Transmission and Physical Properties of the Causal Agent of Mosaico Amarillo del Tomate (Tomato Yellow Mosaic)

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ABSTRACT

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Mosaico amarillo del tomate (MAT) (tomato yellow mosaic) is an important disease affecting tomatoes in Venezuela. The causal agent was mechanically transmitted to several species of the family Solanaceae, with the aid of 0.1 M potassium phosphate alone or containing 1% magnesium trisilicate. The use of 2-mercaptoethanol, dithiothreitol, and sodium diethyldithio-carbamate in the transmission experiments also increased the probability of transmission and kept the inoculum infectious for a longer period of time. The MAT agent is very labile; longevity in vitro is no longer than 15 min. The thermal inactivation point is between 40 and 42 C. The white fly *Bemisia tabaci* is an efficient vector of the agent. After an acquisition period of 2 hr and a latent period of 20 hr, the vectors were able to transmit the agent sporadically for a maximum of seven days.

Additional key words: pathogens transmitted by white flies.

Tomatoes in Venezuela are affected by several diseases (7), one of the most prevalent being the mosaico amarillo del tomate (MAT) (tomato yellow mosaic). Frequently 90-100% of the tomato plants in commercial fields are affected at flowering time by this disease. Mosaico amarillo del tomate was first reported in Venezuela during 1963 (4) as a virus transmitted by the tobacco white fly *Bemisia tabaci* Gennadius. However, it was reported as not mechanically transmissible. Recently, we succeeded in demonstrating the mechanical transmission of the causal agent of this disease. This characteristic places MAT in a small group of white fly transmitted agents which share the same property (2). In this paper, we report several ways by which the normally low percentage of mechanical transmission can be enhanced, the characteristics of insect transmission, and some physical properties of the causal agent.

MATERIALS AND METHODS

The agent was first isolated from field tomatoes by means of the vector *B. tabaci.* Mechanical transmission was carried out by grinding young leaves of infected plants in several buffers, antioxidants and inoculation aids in order to determine their potential for increasing the level of mechanical transmission. The inoculum was applied to cotyledons and first true leaves previously dusted with 600-mesh Carborundum. Inoculated plants were rinsed with distilled water and placed in an insect-proof greenhouse kept at a temperature range of 20-30 C for further examination. In the host range experiments all test plants were used for back inoculation to tomatoes after 30 days, to recover the agent and confirm its identity.

Whiteflies used in transmission experiments were obtained from a healthy colony kept on *Pseudoconiza lyrata* (H.B.K.), a plant which was found in nature to be heavily colonized by *B. tabaci.* To test the susceptibility of plants to the disease, 50 insects were placed in small plastic and nylon cages, and allowed to feed for 24, 48, and 72 hr on diseased plants. Afterwards, they were placed on healthy plants and then transferred daily to new plants. To study the vector relationships, one insect per plant was used, with several replications. After the insects were fasted for three hours, the following acquisition periods were used: 10, 15, and 30 min and 1, 2, 6, 12, and 24 hr. To study the latent period, insects were kept for 12 hr on a tomato plant and then were transferred every hour for the next 12 hr to new plants; afterwards they were transferred every 24 hr. For the persistence experiments insects were transferred to new plants every 24 hr after the latent period.

RESULTS

Stability.—The infectivity of sap expressed from infected leaves was lost in 15 min when kept at room temperature. Different buffers were used in order to try to stabilize infectivity. The following buffers stabilized infectivity to some extent: 0.01 and 0.1 M sodium phosphate pH 8.0; 0.01 and 0.1 M Tris-HCl (Tris-hydroxymethyl aminomethane) pH 8.0. The infectivity also was stabilized by 0.01 and 0.1 M K_2HPO_4 containing 1% magnesium trisilicate adjusted to pH 8.0 with KOH (6). Infected plant sap extracted with 0.1 M phosphate buffer pH 8.0 and 0.1 M K_2HPO_4 + 1% magnesium trisilicate pH 8.0 still retained a quarter of the initial infectivity after 2 hr (Table 1). Consequently, one of these two buffers was used regularly for all subsequent mechanical inoculations.

