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Phytoplasmas: Genetics, Diagnosis and Relationships with the Plant and Insect Host

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1. ABSTRACT

Phytoplasmas cannot be cultivated *in vitro*, and remain the most poorly understood plant pathogens Despite this limitation, the investigation of their nature with the aid of modern tools has produced noteworthy results during the last 20 years. Using biochemical and molecular approaches, the phylogeny of the phytoplasmas has been described, their chromosomal and extrachromosomal components are being studied, and information on the localization, movement, and metabolic interference occurring in their insect and plant hosts accumulated. At the same time, the application of the new findings in phylogeny and genetics has aided the development of powerful diagnostic tools that have improved the ability to manage diseases which are induced by phytoplasmas.

2. INTRODUCTION

Phytoplasmas have the smallest genome among bacteria. Despite their reduced genomic size, they infect plants circumventing the defence response, colonize insect guts, and actively move to insect salivary glands. The complex array of symptoms they induce in plants suggests an intimate, specific relationship with the hosts; however, some phytoplasmas have an extremely wide host range, as in the case of '*Ca.* Phytoplasma asteris', which is found associated with more than 80 plant species, and can be transmitted experimentally by more than 30 species of insect vectors to more than 200 different plant species (1-4). The phytoplasmas are therefore unique among plant pathogens, and the molecular mechanisms underlying their biological cycle have stimulated interest and investigations.

Unfortunately the phytoplasmas cannot be cultivated *in vitro*. This severe handicap has dramatically slowed understanding of the phytoplasma biology, and study has been mostly limited to their role as plant pathogens, focusing on their epidemiology, spread, economic impact, and diagnosis. Only in the last 20 years phytoplasmas have been investigated using the methodologies appropriate for their bacterial nature. The advent of molecular biology techniques had a tremendous impact on the field and biochemical, physiological and genetic information were achieved. Nowadays these advances have resulted in a much deeper insight into the characteristics of the phytoplasmas, which, on turn, significantly improved understanding of the plant/phytoplasma/insect relationship.

3. PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES

Due to the inability to cultivate phytoplasmas under axenic conditions their physiological and genetic characteristics remained poorly understood. In the late nineteen eighties, however, a number of elegant experiments and innovative ideas pursued by the teams lead by B.B. Sears, B.C. Kirkpatrick, and C. Hiruki, unveiled the nature of these plant pathogenic wall-less prokaryotes.

Major goals were accomplished by exploiting the putative taxonomic relatedness with other mycoplasmas, postulated on the basis of micromorphology and sensitivity to tetracycline treatment of the host. If the "mycoplasma-like organisms" (as they were referred to) were actually related to the *Mollicutes*, then they should have a genome with a low content of guanine and cytosine, a feature common to all members of the class (although to different extents from species to species). The successful separation of phytoplasmal DNA by repeated CsCl buoyant density gradient centrifugation from the insect or plant host DNA (5-7) led to the first cloning of phytoplasma DNA (5) and to the estimation of its guanine plus cytosine percent molar content (G+C mol%). The G+C mol% was estimated for a number of phytoplasmas, including those associated with Aster Yellows ('*Ca.* Phytoplasma asteris'), Rape Virescence, Periwinkle virescence, Diplotaxis phyllody and Apple proliferation ('*Ca.* Phytoplasma mali'), and resulted between 23.0% and 26.2% (8).

In other key experiments carried out on an *Oenothera hookeri* leaf tip culture infected by phytoplasmas (9), Lim and Sears showed that the membrane of the phytoplasmas resembles that of the non-sterol requiring acholeplasmas and not that of the sterol-requiring mycoplasmas (10), that these mollicutes use UGA as a stop codon and not as tryptophan like the members of the genera *Spiroplasma* and *Mycoplasma* (11), and that the size of their genome was smaller than that of the spiroplasmas but comparable to that of the animal mycoplasmas (12). These results, together with the first complete 16S rDNA sequences of the *Oenothera hookeri* phytoplasma (13) and the Western Aster Yellows phytoplasma (14), depicted the evolutionary history of this group of plant pathogens (15, 16). Accordingly, the phytoplasmas are phylogenetically most closely related to the genus *Acholeplasma*, which is characterized by similar features, such as the lack of cholesterol in the membrane and the use of UGA as a stop codon. The phytoplasmas' ancestors were presumably insect inhabiting prokaryotes similar to the acholeplasmas. It is possible, although speculative, that the divergence of the phytoplasma branch was determined by the appearance of the angiosperms, which occurred, based on recent estimates (17), between 140 and 210 million years ago (Ma.). Interestingly, using the 16S rDNA sequence as molecular clock, Maniloff (18) calculated that the phytoplasmas diverged from the acholeplasmas 180 Ma.

While the evolutionary history of the phytoplasmas was being unveiled, Deng and Hiruki (19) provided the first PCR amplification of a phytoplasmal 16S rRNA gene, which paved the way to selective PCR, nowadays the method of choice for detection and taxonomic identification of phytoplasmas. At the moment of writing, more than 200 different, nearly full length sequences of phytoplasma 16S rRNA genes have been deposited on public databases (20-22), almost all obtained by phytoplasma-selective PCR in the presence of an excess of host nucleic acids.

The research results published in the early nineteen nineties not only clarified the origin of these organisms, as relatives of the acholeplasmas, but also provided the tools which were then exploited for diagnosis, in the investigation of diversity and the epidemiology of the phytoplasmas.

4. GENES AND GENOMES

4.1. Chromosome

With the advent of DNA techniques the analysis of the genome become the most obvious approach to study the biological features of organisms that cannot be cultivated *in vitro* and therefore their biochemical and physiological characters cannot be tested under axenic conditions.

Unfortunately a pure preparation of the phytoplasmas proved to be difficult. Not only these organisms are usually present at low titres in the host tissues, but also their size is similar to several cellular organelles of the eukaryotic cell. Moreover, their lack of a cell wall makes the mollicutes very sensitive to changes in the osmotic environment. Thus, the early attempts to isolate phytoplasmas by differential and gradient centrifugation were laborious and often inefficient with reference to both purity and yield (23-25).

A significant technological advance was the development of a technique based on Pulsed Field Electrophoresis (PFGE) for the visualization (26) and, successively, the purification and analysis (27, 28) of intact or digested chromosomes prepared in agarose plugs. Using this technique, the size of about 70 different phytoplasmas was calculated (29) and the physical maps of the chromosomes of the Western X disease (27), Apple proliferation (30), Sweet Potato Little Leaf (31) and

European Stone Fruit Yellows (32) phytoplasmas were determined.

The estimation of the size of the genomes gave noteworthy results. '*Ca.* P. cynodontis' has a genome of only 530 kb and therefore is the bacterium with the smallest genome so far determined (29). Conversely, a tomato strain of the stolbur phytoplasma has a genome of 1350 kb, i.e. more than 2.5 times larger. Huge variations are observed even between closely related strains: for example, the Rape virescence phytoplasma and the Hydrangea phyllody (strain from Belgium) differ greatly in their genome size [1130 kb vs. 660 kb according to (29)] despite the fact that they belong to the same subgroup of the aster yellows clade and differ little in their biological characteristics. In the mollicute chromosomes, differences in size are usually due not to a different rate of reduction, but to the occurrence of gene duplication and redundancy. *Mycoplasma pneumoniae* and *M. genitalium* differ significantly in their genome size, but the difference is largely due to gene duplication and to the presence of repetitive sequences in *Mycoplasma pneumoniae*. In the genome of the Onion Yellows (OY) phytoplasma (33), it was estimated that 18% of the total genes are multiple redundant copies of only five genes: *uvrD* (ATP-dependent DNA helicase , 3117 nt., 7 copies), *hflB* (ATP-dependent Zn protease, 1551 nt., 17 copies) , *tmk* (thymidylate kinase, 624 nt., 6 copies), *dam* (DNA methylase, 660 nt., 4 copies) , and *ssb* (single-stranded DNA-binding protein, 345 nt., 15 copies), all of which are generally single copies (if they exist at all) in the other mollicutes whose genome has so far been sequenced (the only exceptions are *dam*, 3 copies in *M. penetrans*, and *uvrD*, 2 copies in *M. gallisepticum*). In addition, 5 genes encoding elements of transporter systems have multiple copies, presumably not all functional. Multiple copies of insertion sequence – like elements are also present in the genome of the OY and other phytoplasma strains (34).

From the evolutionary point of view, the ancestors of the class *Mollicutes* split early on into two branches, the so called AAA (*Asteroplasma, Acholeplasma, Anaeroplasma*) and SEM (*Spiroplasma, Entomoplasma, Mycoplasma*). Both branches independently underwent genome size reduction. However, the size reduction was more modest in the spiroplasmas, which are regarded as evolutionary early mollicutes, and their genome size varies between 780 and 2200, depending on the species (35). Conversely, the phytoplasmas lost three fourths or more of their genes during their evolution from the *Bacillus*/*Clostridium* ancestor bacterium. It is likely that their genome shrank to 500-700 kb, then in some strains enlarged again by gene duplication to 1000-1400 kb. Phytoplasmas and spiroplasmas share a common life style, being phloem inhabiting plant pathogens transmitted by insects, which are in turn actively colonized. However, the spiroplasmas can be cultivated in artificial media; conceivably, in their reductive evolution the phytoplasmas lost mostly genes relevant for their basic metabolism, but retained the set of functions necessary for survival in phloem and colonization of insect bodies that they share with their distant relatives, the spiroplasmas.

It is well known that in their reductive evolution all mollicutes lost some genes essential for important biochemical pathways (35). All genes involved in the synthesis of the cell wall, the biosynthesis of aminoacids and cofactors were lost, with few exceptions. The sets of genes involved in lipid and nucleotide metabolism were severely reduced. Thus the mycoplasmas are host-dependent for fatty acids, from which they generally synthesize their own phospholipids and glycolipids, although in some cases preformed host phospholipids are directly incorporated into the cellular membrane. Possibly due to their parasitic nature, use of host cell components and therefore limited biosynthetic needs, mollicutes lack many genes for energy metabolism. There is no tricarboxylic acid cycle, no quinones and cytochromes in the mollicute genomes so far sequenced, and it is therefore believed that they depend, for the production of ATP, on mechanisms other than oxidative phosphorylation (35).

The mollicutes are then obliged to obtain basic nutrients from their host or from complex growth media (35). Being intracellular parasites, phytoplasmas have a more pronounced dependence on the host and apparently receive some metabolites which cannot be easily supplied as a growth media.

The analysis of the genome sequence of the OY phytoplasma (33), the only complete sequence available at the time of writing, shed some light on the nutritional requirements of these fastidious bacteria. The genome of the OY phytoplasma is about 861 kb and contains 754 ORFs, corresponding to 73% coding capacity. As noted for the other mollicutes, the reductive evolution of the phytoplasma genome did not result in a gene density higher than in other prokaryotes. Like other mollicutes, the OY phytoplasma lacks genes for the biosynthesis of the aminoacids and fatty acids, the TCA cycle, and oxidative phosphorylation (33). While the reduction of biosynthetic genes appears to be the rule for all mollicutes, genes for energy yielding pathways have been differently reduced in the various genera. The mollicutes are traditionally subdivided into fermentative and non fermentative (35). The fermentative mycoplasmas produce acids from carbohydrates, decreasing the pH of the medium; the sequencing project of the *M. genitalium* (36) and *M. pneumoniae* (37) genomes identified in these organisms the genes for all enzymes of the Embden-Meyerhof-Parnas pathway, and an incomplete pentose phosphate pathway. The nonfermentative mycoplasmas may produce ATP through the arginine dihydrolase pathway, raising the pH of the medium (35). Others oxidize organic acids such as lactate and pyruvate to acetate and CO_2 (38). Moreover, the ureaplasmas conceivably produce energy through a F_0F_1-type ATPase which uses the transmembrane potential resulting from urea hydrolysis (39).

According to the genome sequence data, in OY phytoplasma the gene set for complete functional phosphotransferase system could not be found and the pentose phosphate pathway, the arginine dehydrolase pathway and the ATPase systems were all missing.

It is possible that some of the genes of unknown function (which account for 33% of the ORFs) are used to complement these functions, but due to their high sequence conservation this is unlikely. Thus, the phytoplasmas may depend completely on glycolysis for their energy needs. Alternatively, they might import ATP from the host, but an ADP/ATP translocase (such as that identified in *Chlamydia trachomatis*) was not found in the OY phytoplasma genome, and therefore the use of an unknown mechanism has to be hypothesised.

Energy metabolism is certainly a key topic for understanding phytoplasma biology and pathogenesis, as the results of work carried out on *Spiroplasma citri* clearly indicates that these are central aspects in the physiopathology of phloematic diseases. Using newly developed tools for transposon mutagenesis, Foissac and co-workers (40) identified several *S. citri* mutants impaired in virulence and insect transmission. Mutant GMT553 which contained a single transposon insertion within the fructose utilization operon, was unable to use fructose, and did not induce symptoms when inoculated into plants (41). Fructose depletion can therefore be regarded as a primary cause of symptoms in spiroplasma associated yellows diseases. Lepka *et al*. (42) and Maust *et al*. (43) have reported the occurrence of changes in the concentration of carbohydrates in phloem, root and leaves of phytoplasma-infected plants, as compared to the healthy control. Given the large array of symptoms that characterize phytoplasma diseases, it can be speculated that nutrient depletion may not be restricted to fructose, but might include other compounds depending on the pathogen involved. This is confirmed by the discovery that reduction in the concentration of photosynthetic pigments (44-46) and total soluble proteins (44-47), as well as alterations in hormone balance (43, 48-50) and aminoacid transport (42) are also effects of infection of herbaceous and woody hosts by different phytoplasmas. Whether these physiological changes are directly caused by phytoplasma uptake or are downstream events remains to be established.

Nucleic acid metabolism may also interfere with that of the host plant. In fact, the OY phytoplasma differs from all other mollicutes whose genome has been sequenced to date in lacking phosphoribosyltransferases (33), implying that this phytoplasma may be unable to synthesize the ribose-5-phosphate necessary for the synthesis of its own nucleic acids. It is possible that the phytoplasmas are completely dependent on the host for nucleic acids supply; they may have to be imported from the environment, and recycled. Thus, the recombination of phytoplasma DNA with exogenous nucleic acids, as suggested by the analysis of extrachromosomal DNA sequences (see next chapter) may have a physiological basis.

Analyzing the recent data of the genome sequencing project, Bai and coworkers (51) identified four genes for proteins which are present in the Aster Yellows – Witches' Broom phytoplasma and in *S. kunkelii*, but not in six human mycoplasmas belonging to the genus *Mycoplasma* and *Ureaplasma*. Three of these proteins are involved in nucleic acids catabolism (PNPase, Cytosine deaminase) or replication (CFB) and the fourth is an RNA binding protein. These findings reinforce the notion of a central role of nucleic acids metabolism in the phytoplasma biology.

4.2. Extrachromosomal DNA

The presence of extrachromosomal DNA molecules (EC-DNA) in the cytoplasm of phytoplasmas has been postulated since the time that early electron microscopy (EM) observations (52, 53) reported the detection of virus-like particles in the phytoplasmas. However the preliminary reports were not later confirmed. Therefore, when several investigators (54-60) found phytoplasma-associated DNA molecules that move fast in agarose gel electrophoresis they were generally considered of plasmid nature (61). Even after several studies, carried out by cloning, sequencing and Southern blotting (61-75) the identity and function of these molecules has not yet been completely clarified.

To date, several extrachromosomal molecules have been sequenced in full or in part: they include several EC-DNAs from different variant substrains of the OY strain of '*Candidatus* Phytoplasma asteris' (67, 70-72, 75), from the Severe strain of the Aster Yellows phytoplasma (63), the Sugarcane White Leaf (SCWL) phytoplasma (66), the Beet Leafhopper Transmitted Virescence agent (BLTVA) (74), the Vaccinium witches' broom (VAC) phytoplasma (68), and the Peanut witches' broom (PWB) phytoplasma (73). According to the available sequence data, the EC-DNA can be subdivided into two classes, here named type I and type II. It should be noted, however, that EC-DNA was detected by hybridization in a larger number of strains than have been cloned and sequenced, and therefore this subdivision may not be exhaustive.

Type I EC-DNAs include the fully sequenced pOYW plasmid (75) from the wild type strain of the OY phytoplasma, and its variants found in the mutant strains pOYNIM and pOYM (70). These are true plasmids, as they possess an expressed gene with significant similarity to the replication initiator (Rap) of the plasmids of the pLS1/pMV158 family (75). They also possess a SSB (single stranded DNA binding protein), which is consistent with Rolling Circle Replication (RCR), resembling plasmids commonly found in gram positive bacteria and particularly in members of the *Clostridium*/*Bacillus* phylogenetic clade, which are phylogenetically related to the mollicutes. Rolling circle replication (RCR)-type plasmids are widespread among Gram-positive bacteria and a plasmid of the pMV158 family (named pKMK1) has been reported to occur in *Mycoplasma mycoides* subsp. *mycoides* (76). However the replication initiator protein gene of the plasmids found in the phytoplasmas codes an extra, C-terminal 100 a.a. whose sequence resembles a virus-like helicase domain, most similar to that of circoviruses (75), which has never been reported in any replication protein from RCR-type plasmids, including those of the pMV158 family.

