Use of the antibiotics, streptomycin and terramycin, as growth stimulants for orchid seedlings.

Margaret W. O'Neill

USE OF THE ANTIBIOTICS - O'NEILL

NAME AND ADDRESS	DATE

USE OF THE ANTIBIOTICS, STREPTOMYCIN AND TERRAMYCIN, AS GROWTH STIMULANTS FOR ORCHID SEEDLINGS

By

Margaret W. O'Neill

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This thesis, directed and approved by the candidate's committee, has been accepted by the Graduate Committee of the University of New Mexico in partial fulfillment of the requirements for the degree of

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May 30, 1958
DATE

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MASTER OF [illegible]

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ACKNOWLEDGEMENT

Among the many friends who have contributed assistance in this work, I wish especially to express my sincere appreciation to Dr. Howard J. Dittmer, under whose direction this study was carried out. His useful suggestions on the approach to this problem and his guidance throughout have been valuable. I would also like to acknowledge the help and criticisms of Dr. R. C. Jackson during this study and the assistance of Dr. W. J. Koster in the writing of this report.

In addition, my thanks are extended to Dr. J. H. Dettweiler for supplying the streptomycin and terramycin used in this study and to Mary Snodgrass for typing this thesis.

TABLE OF CONTENTS

CHAPTER I

INTRODUCTION

Antibiotics first came to the public's attention in 1945, when penicillin and streptomycin were released by the United States government to fight human diseases. Later, other antibiotics were released and experimental work was done on control of plant and animal diseases. By 1948 plant pathologists found that certain plant diseases such as walnut blight, pear fireblight, bean halo blight, peach and celery blight, tomato and pepper bacterial spot, soft rot, cucumber downy mildew, chrysanthemum bacterial wilt, and lilac bacterial blight (Ark, 1954, pp.7-8) could be controlled by antibiotics.

While working with diseases of plants, some pathologists noticed a definite growth response on the part of the host plant. Nickell (1952, pp.11-12), working with sweet corn, sorrel, radish, and other plants found a definite increase in growth when he used low concentrations of terramycin with vitamin B. Jorgenson (1954, p. 82), working with roses, obtained phenomenal growth of these plants by using very low concentrations of terramycin alone and terramycin and streptomycin combined. He found that 1 ppm concentrations were as effective in promoting growth as higher concentrations and no yellowing of the leaves (chlorosis) was observed in the lesser concentrations. However,

CHAPTER I

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both Nickell (1952, p. 12) and Jorgenson (1955, p. 5) found that in order to maintain satisfactory growth with antibiotics, supplemental food containing the three basic nutrients, trace elements, and vitamin B_1 must be supplied.

However, other investigators have experimented with these and other antibiotics and obtained entirely different results. De Ropp (1948, p. 210) concluded from his work with tomatoes and beans that streptomycin is probably a general inhibitor of growth of embryonic plant tissue. Mitchell, et al (1952, p. 115) found that streptomycin is absorbed by the stems and roots of beans and is translocated to the leaves in amounts sufficient to prevent blight and disease but caused some inhibition of growth. In investigating tomatoes, soybeans, and wheat, Gray (1955, p. 331) found that streptomycin in concentrations of 20 ppm inhibited root growth although at 2.5 ppm there seemed to be a slight stimulation of root growth.

In tests run by Norman (1955, p. 585), the roots of cucumbers, corn, flax, and barley treated with terramycin at 5-10 ppm showed inhibited elongation, no evidence of stimulation of elongation by low concentrations, no stimulation of the rate of weight increase in barley roots, and no difference in percentage of germination of the seeds or time of emergence of the hypocotyl. In field tests on corn, terramycin did not affect the survival rate, but it did increase the number of larger seedlings. Repeated waterings with terramycin were no more effective than a single treatment. Norman found that terramycin probably exercised a protective

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effect on seedlings by suppressing the development of soil organisms that might have caused injury.

Iyengar and Starkey (1953, p. 359) found that terramycin in concentrations of 5-25 ppm reduced the growth of roots and hypocotyl, but streptomycin had little effect.

