Stage Specificity in Streptomycin Action Against Some Plant Pathogenic Peronosporales

Yigal Cohen and Meir Perl

Department of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel, where most of the work reported here was done by both authors. The senior author is currently on leave from Bar-Ilan at the Department of Plant Pathology, Macdonald College of McGill University, Quebec, Canada where he did part of this work.

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ABSTRACT

Streptomycin inhibits sporangial germination of *Plasmopara halstedii*, the causal organism of sunflower downy mildew, but not that of *Pseudoperonospora cubensis*, the causal agent of cucumber downy mildew, nor of *Phytophthora infestans*, the potato late blight agent. Infection of all three hosts by their respective pathogens is inhibited by streptomycin at 10-100 μg/ml in the inocula; however, if applied 24 hr after inoculation, streptomycin has no effect either on symptom expression or on fungal development. Sporulation of all three pathogens on fully developed lesions is not affected by streptomycin. Although fungal development is stopped when streptomycin is added at the initial inoculation, a lesion restricted to the area inoculated is observed on cucumber and potato leaves ("printing"-type lesions). Mycelial growth of *Ph. infestans* in vitro is greatly reduced by streptomycin. No such reduction occurs if the antibiotic is added to the medium of 48 hr, or older, cultures. It is suggested that streptomycin acts on the fungus rather than on the host, and the later "resistance" to the antibiotic is attributed to some intracellular changes occurring in the fungus after the initial stages of growth.

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Streptomycin is known to control a number of downy mildew diseases such as those of hop [caused by *Pseudoperonospora humuli* (Miy. & Tak.) Wilson] (10), cucumber [caused by *Pseudoperonospora cubensis* (Berk. & Curt.) Rost.] (2, 3), tobacco (caused by *Peronospora tabacina* Adam) (1, 15), and potato [caused by *Phytophthora infestans* (Mont.) d By.] (11, 21). The manner in which this control is accomplished is not clear. It has been shown that streptomycin markedly reduces sporangial and zoospore germination (14, 18), decreases infection rate (2, 11), and inhibits spread of the pathogen in host tissue (21). It has been suggested that a reduction in sporulation of *Ps. humuli* is probably the main controlling effect of streptomycin (14).

This paper presents data showing the effect of streptomycin on different stages in the life cycles of three fungi of the Peronosporales that are plant pathogens. The data also show that post-infectional development of the fungi is not affected by the antibiotic.

MATERIALS AND METHODS.—*Plants, pathogens, and inoculation techniques.*—Cucumber (*Cucumis sativus* L. 'Bet-Alpha') was used for inoculation with *Pseudoperonospora cubensis* (Curt. & Berk.) Rost. The experiments were performed with plants in their cotyledonary and second-true-leaf stage. Cotyledons (60-90 per treatment unless otherwise stated) were inoculated by placing 6-mm diam disks of filter paper, saturated with 0.02 - 0.025 ml of a water suspension of 8-12 X 10^4 sporangia/ml of *Ps. cubensis* in the centre of the adaxial leaf surface (22). First and second true leaves (12-18 per treatment) were inoculated on their adaxial surface with 800 ± 10% sporangia on a 4-cm^2 target (7) using a Schein inoculator (24). All inoculated plants were kept for 24 hr at 15 C in a dark moist chamber and then transferred for 5 days or more into growth chambers maintained at 20 ± 0.5 C and 50-70% relative humidity with a 12-hr photoperiod (Sylvania VHO fluroescent lamps) at 16,800 lx (1,560 ft-c) (measured at plant level).

Lesion development on true leaves was evaluated by visual index (5), and disease incidence on cotyledons, by calculating the percentage of infected leaves.

Sporulation of *Ps. cubensis* was measured on infected leaves kept for 24 hr in a dark moist chamber at 15 C. Lesions were detached and shaken in vials containing 2 ml FAA for 10 min and the sporangia were counted with the aid of a cytometer (6).

Sunflower (*Helianthus annuus* L. 'Sunrise') was inoculated with *Plasmopara halstedii* (Farl.) Berl. et de Toni by two methods: "whole seedling immersion" (WSI), and "bud inoculation" (8, 9). For WSI, three-day-old seedlings were soaked in a sporangial suspension of 10^4 sporangia/ml for 6 hr at 15 C in the light, then removed, washed with about 500 ml tap water, and potted (five plants/10-cm diam pot) in pasteurized soil, and grown at 20 ± 1 C [23,650 lx (2,200 ft-c) 14 hr light photoperiod]. After 7-10 days the plants were covered with polyethylene bags to induce sporulation. Sporulation on cotyledons was rated visually on a 0-4 scale (8, 9). "Bud inoculations" were made by placing disks of

filter paper, 6 mm diam, saturated with 0.02 - 0.025 ml of a sporangial suspension (10^4/ml) on the growing point of plants in the two to four leaf stage. Inoculated plants were covered with polyethylene bags and kept at 15 C for 24 hr, then they were uncovered and transferred to a 20 ± 1 C cabinet for 2 weeks for symptom development. WSI-inoculated plants that had to be treated with streptomycin 24 hr after inoculation, were soaked in antibiotic solutions for 6 hr at 15 C, then washed and potted. Bud-inoculated plants were treated by attaching 6-mm diam filter paper, soaked with streptomycin solutions, to their apical buds and covering them for 24 hr at 15 C. Control inoculated plants were treated with distilled water in the same way. The infection was considered to be systemic when the spreading symmetrical pattern of chlorosis, typical of this disease, developed on true leaves.

The potato cultivar 'Up-to-date' (*Solanum tuberosum* L.) was used for inoculation with *Phytophthora infestans* (race 0). Plants were grown as described elsewhere (23) and were inoculated at age of 5 weeks by placing two filter paper disks, soaked in a sporangial suspension (6-10 × 10^3 sporangia/ml) on the adaxial side of each leaflet, one disk on each side of the main vein, 20-40 leaflets/plant, four plants per treatment. Plants were covered with polyethylene bags and kept at 15 C for 24 hr, then uncovered and transferred to a 20 C cabinet, as described for cucumbers. Lesion development was evaluated on the 4th day (unless otherwise stated) using the following 0-4 visual scale: 0 = no infection; 1 = lesions of up to 10 mm in diam; 2, 3 = lesions of 10- and 20-mm diam, respectively; and 4 = lesions of more than 20-mm diam. "Printing"-type lesions refer to separated necrotic lesions (about 1-mm diam) restricted to the inoculated area.

