Effect of Temperature on Concentration of Threadlike Particles, Stem Pitting, and Infectivity of Budwood from Tristeza-infected Palestine Sweet Lime

M. Bar-Joseph and G. Loebenstein

Palestine sweet lime is widely used as a rootstock for Shamouti orange in eastern Mediterranean countries. Sweet lime stock reacts to tristeza infection with pitting of the stem followed by decline after several years' good growth.

Infection by tristeza is accompanied with threadlike particles (TLP), approximately 10 to 12 × 2,000 nm (*5*). Similar particles have not been detected in noninfected plants. This paper reports a study of TLP concentration in relation to symptoms and infectivity of budwood at different temperatures.

MATERIALS AND METHODS

Sweet lime seedlings were grown in a screened greenhouse, in cans containing a 5-kg mixture of sandy loam, sand, and peat (2:1:1), for 18 months. Plants were inoculated by side grafting with two buds, 10 cm apart, carrying a strain of tristeza (ST) described previously (*2*). One week later the plants were topped at 50 cm, and after an additional two weeks they were transferred to greenhouse chambers, at temperatures of approximately 22, 27, 31 and 36° C. A single shoot was allowed to develop on each plant.

Threadlike particles were partially purified from 25 gm of bark, and counts were made in the electron microscope. Different concentrations of tobacco mosaic virus (TMV) served as a reference (*1*). Threadlike particle concentration per gm of bark was established by use of the following formula (*8*):

$$TLP\ \mu g/g = \frac{A \times MW_{TLP}}{MW_{TMV}}$$

A = Concentration of *TMV* (μg/ml), where the number of particles on the grid equals the number of *TLP*.

MW_{TLP} = Molecular weight of one *TLP* with an average length (1,000 mμ) = 70×10^6 (*2*).

MW_{TMV} = Molecular weight of *TMV* = 40×10^6.

RESULTS

Concentration of TLP. TLP content in bark of sweet lime plants, kept at 22° C, was determined at monthly intervals, starting 60 days after inoculation. Averages from two experiments, each with five to eight plants, are summarized in figure 1. Highest content was observed six months after inoculation, followed by a gradual decrease.

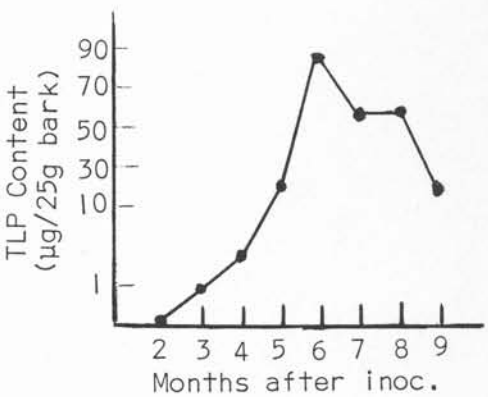

Fig. 1. Concentration of threadlike particles (TLP) at different time intervals after inoculation in 25 gm of bark from sweet lime plants kept at 22° C.

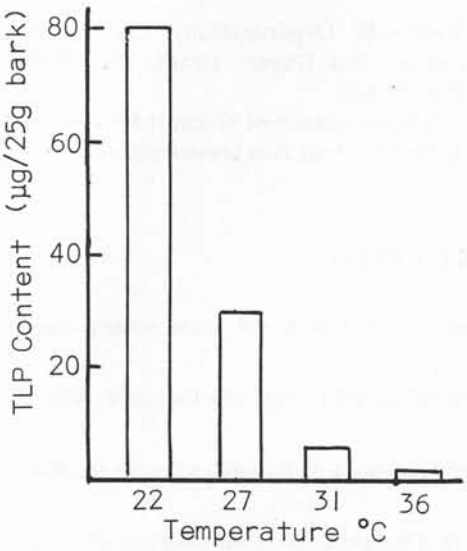

Fig. 2. Concentration of threadlike particles (TLP) in 25 gm of bark from sweet lime plants kept at four different temperatures and sampled six months after inoculation.

No differences in length or structure of TLP were observed, suggesting that lower content was not the result of TLP degradation.

To determine the effect of different temperatures, two groups of five plants each, for each temperature treatment, were sampled six months after inoculation (fig. 2). TLP content was highest in plants kept at 22° C, and decreased with increasing temperatures.

