Association of Mycoplasmalike Organisms with Bermudagrass Yellow Leaf

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ABSTRACT

Round and elongated-curved mycoplasmalike organisms (MLO) were constantly found in Bermudagrass affected by yellow leaf disease. Temporary remission of symptoms associated with disappearance of MLO was obtained by tetracycline treatment. Chlorophyll measurements were used to evaluate this recovery quantitatively.

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A disease causing small leaves and short rhizomes on Bermudagrass (*Cynodon dactylon* L. Pers.) in Israel has recently been reported (6). Diseased plants, singly or in small patches, were subsequently found in most parts of the country.

Here we present evidence from electron microscopy of thin sections and chemotherapy for the association of mycoplasmalike organisms (MLO) with Bermudagrass yellow leaf. The content of the chlorophyll was also determined, to give a quantitative assay for symptom remission following chemotherapeutic treatments.

MATERIALS AND METHODS.—Naturally infected plants were collected near Bet Dagan; the rhizomes were washed after removing the leaves and cut into approximately 3-cm pieces, with a single nodal bud. For each chemotherapeutic treatment, 40 cuttings were soaked with stirring for 120 minutes in 200 ml of the respective tetracycline-HCl (TH) solution, at room temperature. The cuttings, as well as comparable healthy controls, were then planted in 10-cm diameter pots containing a mixture of sand, loam and peat (1:2:1, v/v). Total chlorophyll in the second apical leaf was determined 1, 2, and 3 months after treatment (5).

For electron microscopy, procedures previously described for detecting MLO in periwinkle leaves, were used (7). Thus, in brief, small leaf segments were fixed in

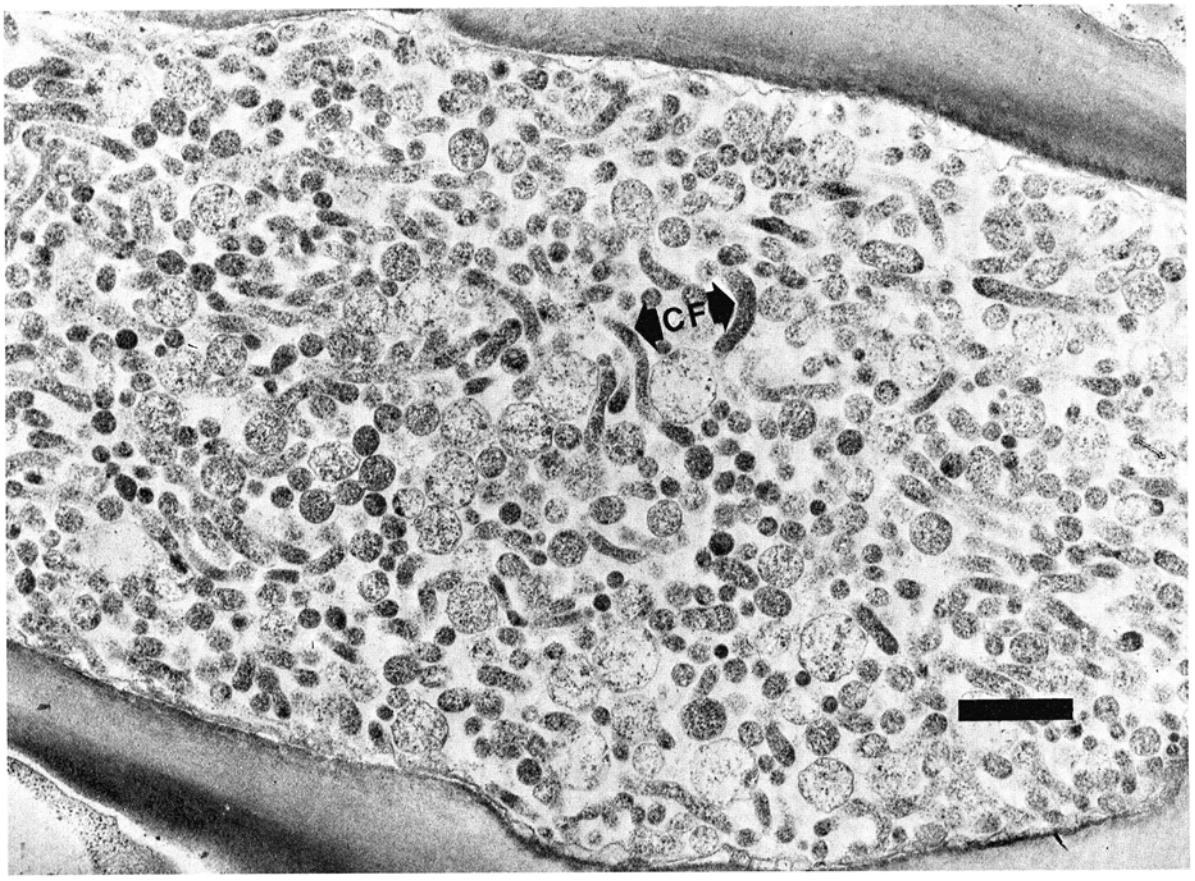

Fig. 1. Densely packed pleomorphic mycoplasmalike organisms in a sieve tube of Bermudagrass affected with yellow leaf. ×30,300. Note elongated curved forms (CF). Scale bar = 500 nm.