Phytophthora infestans was cultured in vitro on liquid wheat seed medium (modified Hodgson & Grainger medium) (16) made of 200 g steamed wheat seeds, 0.02 g $FeCl_3$, 20 g sucrose and distilled water to make 1,000 ml. Two percent agar was added if a solid medium was needed. Cultures were kept at 20 C in the dark.

The pathogen was initially isolated from infected potato leaves. It maintained its germinability and pathogenicity throughout the experimental period.

All inoculation tests were repeated four times or more.

Zoospore release, zoospore germination, and sporulation.—Sporangia were collected and deposited on 5-μ Millipore membranes, then resuspended in distilled water or in streptomycin solutions, and kept in 5-cm diam petri dishes, at 10-15 C for 4-24 hr (unless otherwise stated). Sporangia were scored microscopically and the percentage of empty ones, out of the 200-500 observed, was considered the percentage which had released zoospores. For zoospore-germination tests, zoospores were separated from sporangia, by filtration through Whatman No. 4 paper, and kept in distilled water or in streptomycin solutions at 10-15 C for 24 hr. Germination of *Ps. cubensis* zoospores was carried out in a 0.1 M sucrose solution. To check the effect of streptomycin on sporulation, on germinability of sporangia, and on infectivity of liberated zoospores, infected leaves were floated abaxial surface down, on 10 ml streptomycin solutions in closed petri dishes and kept at 15-20 C for 24 hr in the dark. For germinability and infectivity tests, sporangia were removed carefully (using a hairbrush), washed 25 times with 20 ml of distilled water and resuspended in distilled water (or in 0.1 M sucrose solution for *Ps. cubensis*). Sporulation intensity of *Ps. cubensis* and

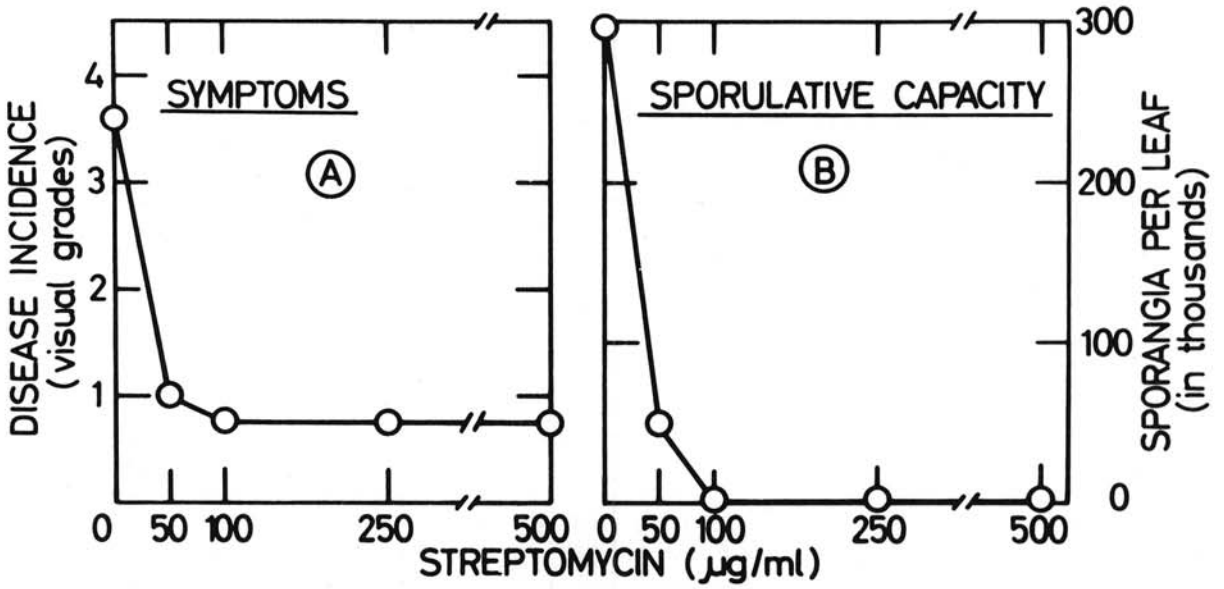

Fig. 1. Effect of streptomycin mixed in the inocula, on downy mildew disease development on true leaves of cucumber. Evaluation was done by visual symptoms (A) and by measuring the sporulative capacity of *Pseudoperonospora cubensis* in lesions (B) on the 6th day after inoculation.

