Sugar Tech

Vol. 4 (3&4) : 79 - 85 (2002)

REVIEW ARTICLE

Phytoplasma Diseases of Sugarcane

C. Marcone

Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Campus Macchia Romana, I-85100 Potenza, Italy

Sugarcane is severely affected by yellows and decline diseases of phytoplasmal etiology. These diseases which are of considerable economic importance, cause similar symptoms but differ in the identity of the associated phytoplasmas, vectorship and geographic distribution. Sugarcane white leaf (SCWL) and sugarcane grassy shoot (SCGS) occur only in the south-east Asian region and not in other sugarcane growing areas of the world. Both are caused by a single phytoplasma type that is a member of the SCWL group and appears to infect only sugarcane. However, SCWL and SCGS phytoplasmas are closely related organisms and could be differentiated by RFLP analysis of rDNA using suitable restriction endonucleases. Sugarcane yellow leaf syndrome (SCYLS) disease has been reported from several African countries, Cuba and Australia, and is associated with distinctly different phytoplasmas which are not specific pathogens. They include members of the X-disease, faba bean phyllody, aster yellows, SCWL, LfWB and CirP groups which are known to infect a wide range of wild and cultivated plants and have a low insect vector specificity. Ramu stunt disease of sugarcane (SCRS) is known to occur in Papua New Guinea. This disease is associated with a SCWL-related phytoplasma and is presumed to be naturally spread by the leafhopper *Eumetopina flavipes*. Sugarcane green grassy shoot (SCGGS) is a newly discovered phytoplasmal disease of sugarcane in Thailand.

KEYWORDS : Phytoplasmas, sugarcane, ribosomal DNA, RFLP analysis, sugarcane white leaf, sugarcane grassy shoot, sugarcane yellow leaf syndrome.

INTRODUCTION

Plant-pathogenic phytoplasmas (formerly called mycoplasma-like organisms [MLOs]) are nonculturable, wall-less prokaryotes of the class Mollicutes with a small genome size ranging from 530 to 1350 kilobases (kb) (Marcone *et al.*, 1999). They incite diseases in several hundred plant species (McCoy *et al.*, 1989; Seemüller *et al.*, 1998). The symptoms shown by infected plants include yellowing and reddening of the leaves, reduced leaf size, stunting, proliferation of axillary shoots resulting in a witches'-broom appearance, virescence, phyllody, sterility of flowers, decline and death. Phytoplasmas are phloem parasites which resides almost exclusively in the sieve tube elements. They are spread to their plant hosts by phloem-feeding insects, mainly leafhoppers and planthoppers (Auchenorrhynca), and less frequently psyllids (Stenorrhyncha). Although some phytoplasmas do occur in temperate climate, the most important are found in tropical and sub-tropical countries (McCoy *et al.*, 1989).

By the introduction of molecular methods into plant mycoplasmology, specific and sensitive detection methods have been developed, mainly based on polymerase chain reaction (PCR) assays (Ahrens and Seemüller, 1992). It also became possible to differentiate, characterize and classify the phytoplasmas on a phylogenetic basis, using sequence and restriction fragment length polymorphism (RFLP) analysis of ribosomal DNA (rDNA) (Lee *et al.*, 1993, 1998, 2000; Schneider *et al.*, 1993; Seemüller *et al.*, 1998). In this way, several phylogenetic groups could be established which include the sugarcane white leaf (SCWL) group, the X-disease group, the aster yellows (AY) group and the faba bean phyllody (FBP) group.

This article summarized information about phytoplasmal diseases of sugarcane.

PHYTOPLASMAL DISEASES OF SUGARCANE

Sugarcane (*Saccharum* spp. and hybrids) is severely affected by yellows and decline diseases of phytoplasmal etiology. These diseases cause similar symptoms but differ in the identity of the associated phytoplasmas, vectorship and geographic distribution.

Sugarcane white leaf

Sugarcane white leaf (SCWL) is one of the most destructive sugarcane diseases in Thailand. It was first

Corresponding author : C. Marcone
Fax : 0039-971-205503; **e-mail :** ma513agr@unibas.it

observed in 1954 in the Lumpang province in the northern part of Thailand (Mangelsdorf, 1962). Only four years later, SCWL was discovered in Taiwan (Ling, 1962). In Thailand, the disease subsequently spread to all important sugarcane-growing areas in the north, northeast and east, resulting in one of the most lethal disease of sugarcane. Currently, it seems present in all areas where the crop is grown (Rishi and Chen, 1989; Sarindu and Clark, 1993; Nakashima *et al.*, 1994, 1996; Wongkaew *et al.*, 1997; Rao and Ford, 2001). In Taiwan, SCWL spread from the Pingtung district into all sugarcane-growing areas except Chifu, Taichung and Yaemei districts. However, due to intensive control campaigns, the disease now occurs sporadically only in areas such as Pingtung, Chishan, Yuching and Wushulin districts (Rishi and Chen, 1989). SCWL was also recorded in 1986 in Japan, in the Tanegashima island, but later disappeared (Nakashima and Murata, 1993; Nakashima *et al.*, 2001).

