

# Witches' Broom Disease of Small-Fruited Acid Lime Trees in Oman: First MLO Disease of Citrus

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**ABSTRACT.** Lime trees are grown on a large scale in the Sultanate of Oman along the coast of the Gulf of Oman in the northern part. In the areas of El-Morer, Shinas and Liwa, many lime trees are affected by a disease which practically kills the trees within a few years and several growers have had to pull out whole orchards. The symptoms of the disease start with the appearance of a witches' broom (WB) and soon, within 3-4 yr, the tree is covered with many WBs. WBs are very conspicuous and characterized by their very small, pale-green leaves, which eventually dry up. Fruit production ceases.

Electron microscopy revealed the presence of numerous mycoplasma-like organisms (MLO) in the sieve tubes of WB leaves. The MLOs completely fill some of the sieve tubes.

The disease appeared some 10 yr ago, but its origin is unknown. Growers became aware of it about 5 yr ago, and now the situation is truly alarming. The disease seems to be spreading along the Coast from North to South. Since MLOs are involved, leafhoppers or psyllas are potential insect-vectors. Lime is the only citrus grown in the affected area, and nothing is known as yet about the susceptibility of other citrus species.

WB disease is associated with a nonhelical, mycoplasma-like organism which has not yet been cultured. WB is the first disease of citrus associated with MLOs.

*Index words.* mycoplasma-like organism, Oman, Mexican lime.

This report is the result of a survey in the Sultanate of Oman from April 5 to 13, 1986 to study a so-called "witches' broom disease" affecting small-fruited acid limes. The disease is restricted so far to the Northern part of the country along the coastal plain (North Batina) and extends from the border with the United Arab Emirates to Khaboura which appears to be the southernmost limit of the affected region.

## SYMPTOMATOLOGY

Affected trees are characterized by the presence of witches' brooms. The witches' brooms that characterize witches' broom disease of lime (WBDL) are easily detected by their compactness and the small to very small leaves, which are often pale green to yellow. These witches' brooms contrast strongly with the normal foliage of the tree.

In the early stages of the disease, the tree shows only one, then a few, witches' brooms, with the other parts of the tree remaining totally symptomless. These early witches' broom are soon followed by many

others in various parts of the tree. In the advanced stages of the disease, the leaves of the older witches' brooms die, but remain attached. Eventually, the dead leaves fall leaving dead twigs and shoots as the only evidence of former witches' brooms. In the final stage of the disease, the trees have many dead twigs, shoots and branches with only a few witches' broom left. At this stage, the trees are almost dead, and farmers pull them out and eventually plant banana trees.

The progress of the disease from the first witches' broom(s) to the last stage is very fast. Once the first symptoms (witches' broom) appear, the trees seem to decline very rapidly and, within a few years, they are totally unproductive and nearly dead.

Practically no flowers or fruits are produced on witches' brooms. Hence, fruit production decreases with increasing numbers of witches' brooms. Bunches of flowers and fruits produced on certain shoots are much reduced in size, and quickly die. No flower virescence seems to occur. Fruit on symptomless parts of affected trees are normal.

No specific symptoms have been observed on trunks and branches, but the shoots that compose a witches' broom are excessively ramified, have short internodes, are thin and fragile; and pale-green to whitish at the distal end.

### HOST RANGE OF WBDL

So far, WBDL is restricted to acid lime trees. In one orchard, where the acid lime trees were badly affected, adjacent sweet lime trees were symptomless. In the affected areas no citrus species other than acid lime and sweet lime are grown and nothing is known about the reaction of sweet orange, mandarin, etc, to WBDL. Graft-inoculation experiments must be conducted to determine the host range.

### ETIOLOGY

Eight samples, essentially 1 to 2 mm long pieces of leaf midribs, were taken on four witches' brooms and fixed the same day (April 6, 1986) in 2% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4; they remained in the fixative until they were processed in Bordeaux.

Five witches' brooms and one normal shoot from an affected tree were collected on April 12, 1986, carried to Bordeaux in plastic bags, and kept in the refrigerator until April 14, when leaf samples were taken and fixed as described above. After fixation, all 17 samples were post-fixed with osmium tetroxide, dehydrated, thin-sectioned and observed in the electron microscope.

All the witches' brooms typical of WBDL contained many microorganisms in their sieve tubes. These organisms were surrounded by a single cell membrane. They had no cell wall and were similar in all respects to the mycoplasma-like organisms (MLO) seen in other plants (1). The MLOs were often extremely numerous in the sieve tubes of midrib samples taken on very small leaves (0.5-2 cm long); some of the sieve

tubes of such samples were literally filled with MLOs. In larger leaves from witches' brooms, the MLOs were less numerous; sometimes they were not detected, even though smaller leaves of the same witches' broom contained high numbers of MLOs. In one witches' broom with many large leaves, MLOs could not be detected in leaves 4-5 cm long.

Finally, no MLOs were detected in the leaf samples from symptomless trees nor in leaves of normal shoots from affected trees. Similarly, no MLOs were seen in the sieve tubes from the symptomless sweet lime trees growing adjacent to affected acid lime trees.

These results show unambiguously that MLOs are associated with witches' broom disease of lime trees and that the severity of the symptoms is positively related to the number of MLOs in the sieve tubes.

### DISCUSSION

As already indicated, MLOs are clearly involved in WBDL. They are very probably the causal agent of the disease, but absolute proof for this must await fulfillment of Koch's postulates. As long as the MLOs are not available in culture, these postulates cannot be fulfilled.

Trees of any age may show the disease. The fact that young trees are affected shows that the incubation period for an infected tree to show symptoms, is short. Hence, the 10, 15, 25, 35 or 50-yr old trees that began to show symptoms around 1981-1982, became infected only a relatively short time before. If they were infected at a young age, they would have shown symptoms earlier. This logically means that they must have become infected through vector transmission of the disease agent. It is well-known that MLO diseases of plants are transmitted in nature by insect vectors, especially leafhoppers (1), and also by psyllas (in the case of pear decline for instance).

WBDL is a new disease, reported for the first time from the Sultanate

of Oman. Apparently, the disease has not been described elsewhere, and during a recent survey no symptoms

of the disease were seen in the United Arab Emirates, Pakistan and South Yemen.

### LITERATURE CITED

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