Type II EC-DNAs are unique to phytoplasmas.

They are characterized by a gene, expressed *in planta* (69), with sequence similarity to the replication associated protein (Rep) of the geminiviruses (68). The EC-DNAs of the type II showed prokaryotic features, such as Shine-Dalgarno, promoter sequences, and a gene similar to the *cop* of pIP404 which regulates plasmid copy number in *Clostridium perfringens* (69). In addition, they have eukaryotic polyadenylation signals and TATA boxes, as previously reported for the geminivirus abutilon mosaic virus (77). The relatively modest similarity between the replication associated proteins of type I and type II suggest an independent origin.

The presence of a circovirus-like helicase domain in the C-terminal region of the replication initiation protein of the phytoplasma type I EC-DNAs and the similarity of the entire replication associated protein of phytoplasma type II EC-DNAs to the geminivirus Rep protein suggest recombination events between plant viruses and gram positive bacterial plasmids within the phytoplasma cell.

The geminiviruses are single stranded DNA (ssDNA) viruses which colonize the plant phloem and are vectored by various insects, including leafhoppers, which also transmit phytoplasmas belonging to several phylogenetic groups. The common habitat could have facilitated gene exchange between the viruses and the phytoplasmas, which may have occurred in either the plant or the insect.

Most plant viruses have a ssRNA genome, and DNA viruses are restricted to the families *Geminiviridae* and *Nanoviridae*. Koonin & Ilyina (78) have hypothesized that the geminiviruses may have originated from prokaryotic ssDNA plasmids, on the basis of the occurrence of moderately similar motifs in the RCR initiator domain (N-terminal region) of the Rep proteins of the geminivirus and the RCR initiator protein of plasmids of the pUB110 and pMV158 families. Therefore it is tempting to speculate that the phytoplasmas had a role in the evolution of geminiviruses, and that the phytoplasma EC-DNAs are the remnants of a molecule ancestral to the virus nucleic acids. At present no evidence has been found that could indicate whether the type II EC-DNAs are the ancestors of geminiviruses or the product of a recombination event between phytoplasmal type I EC-DNA and ssDNA viruses. However, there is evidence that recombination is a frequent event among the phytoplasma EC-DNAs. In some OY phytoplasma strain variants molecules which are almost certainly the product of recombination of a type I and a type II EC-DNA have been detected. Moreover ssDNA binding protein genes, highly similar to that of pOYW, have been found in several copies in the genome of the OY phytoplasma (33). Moreover, it would be difficult to explain the presence of an eukaryotic signal without postulating a recombination event.

The high sequence similarity observed among EC-DNAs of phytoplasmas of very different geographical origin and with different plant and insect hosts suggests an ancient common origin. Thus, the EC-DNA function and possible involvement in pathogenesis, transmission or specificity determination has been considered. Denes and Shina (62) detected EC-DNA rearrangements in a strain which had lost insect transmissibility, but chromosomal changes could not be evaluated. Namba and coworkers (79) found that a highly pathogenic and a mild strain of the OY phytoplasma (named OY-W and OY-M) differed in their EC-DNA content, the OY-M EC-DNA being smaller than that of OY-W, and speculated that the difference could be correlated with pathogenicity. However the strains OY-W and OY-M differed significantly in their chromosomes.

The hypothesis that EC-DNA rearrangements could be involved in the modulation of virulence contrasts with the high variability in size and number of EC-DNA molecules that is usually observed among closely related strains or even within a single field population. Rekab and coworkers (68) characterized several strain variants of the Italian Clover Phyllody phytoplasma from different weeds, which had different EC-DNA patterns. When one isolate from carrot and one from clover were insect inoculated on both carrot and clover, no difference was found in the severity of the disease and in the transmission characteristics. Therefore at present the hypothesis that the EC-DNAs have a role in plant specificity and pathogenicity lacks experimental support. On the other hand, a function for the EC-DNA cannot be ruled out, as these molecules, despite their ancient origin, were not lost during evolution. The structure and size of the EC-DNA appears to vary greatly among field collected plants (68, 74, 80), possibly depending on the stage of infection. The BLTVA phytoplasma contains pBLTVA-1, 10.8 kb in size, and several smaller derivative EC-DNAs, including pBLTVA-2, of only 2.6 kb. According to Southern blot analysis, only pBLTVA-1 was detected in DNA extracted from recently infected periwinkle shoots and the concentrations of pBLTVA-2 increased significantly late in the infection process (80). A consequence of this observation is that full size molecules, or the phytoplasma cells carrying them, should be positively selected in the insect to provide the prevalence of full sized EC-DNAs at the beginning of the next infection and their conservation over time. This would also be consistent with the reports of size defective EC-DNAs in non-insect-transmissible phytoplasma strains (62, 70).

It is known that not all phytoplasmas bear EC-DNA: to date, type I EC-DNA has been found in phylogenetic groups 16SrI, 16SrII, and 16SrXIV while type II EC-DNA has been detected in phylogenetic groups 16SrI and 16SrIII, [groups according to reference (21)]. In some phylogenetic groups such as the 16SrX (Apple proliferation phytoplasma clade) EC-DNAs have been detected by hybridization (61) but the lack of sequence information hinders their classification. Moreover, only a minority of the phytoplasmas has been examined for EC-DNA content, thus the possibility that other phylogenetic groups include EC-DNA bearing phytoplasmas cannot be excluded.

5. RELATIONSHIPS WITH THE PLANT AND THE INSECT HOST

5.1. Plant

A wealth of information on phytoplasma

localization, movement and multiplication in plant and insect hosts, and the alterations induced in both hosts, has been accumulated over time. Though often contradictory, presumably due to the use of different host/pathogen systems, the results of previous research are now being reinterpreted in the light of genetic data, aiming at the identification of plant and phytoplasma features potentially involved in host/pathogen relationships (for an updated picture see the recently published ref. (81) and (82).

Early EM observations of thin sections revealed, in the sieve tubes of vascular tissues of many plant species, phytoplasma cells with different shapes (83-98). Similar structures were also found when immunotrapping and negative stain (99) or EM analyses of cryosections (100) were used. In their early study, Sinha and Paliwal (101) suggested that the different cellular shapes could represent different developmental stages in the life of the bacteria. The inability to culture phytoplasmas *in vitro* and the absence of markers for the different stages in the phytoplasma cycle have so far hampered further considerations on phytoplasma morphology and a putative bacterial life cycle in the host.

While a morphologically defined cellular life cycle lacks experimental support, there is evidence of a disease cycle, in the sense of a host organ colonization pattern. The organ tropism of a phytoplasma may depend on the phytoplasma-host plant combination, especially in the case of woody hosts (81). A further complication is that the phytoplasma concentration varies greatly from plant to plant. Recently, competitive PCR has made it possible to estimate the phytoplasma titre in different host plants; periwinkle was confirmed as a high-concentration species irrespective of the phytoplasma's taxonomic affiliation, while other species should be considered medium- or even low-concentration hosts (102). Apple Proliferation-resistant rootstocks were among the latter, confirming the microscopic observations made, a decade before, on decline-tolerant *Malus* taxa (103).

Taxa with different susceptibility to phytoplasma infection have been reported for a number of plants such as apple (103, 104), pear (105) *Prunus* spp. (106-110), date palms (111), and rice (112) among others, although a quantitative correlation between the concentration of the phytoplasma in the phloem and the severity of symptom expression has not been established. Using different approaches, a reduction/suppression of phytoplasma multiplication has been suggested by fluorescence microscopy (103, 104) or grafting (113, 114) when susceptible cultivars were grafted on resistant or tolerant rootstocks. More recently, the disappearance of phytoplasmas from the canopy but not from the roots of recovered apple proliferation-infected trees has been reported (115).

Host susceptibility is not the only factor relevant in triggering pathogen concentration. Different strains of the same phytoplasma may reach different concentrations in the infected host as reported for aster yellows (116) and ash yellows (117). In a co-inoculation experiment, Sinclair and Griffiths (117) showed that the aggressive strain rDNA restriction profile of Ash yellows was detected by PCR sooner and more frequently than a milder one, unless the latter was inoculated well before the aggressive strain. While the concept of preemptive dominance can explain the latter results, a quantitative approach to study multiplication of the phytoplasmas, especially in woody hosts is still needed. As reported for pear decline (118-120), apple proliferation (120), European stone fruit yellows (106, 121), X-disease (122), Bois Noir (123), jujube and walnut witches' broom (124, 125) and AusGY (126) phytoplasma concentration in woody hosts may also differ according to the season.

Accurate studies on the dynamics of phytoplasma multiplication and movement in plants have been conducted on herbaceous hosts (87, 116, 127, 128). Following inoculation by the insect vector, the phytoplasma is rapidly transported to actively growing areas, such as shoots and root tips. In most cases, the phytoplasma titre in the root is higher than in the shoot apex (87, 128). The movement of phytoplasmas probably occurs following the nutrient flux in the plant towards sink organs. EM analysis has sometimes shown phytoplasma cells in sieve tubes very close to sieve plates (84, 89, 129, 130) and if this is not an artefact due to a sudden release of pressure in the phloem during sample collection, it is possible that phytoplasmas cross the sieve pores. Callose deposition resulting in necrosis of the phloem, observed for example in flavescence dorée-infected broad bean (87), can then stop their movement. Necrosis of the phloem due to other causes such as winter chilling has been reported for several woody plants (84, 85, 87, 131-133) and can also reduce phytoplasma movement. In this case, phytoplasmas in the aerial part of the tree may degenerate and recolonization of the plant has to start again in the spring, as suggested for apple proliferation (134) and pear decline (120) in Germany, and elm yellows (84) in North America. Interestingly, in infected woody hosts, roots often sustain a high concentration of phytoplasma cells (85-87, 89, 133, 135-138). Seasonal movement in woody hosts is not a rule, since reports differ for other phytoplasma-woody plant combinations and climatic conditions. In the case of the X-disease phytoplasma infecting chokecherry or peach, overwintering of the bacteria in dormant buds has been reported (122, 139), while in *Prunus* spp., European stone fruit yellows phytoplasmas are absent in the phloem of newly developed leaves in spring, but easily detected in off-season leaves in winter (106, 121). Also mild winters are thought to be the reason for the survival of pear decline phytoplasma in the aerial part of affected pear trees in Spain (118).

5.2. Insect

Phytoplasmas are transmitted in a persistent manner by insects belonging to the families C*icadellidae, Cixidae, Psyllidae, Delphacidae,* and *Derbidae* (140, 141). The vector acquires the phytoplasma by feeding on an infected source plant (acquisition access period, AAP) and then transmits the pathogen to a healthy one (inoculation access period, IAP) only after completion of the latent period (LP), during which phytoplasmas multiply in the

midgut, haemocytes and salivary glands of the vector (3). Factors influencing the length of the AAP, LP and IAP as well as the efficiency of transmission have recently been reviewed (81). Once a vector becomes infectious, infectivity is retained for life (142, 143), although some discontinuities in vectoring abilities have been reported for several phytoplasma – vector associations (142, 144). Some factors influence transmission, among which are life stage (142, 145), gender (145-149), presence of associated symbionts (150), flight behaviour (146-148), weed control measures (151-155), temperature (156), phytoplasma strain (150, 156), source (157, 158) and recipient plant species (157) [reviewed in (81)].

Evidence of transovarial transmission has been reported for SCWL phytoplasma in the vector *Matsumuratettix hieroglyphicus* (Matsumura) (159), for Mulberry dwarf phytoplasma in *Hischimonoides sellatiformis* (160) and for an aster yellows strain in strain in *Scaphoideus titanus* (161), although the ability of phytoplasma-infected leafhoppers progeny to infect healthy hosts has not been verified.

Multiplication of phytoplasmas in the insect body has recently been confirmed using quantitative approaches based on different techniques (162-167). Multiplication probably only occurs in some organs/tissues such as the midgut, salivary glands and haemolymph (168), although the brain has also been reported as an active site of Western X phytoplasma multiplication (169). Pathogenicity effects on different organs or even reduction of longevity and fecundity have been reported in some phytoplasma-infected vectors (170-174) although pathogenicity of the phytoplasma for the insect is not the rule. In fact, in other phytoplasma-vector combinations moderate positive to mutualistic effects have been reported (175-178), suggesting that a general model cannot cover all phytoplasma-vector associations.

Ingested phytoplasmas pass into the midgut, then adhere to the midgut epithelium cells, pass through the epithelial cells, invade the haemolymph and are transported to different organs or tissues, including salivary glands from where they can be excreted with saliva during feeding (3). Although phytoplasmas have been detected by different techniques in the various organs or tissues of the vectors (167, 179, 180) the existence of two barriers have been suggested: the midgut and the salivary glands. There are reports of phytoplasma multiplication in the midgut of non-vector insects (171, 178, 181, 182) clearly indicating that there are cases where phytoplasmas colonize the insect but are not transmitted. Attachment of FD phytoplasmas to dissected or cryo-sectioned organs of *Euscelidius variegatus* and *Scaphoideus titanus* has been demonstrated (183), but no phytoplasma-specific receptor sites in the organs of the insect vectors have been found, although there is indirect evidence of specific receptor sites on the midgut and salivary glands of *S. titanus* (184). A time course for the colonization of different organs of the vector has been demonstrated only in a few phytoplasma – insect combinations [reviewed in (81)]. It should also be remembered that, in some cases, even the host plant may influence the outcome of transmission. In fact certain plant species may be infected with phytoplasmas by feeding insects, but are unsuitable for further acquisition, at least with some vector species (158, 185) reviewed in (81).

6. METHODS USED IN PHYTOPLASMA DIAGNOSTICS

As mentioned, the colonization by the phytoplasmas may depend on the season, organ, host and pathogen species, and consequently result in a variety of different symptoms with complex interference to the host physiology. Thus, although they may represent a major threat to the cultivation of economically important species, such as fruit trees, palms and grapevine, the prompt identification of phytoplasma diseases is by no means trivial. Sensitive and accurate diagnosis of these microorganisms is therefore a prerequisite for the management of phytoplasma-associated diseases.

Following their discovery (186) phytoplasmas have been difficult to detect due to their low concentration especially in woody hosts (102) and their erratic distribution in the sieve tubes of the infected plants (187). EM observation of thin (83-86, 89, 188-193) or thick cryosections (100) and less frequently scanning EM (194-197) were the only diagnostic techniques until staining with DNA-specific dyes such as DAPI was developed (198, 199) and used with success for the diagnosis of different phytoplasmas even in woody hosts such as coconut palms (200), ash (133, 201), pear (202) and sandal trees (203). Lately, protocols for the production of enriched phytoplasma-specific antigens have been developed, thus introducing serological-based detection techniques such as immunogold labeling or immunosorbent EM for the study of these pathogens in plants (204, 205) and insect vectors (99, 206).

The establishment of EM-based techniques represents an alternative approach to the traditional indexing procedure for phytoplasmoses based on graft transmission of the pathogen to healthy indicator plants. In fact, transmission by grafting (207, 208) or dodder (209-215) to healthy plants belonging to the same or other herbaceous species such as periwinkle is time- and space-consuming and only a few cases have been reported. In many others instances the aetiology of the disease is assessed by a combination of quicker techniques, such as serology (216-219). Moreover, the availability of phytoplasma-specific antibodies has led to the improvement of EM-based diagnostic techniques and immunofluorescence procedures have been designed to specifically detect phytoplasmas in different plant (220-222) and insect hosts (223).

6.1. Serology

In the early nineteen eighties, the first polyclonal antibodies against different phytoplasma-associated antigens were developed (24, 224-227). Purification of the antigenic fraction was a major difficulty and it soon became clear that improved protocols were needed to reduce plant contamination. Different protocols for partial

purification of phytoplasmas were then assayed with some success (23, 226).

In other cases, especially with phytoplasmas such as Flavescence dorée and peach eastern X-disease infecting woody plants, alternative herbaceous hosts (broad bean and celery) were experimentally infected to achieve higher phytoplasma antigen concentrations (228, 229). In general, non-specific reactions against plant material in ELISA analysis has been reduced by including a cross-absorption step of the antiserum with a concentrated preparation of healthy plant antigens. Immunoaffinity purifications of phytoplasmas from plant extracts (230) or of plant antigenic contaminants from phytoplasma-enriched extracts (23, 231) were also used to reduce contamination in the production of monoclonal antibodies against FD (230), primula yellows (23), faba bean phyllody (231), sugar-cane whiteleaf and Bermuda-grass whiteleaf (232).

F(ab')2 fragments of the IgG are extremely efficient in trapping phytoplasma antigens from crude preparations; coating of the ELISA plate with these fragments has minimized cross-reaction with plant contaminants in the diagnosis of faba bean phyllody (233).

Polyclonal antibodies against phytoplasmas belonging to different taxonomic groups have been developed and are very efficient in immunosorbent electron microscopy (IEM) (225), fluorescence microscopy analysis (23, 24, 234-236), western blots (23, 231), and ELISA (23, 24, 227, 232, 233, 236, 237), although, in some cases, they showed cross reactions with other phytoplasmas (23, 225) or even with the healthy controls (235).

