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Effect of tiazofurin on tomato plants infected with tomato spotted wilt virus

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Summary

Tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide) was examined for its activity against tomato-spotted wilt virus (TSWV) in tomato plants. Solutions containing 50, 100, 200, 400 and 800 mg/l of the drug were sprayed onto the leaves.

The results showed that 100 and 200 mg/l were the most efficient concentrations to suppress TSWV infection, thereby delaying the appearance of systemic symptoms. The drug was more effective in controlling TSWV infection when applied after than before virus inoculation. The results suggest that tiazofurin can be used as an efficient antiviral drug in the treatment of TSWV-infected tomato plants.

plant virus chemotherapy; tomato spotted wilt virus; tiazofurin

Introduction

Recently, some drugs with antiviral activity were tested on plant viruses. Among them, Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) showed an antiviral effect against a number of plant viruses such as alfalfa mosaic virus, apple chlorotic leaf spot virus, cucumber mosaic virus, potato virus X, red clover mottle virus, tobacco mosaic virus, tomato spotted wilt virus and tomato white necrosis virus [1–3,5,9–11,13,15,16].

The nucleoside 2-β-D-ribofuranosylthiazole-4-carboxamide = Tiazofurin (TR), structurally related to Ribavirin (Fig. 1) and synthesized in 1976 [6] was reported to be less active than Ribavirin as an antiviral compound [17] but equally potent as an antitumor agent [12]. However, recent in vitro studies have indicated that TR does indeed have a broad spectrum activity against RNA and DNA animal viruses, being in some cases even more efficient than Ribavirin [8].

In the present paper some experiments are described, in which TR was applied on tomato plants inoculated with TSWV.

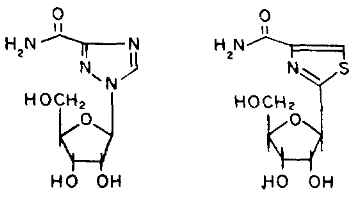

Fig. 1. Structures of ribavirin and tiazofurin.

Materials and Methods

Individually potted tomato plants (*Lycopersicon esculentum* Mill.) cv. Santa Cruz with 4–5 leaves were used in the tests. All plants were sprayed before or after virus inoculation with TR at different concentrations in water solutions. One drop of Tween 20 was added to each 200 ml used. The non-treated (controls) and the treated plants were mechanically inoculated with TSWV and kept in the greenhouse until the end of the experiment. Ten tomato plants were used for each treatment and the experiment was repeated twice.

To evaluate the effect of the drug on production of fruits, the plants were kept in the greenhouse, in pots of 19 cm in diameter for 4 months, after which the number and weight of the fruits were registered, as well as the number of seeds per fruit, fresh weight of 100 seeds and percentage of seed germination. These data were submitted to *t*-test at the 5% confidence limit. The effect of time interval between TR treatment and TSWV inoculation was also evaluated.

Results

Effect of TR on symptom development

The plants were sprayed with either 50, 100, 200, 400 or 800 mg/l of TR 30 min after virus inoculation. The concentrations of 400 and 800 mg/l induced a reduction in plant growth and a phytotoxic reaction shown as chlorosis and malformation of the youngest leaves, which remained evident until the end of the experiment. This phytotoxicity was observed in both non-inoculated and inoculated plants.

Typical systemic TSWV symptoms were observed 15 days after virus inoculation in 80, 40 and 40% of the plants treated with 50, 100 and 200 mg TR/l respectively (Fig. 2), whereas all control plants produced symptoms. A delay in the appearance of systemic symptoms was observed at the three concentrations.

Effect of the interval of time between TR treatment and virus inoculation

As the TR concentrations of 100 and 200 mg/l showed almost the same inhibitory effect on the appearance of TSWV symptoms the concentration of 100 mg/l was chosen for the following experiments.

