Simultaneous Infections of Citrus Leaves with Tristeza Virus and Mycoplasmalike Organism

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ABSTRACT

Likubin-diseased citrus trees in orchards are frequently affected by both the Likubin pathogen and tristeza virus. Electron microscopy of Likubin-diseased Tankan leaves revealed the occurrence of both pathogens. Mycoplasmalike organisms and tristeza virus particles were restricted to phloem tissue cells. Within the phloem tissues, the pathogens were encountered both in widely separated or in adjacent cells. Cells rarely contained both pathogens. No intimate association of the pathogens was observed.

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In Taiwan, Likubin-diseased Ponkan (*Citrus ponki* Hort. ex Tan.) and Tankan (*C. tankan* Tan.) in orchards are frequently affected not only by the Likubin pathogen but also by the tristeza virus (10). Ponkan and Tankan commonly are tolerant to the tristeza virus, and infected plants do not show conspicuous external symptoms. Martinez (9) pointed out, however, that a mixed infection of tristeza seedling-yellows and citrus greening induced more severe leaf symptoms, more stunting, and a greater number of plants undergoing an early decline than were observed for plants infected with the greening

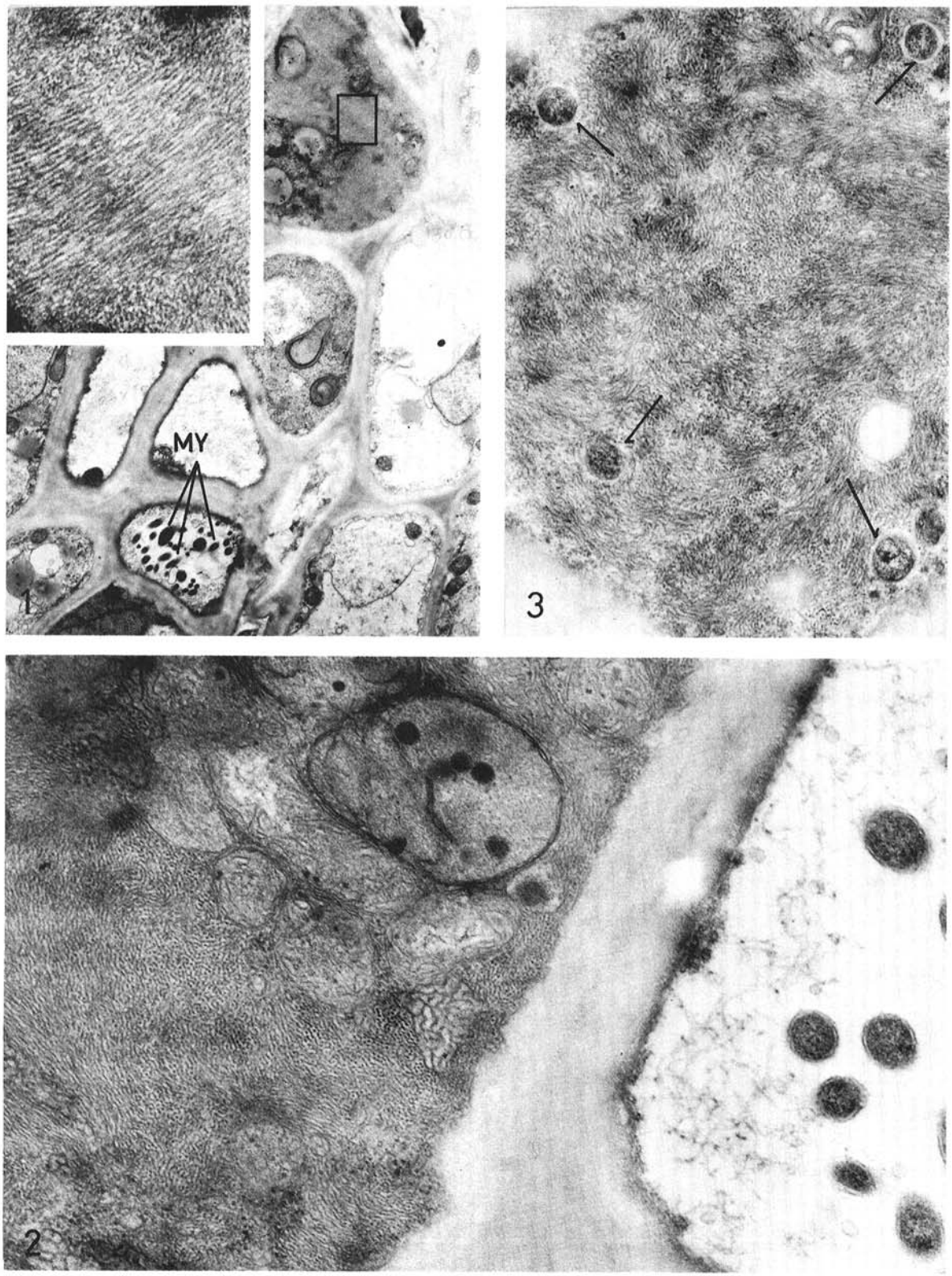
MY
1
2
3

Fig. 4. Detailed profiles of mycoplasmalike organism in a phloem cell of *Citrus tankan* (× 52,000).

pathogen alone. Accordingly, it was felt that the influence of mixed infections with tristeza virus and a mycoplasmalike organism on citrus plants should be considered in investigations on Likubin. Electron microscopy of tristeza virus and a mycoplasmalike organism in Ponkan or Tankan were undertaken and have been reported (2, 3). This paper deals with the relationship between those pathogens in citrus leaves.

