*Antiviral Research*, 3 (1983) 109–113
Elsevier



# Inhibitory effect of Distamycin-A and a pyrazino–pyrazine derivative on tomato spotted wilt virus

Gilda De Fazio* and Massae Kudamatsu

*Seção de Virologia Fitopatológica, Instituto Biológico, Caixa Postal, 7119, São Paulo, Brazil*

(Received 15 December 1982; accepted 15 February 1983)

Distamycin-A hydrochloride, a synthetic antibiotic, and 2,3-bromo-dihydroxy-6-bromo-pyrazino (2,3-β) pyrazine derivative, were used against tomato spotted wilt virus (TSWV) in tobacco plants. The drugs were applied to the leaves at concentrations of 200 and 400 mg/l.

The results showed that both drugs delayed virus spread within the plant, retarding the appearance of systemic symptoms. A virus recovery test, carried out on primary leaves of *Phaseolus vulgaris* cv. Manteiga, showed that TSWV replication was markedly inhibited by the pyrazino–pyrazine derivative at concentrations of 200 and 400 mg/l and, to a lower extent, by Dystamycin-A at 400 mg/l.

plant virus chemotherapy; tomato spotted wilt virus; Distamycin-A; pyrazino–pyrazine derivative

## Introduction

The use of antiphytoviral drugs is still at the experimental level. Nevertheless, several drugs of a most different chemical nature have been tested against plant viruses [6,7]. Some of these drugs, such as Virazole (Ribavirin), were tested against plant viruses after being successfully used against broad spectrum DNA and RNA animal viruses [3,4,7,8,10].

## Experimental section

In the work reported here tomato spotted wilt virus (TSWV) was chosen to test the inhibitory effect of two antiviral substances, Distamycin-A hydrochloride (DA), a synthetic antibiotic, and 2,3-dihydroxy-6-bromo-pyrazino (2,3-β) pyrazine (PP), a pyrazine derivative. Both substances, synthesized by Farmitalia-Carlo Erba, Milan, Italy, have been successfully used against some animal viruses [1,11].

TSWV is a single-stranded RNA virus, with a spherical, enveloped particle, approximately 82 nm in diameter. It is the type member of its own group [9]. Until recently it was considered a pleomorphic myxovirus for its similarity in size, shape and

* To whom correspondence should be addressed.

0166-3542/83/$03.00 

internal structure to influenza virus. It causes a severe tomato disease and is widespread all over the world.

Aqueous solutions of both drugs were prepared at concentrations of 200 and 400 mg/l and Tween 20 was added to all solutions, including water controls, at the concentration of 1 drop/100 ml as a wetting agent. Individually potted 2-mth-old tobacco plants (*Nicotiana tabacum* cv. White Burley) were used as hosts for TSWV. The solutions were brushed on all leaves of each plant with a soft brush, 2–3 h before virus inoculation. The plants were then mechanically inoculated with TSWV as previously described [5] and kept in a greenhouse for 40 days. The test was repeated 3 times using 10 plants for each treatment.

When 20% of control plants showed typical systemic symptoms, approximately 8 days after inoculation, a local lesion test was performed on primary leaves of *Phaseolus vulgaris* cv. Manteiga to evaluate virus concentration in the treated and control tobacco plants. For this test the third secondarily infected tobacco leaf was used, counted from the lowest leaf.

None of the treated plants showed any phytotoxic symptoms. All inoculated tobacco plants showed local reaction to TSWV, developing typical local lesions 5–7 days after inoculation. The plants which received the highest dosage of both drugs showed a reduction in the number of the local lesions, when compared with controls.

The antiviral effect of both substances at different concentrations on TSWV systemic infection is shown in Table I and Fig. I.

TABLE I

Effect of Distamycin A and a pyrazino-pyrazine derivative on TSWV in tobacco plants

| Treatment[a] | Percentage of systemically infected plants | | Average number of local lesions on *P. vulgaris* L. 'Manteiga' leaves[b] | Inhibition percentage (IP)[c] |
|---|---|---|---|---|
| | 8 days | 18 days | | |
| Control (water) | 20 | 80 | 70 | 0 |
| Distamycin A 200 mg/l | 48 | 80 | 96 | 0 |
| Distamycin A 400 mg/l | 0 | 40 | 36 | 49% |
| Pyrazino-pyrazine derivative 200 mg/l | 0 | 40 | 0 | 100% |
| Pyrazino-pyrazine derivative 400 mg/l | 0 | 10 | 0 | 100% |

[a] 10 tobacco plants/treatment.

