# Evaluation of tiazofurin antiviral effect on tomato spotted wilt virus (TSWV) in field experiments

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ABSTRACT

Field experiments were carried out over two years to evaluate the inhibitory effect of Tiazofurin (TR) against tomato spotted wilt virus (TSWV) in tomato plants. The plants, mechanically inoculated with TSWV one week after transplantation, were sprayed with 100 mg/l TR solution. It was observed that two sprays, one applied immediately and the other 24 h or 120 h after inoculation, were efficient to control the virus. The productivity of inoculated, treated tomato plants, evaluated by total weight of fruits, was almost twice that of those inoculated but nontreated. TR antiviral effect could be observed in both experiments and no phytotoxic effect was verified. Although TR residual effect has not been evaluated to date, the drug demonstrated an efficient inhibitor effect against TSWV, suggesting the possible control of the virus under field conditions.

Key words: Antiviral chemotherapy, tomato spotted wilt virus (TSWV), Tiazofurin.

RESUMO

Experimentos de campo foram realizados durante 2 anos a fim de avaliar o efeito inibidor do Tiazofurin (TR) sobre o vírus do vira-cabeça do tomateiro (TSWV) em plantas de tomate. Os tomateiros, inoculados mecanicamente 1 semana após o transplante, foram pulverizados 2 vezes com TR (100 mg/l). Observou-se que 2 pulverizações, uma aplicada imediatamente e a outra 24 h ou 120 h após a inoculação, foram eficientes no controle do TSWV. A produtividade das plantas inoculadas e tratadas, avaliada pelo peso total dos frutos, foi quase o dobro daquelas inoculadas e não tratadas. O efeito antiviral do TR pôde ser verificado nos dois experimentos, não tendo sido observado efeito fitotóxico. Embora o efeito residual do TR não tenha ainda sido avaliado, o quimioterápico mostrou-se um eficiente inibidor do TSWV, sugerindo um possível controle dessa virose, em condições de campo.

Palavras-chave: Quimioterápico antiviral, vírus do vira-cabeça do tomateiro (TSWV), Tiazofurin.

## INTRODUCTION

Tiazofurin (2 - *B* - D - ribofuranosylthiazole - 4 -carboxamide)= TR, an antiviral compound structurally related to Ribavirin (Streissle *et al.* 1985), was tested against both human and plant viruses (Caner *et al.* 1984; Koening 1981; Srivastava *et al.* 1977). Although very few tests were carried out using plant viruses, it was demonstrated, under glasshouse conditions, that TR had an inhibitory effect on tomato spotted wilt virus (TSWV) in tomato and tobacco plants, potato virus X in tobacco plants, and barley stripe mosaic virus in barley and wheat plants (Caner *et al.* 1984; Hansen 1989; Srivastava *et al.* 1977). However, the results of TR under field conditions have been restricted to date. In preliminary results, 95% of tomato plants inoculated with TSWV and sprayed with 100 mg/l TR solution remained without symptoms (Caner *et al.* 1986a). In order to confirm these results, tomato plants under field conditions were inoculated with TSWV and then treated with TR, to evaluate the antiviral effect of this chemical. The experiment was repeated over two years.

## MATERIAL AND METHODS

Tomato plants (*Lycopersicon esculentum* Mill. "Santa Cruz") were chosen as systemic hosts for tomato spotted wilt virus (TSWV). Two groups of one month-old tomato plants were mechanically inoculated with TSWV one week after transplantation to the field. The virus inoculum consisted of a crude-sap TSWV preparation in the proportion of 1 g infected tomato leaves to 5 ml sodium sulphite at 0.5%. The plants were distributed in the field according to the culture prescriptions in random

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blocks. During the inoculation, the inoculum was kept at a low temperature to maintain virus infectivity.

Tiazofurin (TR) was diluted to 100 mg/l with distilled water. Tween-20 was added as wetting agent, in the proportion of one drop per 100 ml of solution. TR was sprayed on all tomato leaves until dripping occurred. The treatments with TR consisted of two sprays, the first immediately after inoculation, and the second 24 h or 120 h after the first one (Tables 1 and 2).