The most characteristic symptoms of SCWL are the presence of leaves with total chlorosis, proliferating tillers and pronounced stunting. The leaves are narrower and smaller than those of healthy plants, with a soft texture and borne on slender chlorotic shoots. Severely diseased plants fail to set fruits, decline and do not produce millable canes.

SCWL is naturally transmitted by the leafhopper *Matsumuratettix hiroglyphicus* Matsumura (Matsumoto *et al.*, 1968). The minimum acquisition and inoculation feeding periods are 3 h and 30 min, respectively (Chen, 1978). The incubation period of SCWL phytoplasma in the insect vector is 25-35 days while in the host plant is 70-90 days (Matsumoto *et al.*, 1968). Transovarial transmission is not known to occur. Lee and Chen (1972) reported the optimum temperature for vector transmission at 25°C. According to the studies of Chen (1978), female adults seem to be more efficient than the males in transmission of SCWL disease. *M. hiroglyphicus* is widely distributed in central and southern parts of Taiwan and in Thailand. Sugarcane and *S. spontaneum* L. (wild cane) are the preferred hosts. In sugarcane fields, the vector population is particularly abundant from July to October. The population declines rapidly in December and remains low until April. Six generations may occur in a year, with overlapping between generations (Yang and Pan, 1979). Disease incidence is correlated with the population trend of the vector in the field. Cuttings planted from July to October are more severely affected than those planted from December to March (Lee, 1970). The females of *M. hiroglyphicus* usually lay their eggs in the soil to a depth of about 0.5 cm, but sometimes eggs are laid in the leaf sheath near the ground. Sandy soils are preferred for oviposition and this may be one of the reasons why the disease is often more severe on sandy soils.

Records on mechanically transmission as well as on transmission by aphids have not been confirmed (Rishi and Chen, 1989).

The causal agent, the SCWL phytoplasma, is a member of the phylogenetic SCWL group, which includes other important phytoplasmas infecting plants of the Poaceae family, such as rice yellow dwarf (RYD), sugarcane grassy shoot (SCGS) and sorghum grassy shoot (SGS) phytoplasmas as well as the strain BVK obtained from the leafhopper *Psammotettix cephalotes* in Germany (Namba *et al.*, 1993; Lee *et al.*, 1998, 2000; Seemüller *et al.*, 1998; Marcone *et al.*, 1997, 2001; Wongkaew *et al.*, 1997; Schneider *et al.*, 1999). The SCWL agent shows a 16S rDNA sequence similarity of 97.7% and 97.3% to RYD and BVK phytoplasmas, respectively. It is lacking the TaqI site following position 228 of the 16S rRNA gene, which is present in BVK and RYD phytoplasmas. Sequence analysis of a less conserved sequence, the region between the 16S and 23S rRNA genes (16S/23S rDNA spacer) of SCWL, RYD and BVK, resulted in a classification scheme similar to that based on full-length or nearly full-length 16S rDNA sequences. The SCWL phytoplasma was assigned to the same subclade of BVK and RYD agents (Kirkpatrick *et al.*, 1994; Schneider *et al.*, 1995). SCWL agent can be differentiated from BVK, RYD, SCGS and SGS phytoplasmas using RFLP analysis of PCR-amplified rDNA with AluI, Sau3AI, HaeIII, MseI, TaqI and HinfI restriction endonucleases (Nakashima *et al.*, 1996; Marcone *et al.*, 1997, 2001, Lee *et al.*, 1998; Tran-Nguyen *et al.*, 2000).

Sugarcane grassy shoot

Sugarcane grassy shoot (SCGS) is one of the most important disease of sugarcane in India. It was first observed near Belapur in the Ahmadnagar district of Bombay (India) in 1949 (Chona, 1958). Similar diseases were also reported from other parts of the country and described under different names such as "new chlorotic disease", "yellowing disease", "albino disease", "bunchy disease" or "leafy tuft" (for review see Marcone *et al.*, 2001). Studies by Rane and Dakshindas (1962) showed that grassy shoot, yellowing and albino symptoms are associated with the same disease and subsequently the term "grassy shoot" was accepted as common name. SCGS has been recorded in most sugarcane-growing areas of India and is known to occur also in Thailand (Wongkaew *et al.*, 1997; Sdoodee *et al.*, 1999; Sdoodee, 2001). Symptoms similar to those of SCGS have been observed in Bangladesh, Malaysia, Nepal, Pakistan, Sri Lanka and Sudan (Rishi and Chen, 1989; Viswanathan, 1997, 2001).