Barton and MacNab (1954, p. 433) pointed out that streptomycin sulfate in low concentrations did not affect tomatoes, oats, buffalo grass, or madrid sweet clover. Terramycin at 10 ppm, on the other hand, stimulated root growth in these plants and increased germination of their seeds. At concentrations of 10 ppm of terramycin germination was hastened in the early stages, but the initial advantage later disappeared. These investigators found no increase in survival or growth of seedlings when using terramycin on plants in the field.

Shaw (1953, p. 649) used aureomycin in concentrations of 1000 ppm on orchid seedlings and found a toxic, inhibitory effect which he felt was possibly due to the high concentration used. This is the only published information on the effects of antibiotics on orchids, either for seed germination, disease control, or growth-impetus studies (Withner, 1956, p. 401). With this in mind, the present study was undertaken to determine whether certain antibiotics would shorten the growth period and produce more vigorous plants with fewer casualties than usual. The length of time required to produce a flower from seed of Cattleya is approximately seven years, and during this time many seedlings succumb (White, 1948, p. 118).

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The following outline of growth pattern of a <u>Cattleya</u> from seed to mature plant is given in order better to demonstrate and evaluate the data obtained. Seeds are sown on nutrient agar in flasks under sterile conditions. These flasks are closed with cotton plugs and set in a warm, moderately illuminated location where the seeds are allowed to grow (Northen, 1950, pp. 110-113). Just before germination, the embryo is composed of cells with dense protoplasm and many oil globules. As the embryo enlarges, chlorophyll develops within the seed in from one to two weeks. The embryo later emerges as a rounded mass called a protocorm. As the protocorm grows during the next twelve months, six to seven leaves and three to four adventitious roots are produced. At this time the seedling is removed from the flask and transplanted, along with 100 to 200 other seedlings, into a pot of finely chopped osmunda fiber (Fink, 1957, p. 704).

The plants are usually transplanted six times before maturity is reached. These six times can be called six stages according to Shushan (1949, pp. 9-13). In the first stage from 100 to 200 seedlings, ranging from .5 to 2 cm. tall, are planted in a community pot, where they grow for 14-16 months. During this time six to nine leaves may develop. At the end of this period, the seedlings range from 1-12 cm. tall.

In the second stage, if the plants are at least 2.5 cm. tall and branched once, 15 to 20 are planted into another community pot, where they remain for 12-16 months. It is during this phase that terminal growth ceases and all but the upper two leaves die. There is one

The following [illegible] of [illegible] growth [illegible] [illegible] from

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branching and occasionally two at this time. (Branch is defined as the stem arising from a bud at the base of a previous growth. It is made up of several basal sheaths or leaves which are dry and papery and one that is expanded into a typical leaf that is photosynthetic. Thus the stem or branch appears single-leafed. At the terminal end of the branch several bracts appear, surrounded by a floral sheath. The flowers arise in the axils of these bracts. One bract remains as a small spur which is the terminal point of the branch (Shushan, 1949, p. 6).

In the third stage, the most vigorous plants are potted into $1\frac{1}{2}$-inch (thumb) pots where they grow for another 12-16 months. By the end of this period the original stem is dead, and two to four branches, ranging from 10 to 20 cm. tall may have developed. Each succeeding branch is slightly larger and the pseudobulb (the basal enlarged portion of the stem) increased in size. Aerial roots arise from the basal nodes of each branch.

At stage four, the seedlings are transplanted to three-inch pots where they remain for 12-16 months. At the end of this time, four to six branches have usually developed.

At stage five, the plants are five to six years old and are in $4\frac{1}{2}$-inch pots. A few flowers may be produced in this stage, although this is not common.

At stage six, the plants are transferred to six-inch pots, where they may remain for several years. Each new branch usually produces flowers and there may be more than one new branch a year.

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stem arising from [illegible]

up of several [illegible]

[illegible] expanded into a [illegible]

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However, many Cattleya and Cattleya hybrids produce a single growth per year except where two or more basal buds develop simultaneously. This type of growth is distinctive in Cattleyas (Northen, 1953, p. 589).

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type of growth in distinction to *Catlings* [illegible] 1973, [illegible]

CHAPTER II

METHODS

Because of the time element involved it was not considered practical to start with seed. Seedlings of a Cattleya hybrid of thumb-pot size (approximately 2½ years old) were used. Stages three and four, as set forth in the introduction, are the ones concerned in this experiment.