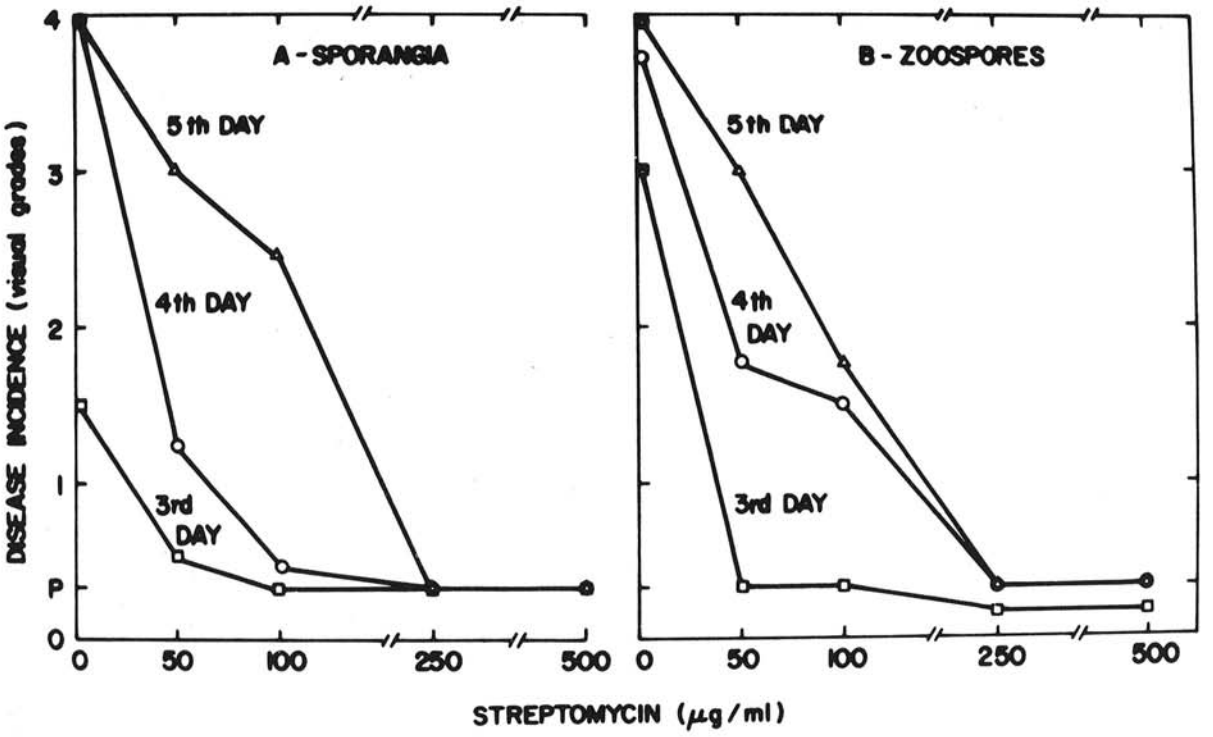

Fig. 2. Patterns of potato late-blight lesion development as affected by streptomycin. Plants were inoculated with sporangial suspensions (A) or with zoospore suspensions (B), containing 0-500 µg/ml streptomycin. Plants kept at 20 C and disease incidence was recorded on the 3rd, 4th, and the 5th day.

Ph. infestans was determined with the aid of a cytometer, whereas that of *Pl. halstedii* was evaluated visually (9). Each of these experiments was repeated three times or more.

Antibiotic.—Streptomycin sulfate (Sigma) solutions in distilled water were used, unless otherwise stated. For germination tests, and for inoculations, the antibiotic was added to sporangial or zoospore suspensions in different final concentrations without changing the fungal propagule concentration.

RESULTS.—*Toxicity of streptomycin to the host plants.*—No damage was seen on cucumber or potato plants sprayed with up to 500 µg/ml within the experimental period (usually one week). Chlorotic symptoms developed 2-3 weeks after treatment.

Sunflowers were much more sensitive to the antibiotic. Chlorotic lesions were seen on leaves 3 days after treatment with streptomycin at a concentration of 100 µg/ml or more.

Effect of streptomycin on infection.—Streptomycin strongly restricted disease development, if it was applied to the host plants with the inoculum mixture. No infection of sunflower, cucumber, or potato leaves by their respective pathogens occurred when they were inoculated with sporangial suspensions containing 10, 100, and 250 µg/ml streptomycin, respectively (Fig. 1-A, 2, Table 1). Potato tuber-slice infection with *Ph. infestans* was restricted by mixing 10 µg/ml streptomycin with the inoculum (Table 2), and no mycelial growth of the fungus occurred on wheat seed medium containing 10 µg/ml streptomycin (Table 3).

However, inoculations of true leaves of cucumber and potato with sporangial suspensions containing 100 or 250 µg/ml streptomycin, respectively, resulted in the development of a special type of lesion, consisting of yellowish-green (cucumber) or necrotic (potato) spots, about 1 mm in diam restricted to the inoculated area (Fig. 3). These are referred to as "printing"-type lesions (P) and were given a score of 0.25 (Fig. 2) and 0.75 (Fig. 1) for potato and cucumber, respectively. No sporulation could be

TABLE 1. The effect of streptomycin applied at time zero and 24 hr after inoculation on infection of sunflowers with *Plasmopara halstedii*

Streptomycin (µg/ml)	Cotyledon infection index[a]		% systemically infected plants[b]	
	0	24 hr	0	24 hr
0	2.85	2.80	100	100
1	0.75	2.00	100	88
10	0.0	2.10	0	86
100	0.0	0.35	0	80

[a] Whole seedling immersion method. Maximum infection index = 4.00. Twenty plants/treatment.

[b] Bud-inoculation method. Fifteen plants/treatment.

TABLE 2. Development of *Phytophthora infestans* on surfaces of potato tuber slices as affected by time of application of streptomycin

Time after inoculation (hours)	Sporangia × 10^3 per cm^2 of tuber slice surface[a] ± S.E.						
	Streptomycin concentration (μg/ml)						
	0	10	25	50	100	250	500
0		0.1	0	0	0	0	0
3	53.5 ± 18.6	2.3	0	0	0	0	0
24		18.3 ± 8.0	38.9 ± 9.1	3.1 ± 5.0	0	0	0
48	37.4	33.8 ± 17.0	36.4 ± 6.0	28.2 ± 7.5	11.0 ± 5.6	23.2 ± 8.1	15.3 ± 2.5
72	57.6 ± 7.2	41.7 ± 12.0	55.8 ± 12.0	54.1 ± 3.7	39.3 ± 5.2	34.7 ± 6.6	44.1 ± 8.7

[a] Eight tuber-slices/treatment. Sporangia collected after 10 days of incubation at 20 C. Initial fungal development was seen on the 4th day.

detected on "printing"-type lesions (Fig. 1-B). (No histological investigations were conducted with "printing"-type lesions.) Similar lesions developed when true leaves of cucumber were inoculated on their adaxial surface and the antibiotic was applied simultaneously to the abaxial surfaces (Fig. 4), whereas no infection was seen on cotyledons treated in the same way.

In order to determine whether the "printing" areas on cucumbers are resistant to a new mildew invasion, true leaves were inoculated with streptomycin-exposed sporangia (100 μg/ml for 1 hr, see below); then the same targets were inoculated with fresh, nontreated sporangia 24 hr or 5 days later. Lesion development was recorded on the 10th day after the first inoculation. All leaves inoculated twice became normally infected, whereas those which were inoculated only with streptomycin-exposed sporangia showed only the "printing" pattern of lesion development.

