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International Organization of Citrus Virologists Conference Proceedings (1957-2010)

Title

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Permalink

<https://escholarship.org/uc/item/9jj240m2>

Journal

International Organization of Citrus Virologists Conference Proceedings (1957-2010), 11(11)

ISSN

2313-5123

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Publication Date

1991

DOI

10.5070/C59jj240m2

Peer reviewed

Investigations Into "Acorn Fruit" Disease Observed in Sweet Oranges in Sicily

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ABSTRACT. Acorn fruits of Tarocco and Golden Buckeye sweet orange have been observed in different citrus growing areas in Sicily. Anomalies and chlorotic patterns resembling zinc deficiency, were also present on leaves. The disease is transmitted by budding and was described previously as stubborn. Transmission tests to indicator plants carried out by bark patches, budsticks, leaves and fruit bearing stems have always been negative. Assays to detect *Spiroplasma citri* by ELISA test from samples collected in field or in greenhouse gave negative results. Attempts to culture *S. citri* from infected citrus trees were unsuccessful. Periwinkle plants left close to affected trees remained symptomless after 2 yr. Shoot-tip grafting from affected plants allowed the elimination of leaf symptoms.

Index words. acorn fruit, stubborn disease.

During field surveys in Sicily for citrus tristeza virus (CTV) many trees of Tarocco and Golden Buckeye sweet orange with acorn shaped fruits were observed. This disease was reported previously in Sicily (8) and in Sardinia (9) and was attributed to stubborn on the basis of symptoms. However, *Spiroplasma citri* has never been isolated from affected plants. The purpose of this study was to further investigate the acorn disease and determine its possible relationship to the stubborn disease by tests like ELISA, culture and transmission of the pathogen and elimination of the pathogen by shoot-tip grafting.

MATERIALS AND METHODS

Field observations. Field observations and surveys were carried out for 3 yr in two different areas near Catania. Each year Tarocco and Golden Buckeye sweet orange trees were observed for symptoms on fruit and leaves and to determine the incidence of abnormal fruit and leaves. In order to carry out the investigation on this malady budsticks from symptomatic trees were grafted on sour orange and Volkamer lemon and plants were grown in a greenhouse at 28-32 C.

Biological assays. Seedlings of nucellar Tarocco and Madame Vinous sweet orange, 15 for each cultivar, were inoculated with bark patches taken from stems with acorn fruits or with 10 cm long budsticks carrying an acorn fruit.

The indicator plants were grown in a greenhouse at 28-32 C.

Detection by culture. Since symptoms of acorn disease were attributed to stubborn disease, we attempted to isolate *S. citri* on liquid medium 3G (2). A total of 30 trees were tested in August, September, October and November of each year for three years. Isolations were made using old and young leaves and fruits (albedo, columella and seeds). A strain of *S. citri* on pummelo served as a positive control. This strain was from an experimental field of the Department of Plant Pathology of the University of California, Riverside. The pummelo was grafted onto Volkamer lemon rootstock and grown at 28-30 C to assure good replication of *S. citri*.

ELISA test. ELISA tests were carried out by conventional procedures (4) using antisera prepared against *S. citri* Moroccan strain R₈A₂ either kindly supplied by Prof. J.M. Bové (Bordeaux, France) or purchased from Sanofi Santé Animal (France). Tested tissues were: a) midveins of young or old leaves of trees grown in the field or greenhouse; b) albedo and columella of fruits from the field; c) normal and aborted seeds from the field after a pectinase treatment (0.16% pectinase in 0.2% EDTA) at 37 C for 4 hr; d) fruitlets (0.5-0.8 mm). The positive control was young pummelo leaves as above.

Natural transmission in the field. Periwinkle plants, an indicator of stubborn disease (6) were placed around

trees with acorn fruit. They were planted at the two-three leaf stage in spring in two different fields and were left in place for 2 yr. The periwinkle plants were inspected every two months for possible symptoms of stubborn disease. In August, September, October and November of both years some leaves were collected from these periwinkles and assayed by ELISA or used in isolation tests for *S. citri*. A total of 300 periwinkle plants were used in this experiment.

Shoot-tip grafting (STG). Our approach to the STG technique involved some modifications (5) of standard procedures. Shoots for grafting were obtained from affected greenhouse grown trees. Plants obtained by STG were grown in a greenhouse at 28-32 C and inspected for leaf symptoms.

RESULTS

Trees affected by the "acorn fruit" disease showed slight to severe stunting, with abnormally dense, bunched and upright foliage. Dieback of branches was observed in some trees. All trees gave a poor fruit set and low yield. Leaves showed various patterns of chlorosis or mottling, resembling zinc or other nutritional deficiencies, and were of abnormal thickness and cupped (Fig. 1), generally small in size with short internodes. A high percentage of the leaves showed these symptoms every year. Fruits were from normal to severely deformed (Fig. 2). Other symptoms on fruits were: irregular or inverse coloring with retention of green near the peduncle area, small lopsided fruits with curved columella and acorn shape. No blue albedo of fruits was found during the 3 yr of observation. Each year about 80-90% of the fruits showed severe symptoms. Tarocco and Golden Buckeye sweet orange generally have only a few seeds, but in some cases we found aborted seeds. None of citrus plants inoculated for biological assays have showed symptoms referable to stubborn disease.