For these reasons, many research groups started the production of monoclonal antibodies. Specific monoclonal antibodies have been developed against a limited number of phytoplasmas such as AY (238, 239), primula yellows (23), peach eastern X-disease (229), apple proliferation (216), tagetes witches' broom agent (240), grapevine yellows (236), FD (164, 230, 241), peach yellow leafroll (237) , maize bushy stunt (176), rice yellow dwarf (242), brinjal little leaf (243), X-disease (244), tomato stolbur and clover phyllody (245) and sweet potato witches' broom (246). The presence of contaminant host proteins was a serious problem for the screening of phytoplasma-specific monoclonal antibodies. To reduce the labour-intensive work of selecting specific hybridomas, Hsu and coworkers developed a specific procedure by using mice neonatally injected with nontarget (healthy plant proteins) antigens before the effective immunization (247). In another approach, phytoplasma antigens obtained from dissected salivary glands of AY-infected insect vectors were used to produce monoclonal antibodies that were then screened with a pathogen antigenic preparation obtained from infected plants (238). Despite its success, the method has been rarely used since it is extremely time- and labour-consuming and for many phytoplasmas the vector species are unknown. In general, monoclonal antibodies always showed a clear advantage over polyclonal ones in providing low background values for the healthy extract controls in ELISA (23) and immunofluorescence microscopy (235). Monoclonal antibodies have been used to differentiate phytoplasmas belonging to different subclusters, such as in the AY strain cluster (248) or in the groups 16Sr-V (230) or 16Sr-X (216) [named according to (21)]. ELISA tests based on monoclonal or polyclonal antibodies have been optimized for phytoplasmas belonging to different taxonomic groups and are now commercially available for the diagnosis of economically important phytoplasma-associated diseases such as Flavescence dorée and Apple proliferation. Serological tools have also been used with success to detect different phytoplasmas in leafhopper vectors or potential vectors, by immunofluorescence (28, 162, 223, 249-251), immunosorbent electron microscopy (99, 250, 252), dot blot (162) or ELISA (28, 87, 162, 253). In other approaches, tissue blotting with direct or indirect antigen detection has been used for the specific diagnosis of phytoplasmas (254). In more recent years, antibodies have been prepared to partial sequences of the major immunodominant proteins of some phytoplasmas (255-257, 258, 259) expressed as fusion proteins in *Escherichia coli*. In another case, an antibody to SecA, an essential component of the bacterial Sec protein translocation system in OY phytoplasma, has been produced (260). Although its use for diagnostic purposes is questionable, its good labelling properties in immunohistochemical studies (260) has clearly indicated that other proteins besides the major immunodominant ones can be considered as potential targets for serological detection. Serological-based diagnosis is easy and inexpensive for large scale screening, but lacks sensitivity especially when the pathogen titre is low; therefore in the last 15 years major efforts have been made to develop nucleic acid-based tools, such as hybridization probes and PCR reagents.

6.2. Nucleic acid technology

Phytoplasma detection is now routinely done by nucleic acid-based techniques. The success of this approach is largely dependent on obtaining total nucleic acid preparations of good quality and enriched in phytoplasma DNA, but this has always been a hard task. Different protocols for total DNA extraction have been reported for the detection of these plant pathogens (261-264). The main goal of each protocol was to concentrate phytoplasma DNA while reducing enzyme-inhibitory plant polyphenolic and polysaccharide molecules. This is generally attained by including a phytoplasma enrichment step. The complexity of most DNA extraction procedures limits the number of samples that can be processed and therefore simpler protocols have been developed (265-267) even using commercially available microspin column matrices (264). The success of each protocol depends on the plant host species (268, 269) as well as on the sampling procedures (270) or storage conditions of collected samples (271). An enrichment step also improves phytoplasma detection in insect vectors, although, possibly due to the high titre of the bacteria in the insect body, diagnostic PCR sometimes produces acceptable results even when total DNA is prepared with a quick boiling procedure (63, 272).

In the nineteen nineties, following the first cloning of phytoplasma DNA (5), nucleic acid-based

probes (randomly cloned DNA or its complementary RNA) were widely applied in different assays to detect and identify phytoplasmas in plants and vectors (56, 125, 273-295). In the same years probes based on cloned phytoplasma-specific extrachromosomal DNAs (54, 55, 57, 60, 64, 201, 296) as well as genomic sequence-specific oligonucleotides were also developed for diagnostic purposes (297, 298) or *in situ* hybridization analysis (180, 299). Cloned DNA probes were soon exploited to study the genetic relatedness of different phytoplasmas (56, 255 , 275, 300-305). The hybridization results obtained with genomic probes, often combined with other analytical techniques such as restriction fragment length polymorphism, were the basis for the first classification of these plant pathogens in several genomic strain clusters (277, 302). In most cases, the sensitivity of phytoplasma detection by hybridization exceeds that by ELISA (236, 276), but it is dramatically improved by PCR-based protocols (236, 306-309), which are now regarded as the most suitable diagnostic techniques.

Following the successful isolation and characterization of phytoplasma DNA from infected *Oenothera hookeri* leaf tip cultures (6), sequence information on the pathogen DNA became available. At first, in the effort to define the evolutionary relationships of these microorganisms, the ribosomal RNA operon was the preferred target for sequencing (13). As a consequence primers were identified in different positions of the ribosomal RNA operon to amplify phytoplasma-specific fragments from total DNA of infected plants and vectors (19, 261, 301, 310-318). In the meanwhile comparison of the phytoplasma-specific 16SrRNA gene sequences (151, 298, 310, 315, 319-326) and the variable (327-329) 16S-23S intergenic regions (315, 318, 330) of phytoplasmas belonging to different strain clusters provided new information for the development of group-specific primers for a quicker preliminary characterization of the pathogen. Ribosomal sequence-based primers are probably the most used for routine diagnosis of phytoplasmas despite the presence of bacterial sequences interfering during the amplification process when using these primers (331), especially in DNA extracts from field-collected woody hosts. Ribosomal protein genes were also among the first phytoplasma-specific targets for the location of universal and group-specific primers (11, 15, 261, 312, 313, 332-334). In the meanwhile different laboratories produced sequence data on phytoplasma-specific DNA fragments most of which were obtained by random cloning of phytoplasma-enriched and purified DNA. Universal or group-specific primers located on chromosomal sequences other than the ribosomal operon such as the *tuf* gene (335), nitroreductase gene (336, 337), gyrase genes (338), and even sequences to which no obvious function had been predicted (236, 263, 324, 339, 340) became available. Other authors have designed universal (63) or specific (72) primers for detection, located on the sequence of plasmids hosted by phytoplasmas. Specific reagents and protocols have been published for the detection and identification of many phytoplasmas in potential vectors (151, 181 , 309, 341, 342 , 343, 344 , 345, 346 , 347 , 348), different crops (307, 349-353) and woody hosts (354-361). In the latter cases, since phytoplasmas occur in low concentrations in the host tissues and their number is subject to seasonal fluctuations, and even the presence of PCR inhibitor compounds in the extracts can vary throughout the year (264, 362), routine diagnostic protocols usually involve the use of nested PCR.

6.3. Future prospects in the diagnosis of phytoplasma diseases

The continuous effort to ameliorate of the diagnostic procedures aims at a quicker and more economic and robust methods. Sensitivity is not an issue *per se*, as the current nested PCR protocols are extremely sensitive, but the achievement of high levels of sensitivity without the risk of false positive results that can be associated with nested PCR is highly desirable. The recent introduction of diagnostic assays based on real time PCR fulfils these requirements: due to the high sensitivity and direct reading of the results which reduce the risk of amplicon contamination and the need for a gel-based post PCR analysis, real time PCR is candidate for replacing standard PCR in routine testing. At the moment of writing, real time protocols have been used for the detection of the Apple proliferation phytoplasma (166, 363), the grapevine yellows phytoplasmas (364-366), and the quantification of pathogen cells in chrysanthemum (164), periwinkle, and poinsettia (367) infected with different phytoplasmas.

In the last few years, several other procedures have been proposed for the analysis of the PCR amplification products from phytoplasma infected plants, including PCR-ELISA (368), PCR-dot blot (309), heteroduplex mobility assay (369, 370), 16S-23S spacer length polymorphism (328), microarray (371) and nanobiotransducer hybridization (372). Although these techniques may not have the characteristics of speed, sensitivity, and robustness of real time PCR, they are nevertheless interesting for developing future assay methods with a higher multiplexing potential, thus improving the efficiency or ability to detect multiple phytoplasmas in a single step.

It should be noted, however, that the major limitation to the development of high throughput, robust diagnostic assays for phytoplasmas remains the difficulty in developing a rapid and cost/labour effective preparation of representative nucleic acids extracts. It is well known that the phytoplasmas may be distributed very irregularly in infected plants. The most reliable diagnostic protocols, therefore, include the collection of samples as pools of subsamples taken from different parts of the individual plant to be tested. In order to reduce the amount of material to be processed usually the samples are enriched for phytoplasma and/or phytoplasma containing tissues (i.e. phloem) before proceeding with nucleic acid extraction. Although this is a lengthy step, its suppression would lead to the occurrence of an unacceptable number of false negatives.

Although methods to rapidly obtain phytoplasma enrichment, such as immunocapture PCR (373), have been

developed, to our knowledge they have never been tested in comparative studies in order to assess whether or not they compare favourably with the conventional methods. Due to the intrinsic characteristics of phytoplasma diseases, i.e. the low concentration and irregular distribution of the pathogens, it is unlikely that the field of diagnostics will see another boost such as that given by the introduction of the PCR at the beginning of the nineteen nineties, before the problem of sample representativeness has completely solved.

7. CONCLUDING REMARKS

The application of nucleic acid technology had a major impact on the field of phytoplasmology both regarding disease comprehension and diagnosis. The diagnostic procedures developed in the last 15 years are now used routinely and are adequate for detecting phytoplasma infection in plant propagation material and identifying insect vectors, thus preventing the spread of the diseases and their economical impact.

Even more promising is the increase in understanding the phytoplasma disease mechanism, with the aim to find an environmentally friendly cure. At present, insect vector control using pesticides is the tool of choice for limiting outbreaks of phytoplasma diseases. Apart from environmental considerations, the efficacy of this approach is far from satisfying, and diseases such as Apple Proliferation continue to be of economic concern in some areas of the world, despite the large use of insecticide treatments. Recent research is shedding new light on several aspects of the phytoplasma biology and host relationships. Interference with the phytoplasma colonization of the insect body or with their nutrient uptake in the plant phloem is primary targets for plant protection without the use of toxic compounds. Identification of barriers to phytoplasma colonization of the insect body is a prerequisite to the development of strategies to reduce the infectivity of vector populations. On the other hand, studies aimed at elucidating the effects of the application of genetic pressure on the evolution of phytoplasma populations are urgent when considering control strategies other than insecticide treatments. Alternatively, phytoplasma nutrient uptake from the plant phloem may be targeted to reduce pathogen multiplication and/or symptom expression in the host. Hopefully these approaches will lead to the protection of plants without the use of toxic compounds.

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9. REFERENCES

1. Kunkel, L. O.: Studies on aster yellows. *Am J Bot*, 23, 646-705 (1926)
2. Brcák, J.: Leafhopper and planthopper vectors of plant disease agents in central and southern Europe. In: Leafhoppers and Plant Disease Agents. Eds: K. Maramorosch & K. F. Harris. Academic Press, New York (1979)
3. Tsai, J. L.: Vector transmission of mycoplasmal agents of plant diseases. In: The Mycoplasmas. Eds: R. F. Whitcomb & J. G. Tully. Academic Press, New York (1979)
4. McCoy, R. E., A. Caudwell, C. J. Chang, T. A. Chen, L. N. Chiykowski, M. T. Cousin, J. L. Dale, G. T. N. de Leeuw, D. A. Golino, K. J. Hackett, B. C. Kirkpatrick, R. Marwitz, H. Petzold, R. C. Sinha, M. Sugiura, R. F. Whitcomb, I. L. Yong, B. M. Zhu & E. Seemüller: Plant diseases associated with mycoplama-like organisms. In: The Mycoplasmas. Eds: R. F. Whitcomb& J. G. Tully. Academic Press, New York (1989)
5. Kirkpatrick, B. C., D. C. Stenger, T. J. Morris & A. H. Purcell: Cloning and detection of DNA from a nonculturable plant pathogenic mycoplamsa-like organism. *Science* 238, 197-199 (1987)
6. Sears, B. B., P. Lim, N. Holland, B. C. Kirkpatrick & K. L. Klomparens: Isolation and characterization of DNA from mycoplasmalike organism. *Mol Plant-Microbe Interact*, 2, 175-180 (1989)
7. Kollar, A., E. Seemüller, F. Bonnet, C. Saillard & J. M. Bové: Isolation of the DNA of various plant pathogenic mycoplasmalike organisms from infected plants. *Phytopathology* 80, 233-237 (1990)
8. Kollar, A. & E. Seemüller: Base composition of the DNA of mycoplasma-like organisms associated with various plant diseases. *J Phytopathol* 127, 177-186 (1989)
9. Sears, B. B. & K. L. Klomparens: Leaf tip cultures of the evening primrose allow stable, aseptic culture of mycoplasma-like organism. *Can J Plant Pathol* 11, 343-348 (1989)
10. Lim, P. O. & B. B. Sears: Membrane properties of a plant-pathogenic mycoplasmalike organism. *J Bacteriol* 174, 682-686 (1992)
11. Lim, P. O. & B. B. Sears: DNA sequence of the ribosomal protein genes rpl2 and rps19 from a plant-pathogenic mycoplasmalike organism. *FEMS Microbiol Lett* 84, 71–74 (1991)
12. Lim, P. O. & B. B. Sears: The genome size of a plant-pathogenic mycoplasma-like organism resembles those of animal mycoplasmas. *J Bacteriol* 173, 2128-30 (1991)
13. Lim, P. O. & B. B. Sears: 16S rRNA sequence indicates that plant-pathogenic mycoplasmalike organisms are evolutionarily distinct from animal mycoplasmas. *J Bacteriol* 171, 5901-5906 (1989)
14. Kuske, C. R. & B. C. Kirkpatrick: Phylogenetic relationships between the western aster yellows mycoplasmalike organisms and other prokaryotes established by 16S rRNA gene sequence. *Int J Syst Bacteriol* 42, 226–33 (1992)
15. Lim, P. O. & B. B. Sears: Evolutionary relationships of

