Streptomycin Residue Determination in Field-Grown Tomatoes

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Homestead 24 tomatoes were sprayed ten times using a 5-day spray schedule with agricultural streptomycin (Agrimycin-17) at levels of 200 and 400 ppm streptomycin activity, terminating 45 days prior to harvest. Biological assay determinations of fruit from the 200-ppm treatment showed the absence of antibiotic activity, while the 400-ppm treatment exhibited trace activity in only one of the 45 fruit samples assayed. Foliage samples showed a slight residue of activity 1 month after the final spray.

In 1952 tests were initiated to study the effect of agricultural streptomycin on bacterial spot [*Xanthomonas vesicatoria* (Doidge) Dowson], a serious disease of tomatoes and peppers grown along the Florida lower east coast (Conover, 1954a).

Experiments in 1954 and 1955 with agricultural streptomycin sprays provided good control of bacterial spot on seedling tomatoes (Conover, 1954b, 1955). Most sprays were applied commercially at 200 ppm with control varying from excellent to poor (Conover, 1957). The poor results were first attributed to poor spray coverage and to delaying the first spray until bacterial infection had taken place. The same results were noted in other experiments with agricultural streptomycin (Stall, 1959). The suspicion of a resistant strain of the infecting organism was confirmed by three studies in the south Florida tomato fields (Conover, 1961; Stall and Thayer, 1962; Thayer and Stall, 1960, 1961).

There are indications that the resistant strain is not very competitive with the parent or susceptible strain. Thus, many growers presently use Agrimycin-17 (agricultural streptomycin, Pfizer Inc.) in mixture with, or an alternative spray treatment of, copper and maneb.

Since growers were continuing the use of Agrimycin-17 on seed beds and possibly in the field, some concern for residue levels of streptomycin on and in the fruit was expressed by Pfizer Inc. Thus, a field study was conducted in the fall of 1970 to determine the possible presence of streptomycin residues at time of harvest and to test the efficiency of Agrimycin-17 throughout the growing season.

EXPERIMENTAL

Field Application. Ten spray treatments of 200 and 400 ppm streptomycin activity, with a hypro-pump at 500 psi, were applied to 50 test plants each treatment, using a 5-day spray schedule. Initial spray was applied on October 1, 1970, and final application was done on November 15, 1970. Foliage was sampled directly after final spray dried on November 15, 1970, and also 1 month after final spray. All fruit samples were harvested at green mature stage on December 31, 1970; interval between last spray and harvest was approximately 45 days. The tomato variety Homestead 24 was used throughout the test.

Fruit and leaf samples were harvested at random for residue analysis. All samples were frozen until assayed for streptomycin residues.

During the course of the experiment the plots were monitored for possible physiological effects, particularly at the 400-ppm level. No differences were observed between treated and nontreated plants.

Disease Control. Owing to near-drought conditions in southeast Florida in the fall of 1970, no bacterial spot was detected in the treated or untreated field plots.

Determination of Test Sensitivity. Cut control fruit into approximately 1-in. cubes and accurately weigh a 100-g sample. Transfer sample to the homogenizer and add 150 ml of 0.1 *M* phosphate buffer, pH 8. Add a known amount of streptomycin sulfate to the mixture and homogenize for 3 min. Adjust pH of the homogenate to pH 7.8 ± 0.2. Filter through fine mesh to remove most of the pulp and centrifuge filtrate at 2000 rpm to obtain a clear supernatant.

Assay. Assay the supernatant for recoverable streptomycin activity by the modified (Grove and Randall, 1955) cylinder plate assay method using *Bacillus subtilis* ATCC 6633 (sensitivity 0.06 μg/ml).

Test Inoculum Preparation and Medium. Prepare a spore suspension by inoculating 100 ml of culture broth (Grove and Randall, 1955, Medium No. 6) from a stock slant (Baltimore Biological Laboratories, Seed Agar #20822). Incubate at 26°C for 6 days on a reciprocal shaker. Centrifuge the culture, remove the supernatant, and reconstitute the cells to 30% of the original broth volume with sterile saline. Heat-shock the spore suspension by immersing in a water bath at 70°C for 30 min.

Standard Preparation. Dry an appropriate amount of the streptomycin sulfate working standard (approx. 260 mg) for 3 hr at 60°C at a pressure of 5 mm or less. Determine the dry weight, dissolve, and quantitatively dilute in sufficient 0.1 *M* phosphate buffer (Code of Federal Regulations, 1971), pH 8.0, to give a final concentration of 100 μg/ml. Perform this in triplicate.

