Natural Incidence of Stubborn in Field-grown Citrus Seedlings and Budlings

E. C. Calavan, M. K. Harjung, A. E.-S. A. Fudl-Allah, and J. W. Bowyer

Natural spread of the stubborn pathogen has been suspected for many years but no vector has been identified and, until recently, natural spread was believed to be slow (3). The presence of infection in occasional ungrafted seedling trees and in some budlings from healthy parent trees suggested the probable importance of natural spread. This paper reports preliminary results indicating extensive natural spread of stubborn in field plots of indicator plants.

MATERIALS AND METHODS

Healthy Madam Vinous sweet orange and Sexton tangelo seedlings, and budlings of Madam Vinous sweet orange on Rough lemon rootstock were planted in 1968 and 1969 in five field plots.

Plot locations. Plots were established in central California at the University of California's Lindcove Field Station on the east side of the San Joaquin Valley and Westside Field Station on the west side of the valley. In southern California, plots were established at the University's South Coast Field Station, 20 km from the sea, the Riverside campus, 62 km from the sea, and at the University's Moreno Farm, about 15 km east of Riverside.

Climate in areas where plots were grown varied considerably, as did the plots' proximity to other citrus trees and to buildings. The Lindcove plot, hot in summer and cool in winter, was planted in a nursery at the edge of a grassy wooded area and was within 200 m of 20 or more large, stubborn-infected navel orange trees. The Westside plot, hot and windy in summer and cold in winter, was within 15 m of buildings and gardens containing 15 small, frost-damaged citrus trees of undetermined health, and was many kilometers from commercial citrus. The South Coast plot, mild most of the year, was located close to buildings and within 0.5 km of many large, stubborn-infected citrus trees. The Riverside plot, hot in summer and mild in winter, was planted between rows of large, mostly stubborn, Valencia orange trees in a district where stubborn is known to have spread naturally more than 10 years ago. The Moreno plot, hot in summer and mostly mild in winter, was adjacent to isolated buildings and a garden, about 1 km from other citrus, but in a district where stubborn incidence has been very high for about 20 years in commercial orchards of grapefruit and sweet orange.

Experimental plants. In May, 1968, healthy six-month-old Rough lemon seedlings from outdoor seedbeds in the Coachella Valley were planted at Lindcove, Riverside, and Moreno. Scions from healthy, glasshouse-grown Madam Vinous seedlings were budded on them in the fall of 1968, and forced to grow early in 1969. Healthy, glasshouse-grown seedlings of Rough lemon were planted at South Coast in October, 1968, and budded with healthy Madam Vinous seedling scions in April, 1969. Healthy, glasshouse-grown Sexton seedlings were planted in all plots except Westside in May 1969. No weak or diseased plants were planted and no graft inoculations were made.

Tree spacing was about 1.8 × 0.7 m, slightly greater at Westside, and irrigation was by furrow. Culture was routine

except that minimal pest control was used at Lindcove, Moreno, and Westside. All plants were examined twice each growing season for stubborn symptoms. Many symptomless and diseased plants were indexed by side graft (*2*) or leaf-piece (*4*) techniques and/or by *in vitro* culturing (*8*).

RESULTS

The first symptoms appeared in a Madam Vinous plant at Riverside in the fall of 1969, about 18 months after planting. By the fall of 1970, several Madam Vinous and Sexton plants at Lindcove, Riverside, and Moreno were apparently affected. Presence of the stubborn pathogen was confirmed, by grafting, late in 1970, for one tree at Riverside and for seven trees in Lindcove. Incidence of stubborn then seemed very low in all plots.

Several Madam Vinous seedlings at Westside, severely frozen in the winter of 1969–1970, showed mild symptoms of stubborn before being frozen again in 1970–1971. The Westside plot was terminated in December, 1971.

Many new cases of stubborn appeared in the Moreno plot during the summer of 1971. Most of these were on the south side of the plot, in Madam Vinous seedlings planted in 1969 adjacent to the garden. There, incidence of stubborn was above 40 per cent; by late summer, 1972, it had reached 90 per cent in the same rows. *Spiroplasma citri* (*1*) was readily cultured from 16 of 20 plants with symptoms, but could not be obtained from any of 20 normal controls. Cultures were also obtained from infected plants in the Lindcove, Riverside, and Westside plots. Sexton tangelo seedlings and Madam Vinous budlings at Moreno had a much lower incidence of stubborn than did Madam Vinous seedlings. This difference was noted also at Lindcove and Riverside.

