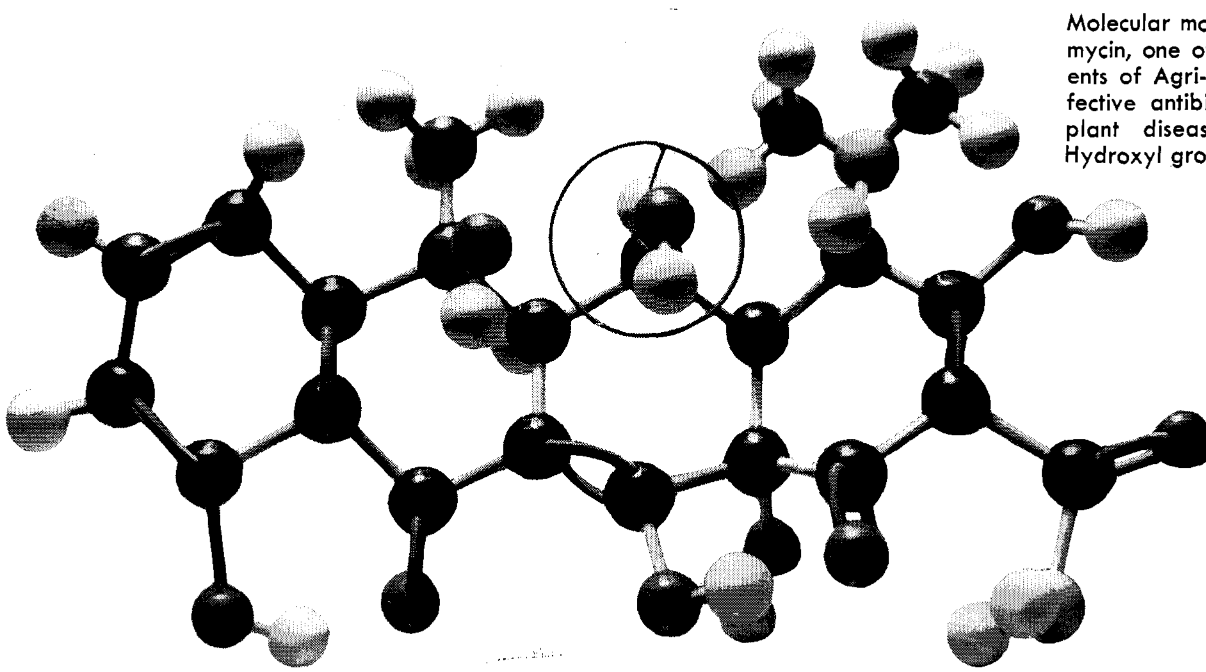

Molecular model of Terramycin, one of the constituents of Agri-mycin, an effective antibiotic agent in plant disease treatment. Hydroxyl group is circled

# Antibiotics and Plant Health

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ANTIBIOTICS for control of plant diseases have been investigated for the past eight or nine years, but only recently has widespread, keen interest in this field developed. Prior to 1951 most of the studies dealt with screening plant pathogens in vitro to determine their sensitivity to antibiotics. Since then, in addition to expanding such work investigators have been testing antibiotic materials in the greenhouse and field for the control of many plant diseases. Although screening tests in vitro have shown that a number of antibiotics are effective against many pathogens, especially bacteria, streptomycin appears to be the most promising for the control of certain bacterial diseases of plants.

Today two commercial formulations of partially purified streptomycin—Agri-mycin (Chas. Pfizer & Co., Inc.) and Agristrep (Merck & Co., Inc.)—are on the market. They are recommended for the control of fireblight of pears and apples, walnut blight, wildfire of tobacco, and bacterial spot of peppers and tomatoes. These compounds are water-soluble, nontoxic to plants at the recommended concentrations, and relatively stable. It has been reported that Agristrep stored for 10 weeks at 60° C. lost less than 5% in potency (*5*). Agri-mycin contains streptomycin and terramycin as its active ingredients in a ratio of 10 to 1. Preliminary reports have shown that it is compatible with most commercial fungicides. Tests in vitro have also been reported to show that the inclusion of Terramycin in the formulation prevents or greatly retards the development of streptomycin-resistant strains of bacteria (*4*).

