

ULTRASTRUCTURAL MODIFICATIONS OF SUGARCANE LEAVES OF PLANTS SUFFERING THE YELLOW LEAF SYNDROME. Dolores Piñón¹,

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Yellow leaf syndrome (YLS) of sugarcane has been found in many different countries and in a wide range of cultivars. In the Mediterranean area, YLS of sugarcane has been detected in Morocco (Abdelmajid *et al.*, 1999) and neighbouring countries. Plants displayed characteristic symptoms of yellow and small leaves and a great impoverishment of the root system. Arocha *et al.* (1999) found that phytoplasma appears associated to viruses in diseased plants in Cuba. Ultrastructural changes associated to YLS have not been yet studied. Thus, we attempt in this paper to study ultrastructural alterations of leaves associated to YLS in order to obtain a more complete knowledge of this sugarcane disease. In this work *Saccharum officinarum* (L.), cv. Louissiana 55-5, field grown, was used. Plants were developed from agamic seeds, obtained from healthy or YLS diseased plants, and cultured for 8 months. The ultrastructure of sugar cane leaves obtained from healthy or diseased plants was examined by conventional SEM. Samples were fixed in 2% glutaraldehyde (v/v) in 0.1 M phosphate buffer, pH 7.2, post-fixed with osmium tetroxide, washed, dehydrated in acetone, critical-point dried, sputter-coated with gold/palladium and scanned at 20 kV by SEM using a Jeol JSM 6400 (Japan). Digital images were obtained by using an INCA (Oxford) program incorporated to the equipment. The upper and the lower epidermal layers of sugarcane leaves are made up of brick-shaped cells with their long axes parallel to the leaf. Abaxial epidermis of diseased leaves shows a large amount of adhered superficial bodies, mainly located between parallel thorny ribs. Some bacteria can be observed as well as fibrillar material, similar to fungal hyphae. Some stomata appear occluded by plugging material whereas the perimeter of the stomatal pore is more or less winding in other (Fig. 1A). In contrast, healthy leaves show well-defined stomata and many regular granules or ribbons (Fig. 1B) that characterized epicuticular waxes of n-aldehyde nature (Holloway and Challen, 1966). Mesophyll cells between veins do not suffer significant changes in diseased leaves, although a thick layer of amorphous material, similar to wax, displaces subepidermic tissue towards deeper zones (Figs. 1C and D). These thin-walled cells of this monocotyledon, the size of which is similar to that of brick-shaped epidermal cells, are confined to the adaxial epidermis (Fig. 1D). When they lose water, they contribute to the rolling of the leaf. The conducting tissues are found within the circular to oval-shaped groups of cells. The bundles are composed of three-sized classes of cells, large, medium and small, the first two being rhomboid to oval in shape, while as a rule, the small type is rather circular. A small, round bundle always lies next to a large vascular bundle which usually extends from the upper to the lower epidermis of the leaf (Fig. 2A and B). YLS-affected leaves show several large xylem vessels filled by amorphous material and debris of the end wall can be observed (Fig. 2C). Sometimes, spherical bodies similar to phytoplasma can be observed in the intercellular spaces of bundle sheath cells (Fig. 2D) whereas healthy leaves show primary xylem elements absolutely empty and none spherical adherence has been seen (Fig. 2E). It is surprising the localization of the phytoplasma-like particles observed here (Fig. 2D) different to that described by Cronje *et al.* (1998).

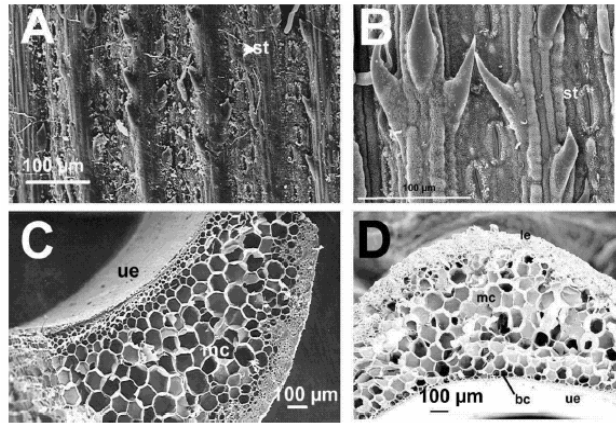


Fig. 1. General ultrastructure of sugarcane leaves from healthy and YLS-diseased plants. (A) View of the surface of the lower epidermis of a YLS-affected leaf of sugarcane Stomata (st). (B) View of the surface of the lower epidermis of a leaf of healthy sugarcane. Stomata (st). (C) Cross-section of a diseased leaf of sugarcane showing the upper epidermis (ue); mesophyll cells (mc). (D) Cross-section of a leaf of healthy sugarcane plants. Upper epidermis (ue); bulliform cells (bc); mesophyll cells (mc); lower epidermis (le).

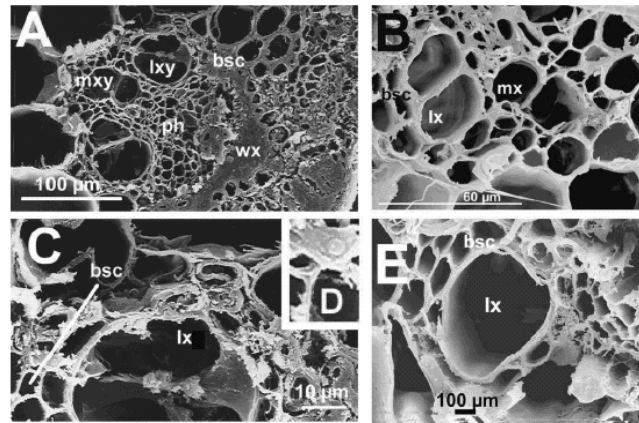


Fig. 2. Scanning electron microscopy of both phloem and xylem of leaves from healthy and YLS-diseased sugarcane plants. (A) Cross-section of an YLS-affected leaf of sugarcane. Large (lxy) and middle xylem elements (mxy); phloem elements (ph); amorphous substance (wx); bundle sheath cells (bsc). (B) Similar cross-section obtained from a healthy leaf. Bundle sheath cells (bsc); large (lx) and middle (mxy) xylem elements (lxy). (C) Cross-section of bundle vessels from a diseased leaf. Bundle sheath cells (bsc); large xylem elements (lxy). (D) Magnification of the intercellular space between two bundle sheath cells. (E) Large xylem elements (lxy) and bundle sheath cells (bsc) from a leaf of a healthy plant.

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