In order to determine the effect of an increase of time interval between TR treatment

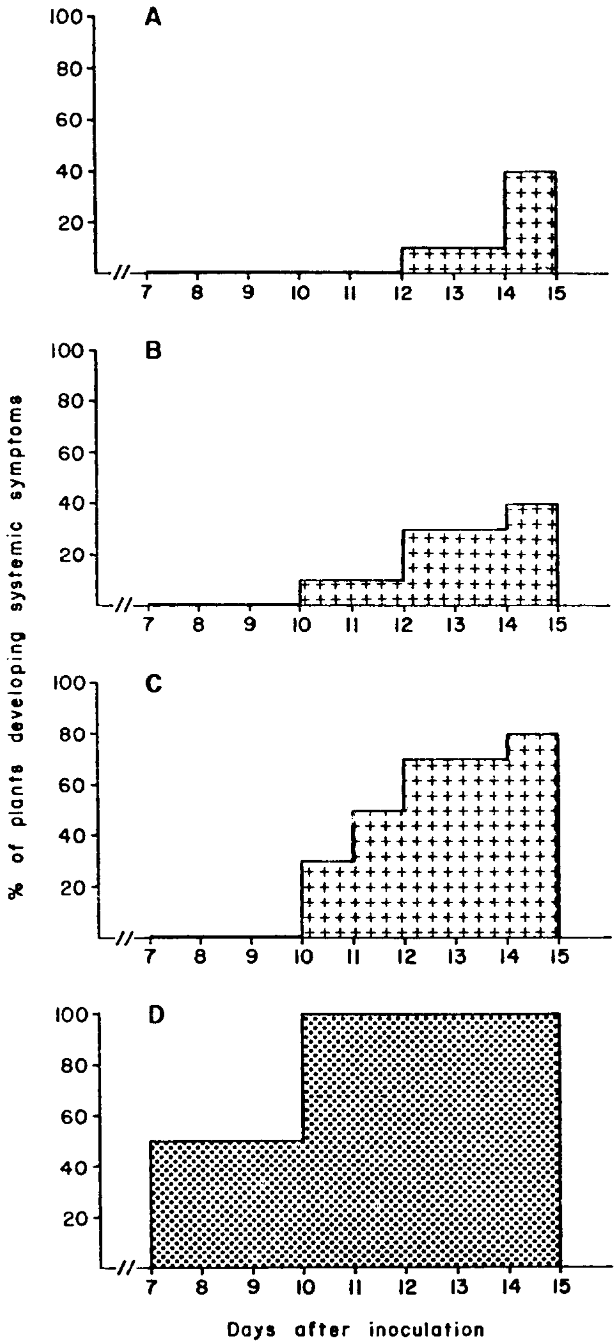

Fig. 2. Effect of different concentrations of TR applied on tomato plants 30 min after TSWV inoculation. A) 200 mg/l. B) 100 mg/l. C) 50 mg/l. D) 0 mg/l (non-treated control plants).

and TSWV inoculation, tomato plants were treated 5 or 9 days before or after inoculation. When TR was applied 5 or 9 days before inoculation, no differences were observed in the number of systemic infections between treated and untreated plants (Fig. 3). However, a remarkable effect was seen when TR was applied 5 days after inoculation. In this case only 20% of inoculated plants showed TSWV systemic symptoms at the end of the experiment, 25 days after inoculation. When inoculated tomato plants were sprayed 9 days after inoculation they grew higher than the non-treated plants, but all of them became systemically infected.

Effect of cumulative TR applications

Only 10% of the tomato plants sprayed twice with TR, 30 min and 5 days after inoculation, produced systemic symptoms 25 days later. Nevertheless, the plants sprayed only once or non-treated (controls) exhibited symptoms in 40% and 100% respectively (Fig. 4). This figure also shows a great delay in the appearance of systemic symptoms in TR-treated plants. All the control plants produced systemic symptoms 18 days after inoculation while treated plants showed no symptoms during the same period.