SCGS disease is characterized by the production of a large number of thin, slender, adventitious tillers from the base of the affected stools. This profuse growth give rise to a dense or crowded bunch of tillers bearing pale yellow or chlorotic leaves which remain thin, narrow, reduced in size and have a soft texture. Each stalk that is produced from the affected stool shows shortened internodes and the development of side shoots

from the bottom to the top. Affected plants do not produce millable canes. The disease is particularly pronounced in the ratoon crop where the clusters of slender tillers with reduced leaves usually growing erect give the appearance of a field full of perennial grass, and from which it has derived its popular name "grassy-shoots".

The vector(s) responsible for the natural spread of SCGS have not been identified. There are reports on transmission of SCGS by three different species of aphids as well as by the fulgorid *Proutista moesta* Westwood (Chona *et al.*, 1960; Edison *et al.*, 1976). However, these reports have not been confirmed (Rishi and Chen, 1989). Also, attempts by Chen (cited in Rishi and Chen, 1989) to transmit the SCGS agent from diseased sugarcane plants belonging to varieties Co 419 and Co 740 to healthy sugarcane of the variety F 160 by means of the leafhopper *M. hiroglyphicus* were not successful.

Sequence analysis of 16S/23S rDNA spacer region, RFLP analysis of PCR-amplified 16S rDNA and 16S/23S rDNA spacer sequences and serological tests revealed that SCGS disease is associated with an organism that is closely related to the SCWL phytoplasma (Wongkaew *et al.*, 1997; Sdoodee *et al.*, 1999; Sdoodee, 2001). The SCGS phytoplasma can be distinguished from the SCWL agent using RFLP analysis of rDNA with MseI and HpaII restriction endonucleases (Wongkaew *et al.*, 1997; Sdoodee *et al.*, 1999; Sdoodee, 2001). However, serological comparisons did not allow discrimination between SCGS and SCWL phytoplasmas (Sarindu and Clark, 1993; Viswanathan, 1997, 2001). It is not known whether the SCGS phytoplasma could be distinguished from the SGS agent by RFLP analysis. The SGS agent is known to occur in Australia where it is associated with a yellows disease of *Sorghum stipoideum* (Schneider *et al.*, 1999; Tran-Nguyen *et al.*, 2000). In PCR assays using the primers fSCWL/rSGS, designed for specific detection of the SGS agent, phytoplasmal DNA was amplified from both SGS and SCWL agents. However, only weak PCR signals were sometimes obtained from SCGS phytoplasma (Tran-Nguyen *et al.*, 2000). On the other hand, phylogenetic studies based on sequence analysis of 16S/23S rDNA spacer region, showed that SCGS phytoplasma is more closely related to the SGS agent that to other phytoplasmas (Tran-Nguyen *et al.*, 2000).

Sugarcane yellow leaf syndrome

Sugarcane yellow leaf syndrome (SCYLS) is a disorder of sugarcane characterized by similar symptoms but differing in etiology. It was first reported from Hawaii in 1989. Later, the disease has been reported to occur in Louisiana, Florida, Texas, Australia, Brazil, Cuba and several African countries including South Africa and Mauritius (for reviews see Tran-Nguyen *et al.*, 2000; Schenck, 2001).

The symptoms described from various geographic areas are similar. They include a yellow discoloration of the leaf midrib which is particularly evident on the lower leaf surface. This discoloration gradually extends to the leaf blade, and sometimes is accompained by shortening of the upper internodes, producing a fan-leaf appearance. Necrosis starts from the leaf tips and then spreads down the blade until the whole leaf is affected. Sometimes red discoloration is also present. Usually the youngest leaves are symptomless, but yellowing appears on the fourth or fifth and older leaves. Young plants are not usually affected in the field.

Numerous abiotic and biotic factors are reported to be associated with SCYLS disease. In Hawaii, SCYLS most often appears in the summer months along with water stress. In Florida, symptoms were associated with drought, waterlogging and cool winters while in Australia and Brazil the disease was most evident during cooler months (for review see Schenck, 2001). A virus, member of the luteovirus group, has been fount to be associated with the disease in many sugarcane-growing areas worldwide (Schenck, 2001). However, during the last few years, phytoplasmas were detected in SCYLS-affected sugarcane plants in several countries using electron microscope observations and PCR-based methods. In some cases, the affected plants were doubly infected with both viruses and phytoplasmas and latent infections have also been observed. Stress conditions seem to exacerbate the symptom expression incited by viral and phytoplasmal infections (Cronjé *et al.*, 1998).

Recent studies employing sequence analysis of 16S/23S rDNA spacer region and RFLP analysis of PCR-amplified 16S rDNA sequences revealed that two different phytoplasmas are associated with SCYLS disease in nine African countries, although the plants were symptomatically similar (Cronjé *et al.*, 1998; Cronjé and Bailey, 1999; Aljanabi *et al.*, 2001). The prevalent agent is a member of the X-disease group which showed a sequence similarity of 98,8% with the western X-disease phytoplasma. This prokaryote was consistently detected in leaf samples of more than 50 sugarcane varieties and a significant correlation between its presence and SCYLS symptoms was observed. The less frequently detected phytoplasma proved to be a member of the SCWL group.

