex-ratio agent in *Drosophila.* Science 133:1489-1490, 1961
7. Williamson DL, Whitcomb RF: Helical, wall-free prokaryotes in *Drosophila,* leafhoppers and plants. Colloq Inst Natl Sante Rech Med 33:283-290, 1974
8. Chen TA, Liao CH: Corn stunt spiroplasma: isolation, cultivation, and proof of pathogenicity. Science 188:1015-1017, 1975
9. Williamson DL, Whitcomb RF: Plant mycoplasmas: a cultivable spiroplasma causes corn stunt disease. Science 188:1018-1020, 1975
10. Tully JG, Whitcomb RF, Bové JM, et al: Plant mycoplasmas: serological relation between agents associated with citrus stubborn and corn stunt diseases. Science 182:827-829, 1973
11. Bové JM, Saillard C: Cell biology of spiroplasmas. In The Mycoplasmas, Vol 3, Plant and Insect Mycoplasmas. Edited by RF Whitcomb, JG Tully. New York, Academic Press, 1979, pp 83-153
12. Raju BC, Nyland G, Meickle T, et al: Helical, motile mycoplasmas associated with flowers and honey bees in California. Canad J Microbiol 27:249-253, 1981
13. Garcia-Jurado O: Evaluation of an immunoenzymatic technique (ELISA) for the detection of spiroplasma and mycoplasma-like organisms in plants. Zbl Bakt Hyg I Abt Orig A 241:229-230 (Abstract), 1978
14. Saillard C, Garcia-Jurado O, Bové JM, et al: Application of ELISA to the detection of *Spiroplasma citri* in plants and insects. Proc 8th Conf Int Org Citrus Virologists. Edited by EC Calavan, SM Garnsey, LW Timmer. Riverside, University of California, 1980, pp 143-152
15. Williamson DL, Whitcomb RF, Tully JG: The spiroplasma deformation test, a new serological method. Curr Microbiol 1:203-207, 1978
16. Williamson DL, Tully JG, Whitcomb RF: Serological relationships of spiroplasmas as shown by combined deformation and metabolism inhibition tests. Int J Syst Bacteriol 29:345-351, 1979
17. Brinton LP, Burgdorfer W: Cellular and subcellular organization of the 277F agent, a spiroplasma from rabbit tick *Haemaphysalis leporispalustris* (Acari: Ixodidae). Int J Syst Bacteriol 26:554-560, 1976
18. Pickens EG, Gerloff RF, Burgdorfer W: Spirochete from the rabbit tick *Haemaphysalis leporispalustris* (Packard). I. Isolation and preliminary characterization. J Bacteriol 95:291-299, 1968
19. Clark HF, Rorke LB: Spiroplasmas of tick origin and their pathogenicity. In The Mycoplasmas, Vol 3, Plant and Insect Mycoplasmas. Edited by RF Whitcomb, JG Tully. New York, Academic Press, 1979, pp 155-174
20. Zeigel RF, Clark HF: Electron microscopy of the suckling mouse cataract agent, a noncultivable animal pathogen possibly related to mycoplasma. Infect Immun 9:430-443, 1974
21. Tully JG, Whitcomb RF, Williamson DL, et al: Suckling mouse cataract agent is a helical wall-free prokaryote (spiroplasma) pathogenic for vertebrates. Nature 259:117-120, 1976
22. Tully JG, Whitcomb RF, Clark HF, et al: Pathogenic mycoplasmas: cultivation and vertebrate pathogenicity of a new spiroplasma. Science 195:892-894, 1977
23. Clark TB: *Spiroplasma* sp., a new pathogen in honey bees. J Invert Pathol 29:112-113, 1977
24. Clark TB: Spiroplasmas: diversity of arthropod reservoirs and host-parasite relationships. Science 217:57-59, 1982
25. Junca P, Saillard C, Tully JG, et al: Caractérisation de spiroplasmes isolés d'insectes et de fleurs de France continentale, de Corse et du Maroc. Proposition pour une classification des spiroplasmes. C R Acad Sci (Paris) Ser D 290:1209-1212, 1980

26. Whitcomb RF, Tully JG, Clark TB, et al: Revised serological classification of spiroplasmas, new provisional groups, and recommendations for serotyping of isolates. Curr Microbiol 7:291–296, 1982
27. Whitcomb RF, Clark TB, Tully JG, et al: Serological classification of spiroplasmas. Yale J Biol Med 56:453–459, 1983
