PRELIMINARY EVIDENCE FOR THE ASSOCIATION OF A PHYTOPLASMA WITH YELLOW LEAF SYNDROME OF SUGARCANE IN AFRICA

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Abstract

Evidence for the presence of a phytoplasma in sugarcane leaves with symptoms of yellow leaf syndrome (YLS) has been obtained. The phytoplasma was demonstrated consistently in YLS leaves from a large number of varieties obtained from different countries in Africa. The phytoplasma was present in all symptomatic as well as some asymptomatic field grown samples. Plants grown from true seed were free of the phytoplasma. The phytoplasma was observed using transmission electron microscopy (TEM) and scanning electron microscopy (SEM). The identity of the phytoplasma was confirmed with the PCR technique using universal primers. Sequence analyses of products generated with PCR confirmed that the products were phytoplasma specific and not of plant or other pathogen origin.

Keywords: yellow leaf syndrome, YLS, phytoplasma

Introduction

Evidence obtained during 1995 and 1996 (Bailey *et al.*, 1996) indicated that yellow leaf syndrome (YLS) was common in most parts of the South African sugar industry. Evidence was also obtained that YLS may have had effects on cane production and quality ([1]personal communication). This has emphasised the importance of the current SASEX research programme on this new problem. As yet no published information is available on the cause, effects or control of YLS. Field observations and evidence that it can be transmitted through vegetative propagation (Schenck, 1990) indicate that YLS is caused by a pathogen. There is no published information on the effect of YLS on growth or yield of sugarcane. Variety SP71-6163 was severely affected in Brazil and suffered losses of 20-30% ([2]personal communication). In South Africa, some new genotypes undergoing selection develop conspicuous symptoms and are stunted, but most current commercial varieties appear to be relatively tolerant (Bailey *et al.*, 1996).

Research at SASEX has shown that a previously unreported phytoplasma is consistently associated with YLS. This organism has been demonstrated in a large number of varieties from a number of countries within Africa, and more recently in samples from other continents. The organism has been observed by electron microscopy and provisionally identified by PCR technology.

A luteovirus, provisionally called sugarcane yellow leaf virus (ScYLV), has been found associated with YLS symptoms by a research group in the USA (Lockhart *et al.*, 1996). This has not been found in southern Africa to date and the evidence for a viral cause appears weaker than for the phytoplasma. This report deals with the finding of a phytoplasma consistently associated with YLS symptoms in a wide range of sugarcane varieties from Malawi, South Africa, Swaziland and Zimbabwe.

As reported previously (Bailey *et al.*, 1996), the symptoms of YLS and the conditions under which they occur are closely similar to those reported for yellow wilt, a condition of sugarcane that was first recorded in Tanzania in 1968 and was subsequently widespread in east and central Africa into the early 1970s (Ricaud, 1968; Rogers, 1970). No cause of yellow wilt was identified. It seems highly probable that YLS and yellow wilt are the same condition, in which case this is not a new disease of sugarcane but has been present, at least in some African countries, for almost 30 years. It has been speculated that the apparent disappearance of yellow wilt was due to the increasing dominance in the region of variety NCo376, which was known to develop milder symptoms of yellow wilt compared with other important varieties being grown at that time, as is now known also applies to YLS (Bailey *et al.*, 1996).

Methods, Results and Discussion

Phytoplasmas have been found to be the cause of numerous yellows-type diseases previously thought to be caused by viruses (Nienhaus and Sikora, 1979). Extensive use was made of transmission electron microscopy (TEM) and scanning electron microscopy (SEM) during the initial phases of this study. Also tested were various DNA isolation techniques for application of the polymerase chain reaction (PCR) technique using universal primers amplifying sequences from the 16SrDNA region of the phytoplasmas.