To further improve the efficiency of transmission, several additives were tested in combination with 0.01 M

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and 0.1 M phosphate buffer pH 8.0. Samples of extracted plant sap were taken at 0, 1, and 4 hr to check infectivity. Dithiothreitol, 2-mercaptoethanol, and sodium diethyldithiocarbamate (dieca) all were useful in preserving infectivity (Table 2).

Factors affecting mechanical inoculation.—Apical leaves showing symptoms of the disease were a better source of inoculum than bottom leaves from the same plant. Tomatoes inoculated with sap extracted from apical leaves showed 90-100% infection, but only 50-70% of the test plants inoculated with sap from old leaves became infected. To test the change of susceptibility with age, twenty tomato plants 10, 12, 14, 15, 17, 20, 22, 25, and 27 days after seeding were inoculated simultaneously with the same inoculum. Young tomato plants were most susceptible to the disease, and this susceptibility gradually was lost with age. Ninety to 100% of the 10- to 12-day-old plants became infected, but only 10% of the 26-day-old plants became infected.

The weight/volume ratio of infected plant material to buffer was found to be important in mechanical inoculation. Different ratios of 0.1 M K_2HPO_4 + 1% magnesium trisilicate and plant tissue were tried. Twenty plants were inoculated for each ratio tested. The w/v ratios tested and the percent of infected plants (in parentheses) were: 1:1 (55%); 1:2 (50%); 1:3 (70%); 1:4 (75%); 1:5 (80%); 1:8 (45%); and 1:10 (30%).

Several preinoculation treatments were tried in order to study their effect on susceptibility. Plants to be inoculated were held 24 and 48 hr at a temperature of 30-40 C or at our normal greenhouse temperature of 20-30 C. After inoculation of twenty plants with three repetitions, 63% of the plants held for 24 hr at 20-30 C became infected and 55% of the plants kept for 48 hr at 20-30 C were infected. When plants were kept at 30-34 C for 24 hr 96% became infected and 82% of the plants kept for 48 hr were infected. In another experiment the plants were placed for the same period of time in dark or light (1,200 lux) conditions. After 24 and 48 hr treatments only 52% of the plants kept in the dark became infected; those kept under light showed 80% infection. High temperature increased the percentage of the plants being infected; darkening of the plants before inoculation resulted in fewer infected plants.

Host range and symtomatology.—The pathogen was transmitted mechanically to the following plants: *Lycopersicon esculentum* Mill., *Lycopersicon pimpinellifollium* (Jusl.) Mill., *Lycopersicon hirsutum*

TABLE 1. Effect of extraction buffer on the stability of the mosaico amarillo del tomate (tomato yellow mosaic) (MAT) agent

Buffers[a]	Plants infected[b] by MAT agent at extraction plus: 0 hr (%)	1 hr (%)	2 hr (%)
Control[c]	0	0	0
0.01 M phosphate	100	0	0
0.1 M phosphate	90	55	25
0.01 M K_2HPO_4 + 1% $Mg_2Si_3O_8$	80	25	0
0.1 M K_2HPO_4 + 1% $Mg_2Si_3O_8$	90	35	30
0.01 M Tris-HCl	40	10	15
0.1 M Tris-HCl	85	75	10

[a]All buffers were adjusted to pH 8.0.
[b]Twenty test plants inoculated for each treatment and time period.
[c]Distilled water adjusted to pH 7.

TABLE 2. Efficacy of agents in preserving the infectivity of sap extracted from tomato plants infected with mosaico amarillo del tomate (tomato yellow mosaic) (MAT) agent

Molarity of phosphate buffer[a]	Agent	Concentration	Plants infected[b] by MAT 0 hr (%)	1 hr (%)	4 hr (%)
0.01 M	2-Mercaptoethanol	0.01 M	95	95	90
0.1 M	2-Mercaptoethanol	0.01 M	85	85	70
0.01 M	Dithiothreitol	0.02 M	85	55	55
0.1 M	Dithiothreitol	0.02 M	65	65	35
0.01 M	Nicotine	2%	40	5	0
0.1 M	Nicotine	2%	10	0	0
0.01 M	DIECA	0.01%	85	80	80
0.1 M	DIECA	0.01%	75	50	35
0.01 M	Control[c]	...	80	10	0
0.1 M	Control[c]	...	75	20	0

[a]All buffers were adjusted to pH 8.0.
[b]Twenty test plants were inoculated for each treatment and time period.
[c]Distilled water adjusted to pH 7.0.