Detection and molecular characterization of phytoplasmas from SCYLS-diseased sugarcane plants have also been reported from Cuba (Arocha *et al.*, 1999). In this case, an organism of the AY group, subgroup 16SrI-A, was identified on the basis sequence analysis of 16S/23S rDNA spacer region and RFLP analysis of PCR-amplified 16S rDNA sequences using AluI, RsaI and HaeIII.

In Australia, a great genetic diversity among phytoplasmas associated with SCYLS disease has been found (Tran-Nguyen *et al.*, 2000). A total of twenty-five phytoplasma isolates was evidenced by RFLP and

sequence analyses of PCR-amplified rDNA. Most of the sugarcane plants examined proved to be infected by the tomato big bud (TBB) phytoplasma. Less frequently detected were phytoplasmas which proved to be most closely related on the basis of 16S rDNA sequence homology to the previously characterized phytoplasma reference strains TBB, FBP, sunnhemp witches'-broom (SUNHP) of the FBP group, stylosanthes little leaf (StLL) of the loofah witches'-broom (LfWB) group and Maryland aster yellows (AY1) of the AY group, respectively. Phytoplasmas which slightly differed in RFLP patterns from either the mentioned phytoplasma reference strains or the galactia little leaf (GaLL) agent of the cirsium phyllody (CirP) group and waltheria little leaf (WaLL) phytoplasma of the FBP group as well as the pigeon pea little leaf (PPLL) agent of the FBP group were also infrequently detected. Multiple infections with two or more distinctly different phytoplasma isolates in a single sugarcane plant were also recorded. There was a weak relationship between phytoplasma infections and symptom expression. The less frequently detected phytoplasma isolates occurred mainly in asymptomatic plants while the TBB phytoplasma which is particularly widespread in Australia (Schneider *et al.*, 1999) was detected with approximately equal frequency in both symptomatic (42%) and asymptomatic (37%) sugarcanes.

Ramu stunt disease of sugarcane

Ramu stunt disease of sugarcane (SCRS) was first observed in the late 1980s in the Ramu valley of Papua New Guinea where resulted a devastating disease causing severe crop losses in commercial sugarcane varieties (Eastwood, 1990). The cultivar Ragnar proved to be highly susceptible. Moderately susceptible were the cvs. Q90 and Yasawa while cvs. Cadmus and Q107 were resistant. Since that time, the replacement of susceptible cultivars with resistant ones has kept the disease under control. At moment, SCRS disease seems to be restricted to Papua New Guinea (Braithwaite, 2001).

The most common symptom of the disease is a pronounced stunting. Leaves show a yellow mottled striping. They are short, erect and have a stiff texture. In some cultivars excessive tillering and grassy shoot appearance are also present. Affected plants die within one year after appearance of the first symptoms.

The rapid spread of the disease during the late 1980s in the Ramu valley suggested that an insect vector is involved. Kuniata *et al.* (1994) reported on the experimental transmission of SCRS agent by the leafhopper *Eumetopina flavipes* Muir. Such leafhopper is particularly abundant on commercial sugarcanes as well as on wild cane in Papua New Guinea. The SCRS causal agent was originally thought to be a virus. However, attempts by Jones *et al.* (1989) have not conclusively proved the viral etiology. Recently, Cronjé *et al.* (1999) reported on detection of phytoplasmal infection in SCRS-affected sugarcane plants as well as in samples of the putative vector *E. flavipes* collected in the Ramu valley. A SCWL-related organism was identified on the basis of sequence analysis of 16S/23S rDNA spacer region and RFLP analysis of PCR-amplified 16S rDNA sequences using RsaI and HaeIII restriction enzymes. The sugarcane phytoplasma showed a sequence homology of 95,98% with the SCWL agent.

Sugarcane green grassy shoot

Sugarcane green grassy shoot (SCGGS) is a newly discovered phytoplasmal disease of sugarcane. It has been observed in Thailand (Pliansinchai and Prammanee, 2000; Rao and Ford, 2001). The symptoms are very similar to those of SCGS disease. However, in SCGGS-affected sugarcane plants the leaves do not become chlorotic. The result from PCR detection showed that sugarcane green grassy shoot disease has their genetic partly related to phytoplasma infected periwinkle and white leaf disease (Prammanee *et al.*, 2000).

DETECTION OF SUGARCANE PHYTOPLASMA INFECTIONS

Sugarcane phytoplasma infections can be detected by microscopic examination of phloem tissue sections stained with the DNA fluorochrome 4'-6-diamidino-2-phenylindole (DAPI) (Seemüller, 1976; Sarindu and Clark, 1993). This procedure is simple, rapid and not much expensive. However, it is limited when the phytoplasma population is very low and unevenly distributed among the plant host organs, as is often true for sugarcane. Moreover, microscopic methods are not appropriate in epidemiological studies to identify plant reservoirs or insect vectors for a given phytoplasma because they do not attain pathogen identification.