28. Davis RE: Spiroplasma associated with flowers of the tulip tree (*Liriodendron tulipifera* L.). Canad J Microbiol 24:954–959, 1978
29. McCoy RE, Williams DS, Thomas DL: Isolation of mycoplasmas from flowers. Proc ROC-US Coop Sci Seminar on Mycoplasma Diseases of Plants. Edited by HJ Su, RE McCoy. NSC Symposium Ser 1. Taipei, National Science Council, 1979, pp 75–81
30. Mouches C, Candresse T, McGarrity GJ, et al: Analysis of spiroplasma proteins: contribution to the taxonomy of group IV spiroplasmas and the characterization of spiroplasma protein antigens. Yale J Biol Med 56:431–437, 1983
31. Mouches C, Bové JM, Albisetti J, et al: A spiroplasma of serogroup IV causes a May-disease-like disorder of honey bees in Southwestern France. Microbial Ecol 8:387–399, 1982
32. Mouches C, Bové JM, Tully JG, et al: *Spiroplasma apis,* a new species from the honey bee (*Apis mellifera*). Ann Microbiol Inst Pasteur 134A:383–397, 1983
33. Oldfield GN, Kaloostian GH: Vectors and host range of the citrus stubborn disease pathogen *Spiroplasma citri.* Proc ROC-US Coop Sci Seminar on Mycoplasma Diseases of Plants. Edited by HJ Su, RE McCoy. NSC Symposium, Ser 1. Taipei, National Science Council, 1979, pp 119–125
34. Saillard C, Vignault JC, Fos A, et al: *Spiroplasma citri* induced lethal wilting of periwinkles is prevented by prior infection of the periwinkles by an MLO. Proc 4th Int Conf Int Org Mycoplasmology, Tokyo (abstract), 1983
35. Lei JD, Su HJ, Chen TA: Spiroplasmas isolated from green leaf bug *Trigonotylus ruficornis* Geoffroy. Proc ROC-US Coop Sci Seminar on Mycoplasma Diseases of Plants. Edited by HJ Su, RE McCoy. NSC Symposium Ser 1. Taipei, National Science Council, 1979, pp 89–98
36. Whitcomb RF, Tully JG, Rose DL, et al: Wall-less prokaryotes from fall flowers in central United States and Maryland. Curr Microbiol 7:285–290, 1982
37. Eden-Green SJ, Waters H: Isolation and preliminary characterization of a spiroplasma from coconut palms in Jamaica. J Gen Microbiol 124:263–270, 1981
38. Lee IM, Davis RE: DNA homology among diverse spiroplasma strains representing several serological groups. Canad J Microbiol 26:1356–1363, 1980
39. Bové JM, Saillard C, Junca P, et al: Guanine-plus-cytosine content, hybridization percentages and EcoRI restriction enzyme profiles of spiroplasmal DNA. Rev Infect Dis 4 (Suppl):S129–S136, 1982
40. Aulakh GS, Tully JG, Barile MF: Differentiation among some acholeplasmas by nucleic acid homology. Curr Microbiol 2:91–94, 1979
41. Archer DB: Extrachromosomal DNA in spiroplasmas. Soc Gen Microbiol Quart 8:100 (Abstract), 1981
42. Bove JM: Les spiroplasmes: nouveaux mycoplasmes pathogenes des végétaux et des animaux. Troisièmes Journées Francaises d'Etudes et d'Information, ACTA, Paris, France: 379–427, 1982
43. Ranhand JM, Mitchell WO, Popkin TJ, et al: Covalently closed circular deoxyribonucleic acids in spiroplasmas. J Bacteriol 143:1194–1199, 1980
44. Mouches C, Bové JM: A plasmid from *S. citri* strain M4 hybridizes with extrachromosomal DNAs from other spiroplasmas, including corn stunt spiroplasma E275, tick spiroplasma 277F, and cocos spiroplasma N525. Yale J Biol Med 56:723–727, 1983
45. Mouches C, Menara A, Tully JG, et al: Polyacrylamide gel analysis of spiroplasmal proteins and its contribution to the taxonomy of spiroplasmas. Rev Infect Dis 4 (Suppl):S141–S147, 1982
46. Mouches C, Vignault JC, Tully JG, et al: Characterization of spiroplasmas by one and two dimensional protein analysis on polyacrylamide slab gels. Curr Microbiol 2:69–74, 1979
47. Eden-Green SJ, Waters H: Isolation of corn stunt spiroplasmas in Jamaica, and probable dual infection with maize mosaic virus. Ann Appl Biol 99:129–134, 1981