Stem pitting and infectivity of budwood. When plants were kept at 22° C for six months after inoculation, the number of stem pittings averaged 30 per 100-cm branch, and decreased to 7, 0, and 0 in plants kept at 22, 31, and 36° C, respectively. For infectivity assays, four plants were held at each temperature, and eight buds from each plant were indexed on Egyptian lime by inserting two buds into each indicator plant. All indicator plants became infected, and no difference in time of symptom appearance was noted. No differences in the infectivity of budwood were observed, when samples were taken from plants kept for six months after inoculation at 22, 27, or 31° C. Only two out of 15 indicators reacted positively, however, when budwood originated from plants kept at 36° C, and symptoms in indicator plants seemed to appear somewhat later.

Location of TLP in girdled plants. In an experiment aimed at associating TLP with infectivity, sweet lime plants were girdled immediately after graft inoculation, either above or below the grafts. Four and eight months later, bark was extracted and examined for TLP. In plants inoculated above the girdle, TLP were found only above the ring, and in plants inoculated below the girdle, only below the ring. This suggests that, as with infectivity (*3, 7, 9*), TLP do not pass across severed phloem.

DISCUSSION

Content of TLP in the bark of plants kept at 22° C increased until six months after inoculation, and then declined gradually. This decrease did not seem to result from degradation of particles, as observed recently in *Tetragonia* plants infected with beet yellows virus (*4*), because no differences in structure or length of TLP were observed. Virus increase followed by a decrease is known in many virus-host combinations (*6*). Differences in TLP concentration from plants grown at 22, 27 or 31° C did not affect budwood infectivity. In plants grown at 36° C, low TLP content was correlated with a marked decrease in the infectivity of budwood. It should be mentioned that indexing of such plants by the lime test (*10*), using four indicator plants, would probably be negative. Conversely, enough TLP would be present to make diagnosis by electron microscopy positive.

ACKNOWLEDGMENTS

This paper represents part of a Ph.D. thesis presented by the senior author to the Hebrew University of Jerusalem.

The study was made possible in part by a contribution from the Agricultural Research Organization, the Volcani Center, Bet Dagan, Israel. 1972 Series, No. 2156-E.

The assistance of Shlomit Shilling and J. Ben-Shalom is acknowledged.

LITERATURE CITED

1. BAR-JOSEPH, M., G. LOEBENSTEIN, AND J. COHEN
   1970. Partial purification of viruslike particles associated with the citrus tristeza disease. Phytopathology **60:** 75–78.
2. BAR-JOSEPH, M., G. LOEBENSTEIN, AND J. COHEN
   1974. Further purification and characterization of threadlike particles associated with the citrus tristeza disease. Virology (in press).
3. COSTA, A. S., T. J. GRANT, AND S.MOREIRA
   1949. Investigacoes sobre á tristeza dos citrus. II. Conceitos e dados sobre a reacáo das plantas cítricas á tristeza. Bragantia **9:** 59–80.
4. ESAU, K., AND L. L. HOEFERT
   1971. Cytology of beet yellows virus infection in Tetragonia. III. Conformations of virus in infected cells. Protoplasma **73:** 51–65.
5. KITAJIMA, E. W., D. M. SILVA, A. R. OLIVEIRA, G. W. MÜLLER, AND A. S. COSTA
   1964. Thread-like particles associated with tristeza disease of citrus. Nature **201:** 1011–12.
6. LOEBENSTEIN, G.
   1972. Inhibition, interference and acquired resistance in plant viruses. *In:* Principles and techniques in plant virology. (C. I. Kado and H. O. Agrawal, eds.) New York: Van Nostrand Reinhold Co., pp. 32–61.
7. NOUR-ELDIN, F., AND M. T. EL-BANNA
   1965. Distribution and movement of psorosis and tristeza viruses in citrus plants. *In:* Proc. 3rd Conf. Intern. Organ. Citrus Virol. (W. C. Price, ed.) Gainesville: Univ. Florida Press, pp. 272–75.
8. POLSON, A., L. STANNARD, AND D. TRIPCONEY
   1970. The use of haemocyanin to determine the molecular weight of *Nudaurelia cytherea capensis* β virus by direct particle counting. Virology **41:** 680–87.
9. PRICE, W. C., AND L. C. KNORR
   1960. Isolation, characterization and interrelationship of citrus viruses. Univ. Florida Exp. Sta. Rept., p. 204.
10. WALLACE, J. M.
   1968. Tristeza and seedling yellows. *In:* Indexing procedures for 15 virus diseases of citrus trees. (J. F. L. Childs, chmn.) Washington D.C.: USDA Agr. Handbook No. 333, pp. 20–27.