Polyclonal antisera have been produced against partially purified antigen preparations from SCWL- and SCGS-affected sugarcane plants (Sarindu and Clark, 1993; Viswanathan, 1997, 2001). Both SCWL and SCGS antisera were successfully used in ELISA tests for detecting their respective homologous phytoplasma antigens in crude tissue extracts of diseased sugarcane. However, cross-reactions were observed in reciprocal tests between these two sugarcane phytoplasma sources and their antisera. Due to lack of the necessary specificity and sensitivity, serology-based techniques are still not widely employed in phytoplasmology for diagnostic purposes (Seemüller *et al.*, 1998; Adams *et al.*, 2001).

Randomly cloned fragments of chromosomal and extrachromosomal DNA of the SCWL phytoplasma have been used in dot and Southern blot hybridization assays as probes to detect phytoplasmal infections (Klinkong and Seemüller, 1993; Nakashima *et al.*, 1994, 2001; Nakashima and Hayashi, 1995). However, these probes showed a considerable broad detection range. They hybridized with total DNA from samples of

SCWL-infected sugarcane plants and the insect vector *M. hiroglyphicus* as well as with DNA from plants infected by phytoplasmas associated with other different plant diseases such as RYD, Bermuda grass white leaf, Brachiaria white leaf and Dactyloctenium white leaf (Nakashima and Hayashi, 1995; Nakashima *et al.*, 2001).

The major problem that detection of phytoplasma infections by DNA hybridization assays may encounter is the insufficient sensitivity of the probes. Usually, no hybridization signals are obtained when phytoplasmas cannot be detected by DAPI fluorescence tests (Seemüller and Kirkpatrick, 1996). Both dot and Southern blot hybridization assays which were used in phytoplasma diagnosis for several years, are now nearly completed replaced by PCR-based assays.

For detection and identification of sugarcane phytoplasmas, the powerful PCR technology has widely been employed in several laboratories (Nakashima *et al.*, 1996, 2001; Wongkaew *et al.*, 1997; Cronjé *et al.*, 1998, 1999; Arocha *et al.*, 1999; Cronjé and Bailey, 1999; Sdoodee *et al.*, 1999; Sdoodee, 2001; Tran-Nguyen *et al.*, 2000; Aljanabi *et al.*, 2001). It offers several advantages over other methods including versatility, relative semplicity, specificity and high sensitivity. Universal phytoplasma primers as well as group-specific primers have been designed, directed to ribosomal and/or non-ribosomal DNA sequences. Primers amplifying rDNA sequences proved most suitable for PCR. It may be performed as one-round PCR using universal or group-specific primer pairs, or by reamplifying the DNA fragments obtained in the first amplification using internal primers (nested PCR). Very often in affected sugarcanes the phytoplasma numbers are so low that infections could be identified only through the highly sensitive nested PCR assay (Tran-Nguyen *et al.*, 2000; Aljanabi *et al.*, 2001).

CONCLUSION

The phytoplasmal diseases of sugarcane are more widespread than previously known and are of considerable economic importance. SCWL and SCGS diseases seem to occur only in the south-east Asian region and not in other sugarcane growing areas of the world. Both are caused by a single phytoplasma type. The SCWL and SCGS agents have never been identified in plants other than sugarcane and seem to have a strict insect vector specificity. In contrast, SCYLS disease occurs at least in three continents and is associated with distinctly different phytoplasmas which are not specific pathogens. They include mostly members of the FBP, X-disease and AY groups which are known to infect a wide range of wild and cultivated plants and have a low insect vector specificity. There are many reports in which different phytoplasmas may induce similar symptoms in a given host plant. Examples are the grapevine yellows phytoplasmas, the spartium witches'-broom agents and phytoplasmas associated with stolbur/big bud disease of tomato (Seemüller, 1998; Lee *et al.*, 2000).

The current information about phytoplasma types associated with sugarcane diseases worldwide is likely to change with future research. For instance, there is still very little known about the occurrence of phytoplasmas in SCYLS-diseased sugarcane plants from the American continent.

REFERENCES

Adams, A.N., Davies, D.L. and Kirby, M.J. (2001). Virus and phytoplasma detection in fruit trees. Outlook Agric., **30** : 45-54.

Ahrens, U. and Seemüller, E. (1992). Detection of DNA of plant pathogenic mycoplasmalike organisms by a polymerase chain reaction that amplifies a sequence of the 16S rRNA gene. Phytopathology, **82** : 828-832.

Aljanabi, S.M., Parmessur, Y., Moutia, Y., Saumtally, S. and Dookun, A. (2001). Further evidence of the association of a phytoplasma and a virus with yellow leaf syndrome in sugarcane. Plant Pathol., **50** : 628-636.

