Proceedings of the Iowa Academy of Science

Volume 69 | Annual Issue Article 33

1962

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Antibiotics Stimulating or Inhibiting Germination and Growth of Rice

ROBERT C. GOSS[1]

Abstract. This report is concerned with the effect of antibiotics on germination and translocation of native chemicals in the laboratory and on the plant's reaction to certain antibiotics used as soil drenches under greenhouse conditions.

The seeds treated with antibiotics active against Gram positive organisms were deficient in both carbohydrates and proteins. The seeds treated with antibiotics active against Gram negative organisms had a delayed germination but a normal physiological development. Streptomycin had the most adverse effect on translocation but stimulated germination. Analysis of variance of germination indicated that seed germination was dependent upon the antibiotic used. The analysis for the translocation resulted in the means being significantly different.

No adverse effects, caused by the soil treatment, were noted for germination. Neomycin, penicillin, and streptomycin appear to stimulate germination; tyrothricin and polymyxin inhibit germination the first week but by the second week have a higher germination percentage than the control.

Streptomycin, polymyxin and neomycin drenches have the most beneficial effect on the growth of Nato, Magnolia, and Upland varieties. A trigger agent is apparently released which stimulates growth from the first week until termination of the experiment. The three antibiotics inhibit the growth of Blue bonnet.

INTRODUCTION

The word antibotic has been devised to indicate a substance formed by one organism which is injurious to another organism. This phenomenon is not confined to microorganisms but is found, at least to some extent, in the higher plants and also in animals. As experience with the practical utilization of antibiotics has increased, and as more research has been made for new ones, the meaning has shifted to the point where antibiotic now means almost any substance isolated from a culture of an organism, and the activity is not only inhibitory but in some cases the substance can be growth-stimulating or growth-changing in nature.

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The attention of the botanist has been directed to the antibiotics. Among the reasons for this trend is the hope of discovering a compound that will exhibit a minimum toxicity to plants, maximum toxicity against parasites, and the property of penetrating into plant cells and accumulating there in amounts detrimental to the parasite (1). The toxicity of many antibiotics to parasitic forms of life and to plants is of considerable interest for reasons both physiological and economical.

Much atention has been directed to the use of antibiotics as biocides in sprays or dusts (2, 3) and to the translocation of the antibiotic once inside the plant (4, 5). This report is concerned with the effect of certain antibiotics on germination and translocation within the seed in the laboratory and on the plant's reaction to certain antibiotics used as soil drenches under greenhouse conditions. The over-all effect of the soil treatment would be influenced by the activities of the native soil microflora and by the soil organic matter. This phase will be reported at a later date.

Materials and Procedures

The antibiotics tested were bacitracin, polymyxin, tyrothricin, penicillin, neomycin, streptomycin, usnic acid and zinc bacitracin. The rice varieties used in various phases of the investigation were Rexora, Upland, Toro, Magnolia, Blue bonnet, Nato and Zenith. A preliminary laboratory screening of 50 different seed varieties indicated that rice was the most reactive to the conditions of the experiment (6).

Ruled filter paper (S & S #8) was placed in Petri plates (100 mm x 15 mm) for the germination experiments. Five cc of a 100 ppm solution, on a weight basis, of the test material was added. Five seeds were placed in each plate. The seeds were free of any type of previous chemical treatment. Incubation was at 31°C ($\pm$1) and lasted for ten days. Fifty seeds were used for each antibiotic. The varieties used were Zenith and Rexora. Germination percentages and stem-root ratios were determined at 2 day intervals.

In the greenhouse a soil mixture of ⅓ acid peat moss, ⅓ sand, and ⅓ muck was made and placed 4 inches deep in 12″ x 20″ flats. The soil was then drenched with 200 cc of a 100 ppm solution, on a weight basis, of the test chemical. Three days later 4 rows of 4 seeds each were uniformly planted in each flat to a depth of one inch. Sixty-four seeds were used for each treatment. One week after planting germination counts of all plants and random measurements of ⅓ of the plants were made. These determinations were made at weekly intervals for 4 weeks. The plants were watered daily.

Results and Discussion

The development of any organ of a germinating seed is often influenced by the physiological process or the physico-chemical conditions prevailing in other organs of the same seed. Growth correlations are not only exerted from one organ to another but also between tissues and between cells. The normal development of the plant body is controlled by correlative influences operating from one organ to another organ, tissue to tissue, and cell to cell. Consequently, indirect methods of determination are usually the only means of answering the question of what substances are present in growing plant tissue.