a plant-pathogenic mycoplasmalike organism and *Acholeplasma laidlawii* deduced from two ribosomal protein gene sequences. *J Bacterio*l 174, 2606-2611 (1992)
16. Sears, B. B. & B. C. Kirkpatrick: Unveiling the evolutionary relationships of plant pathogenic mycoplasmalike organisms. *ASM News* 60, 307–12 (1994)
17. Aoki, S., K. Uehara, M. Imafuku, M. Hasebe & M. J. Ito: Phylogeny and divergence of basal angiosperms inferred from APETALA3- and PISTILLATA-like MADS-box genes *Plant Research* 117, 229-44 (2004)
18. Maniloff, J.: Reconstructing the timing and selective events of mycoplasma evolution. *Proceedings of the 13th International Congress of the International Organization for Mycoplasmology (IOM)*, 65 (2000).
19. Deng, S. & C. Hiruki: Amplification of 16S rRNA genes from culturable and nonculturable mollicutes. *J Microbiol Meth* 14, 53-61 (1991)
20. Seemüller, E., C. Marcone, U. Lauer, A. Ragozzino & M. Göschl: Current status of molecular classification of the Phytoplasmas. *J Plant Pathol* 80, 3-26 (1998)
21. Lee, I. -M., R. E. Davis & D. E. Gundersen-Rindal: Phytoplasma: Phytopathogenic mollicutes. *Annu Rev Microbiol* 54, 221-255 (2000)
22. IRPCM Phytoplasma/Spiroplasma Working Team – Phytoplasma taxonomy group (2004).: '*Candidatus* Phytoplasma', a taxon for the wall-less, non-helical prokaryotes that colonize plant phloem and insects. *Int J Syst Evol Microbiol* 54, 1243-1255 (2004)
23. Clark, M. F., A. Morton & D. L. Buss: Preparation of MLO immunogens from plants and a comparison of polyclonal and monoclonal antibodies made against primula yellows MLO-associated antigens. *Ann Appl Biol* 114, 111–24 (1989)
24. Clark, M. F., D. J. Barbara & D. L. Davies: Production and characteristics of antisera to *Spiroplasma citri* and clover phyllody-associated antigens derived from plants. *Ann Appl Biol* 103, 251-259 (1983)
25. Chen, T. A.: Isolation and cultivation of spiroplasmas and mycoplasmalike organisms: approaches and perspectives. In: Tree mycoplasmas and mycoplasma diseases. Ed: C. Hiruki. University of Alberta Press, Canada (1988)
26. Neimark, H. & B. C. Kirkpatrick: Isolation and characterization of full-length chromosomes from non-culturable plant-pathogenic mycoplasma-like organisms. *Mol Microbiol* 7, 21-28 (1993)
27. Firrao, G., C. D. Smart & B. C. Kirkpatrick: Physical map of the western X disease phytoplasma chromosome. *J Bacteriol* 178, 3985–88 (1996)
28. Liefting, L. W. & B. C. Kirkpatrick: Cosmid cloning and sample sequencing of the genome of the uncultivable mollicute, Western X-disease phytoplasma, using DNA purified by pulsed-field gel electrophoresis. *FEMS Microbiol Lett* 221, 203-211 (2003)
29. Marcone, C., A. Neimark, A. Ragozzino, U. Lauer & E. Seemüller: Chromosome sizes of phytoplasmas composing major phylogenetic groups and subgroups. *Phytopathology* 89, 805–10 (1999)
30. Lauer, U. & E. Seemüller: Physical map of the chromosome of the apple proliferation phytoplasma. *J Bacteriol* 182, 1415-1418 (2000)
31. Padovan, A. C., G. Firrao, B. Schneider & K. S. Gibb: Chromosome mapping of the sweet potato little leaf phytoplasma reveals genome heterogeneity within the phytoplasmas. *Microbiology* 146, 893-902 (2000)
32. Marcone, C. & E. Seemüller: A chromosome map of the European stone fruit yellows phytoplasma. *Microbiology* 147, 1213-1221 (2001)
33. Oshima, K., S. Kakizawa, H. Nishigawa, W. W. Jung H.Y., S. Suzuki, R. Arashida, D. Nakata, S. Miyata, M. Ugaki & S. Namba: Reductive evolution suggested from the complete genome sequence of a plant-pathogenic phytoplasma. *Nat Genet* 36, 27-29 (2004)
34. Lee, I.-M., Y. Zhao & K.D. Bottner: Novel insertion sequence-like elements in phytoplasma strains of the aster yellows group are putative new members of the IS3 family. *FEMS Microbiol Lett* 242, 353-360 (2005)
35. Razin, S., D. Yogev & Y. Naot: Molecular biology and pathology of mycoplasmas. *Microbiol Mol Biol R* 62, 1094– 156 (1998)
36. Fraser, C. M., J. D. Gocayne, O. White, M. D. Adams, R. A. Clayton, R. D. Fleischmann, C. J. Bult, A. R. Kerlavage, G. Sutton, J. M. Kelley, R. D. Fritchman, J. F. Weidman, K. V. Small, M. Sandusky, J. Fuhrmann, D. Nguyen, T. R. Utterback, D. M. Saudek, C. A. Phillips, J. M. Merrick, J. F. Tomb, B. A. Dougherty, K. F. Bott, P. C. Hu, T. S. Lucier, S. N. Peterson, H. O. Smith, C. A. Hutchison, 3rd & J. C. Venter: The minimal gene complement of *Mycoplasma genitalium*. *Science* 270, 397-403 (1995)
37. Himmelreich, R., H. Hilbert, H. Plagens, E. Pirkl, B. C. Li & R. Herrmann: Complete sequence analysis of the genome of the bacterium *Mycoplasma pneumoniae*. *Nucleic Acids Res* 24, 4420-49 (1996)
38. Taylor, R. R., H. Varsani & R. J. Miles: Alternatives to arginine as energy sources for the non-fermentative *Mycoplasma gallinarum*. *FEMS Microbiol Lett* 115, 163–168 (1994)
39. Romano, N., G. Tolone, F. Ajello & R. La Licata: Adenosine 5'-triphosphate synthesis induced by urea hydrolysis in *Ureaplasma urealyticum*. *J Bacteriol* 144, 830–832 (1980)
40. Foissac, X., C. Saillard, J. L. Danet, P. Gaurivaud, C. Paré, F. Laigret & J. M. Bové: Mutagenesis by insertion of transposon Tn4001 into the genome of *Spiroplasma citri*: Characterization of mutants affected in plant pathogenicity and transmission to the plant by the leafhopper vector *Circulifer haematoceps*. *Mol Plant-Microbe Interact* 10, 454-461 (1997)
41. Gaurivaud, P., J. L. Danet, F. Laigret, M. Garnier & J. M. Bové: Fructose utilization and pathogenicity of *Spiroplasma citri*. *Mol Plant-Microbe Interact* 13, 1145-1155 (2000)
42. Lepka, P., M. Stitt, E. Moll & E. Seemüller: Effect of phytoplasmal infection on concentration and translocation of carbohydrates and amino acids in periwinkle and tobacco. *Physiol Mol Plant Pathol* 55, 59-68 (1999)
43. Maust, B.E., F. Espadas, C. Talavera, M.Aguilar, J.M. Santamaria & C. Oropeza: Changes in carbohydrate metabolism in coconut palms infected with the lethal yellowing phytoplasma. *Phytopathology* 93, 976-981 (2003)
44. Chang, C. J.: Pathogenicity of aster yellows phytoplasma and *Spiroplasma citri* on periwinkle.

Phytopathology 88, 1347–50 (1998)
45. Bertamini, M., N. Nedunchezhian, F. Tomasi & M. S. Grando: Phytoplasma [Stolbur-subgroup (Bois Noir-BN)] infection inhibits photosynthetic pigments, ribulose-1,5-bisphosphate carboxylase and photosynthetic activities in field grown grapevine (*Vitis vinifera* L. cv. Chardonnay) leaves. *Physiol Mol Plant Pathol* 61, 357-366 (2002)
46. Bertamini, M., M. S. Grando, K. Muthuchelian & N. Nedunchezhian: Effect of phytoplasmal infection on photosystem II efficiency and thylakoid membrane protein changes in field grown apple (*Malus pumila*) leaves. *Physiol Mol Plant Pathol* 61, 349-356 (2002)
47. Musetti, R., M. A. Favali & L. Pressacco: Histopathology and polyphenol content in plants infected by phytoplasmas. *Cytobios* 102, 133-147 (2000)
48. Favali, M. A., R. Musetti, S. Benvenuti, A. Bianchi & L. Pressacco: *Catharanthus roseus* L. plants and explants infected with phytoplasmas: alkaloid production and structural observations. *Protoplasma* 223, 45-51 (2004)
49. Choi, Y. H., E. C. Tapias, H. K. Kim, A. W. M. Lefeber, C. Erkelens, J. T. J. Verhoeven, J. Brzin, J. Zel & R. Verpoorte: Metabolic discrimination of *Catharanthus roseus* leaves infected by phytoplasma using 1H-NMR spectroscopy and multivariate data analysis. *Plant Physiol* 135, 2398-2410 (2004)
50. Jagoueix-Eveillard, S., F. Tarendeau, K. Guolter, J. L. Danet, J. M. Bové & M. Garnier: *Catharanthus roseus* genes regulated differentially by mollicute infections. *Mol Plant-Microbe Interact* 14, 225-233 (2001)
51. Bai, X. D., J. H. Zhang, I. R. Holford & S. A. Hogenhout: Comparative genomics identifies genes shared by distantly related insect-transmitted plant pathogenic mollicutes. *FEMS Microbiol Lett* 235, 249-258 (2004)
52. Ploaie, P. G.: Particles resembling viruses associated with mycoplasma-like organisms in plants. *Rev Roum Biol Botanique* 16, 3 (1971)
53. Gourret, J. P., P. L. Maillet & J. Gouranton: Virus-like particles associated with the mycoplasmas of clover phyllody in the plant and in the insect vector. *J Gen Microbiol* 74, 241-249 (1973)
54. Davis, M. J., J. H. Tsai, R. L. Cox, L. L. McDaniel & N. A. Harrison: Cloning of chromosomal and extrachromosomal DNA of the mycoplasmalike organism that causes maize bushy stunt disease. *Mol Plant-Microbe Interact* 1, 295-302 (1988)
55. Davis, R. E., I. -M. Lee, S. M. Douglas & E. L. Dally: Molecular cloning and detection of chromosomal and extrachromosomal DNA of the mycoplasmalike organism (MLO) associated with little leaf disease in periwinkle (*Catharanthus roseus*). *Phytopathology*, 80, 789–93 (1990)
56. Bertaccini, A., R. E. Davis, I. -M. Lee, M. Conti, E. L. Dally & S. M. Douglas: Detection of chrysanthemum yellows mycoplasmalike organism by dot hybridization and Southern blot analysis. *Plant Dis*, 74, 40–43 (1990)
57. Kuske, C. R. & B. C. Kirkpatrick: Identification and characterization of plasmids from the western aster yellows mycoplasmalike organism. *J Bacteriol* 172, 1628-1633 (1990)
58. Kuske, C. R., B. C. Kirkpatrick, M. J. Davis & E. Seemüller: DNA hybridization between western aster yellows mycoplasmalike organism plasmids and extrachromosomal DNA from other plant pathogenic mycoplasmalike organisms. *Mol Plant- Microbe Interact* 4, 75–80 (1991)
59. Chen, J. C., C. J. Chang, R. Jarret & A. Gawel: Isolation and cloning of DNA fragments from mycoplasmalike organism associated with walnut witches'-broom disease. *Phytopathology* 82, 306–9 (1992)
60. Harrison, N. A., J. H. Tsai, C. M. Bourne & P. A. Richardson: Molecular cloning and detection of chromosomal and extrachromosomal DNA of mycoplasmalike organisms associated with witches'-broom disease of pigeon pea in Florida. *Mol Plant-Microbe Interact* 4, 300–7 (1991)
61. Schneider, B., R. Maurer, C. Saillard, B. C. Kirkpatrick & E. Seemüller: Occurrence and relatedness of extrachromosomal DNAs in plant pathogenic mycoplasmalike organisms. *Mol Plant-Microbe Interact* 5, 489-495 (1992)
62. Denes, A. S. & R. C. Sinha: Alteration of clover phyllody mycoplasma DNA after in vitro culturing of phyllody-diseased clover. *Can J Plant Pathol* 14, 189-196 (1992)
63. Goodwin, P. H., B. G. Xue, C. R. Kuske & M. K. Sears: Amplification of plasmid DNA to detect plant pathogenic mycoplasmalike organisms. *Ann Appl Biol* 124, 27–36 (1994)
64. Klinkong, S. & E. Seemüller: Detection and differentiation of the mycoplasma-like organism association with sugarcane white leaf disease using cloned extrachromosomal DNA probe. *The Kasetsart Journal: Natural Sciences* 27, 98-103 (1993)
65. Nakashima, K. & T. Hayashi: Extrachromosomal DNAs of rice yellow dwarf and sugarcane white leaf phytoplasmas. *Ann Phytopathol Soc Japan* 61 (5), 456-462 (1995)
66. Nakashima, K. & T. Hayashi: Sequence analysis of extrachromosomal DNA of sugarcane white leaf phytoplasma. *Ann Phytopathol Soc Jpn* 63, 21–25 (1997)
67. Kuboyama, T., L. X. Y. Huang C.C, T. Sawayanagi, T. Kanazawa, T. Kagami, I. Matsuda, T. Tsuchizaki & S. Namba: A plasmid isolated from phytopathogenic onion yellows phytoplasma and its heterogeneity in the pathogenic phytoplasma mutant. *Mol Plant-Microbe Interact* 11, 1031-1037 (1998)
68. Rekab, D., L. Carraro, B. Schneider, E. Seemüller, J. C. Chen, C. J. Chang, R. Locci & G. Firrao: Geminivirus-related extrachromosomal DNAs of the X-clade phytoplasmas share high sequence similarity. *Microbiology* 145, 1453-1459 (1999)
69. Nishigawa, H., S. I. Miyata, K. Oshima, T. Sawayanagi & A. Komoto: In planta expression of a protein encoded by the extrachromosomal DNA of a phytoplasma and related to geminivirus replication proteins. *Microbiology* 147, 507-513 (2001)
70. Nishigawa, H., K. Oshima, S. Kakizawa, H. Y. Jung, T. Kuboyama, S. Miyata, M. Ugaki & S. Namba: A plasmid from a non-insect-transmissible line of a phytoplasma lacks two open reading frames that exist in the plasmid from the wild-type line. *Gene* 298, 195-201 (2002)
71. Nishigawa, H., K. Oshima, S. Kakizawa, H. Y. Jung, T. Kuboyama, S. Miyata, M. Ugaki & S. Namba: Evidence of intermolecular recombination between extrachromosomal DNAs in phytoplasma: a trigger for the biological diversity

of phytoplasma? *Microbiology* 148, 1389-1396 (2002)
72. Nishigawa, H., K. Oshima, S. Miyata, M. Ugaki & S. Namba: Complete set of extrachromosomal DNAs from three pathogenic lines of onion yellows phytoplasma and use of PCR to differentiate each line. *J Gen Plant Pathol* 69, 194-198 (2003)
73. Wei, H. C. & C. P. Lin: Cloning and sequencing of extrachromosomal DNA and insertion sequence of phytoplasma associated with peanut witches' broom using random sequencing. *Plant Pathol Bull* 13, 143-154 (2004)
74. Liefting, L. W., M. E. Shaw & B. C. Kirkpatrick: Sequence analysis of two plasmids from the phytoplasma beet leafhopper-transmitted virescence agent. *Microbiology* 150, 1809-1817 (2004)
75. Oshima, K., S. Kakizawa, H. Nishigawa, T. Kuboyama, S. I. Miyata, M. Ugaki & S. Namba: A plasmid of phytoplasma encodes a unique replication protein having both plasmid- and virus-like domains: clue to viral ancestry or result of virus/plasmid recombination? *Virology* 285, 270-277 (2001)
76. King, K. W. & K. Dybvig: Nucleotide sequence of *Mycoplasma mycoides* subspecies Mycoides plasmid pKMK1. *Plasmid* 28, 86-91 (1992)
77. Frischmuth, T., G. Zimmat & P. A. Jeske: The nucleotide sequence of abutilon mosaic virus reveals prokaryotic as well as eukaryotic features. *Virology* 178, 461-46 (1990)
78. Koonin, E. V. & T. V. Ilyina: Geminivirus replication proteins are related to prokaryotic plasmid rolling circle DNA replication initiator proteins. *J Gen Virol* 73, 2763-2766 (1992)
79. Kuboyama, T., C. C. Huang, X. Lu, T. Sawayanagi, T. Kanazawa, T. Kagami, I. Matsuda, i. T. Tsuchizak & S. Namba: A plasmid isolated from phytopathogenic onion yellows phytoplasma and its heterogeneity in the pathogenic phytoplasma mutant. *Mol Plant-Microbe Interact* 11, 1031–37 (1998)
80. Shaw, M. E.: Biological and molecular characterization of virescence agents infecting herbaceous crops in California. PhD. Thesis. University of California, Davis, USA (1991).
81. Marzachì, C., R. G. Milne & B. E. Bosco: Phytoplasma-plant-vector relationships. In: Recent Research Developments in Plant Pathology. Eds: S. G. Pandalai & A. Gayathri. Research Signpost, Kerala, India (2004)
82. Namba, S., K. Oshima & K. Gibb: Phytoplasma genomics. In: Mycoplasmas molecular biology pathogenicity and strategies for control. Eds: A. Blanchard & G. Browning. Horizon Bioscience, Wymondham, U.K. (2005)
83. Jones, A. L., G. R. Hooper & D. A. Rosenberger: Association of mycoplasmalike bodies with little peach and X-disease. *Phytopathology* 64, 755-756 (1974)
84. Braun, E. J. & W. A. Sinclair: Phloem necrosis of elms: symptoms and histopathological observations in tolerant hosts. *Phytopathology* 69, 54-358 (1979)
85. Schneider, H.: Indicator hosts for pear decline: symptomatology, histopathology, and distribution of mycoplasmalike organisms in leaf veins. *Phytopathology* 67, 592-601 (1977)
86. Siddique, A. B. M., J. N. Guthrie, K. B. Walsh, D. T. White & P. T. Scott: Histopathology and within-plant distribution of the phytoplasma associated with Australian papaya dieback. *Plant Dis* 82, 1112-1120 (1998)
87. Lherminier, J., M. Courtois & R. Caudwell: Determination of the distribution and multiplication sites of flavescence dorée mycoplasma-like organisms in the host plant *Vicia faba* by ELISA and immunocytochemistry. *Physiol Mol Plant Pathol* 45, 25-138 (1994)
88. Parthasarathy, M. V.: Mycoplasmalike organisms in the phloem of palms in Florida affected by lethal yellowing. *Plant Dis Reptr* 57, 861-862 (1973)
89. Parthasarathy, M. V.: Mycoplasmalike organisms associated with lethal yellowing disease of palms. *Phytopathology* 64, 667-674 (1974)
90. Carling, D. E. & D. F. Millikan: Banded filaments associated with the aster yellows MLO in *Vinca rosea*. *Can J Microbiol* 24, 1417-1418 (1978)
91. Abdulsalam, K. S., M. I. Abdel-Megeed, M. A. Rezk & M. A. Nageeb: The influence of oxytetracycline on wijamed date palm trees. *Ann Agricult Sci (Cairo)* 38, 301-309 (1993)
92. Alivizatos, A. S.: Association of mycoplasma-like organisms with tomato big bud disease in Greece. *Plant Pathol* 42, 158-162 (1993)
93. Hirumi, H. & K. Maramorosch: Intracytoplasmic mycoplasmalike bodies in phloem parenchyma cells of aster yellows-infected Nicotiana rustica. *Phytopathol Z* 77, 71-83 (1973)
94. Magarey, P. A., B. Plavsic & M. F. Wachtel: MLO associated with Australian grapevine yellows diseased phloem cells. *Internat J Trop Plant Diseases* 6, 175-179 (1988)
95. Meignoz, R., E. Boudon-Padieu, J. Larrue & A. Caudwell: Grapevine flavescence dorée. Presence of MLO and associated cytopathological effects in grapevine phloem. *J Phytopathol* 134, 1-9 (1992)
96. Parente, A. M., I. Abreu & R. Salema: Mycoplasma-like organisms associated with phloem cells of diseased grapevines in northern Portugal. *Z Pflanzenk Pflanzen* 101, 124-127 (1994)
97. Seliskar, C. E.: Mycoplasmalike organism found in the phloem of bunch-diseased walnuts. *Forest Sci* 22, 144-148 (1976)
98. Seliskar, C. E., C. L. Wilson & C. E. Bourne: Mycoplasmalike bodies found in phloem of Black Locust affected with witches'-broom. *Phytopathology* 63, 30-34 (1973)
99. Vera, C. & R. G. Milne: Immunosorbent electron microscopy and gold label antibody decoration of MLOs from crude preparations of infected plants and vector insects. *Plant Patholol* 43, 90-199 (1994)
100. D'Agostino, G. G. A.: Ultrastructural features of primula yellows mycoplasma-like organism (MLO) in cryosections of *Catharanthus roseus* leaves. *J Struct Biol*, 107, 56-64 (1991)
101. Sinha, R. C. & Y. C. Paliwal: Association, development, and growth cycle of mcoplasma-like organisms in plant affected with clover phyllody. *Virology*, 39, 759-767 (1969)
102. Berges, R., M. Rott & E. Seemüller: Range of phytoplasma concentrations in various plant hosts as determined by competitive polymerase chain reaction.