The trees replicated in greenhouse showed the same symptomatology observed in field.

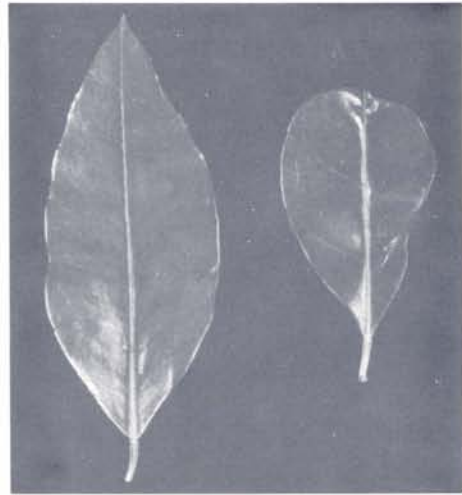


Fig. 1. On the right, abnormal and cupped leaf of Tarocco sweet orange collected in the field, on the left a normal leaf.

We made 300 attempts to culture *S. citri* from leaves, shoots, and fruits of field grown trees and 85 attempts to culture from shoots of inoculated greenhouse grown Tarocco and Madame Vinous and from Tarocco and Golden

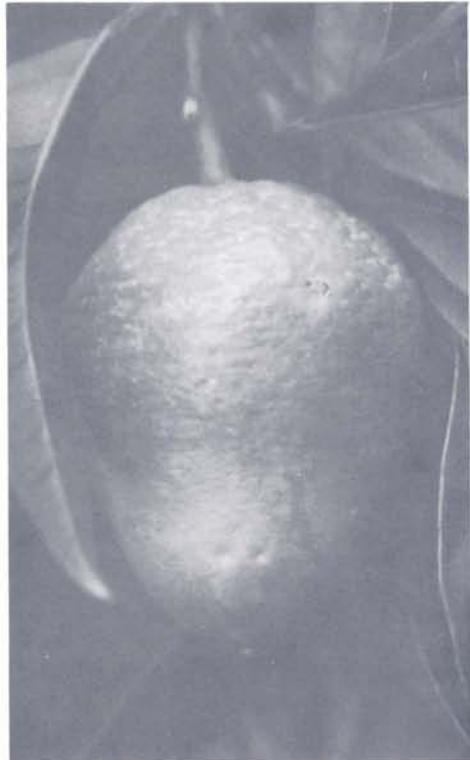


Fig. 2. Acorn fruits of Tarocco sweet orange collected in the field.

Buckeye sweet oranges. We also carried out 50 isolations from shoots of positive control plants. *S. citri* growth was obtained in 29 of 50 positive controls, but no *S. citri* was obtained from any other source. Over 600 ELISA tests for *S. citri* using acorn disease affected tissues collected from field or greenhouse grown plants were positive. But ELISA tests were positive using the known infected young shoots of pummelo collected in greenhouse.

Attempts to culture *S. citri* and ELISA test from periwinkle gave negative results. The periwinkle plants remained symptomless for two years in the field.

None of the trees obtained by STG showed symptoms on leaves (zinc deficiency, abnormal thickness and cupping, etc.) so far after 2 yr.

DISCUSSION

Tarocco and Golden Buckeye sweet oranges in field show acorn fruit symptoms previously attributed to stubborn disease. Our tests show that this malady is graft transmissible.

But attempts to isolate *S. citri* and detect it by ELISA were negative. These results indicate that acorn dis-

ease is due to some agent other than *S. citri*. Other attempts to isolate *S. citri* carried out in Sicily from symptomatic citrus trees (Cartia and Rana, unpublished data) and from *Sal-sola kali* (Davino, unpublished data) both field collected, gave negative results. It is possible to assert that *S. citri* is not present in Sicily. Also attempts to infect periwinkles in field have always given negative results although many leafhoppers, natural vectors of *S. citri* were present in the same fields. Natural infection of periwinkles in Arizona and California with spiroplasmas reported (1, 6). More recently natural transmission of *S. citri* was reported in Morocco (7).

STG demonstrates the infectious nature of this new alteration. On the ground of our results we can assert that acorn fruit is not a symptom specific to stubborn disease but caused by an infectious agent. Further research is necessary to clarify this question.

ACKNOWLEDGMENTS

The authors acknowledge C.N. Rois-tacher for supplying the *Spiroplasma citri* strain and J. M. Bové for antiserum to *S. citri*.

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