

INTRODUCTION

'Marchitez letal', caused by an unidentified microorganism, is a major disease affecting oil palm and has been observed with increasing frequency in Colombia. Incidences of up to 30% have been recorded in several commercial fields in production areas of Villanueva and Casanare. Its symptoms are similar to those caused by infection with phytoplasma and include leaf discoloration, lower leaves will turn brown and hang downwards like a collapsed umbrella (Figure 1).

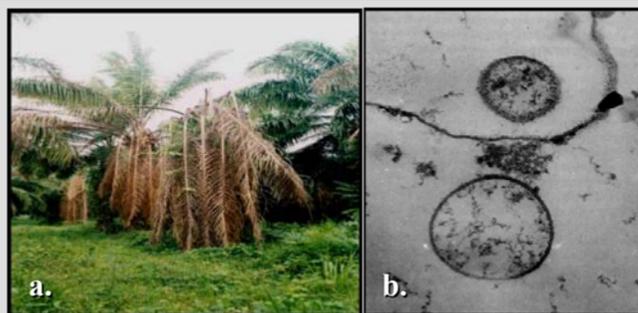


Figure 1a. 'Marchitez letal', a disease affecting oil palm crops, Eastern Plains, Colombia.

Figure 1b. MLO in a phloem cell (EM photo: P. Jones).

The occurrence of what seems to be a phytoplasma different from that occurring in coconut and other palm species has been recorded in an oil palm plantation in the West New Britain area of Papua New Guinea⁽¹⁾, where electron microscopy has played an important role in detecting phytoplasmas in infected tissue (Figure 1b), and in Kerala, India⁽²⁾.

MATERIALS AND METHODS

Plant tissue. Leaf bases, inflorescences, and meristem tissue from infected and healthy oil palm plants were used in this study.

Insect survey. Leafhoppers were collected from the leaves of palms affected by 'marchitez letal'. The presence of the phytoplasma was verified by nested PCR.

Staining methods. Two staining methods were used as follows: DAPI, which stains the phloem (Sinclair et al., 1989), and Dienes' stain, which metabolizes and produces a blue color (Deeley et al., 1979).

DNA extraction. Total DNA was extracted as described by Gilbertson and Dellaporta (1983).

Nested PCR analysis. The primer pairs P1/P7 or R16mF2/R16mR1 were used for the first amplification, with an annealing temperature of 55°C. For the nested PCR, diluted PCR products were used for amplification, with the primer pair R16F2n/R16R2, at an annealing temperature of 50°C. PCR products were analyzed by electrophoresis on 1.2% agarose gel.

RFLP analyses. The amplified PCR products were digested with the restriction endonucleases *MseI* and *AluI*. The restriction products were analysed by electrophoresis on 5% polyacrylamide gel.

Cloning and sequencing of 16S rRNA and tRNA genes. The amplified fragment was cloned and sequenced with primer T7 and Sp6.

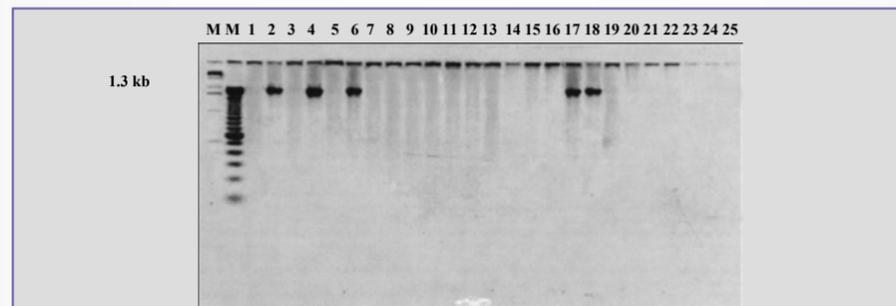
Transmission. Transmission was performed by grafting.

Control treatment. Oxytetracycline antibiotic was applied to oil palm by liquid injection into the trunk (5.0 g per palm) over a 4-month period.

RESULTS

Nested PCR analysis. Phytoplasmas were detected by nested PCR.

The specific primers R16mF2/R16mR1 and R16F2n/R16R2 were successfully used in a nested-PCR assay (Figure 2) to detect and confirm that phytoplasmas were associated with 'marchitez letal'.