Phytopathology 90, 1145-1152 (2000)
103. Kartte, S. & E. Seemüller: Susceptibility of grafted Malus taxa and hybrids to apple proliferation disease. *J Phytopathol* 131, 137-148 (1991)
104. Kartte, S. & E. Seemüller: Histopathology of apple proliferation in Malus taxa and hybrids of different susceptibility. *J Phytopathol* 131, 149-160 (1991)
105. Seemüller, E., K. H. Lorenz & U. Lauer: Pear Decline Resistance in *Pyrus communis* rootstocks and progenies of wild and ornamental *Pyrus* taxa. *Acta Hortic* 472, 681-690 (1998)
106. Seemüller, E., H. Stolz & H. Kison: Persistence of the European stone fruit yellows phytoplasma in aerial parts of *Prunus* taxa during the dormant season. *J Phytopathol* 146, 407-410 (1998)
107. Jarausch, W., J. P. Eyquard, K. Mazy, M. Lansac & F. Dosba: High level of resistance of sweet cherry (*Prunus avium* L.) towards European stone fruit yellows phytoplasmas. *Advan Hort Sci* 13, 108-112 (1999)
108. Uyemoto, J. K., B. C. Kirkpatrick, J. N. Cummins & C. F. Luhn: Susceptibility of selected cherry clones and related species to Western X-disease. *Hortscience* 26, 1510-1511 (1991)
109. Audergon, J., C. Castelain, G. Morvan & M. G. Chastelliere: Apricot varietal susceptibility and genetic variability to apricot chlorotic leaf roll disease. *Acta Hortic* 235, 205-13 (1989)
110. Giunchedi, L., C. Poggi Pollini, R. Bissani, A. R. Babini & V. Vicchi: Etiology of a pear decline disease in Italy and susceptibility of pear variety and rootstock to phytoplasma-associated pear decline. *Acta Hortic* 386, 89-495 (1995)
111. Howard, F. W.: Lethal yellowing susceptibility of date palms in Florida. *Principes* 36, 217-222 (1992)
112. Pun, K. B. & R. K. S. M. Baruah: Susceptibility of some modern rice genotypes and indigenous rice germplasm to rice yellow dwarf disease. *Indian J Virol* 15, 53-55 (1999)
113. Seemüller, E., U. Schaper & L. Kunze: Effect of pear decline on pear trees on 'Quince A' and *Pyrus communis* seedling rootstocks. *Z Pflanzenk Pflanzen* 93, 44-50 (1986)
114. Pastore, M., M. Santonastaso, I. -M. Lee, M. Vibio, A. Bertaccini & F. La Cara: Susceptibility to phytoplasma infection of three pear varieties grafted on different rootstocks. *Acta Hortic* 472, 673-680 (1998)
115. Carraro, L., P. Ermacora, N. Loi & R. Osler: The recovery phenomenon in apple proliferation-infected apple trees. *J Plant Pathol* 86, 141-146 (2004)
116. Kuske, C. R. & B. C. Kirkpatrick: Distribution and multiplication of western aster yellows mycoplasmalike organisms in *Catharanthus roseus* as determined by DNA hybridization analysis. *Phytopathology* 82, 457-62 (1992)
117. Sinclair, W. A. & H. M. Griffiths: Variation in aggressiveness of ash yellows phytoplasmas. *Plant Dis* 84, 282-288 (2000)
118. Errea, P., V. Aguelo & J. I. Hormaza: Seasonal variations in detection and transmission of pear decline phytoplasma. *J Phytopathol* 150, 439-443 (2002)
119. Garcia-Chapa, M., V. Medina, M. A. Viruel, A. Lavina & A. Batlle: Seasonal detection of pear decline phytoplasma by nested-PCR in different pear cultivars. *Plant Pathol* 52, 513-520 (2003)
120. Seemüller E, U. Schaper & F. Zimbelmann: Seasonal variation in the colonization patterns of mycoplasmalike organisms associated with apple proliferation and pear decline. *J Plant Dis Protect* 91, 371-382 (1984)
121. Jarausch, W., M. Lansac & F. Dosba: Seasonal colonization pattern of European stone fruit yellows phytoplasmas in different Prunus species detected by specific PCR. *J Phytopathol* 147, 47-54 (1999)
122. Rosenberg, D. A. & A. L. Jones: Seasonal variation in infectivity of inoculum from X-diseased peach and choke-cherry plants. *Plant Dis Reptr* 61, 1022-1024 (1977)
123. Skoric, D., A. Saric, M. Vibio, E. Murari, M. Krajacic & A. Bertaccini: Molecular identification and seasonal monitoring of phytoplasmas infecting Croatian grapevines. *Vitis*, 37, 171-175 (1998)
124. Yi, J., T. Lim & B. Cha: Changes in phytoplasma densities in witches' broom-infected jujube trees over seasons. *Plant Pathol J*, 17, 295-299 (2001)
125. Chen, J. C., C. J. Chang & R. L. Jarret: DNA probes as molecular markers to monitor the seasonal occurrence of walnut witches' broom mycoplasmalike organism. *Plant Dis* 76, 1116-1119 (1992)
126. Constable, F. E., K. S. Gibb & R. H. Symons: Seasonal distribution of phytoplasmas in Australian grapevines. *Plant Pathol* 52, 267-276 (2003)
127. Sinha, R. C.: Relative concentration of mycoplasma-like organisms in plants at various times after infection with aster yellows. *Can J Plant Pathol* 5, 7-10 (1983)
128. Wei, W., S. Kakizawa, S. Suzuki, H. Y. Jung, H. Nishigawa, S. Miyata, K. Oshima, M. Ugaki, T. Hibi & S. Namba: In planta dynamic analysis of onion yellows phytoplasma using localized inoculation by insect transmission. *Phytopathology* 94, 244-250 (2004)
129. Rudzínska- Langwald, A. & M. Kamínska: Cytopathological evidence for transport of phytoplasma in infected plants. *Acta Soc Bot Pol* 68, 261-266 (1999)
130. Rudzínska-Langwald, A. & M. Kaminska: Ultrastructural changes in aster yellows phytoplasma affected *Limonium sinuatum* Mill. plants. I. Pathology of conducting tissues. *Acta Soc Bot Pol* 70, 173-180 (2001)
131. Credi, R.: Mycoplasma-like organisms associated with a grapevine yellows disease occurring in Italy. *J Phytopathol* 141, 113-120 (1994)
132. Schaper, U. & E. Seemüller: Condition of the phloem and the persistence of mycoplasmalike organisms associated with apple proliferation and pear decline. *Phytopathology* 72, 736-742 (1982)
133. Sinclair, W. A., R. J. Iuli, A. T. Dyer & A. O. Larsen: Sampling and histological procedures for diagnosis of ash yellows. *Plant Dis* 73, 432-435 (1989)
134. Schaper, U. & E. Seemüller: Recolonization of the stem of apple proliferation and pear decline-diseased trees by the causal organisms in spring. *Z Pflanzenk Pflanzen* 91, 608-613 (1984)
135. Dyer, A. T. & W. A. Sinclair: Root necrosis and histological changes in surviving roots of white ash infected with mycoplasmalike organisms. *Plant Dis* 75, 814-819 (1991)
136. Seemüller, E.: Colonization patterns of mycoplasma-like organisms in trees affected by apple proliferation and pear decline. In: Tree mycoplasmas and mycoplasma diseases. Ed: C. Hiruki. University of Alberta Press,

Canada (1988)
137. Seemüller, E., L. Kunze & U. Schaper: Colonization behaviour of MLO, and symptom expression of proliferation-diseased apple trees and decline-diseased pear trees over a period of several years. *Z Pflanzenk Pflanzen* 91, 525-532 (1984)
138. Sinha, R. C. & L. N. Chiykowski: Transmission and morphological features of mycoplasmalike bodies associated with peach X-disease. *Can J Plant Pathol* 2 119-124, 119-124 (1980)
139. Douglas, S. M.: Detection of mycoplasmalike organisms in peach and chokecherry with X-disease by fluorescence microscopy. *Phytopathology* 76, 784-787 (1986)
140. D'Arcy, C. J. & L. R. Nault: Insect transmission of plant viruses and mycoplasmalike and rickettsialike organisms. *Plant Dis*, 66, 99-104 (1982)
141. Weintraub, P.G. & L. Beanland: Insect Vectors of Phytoplasmas. Ann. Rev. Entomol., in press (2006)
142. Palermo, S., A. Arzone & D. Bosco: Vector-pathogen-host plant relationships of chrysanthemum yellows (CY) phytoplasma and the vector leafhoppers *Macrosteles quadripunctulatus* and *Euscelidius variegatus*. *Entomol Exp Appl* 99, 347-354 (2001)
143. Carraro, L., N. Loi & P. Ermacora: Transmission characteristics of the European stone fruit yellows phytoplasma and its vector *Cacopsylla pruni*. *Eur J Plant Pathol* 107, 695-700 (2001)
144. Chiykowski, L. N. & R. C. Sinha: Some factors affecting the transmission of eastern peach X-mycoplasmalike organism by the leafhopper *Paraphlepsius irroratus*. *Can J Plant Pathol*, 10, 85-92 (1988)
145. Swenson, K. G.: Relation of age, sex and mating of *Macrosteles fascifrons* to transmission of Aster yellows. *Phytopathology*, 61, 657-659 (1971)
146. Beanland, L., C. W. Hoy, S. A. Miller & L. R. Nault: Leafhopper (Homoptera: Cicadellidae) transmission of Aster Yellows phytoplasma: Does gender matter? *Environ Entomol*, 28, 1101-106 (1999)
147. Lessio, F. & A. Alma: Seasonal and daily movement of *Scaphoideus titanus* ball (Homoptera : Cicadellidae). *Environ Entomol* 33, 1689-1694 (2004)
148. Hoy, C. W., X. Zhou, L. R. Nault, S. A. Miller & J. A. Styer: Host plant, phytoplasma, and reproductive status effects on flight behavior of aster leafhopper (Homoptera: Cicadellidae). *Ann Entomol Soc Am* 92, 523-528 (1999)
149. Garcia-Chapa, M., J. Sabate, A. Lavina & A. Batlle: Role of *Cacopsylla pyri* in the epidemiology of pear decline in Spain. *Eur J Plant Pathol* 111, 9-17 (2005)
150. Purcell, A. H. & K. G. Suslow: Pathogenicity and effects on transmission of a mycoplasmalike organism of a transovarially infective bacterium on the leafhopper *Euscelidius variegatus* (Homoptera: Cicadellidae). *J Invert Pathol* 50, 285-290 (1987)
151. Maixner, M., U. Ahrens & E. Seemüller: Detection of the German grapevine yellows (Vergibungskrankheit) MLO in grapevine, alternative hosts, and a vector by a specific PCRprocedure. *Eur J Plant Pathol*, 101, 241–50 (1995)
152. Sforza, R., D. Clair, X. Daire, J. Larrue & E. Boudon-Padieu: The role of *Hyalesthes obsoletus* (Hemiptera: Cixiidae) in the occurrence of bois noir of grapevines in France. *J Phytopathol* 146, 549-556 (1998)
153. Langer, M. & M. Maixner: Molecular characterisation of grapevine yellows associated phytoplasmas of the stolbur-group based on RELP-analysis of non-ribosomal DNA. *Vitis* 43, 191-199 (2004)
154. Tedeschi, R., D. Bosco & A. Alma: Population dynamics of *Cacopsylla melanoneura* (Homoptera: Psyllidae), a vector of apple proliferation phytoplasma in northwestern Italy. *J Econ Entomol* 95, 544-551 (2002)
155. Carraro, L., R. Osler, N. Loi, P. Ermacora & E. Refatti: Transmission of European stone fruit yellows phytoplasma by *Cacopsylla pruni*. *J Plant Pathol* 80, 233-239 (1998)
156. Murral, D. J., L. R. Nault, C. W. Hoy, L. V. Madden & S. A. Miller: Effects of temperature and vector age on transmission of two Ohio strains of aster yellows phytoplasma by the aster leafhopper (Homoptera: Cicadellidae). *J Econ Entomol* 89, 1223-1232 (1996)
157. Bosco, D., C. Minucci, G. Boccardo & M. Conti: Differential acquisition of chrysanthemum yellows phytoplasma by three leafhopper species. *Entomol Exp Appl* 83, 219-224 (1997)
158. Alma, A., C. Marzachì, M. d'Aquilio & D. Bosco: Cyclamen (*Cyclamen persicum* L.): a dead-end host species for 16Sr-IB and -IC subgroup phytoplasmas. *Ann Appl Biol* 136, 173-178 (2000)
159. Hanboonsong, C., S. Choosai, D. Panyim & S. Damak: Transovarial transmission of sugarcane white leaf phytoplasma in the insect vector *Matsumuratettix hiroglyphicus* (Matsumura). *Insect Mol Biol* 11 (1), 97- 103 (2002)
160. Kawakita, H., T. Saiki, W. Wei, W. Mitsuhashi, K. Watanabe & M. Sato: Identification of mulberry dwarf phytoplasmas in the genital organs and eggs of leafhopper *Hishimonoides sellatiformis*. *Phytopathology* 90, 909-914 (2000)
161. Alma, A., D. Bosco, A. Danielli, A. Bertaccini, M. Vibio & A. Arzone: Identification of phytoplasmas in eggs, nymphs and adults of *Scaphoideus titanus* Ball reared on health plants. *Insect Mol Biol* 6, 115-121 (1997)
162. Boudon-Padieu, E., J. Larrue & A. Caudwell: ELISA and Dot-blot detection of Flavescence dorée-MLO in individual leafhopper vectors during latency and inoculative state. *Curr Microbiol* 19, 357-364 (1989)
163. Lefol, C., J. Lherminier, E. Boudon-Padieu, J. Larrue, C. Louis & A. Caudwell: Propagation of Flavescence dorée MLO (mycoplasma-like organism) in the leafhopper vector *Euscelidius variegatus* Kbm. *J Invert Pathol*, 63, 285-293 (1994)
164. Marzachì, C. & D. Bosco: Relative quantification of Chrysanthemum yellows (16Sr I) phytoplasma in its plant and insect host using real-time polymerase chain reaction. *Mol Biotechnol* 30, 117-127 (2005)
165. Nakashima, K. & T. Hayashi: Multiplication and distribution of rice yellow dwarf phytoplasma in infected tissues of rice and green rice leafhopper *Nephotettix cincticeps*. *Ann Phytopathol Soc Jpn* 61, 451-5 (1995)
166. Jarausch, W., T. Peccerella, N. Schwind, B. Jarausch & G. Krczal: Establishment of a quantitative real-time PCR assay for the quantification of apple proliferation phytoplasmas in plants and insects. *Acta Hortic* 657, 415-420 (2004)