At the end of this experiment, 120 days after inoculation, 90% of the plants sprayed twice with TR and 50% of the plants treated only once were healthy. By that time, all control plants had died.

Effect of TR on production

Evaluations of the number of fruits, number and weight of seeds and percentage of

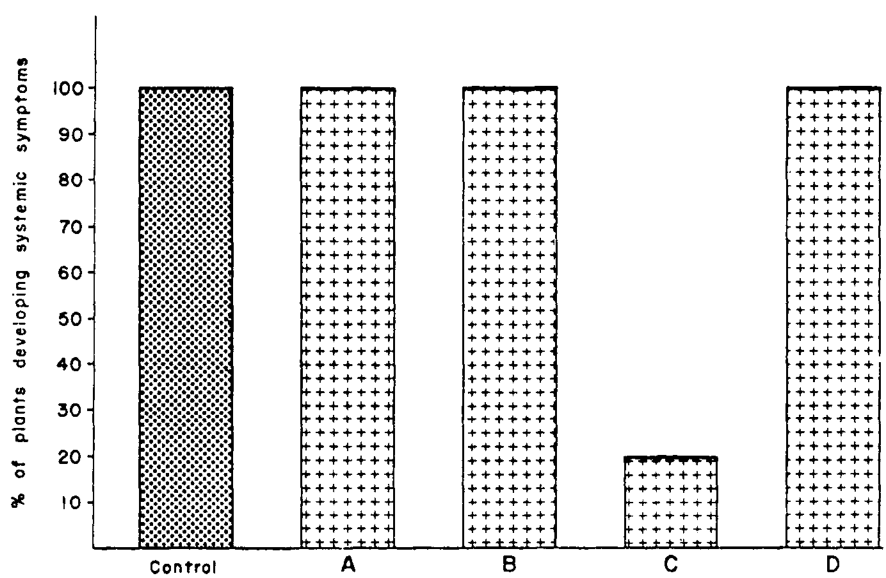

Fig. 3. TR-treated tomato plants showing TSWV systemic symptoms 25 days after inoculation. Treatment A) 5 days before inoculation; B) 9 days before inoculation; C) 5 days after inoculation; D) 9 days after inoculation.