Arocha, Y., Gonzalez, L., Peralta, E.L. and Jones, P. (1999). First report of virus and phytoplasma pathogens associated with yellow leaf syndrome of sugarcane in Cuba. Plant Dis., **83** : 1177.

Braithwaite, K.S. (2001). New virus and virus-like diseases of sugarcane – an overview. p. 3-24. In: G.P. Rao, R.E. Ford, M. Tosic, D.S. Teakle (eds). Sugarcane Pathology. Volume II: Virus and Phytoplasma Diseases. Enfield (NH), USA: Science Publishers, Inc.

Chen, C.T. (1978). Vector-pathogen relationships of sugarcane white leaf disease. Taiwan Sugar, **25** : 50-54.

Chona, B.L. (1958). Some diseases of sugarcane reported from India in recent years. Indian Phytopathol., **11** : 1-9.

Chona, B.L., Capoor, S.P., Varma, P.M. and Seth, M.L. (1960). "Grassy-shoot" disease of sugarcane. Indian Phytopathol., **13** : 37-47.

Cronjé, C.P.R., Tymon, A.M., Jones, P. and Bailey, R.A. (1998). Association of a phytoplasma with a yellow leaf syndrome of sugarcane in Africa. Ann. appl. Biol., **133** : 177-186.

Cronjé, C.P.R. and Bailey, R.A. (1999). Association of phytoplasmas with yellow leaf syndrome of sugarcane. Proc. Int. Soc. Sugarcane Technol., **23** : 373-381.

Cronjé, C.P.R., Bailey, R.A., Jones, P. and Suma, S. (1999). The phytoplasma associated with Ramu stunt disease of sugarcane is closely related to the white leaf phytoplasma group. Plant Dis., **83** : 588.

Eastwood, D. (1990). Ramu stunt disease. Development and consequences at Ramu Sugar Ltd. Sugar Cane, **2** : 15-19.

Edison, S., Ramakrishnan, K. and Narayanasamy, P. (1976). Comparison of grassy shoot disease (India) with the white leaf disease (Taiwan) of sugarcane. Sugarcane Pathol. Newsl., **17** : 30-35.

Jones, P., Antoniw, J.F. and Eastwood, D. (1989). Investigations into the aetiology of Ramu stunt, a new sugarcane disease in Papua New Guinea. L'Agronomie Tropicale, **44** : 179-184.

Kirkpatrick, B., Smart, C., Gardner, S., Gao, J.-L., Ahrens, U., Mäurer, R., Schneider, B., Lorenz, K.-H., Seemüller, E., Harrison, N., Namba, S. and Daire, X. (1994). Phylogenetic relationship of plant pathogenic MLOs established by 16/23S rDNA spacer sequences. IOM Letters, **3** : 228-229.

Klinkong, S. and Seemüller, E. (1993). Detection and differentiation of the mycoplasmalike organism associated with sugarcane white leaf disease using cloned extrachromosomal DNA probe. Kasetsart J., 27: 98-103.

Kuniata, L.S., Young, G.R., Pais, E., Jones, P. and Nagaraja, H. (1994). Preliminary observations on Eumetopina sp. (Hemiptera: Delphacidae) as a vector of Ramu stunt, a new sugarcane disease in Papua New Guinea. J. Austr. Entomol. Soc., **33** : 185-186.

Lee, C.S. (1970). Studies on the sugarcane white leaf disease. Annu. Rep. Taiwan Sugar Exp. Stn., p. 100-101.

Lee, C.S. and Chen, C.T. (1972). Preliminary studies on transmission characteristics of sugarcane white leaf disease by *M. hiroglyphicus* Mats. Rept. Taiwan Sug. Exp. Stn., **56** : 57-62.

Lee, I.-M., Hammond, R.W., Davis, R.E. and Gundersen, D.E. (1993). Universal amplification and analysis of pathogen 16S rDNA for classification and identification of mycoplasmalike organisms. Phytopathology, **83** : 834-842.

Lee, I.-M., Gundersen-Rindal, D.E., Davis, R.E. and Bartoszyk, M. (1998). Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences. Int. J. Syst. Bacteriol., **48** : 1153-1169.

Lee, I.-M., Davis, R.E. and Gundersen-Rindal, D.E. (2000). Phytoplasma: phytopathogenic mollicutes. Ann. Rev. Microbiol., **54** : 221-255.

Ling, K.C. (1962). White leaf disease of sugarcane. Taiwan Sugar, **9** : 1-5.

Mangelsdorf, A.J. (1962). A research program for the Thailand sugar industry. Bangkok: Department of Agriculture.

Marcone, C., Ragozzino, A. and Seemüller, E. (1997). Detection of Bermuda grass white leaf diseases in Italy and characterization of the associated phytoplasma by RFLP analysis. Plant Dis., **81** : 862-866.