Fruit Sample Preparation. Fruits were selected at random and cut into approximately 1-in. cubes. Samples of 100 g were accurately weighed, homogenized in 150 ml 0.1 *M* phosphate buffer, pH 8.0, filtered, and centrifuged at 2000 rpm. All samples were assayed using method of assay described.

Foliage Sample Preparation. Samples from 400 ppm-treated foliage harvested directly after final spray were dried and 1 month after final spray were cut in shredded form and accurately weighed into 10-g samples. Fifteen milliliters of standard 0.1 *M* phosphate buffer, pH 8.0, were added to sample, which was held for 2 hr at room temperature to effect optimum penetration of tissue and solubilization of available antibiotic into solution. Samples were centrifuged at 2000 rpm to separate tissue from supernatant. All samples were assayed using method of assay described.

Preparation of Plates. SINGLE LAYER. Pipet 10 ml of

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Table I. Bioassay Sensitivity Tests of Streptomycin in Tomatoes

Streptomycin added, μg/g tomato[a]	Streptomycin recovered, μg/g[a]	Streptomycin recovered, %
Control	No zone	None
0.25	0.06	Trace
0.35	0.06	Trace
0.5	0.16	32
1.0	0.33	33
1.5	0.51	34

[a] Average of eight samples per treatment.

inoculated test medium (Baltimore Biological Laboratories, Streptomycin Assay Agar #11658) into each plate. Add 2.5 ml of inoculum to each 1000 ml of test medium. Spread the agar evenly over the plate. Refrigerate the plates at least 1 hr before using.

Procedure. STANDARD CURVE. Quantitatively dilute each working standard to 0.06, 0.07, 0.08, 0.09, 0.10, 0.25, 0.50, and 1.0 μg/ml with 0.1 *M* phosphate buffer, pH 8.0. Dilute one of the working standards again to 0.25 μg/ml and use as the reference standard. Place five cylinders equidistant on each plate. Use nine plates to determine each concentration. Fill two cylinders with reference standard and three cylinders with sample. Incubate 16–18 hr at 37°C. After incubation, measure the diameter of the zones of inhibition, average standard readings and sample reading, plot corrected standard values, and calculate potency.

Calculation. $A \times$ dilution = μg of streptomycin/ml, where A = μg/ml of streptomycin in the final sample solution determined by the standard curve.

RESULTS AND DISCUSSION

The bioassay sensitivity tests of streptomycin recovered from the fruit were quantitative at a level of 0.5 ppm and detected qualitatively at levels as low as 0.25 ppm (Table I). Using this technique approximately 30 to 35% of the original antibiotic added can be detected upon plate assay. This average percent recovery does not vary with concentration, which indicates that the volume of final sample of 250 g influences final recovery.

Determination of streptomycin residues in fruit is complicated by the presence of soluble carbohydrates. These substances stimulate the test organism, influence the biological activity of streptomycin, increasing the viscosity of the test solutions, and interfere with the normal movement of the antibiotic. Further dilution of a sample, particularly at higher levels of addition, would increase percent recoveries.

Forty-five individual fruit samples were assayed from each treatment of 200 and 400 ppm of streptomycin. These samples were harvested from plants sprayed a total of ten times during the season under conditions of near-total lack of rainfall. None of the 200-ppm treated tomatoes showed any antibiotic activity, while only one sample from the 400-ppm treated exhibited trace activity.

Table II. Streptomycin Residues in Tomato Foliage Treated with 400 ppm of Agrimycin 17

Sample	Streptomycin, μg/g
Directly after final spray	
1	2.5
2	3.1
3	2.1
4	3.5
5	3.1
6	3.6
7	2.4
8	1.3
One month after final spray	
9	0.245
10	0.192
11	0.147
12	0.115
13	0.110
14	0.162
15	0.171
16	0.147
17	0.171
18	0.185
19	0.192
20	0.320

High levels of antibiotic were found in and on foliage samples directly after final spray application of 400 ppm (Table II). An average of 2.7 ppm of streptomycin was detected in these samples. One month after final spray the antibiotic titer had decreased to an average level of 0.179 ppm or approximately one-fifteenth of the activity found 1 month earlier. It is interesting to find that the foliage showed a slight residue after 1 month.

In other studies, not reported here, plants treated at 200 ppm of streptomycin showed approximately 1-ppm residues in the foliage sampled directly after the final spray. These results appear to be in line with the data obtained at the 400-ppm level of treatment.

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Received for review January 21, 1972. Accepted March 24, 1972. Florida Agricultural Experiment Station Journal Series No. 4292.