Persistence and Lack of Absorption and Translocation of Streptomycin Sulfate in Passion Fruit Leaves[1]

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ABSTRACT

Using passion fruit (*Passiflora edulis*) as a test plant, absorption, translocation and persistence of the antibiotic streptomycin sulfate in the plant tissue was investigated using bioelectrophoresis as bioassay. The antibiotic was neither absorbed nor translocated in the leaf tissue. However, under greenhouse conditions, the antibiotic persisted on the leaf surface for more than two weeks, behaving consequently as a non-systemic bactericide.

COMPENDIO

Se estudió la absorción, translocación y persistencia del antibiótico sulfato de estreptomicina en hojas de maracuyá (*Passiflora edulis*), por medio de bioeletroforesis. El producto no fue absorbido ni translocado en el tejido foliar. Sin embargo, en condiciones de invernadero, el antibiótico persistió en la superficie foliar por más de dos semanas, actuando así como un bactericida no-sistémico.

INTRODUCTION

Antibiotics have been recommended to control bacterial plant diseases under field conditions (4, 9), as well as for seed treatment (4, 5). Antibiotics such as terramycin (2), vancomycin (8) oxytetracyclin (11) have been used. Nevertheless, the antibiotic most recommended for controlling bacterial plant diseases is streptomycin, mainly in the sulfate form.

Antibiotics are reported to be absorbed and translocated in bean leaves (7), in *Chrysanthemum* stems (10) and in soybean seedlings (3).

In Brazil, passion fruit (*Passiflora edulis*) is cultivated by juice-processing industries, and bacterial blight caused by *Xanthomonas campestris* pv *passiflorae* is a serious problem. Commercial preparations of streptomycin sulfate have been recommended as sprays. The antibiotic does not always work, even though isolates of the pathogen were sensitive to the antibiotic *in vitro*. The absorption translocation and persistence of streptomycin sulfate on passion fruit leaves were thus investigated.

MATERIALS AND METHODS

Passion fruit seedlings (*P. edulis* 'Amarelo') with four to six definite leaves were obtained by planting seeds in greenhouse-soil mix under greenhouse conditions.

Bioassay

Gel plates were prepared on glass slides (20 cm x 20 cm x 0.1 cm) which were previously washed with water and soap, rinsed several times with distilled water, dried, and rinsed with acetone. Plates were then laid onto a horizontal surface and surrounded with pieces of glass 0.2 cm in height. The four edges of the square slide were sealed with melted 2 per cent water-agar to avoid leaking during the gel dispensing process.

Melted (50 °C) 0.8 per cent agar in 0.025 M sodium phosphate buffer (pH = 7.0) was poured onto the surface of the square glass plate and immediately spread with a glass rod, so that the agar layer, after solidification, was 0.1 cm thick. Then, 0.8 cm diameter holes were punched in the gel layer using a cork-borer and the agar gel disks removed. Six to seven holes per plate were made, about 2 cm from one of the edges of the gel plate.

The plate was set in an electrophoresis apparatus with the electrode chamber containing 0.05 M potassium phosphate buffer (pH = 7.0). It was positioned in such a way that the edge near the holes was connected to the positive electrode, so that the antibiotic would run toward the negative electrode. The gel was connected with the buffer by two pieces of Whatman No. 1 filter paper. Samples (10 μl) were

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then applied to the holes in the gel and the electrophoretic run was performed in a cold chamber (4 °C) for 120 minutes

Bioautography was carried out using 24-hr-old liquid cultures of *Bacillus subtilis* (OD_{550} = 0.5). One ml of culture was transferred to 100 ml of melted and cooled (50 °C) semi-solid agar. The medium, containing approximately 10^7 cells/ml was then sprayed onto the surface of the gel plate. Care was taken to perform the spraying as uniformly as possible and about 10 ml of *Bacillus* containing the medium was sprayed on each plate. After spraying, plates were incubated at 28 °C in a moist chamber to avoid drying of the gel layer

Standard curve

To determine the size of the halo diameter as a function of the amount of streptomycin present in the sample, known concentrations of streptomycin were applied and the bioautograph run. The diameter of the halo was then plotted against the log of the concentration of streptomycin (Fig. 1). This curve could then be used to determine the concentration of streptomycin present in the extracts or wash water of treated leaves