[b] 4 *Phaseolus vulgaris* L. 'Manteiga' primary leaves per individual tobacco plant. Tests made 8 days after inoculation.

[c] IP = (1 - A/B) × 100%, in which: A = average number of lesions produced by treated tobacco plants; B = average number of lesions produced by untreated tobacco plants.

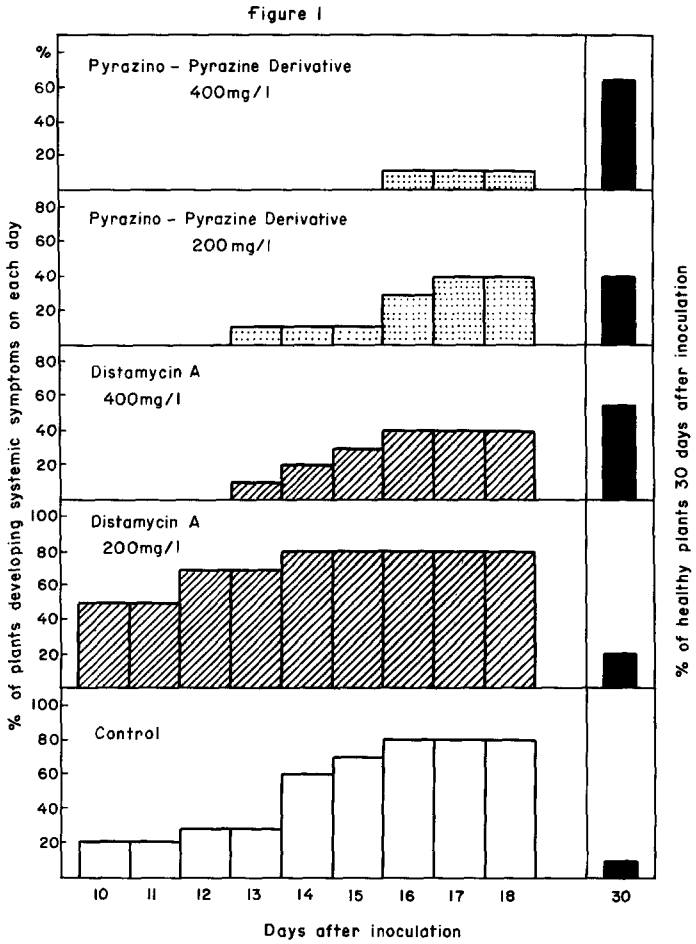


Fig. 1. Effect of Distamycin A and a pyrazino-pyrazine derivative on TSWV in tobacco plants.

Table I presents the percentage of systemically infected tobacco plants and the results of the recovery test on *P. vulgaris* cv. Manteiga primary leaves. This enables us to evaluate virus concentration and to calculate the inhibition percentage. It was observed that, 18 days after inoculation, 80% of the control plants showed typical and severe symptoms of TSWV systemic infection, as did the plants which received DA at 200 mg/l. In this case an increase in the number of local lesions was observed. DA at 400 mg/l inhibited virus replication at a rate of 49% and a drastic reduction in virus concentration was induced after PP treatment at both dosages. The inhibition obtained with the two dosages of this drug was 100%, i.e., no local lesions could be detected on *P. vulgaris* test plants.

Figure 1 shows that DA at 200 mg/l caused a slight stimulation of virus replication, from the tenth to the fifteenth day after inoculation. After this period, the percentage of plants showing systemic symptoms was similar to the control. This effect is also

evident from the number of infected tobacco plants and the average number of local lesions induced on *P.vulgaris* primary leaves (Table I).