MATERIALS AND METHODS.—Likubin-diseased leaves of *Citrus tankan* Tan. were collected in a commercial orchard near Taipei. The leaves showed faint interveinal chlorosis. Their lateral vein tissues were observed by a thin-sectioning procedure described previously (2).

RESULTS AND DISCUSSION.—In the present study, tristeza virus and a mycoplasmalike organism were restricted to some of the phloem tissue cells. The cells containing tristeza virus particles were larger in number than those that contained a mycoplasmalike organism. Most thin sections prepared from veinal tissue revealed either pathogen alone or no pathogen; however, a few thin sections contained both pathogens. In some sections, tristeza virus particles and the mycoplasmalike organism were encountered in widely separated cells (Fig. 1). In other sections, they were detected in adjacent cells (Fig. 2). Cells in which both pathogens were detected together occurred rarely (Fig. 3). There were no differences in the intracellular appearance of tristeza virus particles observed in cells that contained both pathogens and those infected with tristeza virus alone (5, 11, 12, 13). Frequent occurrence of necrotic cells and an abundance of tubular structures within the phloem tissues associated with tristeza virus infection (13) were also encountered in the present study. According to Lafléche & Bové (8), the mycoplasmalike organism associated with citrus stubborn disease differed somewhat from those

Fig. 1-3. 1) Tristeza virus particles and a mycoplasmalike organism restricted to widely separated phloem cells of *Citrus tankan;* MY = mycoplasmalike organism (× 5,800); inset is a part of the cell marked by a square in Fig. 1, showing tristeza virus particles (× 40,000). **2)** Tristeza virus particles and a mycoplasmalike organism were detected in adjacent cells (× 37,000). **3)** Tristeza virus particles and a mycoplasmalike organism (arrows) were observed together in the same cell (× 30,000).

associated with citrus greening, citrus leaf mottle, and citrus dieback diseases, because the agent of citrus stubborn disease was surrounded by a typical unit membrane of ca. 10-nm width, whereas the agents of the latter diseases had an outer membrane system with a width of about 20 nm. The mycoplasmalike organism associated with Likubin, like the pathogens of citrus stubborn disease, were surrounded by thin double-membranes 7-10 nm in width (Fig. 4). The intracellular appearance of cells of citrus leaves infected singly with tristeza virus or mycoplasmalike organism (2, 4, 5, 6, 7, 11, 12, 13) did not differ from those of leaves infected with both pathogens. That both pathogens were observed only rarely in the same thin sections probably was a result of the small number of cells bearing tristeza virus particles or mycoplasmalike organisms, though intracellular concentration of these pathogens was not always low. Furthermore, it is likely that the rare occurrence of both pathogens in the same cell derives from the fact that the host cells may be affected differently by the two pathogens, since the tristeza virus in synthesized via the host cells in response to the viral genome, whereas mycoplasmalike organisms have self-reproducing ability. Although Atanasoff (1) suggested recently that mycoplasma may be a vector of viruses, tristeza virus particles were not observed within the mycoplasmalike organism, and there was no intimate association between them observed in the present study.

Although the present study provides no histological or cytological basis to explain the increase in symptoms induced by mixed infections with both pathogens, the severity of external symptoms often is not directly related to the amount or appearance of internal symptoms. For instance, intracellular modifications that occurred within the leaf phloem tissues of *C. aurantifolia, C. hassaku, C. sinensis,* or *C. paradisi* infected with tristeza virus (5, 11, 12, 13) were similar to those of infected *C. ponki* or *C. tankan,* though the former species are intolerant and the latter are tolerant to tristeza virus infection. Little is known about the anatomy and physiology of plants doubly infected with a mycoplasmalike organism and a virus. In herbaceous plants, however, one pathogen frequently changes a host's susceptibility to other pathogens. Furthermore, the mycoplasmas isolated from man or other animals occur commonly as contaminants in tissue cultures, and frequently change the host cell growth or susceptibility to virus infection (14). In any case, it is clear from the present study that two different pathogens are actually present in the same tissues or cells, and therefore the responses to tristeza virus infection of citrus plants already infected with a mycoplasmalike organism, or vice versa, should be examined in any investigations on Likubin.

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