Streptomycin and terramycin in low concentrations were the two antibiotics chosen because of the previous work done with them on other plants. Dilute solutions of nutrients were used on orchids since strong solutions have proved detrimental (Craig, 1958, p. 108) and likely to endanger the general health of the plants (Rittershausen, 1953, p. 48).

Terramycin (oxytetracycline) and streptomycin sulfate were used with "Ra-Pid-Gro", a foliar food supplement with an approximate neutral pH containing 23% total nitrogen, 21% phosphoric acid, 17% potash, several trace elements, thiamin (B_1), and riboflavine (B_2). A supplemental food was used with the B vitamins following the method of Jorgenson (1955, p. 5).

Forty Cattleya hybrid seedlings of R-1796 (Rivermont Orchids) L. C. Abydos II X C. Mossiae var. R. E. Patterson were used. These were divided into groups of three, except for the control group of

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Forty *Cattleya* hybrid seedlings ([illegible]) L. C. Abydos II X C. [illegible] were divided into groups of [illegible]

four, and were marked for treatment as follows:

Group 1 Terramycin, 1 ppm applied weekly, monthly, and semiannually.

Group 2 Streptomycin, 1 ppm applied weekly, monthly, and semiannually.

Group 3 Terramycin, 10 ppm applied weekly, monthly, and semiannually.

Group 4 Streptomycin, 10 ppm applied weekly, monthly, and semiannually.

Group 5 No antibiotics. Control.

Each plant was watered as scheduled with its proper concentration of antibiotic. Plain tap water was used whenever the plants were dry. Every two weeks each plant received a foliar feeding of liquid "Ra-Pid-Gro" with the excess allowed to run into the substrate.

All plants were in osmunda fiber while in the thumb pots. At nine months, they were transferred to 2½-inch pots containing a medium-coarse grade of fir bark as the potting medium, following the method of Hawkinson (1956, p. 247). Again at eighteen months, they were transferred to three-inch pots with the same medium of fir bark. All plants were 4 cm. tall with the original stem and two leaves when this study was started.

Observations were made and data recorded every month for the two-year period. Measurements of leaves, counts of the number of visible roots, number of new branches formed, and the general appearance of the plants were noted.

CHAPTER III

RESULTS

TABLE I. Average length in centimeters of largest mature leaf of Cattleya seedlings, treated with concentrations of 1 ppm of terramycin and streptomycin.

Treated	Length at Start	1 month	6 months	1 year	18 months	2 years
Group 1 Terramycin						
weekly	4	4	7.6	7.7	8.25	9
monthly	4	4.5	7.6	7.8	9.25	10
semiannually	4	4	5.7	7	3 dead	
Group 2 Streptomycin						
weekly	4	4.5	8	8.3	8.5	10.4
monthly	4	4.5	8.3	10	10.5	10.5
semiannually	4	4	4.8	5.2	7.25	10
Group 5 Control	4	4	7.7	8	12.5	13.5

After one month there was no evidence of appreciable growth. The streptomycin-treated plants showed slight elongation of the leaves. At six months, the leaves of the terramycin-treated plants had made equal growth with the control group and the leaves of the streptomycin-treated plants were slightly more elongated. By eighteen months the control group had made better growth in leaf length than the treated plants and continued to do so for the remainder of the two years. At eighteen months, there was one plant dead in each of the treated groups

except for those which received terramycin semiannually in which all three of the orchids were dead.

TABLE II. Average number of branches or stems of *Cattleya* seedlings treated with concentrations of 1 ppm of terramycin and streptomycin.

Treated	Number at Start	1 month	6 months	1 year	18 months	2 years
Group 1 Terramycin						
weekly	1	1	1	1	2	4
monthly	1	1	2	2	2	3
semiannually	1	1	2	3	3 dead	
Group 2 Streptomycin						
weekly	1	1	1	2	3	4
monthly	1	1	2	3	3	4
semiannually	1	1	2	2	3	4
Group 5 Control	1	1	2	3	4	6

The average number of branches remained constant the first year for both the treated groups and the control group. After this period the untreated plants produced more new branches and larger branches and had a healthier appearance.