Attached leaves of potato were inoculated on their adaxial surfaces and streptomycin was applied simultaneously to the abaxial surfaces, and vice versa. No inhibition of infection and no inhibition of lesion development was caused by streptomycin, even at 5 mg/ml; nor were infection and lesion development affected by spraying streptomycin solutions (up to 500 μg/ml) on the leaf surface 0-24 hr before inoculating the unsprayed side of the leaves. As will

TABLE 3. In vitro growth of *Phytophthora infestans* on wheat seed medium as affected by streptomycin applied various times after inoculation

Time of application (days)	mg fungus per colony (dry weight)[a]			
	Streptomycin concentration (μg/ml)			
	CK[b]	0	10	50
0	0	12.5 ± 2.1	0	0
2	0	10.0 ± 7.0	0.65 ± 0.35	0
4	1.0 ± 0.57	8.0 ± 4.2	3.7 ± 1.7	4.0 ± 0.86
6	8.2 ± 1.2	13.3 ± 4.0	12.8 ± 4.0	11.9 ± 3.3

[a] Average of four replicates, weighed on the 16th day after inoculation.

[b] Dry weight at time of applying the antibiotic.

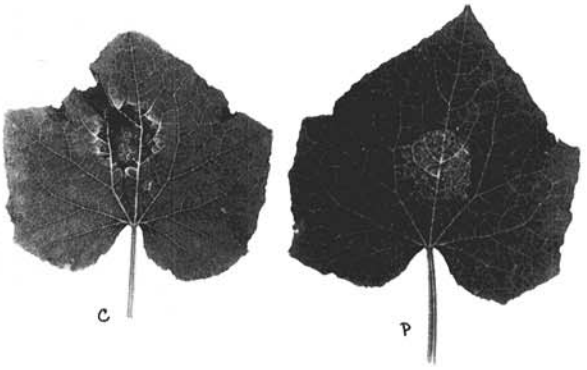

Fig. 3. "Printing"-type lesion (P) and normal downy mildew lesion (C) on cucumber true leaves a week after inoculation with streptomycin-free sporangial suspension (C) or with sporangial suspension containing 100-500 μg/ml streptomycin (P).

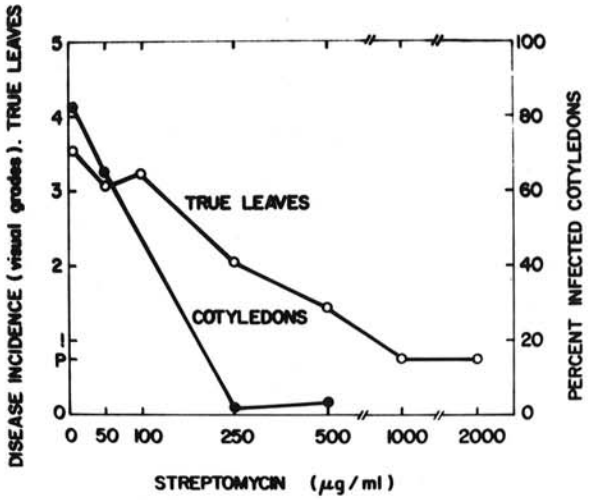

Fig. 4. Effect of streptomycin applied to the abaxial surfaces of cucumber leaves at zero-time after inoculating the adaxial surfaces, on the subsequent lesion development of *Pseudoperonospora cubensis*.

TABLE 4. Infection of cucumber cotyledons treated with streptomycin up to nine days prior to inoculation with *Pseudoperonospora cubensis*

Period prior to inoculation (days)	% infected cotyledons[a]	
	Water control	Streptomycin (250 μg/ml)
1	74.1	0
2	61.4	0
3	76.2	1.7
7	91.8	0
8	87.8	11.1
9	83.6	35.3

[a] Number of leaves/treatment ranged from 54 to 115.

be shown later, this lack of inhibition probably resulted from failure of the antibiotic to penetrate the leaf tissue (21). However, floating detached potato leaves (abaxial side down) on a streptomycin solution of 250 μg/ml 12-24 hr prior to inoculation inhibited infection; only three to 12 hr was needed if the concentration of the antibiotic was 500 μg/ml.

The ability of streptomycin to persist on plants, and to protect them from infection, was investigated using cucumber plants at their cotyledonary stage. Plants were sprayed with streptomycin solution of 250 μg/ml at zero time, and inoculated at various times thereafter. The plants were protected against the pathogen for a week (Table 4); infection gradually increased after this time.

The time needed for the antibiotic to penetrate the leaves was tested on cucumber plants at their cotyledonary stage. Plants were sprayed with streptomycin solutions of 0, 50, 250, and 500 μg/ml, covered with plastic bags for periods up to 24 hr prior to inoculation, then uncovered, washed with water and inoculated. Infection was found to be correlated with concentration and period of contact of the antibiotic with the host plant: the higher the streptomycin concentration used, the shorter the time needed to protect the plants from infection. Thus, to limit infection to 10% of the control, 24 hr were needed for streptomycin at 50 μg/ml, 6 hr for 250 μg/ml, and 3 hr for 500 μg/ml.

Pretreatment of 3-day-old seedlings of sunflower with streptomycin protected them from mildew infection. Plants were immersed in streptomycin solutions for five min, removed, washed thoroughly with water, and inoculated (WSI method). The infection index of cotyledons a week later was: 2.85, 2.40, 0.65, and 0.40 for 0, 10, 100, and 1,000 μg/ml streptomycin solution treatments, respectively.