Humb and Bompl., *Datura stramonium* L., *Nicotiana glutinosa* L., *Petunia hybrida* Vilm., *Nicandra physaloides* L., *Physalis peruviana* L., and *Nicotiana tabacum* L. 'Samsun', 'White Burley', and 'Virginia'. The symptoms were similar in the *Lycopersicon* species and consisted of a bright yellow mosaic, curling of leaves, and stunting of the plant (Fig. 1). Plants of the other species which became infected showed faint yellow spots and vein-clearing followed by a very pale yellow mosaic. In the field, the pathogen was only found on and isolated from tomatoes.

The following species and cultivars apparently were immune to the pathogen since they did not develop symptoms after mechanical inoculation and *B. tabaci* transmission, and the pathogen could not be recovered from inoculated plants: Solanaceae: *Capsicum frutescens* L. 'Yolo Wonder' and 'Conoides'; *Datura metel* L.; and *Solanum melongena* L.. Leguminosae: *Arachis hypogaea* L. 'Roxo' and 'Virginia'; *Phaseolus vulgaris* L. 'Stringless Green Pod', 'Kentucky Wonder', 'Top Crop', 'Coche', 'Cubagua', and 'Bountiful'; *Pisum sativum* L. 'Tall Alderman' and 'Alaska'; *Canavalia ensiformes* DC.; *Lens culinaria* Medic.; *Vicia faba* L.; *Vigna sinensis* L. 'SR' and 'RS'. Malvaceae: *Gossypium hirsutum* L.; *Althaea rosea* L.; *Malva parviflora* L.; *Sida rhombifolia* L.; *S. triloba* L.; *Abutilon hirsutum* (Lam.) Sweet. Chenopodiaceae: *Chenopodium quinoa* Willd.; *C. amaranticolor* Coste & Reyn., and *C. album* L.. Cruciferae: *Raphanus sativus* L.; and *Brassica oleracea* L.. Euphorbiaceae: *Euphorbia prunifolis* Jacq. Cucurbitaceae: *Bemincasa hispida* Cogn.; *Bryonia alba* Jacq.; *Sicana odorifera* (Vell.) Naud.; *Momordica charantia* L.; *Luffa acutangula* L. (Roxb.); *Ecbalium elterium* L. (Rich); *Cucumis sativus* L.; and *Cucurbita pepo* L. Labiatae: *Leonorum sibiricus* L.

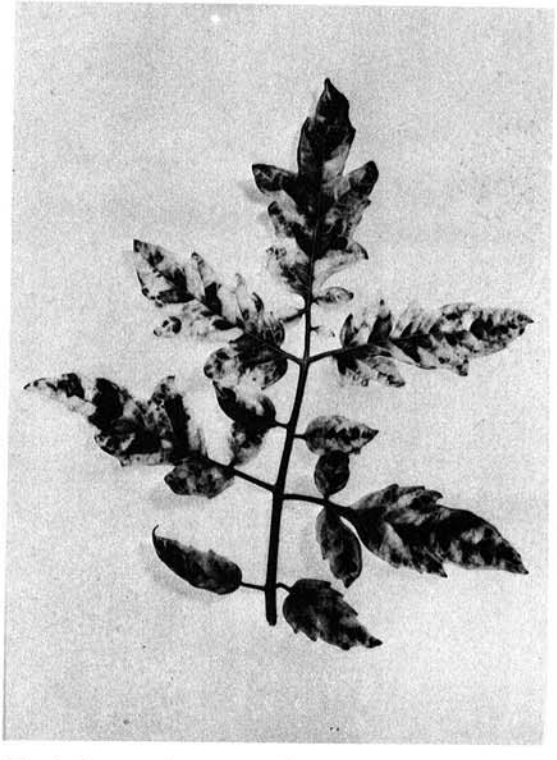

Fig. 1. Leaves of tomato cultivar Marglobe infected with mosaico amarillo del tomate (tomato yellow mosaic) showing bright yellow mosaic and slight curling of the leaflets.