The third antibiotic formulation on the market is Actidione (Upjohn Co.), cycloheximide, which is recommended for the control of certain fungus diseases. It is slightly soluble in water and stable in neutral or acid solutions, but rather phytotoxic at relatively low concentrations. Actidione Ferrated, the formulation used for the treatment of turf diseases, contains 4% cycloheximide and 96% exsiccated ferrous sulfate.

This paper reviews the more recent work on antibiotic control of plant diseases on crops other than orchard trees. Particular emphasis is placed on the results obtained under greenhouse and field conditions.

### Halo Blight of Beans

Halo blight of beans, caused by *Pseudomonas phaseolicola*, was one of the first bacterial diseases of vegetables to be effectively controlled with an antibiotic under field conditions. This disease may be found wherever rain falls frequently during the growing season. The symptoms of halo blight occur on the leaves, stems, and pods. When the disease is widespread, it may cause considerable loss. In 1952 Mitchell, Zaumeyer, and Anderson (*10*) showed in greenhouse tests that bean seedlings were protected from halo blight by application of a small amount of streptomycin sulfate to the stems of plants before inoculation with the causal organism. The stems absorbed the antibiotic and translocated it upward into the primary leaves in sufficient amounts to protect the plants from infection.

Later field studies (*17, 18*) showed that when bean plants were sprayed three times at weekly intervals with a water solution containing 1000 p.p.m. of streptomycin they were protected from infection. The plants were artificially inoculated three days after the first spraying. Forty-one per cent became infected in the plots that were sprayed once with streptomycin, 10% in those sprayed twice, and none in those sprayed three times. In the untreated control, 93% of the plants became infected. In plots sprayed four times with ferbam and captan, 88 and 91%, respectively, became infected. Still later tests indicated that control was equally good when the concentration of streptomycin was lowered to 250 p.p.m.

In 1954 field tests indicated that the halo blight organism can be largely eradicated by the use of an Agri-mycin spray. In a series of plots, bean plants that had been artificially inoculated with the halo blight organism were sprayed one to three times with a solution containing 200 p.p.m. of Agri-mycin, beginning at the time the first symptoms of the halo blight appeared. In a second series similar plots were treated three times with the antibiotic at 500 p.p.m. at weekly intervals, beginning after the symptoms of the disease were much more advanced than in the first series.

In the first series of plots, 1.1% of the plants receiving one spray and 0.8% of those receiving three sprays were infected. No active lesions were observed on any of the sprayed plants. In the unsprayed plots, 19% of the plants were infected and all had active lesions.

In the series in which the plants were treated only after the halo blight symptoms were rather advanced, infection was reduced so that only 1.8% of the plants receiving one spray had active lesions and there were no active infections on those sprayed three times. In the unsprayed plot, 26.5% of the plants had active lesions. In all plots, the plants that had been seriously infected at any time remained stunted, even though no active lesions were evident after treatment. Most of the organisms in the sprayed plots apparently were killed by the treatments since in most cases the percentages of secondary infections were appreciably reduced and in the plot sprayed three times completely eliminated.

The translocation of streptomycin in bean plants was investigated in detail by Mitchell, Zaumeyer, and Preston (*11*). When the antibiotic was applied in a lanolin paste to the stems of young bean plants, streptomycin was detected in the juice of nearby leaves. When filter paper disks impregnated with this juice were placed on plates of Thaxter agar seeded with *Xanthomonas phaseoli*, the cause of common bean blight, inhibition zones developed around the disks. Similarly, a streptomycin-dependent strain of *Escherichia coli* grew on agar that contained the diffusate from the juice of plants whose stems had been treated with streptomycin sulfate. *E. coli* failed, however, to grow on agar that contained diffusate from the juice of untreated plants. These experiments indicated that the antibiotic translocated in the plants was streptomycin.

Streptomycin was rapidly absorbed by the bean stems and translocated to primary leaves, as measurable amounts of the free antibiotic were detected within eight hours after treatment. Concentration of the free antibiotic was greatest during the third day. It was shown that as much as 67% of the total streptomycin applied to the stems was translocated to the primary leaves as free streptomycin during the five days immediately following treatment.