R16MF2, R16MR1/ R16F2N, R16R2

Figure 2. A 1.3-kb fragment was amplified from diseased samples by nested PCR. Lane M = DNA marker.

RFLP analysis. Restriction digestion with *MseI* and *AluI* of the amplified product showed similar restriction patterns.

Sequence analysis of the 16S rRNA genes revealed that the oil palm phytoplasma was similar to the aster yellows group, with a sequence homology of 99% regarding phytoplasma from oil rape seed (GenBank accession no. U89378).

REFERENCES

1. Jones, P. and Turner, P.D. 1979. The occurrence of mycoplasma-like organisms in oil palm (*Elaeis guineensis*). West New Britain (Papua New Guinea).
2. Babu and Nair. 1993. Distribution of spear rot disease of oil palm and its possible association with MLO disease of palms in Kerala, India.

Staining methods. The presence of phytoplasmas in meristem, inflorescence, and leaf tissue was confirmed by DAPI and Dienes' stain (Figures 3 and 4).

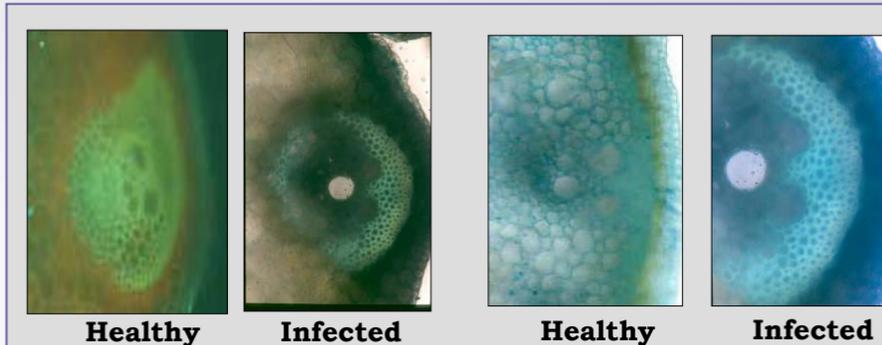
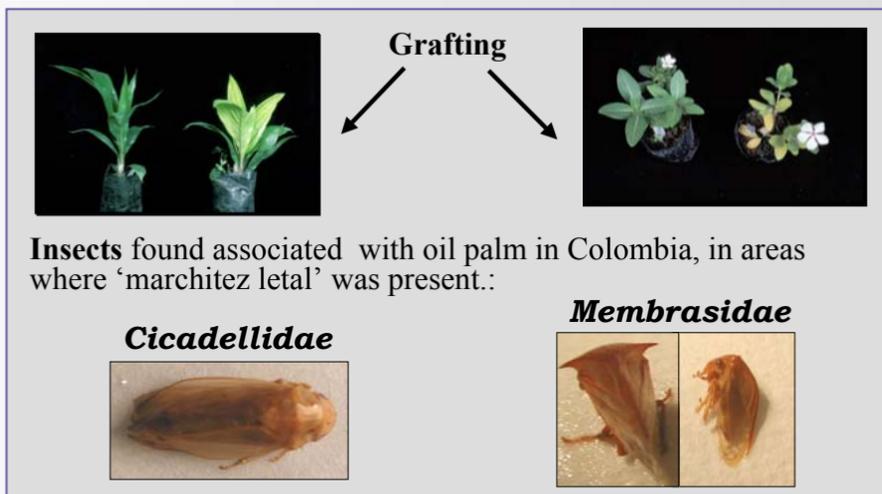


Figure 3 . DAPI staining.

Figure 4. Dienes' staining.



Insects found associated with oil palm in Colombia, in areas where 'marchitez letal' was present.:

Cicadellidae

Membrasidae

CONCLUSIONS

Phytoplasmas were detected in 'marchitez letal' infected oil palm meristems, inflorescences and leaf bases.

PCR is a very sensitive method for phytoplasma detection, identification and classification.

On the basis of DNA sequences, the oil palm phytoplasma was classified as a member of the 16 SrI in the aster yellows group. This is the first report of a phytoplasma in oil palm in Colombia.

Insects associated with 'marchitez letal' were identified as Cicadellidae and Membrasidae.

Transmission from diseased oil palm to either healthy oil palm or *Catharanthus roseus* was achieved by grafting.