The sem-root ratio has been used to determine what effect the antibiotic treatment has on the movement of the native chemicals within the seed. The ratio is governed by correlative influences operating between the aerial parts and the roots of the plant.

Under certain conditions the stem-root ratio can be misleading. A seed which is naturally slow could have the same ratio as a fast germinating seed. Preliminary data indicated that the ratio for rice would be unreliable until the 3rd day. At this time approximately 80% of the germinated seeds have comparable root and stem measurements. The comparison increased with incubation. Furthermore, the measurement is relatively simple to obtain. The first outward indication of germination is the emergence of the root. A short time after the appearance of the root the plumule begins to emerge. The unfolding of the plumule is rather slow while the development of the root is comparatively rapid. The seed remains attached and separates the root from the stem.

The following interpretation has been placed on the stem-root ratio. A low ratio would indicate that the growth of the aerial portions of the plant are relatively slow. This indicates that the plant is deficient in proteins. The synthesis of amino acids is dependent on the nitrates present. The amino acids are used in the synthesis of protoplasmic proteins during the growth of the roots. The carbohydrates necessary for the formation of amino acids will be translocated downward from the embryonic leaves. A normal ratio would indicate a relatively high rate of protein synthesis and would be considered the characteristic physiological process of cell division. A high ratio would indicate that the roots are developing at a slower rate than the stem. The roots are likley to be deficient in both carbohydrates and proteins since the synthesis of proteins requires carbohydrates. Proteins are not consumed in respiration but are utilized in the synthesis of the organic nitrogen compounds of the growing embryo.

The analysis of variance for germination indicated that germination was dependent upon the antibiotic used. The second day is highly significantly different (Table 1). The analysis of variance for translocation resulted in the means being significantly different (Table 2).

Table 1. The effect of antibiotics on the cumulative germination percentage of Rexora and Zenith.

		Germination Percentage time in days				
Antibiotic	Seed Variety	2	4	6	8	10
Bacitracin	Rexora	40	25	15	0	0
	Zenith	85	5	0	5	0
Penicillin	Rexora	55	25	0	0	0
	Zenith	30	5	15	10	0
Polymyxin	Rexora	0	55	10	10	0
	Zenith	65	0	15	5	0
Streptomycin	Rexora	75	10	0	0	0
	Zenith	50	5	0	10	0
Tyrothricin	Rexora	40	30	0	0	0
	Zenith	60	10	0	5	0
Usnic acid	Rexora	60	20	0	0	0
	Zenith	50	10	10	5	0
Zinc bacitracin	Rexora	80	15	0	0	0
	Zenith	80	10	0	0	0
Distilled water	Rexora	45	45	0	5	0
	Zenith	35	30	5	5	0
Means		65	33	2.8	0.6	0.0
X2 (P=0.05)=36.4						

Table 2. The effect of antibiotics on the stem-root ratio of rice varieties Rexora and Zenith.

Antibiotic	Seed Variety	2	4	6	8	10
Bacitracin	Rexora	0.0	.5	2.7	.9	1.2
	Zenith	.4	.5	.6	1.0	1.1
Penicillin	Rexora	1.1	.5	.5	.8	.9
	Zenith	.3	.3	.4	.6	.7
Polymyxin	Rexora	0.0	1.0	.9	1.2	1.1
	Zenith	.5	.5	.6	.9	1.1
Streptomycin	Rexora	.2	2.7	2.8	3.7	4.0
	Zenith	1.4	5.5	6.6	6.4	6.9
Tyrothricin	Rexora	0.0	1.5	3.2	1.9	5.4
	Zenith	.5	2.2	1.1	1.5	2.2
Usnic acid	Rexora	.5	.4	.8	.8	.9
	Zenith	.4	.5	.6	.8	.8
Zinc bacitracin	Rexora	.7	3.4	1.0	.9	.9
	Zenith	.6	.8	1.5	1.6	1.6
Distilled water	Rexora	.2	.4	.5	.7	.7
	Zenith	.1	.3	.7	.7	.7

The seeds treated with antibiotics active against Gram positive organisms were deficient in both carbohydrates and proteins. The seeds treated with antibiotics active against Gram negative organisms had a delayed germination but a normal physiologic development. Streptomycin had the most adverse effect on translocation but stimulated germination. The chemical stimulates leaf elongation but inhibits root development. Chlorosis was noticeable on all stems of plants treated with streptomycin. The phytoxoic symptoms caused by streptomycin agree with those reported by Rosen (7) except for leaf elongation.