Primary leaves inoculated with the halo blight organism about 10 minutes after the stems were treated with streptomycin failed to develop visible symptoms of halo blight. One hundred $\gamma$ of streptomycin per plant applied to bean stems completely protected the primary leaves from halo blight infection. Lower doses delayed the appearance of initial symptoms.

Streptomycin was shown to be translocated only in an upward direction through the stems and from the base of the leaves to the tip. Translocation of the antibiotic from the leaves and stems into the pods was not observed.

***Plant diseases have taken a heavy toll from agricultural crops. Antibiotics, only now emerging from the research into the commercial stage, offer hope in areas previously almost defenseless***

### Bacterial Spot of Tomato and Pepper

Bacterial spot, caused by *Xanthomonas vesicatoria*, frequently causes considerable loss to tomatoes in the Middle and South Atlantic, Central, and Gulf States. It is most common in seasons when rainy periods are frequent. Peppers also are subject to the disease and often are seriously damaged. For many years it has been a serious disease of these crop plants in the vegetable growing areas along the lower east coast of Florida. Seedlings grown in plant beds for transplanting the fall and winter crops are frequently so seriously infected as to be

**Demonstration of bacterial spot of peppers. Experimental work has shown effectiveness of Agri-mycin against this disease**

unfit for this purpose. The less seriously infected seedlings are often used, and as a result the disease is introduced into the field where it causes considerable loss when prolonged rainy periods occur. Control of bacterial spot should start in the seed bed and effective control there will result in much less field damage. Prior to the use of antibiotics there was no effective control of this disease.

Conover (*2*) in 1954 reported good control of the bacterial spot in tomato plant beds with a water spray containing 200 p.p.m. of Agri-mycin. The first application was made when most of the seedlings had two expanding leaves and the third leaf was emerging. Five applications were made, the first three at four-day intervals and the last two at two-day intervals. The intervals between the last two applications were shortened because hard driving rains occurred. Of the treated seedlings, 74% were disease-free and only 0.4% were severely infected. In the untreated beds 12.8% of the plants were disease-free and 34.8% were severely diseased. In the treated beds, 95% of the plants were suitable for transplanting whereas in the untreated beds only 27% were usable. No injury to the seedlings by the treatment was observed.

Conover's (*2*) experiments on control of bacterial spot on pepper seedlings were not as extensive as those on tomato seedlings, but similar results were obtained. Five applications of Agri-mycin at 200 p.p.m. were as effective in controlling the disease on peppers as on tomatoes. Cox *et al.* (*3*) reported that three applications of streptomycin at 500 p.p.m. eradicated the disease in severely infected field-grown peppers.

### Tobacco Wildfire

Damage by wildfire, caused by *Pseudomonas tabaci*, a bacterial organism, varies greatly in different years and areas. In certain years and in all major tobacco-growing areas this disease is extremely destructive. Recently, it has been very prevalent and destructive throughout the burley tobacco–growing areas of Kentucky and Tennessee.

Heggestad and Clayton (*7*) reported that streptomycin sulfate gave excellent control of wildfire in tobacco plant beds that had been artifically inoculated with the causal organism. Spray treatments were more effective than drench or dust treatments; the last was the least effective. They used Agristrep, Agri-mycin, streptomycin chloride, an insoluble salt of streptomycin (Merck STB), Bordeaux, and tribasic copper sulfate. First treatments were applied when the plants were in the two-leaf stage and three additional sprays were made at weekly intervals. All spray treatments were made at the rate of 10 gallons per 100 square yards.

The best control resulted from Agri-mycin and Agristrep sprays at the rates of 200 and 400 p.p.m., the former strength being as effective as the latter. In the plant beds treated with these antibiotics, no plants were killed by the disease whereas 7% of the plants treated with Bordeaux and 20% of those treated with tribasic copper sulfate died. In the untreated checks, 30% of the plants died. Streptomycin chloride and the insoluble salt formulation of streptomycin were inferior to Agristrep or Agri-mycin. Leaf infection of the surviving plants was practically negligible in the plots treated with Agristrep and Agri-mycin while in those treated with the copper compounds there was a much higher incidence of infection.

