A NONCIRCULATIVE WHITEFLY-BORNE VIRUS AFFECTING TOMATOES IN ISRAEL

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A rod-shaped virus, 700 nm long, was found to be associated with chlorosis in tomatoes grown in the northern Negev of Israel. The viral agent could be transmitted mechanically to hosts of the Solanaceae family only. The virus was found to have a noncirculative relationship with the insect vector, the tobacco whitefly *Bemisia tabaci* Gennadius. This report presents some information on the viral agent, and its relationship with the insect vector.

KEY WORDS: Virus-vector relationship; Tomato Pale Chlorosis Disease; *Bemisia tabaci*.

INTRODUCTION

In the course of a survey carried out in tomato fields and greenhouses in the northern Negev region of Israel, a few plants with normal development but which were pale and chlorotic, were noted during the autumn season. The disease was named Tomato Pale Chlorosis Disease (TPCD). The present work was initiated to verify the nature of the causal agent of this new disease.

MATERIALS AND METHODS

Virus and vector culture

The virus isolate was obtained from naturally infected greenhouse-grown tomatoes. Cultures of the virus were maintained on *Datura stramonium* L. or on *Nicotiana glutinosa* L. plants, and renewed every 3 weeks by insect and mechanical inoculations alternately. Test plants were grown in an insect-proof greenhouse regularly fumigated with nicotine sulfate. Mechanical inoculations were made on 400-mesh carborundum-dusted plants. The procedure for the maintenance of whiteflies was described previously (3). The aphid *Myzus persicae* Sulz. was cultured on Japanese cabbage, and *Aphis gossypii* Glover was reared on cucumber seedlings. Insects were
allowed an acquisition feeding on *D. stramonium* plants immediately after the appearance of symptoms. For inoculation feeding, insects were transferred to *D. stramonium* seedlings when the first true leaves were fully expanded.

**Transmission by insects**

Several hundred individuals of *M. persicae* and *A. gossypii* were given different acquisition and inoculation feeding periods ranging from 1 minute to several days. Whitefly females held in leaf cages were allowed different periods of acquisition feed and were then transferred to healthy test plants for different periods of inoculation feed. For persistence tests, experiments were conducted as follows: Each leaf cage containing 60 whitefly females was attached for 24 h to young leaves of *D. stramonium* infected with TPCD. At the end of this period the cages were removed and divided at random into seven groups. For the first inoculation feed, four groups were allowed to feed for 2, 4, 6 and 24 h, respectively. Upon completion of its respective inoculation period, each group was immediately transferred to a second inoculation feed lasting 24 h. At the same time, the three remaining groups were starved for 2, 4 and 6 h before being transferred for a 24-h inoculation feeding period.

Results of insect transmission trials were analyzed by nonparametric tests (8).

**Electron microscopy**

For detection of virus particles, pieces of the lower epidermis of *N. glutinosa* leaves showing typical vein-clearing symptoms were immersed in a drop of 0.2 M phosphate buffer, pH 7, mounted on a grid. Particles were negatively stained with 1% ammonium molybdate dissolved in 0.05 M ammonium acetate, pH 7. For determination of normal length, particles were photographed at an original magnification of 57,143 as calibrated with a 54,864 lines/inch grating replica. Using the decoration technique (6), serial dilutions of Sweet Potato Mild Mottle Virus (SPMV) antiserum were incubated with virus particles adsorbed to grids. Hollings' SPMV antiserum was kindly provided by Dr. M. Bar-Joseph, ARO, The Volcani Center, Israel. For fixation of tissue, small portions of systemically infected *N. glutinosa* leaves were treated with 4% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.4, post-fixed in 2% OsO₄, and then dehydrated through graded ethanol and embedded in epon. The tissue was sectioned on an LKB microtome with a glass knife. Pieces of healthy tissue served as controls.

All microscopic examinations were made with a JEM 7A electron microscope operating at 100 KV.

**RESULTS**

**Host range**

The following plant species and varieties developed symptoms when mechanically inoculated with TPCD and by using the insect vector: *Datura stramonium* L. – Vein clearing followed by systemic mosaic. These symptoms are accompanied by small necrotic local lesions (in winter only) and distortion of the leaves. *Lycopersicon*