TABLE III. Average number of visible roots of Cattleya seedlings, treated with concentrations of 1 ppm of terramycin and streptomycin.

Treated	Number at Start	1 month	6 months	1 year	18 months	2 years
Group 1 Terramycin						
weekly	1	1	3	3	3	6
monthly	1	1	5	3	3	6
semiannually	1	1	4	3	3 dead	
Group 2 Streptomycin						
weekly	1	1	4	3	3	6
monthly	1	1	5	3	4	7
semiannually	1	1	5	3	4	7
Group 5 Control	1	1	2	3	4	7

In the instances where there were more roots visible at one time than at a later one, the damage was attributed to the antibiotic. The damaged roots were short, stubby, and brownish in color. However, all groups treated with the antibiotics made better root growth during the first six months than the control group, but the treated plants lost some of their roots by the end of the first year. Ultimately, the number of visible roots for both the treated plants and the untreated plants was approximately the same at the end of the two years.

TABLE IV. Average length in centimeters of largest mature leaf of Cattleya seedling, treated with concentrations of 10 ppm of terramycin and streptomycin.

Treated	Start	1 month	6 months	1 year	18 months	2 years
Group 3 Terramycin						
weekly	4	4.8	5.5	10	10.5	13.2
monthly	4	4.8	6	6.6	7.25	7.25
semiannually	4	4.5	5.5	7.3	9	9.8
Group 4 Streptomycin						
weekly	4	4	6.7	6.7	9	9
monthly	4	4	8.3	8.3	8.7	8.8
semiannually	4	4	8	8	8	9.4
Group 5 Control	4	4	7.7	8	12.5	13.5

The group receiving terramycin in 10 ppm concentration showed a definite stimulation of leaf elongation at the end of one month; however, this advantage was lost by the end of six months and the streptomycin and the control group caught up. After one year the control group made steady expected growth whereas growth in the treated groups slowed down. At the end of one year, two plants treated weekly with terramycin at 10 ppm were dead, but the remaining plant continued to grow very well. At the end of eighteen months one plant in each group was dead except for the group treated with streptomycin monthly and the control group in which all were living.

TABLE V. Average number of branches of Cattleya seedlings, treated with concentrations of 10 ppm terramycin and streptomycin.

Treated	Number at					
	Start	1 month	6 months	1 year	18 months	2 years
Group 3 Terramycin						
weekly	1	1	2	3	4	5
monthly	1	1	2	2	3	3
semiannually	1	1	2	3	4	5
Group 4 Streptomycin						
weekly	1	1	1	2	3	4
monthly	1	1	2	3	4	5
semiannually	1	1	1	3	4	6
Group 5 Control	1	1	2	3	4	6

The number of branches produced by both the treated and the control groups remained fairly constant. However, the plants in both groups treated with terramycin and streptomycin were chlorotic and were not vigorous in appearance.

TABLE VI. Average number of visible roots of Cattleya seedlings, treated with concentrations of 10 ppm terramycin and streptomycin.

Treated	Number at					
	Start	1 month	6 months	1 year	18 months	2 years
Group 3 Terramycin						
weekly	1	1	6	3	5	9
monthly	1	1	5	5	5	6
semiannually	1	1	5	2	5	6
Group 4 Streptomycin						
weekly	1	1	3	3	3	7
monthly	1	1	4	3	5	5
semiannually	1	1	5	2	3	6
Group 5 Control	1	1	2	3	4	7

The number of roots averaged about the same for the treated groups as for the control. However, the roots of the control group were longer and thicker, while those of the treated groups were short, stubby, and stunted.

CHAPTER IV

DISCUSSION

Cattleya plants are very long-lived and retain their initial vigor for years if there is no cultural impairment (Erickson, 1957, p. 560). Seedlings quickly show the effects of mismanagement. In this study such was the result.