To check whether streptomycin could stop invasion of newly developing leaves of sunflower (17) by *Pl. halstedii*, healthy and infected plants (20/treatment, WSI method) were sprayed, at the early two-leaf stage, with water or with streptomycin solutions of up to 1,000 μg/ml. A week after spraying (early four-leaf stage), 10 plants of each treatment were moved to moist chambers to induce sporulation, and the remaining 10 plants of each initial treatment were again sprayed with the antibiotic; sporulation was induced on these plants 7 days later at the early six-leaf stage. The pathogen sporulated abundantly on all leaves of all plants treated once with the antibiotic. A slight decrease in sporulation was observed on some of the leaves treated twice with 1,000 μg/ml streptomycin solution. All leaves showed the typical systemic symptom pattern. Similar results were obtained with apical bud inoculations.

Effect of timing of streptomycin applications.—In another series of experiments streptomycin was administered to test plants at intervals up to 24-48 hr after inoculation. Cucumber true leaves were inoculated on their adaxial surface at time zero and streptomycin was applied to the inoculated (adaxial) surfaces 0, 3, 6, 12, and 24 hr thereafter. In another experiment, cotyledons were inoculated on their adaxial surface at zero time and streptomycin solutions were sprayed on their abaxial surfaces at intervals after inoculation. In both cases, streptomycin was found to restrict disease development if applied shortly after inoculation. Inoculated true leaves treated with the antibiotic up to 12 hr subsequent to inoculation showed only the "printing" pattern development of lesions (Fig. 5). If applied at 24 hr, however, the only effect of the streptomycin was to slow the rate of infection. In the water control, the pathogen reached its maximal sporulating potential (7) on the 5th day; on leaves treated with streptomycin after 24 hr, it was not reached until the 11th day. No sporulation occurred on the leaves treated 0-12 hr after inoculation. Cotyledons treated at time zero with streptomycin at 250 or 500 μg/ml showed almost no infection. Those treated at the third hour and later, showed gradual increase in percentage of infection. Streptomycin at 50 μg/ml had no effect.

The antibiotic markedly limited infection of sunflower cotyledons when applied at 1 μg/ml level with the inoculum; however, if applied at 10 μg/ml 24 hr after inoculation, it did not affect infection rates. At a concentration of 100 μg/ml it decreased infection of cotyledons but not of bud-inoculated plants (Table 1).

Detached potato leaves were placed, adaxial surface upward, on wet filter paper inside petri dishes at 20 C. The adaxial leaf surfaces were inoculated with a block (3 by 3 mm) of wheat seed-agar culture of *Ph. infestans*. At 24 or 48 hr after inoculation, 50 μl of 0-500 μg/ml streptomycin solutions were applied to the adaxial surfaces of each leaf. Diameters of lesions were measured a week later. A similar experiment was carried out using potato tuber slices; sporulation on the slice was the criterion of fungal development. Results show that streptomycin restricted lesion development ("printing" type lesions) and fungal development (Table 2) if applied 0-24 hr after inoculation. No effect was seen on leaves or on tuber slices (Table 2) treated with the antibiotic after 24 hr.

Timing experiments were also performed in an in vitro system. Petri dishes containing 10 ml of liquid wheat seed medium were inoculated with 2- by 2-mm

blocks of wheat-agar cultures of *Ph. infestans* at zero time. The original medium was aseptically removed by suction 0, 2, 4, and 6 days after inoculation, and replaced with liquid wheat seed media, either containing streptomycin, or streptomycin-free. On the 16th day, colonies were collected on 5-μ Millipore membranes, washed, dried (overnight at 90 C), and weighed. Streptomycin was found to completely inhibit fungal development if applied at time zero (Table 3). However, it only partially inhibited fungal growth if applied on the second day (10 μg/ml) or on the fourth day (50 μg/ml), and no differences in growth were detected if the antibiotic was applied on the sixth day (Table 3). The same results were obtained when dihydrostreptomycin was used.

Effect of streptomycin on zoospore release and zoospore germination.—Zoospore release of *Ps. cubensis* (8-12 X 10^4 sporangia/ml) was almost unaffected by concentrations of streptomycin up to 500 μg/ml (the highest tested) (Fig. 6-A). The percentage of germinated zoospores as determined in 0.1 M sucrose solutions (zoospore germination does not occur in distilled water) was only slightly affected by the antibiotic (Fig. 6-B). Both zoospore release and zoospore germination of *Ph. infestans* were affected by the antibiotic. Percentage of release and germination decreased with increasing concentration of the antibiotic. However, even in a streptomycin concentration as high as 500 μg/ml, about 30% of the sporangia and of the zoospores, respectively, did germinate. Inoculation of potato leaves with a mixture of germinated zoospores (obtained at 10 C, 20 hr after separation from sporangia) and

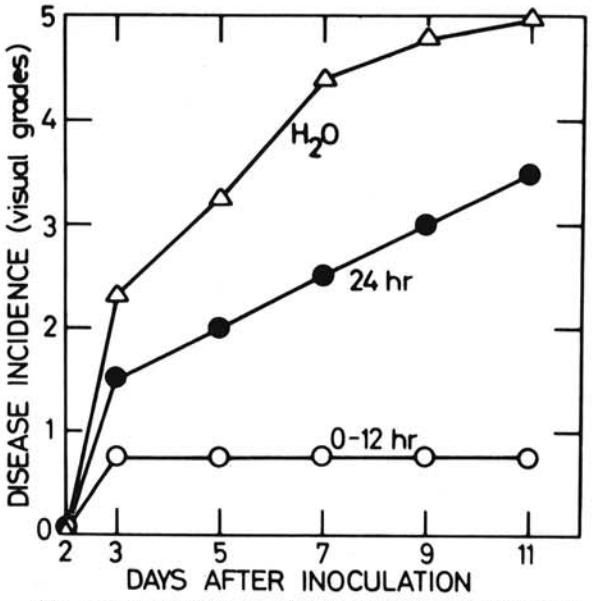

Fig. 5. Effect of timing of streptomycin application on development of cucumber downy mildew symptoms. True leaves of cucumbers were inoculated with *Pseudoperonospora cubensis* at zero time; thereafter, at varying times 0.7 ml of 100 μg/ml streptomycin solution was applied. The 0-12 hr curve is representative of treatments at 0, 3, 6, or 12 hr.