Marcone, C., Neimark, H., Ragozzino, A., Lauer, U. and Seemüller, E. (1999). Chromosome sizes of phytoplasmas composing major phylogenetic groups and subgroups. Phytopathology, **89** : 805-810.

Marcone, C., Ragozzino, A. and Seemüller, E. (2001). Sugarcane white leaf and sugarcane grassy shoot diseases: Biology and taxonomic characterization of the associated phytoplasmas. p. 83-97. In: G.P. Rao, R.E. Ford, M. Tosić, D.S. Teakle (eds). Sugarcane Pathology. Volume II: Virus and Phytoplasma Diseases. Enfield (NH), USA: Science Publishers, Inc.

Matsumoto, T., Lee, C.S. and Teng, W.S. (1968). Studies on sugarcane white leaf disease of Taiwan, with special reference to transmission by a leafhopper, *Epitettix hiroglyphicus* Mats. Proc. Int. Soc. Sugar Cane Technol., **13** : 1090-1098.

McCoy, R.E., Caudwell, A., Chang, C.J., Chen, T.A., Chiykowski, L.N., Cousin, M.T., Dale, J.L., de Leeuw, G.T.N., Golino, D.A., Hackett, K.J., Kirkpatrick, B.C., Marwitz, R., Petzold, H., Sinha, R.C., Sugiura, M., Whitcomb, R.F., Yang, I.L., Zhu, B.M. and Seemüller, E. (1989). Plant diseases associated with mycoplasma-like organisms. p. 545-640. In: R.F. Whitcomb, J.G. Tully (eds). The Mycoplasmas. Vol. V. San Diego, USA: Academic Press.

Nakashima, K. and Murata, N. (1993). Destructive plant diseases caused by mycoplasma-like organisms in Asia. Outlook Agric., **22** : 53-58.

Nakashima, K. and Hayashi, T. (1995). Extrachromosomal DNAs of rice yellow dwarf and sugarcane white leaf phytoplasmas. Ann. Phytopathol. Soc. Jpn., **61** : 456-462.

Nakashima, K., Chaleeprom, K., Wongkaew, P. and Sirithorn, P. (1994). Detection of mycoplasma-like organism associated with white leaf disease of sugarcane in Thailand using DNA probes. JIRCAS J., **1** : 57-67.

Nakashima, K., Hayashi, T., Chaleeprom, W., Wongkaew, P. and Sirithorn, P. (1996). Complex phytoplasma flora in northeast Thailand as revealed by 16S rDNA analysis. Ann. Phytopathol. Soc. Jpn., **62** : 57-60.

Nakashima, K., Wongkaew, P. and Sirithorn, P. (2001). Molecular detection and characterization of sugarcane white leaf phytoplasmas. p. 157-175. In: G.P. Rao, R.E. Ford, M. Tosić, D.S. Teakle (eds). Sugarcane Pathology. Volume II: Virus and Phytoplasma Diseases. Enfield (NH), USA: Science Publishers, Inc.

Namba, S., Oyaizu, S., Kato, S., Iwanami and S., Tsuchizaki, T. (1993). Phylogenetic diversity of phytopathogenic mycoplasmalike organisms. Int. J. Syst. Bacteriol., **43** : 461-467.

Pliansinchai, U. and Prammanee, S. (2000). Green grassy shoot. p. 221-225. In: P. Rott, R.A. Bailey, J.C. Comstock, B.J. Croft, S.A. Saumtally (eds). A Guide to Sugarcane Diseases. CIRAD-ISSCT.

Prammanee, S., Pliansinchai, U., Sarindhu, N. and Lairungreang, C. (2000). Molecular identification of sugarcane green grassy shoot disease in Thailand. Ph. 11. The 6th ISSCT Sugarcane Pathology Workship. Cha-am Islands, Thailand.

Rane, M.S. and Dakshindas, D.G. (1962). The sugarcane disease "albino" or "grassy shoot"? Indian Sugar, **12** : 179-180.

Rao, G.P., and Ford E. (2001). Vectors and phytoplasma diseases of sugarcane – an overview. p. 267-318. In: G.P. Rao, R.E. Ford, M. Tosić, D.S. Teakle (eds). Sugarcane Pathology. Volume II: Virus and Phytoplasma Diseases. Enfield (NH), USA: Science Publishers, Inc.

Rishi, N. and Chen, C.T. (1989). Grassy shoot and white leaf diseases. p. 289-300. In: B.C. Ricaud, B.T. Egan (eds.). Diseases of sugarcane. Amsterdam: Elsevier.

Sarindu, N. and Clark, M.F. (1993). Antibody production and identity of MLOs associated with sugar-cane whiteleaf disease and bermuda-grass whiteleaf disease from Thailand. Plant Pathol., **42** : 396-402.

