The Activity of Thiolutin Against Certain Fungi and Seed-borne Diseases[1]

K. S. Gopalkrishnan and J. A. Jump, University of Notre Dame

Interest in anti-fungal antibiotics has its origin in a number of rather diverse possibilities of application, in addition to purely scientific interest in antagonism as such. The first of these, which has received perhaps the major share of attention up to the present time is the possibility of the development of an antibiotic to combat certain fungi which cause human disease and are resistant or slow to respond to present methods of treatment. Then there is a group of plant pathogens which might be controlled practicably by an antibiotic, assuming that it could be produced at a reasonable cost. Finally there is the possibility that the study of the chemical structure of anti-fungals might lead to valuable synthetic fungicides.

Several antibiotics of antifungal activity have been isolated from strains of *Streptomyces albus*. Jump (2) reported the antifungal activity of strain, the active principle of which is thermostable and very low in phytotoxicity, but which has proved difficult to isolate. Other antibiotics from strains of this same species include Endomycin, isolated by Gottlieb and coworkers (1) and Thiolutin which was isolated by Tanner and associates, and with which this report is chiefly concerned.

The effect of Thiolutin on 9 phytopathogenic fungi was studied *in vitro*. The test organisms were grown in petri plates on potato dextrose agar with various amounts of Thiolutin incorporated. The concentrations of the antibiotic ranged from 0.1 ppm. to 100 ppm. depending upon the resistance of the fungus being tested. Four replicates were run at each concentration, and controls were grown on the same type of agar without Thiolutin. The amount of growth of the colony in each plate was expressed as the average of four measurements of the diameter of the colony, and the data of the four replications were, in turn, averaged. Data were recorded at 24 hour intervals for 8 to 11 days, depending upon the rapidity of growth of the fungus. The experiment was not terminated until the diameter of the control colony was at least 9 cm., which was the diameter of the petri plate.

It was difficult to obtain complete solution of the antibiotic in water at the higher concentrations, so the antibiotic was first dissolved in a small quantity of acetone, and water was added to give the required dilution. In such cases acetone was also added in equal amount to the control. The amount of acetone did not exceed 5% in any case, which had been found by previous experiment to produce no significant difference in the rate of growth of the fungus.

[1] Samples of Thiolutin and Actidione were supplied by Charles Pfizer & Co. and the Upjohn Co. respectively. The authors also wish to express thanks to Dr. M. D. Whitehead of Texas Agricultural and Mechanical College and Dr. A. L. Smith of Alabama Polytechnic Institute for supplies of diseased oat and cotton seed.

The fungi used in these experiments, and the plant diseases of which they are causal agents were: *Alternaria solani* (early blight of potato), *Colletotrichum gossypii* (cotton anthracnose), *Fusarium lycopersici* (tomato wilt), *Helminthosporium sativum* (dry-land foot rot of wheat), *Pythium debaryanum* (damping off), *Phomopsis citri* (citrus stem end rot), *Rhizoctonia solani* (damping off), *Sclerotinia fructicola* (brown rot) and *Sphaeropsis ulmicola* (elm canker). These were grown for inoculum in petri plates for 24-72 hours depending upon the speed of growth. Small disks of agar bearing the fungus mycelium were then cut from the edge of the colony and transferred to the plates containing the antibiotic and to the controls.

All of the fungi tested were inhibited by the antibiotic, but the response and the concentration required for inhibition varied with the fungus. On the basis of these variations the fungi tested could be

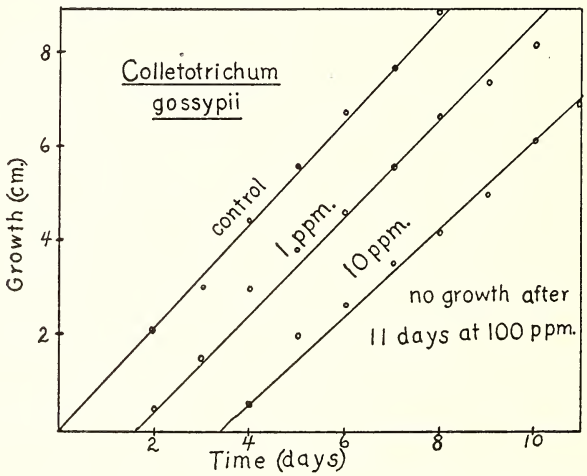

Fig. 1. Growth of *Colletotrichum gossyppii* at various concentrations of thiolutin.

divided into three categories. The highly sensitive ones were inhibited completely by concentrations of 10 ppm or less and included Pythium, Sphaeropsis, and Sclerotinia. Those in the second category of intermediate sensitivity were completely inhibited between 50 and 100 ppm. and included Colletotrichum, Phomopsis and Rhizoctonia. The third category of comparatively resistant fungi showed growth even at 100 ppm. although it was delayed until at least the 7th day. This group included Alternaria, Fusarium and Helminthosporium.