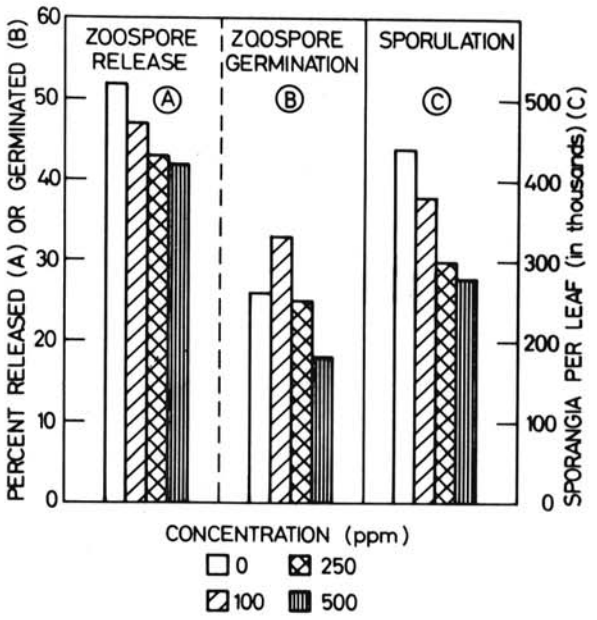

Fig. 6. Effect of streptomycin on zoospore release (A), zoospore germination (B), and on sporulation (C) of *Pseudoperonospora cubensis*. A and B were checked after 24 hr of incubation at 10 C, and C after 24 hr at 15 C in the dark.

streptomycin (100 μg/ml) resulted in development of "printing"-type lesions.

Zoospore germination of *Pl. halstedii* was markedly inhibited by the antibiotic: 90% of the sporangia failed to release zoospores in the presence of 1 μg/ml of streptomycin, and none did so at 10 μg/ml level.

Sporulation, germinability, and infectivity.—In order to ascertain whether streptomycin affects spore formation from fully developed fungal mycelia, infected leaves were detached and floated, abaxial side down, on streptomycin solutions for 24 hr at 15-20 C in the dark. The antibiotic had little effect on sporulation of *Ps. cubensis* (Fig. 6-C); a rather heavy sporulation also occurred on leaves floated on a solution containing 5 mg/ml streptomycin. Sporangia of *Ps. cubensis* collected from leaves floated on streptomycin solutions had high germinability, but had a much lower infective capacity than those collected from leaves floated on water (Table 5).

Streptomycin had no effect on sporulation of *Pl. halstedii*. Infected leaves and cotyledons of sunflower showed heavy sporulation of the mildew, even at the 1,000 μg/ml level of streptomycin. Sporangia produced in the presence of the antibiotic were collected (using a camel's-hair brush) and checked for germination and infective capacity. Although germination was reduced only about one-third, the infective capacity was decreased markedly. About 50% of the sporangia formed in the presence of 1,000 μg/ml streptomycin were able to release zoospores, but they caused systemic infection of less than 5% of the inoculated plants, probably because the

TABLE 5. Infective capacity of sporangia of *Pseudoperonospora cubensis* produced in the presence of streptomycin. Infected cotyledons and true leaves of cucumbers were floated on the antibiotic; sporangia were collected, washed and used for ionculations of healthy cotyledons and true leaves

Streptomycin (μg/ml)	% infected cotyledons	Lesion development on true leaves[a]
	Sporangia from cotyledons	
0	87.2	3.2
500	27.4	2.1
5,000	10.9	P[b]
	Sporangia from true leaves	
0	90.7	3.3
5,000	10.0	P[b]

[a] 0 - 5 visual scale.
[b] "Printing"-type lesions.

TABLE 6. Infective capacity of sporangia of *Pseudoperonospora cubensis* exposed to streptomycin for 1 hr, as evaluated by subsequent lesion development and sporulating potential on cotyledons and true leaves of cucumbers

Plants inoculated	Disease assessment	Streptomycin concentration (μg/ml) 0	100	250	500
Cotyledons	% infection[a]	98.6	100	31.7	3.3
	Sporulating potential[b]	219	200	30	4
True leaves	Lesion development[c]	3.4	1.0	P-1.0	P
	Sporulating potential[b]	250	51	13	0

[a] 42-60 cotyledons/treatment.
[b] Thousands of sporangia/leaf; average of 12 replicates.
[c] Visual scale 0 - 5. P = "printing"-type lesions P-1.0 = green-yellowish lesions of "printing"-type ones. Eighteen leaves/treatment.

protoplasm of many zoospores became disorganized 2-3 hr after release. Although no such protoplasmic disorganization was observed in zoospores treated with 100 μg/ml, their infective capacity was slightly lower and some plants became locally rather than systemically infected.

Sporangia of *Pl. halstedii* are the only ones (among those we worked with) which can be detached by gentle shaking of the sporulating leaf. Therefore, infected true leaves of sunflower were floated on streptomycin solutions for 24 hr, blotted dry, and the sporulating leaves then shaken above distilled water. These sporangial suspensions were used for bud inoculations. Sporangia removed from leaves floated on streptomycin solutions up to 1,000 μg/ml caused 100% infection. This indicates that use of a camel's-hair brush to collect sporangia probably causes some exposure to streptomycin.

Sporulation of *Ph. infestans* on potato leaves was unaffected by streptomycin up to 5 mg/ml. The germinability and the infectivity of the sporangia produced on leaves floating on the antibiotic solution also was unaffected.

Toxicity of streptomycin to sporangia.—Toxicity of streptomycin to sporangia was tested by immersing them in the antibiotic for 1 hr (unless otherwise stated) at 10-20 C. Sporangia were then washed on a 5-μ Millipore filter with 20 × 50 ml of distilled water, and used for germination and inoculation tests. The antibiotic did not affect zoospore release or zoospore germination of *Ps. cubensis*; however, the infective capacity of the treated sporangia was markedly reduced. Thus, exposure of sporangia (1 hr, 10 C) to streptomycin concentrations of 250 μg/ml or more reduces infection of cotyledons almost 70%, and resulted in only "printing"-type lesions on true leaves (Table 6).

