Influence of Temperature on Symptoms of California Stubborn, South Africa Greening, India Citrus Decline, and Philippines Leaf Mottling Diseases

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Symptoms of citrus stubborn and greening diseases are influenced by temperatures at which the affected plants grow. Symptoms of stubborn are severe in hot areas of California, Arizona, and Morocco, while those of greening in South Africa are strong in high, cool areas and less severe in low, hot areas (*10*). In greenhouse studies, cool conditions favored development of greening symptoms (*8*) whereas temperatures between 30° and 35° C were best for symptoms of stubborn (*5*).

To further document the influence of temperature on symptomatology, the two diseases were compared side by side under conditions of controlled temperature and humidity. Philippines leaf mottling and India citrus decline, two diseases thought to be related to South Africa greening (*9*), were included in the comparison.

MATERIALS AND METHODS

This work was conducted in the phytotron, at Gif sur Yvette, France.

California stubborn. Eight Madam Vinous sweet orange seedlings affected by stubborn (California 189) but free of known citrus virus and viruslike diseases, were sent from Riverside, California, in March, 1969. They were planted in 5-liter plastic containers, cut back severely to favor new growth, and transferred to the phytotron in April, 1969.

Mycoplasmalike organisms seen in the sieve tubes of the affected Madam Vinous seedlings have been isolated and obtained in pure culture (*6*), and characterized and described as *Spiroplasma citri* (*7*).

South Africa greening. Eloff sweet orange seedlings infected with greening (Nelspruit strain) by *Trioza erytreae* came from Nelspruit in January, 1967, and served as the source of South Africa greening. Budwood sticks were taken from one of the infected plants and used as inoculum to infect Hamlin sweet orange seedlings.

Budwood sticks from one infected Hamlin sweet orange seedling were propagated on seedling Orlando tangelo stocks in July, 1969. Two batches of eight plants each were transferred immediately to the phytotron.

Filamentous structures resembling microorganisms have been seen in the sieve tubes of the affected plants, and have envelopes 150 to 250 Å thick (*6*).

India citrus decline. A Mosambi sweet orange seedling, infected with India citrus decline (Poona strain) by *Diaphorina citri,* was sent to France in December, 1969, and served as inoculum. In June, 1970, Hamlin sweet orange seedlings were inoculated by the technique of Calavan *et al.* (*1*). Two batches of nine plants each were then cut back and transferred immediately to the phytotron.

In a second experiment, eight Madam Vinous and eight Hamlin sweet orange seedlings were inoculated with leaf patches from the affected Mosambi sweet orange seedling, in October, 1969. They were cut back and transferred to the phytotron the next day.

Filamentous structures with envelopes 150 to 250 Å thick have been observed in the sieve tubes of affected plants (*6*).

Philippines leaf mottling. Ladu mandarin seedlings, infected with Philippines leaf mottling (Lipa strain) by *Di-*

aphorina citri in April, 1970, were sent to France in July, 1970. In October, 1970, they were used to inoculate Hamlin and Madam Vinous sweet orange seedlings by the leaf-piece technique (*1*). Eight days later, four Hamlin and four Madam Vinous sweet orange seedlings were cut back and placed in the phytotron.

Filamentous structures with envelopes 150 to 250 Å thick have been observed in the sieve tubes of affected plants (*6*).

Uninoculated Hamlin and Madam Vinous sweet orange seedlings were used as controls.

All plants were grown in 5-liter, cylindrical, plastic containers with glass wool in the lower one-third and vermiculite in the upper two-thirds. They automatically received complete nutrient solution by the drip method.

Half of the plants were grown in a chamber under cool conditions, the other half in another chamber, under warm conditions. Cool conditions consisted of 22° C for 8-hr nights and 24° C for 16-hr days; warm conditions consisted of 27° C for 8-hr nights and 32° C for 16-hr days. Relative humidity was 80 per cent, except at night, when 60 per cent was maintained in the warm chamber.

The plants in the cooler chamber were exposed to daylight illumination supplemented with mercury-vapor incandescent lamps to achieve a 16-hr light period.

The plants in the warmer chamber were illuminated with artificial light (fluorescent tubes with incandescent lamps) giving an illumination of 20,000 lux at 1 m below the lights, for 16 hours per day.

RESULTS

The results are summarized in table 1. Madam Vinous seedlings affected by stubborn and placed under warm conditions produced new growth within 3 weeks and showed severe symptoms of stubborn within 5 weeks. Seedlings in the cool chamber produced new growth within 3–5 weeks, but showed only mild symptoms after 26 weeks in the phytotron. The typical symptoms of stubborn (small, cupped leaves with pale-green tips and mottling) were obtained only under warm conditions. In the cool environment, the leaves showed unspecific mottling and only moderate reduction in size. After 43 weeks the affected seedlings had an average height of 120 cm at 22°–24° C and 93 cm at 27°–32° C, while the healthy control plants measured 160 cm and 205 cm, respectively, at 22°–24° C and 27°–32° C.

Plants in the warm room were transferred to the cool conditions and *vice versa*. Plants now in the cool environment produced new growth with large leaves, in contrast to the small, cupped leaves from the previous growth under warm conditions. Conversely, small, cupped leaves were obtained under warm conditions on the plants held previously under cool conditions. Seedlings were reciprocally transferred once more and, as expected, they reacted as they had in the beginning.

It should be added that *Spiroplasma citri* (*7*), the spiral shaped, culturable mycoplasmalike microorganism consistently associated with stubborn (*2, 3, 6*), was more abundant in sieve tubes of plants growing in the warm chamber than in those growing under cool conditions (*4*). Also, it is interesting to note that *S. citri* has an optimum growth temperature of about 32° C (7), which is close to the most favorable temperature for development of stubborn symptoms.