The initiation of growth was usually progressively delayed as the concentration of Thiolutin in the medium increased. There was also some variation in the rate of growth among the different fungi. In a graph such as that showing the growth of Colletotrichum (Fig. 1) the slopes of all of the curves are approximately equally steep, indicating that the rate of growth was not retarded by the antibiotic after the

organism had overcome the original inhibition. In other instances, as is seen in the graphs of Pythium and Alternaria there is a progressive flattening of the growth curves with increasing dosage, showing a continuing inhibitory effect which varies directly as the concentration of the antibiotic increases. (Fig. 2, Fig. 3).

When the fungi growing on the higher concentrations of the antibiotic medium were transferred to antibiotic-free medium they grew normally and required the original inhibitory concentration to suppress growth. Their rate of growth, even after several such transfers, was unchanged from that of the original controls. Thus there was evidence neither of increasing resistance nor increasing sensitivity. Since the antibiotic is fungistatic at these levels, the growth of some of the

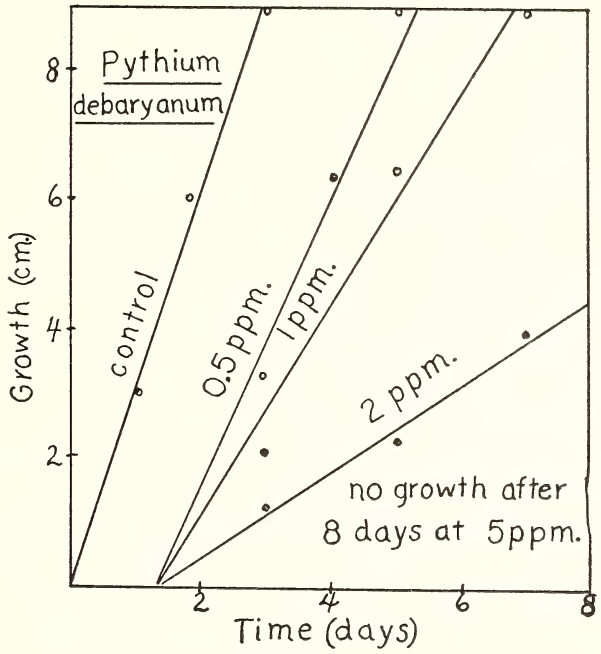

Fig. 2. Growth of *Pythium debaryanum* at various concentrations of thiolutin.

fungi after an initial delay might indicate a gradual reduction in the activity of the antibiotic following incorporation in the medium.

Before attempting to use Thiolutin as a seed protectant, some information was obtained with respect to its phytotoxicity. Young tomato plants were sprayed at the six leaf stage with suspensions and solutions of Thiolutin at concentrations of 6.5, 12, 25, 50, 100 and 200 ppm. No foliar injury was noted, even at the highest concentration. Comparative tests with Actidione, an antifungal antibiotic from *Streptomyces griseus*, indicated that Thiolutin was superior in this respect as

Actidione showed definite phytotoxic symptoms at the higher levels of concentration.

Root systems of young plants of corn, peas, tomatoes, and sunflowers were immersed for a week in nutrient solutions to which had been added 1, 10, 50 and 100 ppm. of Thiolutin and no injury to the root system was noticed except for some evidence of stunting at 100