Schenck, S. (2001). Sugarcane yellow leaf syndrome: history and current concepts. p. 25-35. In: G.P. Rao, R.E. Ford, M. Tosić, D.S. Teakle (eds). Sugarcane Pathology. Volume II: Virus and Phytoplasma Diseases. Enfield (NH), USA: Science Publishers, Inc.

Schneider, B., Ahrens, U., Kirkpatrick, B.C. and Seemüller, E. (1993). Classification of plant-pathogenic mycoplasma-like organisms using restriction-site analysis of PCR-amplified 16S rDNA. J. Gen. Microbiol., **139** : 519-527.

Schneider, B., Seemüller, E., Smart, C.D. and Kirkpatrick, B.C. (1995). Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas. p. 369-380. In: S. Razin, J.G. Tully (eds). Molecular and Diagnostic Procedures in Mycoplasmology. Vol. I. San Diego, USA: Academic Press.

Schneider, B., Padovan, A., De La Rue, S., Eichner, R., Davis, R., Bernuetz, A. and Gibb, K.S. (1999). Detection and differentiation of phytoplasmas in Australia: an update. Aust. J. Agric. Res., **50** : 333-342.

Sdoodee, R. (2001). Recent studies on white leaf and grassy shoot phytoplasmas of sugarcane. p. 235-244. In: G.P. Rao, R.E. Ford, M. Tosić, D.S. Teakle (eds). Sugarcane Pathology. Volume II: Virus and Phytoplasma Diseases. Enfield (NH), USA: Science Publishers, Inc.

Sdoodee, R., Schneider, B., Padovan, A.C. and Gibb, K.S. (1999). Detection and genetic relatedness of phytoplasmas associated with plant diseases in Thailand. J. Biochem. Mol. Biol. and Biophys, **3** : 133-140.

Seemüller, E. (1976). Investigations to demonstrate mycoplasma-like organisms in diseased plants by fluorescence microscopy. Acta Hortic. **67** : 109-112.

Seemüller, E. and Kirkpatrick, B.C. (1996). Detection of phytoplasma infections in plants. p. 299-311. In: J.G. Tully, S. Razin (eds). Molecular and Diagnostic Procedures in Mycoplasmology. Vol. II. San Diego, USA: Academic Press.

Seemüller, E., Marcone, C., Lauer, U., Ragozzino, A. and Göschl, M. (1998). Current status of molecular classification of the phytoplasmas. J. Plant Pathol., **80** : 3-26.

Tran-Nguyen, L., Blanche, K.R., Egan, B. and Gibb, K.S. (2000).

Diversity of phytoplasmas in northern Australian sugarcane and other grasses. Plant Pathol., **49** : 666-679.

Viswanathan, R. (1997). Detection of phytoplasmas associated with grassy shoot disease of sugarcane by ELISA techniques. Z. PflKrankh. PflSchutz, **104** : 9-16.

Viswanathan, R. (2001). Serodiagnosis of phytoplasmas causing grassy shoot disease in sugarcane. p. 209-220. In: G.P. Rao, R.E. Ford, M. Tosić, D.S. Teakle (eds). Sugarcane Pathology. Volume II: Virus and Phytoplasma Diseases. Enfield (NH), USA: Science Publishers, Inc.

Wongkaew, P., Hanboonsong, Y., Sirithorn, P., Choosai, C., Boonkrong S., Tinnangwattana, T., Kitchareonpanya, R. and Damak, S. (1997). Differentiation of phytoplasmas associated with sugarcane and gramineous weed white leaf disease and sugarcane grassy shoot by RFLP analysis and sequencing. Theor. Appl. Genet., **95** : 660-663.

Yang, S.L. and Pan, Y.S. (1979). Ecology of Matsumuratettix hiroglyphicus (Matsumura), an insect vector of sugarcane white leaf disease. Pro. ROC-US Coop. Sci. Sem. on Mycoplasma Dis. of Plants. NSC, **1** : 11-119.

Dr. C. Marcone is a research phytoplasmologist at the University of Basilicata, Potenza (southern Italy). After obtaining his Ph.D. degree in plant pathology in 1994 from the University of Naples "Federico II", he worked as post-doctoral scientist for more than five years (September 1994-March 2000) at Federal Biological Research Centre for Agriculture and Forestry, Dossenheim Institute, Germany, under the guidance of Dr. E. Seemüller. His research interests cover several fields of phytoplasmology including pathology, diagnosis, taxonomy, phylogeny and genomics. Major objectives are phytoplasma diseases and their causal agents of fruit trees, forest trees, vegetable, ornamental and forage crops including weeds in southern Italy. Most of the phytoplasmal diseases discovered in southern Italy by Dr. Marcone were the first records for the world or Europe. He also determined the genome size of phytoplasmas representing nearly all phylogenetic groups and was among the firsts to construct a physical map of a phytoplasma chromosome. Currently, he has also responsibility for teaching a plant pathology course at the University of Salemo. Dr. Marcone is author of more than 70 publications most of them in leading international phytopathological and microbiological journals.