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## Studies of Stubborn Disease in Corsica

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Many 10- to 15-year-old sweet orange trees in Corsica are traceable to budwood from Morocco where stubborn disease is severe and widespread. Yet very few trees in Corsica show stubborn symptoms. Since stubborn is favored by high temperatures (1, 4), the mild tempera-

tures of Corsica could explain the lack of symptoms. The Corsican trees should, however, harbor the stubborn pathogen even though they show no symptoms of the disease, and this paper reports studies made to determine whether such is the case.

### MATERIALS AND METHODS

Material for stubborn indexing was taken from the following sources:

1. Washington navel, Thomson navel, Golden Buckeye, and Navelencia sweet orange trees severely affected by stubborn disease in Morocco were propagated on sour orange seedlings in Corsica in 1960. None of these trees has shown symptoms of stubborn under field conditions in Corsica.

2. In 1970, budwood from Washington navel and Valencia late sweet orange trees, with or without stubborn symptoms, was collected in the Tadla area of Morocco. Budwood was propagated in Corsica in a greenhouse kept at an average day temperature of 30°C and a night temperature of 26°C. Two progeny trees of each source were kept, and each test plant was inoculated with inoculum from the two progeny trees.

3. Stubborn from California was propagated on various sweet orange seedlings and kept in the greenhouse at 26–32°C, and Washington navel sweet orange (stubborn strain C 159) was grown under screenhouse conditions. Inoculum was taken from the nine trees grown in the screenhouse.

4. Frost nucellar Washington navel sweet orange (CES 3033) received from California in 1961 was increased in Corsica in the screenhouse. In 1964 and 1965, 162 trees from this line were planted in the field (plot C7). None of

the trees show typical stubborn symptoms, but very mild, relatively nonspecific leaf symptoms have been observed. No fruit symptoms and no stunting of the trees have been noticed. Inoculum for indexing was taken from 64 trees of plot C7.

Budwood from trees in plot C7 was increased, and progeny trees were planted in plot C5 in 1966. In 1970, severe symptoms of stubborn were observed in plot C5. This plot is located on a slope, protected by windbreaks, with an average day temperature approximately 5°C higher than that in plot C7. Total time above 28°C from July 3 to 23, 1972, in C5 and C7 was 106 and 27 hours, respectively. One tree (T18) in plot C5 shows very severe symptoms of stubborn, 10 trees have severe symptoms, and 68 trees have symptoms that are moderate. Inoculum for indexing has been taken from tree T18.

Four Hamlin or Madam Vinous sweet orange indicator seedlings were inoculated by the leaf-piece graft method (3). Uninoculated seedlings were kept as controls. Plants were grown in 5-liter cans kept in an insect-proof greenhouse maintained at 32°C during the day and 26°C at night.

The presence of *Spiroplasma citri* in some inoculated seedlings was determined by electron microscopy and by isolation of the microorganism (2).

## RESULTS AND DISCUSSION

Stubborn symptoms appeared within seven to 10 months on seedlings inoculated with leaf inoculum from the field, and within two or three months on those with inoculum from the 26° to 32°C greenhouse. The highest percentage of transmission was obtained with field inoculum collected between May and August.

In cases where stubborn symptoms were observed on a source tree in Morocco, the presence of the stubborn pathogen was confirmed by indexing (table 1). The stubborn pathogen was detected in a seven-year-old Washington navel orange tree even though the tree still appeared normal. All trees carrying California stubborn were positive for stubborn.

Table 2 shows that, with the exception of the two Thomson navel trees, all trees of Moroccan origin are infected with the stubborn pathogen. A similar situation is found with Washington navel trees infected with California

stubborn (source C159) (table 2). These trees have always been kept in the screenhouse. Seventy-nine progeny trees of this line have been planted in the field, however, as part of other experiments, and none has shown stubborn symptoms.

With Frost nucellar Washington navel (table 2), none of the trees of plot C7 show typical stubborn symptoms, even though 64 indexed trees are positive for stubborn. This Washington navel line does express strong stubborn symptoms when planted in a warmer location (plot C5). Budwood of this symptomless Washington navel line from C7 was shipped to Morocco in 1970. All progeny trees developed strong stubborn symptoms in the nursery (A. J. Vanderweyen, personal communication). In 1963 and 1964, respectively, 1,555 and 4,000 buds of this Washington navel line were introduced directly from California to Morocco. A high percentage of the progeny trees planted in 1965 showed strong stubborn symptoms in 1970 (J. M. Bové, personal communication).

These results seem to indicate that the prevailing climatic conditions in Corsica discourage development of stubborn disease symptoms, but not of the stubborn pathogen. This further confirms the importance of high temperature for the development of stubborn symptoms.

The Frost nucellar Washington navel line was propagated under screenhouse conditions from seven buds from three different budwood sticks. Records have been kept of the progeny trees from each budwood stick. All trees (64) of plot C7 that were indexed for the stubborn pathogen were infected with it. This very high percentage of infection is likely the result either of perpetuation of the pathogen during propagation of the stock, or, less likely, of later infection of outdoor trees through insect transmission.

TABLE 1  
RESULTS OF INDEXING FOR STUBBORN DISEASE IN PROPAGANTS OF SWEET ORANGE TREES FROM MOROCCO

Source and age of trees in Morocco	Presence (+) or absence (-) of stubborn symptoms	No. trees positive for stubborn/no. trees indexed
Washington navel:		
28 years.....	+	9/9*
7 years†.....	+	2/2*
7 years.....	-	1/1
Valencia late:		
28 years.....	+	2/2
28 years.....	-	0/2
9 years.....	+	1/1
9 years.....	-	0/1

\* Presence of *Spiroplasma citri* in one tree was ascertained by electron microscopy and culture of microorganism.

† Budwood came from 28-year-old trees.

TABLE 2  
RESULTS OF INDEXING FOR STUBBORN DISEASE OF SWEET ORANGE TREES  
IN CORSICA

Source trees in Corsica	Origin of budwood	Presence (+) or absence (-) of field symptoms of stubborn on:		No. trees positive for stubborn/no. trees indexed
		Mother trees in country of origin*	Progeny trees in Corsica	
Washington navel.....	Morocco	+	-	6/6
Thomson navel.....	Morocco	+	-	0/2
Golden Buckeye.....	Morocco	+	-	1/1
Navelencia.....	Morocco	+	-	2/2
C159 in Washington navel.....	California	+	-	9/9‡
Fawcett 8A-29-36.....	California	+	+†	1/1‡
Herman 4506.....	California	+	+†	5/5
C189.....	California	+	+†	7/7‡
Frost Washington navel CES 3033:	California	-		
plot C7.....	California		-	64/64
plot C5.....	California		+	1/1‡

\* At time of budwood collection.

† Grown in heated greenhouse.

‡ Presence of *Spiroplasma citri* was ascertained by electron microscopy and/or culture of the microorganism.

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