At first, it would appear that growth of orchid seedlings is stimulated by streptomycin in 1 ppm and terramycin in 10 ppm concentrations to produce longer leaves. However, as the test progressed the control group showed steady growth whereas the treated groups became weaker with some plants dying. The new growths were neither as long nor as branched as the controls. The differences were enough to postulate inhibition of growth and toxicity to orchid seedlings with repeated use of these two antibiotics over an extended period of time. However the genetic make-up of each of these forty plants is different. As has been found in working with flies, resistance to antibiotics and insecticides can quickly be developed, and because of the genetic diversity, each fly would and could react differently. (Bruce, 1952, pp. 324-325.) There is no reason to suppose that this would not be true for plants. Therefore, in order to have a completely true control,

CHAPTER IV

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divisions of the same clone should be used whereby the antibiotics would be reacting on only plants of the same genotype.

In the work previously done on other plants with these two antibiotics, there are conflicting reports as to whether streptomycin and terramycin inhibit or stimulate plant growth. The reason for these conflicting reports might well be the different genetic make-up of the plants used in the experiments.

Mitchell, et al (1952, p. 115) found that sufficient amounts of streptomycin are absorbed by roots and stems to prevent disease and blight, but these amounts caused some inhibition of growth of the plants. Gray (1955, p. 331) noticed that streptomycin inhibited root growth in concentrations of 20 ppm, but there was a slight stimulation of roots at 2.5 ppm which he considered insignificant. These results were further confirmed by this study in which an initial stimulation was recorded, but with continued use of streptomycin the plants reacted unfavorably.

Using terramycin, Iyengar and Starkey (1953, p. 359) found no consistent difference at 1 ppm, but in concentrations of from 5-25 ppm, root growth was inhibited. Norman (1955, p. 585) obtained definite inhibition of root growth with terramycin at 5 to 10 ppm, but he could find no evidence of stimulation, weight increase, nor percentage of germination increase at lower concentrations. He found that repeated waterings with terramycin were no more effective than a single treatment and postulated that terramycin probably exercised a protective effect on

seedlings by suppressing the development of organisms which might have caused injury. However, in their experiments with beans, Barton and MacNab (1954, p. 433) found that terramycin at 10 ppm stimulated root growth and hastened seed germination in the early stages, but the initial advantage disappeared over longer periods. These results are in accord with this investigation.

The findings obtained in this study and those by Shaw (1953, p. 649) in which he used aureomycin follow the above results of the effects of terramycin on various plants.

In contrast with other works, Nickell (1952, p. 11) and Jorgenson (1954, p. 82; 1955, p. 5; and 1956, p. 5) found that growth response was definitely noticeable. Jorgenson states that "there seemed to be an inseparable link with foliar plant foods in that antibiotics act synergistically, enhancing the effect of the foliar foods." (Jorgenson, 1956, p. 5.)

Nickell (1952, p. 12) indicated that the elimination of microflora by terramycin appeared to be the major factor in growth stimulation. Possibly the bacterial population was altered by the antibiotic to favor increased growth or the antibiotic may stimulate the absorption of certain nutrients. From this study and other reports, it is questionable whether antibiotics have a direct influence as a growth stimulant. Their main benefit may be in killing disease organisms thereby producing a healthier plant. They certainly show promise in the field of plant diseases.

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The use of terramycin and streptomycin can not be recommended for Cattleya seedlings. Possibly the inhibitory and toxic effects observed in this investigation can be attributed to the orchid's slow growth or to an accumulation of salts in the substrate. It may be that repeated use of antibiotics was toxic or excess salts may have increased the pH of the cell sap. However, it is possible that a single application of streptomycin at 1 ppm or terramycin at 10 ppm might show a stimulating effect by retarding deleterious bacterial action on the orchids.

CHAPTER V

SUMMARY

Groups of comparable Cattleya orchid seedlings were treated with terramycin and streptomycin, at 1 and 10 ppm, for a two-year period.

On an average all groups treated with streptomycin and terramycin showed poorer growth of leaves, roots, and branches than the control group.

At least one plant died in each group except those of the controls and also in the group that received streptomycin in 10 ppm monthly. The remaining plants in the treated groups were smaller, had fewer and shorter roots, and were decidely weak.

There was a slight stimulation of leaf elongation and root growth during the first six months, particularly with terramycin at 10 ppm concentration and streptomycin at 1 ppm. The control group kept a steady growth rate as expected under normal cultural conditions, but the treated plants declined after six months. Plants treated with streptomycin and terramycin in concentrations of 1 ppm and 10 ppm over an extended period of treatment showed definite inhibition of growth.

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