**Table 1. Effect of Tiazofurin (TR) on tomato plants.**

| Experiment | Treatment | Percentage of systemically infected plants days after inoculation | | | | |
|---|---|---|---|---|---|---|
| | | 15 | 20 | 25 | 30 | 90 |
| I | TR(2X)*+ TSWV | 0 | 0 | 0 | 5 | 10 |
| | $H_2O$ + TSWV | 80 | 80 | 90 | 100 | 100 |
| | Non-inoculated non-treated | 0 | 0 | 0 | 20 | 20 |

* After inoculation and 120 h after inoculation; 20 plants per treatment.

| | | | | | | |
|---|---|---|---|---|---|---|
| II | TR(2X)** + TSWV | 0 | 0 | 0 | 5 | 11 |
| | H2O + TSWV | 83 | 83 | 83 | 83 | 83 |
| | Non-inoculated non-treated | 0 | 0 | 17 | 17 | 39 |
| | Non-inoculated treated | 0 | 0 | 0 | 17 | 22 |

** After inoculation and 24 h after inoculation; 18 plants per treatment.

**Table 2. Effect of Tiazofurin (TR) on the productivity of tomato plants.**

| Experiment | plants (%) which produced fruits | medium weight of fruits (g) | number of fruits per plant | total weight of fruits per plant (g) |
|---|---|---|---|---|
| I | | | | |
| TR(2X) +TSWV | 100 | 45.2 | 21 | 977.1 |
| $H_20$ + TSWV | 0 | - | 0 | 0 |
| Non-inoculated non-treated | 90 | 39.9 | 28 | 1 117.7 |
| II | | | | |
| TR(2X)+ TSWV | 100 | 71.3 | 15 | 1 060.5 |
| H20 + TSWV | 17 | 74.2 | 8 | 569.5 |
| Non-inoculated non-treated | 83 | 62.5 | 14 | 884.9 |
| Non-inoculated treated | 92 | 70.3 | 16 | 1 160.8 |

Control plants received distilled water plus Tween-20 in the same proportion as treated plants. Noninoculated and nontreated plants were maintained in the field to evaluate the natural virus infection. In one of the experiments, plants noninoculated, but TR-treated were used as controls to observe possible phytotoxic effects and whether the drug interfered with fruit production.

Observations on the number of systemically infected plants were made 15, 20, 25, 30 and 90 days after inoculation. The productivity evaluation, consisting of determining percent of plants which produced fruits, number of fruits per plant, the medium weight of the fruits and the total weight of the fruits per plant, was made at the end of the experiment, three months after inoculation.

To compare by indirect ELISA the viral content in plants submitted to the different treatments, one upper non-inoculated leaflet was collected from each plant and crushed in a mortar in 0.01 M phosphate buffer, pH 7 (1 g/10 ml). Ten plants were chosen at random for each treatment, 20 days after inoculation. Extracts were centrifuged (2500 g - 5 min) and supernatants were diluted 1:100 with 0.05 M sodium carbonate buffer, pH 9.6. Antiserum to TSWV was diluted 1:80 000 with phosphate-buffered saline containing 0.05% Tween-20 and 2% polyvinil pyrrolidone 40 000 ($PBSTP_o$). Viral antibodies were detected by alkaline phosphatase-labeled anti-rabbit IgG diluted 1:1000 with $PBSTP_o$; P-nitrophenyl phosphate, at 1 mg/ml in 0.1 M diethanolamine buffer, with pH 9.8 used as substrate. After 120 min of hydrolysis, 50 ul of 3 M NaOH was added to each well and serological reactions were assessed by readings of absorbance at 405 nm (Melissa *et al.* 1989).

## RESULTS

No phytotoxicity could be observed in tomato plants sprayed twice with TR after inoculation. Table 1 shows the percentage of plants treated with Tiazofurin and systemically infected with TSWV (TR + TSWV), in comparison with control groups. In experiment I, the results showed that TR sprayed twice (immediately and 120 h after inoculation) inhibited TSWV infection for up to 90 days after inoculation, presenting only 10% of plants with systemic symptoms. Inoculated controls presented 80% of infection as early as 15 days after inoculation; the percentage increased to 100% 15 days later. In this experiment, natural field infection was low (20%).