Symptoms of stubborn were milder at Lindcove than at Riverside, and many Lindcove plants improved in 1972 after showing symptoms diagnosed as stubborn in 1971. The incidence of stubborn at Lindcove was much lower than at Moreno for late 1971 and early 1972.

The apparent incidence of stubborn in each group of plants in all plots by late fall, 1971, is shown in table 1. Numbers of suspect plants indexed by tissue grafts or by culturing are shown for each plot in table 2.

TABLE 1
INCIDENCE OF STUBBORN IN YOUNG FIELD PLANTS OF CITRUS PLANTED IN 1968–1969, BASED ON SYMPTOMS IN NOVEMBER, 1971

Plot	No. plants with stubborn/no. plants examined			Stubborn
	MV/RL*	MVS†	STS‡	
				per cent
Lindcove	35/400	47/193	21/203	13
Moreno	51/393	60/179	27/200	18
Riverside	23/397	30/162	11/176	9
South Coast	0/309	0/200	0/200	0
Westside	.....	42/248	.....	17

* MV/RL = Madam Vinous sweet orange on Rough lemon rootstock.
† MVS = Madam Vinous sweet orange seedlings.
‡ STS = Sexton tangelo seedlings.

DISCUSSION AND CONCLUSIONS

Natural incidence of stubborn in several locations where summers are hot could account for much of the current outbreak of stubborn in California. No spread of stubborn was detected in the relatively cool coastal plot despite its presence in adjacent orchards. A mycoplasmalike organism, *Spiroplasma citri,* was easily cultured from stubborn, but not from healthy plants, in all plots except the South Coast.

Natural spread of stubborn in our plots may be attributable to insect vectors, as yet undetected, to seed transmission, or to transmission by a soil-borne organism. The stubborn pathogen is known to be present in the seed coats of aborted seed, but the use of healthy seedlings from healthy trees has minimized the chances of seed transmission. Furthermore, no stubborn has been found among thousands of indicator seedlings, including several hundred from moderately aborted seeds, grown indoors up to three years. The relationship of *Spiroplasma citri* to citrus seed merits further study.

The probability that insect vectors spread stubborn in our plots is indicated by: (1) the existence of insect vectors for pathogens of similar diseases (*5, 6, 9*), especially the corn stunt *Spiroplasma* (*7*), which closely resembles *S. citri;* (2) higher incidence of stubborn on the side of the Moreno plot closest to a garden and buildings; (3) higher incidence of stubborn in the unsprayed Moreno plot than in the Riverside plot where 16 pesticidal sprays were applied from 1969 through 1972; and (4) the apparent absence of stubborn in the coastal plot where the mild climate is favorable to survival of the stubborn agent in infected plants (*3*) but is too cool for optimum symptom expression and optimum growth of *S. citri.*

Clean propagative material of sweet orange, tangelo, and many other stubborn-susceptible varieties cannot be expected to remain clean very long under orchard conditions in the hot citrus areas of central and southern California. We should investigate further the possibilities of preventing natural infection by growing clean materials in a cool coastal location and reducing incidence by regular use of insecticidal sprays in areas where the climate favors spread of the disease. If plants recover after initial infection, as may be inferred from the findings that some plants appeared diseased in 1971 and had apparently recovered in 1972, and that in many cases apparently complete recovery occurred in greenhouse plants used in unrelated experiments, then control of stubborn spread by controlling vectors in young orchards might be possible.

Isolation from other citrus apparently had little effect in slowing stubborn spread, as is indicated by the results obtained at Moreno and Westside. To avoid stubborn infection, clean propagative stock of citrus should be maintained indoors, at least until the possibility of maintaining it in a cool coastal area is more carefully explored.

TABLE 2

INCIDENCE OF STUBBORN, VERIFIED BY GRAFT TRANSMISSION OR BY CULTURING, IN ABNORMAL YOUNG FIELD PLANTS OF CITRUS TWO TO FOUR YEARS OLD

Plot	No. plants with stubborn/no. plants tested	Stubborn
		per cent
Lindcove	13/97	13
Moreno	25/52	48
Riverside	29/178	16
South Coast	0/62	0
Westside	4/18	22

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