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Seasonal and Geographical Variations in Natural Spread of Citrus Stubborn Disease

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The natural spread of stubborn disease was once believed to be slow because for many years little spread was noted and no vector was known (Calavan, 1969). Recently Calavan *et al.* (1974) reported preliminary data on natural incidence of stubborn in young field-grown citrus, indicating moderate to rapid natural spread in some California locations but no spread near the coast. Two species of leafhoppers, *Circulifer tenellus* Baker

(Lee *et al.*, 1973; Calavan and Gumpf, 1974; Rana *et al.*, 1975) and *Scaphytopius nitridus* DeLong (Kaloostian *et al.*, 1975) have been shown capable of vectoring the stubborn pathogen, *Spiroplasma citri* Saglio *et al.* This paper reports the results of a 2-year study of the natural spread of stubborn disease in young sweet orange seedlings growing at three locations in California.

MATERIALS AND METHODS

Healthy Madam Vinous sweet orange seedlings 0.2 to 0.3 m high were planted in June 1973 at the University of California (UC) Moreno Farm near Riverside and at the UC Lindcove Field Station east of Visalia. A similar plot was established near the seacoast in July 1973 at the UC South Coast Field Station southeast of Santa Ana. Stubborn disease was present in adjacent plantings at all three locations.

The experimental plants were observed closely for symptoms resembling those of stubborn: Young shoots were collected from suspect plants for culturing on a medium modified from that of Saglio *et*

al. (1971) (Fudl-Allah *et al.*, 1972). Only plants from which *S. citri* could be cultured and seen by darkfield microscopy were classified stubborn positive.

In August 1973, 10 suspect plants were transferred from the Moreno Farm plot to a glasshouse. Subsequently, groups of 10 to 20 healthy Madam Vinous sweet orange seedlings were exposed in this plot for limited periods to determine the season of natural spread. Cultures were attempted from each plant following field exposure and subsequent incubation in a warm glasshouse for several months.

RESULTS

South Coast plot. No strongly suspect plants were found and none of 21 seedlings cultured yielded *S. citri*.

Lindcove plot. Two strong suspects, of 66 plants cultured, yielded *S. citri*, one in 1974, the other in 1975.

Moreno Farm plot. All 10 suspect seedlings removed from the plot in August 1973 were stubborn positive. From August to November 1973, 66 of 95 additional seedlings cultured were

found stubborn positive. In 1974, another 55 seedlings and up to July 31, 1975, nine additional seedlings were found stubborn positive. By July 31, 1975 only 10 of the original 150 seedlings remained apparently healthy. Table 1 shows the natural spread of stubborn in all three plots from mid-1973 to mid-1975.

The results of temporary exposure of groups of sweet orange seedlings during

TABLE 1
INCIDENCE OF INFECTION BY *SPIROPLASMA CITRI* IN SWEET ORANGE SEEDLINGS
AT THREE LOCATIONS, JUNE 1973 THROUGH JUNE 1975

Plot	No. plants exposed	No. plants cultured	No. plants infected
South Coast	165	21	0
Lindcove	200	66	2
Moreno	150	150	140

various months of the year (Table 2) show that most, or all, natural spread in the Moreno plot occurred during the months of June to September, inclusive, and that natural spread was much greater

there in 1973 than in 1974. In a related experiment at Moreno, only one of 12 Madam Vinous sweet orange seedlings exposed from June 27 to October 29, 1974 became infected with *S. citri*.

TABLE 2
INCIDENCE OF INFECTION BY *SPIROPLASMA CITRI* IN SWEET ORANGE SEEDLINGS
BRIEFLY EXPOSED IN THE MORENO PLOT

Exposure period	No. plants infected/ no. plants exposed
June 11 - Aug. 21, 1973	10/10*
Aug. 21 - Oct. 10	6/10
Oct. 10 - Nov. 9	0/10
Nov. 9 - Dec. 11	0/10
Dec. 11 - Jan. 17, 1974	0/10
Jan. 17 - Feb. 28	0/10
Feb. 28 - Apr. 1	0/10
Apr. 1 - May 1	0/10
May 1 - June 4	0/10
June 4 - June 28	0/10
June 28 - July 31	0/20
July 31 - Aug. 30	0/20
Aug. 30 - Oct. 1	3/20
Oct. 1 - Nov. 4	0/20
Nov. 4 - Dec. 3	0/20
Dec. 3 - Dec. 31	0/20
Dec. 31 - Feb. 6, 1975	0/20
Feb. 6 - Mar. 4	0/20
Mar. 4 - Mar. 31	0/20
Mar. 31 - May 5	0/20
May 5 - June 3	0/20

*Plants of this group only were selected after symptoms appeared.

DISCUSSION AND CONCLUSIONS

The natural incidence of stubborn disease was found to vary greatly with location, year, and season. As previously reported (Calavan *et al.*, 1974) no spread of stubborn was detected near the coast.

Incidence was highest at a hot interior location (Moreno), where substantial numbers of vectors were observed. The incidence of stubborn at Lindcove was much lower than expected, which leads

us to suspect that in a prior experiment at Lindcove (Calavan *et al.*, 1974) not all plants rated stubborn positive solely on the basis of symptoms were really infected with *S. citri*.

The very high incidence of stubborn at Moreno in 1973, confirmed by obtaining *S. citri* from 76 of 105 plants from which cultures were attempted, may have been due in part to an adjacent planting of young citrus containing several hundred naturally infected stubborn trees. Removal of this planting in early 1974 may have been a factor in lowering the natural incidence of stubborn even though, by that time, there were many stubborn-diseased plants within the Moreno plot. Inasmuch as cultures were not attempted in 1973 from 45 of the plants in the Moreno plot, and may have failed to detect *S. citri* present in some others, we suspect that many of the 55 plants in which the pathogen was first found in 1974 may have become infected in 1973.

The data indicate that young citrus plants in the presence of naturally infected trees and substantial vector populations are likely to have a rather high incidence of *S. citri* infection during the

hot season. The natural incidence of *S. citri* infection in sweet orange plants in the Moreno plot was apparently confined to the hot months of summer and early fall, June through October. The appearance of definite symptoms in some plants by the end of July 1973 is believed to indicate infection in June or early July. Incidence during late summer and early fall 1973 was especially high, with 6 of 10 seedlings becoming infected between August 21 and October 10. Late summer and early fall was the only period in 1974 during which *S. citri* was acquired by plants briefly exposed in the Moreno plot. Unfortunately, no plants were exposed during the spring growth flush in 1973 but in 1974 and 1975 no evidence of natural incidence of *S. citri* infection in the spring was found in exposed sweet orange plants.

We conclude that effective control of the insect vectors of *S. citri* during the hot summer months would probably reduce the natural incidence of stubborn disease and that citrus nurseries should be located in areas where there is little or no natural spread of *S. citri*.

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