Several attempts were made to isolate possible viral particles from YLS infected cane leaves using the method of Hammond *et al.* (1983). These did not result in the isolation of any clearly defined luteovirus particles. The majority of these particles did

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not appear virus-like and appeared to have a distinct membrane surrounding an electron-dense core (Figure 1). Some particles appeared to be clumped in groups and were surrounded by a further membrane. The sedimentation coefficient of the particles was higher than the 104-118S reported for the luteoviruses (Waterhouse *et al.*, 1988), since substantial sedimentation occurred even when centrifugation runs at half the recommended speed were used. High sedimentation rates were also noted by Black (1943), Steere (1967) and Lee and Chiyokowski (1963) when attempting to isolate viruses as possible agents of diseases in other crops, and in all these cases the pathogen was later confirmed to be a phytoplasma.

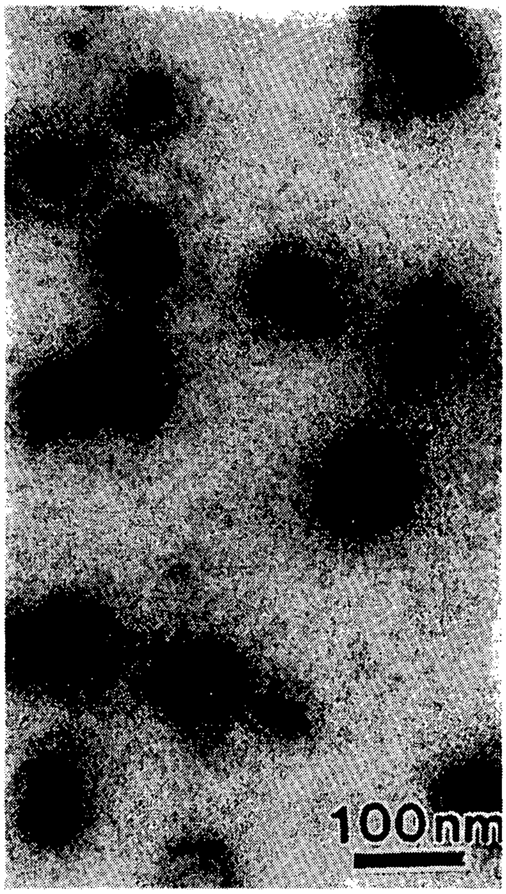

Figure 1. Transmission electronmicrograph of dense membrane-bound bodies isolated from YLS symptomatic material during attempts to isolate virus.

Electron microscopy

Transmission electron microscopy at low magnification (x 800-1 200) showed no obvious differences between YLS and symptomless leaves, except for the vascular bundles. The phloem and phloem companion cells were affected, but no differences were observed in the xylem. A series of changes were observed in the phloem tissue. In symptomless leaves, the phloem cells had a largely empty appearance and the cell walls were not thickened. In YLS leaves, the phloem cell walls were noticeably thickened and showed osmophilic patches after staining. In some of these cells membranous, pleiomorphic structures were seen (Figure 2). Usually between 30-50% of the phloem cells in any given vascular bundle from YLS leaves showed the thickened cell walls, and in about 5% of these the pleiomorphic structures were observed. These observations seemed to be dependent on the age of the material, the severity of symptoms and the sugarcane variety being examined. Generally, cane leaves from plants of six months of age or older showed more pronounced effects, and leaves with conspicuous symptoms showed general degradation of the phloem and only fixed poorly in preparation for microscopy. Varieties with conspicuous symptoms, such as SP71-6163 and H50-7209, showed numerous pleiomorphic bodies whereas varieties N19 and NCo376, which develop milder symptoms, at no stage contained similar numbers of bodies.

Figure 2. Electronmicrograph of an ultra-thin section through a phloem vessel of a YLS infected sugarcane leaf. Note the numerous membrane-bound pleiomorphic bodies and filaments present in the cell lamen.

While phloem cell wall alterations were always observed, the appearance of the pleiomorphic bodies was variable, depending on the section plane and also on plant age. Smaller bodies predominated in samples from young plants, while larger, filament-forming and budding forms were observed in symptomatic leaves from plants 7-12 months old. Generally in older cane, the pleiomorphic bodies seemed to degenerate, although this could have been due to fixation problems with such material.

The pleiomorphic bodies were clearly membrane-bound and contained a fibrous network of varying density. The bodies varied in size from c. 50 nm to more than 880 nm in diameter. Larger bodies sometimes showed evidence of budding or filamentous outgrowths.