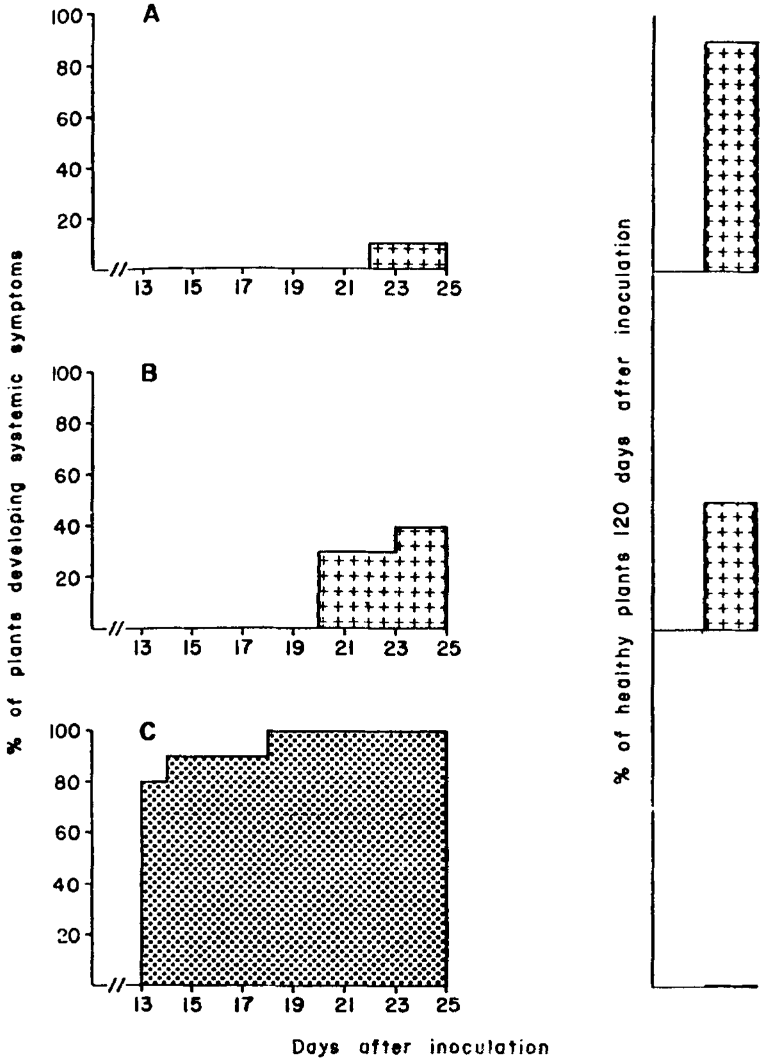

Fig. 4. Effect of cumulative TR (100 mg/l) applications on TSWV inoculated tomato plants. A) Plants sprayed 30 min and 5 days after inoculation. B) Plants sprayed 30 min after inoculation. C) Non-treated control plants.

germination (Table 1) showed that TR did not interfere with the production of the non-inoculated tomato plants. Control plants inoculated with TSWV died before producing fruits. Fruits and seeds from inoculated plants that were treated only once with TR and remained healthy until the end of the experiment (120 days after inoculation), were lighter than those produced by all the other plants including the non-inoculated. No difference could be observed with respect to the other parameters.

TABLE 1

Effect of TR on the production of tomato plants

	Non-inoculated		Inoculated	
	Non-sprayed	TR sprayed once	TR sprayed once	TR sprayed twice
Number of fruits/plant	1.6	2.6	2.4	1.6
Weight of fruits (g)	43.08 ± 6.31[a]	50.53 ± 9.3	29.64 ± 5.48	40.32 ± 9.89
Number of seeds/fruit	71.8 ± 19.6	92.4 ± 21.0	94.5 ± 15.2	71.8 ± 24.3
Weight of 100 seeds (g)	0.41	0.47	0.24	0.50
Seed germination (percentage)	83	86	76	85

[a] *t*-test at the confidence limit of 5%.

Discussion

Comparing the results obtained with TR in the experiments reported here and those of previous papers on the effect of Ribavirin [2,3], TR was more efficient at lower concentrations than Ribavirin in suppressing systemic TSWV infection of the plants (Fig. 2). These TR concentrations did not cause any phytotoxicity. A decrease in the number of systemic infected plants was observed when they were inoculated with TSWV and treated twice with TR (Fig. 4). The same effect was observed in *Nicotiana tabacum* and *N. glutinosa* infected with potato virus X, tobacco mosaic virus and cucumber mosaic virus when treated with Ribavirin and 2,4-dioxo-hexahydro-1,3,5-triazine or both [14].

TR was more effective when sprayed after inoculation, in a stage when local symptoms could be observed (Fig. 3). It seems that there was a better penetration of TR in infected cells. This hypothesis is strengthened by the fact that a higher phytotoxic effect of TR was observed on bean plants infected with bean golden mosaic virus than on healthy controls (unpublished data). In all cases, a delay in the appearance of systemic symptoms was observed in TR-treated tomato plants (Figs. 2 and 4), indicating a decrease in virus spread and virus concentration. Similar observations were made in TSWV-infected tomato plants treated with Distamicin A or a Pyrazino-pyrazine derivate [4]. Furthermore, TR-treated plants, inoculated or not, produced fruits and seeds similar in number to those of the non-treated controls (Table 1).

The mechanism of action of TR on the replication of TSWV is still unknown, but it may be similar to that of Ribavirin [7,18].

The results also suggest the possibility of using TR as an efficient antiviral to control TSWV since it proved effective even after the virus infection was established.

Acknowledgements

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