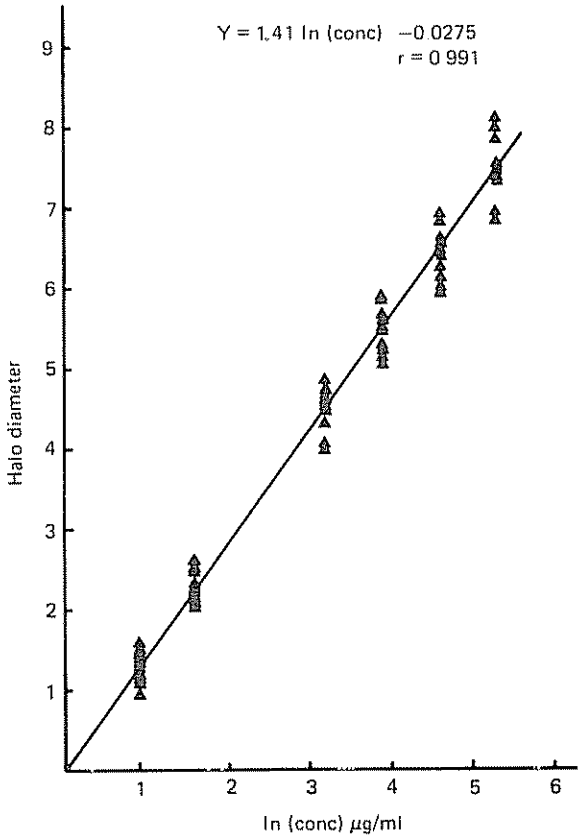

Fig. 1. Standard curve of the natural logarithm of known concentrations of streptomycin, with respect to halo diameter after electrophoresis

Experiments

The persistence of the antibiotic on or in the leaf was determined using ten transplants of passion fruit in pots, with four to six leaves; they were sprayed with an aqueous solution of streptomycin at 200 µg/ml and kept under greenhouse conditions. Sampling was done at 0, 4, 8, 12 and 16 d after spraying by removing two 1 cm leaf disks per leaf on each sampling day. Leaf disks harvested on the same day were combined, weighed and ground by using a mortar and pestle with water (0.5 ml/g of fresh weight). The macerate was then centrifuged at 3000 rpm for 20 min in a clinical centrifuge and the supernatant submitted to bioelectrophoresis

To determine if streptomycin is absorbed by the leaves of passion fruit plants, four to six leaves were each sprayed with aqueous 200 µg/ml streptomycin sulfate. Four and 16 days after spraying, five leaf disks (1 cm in diameter each) were harvested from each plant by using a cork-borer. Disks were washed five times with 2 ml of distilled water each time, the rinsing water pooled and dried under vacuum at 50 °C. The residue was resuspended in 0.85 ml of water and then submitted to bioelectrophoresis. The disks were then thoroughly washed with detergent and tap water to remove any superficial antibiotic residue, and ground with a mortar and pestle in water (0.85 ml/5 disks), the macerate was centrifuged at 3000 rpm for 20 min and the supernatant also submitted to bioelectrophoresis.

To investigate the possibility of acropetal, basipetal or lateral translocation, leaves were shielded with aluminum foil on either the apex, base or side and the unprotected areas were sprayed with 200 µg/ml aqueous streptomycin. Four days after spaying, protected and unprotected areas of leaves were harvested, ground in water at 0.5 ml/g of fresh weight and the macerate centrifuged at 3000 rpm for 30 min. Supernatants were then submitted to bioelectrophoresis.

RESULTS AND DISCUSSION

Significant amounts of streptomycin were found on the leaves even after 16 days (Fig. 2). Although there was a reduction in the amount of the antibiotic from day 0 to day 16, considerable amounts of the antibiotic were still present. Considering that plants were kept in a greenhouse and that care was taken to avoid splashing water on the leaves during irrigation, the chance of the reduction of streptomycin due to

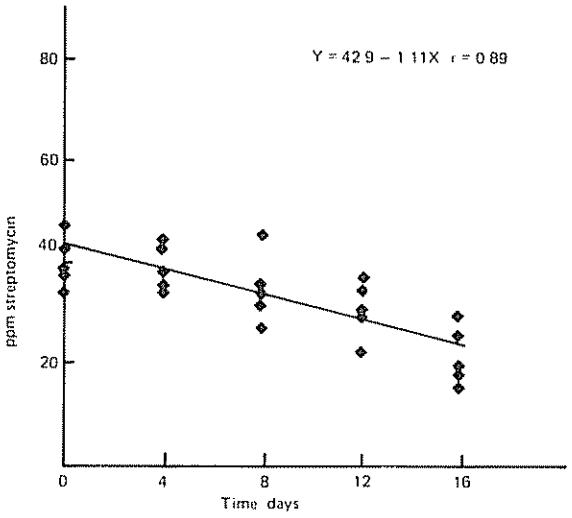

Fig. 2. Persistence of streptomycin on the leaves as analyzed through bio-electrophoresis using the standard curve. Leaves were sprayed on day 0 just before sampling

irrigation is nil. Since streptomycin sulfate is very soluble in water, it is logical that if it is not absorbed by leaves, it may be easily washed by rains.