In experiment II, two TR sprays were applied with a 24 h interval, in an attempt to further reduce TSWV infection. In this case, the percentage of TSWV-infected plants presented a reduction of 72% in relation to inoculated nontreated control plants. The results were very similar to those obtained in experiment I, in which the interval between TR treatments had been 120 h. In experiment II, natural infection was also low (39%) and TR treatments were not phytotoxic. By means of ELISA on leaf extracts of experiment II (Fig. 1), it was demonstrated that virus content of inoculated and treated plants proved to be similar to those of control groups (noninoculated treated plants and noninoculated nontreated plants). In contrast, ELISA of leaf extracts of inoculated and nontreated plants revealed a higher content of TSWV antigens.

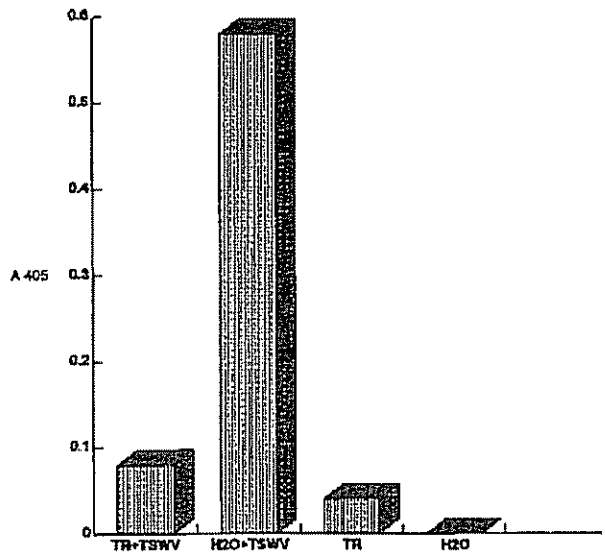


**Fig. 1. Average readings ($A_{405}$) of indirect ELISA of leaf extracts of different treatments (Experiment II).**

In the productivity evaluation, results of experiments I and II showed that TR-treated groups produced a higher a percentage of plants with fruits than nontreated controls (Table 2). Large differences between treated and control groups could also be seen in the total weight of fruits per plant. In both experiments, the number of fruits from treated plants was greater than that of nontreated plants; in experiment I, this effect was much more accentuated. The medium weight of fruits produced

by treated and controls did not show significant differences.

## DISCUSSION

Tiazofurin showed, under field conditions, an antiviral effect against TSWV on mechanically inoculated tomato plants confirming preliminary results (Caner *et al.* 1986a) and also results obtained under glasshouse conditions (Caner *et al.* 1984).

In the experiments where two TR sprays were applied in tomato plants, no visible phytotoxic effect was observed. However, Caner *et al.* (1986a) verified phytotoxicity of TR, consisting of a strong chlorotic and mottling of the leaves, when the drug was applied on tobacco plants through sprays of 100 and 200 mg/l. This shows the selective effect of TR, not only in relation to phytotoxicity, but also in relation to its antiviral activity, since TR was able to control TSWV in tomato and tobacco plants, but not tobacco streak virus and tobacco mosaic virus in tobacco plants (Caner *et al.* 1986b).

Nagy *et al.* (1989), working with TR against barley stripe mosaic virus, applied the drug at various time intervals before and after inoculation and verified that two applications were more efficient in controlling the virus than one, although some phytotoxicity has been observed.

The antiviral effect of TR was shown in the two experiments and it was evident that two sprays resulted in low percentages of systemically infected plants TR-treated, in comparison with nontreated controls. These results were confirmed by ELISA, carried out with leaf extracts in experiment II, which indicated that no virus could be detected after TR treatment in plants with no symptoms.

Productivity of treated groups, as evaluated by the total weight of fruits per plant, was almost twice that of inoculated and nontreated controls. The average weight of the fruits, however, was similar in all groups, since treated and nontreated plants which produced fruits, probably contained either low virus concentrations or no virus.

The biochemical mechanism of TR action has been studied in animal systems, indicating that the drug would indirectly affect glycoprotein biosynthesis by inhibiting the reaction catalyzed by IMP dehydrogenase (Nagy *et al.* 1989). However, since in plant systems, this mechanism was not investigated, these results cannot necessarily be extrapolated to live plants.

Although TR has been used as an experimental drug and experiments related to its residual effects have not been conducted to date. TR proved to be an effective plant virus inhibitor, suggesting its possible use in controlling TSWV under field conditions.

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