167. Liu, H. W., P. H. Goodwin & C. R. Kuske: Quantification of DNA from the aster yellows mycoplasmalike organism in aster leafhoppers (*Macrosteles fascifrons* Stal) by a competitive polymerase chain reaction. *Syst Appl Microbiol* 17, 274-280 (1994)
168. Gouranton, J. & P. L. Maillet: High resolution autoradiography of mycoplasmalike organisms multiplying in some tissues of an insect vector for clover-phyllody. *J Invert Pathol* 21, 158-16 (1973)
169. Nasu, S., Y. Kono & D. D. Jensen: The multiplication of Western X mycoplasmalike organism in the brain of a leafhopper vector, *Colladonus montanus* (*Homoptera: Cicadellidae*). *Appl Entomol Zool* 9, 277-279 (1974)
170. Whitcomb, R. F. & D. D. Jensen: Proliferative symptoms in leafhoppers infected with Western-X disease virus. *Virology* 35, 174-177 (1968)
171. Sinha, R. C. & L. N. Chiykowski: Multiplication of aster yellows virus in a nonvector leafhopper. *Virology* 31, 461-466 (1967)
172. Whitcomb, R. F., D. D. Jensen & B. E. Richardson: The infection of leafhoppers by Western-X disease virus. III. Salivary, neural, and adipose histopathology. *Virology* 31, 539-549 (1967)
173. Jensen, D. D., R. F. Whitcomb & K. and Richardson: Lethality of injected peach Western X-disease virus to its leafhopper vector. *Virology* 31, 532-53 (1967)
174. Bressan, A., V. Girolami & E. Boudon-Padieu: Reduced fitness of the leafhopper vector *Scaphoideus titanus* exposed to Flavescence dorée phytoplasma. *Entomol Exp Appl* 115, 83- 290 (2005)
175. Ebbert, M. A. & L. R. and Nault: Survival in *Dalbulus* leafhopper vectors improves after exposure to maize stunting pathogens. *Entomol Exp Appl* 100, 311-324 (2001)
176. Chen, T. A. & X. F. Jiang: Monoclonal antibodies against the maize bushy stunt agent. *Can J Microbiol*, 34, 6-1 (1988)
177. Beanland, L., C. W. Hoy, S. A. Miller & L. R. Nault: Influence of aster yellows phytoplasma on the fitness of aster leafhopper (Homoptera: Cicadellidae). *Ann Entomol Soc Am* 93, 271-276 (2000)
178. Purcell, A. H.: Increased survival of *Dalbulus maidis*, a specialist on maize, on non-host plants infected with mollicute plant pathogens. *Entomol Exp Appl* 46, 187-196 (1988)
179. Sinha, R. C. & L. N. Chiykowski: Serological detection of mycoplasma-like organisms in plants and vector leafhoppers using polyclonal antibodies. *Recent advances in mycoplasmology. Proceedings of the 7th congress of the International Organization for Mycoplasmology*, G. Stanek, G. H. Cassell, J. G. Tully&R. F. Whitcomb, Eds., 276-279 (1990).
180. Webb, D. R., R. G. Bonfiglioli, L. Carraro, R. Osler & R. H. Symons: Oligonucleotides as hybridization probes to localize phytoplasmas in host plants and insect vectors. *Phytopathology* 89, 894-901 (1999)
181. Vega, F. E., R. E. Davis, P. Barbosa, E. L. Dally, A. H. Purcell & I. -M. Lee: Detection of a plant pathogen in a non-vector insect species by the polymerase chain reaction. *Phytopathology* 83, 621–24 (1993)
182. Khadair, A. H., C. Hiruki & S. F. Hwang: Molecular detection of alfalfa witches' broom phytoplasma in four leafhopper species associated with infected alfalfa plants. *Microbiol Res* 152, 269-275 (1997)
183. Lefol, C., A. Caudwell, J. Lherminier & J. Larrue: Attachment of the flavescence dorée pathogen (MLO) to leafhopper vectors and other insects. *Ann Appl Biol* 123, 611-22 (1993)
184. Lefol, C., J. Lherminier, E. Boudon-Padieu, R. Meignoz, J. Larrue, C. Louis, A. C. Roche & E. Caudwell: Presence of attachments sites accounting for recognition between flavescence dorée MLO and its leafhopper vector. *IOM Letters* 3, 282-283 (1995)
185. Bosco, D., A. Alma & A. Arzone: Studies on population dynamics and spatial distribution of leafhoppers in vineyards (Homoptera: Cicadellidae). *Ann Appl Biol* 130, 1-11 (1997)
186. Doi, M., M. Teranaka, K. Yora & R. and Asuyama: Mycoplasma or PLT- group-like organism found in the floem elements of plants infected with mulberry dwarf, potato witches' broom, aster yellow, or paolownia witches' broom. *Ann Phytopathol Soc Jpn* 33, 259-26 (1967)
187. Martinez, S., I. Cordova, B. E. Maust, C. Trejo, C. Oropeza & J. M. Santamaria: Is abscissic acid responsible for abnormal stomatal closure in coconuts palms showing lethal yellowing? *J Plant Pathol*, 156, 319-322 (2000)
188. Kaminska, M., H. Sliwa & L. Startek: First report of phytoplasma infection in Freesia plant. *Plant Dis*, 85, 336 (2001)
189. Bertaccini, A. & F. Marani: Electron microscopy of two viruses and mycoplasma-like organisms in lilies with deformed flowers. *Phytopathol Medit* 21, 8-14 (1982)
190. Cousin, M. T., A. K. Sharma & S. Misra: Correlation between light and electron microscopic observations and identification of mycoplasmalike organisms using consecutive 350 nm thick sections. *J Phytopathol* 115, 368-374 (1986)
191. Singh, S. J.: Electron microscopic evidence of the association of mycoplasma-like organism with phyllody disease of bottlegourd. *Plant Disease Research* 6, 117-20 (1991)
192. Tanaka, M., S. Osada & I. Matsuda: Transmission of rhus (*Rhus javanica* L.) yellows by *Hishimonus sellatus* and host range of the causal phytoplasma. *J Gen Plant Pathol* 66, 323-326 (2000)
193. Ahrens, U. & E. Seemüller: Detection of mycoplasmalike organisms in declining oaks by polymerase chain reaction. *Eur J For Pathol*, 24, 55-63 (1994)
194. Haggis, G. H. & R. C. Sinha: Scanning electron microscopy of mycoplasmalike organisms after freeze fracture of plant tissues affected with clover phyllody and aster yellows. *Phytopathology* 68, 677–80 (1978)
195. Poghosyan, A. V., V. K. Lebsky, M. Arce-Montoya & L. Landa: Possible phytoplasma disease in papaya (*Carica papaya* L.) from Baja California Sur: diagnosis by scanning electron microscopy. *J Phytopathol* 152, 376-380 (2004)
196. Leeuw, G. T. N. D., M. Frosch, R. A. Samson & J. A. Stalpers: Scanning electron microscopy of rickettsia-like organisms in latent rosette-diseased *Beta vulgaris*. *Z Pflanzenk Pflanzen* 90, 409-414 (1983)
197. Marcone, C. & A. Ragozzino: Comparative ultrastructural studies on genetically different phytoplasmas using scanning electron microscopy. *Petria* 6, 125-136 (1996)

198. Schaper, U. & R. H. Converse: Detection of mycoplasmalike organisms in infected blueberry cultivars by the DAPI technique. *Plant Dis* 69, 193-196 (1985)
199. Hiruki, C. & A. D. Rocha: Histochemical diagnosis of mycoplasma infections in *Catharanthus roseus* by means of a fluorescent DNA-binding agent, 4'-6'-diamidino-2-phenylindole-HC1 (DAPI). *Can J Plant Pathol* 8, 185-188 (1986)
200. Schuiling, M. & A. Forstel-Neuhaus: The use of the fluorochrome DAPI in the diagnosis of lethal disease of coconut palm (*Cocos nucifera*) in Tanzania. *Z Pflanzenk Pflanzen* 99, 614-616 (1992)
201. Sinclair, W. A., H. M. Griffiths, R. E. Davis & I. -M. Lee: Detection of ash yellows mycoplasmalike organisms in different tree organs and in chemically preserved specimens by a DNA probe vs. DAPI. *Plant Dis* 76, 154-158 (1992)
202. Malinowski, T., J. Zandarski, B. Komorowska & B. Zawadzka: Application of DAPI staining and PCR amplification of DNA for the identification of pear decline phytoplasma in declining trees in Poland. *Phytopathol Polonica* 12, 103-110 (1996)
203. Sunil Thomas & Balasundaran, M.: In situ detection of phytoplasma in spike-disease-affected sandal using DAPI stain. *Curr Sci India* 74, 989-993 (1998)
204. Milne, R. G., E. Ramasso, R. Lenzi, V. Masenga, S. Sarindu & M. F. Clark: Pre- and Post-embedding immunogold labeling and electron microscopy in plant host tissues of three antigenically unrelated MLOs: primula yellows, tomato big bud and bermudagrass whiteleaf. *Netherlands J Plant Pathol* 101, 57-67 (1995)
205. Musetti, R., N. Loi, L. Carraro & P. Ermacora: Application of immunoelectron microscopy techniques in the diagnosis of phytoplasma diseases. *Microsc Res Techniq* 56, 462- 464 (2002)
206. Begtrup, J. & L. Lange: Mycoplasma-like organism in phloem elements of *Silene vulgaris* and *Agrostemma githago*. *Phytopathol Z* 90, 164-171 (1977)
207. Jarausch, W., M. Lansac, C. Portanier, D. L. Davies & V. Decroocq: In vitro grafting: a new tool to transmit pome fruit phytoplasmas to non-natural fruit tree hosts. *Advan Hort Sci* 14, 32 (2000)
208. Pastore, M., P. Piccirillo, A. M. Simeone, J. Tian, S. Paltrinieri & A. Bertaccini: Transmission by patch grafting of ESFY phytoplasma to apricot (*Prunus armeniaca* L.) and Japanese plum (*Prunus salicina* Lindl). *Acta Hortic*, 550, 339-344 (2001)
209. Credi, R. & A. Santucci: Dodder transmission of mycoplasma-like organisms (MLOs) from grapevines affected by a flavescence dorée-type disease to periwinkle. *Phytopathol Medit* 31, 154-62 (1992)
210. Kaminska, M., M. Korbin & A. Rudzinska-Langwald: The response of lily seedlings to inoculation with aster yellows phytoplasma by leafhopper and dodder. *Phytopath Polonica* 21, 69-79 (2001)
211. Loi, N., L. Carraro, R. Musetti, I. Pertot & R. Osler: Dodder transmission of two different MLOs from plum trees affected by "leptonecrosis". *Acta Hortic*, 386, 465-470 (1995)
212. Marcone, C., F. Hergenhahn, A. Ragozzino & E. Seemüller: Dodder transmission of pear decline, European stone fruit yellows, Rubus stunt, Picris echioides yellows and cotton phyllody phytoplasmas to periwinkle. *J Phytopathol* 147, 187-192 (1999)
213. Marcone, C., A. Ragozzino & E. Seemüller: Dodder transmission of alder yellows phytoplasma to the experimental host *Catharanthus roseus* (periwinkle). *Eur J For Pathol* 27, 347-350 (1997)
214. Acikgoz, S.: Tuber, graft and dodder transmission of potato disease caused by mycoplasmalike organisms (MLO) in Erzurum region. *J Turkish Phytopath* 18, 31-38 (1989)
215. Carraro, L., R. Osler, N. Loi & M. A. Favali: Transmission characteristics of the clover phyllody agent by dodder. *J Phytopathol* 133, 15-22 (1991)
216. Loi, N., P. Ermacora, L. Carraro, R. Osler & T. A. Chen: Production of monoclonal antibodies against apple proliferation phytoplasma and their use in serological detection. *Eur J Plant Pathol* 108, 81-86 (2002)
217. Saeed, E. M., A. E. Dafalla, J. Roux & M. T. Cousin: Characterization of a MLO phyllody naturally occurred on *Catharanthus roseus* in the Sudan through indirect ELISA using F (ab')2 fragments of polyclonal IgC and serological relatedness with mollicute diseases from other continents. *Internat J Trop Plant Dis* 10, 31-35 (1992)
218. Sasikala, M., V. R. Prakash, R. Ajithkumar & R. ChandraMohanan: Selection of root (wilt) disease free coconut elite mother palms using serological tests for production of quality planting materials for disease prevalent tracts. *Indian Coconut Journal* 35, 16-19 (2004)
219. Sinha, R. C.: Serological detection of mycoplasmalike organisms from plants affected with yellows diseases. In: Tree Mycoplasmas and Mycoplasma Diseases. Ed: C. Hiruki. University of Alberta Press, Canada (1988)
220. Tian GuoZhong, Z. X., Z. C. Zhu ShuiFang & H. W. Luo Fei: Detection of mycoplasma-like organisms in the infected paulownia by indirect immunofluorescence microscopy. *For Res* 9, 1-6 (1996)
221. Cousin, M. T., G. Dafalla, E. Demazeau, E. Theveu & J. Grosclaude: In situ detection of MLOs for Solanaceae stolbur and faba bean phyllody by indirect immunofluorescence. *J Phytopathol* 124, 71-79 (1989)
222. Hiruki, C.: Immunofluorescence microscopy of yellows diseases associated with plant mycoplasma-like organisms. In: Tree mycoplasmas and mycoplasma diseases. Ed: C. Hiruki. University of Alberta Press, Canada (1988)
223. Lherminier, J., T. T. V. Scheltinga, E. Boudon-Padieu & A. Caudwell: Rapid immunofluorescent detection of the grapevine flavescence dorée mycoplasmalike organism in the salivary glands of the leafhopper *Euscelidius variegatus* Kbm. *J Phytopathol* 125, 353-360 (1989)
224. Schwartz, Y., E. Boudon-Padieu, J. Grange, R. Meignoz & A. Caudwell: Obtention d'anticorps monoclonaux spécifiques de l'agent pathogène de type mycoplasme (MLO) de la flavescence dorée de la vigne. *Res Microbiol* 140, 311-32 (1989)
225. Sinha, R. C. & N. Benhamou: Detection of mycoplasmalike organism antigens from aster yellows-diseased plants by two serological procedures. *Phytopathology* 73, 1199-1202 (1983)
226. Jiang, Y. P. & T. A. Chen: Purification of mycoplasma-like organisms from lettuce with aster yellows disease. *Phytopathology* 77, 949-953 (1987)