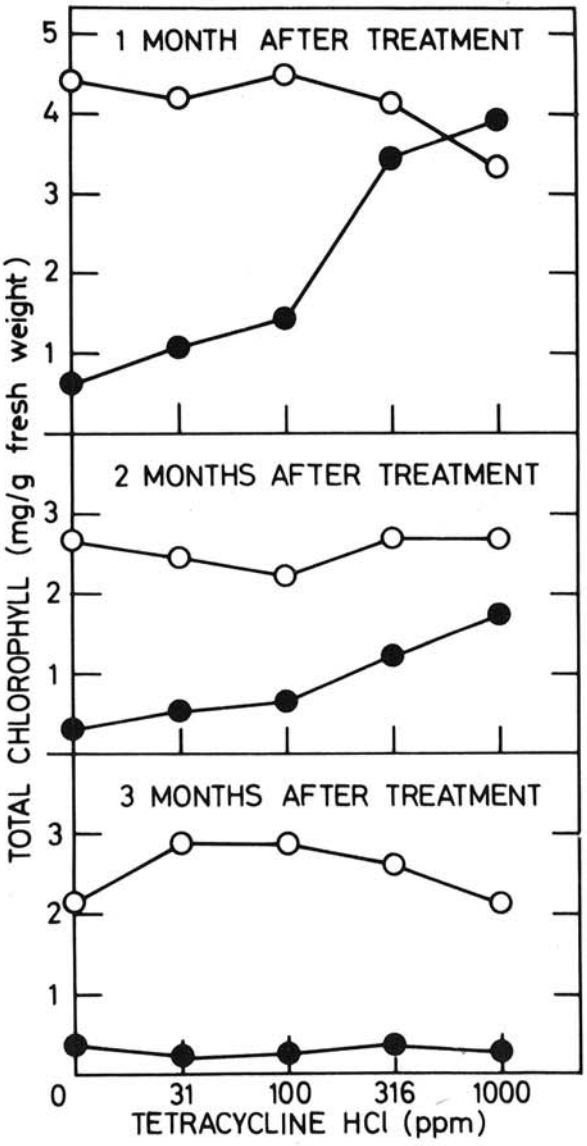

Fig. 2. Effect of different concentrations of tetracycline-HCl on chlorophyll concentration in Bermudagrass leaves; •——• infected, o——o healthy.

glutaraldehyde, postfixed in osmium tetroxide, and embedded in Epon mixture. Ultrathin sections (approximately 60 nm) were then cut with glass knives on an LKB Ultrotome III. Sections were stained with aqueous 6% uranyl acetate, counterstained with lead citrate, and examined with a JEM 7A electron microscope.

RESULTS AND DISCUSSION.—Mycoplasmalike organisms were observed in phloem tissues (Fig. 1) in at least 20 infected plants, some of which were collected from three other locations in Israel. No MLO were found in similar phloem sections from healthy plants. The MLO were bounded by a clearly defined "unit membrane", and contained ribosome-like granules and fine strands presumed to be nucleic acid. Rounded forms, with a diameter of about 200 to 500 nm and low-electron-opacity cytoplasm, and elongated forms with a width of about 80 to 150 nm and electron-dense cytoplasm, were observed. Spherical bodies, with a diameter of about 100 to 150 nm and dense cytoplasm, may represent transverse sections of the elongated forms. No spiroplasmalike forms (3) were observed when sections approximately 300 nm, or negatively contrasted juice expressed from leaves, were examined. Some of the elongated forms, however, were curved (Fig. 1, CF). This does not seem to result from an accidental arrangement of a flexible body under crowded conditions, since similar forms were also seen when only few MLO were present. These forms may represent an intermediate type between spiroplasma and nonhelical mycoplasma.

The effect of tetracycline treatment on chlorophyll concentration after 1, 2, and 3 months is summarized in Fig. 2. One month after treatment with 316 and 1,000 μg/ml, infected plants developed normal leaves and chlorophyll concentrations were similar to those in healthy control leaves. Subsequently, chlorophyll concentration in infected plants decreased again. Two months after treatment, chlorophyll content (even at the highest TH level) was 65% of the control, and after 3 months only 17%. Symptoms also recurred after 3 months. When thin sections were screened, no MLO could be seen one month after TH treatment (1,000 μg/ml), a few were seen after 2 months, and after 3 months many MLO were again observed.

The results are in accordance with others (1, 2, 4) that antibiotics have only a temporary effect on symptom remission in MLO-infected plants. Chlorophyll measurements may, however, be useful to evaluate this effect quantitatively. The presence of MLO in diseased tissue, together with their absence in TH-treated recovered plants, strongly implicate MLO as the causal agent of Bermudagrass yellow leaf.

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