A delay in the appearance of the systemic symptoms was observed after the treatment with DA at 400 mg/l and PP at 200 mg/l. In these cases the symptoms became detectable only 13 days after inoculation and the percentage of systemically infected plants reached 40%. Plants treated with PP at 400 mg/l showed systemic symptoms only 16 days after inoculation and were restricted to only 10% of the inoculated plants.

Control plants and plants tested with DA at 200 mg/l reacted to the virus infection with severe systemic symptoms and died approximately 1 mth after inoculation; only about 20% of these plants remained healthy. The plants submitted to the other treatments showed mild systemic symptoms and could overcome virus infection 30 days after inoculation, developing symptomless young leaves (Fig. 1).

The disease caused by TSWV affects tomato plants at any age but it is particularly severe in younger plants, ready for transplantation [2]. For this reason a control measure resulting in a delay of systemic symptoms and virus spread during the early phases of plant development seems to be highly important. It is unpredictable if these substances will be of practical use under field conditions. Nevertheless, they appear useful in laboratory virus research.

Although some promising results arise from these preliminary experiments, they do not allow to assert that the plants which remain virus free up to the end of the experiment will remain virus free until the end of their life cycle. Furthermore, the actual amount of the drugs taken up or retained by the leaves as well as their distribution and metabolization within the plant after absorption is still unknown.

**Acknowledgements**

The authors are indebted to Dr. Maria Antonietta Verini and Dr. Federico Arcamone from Farmitalia-Carlo Erba, Milan, Italy, for providing the drugs used in the experiments, to Dr. Marly Vicente for useful suggestions and to Dr. Victoria Rossetti for revision of the English text.

**References**

1 Casazza, A.M., Fioretti, A., Ghione, M., Soldati, M. and Verini, M.A. (1966) Distamycin A, a new antiviral antiobiotic. Antimicrobial Agents and Chemotherapy 1965, pp. 593–598.

2 Chagas, C.M. and Vicente, M. (1977) Tomato spotted wilt virus. In: Diseases, Pests and Weeds in Tropical Crops. Eds.: Kranz, J., Schmutterer, H.G. and Koch, W. (Paul Parey Verlag, Berlin and Hamburg) pp. 35–37.

3 De Fazio, G., Caner, J. and Vicente, M. (1978) Inhibitory effect of Virazole (Ribavirin) on the replication of tomato white necrosis virus (VNBT). Arch. Virol. 58, 153–156.

4 De Fazio, G., Caner, J. and Vicente, M. (1980). Effect of Virazole (Ribavirin) on tomato spotted wilt virus in two systemic hosts, tomato and tobacco. Arch. Virol. 63, 305–309.

5 De Fazio, G., Kudamatsu, M. and Vicente, M. (1980). Virazole pretreatments for the prevention of tomato spotted wilt virus (TSWV) systemic infection in tobacco plants, *Nicotiana tabacum* L. 'White Burley'. Fitopatologia Bras. 5, 343–349.

6 Hirai, T. (1977) Action of Antiviral Agents. In: Plant Disease, Vol. 1, ch. 15. Eds.: Horsfall, J. and Cowling, E.B. (Academic Press, New York) pp. 285–306.
7 Kluge, S. (1980) Virus multiplication and selected antiphytoviral drugs. In: Endocytobiology, Endosymbiosis and Cell Biology. Vol 1. (W. De Gruyter & Co., Berlin/New York) pp. 606–614.
8 Lerch, B. (1977) Inhibition of the biosynthesis of potato virus X by Ribavirin. Phytopathol. Z. 89, 44–49.
9 Matthews, R.E.F. (1982) Classification and nomenclature of viruses. Fourth Report of the International Committee on Toxonomy of Viruses. Intervirology 17, 123.
10 Schuster, G. (1976) Wirkung von 1-β-D-ribofuranosyl-1,2,4-triazol-3-carboxamid (Virazol) auf die Vermehrung systemischer Viren in *Nicotiana tabacum* 'Samsun'. Berl. Inst. Tabakforsch. Dresden 23, 21–36.
11 Verini, M.A., Fioretti, A., Casazza, A.M., Sanfilippo, A., Palamidessi, G. and Ghione, M. (1975). Antiviral activity of a pyrazino-pyrazine derivative. Chemotherapy 21, 221–230.