227. Hobbs, H. A., D. V. R. Reddy & A. S. Reddy: Detection of a mycoplasma-like organism in peanut plants with witches' broom using indirect enzyme-linked immunosorbent assay (ELISA). *Plant Pathol* 36, 64-167 (1987)
228. Caudwell, A., C. Kuszala & A. Fleury: Antigen preparation from plant tissues of pathogenic mycoplasmas (MLO) causing flavescence dorée. *J Phytopathol* 123, 124-132 (1988)
229. Jiang, Y. P., T. A. Chen, L. N. Chiykowski & R. C. Sinha: Production of monoclonal antibodies to peach eastern X-disease agent and their use in disease detection. *Can J Plant Pathol* 11, 325-331 (1989)
230. Seddas, A., R. Meignoz, X. Daire & E. Boudon-Padieu: Generation and characterization of monoclonal antibodies to flavescence dorée phytoplasma: serological relationships and differences in electroblot immunoassay profiles of flavescence dorée and elm yellows phytoplasmas. *Eur J Plant Pathol* 102, 757-764 (1996)
231. Saeed, E., P. Rage & M. T. Cousin: Determination of the antigenic protein size associated with faba bean phyllody MLO by using (SDS-PAGE) electrophoresis and immunotransfer. *J Phytopathol* 136, 1-8 (1992)
232. Sarindu, N. & M. F. Clark: Antibody production and identity of MLOs associated with sugar-cane whiteleaf disease and bermuda-grass whiteleaf disease from Thailand. *Plant Pathol* 42, 396-402 (1993)
233. Saeed, E. M., J. Roux & M. T. Cousin: Studies of polyclonal antibodies for the detection of MLOs associated with faba bean (*Vicia faba* L.) using different ELISA methods and dot-blot. *J Phytopathol* 137, 33-43 (1993)
234. Cousin, M. T., R. Berges, J. Roux, J. P. Moreau, C. Hiruki & E. Seemüller: *Populus nigra* L. Italica decline in France.Variability of the phytoplasma responsible for the disease in Europe. Results and perspectives. *Acta Hortic* 496, 77-86 (1999)
235. Lin, C. P. & T. A. Chen: Comparison of monoclonal antibodies and polyclonal antibodies in detection of the aster yellows mycoplasmalike organism. *Phytopathology* 76, 45-50 (1986)
236. Chen, K., J. R. Guo, X. Wu, N. Loi, L. Carraro, Y. H. Guo, Y. D. Chen, R. Osler, R. Pearson & T. A. Chen: Comparison of monoclonal antibodies, DNA probes, and PCR for detection of the grapevine yellows disease agent. *Phytopathology* 83, 915-22 (1993)
237. Davies, D. L. & M. F. Clark: Production and characterization of polyclonal and monoclonal antibodies against peach yellow leafroll MLO-associated antigens. *Acta Hortic* 383, 275–83 (1992)
238. Lin, C. P. & T. A. Chen: Monoclonal antibodies against the aster yellows agent. *Science* 227, 1233–35 (1985)
239. Lin, C. P. & T. A. Chen: In vitro and in vivo immunization techniques for the production of monoclonal antibodies against the aster yellows phytoplasma. *Plant Pathol Bull* 5, 28-32 (1996)
240. Loi, N., P. Ermacora, T. A. Chen, L. Carraro & R. Osler: Monoclonal antibodies for the detection of tagetes witches' broom agent. *J Plant Pathol* 80, 171-174 (1998)
241. Seddas, A., R. Meignoz, X. Daire, E. Boudon-Padieu & A. X. Caudwell: Purification of grapevine flavescence dorée MLO (mycoplasma-like organism) by immunoaffinity. *Curr Microbiol* 27, 229-36 (1993)
242. Chang, F. L., C. C. Chen & C. P. Lin: Monoclonal antibody for the detection and identification of a phytoplasma associated with rice yellow dwarf. *Eur J Plant Pathol* 101, 511-518 (1995)
243. Das, A. K. & D. K. Mitra: Detection of brinjal little leaf phytoplasma by monoclonal antibodies. *J Mycol Plant Pathol* 29, 48-51 (1999)
244. Guo, Y. H., Z. M. Cheng, J. A. Walla & Z. Zhang: Diagnosis of X-disease phytoplasma in stone fruits by a monoclonal antibody developed directly from a woody plant. *J Environ Horticulture* 16, 33-37 (1998)
245. Garnier, M., G. Martin-Gros, M. L. Iskra, L. Zreik, J. Gandar, A. Fos & J. M. Bové: Monoclonal antibodies against the MLOs associated with tomato stolbur and clover phyllody. *Recent advances in mycoplasmology Proceedings of the 7th congress of the International Organization for Mycoplasmology*, 263-269 (1990).
246. Shen, W. C. & C. P. Lin: Production of monoclonal antibodies against a mycoplasmalike organism associated with sweetpotato witches' broom. *Phytopathology* 83, 671-5 (1993)
247. Hsu, H. T., I. -M. Lee, R. E. Davis & Y. C. Wang: Immunization for generation of hybridoma antibodies specifically reacting with plants infected with a mycoplasmalike organism (MLO) and their use in detection of MLO antigens. *Phytopathology* 80, 946-950 (1990)
248. Lee, I. -M., R. E. Davis & H. T. Hsu: Differentiation of strains in the aster yellows mycoplasmalike organism strain cluster by serological assay with monoclonal antibodies. *Plant Dis* 77, 815-7 (1993)
249. Boudon-Padieu, E. T., T. Terwisscha Van Scheltinga, J. Lherminier & R. Caudwell: Elisa and immunofluorescence (IF) detection of the MLO agent of the grapevine flavescence dorée (FD) on individual leafhopper vectors. *6th International Congress of the International Organization for Mycoplasmology*, 198 (1986).
250. Lherminier, J., G. Prensier, E. Boudon-Padieu & A. Caudwell: Immunolabeling of grapevine flavescence dorée MLO in salivary glands of *Euscelidius variegatus*: a light and electron microscopy study. *J Histochem Cytochem* 38, 79-83 (1990)
251. Scheltinga, T. T. V., E. Boudon-Padieu & A. Caudwell: Rapid immunofluorescent detection of the grapevine flavescence dorée mycoplasmalike organism in the salivary glands of the leafhopper *Euscelidius variegatus* Kbm. *J Phytopathol* 125, 353-360 (1989)
252. Sinha, R. C. & L. N. Chiykowski: Detection of mycoplasmalike organisms in leafhopper vectors of aster yellows and peach-X disease by immunosorbent electron microscopy. *Can J Plant Pathol* 4, 387-393 (1986)
253. Fos, A., J. Danet, L. Zreik, M. Garnier & J. M. Bové: Use of a monoclonal antibody to detect the stolbur mycoplasmalike organism in plants and insects and to identify a vector in France. *Plant Dis* 76, 1092-6 (1992)
254. Lin, N. S., Y. H. Hsu & H. T. Hsu: Immunological detection of plant viruses and a mycoplasmalike organism by direct tissue blotting on nitrocellulose membranes. *Phytopathology* 80, 824-828 (1990)
255. Berg, M., D. L. Davies, M. F. Clark, H. J. Vetten, G. Maier, C. Marcone & E. Seemüller: Isolation of the gene

encoding an immunodominant membrane protein of the apple proliferation phytoplasma, and expression and characterization of the gene product. *Microbiology* 145, 1937-1943 (1999)
256. Blomquist, C. L., D. J. Barbara, D. L. Davies, M. F. Clark & B. C. Kirkpatrick: An immunodominant membrane protein gene from the western X-disease phytoplasma is distinct from those of other phytoplasmas. *Microbiology* 147, 571-580 (2001)
257. Hong, Y., D. L. Davies, R. V. Wezel, B. E. Ellerker, A. Morton & D. Barbara: Expression of the immunodominant membrane protein of chlorantie-aster yellows phytoplasma in *Nicotiana benthamiana* from a potato virus X-based vector. *Acta Hortic* 550, 409-415 (2001)
258. Mergenthaler, E., O. Viczian, M. Fodor & S. Sule: Isolation and expression of an immunodominant membrane protein gene of the ESFY phytoplasma for antiserum production. *Acta Hortic* 550, 355-360 (2001)
259. Yu, Y. L., K. W. Yeh & C. P. Lin: An antigenic protein gene of a phytoplasma associated with sweet potato witches' broom. *Microbiology* 144, 1257-1262 (1998)
260. Kakizawa, S., K. Oshima, T. Kuboyama, H. Nishigawa, H. Y. Jung, T. Sawayanagi, T. Tsuchizaki, S. Miyata, M. Ugaki & S. Namba: Cloning and expression analysis of phytoplasma protein translocation genes. *Mol Plant- Microbe Interact* 14, 1043-1050 (2001)
261. Ahrens, U. & E. Seemüller: Detection of DNA of plant pathogenic mycoplasma-like organisms by a polymerase chain reaction that amplifies a sequence of the 16S rRNA gene. *Phytopathology* 82, 828-832 (1992)
262. Prince, J. P., R. E. Davis, T. K. Wolf, I. -M. Lee, B. D. Mogen, E. L. Dally, A. Bertaccini, R. Credi & M. Barba: Molecular detection of diverse mycoplasmalike organisms (MLOs) associated with grapevine yellows and their classification with aster yellows, X-disease, and elm yellows MLOs. *Phytopathology* 83, 1130–37 (1993)
263. Daire, X., D. Clair, W. Reinert & E. Boudon-Padieu: Detection and differentiation of grapevine yellows phytoplasmas belonging to the elm yellows group and to the stolbur subgroup by PCR amplification of non-ribosomal DNA. *Eur J Plant Pathol* 103, 507-514 (1997)
264. Green, M. J., D. A. Thompson & D. J. MacKenzie: Easy and efficient DNA extraction from woody plants for the detection of phytoplasmas by polymerase chain reaction. *Plant Dis* 83, 482-485 (1999)
265. Guo, Y. H., Z. M. Cheng & J. A. Walla: Rapid PCR-based detection of phytoplasmas from infected plants. *HortScience* 38, 1134-1136 (2003)
266. Levy, L., I. -M. Lee & A. Hadidi: Simple and rapid preparation of infected plant tissue extracts for PCR amplification of virus, viroid, and MLO nucleic acids. *J Virol Methods* 49, 295-304 (1994)
267. Zhang, Y. P., J. K. Uyemoto & B. C. Kirkpatrick: A small-scale procedure for extracting nucleic acids from woody plants infected with various phytopathogens for PCR assay. *J Virol Methods*, 71, 45-50 (1998)
268. Gibb, K. S. & A. Padovan: A DNA extraction method that allows reliable PCR amplification of MLO DNA from 'difficult' plant host species. *PCR Meth Appl*, 4, 56-58 (1994)
269. Boudon-Padieu, E., A. Bejat, D. Clair, J. Larrue, M. Borgo, L. Bertotto & E. Angelini: Grapevine yellows: comparison of different procedures for DNA extraction and amplification with PCR for routine diagnosis of phytoplasmas in grapevine. *Vitis* 42, 141-149 (2003)
270. Palmano, S.: A comparison of different phytoplasma DNA extraction methods using competitive PCR. *Phytopathol Medit* 40, 99-107 (2001)
271. Bosco, D., S. Palermo, G. Mason, R. Tedeschi, C. Marzachì & G. Boccardo: DNA-based methods for the detection and the identification of phytoplasmas in insect vector extracts. *Mol Biotechnol* 22, 9-18 (2002)
272. Marzachi, C., F. Veratti & D. Bosco: Direct PCR detection of phytoplasmas in experimentally infected insects. *Ann Appl Biol* , 133, 45-54 (1998)
273. Bertaccini, A., R. E. Davis & I. -M. Lee: Distinctions among mycoplasmalike organisms (MLOs) in *Gladiolus, Ranunculus, Brassica*, and *Hydrangea* through detection with nonradioactive cloned DNA probes. *Phytopathol Medit* 29, 107-113 (1990)
274. Lee, I. -M., R. E. Davis & N. D. DeWitt: Nonradioactive screening method for isolation of disease-specific probes to diagnose plant diseases caused by mycoplasmalike organisms. *Appl Environ Microbiol* 56, 1471-5 (1990)
275. Lee, I.-M. & R. E. Davis: Detection and investigation of genetic relatedness among aster yellows and other mycoplasmalike organisms by using cloned DNA and RNA probes. *Mol Plant-Microbe Interact* 1, 303–10 (1988)
276. Davis, R. E., I. -M. Lee, E. L. Dally, N. Dewitt & S. M. Douglas: Cloned nucleic acid hybridization probes in detection and classification of mycoplasmalike organisms (MLOs). *Acta Hortic* 234, 115-122 (1988)
277. Davis, R. E., I.- M. Lee, S. M. Douglas, E. L. Dally & N. E. L. Dewitt: Development and use of cloned nucleic acid hybridization probes for disease diagnosis and detection of sequence homologies among uncultured mycoplasmalike organisms (MLOs). *Recent advances in mycoplasmology Proceedings of the7th congress of the International Organization for Mycoplasmology*, (1988).
278. Bonnet, F., C. Saillard, A. Kollar, E. Seemüller & J. M. Bové: Detection and differentiation of the mycoplasmalike organism associated with apple proliferation disease using cloned DNA probes. *Mol Plant-Microbe Interact* 3, 438-43 (1990)
279. Deng, S. & C. Hiruki: The use of cloned DNA probes for diagnosis of noncultivable plant mollicutes. *P Jpn Acad B-Phys* 66, 58-61 (1990)
280. Deng, S. & C. Hiruki: Localization of pathogenic mycoplasma-like organisms in plant tissue using *in situ* hybridization. *P Jpn Acad B-Phys* 67, 197-202 (1991)
281. Deng, S. & C. Hiruki: Molecular cloning and detection of DNA of the mycoplasmalike organism associated with clover proliferation. *Can J Plant Pathol* 12, 383-8 (1990)
282. Harrison N.A., C. M. Bourne, R. l. Cox, J. H. Tsai & P. A. Richardson: DNA probes for detection of mycoplasmalike organisms associated with lethal yellowing disease of palms in Florida. *Phytopathology* 82, 216– 24 (1992)
283. Davis R.E., W. A. Sinclair, I. -M. Lee & E. L.Dally: Cloned DNA probes specific for detection of a mycoplasmalike organism associated with ash yellows. *Mol*

Plant- Microbe Interact 5, 163–69 (1992)
284. Daire, X., E. Boudon-Padieu, A. Berville, B. Schneider & A. Caudwell: Cloned DNA probes for detection of grapevine flavescence dorée mycoplasma-like organism (MLO). *Ann Appl Biol* 121, 95-103 (1992)
285. Chang, C. J., R. Jarret & N. Gawel: Isolation and cloning of DNA fragments from a mycoplasmalike organism associated with walnut witches'-broom disease. *Phytopathology* 82, 306-309 (1992)
286. Davis, R. E., E. L. Dally, A. Bertaccini, R. Credi, I. -M. Lee, R. Osler, L. Carraro & M. Barba: Cloned DNA probes for specific detection on Italian periwinkle virescence mycoplasmalike organism (MLO) and investigation of genetic relatedness with other MLOs. *Phytopathol Medit* 31, 5-12 (1992)
287. Wu, F. Y., C. P. Lin & C. C. Chen: Development of cloned DNA probes for a mycoplasmalike organism associated with rice yellow dwarf. *Plant Pathol Bull* 2, 128-35 (1993)
288. Ko, H. C. & C. P. Lin: Development and application of cloned DNA probes for a mycoplasmalike organism associated with sweetpotato witches'-broom. *Phytopathology*, 84, 468–73 (1994)
289. Nakashima, K., W. Chaleeprom, P. Wongkaew & P. Sirithorn: Detection of mycoplasma-like organisms associated with white leaf disease of sugarcane in Thailand using DNA probes. *Jircas Journal* 1, 57-67 (1994)
290. Kuan, C. P. & H. J. Su: Development of cloned DNA probes for phytoplasma associated with loofah witches' broom. *J Phytopathol* 146, 309-313 (1998)
291. MengLing. W, C. TunTschu., W.LaingChour & F. ChuenHsu: Rapid detection of phytoplasma associated with paulownia witches'-broom using a nonradioactive DNA probe and a PCR-based assay. *Taiwan J Forest Sci* 172, 123-133 (2002)
292. Bertaccini, A., A. Arzone, A. Alma, D. Bosco & M. Vibio: Detection of mycoplasmalike organisms in *Scaphoideus titanus* Ball reared on flavescence dorée infected grapevine by dot hybridizations using DNA probes. *Phytopathol Medit* 32, 20-24 (1993)
293. Garcia-Chapa, M., A. Batlle, A. Lavina, L. Galipienso & G. Firrao: Pear decline phytoplasma detection in pear trees and insect vectors by dot-blot hybridization and nested-PCR. *Acta Hortic* 657, 431-436 (2004)
294. Rahardja, U., M. E. Whalon, C. Garcia-Salazar & Y. T. Yan: Field detection of X-disease mycoplasmalike organism in *Paraphlepsius irroratus* (Say) (Homoptera: Cicadellidae) using a DNA probe. *Environ Entomol* 21, 81-88 (1992)
295. Vega, F. E., R. E. Davis, E. L. Dally, P. Barbosa, A. H. Purcell & I. -M. Lee: Use of a biotinylated DNA probe for detection of the aster yellows mycoplasmalike organism in *Dalbulus maidis* and *Macrosteles fascifrons* (*Homoptera: Cicadellidae*). *Fla Entomol*, 77, 330-4 (1994)
296. Davis, R. E., E. L. Dally, A. Bertaccini, I. -M. Lee, R. Credi, R. Osler, V. Savino, L. Carraro, B. Di Terlizzi & M. Barba: Restriction fragment length polymorphism analyses and dot hybridisations distinguish mycoplasmalike organisms associated with Flavescence dorée and southern European grapevine yellows disease in Italy. *Phytopathology* 83, 772-776 (1993)
297. Firrao, G., E. Gobbi & R. Locci: Use of polymerase chain reaction to produce oligonucleotide probes for mycoplasmalike organisms. *Phytopathology* 83, 602–7 (1993)
298. Malisano, G., G. Firrao & R. Locci: 16S rDNA-derived oligonucleotide probes for the differential diagnosis of plum leptonecrosis and apple proliferation phytoplasmas. *EPPO Bulletin* 26, 421-428 (1996)
299. Lherminier, J., R. G. Bonfiglioli, X. Daire, R. H. Symons & E. Boudon-Padieu: Oligodeoxynucleotides as probes for in situ hybridization with transmission electron microscopy to specifically localize phytoplasma in plant cells. *Mol Cel Probe* 13, 41-47 (1999)
300. Kuske, C. R., B. C. Kirkpatrick & E. Seemüller: Differentiation of virescence phytoplasmas using western aster yellows mycoplasma-like organism chromosomal DNA probes and restriction fragment length polymorphism analysis. *J Gen Microbiol* 137, 153-159 (1991)
301. Lee, I.-M., R. E. Davis & C. Hiruki: Genetic relatedness among clover proliferation mycoplasmalike organisms (MLOs) and other MLOs investigated by nucleic acid hybridization and restriction fragment length polymorphism analyses. *Appl Environ Microbiol* 57, 3565–69 (1991)
302. Lee, I.-M., Davis, R. E., T. A. Chen, L. N. Chiykowski, J. Fletcher, C. Hiruki & D. A. Schaff: A genotype-based system for identification and classification of mycoplasmalike organisms (MLOs) in the aster yellows MLO strain cluster. *Phytopathology* 82, 977-986 (1992)
303. Ahrens, U., K. H. Lorenz & E. Seemüller: Genetic diversity among mycoplasmalike organisms associated with stone fruit diseases. *Mol Plant-Microbe Interact* 6, 686–91 (1993)
304. Deng, S. & C. Hiruki: Genetic relatedness between two nonculturable mycoplasmalike organisms revealed by nucleic acid hybridisation and polymerase chain reaction. *Phytopathology* 81, 1475-1479 (1991)
305. Nakashima K, K. S. & M. N. Iwanami S: DNA probes reveal relatedness of rice yellowdwarf mycoplasmalike organisms (MLOs) and distinguish them from other MLOs. *Appl Environ Microbio,* 59:1206–12, 9:1206–12 (1993)
306. Mpunami, A., P. Jones, A. Tymon & M. Dickinson: The use of DNA probes and PCR for detection of coconut lethal disease (LD) in Tanzania. *Brighton Crop Protection Conference: Pests & Diseases* 577-582 (1996).
307. Chen, M. F. & C. P. Lin: DNA probes and PCR primers for the detection of a phytoplasma associated with peanut witches'-broom. *Eur J Plant Pathol* 103, 137-145 (1997)
308. Wu, M. L., T. T. Chang, L. C. Wang & C. H. Fu: Rapid detection of phytoplasma associated with paulownia witches'-broom using a nonradioactive DNA probe and a PCR-based assay. *Taiwan J Forest Sci* 17, 123-133 (2002)
309. Bertin, S., S. Palermo, C. Marzachì & D. Bosco: A comparison of molecular diagnostic procedures for the detection of aster yellows phytoplasmas (16Sr-I) in leafhopper vectors. *Phytoparasitica* 32, 141-145 (2004)
310. Namba, S., S. Kato, i. S. Iwanam, H. Oyaizu, H. Shiozawa & T. Tsuchizaki: Detection and differentiation of plant-pathogenic mycoplasmalike organisms using polymerase chain reaction. *Phytopathology* 83, 786–91 (1993)
311. Lee, I. -M., R. W. Hammond, R. E. Davis & D. E.