To test the effect of streptomycin sulfate on the eradication of wildfire, applications were made in growers' beds in which the plants were seriously infected with the disease. The plants were sprayed three times at weekly intervals at a concentration of 200 p.p.m. At the termination of the treatments one third more of the plants were living in the treated areas than in the untreated ones. Only 5% of the leaves examined from the untreated areas were free of wildfire infection, while 79% of the leaves examined from the plots receiving three applications of streptomycin sulfate were free. Similar treatments of diseased tobacco beds made by a number of farmers gave equally striking results. It thus appears that streptomycin is effective both as a protectant against the wildfire organism and as an eradicant.

It was noted that plants treated with streptomycin sulfate sprays at the rate of 200 and 400 p.p.m. had larger and more fibrous roots than plants that survived in the untreated plots and in those sprayed with fixed copper. Severe wildfire infection often causes appreciable injury to the roots and to the tops of plants. Plants with good fibrous root systems survive transplanting better than those with poor root development. The production of a good root system following the application of streptomycin may be an additional advantage of using this antibiotic to control wildfire in tobacco plant beds.

### Blue Mold of Tobacco

Blue mold, caused by *Peronospora tabacina*, is a fungus disease of tobacco in plant beds. If conditions for the development of the causal fungus, such as cool, moist, cloudy weather, are favorable seedling losses may be severe.

Grosso (*6*) reported the control of this disease with streptomycin sulfate in greenhouse tests. Young tobacco plants were sprayed with aqueous solutions of 10 antibiotics at 100 p.p.m. and with Dithane when their leaves were about the size of a silver dollar. Four applications were made at three- or four-day intervals. Beginning the day following the second application, the plants were inoculated on two successive days with spore suspensions of the blue mold fungus.

In one experiment, streptomycin reduced leaf damage due to the fungus to 1.8% as compared with 30% in the untreated check. Damage by blue mold in seedlings sprayed with several other antibiotics ranged from 7% for those treated with Polymixin to 40% for those treated with Bacitracin. Plants treated with Dithane had 1.7% leaf damage.

In a second experiment, plants sprayed with streptomycin at 200 p.p.m. showed

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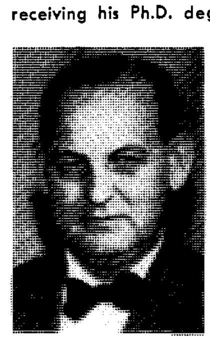

Photo from *Plant Disease Reporter* shows control of tobacco wildfire with streptomycin sulfate spray, right; tribasic copper sulfate spray, left; no treatment, center

1.1% leaf damage caused by the fungus compared with 50% on plants in the untreated check. Plants treated with Dithane showed 12.7% damage. This is the first report of the control of a fungus disease with streptomycin.

### Potato Seed Piece Decay

Seed piece decays caused by *Erwinia atroseptica* and *Pseudomonas fluorescens* are sometimes responsible for extensive losses of stand in the field. The same organisms may also cause heavy losses of potatoes in storage. In the field, the former organism also causes a disease of the plant known as black leg.

Bonde (*1*) conducted greenhouse tests with freshly cut potato seed pieces sprayed with the bacterial pathogens and then dipped in water solutions of streptomycin sulfate, terramycin hydrochloride, or a combination of the two before planting. The first and third treatment controlled these diseases. Treating infected seed pieces for 10 to 30 minutes in solutions containing 25 p.p.m. of either of these compounds practically eliminated seed piece decay and an almost perfect stand of potatoes resulted. Of the untreated infected seed pieces, 80 to 100% decayed. Terramycin hydrochloride alone did not control seed piece decay. No black leg-diseased plants were produced from seed pieces treated with streptomycin sulfate or the combination of this antibiotic and terramycin hydrochloride. From the infected untreated seed pieces and from those treated with terramycin hydrochloride alone, 2 to 5% black leg-infected plants were produced.

In a field experiment Bonde (*1*) treated 100 black leg-infected seed pieces of the Kennebec variety of potato in a 50 p.p.m. streptomycin sulfate solution for 30 minutes before planting. Healthy plants resulted from all of the treated seed pieces whereas infected untreated seed pieces were almost completely destroyed.

### Black Rot of Rutabaga

Black rot of crucifers, caused by *Xanthomonas campestris*, is found wherever these plants are grown except in regions of very low rainfall during the period when the seedlings are being grown. It is a seed-borne disease and its control is difficult.