The antibiotic was not found inside the leaves, but rather in the washing water. Water extracts of ground tissue showed zero ppm streptomycin, while washing water yielded values of 35-50 ppm streptomycin. This indicates that, streptomycin was not absorbed by the leaves, either at four or at 16 d after spraying under natural conditions.

In addition, streptomycin was not translocated in leaves either from one side of the mid rib to the other, from the base to the apex, or from the apex to the base (Fig. 3). Assays for all unsprayed areas yielded zero streptomycin, while the sprayed areas yielded

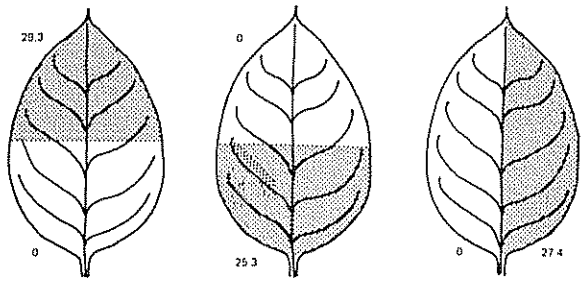

Fig. 3. Translocation of streptomycin basipetally, acropetally or laterally. Leaves were sprayed on one side (shaded) and after four days, both sides were sampled for the presence of streptomycin. The numbers near each leaf half are the ppm streptomycin found.

streptomycin in concentrations which varied from 15-30 ppm. Consequently, at least in passion fruit leaves and under the conditions in which the experiment was carried out, no translocation was observed; the antibiotic is therefore not systemic in leaves of passion fruit within four days after spraying.

The ability of antibiotics to be taken up by plants and then translocated varies. When streptomycin was incorporated into a paste of lanolin plus Tween and applied to bean stems, it was absorbed and translocated; however, without these surfactants no absorption occurred (6). On the other hand, the addition of surfactants enhanced apparent disease control of fireblight although differences were not significant (1). Such methods as placing small balls of lanolin impregnated with streptomycin do not lend themselves to field applications. Therefore, in passion fruit leaves, the nature of action of streptomycin can be considered as protectant rather than systemic.

LITERATURE CITED

1. BEER, S.V. 1976. Fire blight control with streptomycin sprays and adjuvants at different application values. Plant Disease Reporter 60:541-544.

2. CARROL, J.; KEIL, H.L. 1967. Control of peach bacterial spot with terramycin in Maryland. Plant Disease Reported 51:345-349.

3. ELLIS, M.A.; SINCLAIR, J.B. 1974. Uptake and translocation of streptomycin by soybean seedlings. Plant Disease Reporter 58:534-535.

4. GALLI, F.; IOKESHI, H.; CARVALHO, P.C.T.; BALMER, E.; KIMATI, H.; CARDOSO, C.O.N.; SALGADO, C.L. 1968. Manual de Fitopatología (Doencas de Plantas e Seu Controle). São Paulo, Bra., Biblioteca Agronomica Ceres. 640 p.

5. HUMAYDAN, H.S.; HERMAN, G.E.; NEDRON, B.L.; DINITRO, L.V. 1980. Eradication of *Xanthomonas campestris*, the causal agent of black rot from *Brassica* seeds with antibiotics and sodium hypochloride. Phytopathology 70:127-131.

6. MITCHELL, J.W.; ZAUMEYER, W.J.; ANDERSON, W.P. 1952. Translocation of streptomycin in bean plants and its effect on bacterial blights. Science 115:114-115.

7. MITCHELL, J.W.; ZAUMEYER, W.J.; PRESTON, W.H. 1954. Absorption and translocation of streptomycin by bean plants and its effect on the halo and common blight organisms. Phytopathology 44:25-30.

8. PRICE, R.M.; BOYLE, A.M. 1963. Vancomycin prevents crown gall. Phytopathology 53:1272-1275.

9. PRADO FILHO, L.G. 1966. Generalidades sobre o emprego de antibioticos nas plants. Piracicaba, Bra., Instituto Zimotecnico "Prof. Jaime Rocha de Almeida" da USP. 18 p.

10. ROBISON, R.S.; STARKEY, R.L.; DAVIDSON, O.W. 1954. Control of bacterial wilt of chrysanthemums with streptomycin. Phytopathology 44:646-650.

11. WEAVER, L.O.; KEIL, H.L. 1970. Grower trials with oxytetracyclin for control of peach bacterial spot in Maryland. Plant Disease Reporter 54:208-211.