While TEM confirmed the internal structure of the pleiomorphic bodies, and especially the nature of the membrane, further study by SEM was necessary to determine whether the observed structures resembled those reported for phytoplasmas (Waters and Hunt, 1980; Marcone *et al.,* 1996). The bodies observed by SEM were closely associated with the plasmalemma of infected cells. The bodies were interconnected with a fine fibrilar network and binary, fission-like budding and serial budding were seen. Numerous small buds were also observed on the surfaces of most of the bodies (Figure 3). The structures observed were broadly similar to the structures described by Waters and Hunt (1980), although they used models constructed from consecutive serial sections to elucidate the phytoplasmal morphology.

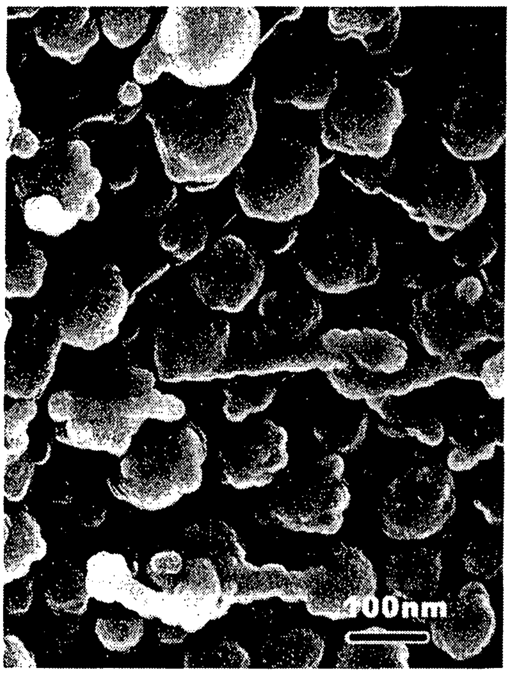

Figure 3. Scanning electronmicrograph of numerous pleiomorphic bodies showing evidence of budding and filament formation in the phloem of YLS infected sugarcane leaves.

PCR identification

Although electron microscopic examination of YLS samples showed ample evidence of phytoplasma-like organisms, confirmation of their phytoplasma identity was desirable. Since universal primers were available for the amplification of the 16SrDNA of the phytoplasmas (Lee *et al.,* 1993; Ahrens and Seemüller, 1992; Deng and Hiruki, 1991), the PCR approach was used for this confirmation. Of the initial primer combinations tested, the Deng and Hiruki primers gave the most consistent results.

Symptomless controls also occasionally yielded products with PCR. This is not surprising since the symptoms of YLS usually appear late in plant development and infection must therefore often be latent. Further evidence of latency is that symptoms can appear in apparently healthy cane within a few days after cold weather, insect damage or other stress conditions. The only material that consistently gave negative results for phytoplasma was obtained from seedlings grown under glasshouse conditions from true sugarcane seed.

The PCR products were sequenced directly and compared with the international database through access to the National Centre for Biological Information, using the BLASTN program, Version 1.4.9 MP (Altschul *et al.,* 1990). All queries submitted in this way confirmed that the products generated by PCR were indeed phytoplasma. Further, all the isolates from different countries appeared to be genetically identical.

Conclusions

Good evidence at both the morphological and genetic levels demonstrate the presence of an hitherto unreported phytoplasma in sugarcane plants with symptoms of YLS. The morphology of the organism is closely similar to the structures reported in numerous descriptions of the ultrastructure of the phytoplasmas (Sinha, 1978; Worley, 1970; Florance and Cameron, 1978; Waters and Hunt, 1980).

Although no claim to pathogenicity is made for the phytoplasma found in sugarcane with YLS symptoms, it is clear that the organism is closely associated with this disease. The phytoplasmas are known to be involved in numerous yellows-type diseases, and it is possible that the phytoplasma associated with YLS of sugarcane might be a member of this group. It has provisionally been named the sugarcane yellows (SCY) phytoplasma.

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