Exposure of sporangia of *Pl. halstedii* to streptomycin (1 hr, 20 C) at 0, 1, 10, 100, and 1,000 μg/ml resulted in 48.9, 5.3, 3.0, 0, and 0 percent germination, respectively; whereas, the numbers of plants (28/treatment) that became systemically infected (2 weeks after bud inoculation) were 28 (100%), 8 (28.5%), 0, and 0 for sporangia exposed to 0, 10, 100, and 1,000 μg/ml streptomycin, respectively. It is of interest to note that chlorotic lesions (typical of streptomycin toxicity) appeared on plants inoculated with sporangia treated with streptomycin at 1,000 μg/ml. Plants inoculated with sporangia treated at 100 μg/ml streptomycin solution formed small (less than 1-mm diam) blisters.

Germinability and infective capacity of sporangia of *Ph. infestans* was not affected by immersing them in streptomycin solutions of 50-500 μg/ml up to 1 hr, nor in 100 μg/ml streptomycin solution for 3 hr.

DISCUSSION.—Zoospore release of *Pl. halstedii* was strongly inhibited by streptomycin while zoospore release and germination of *Ps. cubensis* and *Ph. infestans* were almost unaffected (Fig. 6, and text). Griffin & Coley-Smith (14) found that streptomycin was as toxic to sporangia of *Ph. infestans* as to those of *Pseudoperonospora humuli* (Miy. & Tak.) Wilson, whereas sporangia of *Plasmopara viticola* Berl. & De T. and *Peronospora parasitica* (Pers.) ex Fr. were less sensitive to the antibiotic.

The limited lesion development on the area initially inoculated ("printing"-type lesions) (Fig. 3) indicates that penetration (4, 9, 23) and primary stages of parasitic contact are not inhibited by the antibiotic, but mycelial spread is restricted markedly. This type of lesion development was observed by Muller et al. (21) on streptomycin-treated potato and tomato leaves inoculated with *Ph. infestans*. They suggested (no histological evidence presented) that infection itself was not prevented by presence of the antibiotic in the tissue.

Presence of streptomycin on the host at the time of inoculation or in the inoculum suspension was essential to stop fungal development (Fig. 1, 2, 4, 5 and Tables 1, 2, 3). In all three host-parasite

interactions tested, applying the antibiotic 24 hr subsequent to inoculation had no effect on final lesion or fungal development (Fig. 5, Table 1). Furthermore, essentially the same timing effect was found in growth of *Ph. infestans* on the surface of tuber slices (Table 2) and on wheat seed medium in vitro (Table 3). As far as the authors are aware, this is the first report of a timing effect in streptomycin action upon downy mildews.

Horner (17) found that streptomycin applied to hop plants up to 10 days after inoculation with *Ps. humuli* completely prevented symptoms of secondary systemic infection. He concluded that streptomycin shows a chemotherapeutic action affecting the progression of systemic downy mildew in hops. We found no effect of streptomycin (1,000 μg/ml, applied 24 hr after inoculation or later) upon systemic symptom development nor upon the final sporulating potential of the pathogen in diseased sunflowers (Table 1 and text). These differences cannot be attributed to different streptomycin uptake rates, because healthy sunflower plants treated in the same way showed marked chlorosis (see above.)

Most workers have concluded that sporulation of *Ps. humuli* is markedly decreased by streptomycin (10, 14, 17, 18). Sporulation of the three pathogens we tested was unaffected by the antibiotic (Fig. 6 and text). The studies noted do not appear to have discriminated between sporulating potential (7) and sporulation as such. None of them has investigated the effect of the antibiotic on fully developed mycelia. Therefore, it might appear that the decrease in sporulation they have recorded, a week or so after spraying, resulted from an indirect effect of the antibiotic on the host. Since streptomycin has a bleaching effect and causes inhibition of chlorophyll synthesis and of chloroplast function (13), it might seem reasonable that lack of photosynthetic materials (hexoses) needed for sporulation could cause a decrease of sporulating potential (7, 22).

Whether sporangia formed in the presence of streptomycin are defective (or contain the antibiotic) is still not clear. Using infectivity as a criterion, *Ps. cubensis* (Table 5) and *Pl. halstedii* were affected while *Ph. infestans* was not. However, the fact that sporangia of *Pl. halstedii* were found to be fully infective if removed by shaking (rather than by brushing) implies that a direct exposure of the sporangia to streptomycin might account for the loss of infectivity. Sporangia of *Ph. infestans* were unaffected probably because of the difficulty with which streptomycin moved across the sporulating leaf.

Short-time exposure of sporangia to streptomycin resulted in a loss of germinability of *Pl. halstedii*, loss of infectivity ("printings") of *Ps. cubensis* (Table 6) and in no effect on *Ph. infestans*. Nevertheless, all of them were found to contain the antibiotic subsequent to such exposure. ^{3}H-streptomycin was absorbed by sporangia of *Ps. cubensis* and *Ph. infestans* (*unpublished* and 27), and exposed sporangia of *Pl. halstedii* induced chlorosis in the inoculated host plant. The "resistance" of *Ph. infestans* to such exposure cannot be satisfactorily explained. Mg^{++} ions supplied before or after exposure of sporangia of *Ps. cubensis* to streptomycin were unable to counteract its effect. Similarly Mg^{++} ions mixed with sporangial suspensions containing the antibiotic failed to reverse the streptomycin inhibition (*unpublished*). This indicates that the binding sites of Mg^{++} ions and streptomycin may not be the same in Peronosporales as those reported for plants (25).

The specific sensitivity of fungi having cellulose cell walls (development of cucumber powdery mildew was unaffected on our streptomycin-treated plants; see also 21) suggests that the site of streptomycin action is the fungus rather than the host. The stage specificity in vivo (Fig. 5, Tables 1, 2) and in vitro (Table 3), as well as the limited type of infectivity obtained (i.e., the "printing" phenomenon) suggest a specific process subsequent to in vivo germination or to initial in vitro growth that occurs at an early stage of cellular invasion or of growth.