Sutton and Bell (*15*) reported in 1954 that treating seeds of rutabaga in aqueous solutions of Aureomycin provided effective control of seed-borne infection of the black rot organism. Artificially infected seed was soaked in several concentrations of Aureomycin hydrochloride for 30 minutes and dried for three days. In addition, such treated seed was dusted with Arasan or Spergon. Similar infected seed was immersed in hot water for 25 minutes, the standard seed treatment for this disease, as well as treated with mercuric chloride and a number of other compounds.

When Aureomycin-treated seed was placed on agar plates, no colonies of the pathogen were observed, indicating complete control except in a few instances where Arasan or Spergon was also applied. In the untreated checks, there was 88% infection. Greenhouse soil tests showed that the germination was increased when black rot-infected seed was treated with Aureomycin. At 1-to-2500 and 1-to-1000 dilutions, the organism was eliminated except in one case where Arasan was added. In the untreated check, there was 76% seedling infection. Hot water and chlorine (2%) gave comparable control, but germination of seeds given the latter treatment was lower.

In field trials, similar results were noted. Examination of mature roots of plants from Aureomycin-treated seed showed no evidence of infection, while in those from untreated seed, 30% infection was recorded.

Sutton and Bell (*15*) stated that Aureomycin might replace the hot-water treatment for the control of seed-borne-infection of black rot in crucifers and thus eliminate the objectionable features of that process. Their tests also indicated that the antibiotic is compatible with Arasan and Spergon in storage for as long as nine months and possibly longer without loss of effectiveness.

### Bacterial Wilt of Chrysanthemums

Bacterial wilt, a systemic disease of chrysanthemums, caused by *Erwinia chrysanthemi*, has been shown by Robinson, Starkey, and Davidson (*13*) to be controlled by streptomycin and oxytetracycline. Chrysanthemum cuttings were dipped in water solutions of the antibiotics for six hours, washed and inoculated by dipping in a bacterial suspension of the wilt organism. They were then dusted with a rooting "hormone" powder and placed in flats of sterilized sand. Streptomycin was more effective than oxytetracycline in that it provided protection at lower concentrations and was less toxic to the cuttings. After 25 days, a high percentage of cuttings treated with streptomycin at concentrations of 50 to 600 p.p.m. had survived. All the untreated cuttings had died.

Cuttings inoculated with the wilt organism and later treated with the two antibiotics showed a very high survival after 20 days, whereas all the untreated cuttings were completely killed by the wilt organism. As in the protection experiment, both antibiotics controlled the disease, but streptomycin gave better control and was less toxic than oxytetracycline.

The antibiotics were readily absorbed and translocated in cuttings and less readily in rooted plants. In treated cuttings the antibiotics persisted in sufficient amounts to protect the newly developing plants from infection. Streptomycin remained active in the plants for several weeks. The best concentrations of streptomycin both for disease protection and for rooting of the cuttings were 50 to 150 p.p.m. Higher concentrations interfered with root formation.

Sand treated with streptomycin eliminated established infection in cuttings planted therein. Infection was also controlled when the cuttings were treated with hormone rooting powder containing streptomycin.

### Diseases of Other Crop Plants

Nelson (*12*) reported that a 2% Actidione-sulfur dust was less effective than Dithane Z78-sulfur dust in controlling onion mildew, caused by *Peronospora destructor*. The Actidione-sulfur dust did, however, produce significant yield increases. The Actidione mixture was superior to the Dithane mixture in controlling mint rust, but less effective than Fermate. In spite of this, Nelson suggested that Actidione may be preferred in the control of this disease because the sulfur fungicides leave undesirable residues in the distilled mint oil.

Vaughn and Klomparens (*16*) in 1952 reported that Actidione gave excellent control of dollar spot of turf, caused by *Sclerotinia homoeocarpa*. Actidione was also completely effective for the control of melting out of turf, caused by *Helminthosporium* sp., while phenyl mercury did not check the disease at all.

Howard (*8*) reported this same antibiotic as effective in controlling the fading out disease of turf grasses, caused by *Curvularia lunata*.