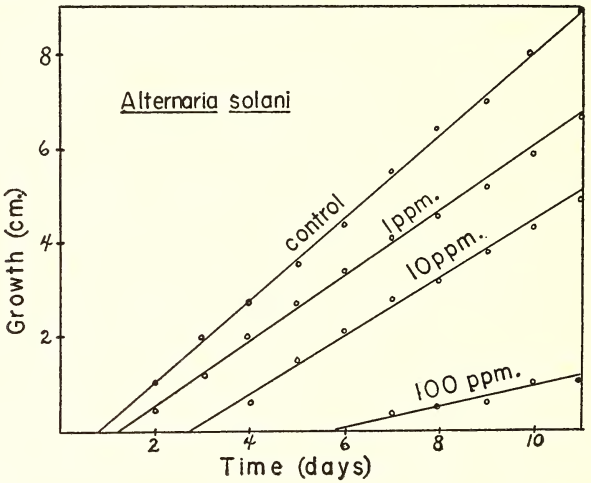

Fig. 3. Growth of *Alternaria solani* at various concentrations of thiolutin.

ppm. A comparative set of experiments with Actidione showed greater phytotoxicity than was encountered with Thiolutin.

The phytotoxic effects of Thiolutin and Actidione on seed germination were evaluated by soaking seeds of corn, peas and radish for 8 hours prior to sowing in solutions of the antibiotics at various concentrations. One hundred seeds were tested of each variety at each concentration, and concentrations of 1, 10, 50 and 100 ppm. were used. Following treatment in the solution, the seeds were rinsed thoroughly in running water and placed to germinate in petri plates between layers of moist filter paper. The results of this experiment are summarized in Table I. Thiolutin does not appear to inhibit germination of the

TABLE I. The Effect of Thiolutin and Actidione upon Seed Germination.

SEED	% GERMINATION								
	Control	Thiolutin (ppm.)				Actidione (ppm.)			
		1	10	50	100	1	10	50	100
Corn	95	95	96	95	95	42	39	21	9
Peas	100	100	100	100	100	33	31	9	0
Radish	100	100	100	98	100	46	12	0	0

seeds tested at any of the levels employed. Actidione, however, is definitely inhibitory. The inhibition by Actidione increases directly as the concentration and appears to effect radish the mostly strongly and corn the least.

The low phytotoxicity of Thiolutin made it appear practicable to test it as a seed protectant. Both dusting and soaking treatments were employed with oat seeds infected with *Helminthosporium* and cotton seeds infected with *Colletotrichum gossypii*. The same technique was used in the soaking treatments that was previously mentioned in the germination experiments, except for changes in the concentration. In the case of dust treatments the antibiotic was placed with the seeds in bottles which were then stoppered and rolled mechanically for 45 minutes to insure uniform coverage. Spergon and New Improved Ceresan were used in other treatments as a basis for evaluation of results, and were applied at concentrations recommended by the manufacturers. The concentrations of Thiolutin ranged from 1/16% to 2% of the seed weight. Following treatment the seeds were planted, and data on germination and disease incidence were recorded. It appears from the results with oats (Table II) that a 1% Thiolutin dust was the most effective treatment, although the soaking treatments even at the lowest concentration were superior to Spergon. In the case of the cotton

TABLE II. Thiolutin as a Seed Treatment for Oats infected with Helminthosporium.

TREATMENT	% GERMINATION	% DISEASE
Control	88	53
1% Thiolutin (soaking)	85	6
1/2% Thiolutin (soaking)	90	14
1/4% Thiolutin (soaking)	90	14
1/8% Thiolutin (soaking)	83	17
1/16% Thiolutin (soaking)	83	24
1% Thiolutin (dusting)	96	3
Spergon (dusting)	95	32

TABLE III. Thiolutin as a Seed Treatment for Cotton Infected with Anthracnose.

TREATMENT	% GERMINATION	% DISEASE
2% Thiolutin (dusting)	80	32
1% Thiolutin (dusting)	80	42
1/2% Thiolutin (dusting)	78	46
Spergon	72	30
New Improved Ceresan	80	6
Control	80	65

seed, although Thiolutin treatments reduce disease in comparison to untreated controls, they do not afford as much protection as Spergon or New Improved Ceresan. The cotton seeds were not delinted and it is possible that improved results might have been obtained by such a procedure, since the effect of the relative volatility of the fungicides would then be less significant. Since Thiolutin was not available at the time in large quantities it was necessary to limit the variety of the treatments.

Literature Cited

1. GOTTLEIB, D. et al. 1951. Endomycin, a new antibotic. Phytopath. **41**: 393-400.
2. JUMP, J. A. 1950. Observations upon a strain of *Streptomyces albus* antagonistic to certain phytopathogenic fungi. Proc. Ind. Acad. Sci. **59**:71-74.