The fate of the antibiotics after they are within the plant tissue was not investigated. A review of the literature indicates that this phase has scarcely been studied. From the investigations that have been reported it would indicate that the fate of the antibiotic in tissue is a external-internal relationship. The absorption of many antibiotics is simply related to time, with others it is proportional to the volume of water transpired. Charles (8) ascribes the failure of streptomycin to move in Cherry Laurel vascular tissue to a binding action of the positively charged antibiotic molecule at negatively charged sites on the surfaces of the xylem vessels. Crowdy and Pramer (9) concluded that the neutral and acid antibiotics are readily translocated whereas the basic and amphoteric ones have provided irregular results. deRopp's (10) data suggests that streptomycin is a general inhibitor of the growth of embryonic tissue.

In order to have a quantitive measure of activity a working mathematic equation was devised. It is called the *Chemical Activity Index* and is as follows

$$CAI = \sum_{i=1}^{i=10} (12 - D_2) \left[(T_T - T_C) + \frac{(G_T - G_C)}{50} \right]$$

where D=time in days; T=stem-root ratio; and G=percent of germination.

A chemical activity index of aruond 1 would indicate normal physiological development. A negative index indicates inhibition of both germination and translocation. A high positive figure indicates a stimulatory effect (Table 3).

Table 3. The chemical activity index of certain antibiotics against Rexora and Zenith.

Antibiotic	Chemical Activity Index Rexora	Zenith
Bacitracin	8.60	7.12
Penicillin	2.61	—3.30
Polymyxin	—3.02	5.86
Streptomycin	22.90	49.34
Tyrothricin	10.43	16.10
Usnic acid	.90	1.38
Zinc bacitracin	24.98	11.52

Under soil conditions there are few organic substances that are so insoluble or so toxic that soil microbes cannot dispose of the chemical. There are though, a number of environmental factors which may limit microbial activity or which may influence rapid destruction of the compound. The principal inactivating factors appear to be (a) the absorption of the material by the soil; (b) unstable pH of the soil; (c) chemical reaction with some soil component; and (d) microbial decomposition.

No adverse effects, caused by the soil treatment, were noted for germination. Neomycin, penicillin, and streptomycin stimulate germination; tyrothricin and polymyxin inhibit germination the first week but by the 2nd week have a higher germination percentage than the control.

Streptomycin, polymyxin, and neomycin drenches have the most beneficial effect on the growth of Nato, Magnolia, and Upland varieties. A trigger agent is apparently released which stimulates growth from the first week until termination of the experiment. The three antibiotics, on the other hand, inhibit the growth of Blue bonnet (Table 4). The results from Hervey's (11) study suggest that a number of factors influencing the microbial population of the soil may be involved in the phenomenon of antibiotic-induced plant growth stimulation.

Table 4. The terminal growth ratio of plants growing in antibiotic treated soils/plants growing in untreated soil.

Antibiotic	Nato	Magnolia	Blue bonnet	Upland
Neomycin	1.5	1.6	1.0	2.1
Penicillin	1.0	1.1	2.0	1.4
Polymyxin	1.3	1.7	1.0	2.1
Streptomycin	1.7	1.6	1.1	1.7
Tyrothricin	1.0	0.6	0.9	1.1

The antibiotics used can be segregated roughly as (1) organic acids, (2) organic bases, and (3) polypeptides. Acids, such as penicillin, and bases, such as streptomycin, form salts easily. In the case of the polypeptide antibiotics, the compound may possess the ability to form salts readily (polymyxin) or may lack such ability (tyrothricin). The ability to form salts easily is a reflection of the fundamental structure of the molecule. Those polypeptides which posses such ability may be assumed to contain free amino or carboxyl groups or other radicals capable of salt formation, and those lacking such ability may be assumed not to possess such free groups.

It is theorized that any observable effect on the growth of the plant under greenhouse conditions would result in part from the general saprophytic activities of the soil microflora, and that toxic or nontoxic metabolites, other than specific antibiotic substances, are involved.

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