Gundersen-Rindal: Universal amplification and analysis of pathogen 16S rDNA for classification and identification of mycoplasmalike organisms. *Phytopathology* 83, 834-842 (1993)
312. Gundersen, D. E., I. -M. Lee, S. A. Rehner, R. E. Davis & D. E. Kingsbury: Phylogeny of mycoplasmalike organisms (phytoplasmas): a basis for their classification. *J Bacteriol* 176, 5244–54 (1994)
313. Yoshikawa, N., H. Nakamura, N. Sahashi, T. Kubono, K. Katsube, T. Shoji & M. Takahashi: Amplification and nucleotide sequences of ribosomal protein and 16S rRNA genes of mycoplasma-like organism associated with paulownia witches' broom. *Ann Phytopathol Soc Jpn* 60, 569-75 (1994)
314. Gundersen, D. E. & I. -M. Lee: Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. *Phytopathol Medit* 35, 144-151 (1996)
315. Smart, C. D., B. Schneider, C. L. Blomquist, L. J. Guerra, N. A. Harrison, U. Ahrens, K. H. Lorenz, E. Seemüller & B. Kirkpatrick: Phytoplasma-specific PCR primers based on sequences of the 16S-23S rRNA spacer region. *Appl and Environ Microbiol* 62, 2988-2993 (1996)
316. Guo, Y. H., Z. M. Cheng & J. A. Walla: Amplification and RFLP analysis of 23S ribosomal DNA from phytoplasmas. *Phytopathology* 88, 35 (1998)
317. Lin, T. C. & C. P. Lin: Evaluation of universal PCR primers for the detection of phytoplasmas. *Plant Pathol Bull* 7, 33-42 (1998)
318. Guo, Y. H., Z. M. Cheng & J. A. Walla: Amplification of the 23S rRNA gene and its application in differentiation and detection of phytoplasmas. *Can J Plant Pathol* 22, 380-386 (2000)
319. Firrao, G., E. Gobbi & R. L. Locci: Rapid diagnosis of apple proliferation mycoplasma-like organism using a polymerase chain reaction procedure. *Plant Pathol* 43, 669-74 (1994)
320. Davis, R. E. & I. -M. Lee: Cluster-specific polymerase chain reaction amplification of 16S rDNA sequences for detection and identification of mycoplasmalike organisms. *Phytopathology* 83, 1008–11 (1993)
321. Rhode, W., A. Kullaya, A. Mpunami & D. Becker: Rapid and sensitive diagnosis of mycoplasmalike organisms associated with lethal disease of coconut palm by a specifically primed polymerase chain reaction for the amplification of 16S rDNA. *Oleagineux* 48, 319-322 (1993)
322. Bianco, P. A., P. Casati & G. Belli: Detection and identification by PCR-based techniques of diverse phytoplasmas infecting grapevine. *Proceedings 4th International Symposium of the European Foundation for Plant Pathology*, 179-182 (1997).
323. Lee, I.-M., D. E. Gundersen, R. W. Hammond & R. E. Davis: Use of mycoplasmalike organism (MLO) group-specific oligonucleotide primers for nested-PCR assays to detect mixed-MLO infections in a single host plant. *Phytopathology* 84, 559– 66 (1994)
324. Lorenz, K. H., B. Schneider, U. Ahrens & E. Seemüller: Detection of the apple proliferation and pear decline phytoplasmas by PCR amplification of ribosomal and nonribosomal DNA. *Phytopathology* 85, 771-776 (1995)
325. Gundersen, D. E., I. -M. Lee, D. A. Schaff, N. A. Harrison, C. J. Chang, R. E. Davis & D. T. Kingsbury: Genomic diversity and differentiation among phytoplasma strains in 16S rRNA groups I (aster yellows and related phytoplasmas) and III (X-disease and related phytoplasmas). *Int J Syst Bacteriol* 46, 64-75 (1996)
326. Schneider, B., U. Ahrens, B. C. Kirkpatrick & E. Seemüller: Classification of plant-pathogenic mycoplasma-like organisms using restriction-site analysis of PCR-amplified 16S rDNA. *J Gen Microbiol* 139, 519-527 (1993)
327. De la Rue, S., A. Padovan & K. Gibb: *Stylosanthes* is a host for several phytoplasmas, one of which shows unique 16S-23S intergenic spacer region heterogeneity. *J Phytopathol* 149, 613-619 (2001)
328. Palmano, S. & G. Firrao: Diversity of phytoplasmas isolated from insects, determined by a DNA heteroduplex mobility assay and a length polymorphism of the 16S-23S rDNA spacer region analysis. *J Appl Microbiol* 89, 744-750 (2000)
329. Hsyu, S. M. & C. P. Lin: Phylogenetic relationships of phytoplasmas on the basis of sequences of 16S-23S rDNA spacers. *Plant Pathol Bull* 11, 199-206 (2002)
330. Wang, K., C. Hiruki & F. Yeh: Molecular evolution of phytoplasmas based on polymorphisms in the 16S rRNA genes and the 16/23S spacer regions. *P Jpn Acad B-Phys* 79, 155-162 (2003)
331. Skrzeczkowski, L. J., W. E. Howell, K. C. Eastwell & T. D. Cavileer: Bacterial sequences interfering in detection of phytoplasma by PCR using primers derived from the ribosomal RNA operon. *Acta Hortic*, 550, 417-424 (2001)
332. Bertaccini, A. & M. Martini: Ribosomal and non-ribosomal primers for sensitive detection and identification of phytoplasmas. *Petria* 9, 89-92 (1999)
333. Jomantiene, R., R. E. Davis, J. Maas & E. L. Dally: Classification of new phytoplasmas associated with diseases of strawberry in Florida, based on analysis of 16S rRNA and ribosomal protein gene operon sequences. *Int J Syst Bacteriol* 48, 269-277 (1998)
334. Lee, I. -M., D. E. Gundersen-Rindal, R. E. Davis & I. M. Bartoszyk: Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences. *Int J Sys Bacteriol* 48, 1153-1169 (1998)
335. Schneider, B., K. S. Gibb & E. Seemüller: Sequence and RFLP analysis of the elongation factor Tu gene used in differentiation and classification of phytoplasmas. *Microbiology* 143, 3381-3389 (1997)
336. Jarausch, W., C. Saillard, F. Dosba & J. M. Bové: Differentiation of mycoplasmalike organisms (MLOs) in European fruit trees by PCR using specific primers derived from sequence of a chromosomal fragment of the apple proliferation MLO. *Appl Environ Microbiol* 60, 2916-2923 (1994)
337. Jarausch, W., M. Lansac, C. Saillard, J. M. Broquaire & F. Dosba: PCR assay for specific detection of European stone fruit yellows phytoplasmas and its use for epidemiological studies in France. *Eur J Plant Pathol* 104, 17-27 (1998)
338. Chuang, J. G. & C. P. Lin: Cloning of gyrB and gyrA genes of phytoplasma associated with peanut witches'broom. *Plant Pathol Bull* 9, 157-166 (2000)
339. Marzachì, C., F. Veratti, M. d'Aquilio, A. Vischi, M.

Conti & G. Boccardo: Molecular hybridization and PCR amplification of non-ribosomal DNA to detect and diferenciate stolbur phytoplasma isolates from Italy. *J Plant Pathol* 82, 201-212 (2000)
340. Davis, R. E., J. P. Prince, R. W. Hammond, E. L. Dally & I. -M. Lee: Polymerase chain reaction detection of Italian periwinkle virescence mycoplasmalike organism (MLO) and investigation of genetic relatedness with other MLOs. *Petria* 2, 184-193 (1992)
341. Blomquist, C. L. & B. C. Kirkpatrick: Identification of phytoplasma taxa and insect vectors of peach yellow leaf roll disease in California. *Plant Dis* 86, 759-763 (2002)
342. Jarausch, W., J. L. Danet, J. M. Broquaire, C. Saillard, M. Garnier & F. Dosba: Apricot chlorotic leaf roll in Roussillon: specific detection of the pathogen, search for alternative host plants, attempts to determine infection periods and attempts to identify the insect vector. *Acta Hortic* 488, 739-744 (1999)
343. Jarausch, W., J. L. Danet, G. Labonne, F. Dosba, J. M. Broquaire, C. Saillard & M. Garnier: Mapping the spread of apricot chlorotic leaf roll (ACLR) in southern France and implication of *Cacopsylla pruni* as a vector of European stone fruit yellows (ESFY) phytoplasmas. *Plant Pathol* 50, 782-790 (2001)
344. Jarausch, B., N. Schwind, W. Jarausch, G. Krczal, E. Dickler & E. Seemüller: First report of *Cacopsylla picta* as a vector of apple proliferation phytoplasma in Germany. *Plant Dis* 87, 101 (2003)
345. Krczal G, K. H. & L. J. Kunze: *Fieberiella florii* (Stal), a vector of apple proliferation agent. *Acta Hortic* 235, 99-106 (1989)
346. Maixner, M. & W. Reinert: *Oncopsis alni* (Schrank) (*Auchenorrhyncha: Cicadellidae*) as a vector of the alder yellows phytoplasma of *Alnus glutinosa* (L. Gaertn.). *Eur J Plant Pathol* 105, 87-94 (1999)
347. Tanne, E., E. Boudon-Padieu, D. Clair, M. Davidovich, S. Melamed & J. K. Klein M: Detection of phytoplasma by polimerase chain reaction of insect feeding medium and its use in determining vectoring ability. *Phytopathology* 91 (8), 741-746 (2001)
348. Weintraub, P. G. & S. Orenstein: Potential leafhopper vectors of phytoplasma in carrots. *Internat J Trop Insect Sci*, 24, 228-235 (2004)
349. Harrison, N. A.: PCR assay for detection of the phytoplasma associated with maize bushy stunt disease. *Plant Dis*, 80, 263–69 (1996)
350. Harrison, N. A., P. A. Richardson, J. H. Tsai, M. A. Ebbert & J. B. Kramer: PCR assay for detection of the phytoplasma associated with maize bushy stunt disease. *Plant Dis* 80, 263-269 (1996)
351. Andersen, M. T., R. E. Beever, A. C. Gilman, L. W. Liefting, E. Balmori, D. L. Beck, P. W. Sutherland, G. T. Bryan, R. C. Gardner & R. L. S. Forster: Detection of phormium yellow leaf phytoplasma in New Zealand flax (*Phormium tenax*) using nested PCRs. *Plant Pathol* 47, 188-196 (1998)
352. Ho Kuo Chieh, H.: Cloning and nucleotide sequence analysis of a tuf gene from loofah witches' broom phytoplasma. *Taiwania*, 46, 285-294 (2001)
353. Khadhair, A. H., I. R. Evans & B. Choban: Identification of aster yellows phytoplasma in garlic and green onion by PCR-based methods. *Microbiol Res*, 157, 161-167 (2002)
354. Harrison, N. A., P. A. Richardson, J. B. Kramer & J. H. Tsai: Detection of the mycoplasmalike organism associated with lethal yellowing disease of palms in Florida by polymerase chain reaction. *Plant Pathol* 43, 998–1008 (1994)
355. Jarausch, W., C. Saillard, F. Dosba & J. M. Bové: Specific detection of mycoplasma-like organisms in European fruit trees by PCR. *Bulletin OEPP* 25, 219-25 (1995)
356. Nakamura, H., N. Yoshikawa, T. Takahashi, N. Sahashi, T. Kubono & T. Shoji: Evaluation of primer pairs for the reliable diagnosis of Paulownia witches'-broom disease using a polymerase chain reaction. *Plant Dis*, 80, 302-305 (1996)
357. Gibb, K. S., B. Schneider & A. C. Padovan: Differential detection and genetic relatedness of phytoplasmas in papaya. *Plant Pathol* 47, 325-332 (1998)
358. Tymon, A. M., P. Jones & N. A. Harrison: Phylogenetic relationships of coconut phytoplasmas and the development of specific oligonucleotide PCR primers. *Ann Appl Biol* 132, 437-452 (1998)
359. Guo, Y. H., Z. M. Cheng & J. A. Walla: Characterization of X-disease phytoplasmas in chokecherry from North Dakota by PCR-RFLP and sequence analysis of the rRNA gene region. *Plant Dis* 84, 1235-1240 (2000)
360. Orenstein, S., T. Zahavi & P. Weintraub: Distribution of phytoplasma in grapevines in the Golan Heights, Israel, and development of a new universal primer. *Vitis* 40, 219-223 (2001)
361. Al-Awadhi, H. A., S. Hanif A, P. Suleman & S. Montaser: Molecular and microscopical detection of phytoplasma associated with yellowing disease of date palms *Phoenix dactylifera* L. in Kuwait. *Kuwait J Sci Eng* 29, 87-109 (2002)
362. Maliyakal, E. J.: An efficient method for isolation of RNA and DNA from plants containing polyphenolics. *Nucleic Acids Res* 20, 2381 (1992)
363. Baric, S. & J. Dalla-Via: A new approach to apple proliferation detection: a highly sensitive real-time PCR assay. *J Microbiol Meth* 57, 135-145 (2004)
364. Bianco, P. A., P. Casati & N. Marziliano: Detection of phytoplasmas associated with grapevine flavescence dorée disease using real-time PCR. *J Plant Pathol*, 86, 257-261 (2004)
365. Marzachì, C., L. Galetto & D. Bosco: Real-Time PCR detection of Bois Noir and Flavescence Dorée from field collected symptomatic grapevines. Proc. *14th ICVG Conference, Locorotondo, 12-17th September* 56-57 (2003).
366. Galetto, L., D. Bosco & C. Marzachì: Universal and group-specific Real Time PCR diagnosis of flavescence dorée (FD, 16Sr-V), bois noir (BN, 16Sr-XII) and apple proliferation (AP, 16Sr-X) phytoplasmas from field-collected plant hosts and insect vectors. *Ann Appl Biol*, in press, (2005)
367. Christensen, N. M., M. Nicolaisen, M. Hansen & A. Schulz: Distribution of phytoplasmas in infected plants as revealed by real-time PCR and bioimaging. *Mol Plant-Microbe Interact*, 17, 1175-1184 (2004)
368. Poggi Pollini, C., L. Giunchedi & R. Bissani: Immunoenzymatic detection of PCR products for the

identification of phytoplasmas in plants. *J Phytopathol*, 145, 371-374 (1997)
369. Wang, K. & C. C. Hiruki: Heteroduplex mobility assay detects DNA mutations for differentiation of closely related phytoplasma strains. *J Microbiol Meth*, 41, 59-68 (2000)
370. Wang, K. & C. C. Hiruki: Use of heteroduplex mobility assay for identification and differentiation of phytoplasmas in the aster yellows group and the clover proliferation group. *Phytopathology*, 91, 546-552 (2001)
371. Frosini, A., P. Casati, P. A. Bianco, R. Bordoni, C. Consolandi, B. Castiglioni, A. Mezzelani, E. Rizzi, C. Battaglia, G. Belli, L. Rossi Bernardi & G. De Bellis: Ligase detection reaction and universal array as a tool to detect grapevine infecting phytoplasmas. *Minerva Biotecnol*, 14, 265-26 (2002)
372. Firrao, G., M. Moretti, M. Ruiz Rosquete, E. Gobbi & R. Locci: Nanobiotransducer for detecting flavescence dorée phytoplasma. *J Plant Pathol* , 87, 101-107 (2005)
373. Heinrich, M., S. Botti, L. Caprara, W. Arthofer, S. Strommer, V. Hanzer, H. Katinger, A. Bertaccini & M. Laimer da Câmara Machado: Improved detection methods for fruit tree phytoplasmas. *Plant Mol Biol Reptr*, 19, 169-179 (2001)

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