Longevity in vitro.—Sap extracted from infected plants was divided into 1-ml aliquots. Samples were kept at room temperature (20-22 C) and inoculated into tomato plants at 5, 10, 15, and 20 min and 1 and 4 hr. The infectivity was completely lost within 15 min.

Thermal inactivation.—Since infectivity is rapidly lost, infectious plant sap was simultaneously treated in five water baths at 39, 40, 41, 42, and 43 C for 10 min. After treatment, the plant sap was cooled on an ice bath and immediately inoculated to tomato plants. The thermal inactivation point of the MAT agent was between 41 and 42 C.

Insect transmission.—The minimum acquisition period for *B. tabaci* was 2 hr. In 10 different experiments it was not possible to produce transmission with insects which had fed for less time on infected tomatoes. A 20-hr latent period was needed for the insect to transmit the disease. Thereafter, the insect remained infectious for a maximum of 7 days. The transmission pattern was not consistent. After successfully transmitting the disease agent several times, insects failed to do so on successive days, but recovered the ability to transmit the pathogen during the following days.

The efficiency of transmission was 93% when the insects were kept in a 30-34 C range. When they were kept at 20-30 C only 75% transmission was achieved. Females were more efficient than males as transmitters of the causal agent. When females were used exclusively in the transmission experiments 95% successful transmission was obtained against 75% when only males were used.

Seed transmission.—Seeds were collected from four different groups of infected tomato plants grown in the greenhouse. Seeds were planted 1 to 6 mo after collection and total of 1,500 plants were allowed to grow for 45 days. Plants were checked periodically for symptoms of the disease. None of these plants showed symptoms of MAT during this period.

DISCUSSION

Even though the MAT disease has been reported not to be mechanically transmitted (4), we were able to accomplish this with a high percentage of efficiency. The failure to obtain mechanical transmission (4) could be due to the lability of the causal agent in extracted plant sap. With the help of a buffer and an antioxidant, and by using young plants, we obtained close to 100% transmission. The fact that an inoculum/buffer ratio of 1/5 (g/ml) yielded a higher percentage of infected plants than did a lower ratio could indicate the presence of inhibitors in the tomato plant sap.

There are only a few reports of mechanical transmission of white fly transmitted agents. Abutilon mosaic virus (AMV), bottle gourd mosaic virus (BoGMV), bean golden mosaic virus (BGMV), and Euphorbia mosaic (EM) (2) have been reported to be mechanically transmissible. Because of the difficulty in mechanical transmission of these agents, little is known

about their physical properties. Longevity in vitro was reported to be 24 hr for AMV and 48 hr for BGMV (2). In contrast, we found that MAT has a very short longevity in vitro (only 15 min) and this probably was one of the reasons why it was not successfully transmitted by earlier workers. Thermal inactivation also was lower than those reported for AMV, BoGMV, and BGMV which are in the range of 52-55 C (2).

Bemisia tabaci is an efficient vector of the agent, especially under high temperature conditions. Acquisition, incubation, and persistence of the agent in the insect is well within the range of several white-fly-transmitted agents (2). It was suggested by Debrot et al. (4) that MAT might be related to AMV or tobacco leaf curl (TLC). Even though AMV also is mechanically transmitted, the main hosts for this agent are *Althea rosea* L., *Malva parviflora* L., *Sida rhombifolia* L. and *Gossypium hirsutum* L. (3), which are immune to MAT whereas the TLC agent has not been proved to be mechanically transmissible. Furthermore, the symptoms caused by the TLC agent on tobacco differ considerably from the MAT symptoms.

There are several tomato diseases that have symptoms similar to MAT. They are tomato yellow leaf curl found in Israel (1), a yellow mosaic on tomatoes in India which the authors suggested could be related to the disease found in Israel (9), and a viruslike disease of tomatoes recently reported in Nigeria (5). These diseases may be related to MAT since they share similar symptoms and the same vector. However, none of the pathogens of these diseases has been mechanically transmitted and the host range was somewhat different. A similar disease of tomatoes was found in Brasil and it was called mosaico dourado do tomateiro (MDT) (8). It was possible to transmit the MDT agent mechanically from tomato to tobacco and to a *Datura* sp., but not from tomato to tomato (2). However, since the symptomatology is similar to MAT and the agent also is mechanically transmitted, the disease found in Venezuela may be related to the Brasilian disease.

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