Investigations on the inhibitory action of antibiotics on plant viruses are very meager. Leben and Fulton (*9*) tested nine antibiotics for inhibitory action against three plant viruses under laboratory conditions. Detached leaves of test plants were inoculated separately with the viruses of tobacco mosaic, tobacco necrosis, and tobacco ring spot and placed on an agar medium containing the various antibiotics. Streptomycin and Terramycin prevented the production of local lesion symptoms on cowpea leaves by tobacco necrosis and tobacco ring spot viruses. Increase of the tobacco mosaic virus in detached tobacco leaves on agar containing Terramycin was only partially inhibited.

Schlegel and Rawlins (*14*) studied the effect of an antibiotic, MK61 (Merck & Co., Inc.), produced by an actinomycete, previously classified as *Nocardia formica*, on tobacco mosaic virus multiplication in leaf disks floating on solutions of this antibiotic. The concentration of the virus in the disks was determined spectrophotometrically. The percentage of inhibition was based on the amount of virus produced in the same number of infected leaf disks floated on distilled water.

Quantitative data showed that MK61 at 10 p.p.m. and higher produced 69 to 90% inhibition. The inhibitory action was independent of light conditions and its action may be directly on virus multiplication rather than on host metabolism.

### Future of Antibiotics

In the relatively few years in which antibiotics have been investigated for plant disease control, excellent progress has been made. Not more than three years ago this type of research was conducted at only a few institutions but at present investigators at more than 20 are engaged in the evaluation of antibiotics for this purpose. Many of the large pharmaceutical firms have established research laboratories for the screening of new antibiotics against organisms that produce diseases of plants.

Prior to the use of streptomycin, bacterial diseases of plants were extremely difficult to control. Chemical control methods, although used on some crop plants, were not satisfactory. In many cases, the only control measures recommended for annual crop plants such as vegetables were crop rotation and the use of healthy seed produced in areas where the diseases do not occur. Since a number of diseases of several crop plants have been shown to be effectively controlled with antibiotics both as protectants against and eradicants of the causal organisms, there will undoubtedly be a further expansion in this field of research.

### Antibiotics with Antifungal Activity Sought

Few antibiotics have been reported to show any antifungal activity. Actidione appears to be the only one thus far that has shown promise in this respect. It is likely that others will be discovered. With the vast number of fungus diseases that now cost the farmer about $35 million annually for fungicides and with many groups of diseases such as the root rots and others that are unhampered by our best control methods, it is believed that much effort will be made by the chemical industry to produce antibiotics that will control them. It has been estimated that approximately $1 billion in loss, about one half of the losses caused by diseases, could be eliminated if the proper chemicals were available and the growers knew how to use them.

Fungicides are used to protect plant surfaces from infection and applications must be repeated as new foliage develops or if the fungicide is washed off the foliage by rain. A more satisfactory treatment would be to apply materials that are absorbed through leaves or roots and thus provide a systemic protection to all plant parts. This has been partially accomplished by application of certain antibiotics such as streptomycin to stems, leaves, or cuttings. Applications of antibiotics to the soil have proved unsatisfactory thus far, because those available are readily destroyed in the soil. It is possible, however, that there may be produced some which will be stable enough to be applied to the soil where they will be absorbed through the roots and afford systemic protection against many parasites. Then it may be possible to protect against root diseases of crop plants that are caused by soil-inhabiting organisms, most of which are now uncontrollable.

The antibiotics now on the market for the control of bacterial diseases are not economical to use except on high-valued crops or when the control requires only small quantities of spray material such as for seed beds. Antibiotics might be used on lower valued crops if only a single application of the material is necessary to eliminate a trace of bacterial infection in a field so as to prevent destructive secondary dissemination of the pathogen. Effective antibiotics are practical as seed treatments where relatively small quantities of the materials are necessary. It has been estimated that the cost of 100 gallons of Agrimycin at 100 p.p.m. is approximately $9.00. If ground equipment is used this amount would be sufficient to spray about one acre of a vegetable or similar crop. Actidione, which has been shown to be effective against some fungus diseases at very low concentrations, costs approximately 40 cents per 1000 square feet for one application to control turf diseases. When and if a large potential use of antibiotics becomes assured, it is entirely possible that American industry will make use of its vast production methods to produce these products at prices low enough for general agricultural use.

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