With South Africa greening, severe symptoms were obtained under the cool conditions, whereas at 27°–32° C no symptoms developed (table 1). After 30 weeks at 22°–24°C the affected plants measured only 35 cm in height as against 190 cm for those under the

TABLE 1
EFFECT OF TEMPERATURE ON SYMPTOMS IN SWEET ORANGE PLANTS INFECTED WITH STUBBORN, GREENING, CITRUS DECLINE, OR LEAF MOTTLING PATHOGENS

Disease	Treatment duration (weeks)	Symptom severity* at: 22°–24°C	27°–32°C
Stubborn (Calif. 189)	5	0	S
	26	M	S
	43	M	S
Greening (Nelspruit, S. Africa)	18	M	0
	30	S	0
	72	S	0
Citrus decline (Poona, India)	11	M	M
	15	M	M–S
	30	S	S
Leaf mottling (Lipa City, P. I.)	11	M	M
	17	M	S
	35	S	S

* 0 = symptomless; M = mild; S = severe.

warm conditions. When severely diseased plants were transferred to the warm conditions after 40 weeks in the cool chamber, they quickly produced new, vigorous growth, recovered, and remained symptomless during the remaining 10 months of the experiment.

India citrus decline and Philippines leaf mottling reacted similarly. Symptoms were as pronounced at 22°–24° C as at 27°–32° C. Some seedlings produced more growth in the warmer chamber but were as severely affected as those in the cooler room (table 1). After 30 weeks under the warm conditions, the seedlings infected with India citrus decline were 25 cm in height while the healthy controls reached 180 cm. In Philippines leaf mottling, affected plants grown for 17 weeks at 27°–32° C were 40 cm high while the healthy control seedlings measured 140 cm.

DISCUSSION AND CONCLUSIONS

South Africa greening, on one side, and India citrus decline and Philippines leaf mottling on the other, show different effects under similar temperatures. All three diseases, however, are characterized by the presence, in the sieve tubes, of similar filamentous structures resembling microorganisms, with envelopes 150 to 250Å thick—too thick to be unit membranes (*4, 6*). If these structures represent the causal agents of the respective diseases, those associated with heat-sensitive South Africa greening, on the one hand, and those relative to the heat-tolerant India citrus decline and Philippines leaf mottling, on the other, must be different strains since they seem to have different heat sensitivities as judged from the influence of temperature on symptoms. The strain difference may be reflected in the difference in vectors. The heat-tolerant causal agent is transmitted by *Diaphorina citri*, the tropical citrus psylla, whereas the heat-sensitive greening disease agent is transmitted by the African citrus psylla, *Trioza erytreae*. If this theory is correct, we can predict that likubin belongs with citrus decline and leaf mottling, in the heat-tolerant group.

While stubborn is also heat-tolerant, it cannot be part of that group because the structures seen in sieve tubes, presumably the causal agent of stubborn, differ from those associated with greening, citrus decline, and leaf mottling.

LITERATURE CITED

1. CALAVAN, E. C., E. O. OLSON, AND D. W. CHRISTIANSEN
1972. Transmission of the stubborn pathogen in citrus by leaf-piece grafts. *In:* Proc. 5th Conf. Intern. Organ. Citrus Virol. (W. C. Price, ed.). Gainesville: Univ. Florida Press, pp. 11–14.

2. FUDL-ALLAH, A. E.-S. A., E. C. CALAVAN, AND E. C. K. IGWEGBE
1971. Culture of a mycoplasmalike organism associated with stubborn disease of citrus. Phytopathology **61:** 1321.

3. FUDL-ALLAH, A. E.-S. A., E. C. CALAVAN, AND E. C. K. IGWEGBE
1972. Culture of a mycoplasmalike organism associated with stubborn disease of citrus. Phytopathology **62:** 729–31.

4. LAFLÈCHE, D., and J. M. BOVÉ
1970. Mycoplasmes dans les agrumes atteints de "greening," de "stubborn" ou de maladies similaires. Fruits **25:** 455–65.

5. OLSON, E. O., AND B. ROGERS
1969. Effects of temperature on expression and transmission of stubborn disease of citrus. Plant Dis. Reptr. **53:** 45–49.

6. SAGLIO, P., D. LAFLÈCHE, C. BONISSOL, AND J. M. BOVÉ
1971. Isolement, culture et observation au microscope électronique des structures de type mycoplasme associées à la maladie du stubborn des agrumes et leur comparison avec les structures observées dans le cas de la maladie du greening des agrumes. Physiol. Veg. **9:** 569–82.

7. SAGLIO, P., M. LHOSPITAL, D. LAFLÈCHE, G. DUPONT, J. M. BOVÉ, J. G. TULLY, AND E. A. FREUNDT
1974. Characterization of the organism associated with citrus stubborn disease and its description as a new member of the Mycoplasmatales. Jour. Bact. (in press).

8. SCHWARZ, R. E.
1968. Indexing and epidemiology of greening and some other citrus virus diseases in South Africa. D. Sc. thesis, Univ. Pretoria, South Africa.

9. SCHWARZ, R. E.
1972. A review of stubborn and greening diseases of citrus. *In:* Proc. 5th Conf. Intern. Organ. Citrus Virol. (W. C. Price, ed.). Gainesville: Univ. Florida Press, pp. 1–5.

10. SCHWARZ, R. E., AND G. C. GREEN
1972. Heat requirements for symptom suppression and inactivation of the greening pathogen. *In:* Proc. 5th Conf. Intern. Organ. Citrus Virol. (W. C. Price, ed.). Gainesville: Univ. Florida Press, pp. 44–51.