It seems unlikely that haustoria formation was inhibited by the antibiotic, because potato late blight lesions were able to expand (Fig. 2), and sunflower downy mildew was able to spread into healthy tissues in the presence of streptomycin. It seems unlikely that streptomycin-uptake rates differ at different stages of fungal development, or that a streptomycin-degrading enzyme is produced (26, 27), although these possibilities cannot be entirely excluded.

In studies with bacterial systems, considerable evidence has accumulated showing that streptomycin acts at the ribosomal level, causing distortion of ribosomal sites and breakdown of polysomes (20) or codon misreading (12). Recent studies by Miskin & Zamir (19) showed that a rearrangement of ribosomes of *Escherichia coli* is required in order for streptomycin to induce its effect, and it acts not merely by distorting ribosomal sites but by blocking conformational changes which continuously modify the nature of these sites.

To explain the timing effect in streptomycin action both in vivo and in vitro one must assume that some intracellular (structural or biochemical) change(s) has occurred in the fungus making it "resistant" to the antibiotic.

This level of "resistance" is achieved somewhat later in vitro, indicating some effect of the host upon this process. Streptomycin-sensitive sites exist in the sporangium (Table 6), and remain sensitive till after the first stages of establishment in the host.

LITERATURE CITED

1. ANDERSON, P. J. 1956. Streptomycin for control of blue mold and bed rot of tobacco. Phytopathology 46:240 (Abstr.).
2. ARK, P. A., & J. B. THOMPSON. 1957. Control of downy mildew of cucumber with antibiotics. Plant Dis. Reptr. 41:452-454.
3. COE, D. M. 1955. Streptomycin as a control for downy mildew of cucurbits. Plant Dis. Reptr. 39:729-730.

4. COHEN, Y. 1969. Parasitic ecology of cucumber downy mildew disease caused by the fungus Pseudoperonospora cubensis (Berk. and Curt.) Rost. Ph.D. Thesis, Hebrew University of Jerusalem. (Hebrew with English summary). 119 p.
5. COHEN, Y., & J. ROTEM. 1969. The effects of lesion development, air temperature and duration of moist periods on sporulation of Pseudoperonospora cubensis in cucumbers. Israel J. Bot. 18:135-140.
6. COHEN, Y., & J. ROTEM. 1970. The relationship of sporulation to photosynthesis in some obligatory and facultative parasites. Phytopathology 60:1600-1604.
7. COHEN, Y., & J. ROTEM. 1971. Rate of lesion development in relation to sporulating potential of Pseudoperonospora cubensis in cucumber. Phytopathology 61:265-268.
8. COHEN, Y., & W. E. SACKSTON. 1972. Zoosporangia of Plasmopara halstedii in epidemiology of downy mildew of sunflowers. Phytopathology 62:751 (Abstr.).
9. COHEN, Y., & W. E. SACKSTON. 1973. Factors affecting infection of sunflowers by Plasmopara halstedii. Can. J. Bot. 51:15-22.
10. COLEY-SMITH, J. R. 1966. Early-season control of hop downy mildew, Pseudoperonospora humuli (Miy. and Tak.) Wilson, with streptomycin and protectant fungicides in severely infected plantings. Ann. Appl. Biol. 57:183-191.
11. CROSSE, R., R. MC WILLIAM, A. RHODES, & A. T. DUNN. 1960. Antifungal action of streptomycin-copper chelate against Phytophthora infestans on tomato (Lycopersicon esculentum). Ann. Appl. Biol. 48:270-278.
12. DAVIES, J., W. GILBERT, & L. GORINI. 1964. Streptomycin, suppression, and the code. Proc. Nat. Acad. Sci. U.S. 51:883-890.
13. DE DEKEN-GRENSON, M. 1955. Action de la streptomycine sur la formation des chloroplastes. Biochim. Biophys. Acta 17:35-47.
14. GRIFFIN, M. J., & J. R. COLEY-SMITH. 1971. Some effects of streptomycin on Pseudoperonospora humuli, the downy mildew of hop. J. Gen. Microbiol. 69:117-134.
15. GROSSO, J. J. 1954. Control of tobacco blue mold by antibiotics. Plant Dis. Reptr. 38:333.
16. HODGSON, W. A., & P. N. GRAINGER. 1964. Culture of Phytophthora infestans on artificial media prepared from rye seeds. Can. J. Plant Sci. 44:583.
17. HORNER, C. E. 1963. Chemotherapeutic effects of streptomycin on establishment and progression of systemic downy mildew infection in hops. Phytopathology 53:472-474.
18. MAIER, C. R. 1959. Effects of streptomycin sulfate on the hop downy mildew fungus in vivo. Phytopathology 49:545 (Abstr.).
19. MISKIN, R., & A. ZAMIR. 1972. Effect of streptomycin on ribosome interconversion, a possible basis for the action of the antibiotic. Nat. New Biol. 238:78-80.
20. MODOLELL, J., & B. D. DAVIS. 1969. Mechanism of inhibition of ribosomes by streptomycin. Nature 224:345-348.
21. MULLER, K. O., J. H. E. MAC KAY, & JANET N. FRIEND. 1954. Effect of streptomycin on the host-pathogen relationship of a fungal phytopathogen. Nature 174:878-879.
22. PERL, M., Y. COHEN, & J. ROTEM. 1972. The effect of humidity during darkness on the transfer of assimilates from cucumber leaves to sporangia of Pseudoperonospora cubensis. Physiol. Plant Pathol. 2:113-122.
23. ROTEM, J., Y. COHEN, & J. PUTTER. 1971. Relativity of limiting and optimum inoculum loads, wetting durations, and temperatures for infection by Phytophthora infestans. Phytopathology 61:275-278.
24. SCHEIN, R. D. 1964. Design, performance, and use of a quantitative inoculator. Phytopathology 54:509-513.
25. VENIS, M. A. 1969. Streptomycin inhibition of protein synthesis in peas reversed by divalent cations. Nature 221:1147-1148.
26. VÖRÖS, J. 1963. Mode of action of selective fungistatic effect of streptomycin. Nature 199:1110-1111.
27. VÖRÖS, J. 1965. Streptomycin sensitivity of Oomycetes due to the increased absorption of streptomycin by their mycelia. Phytopathol. Z. 54:249-257.