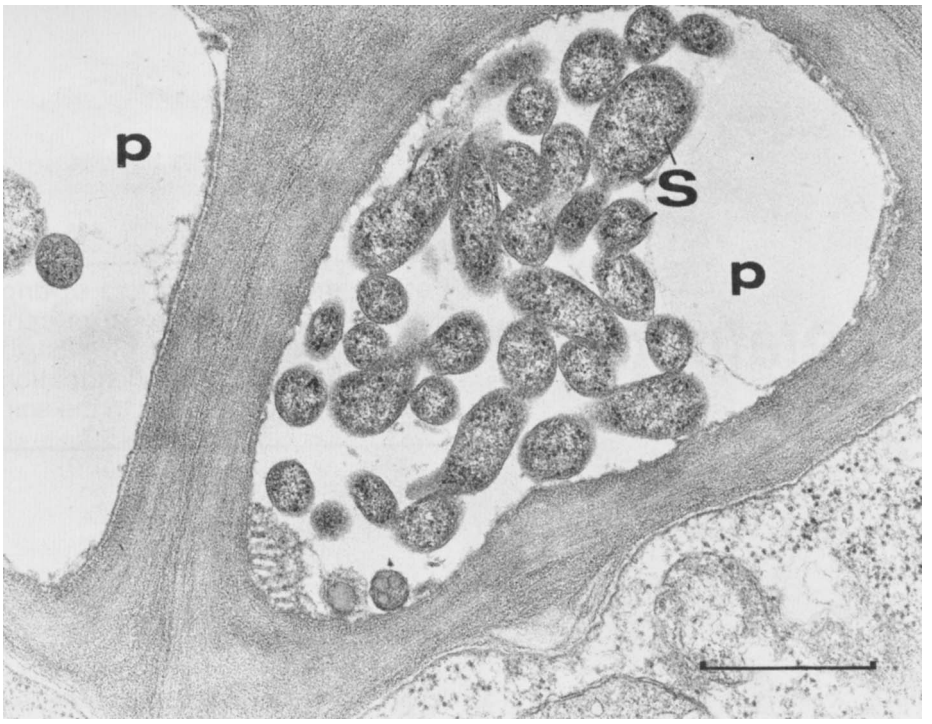

Electron micrograph of *Spiroplasma citri* organisms (S) in phloem cells (p) in a diseased periwinkle plant. Bar is 500 nm.

Occurrence of *SPIROPLASMA*

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Citrus stubborn disease is a serious economic problem in California, in Southwestern United States, and in other arid citrus areas of the world. Knowledge of the disease has rapidly increased since 1969 when a mycoplasma-like organism was found by electron microscopy in thin sections of diseased leaves. In 1970, a mycoplasma, now named *Spiroplasma citri*, was cultured from diseased citrus tissue. Further research has revealed that two leafhoppers (see *California Agriculture*, November 1973) can transmit the stubborn disease organism. Cultured spiroplasma have been fed or injected into these insects and they, in turn, have transmitted stubborn to healthy citrus seedlings. More recently (see *California Agriculture*, February 1975), one of the insects, *Scaphytopius nitridus*, fed on diseased citrus trees was shown to transmit a severe disease to healthy *Vinca rosea* L., periwinkle plants, in controlled greenhouse experiments. This information prompted our investigating the possibility of natural spread of stubborn into periwinkle plants.

Periwinkle around motels, downtown businesses, and homes in Riverside were sampled first. One positive isolation stimulated further search and to date six plants have yielded isolates of *S. citri* (see table). This included collections made in Bakersfield, Exeter, Lindcove, and Visalia in the San Joaquin Valley and in Indio and Palm Springs in the Coachella Valley. In addition, plants were sampled in Laguna Beach, San Juan Capistrano and Tustin in the coastal area southwest of Riverside. Observations were made on over 300 plants, but only 61 chlorotic, wilted or otherwise unhealthy appearing plants were sampled.

One to three growing shoots about 6 inches long were collected. The samples were sealed in a plastic bag and refrigerated or kept over ice until isolations were attempted, within 48 hours. Previous work indicated that *S. citri* could be isolated from known diseased shoots 360 hours after removal from the plant. Leaves were cut from the shoot samples and the shoots together with 2 or 4 leaf midribs were surface sterilized in 1 percent sodium hypochlorite, rinsed in sterile distilled water, and minced with a razor blade. The pieces, now in a sterile petri plate, were flooded with 8 ml growth medium and after 5 minutes the fluid was passed through a 0.45 micron membrane filter. This filtrate was incubated in test tubes for 7 to 21 days at 30°C. If *S. citri* were present, growth of the organism would result in the production of acid which would be indicated

INCIDENCE OF *S. CITRI* IN PERIWINKLE PLANTS IN CALIFORNIA

Riverside	1/21[a]
San Joaquin Valley	2/19
Coachella Valley	3/11
Coastal area	0/10
Moreno Valley experimental plot	4/18

[a]Number of plants with *S.citri*/number of plants sampled.

CITRI in Periwinkle in California

by phenol red in the medium changing to yellow. Any cultures 21 days old as well as those showing the indicated color change were routinely checked in the compound light microscope using dark-field optics. Positive cultures contained numerous thin, motile, spiral strands, unmistakable examples of *S. citri.*

Since most periwinkle is sold as small plants and is transplanted to home gardens, we checked nurseries as a source of diseased periwinkles. None of the collections from nurseries was positive. To further confirm that natural spread was taking place we grew healthy periwinkle in the greenhouse from seed. Eighteen 8-week-old plants were transplanted to an experimental citrus plot in Moreno Valley about 10 miles east of the Riverside campus in an area where there is a high incidence of citrus stubborn. After two months in the field, one plant showed disease symptoms, small flowers and chlorotic leaves. *S. citri* was recovered from this plant as well as from three others later in the season. The plants showed severe water stress and were dead 4 to 6 weeks after initial symptoms. The organism could be recovered from wilted but not from dead plants. Even very wilted shoots cut from a dying plant revived almost completely when immersed in water. The spiroplasma was easily recovered from the revived tissue. The disease was transmitted to healthy periwinkles by grafting with pieces from diseased plants.

Electron microscopy of ultrathin sections of the diseased Moreno Valley plants revealed typical mycoplasma organisms restricted to the phloem (see photograph).

This report indicates that the stubborn disease organism, *S. citri,* can be recovered from periwinkle plants growing in several areas of California. Natural spread was responsible for the disease and death of 22 percent of the healthy periwinkle plants placed in an experimental plot. Leafhoppers are assumed to spread *S. citri* from plant to plant because both *Circulifer tenellus* and *Scaphytopius nitridus* leafhoppers are found in southern California and are known to harbor and transmit the spiroplasma either experimentally or under field conditions.

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