TECHNICAL PAPERS

A New Group of Plant Growth Regulators

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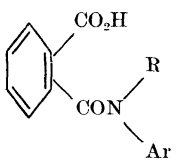
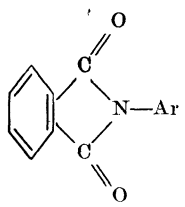
A new group of plant growth regulating chemicals, the N-aryl phthalamic acids, has been discovered. These compounds, which are readily prepared from phthalic anhydride and aromatic amines, can be represented by the general formula I. The esters, amides, and soluble salts of these acids, as well as the N-aryl phthalimides (II) have been found to possess similar activity.

I



II



Where R is a hydrocarbon radical or a hydrogen atom, and Ar is an aromatic group.

The nature and degree of the growth response shown by plants treated with these chemicals depend on the type and age of the plant, and on the concentration and identity of the phthalamic acid derivative used. Data from a field test in which Bonny Best tomato plants having from one to three unset blossom clusters were treated with compounds of this group show the effects obtained (see Table 1). Each plant was sprayed with 100 cc of a solution containing one drop of a commercial wetting agent (Emulphor EL) per 100 mg of chemical used.

TABLE 1

N-aryl phthalamic acid, Aryl group	Approximate minimum concentration in ppm to cause: Chemical fruit set	Formative effect	Pronounced inhibition of fruit set
2-Chlorophenyl	63	20	632
2,5-Dichlorophenyl	63	200	632
2,4,5-Trichlorophenyl	63	632	632
3-Chlorophenyl	63	632	632
4-Chlorophenyl	63	632	200
4-Bromophenyl	200	632	632
o-Tolyl	200	632	2000
4-Nitrophenyl	200	2000	2000
2-Nitrophenyl	632	2000	*
2-Hydroxyphenyl	632	632	*
m-Tolyl	632	632	*
p-Tolyl	2000	200	*
Phenyl	2000	*	*

* The effect was not produced at 2000 ppm, the highest concentration tested.

It will be seen that the active concentrations of different compounds vary greatly. A general pattern of response, however, is evident. At low concentrations fruit set is stimulated, and seedless tomatoes are often produced. A morphological response involving a broadening of the leaves is usually evident. This formative effect does not appear to injure the plant seriously; in fact, in some cases the test plants actually seemed to be more vigorous than the unsprayed check plants.

As the concentration of chemical is increased, the formative effect becomes more pronounced. Fruit set is inhibited, although the fruits which are formed may be seedless. Fruit set may be greatly diminished at concentrations which do not decrease vegetative growth.

At still higher concentrations (about 2000 ppm) tomato plants are definitely injured, although seldom killed, by the more toxic compounds. Even at 2000 ppm, however, many of the fruit-setting chemicals cause no apparent injury. In all of these experiments the whole plant was sprayed; no effort was made to confine the spray to blossom clusters.

The effects of N-1-naphthyl phthalamic acid differ from those of other members of the series. At 0.1 ppm it causes a leaf roll, at 0.31 ppm epinasty, and at 20 ppm stem swelling (greenhouse data). In the field test it completely inhibited fruit set at 20 to 2000 ppm. The vine weight was almost normal at 20 ppm (the lowest concentration tested) but epinastic and morphological effects were severe.

N-aryl phthalimides behave much like the corresponding phthalamic acids. Compounds derived from secondary amines (formula I, where R is a hydrocarbon radical) are less active than the corresponding compounds in which R is a hydrogen atom.

Maleic Hydrazide, a Unique Growth Regulant

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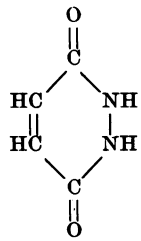
Maleic hydrazide has been found to have a pronounced, but temporary, inhibiting effect on plant growth. The length of inhibition period appears to be directly proportional to the concentration used. To our knowledge this effect is unique in that growth inhibition is obtained with little visible harm to the plants.

Maleic hydrazide, or more properly, 1,2-dihydropyridazine-3,6-dione, has the following structure (*1*):



It is slightly acidic in character and forms salts readily with alkalies. The free compound is completely water-soluble at 2000 ppm (0.2%) but does not dissolve completely at 1%. More concentrated solutions can be made using triethanolamine or other alkalies. Heavy metals precipitate insoluble salts from such solutions. Both the soluble and the insoluble salts function as growth regulants.

Maleic hydrazide, its cupric salt, and its zinc salt were dissolved or suspended in water at a concentration of 2000 ppm. Emulphor EL was used as the wetting agent at the rate of one drop per 100 mg of hydrazide. Six-inch tomato plants, variety Bonnie Best, were sprayed to run-off in duplicate with each suspension. The treated plants, in a greenhouse, failed to grow for a period of about two month. Root growth was also inhibited. The controls grew normally. After about one month some chlorosis developed on the younger leaves, but otherwise the plants were normal in appearance. After the quiescent period, growth resumed mainly from lateral buds. The plants had blossomed before they were discarded. Other tomato plants were not killed by a 1% suspension, nor did they show necrotic spots. A 1000-ppm solution produced a formative effect when regrowth started from the terminal bud. This effect was not pronounced in growth from the lateral buds.

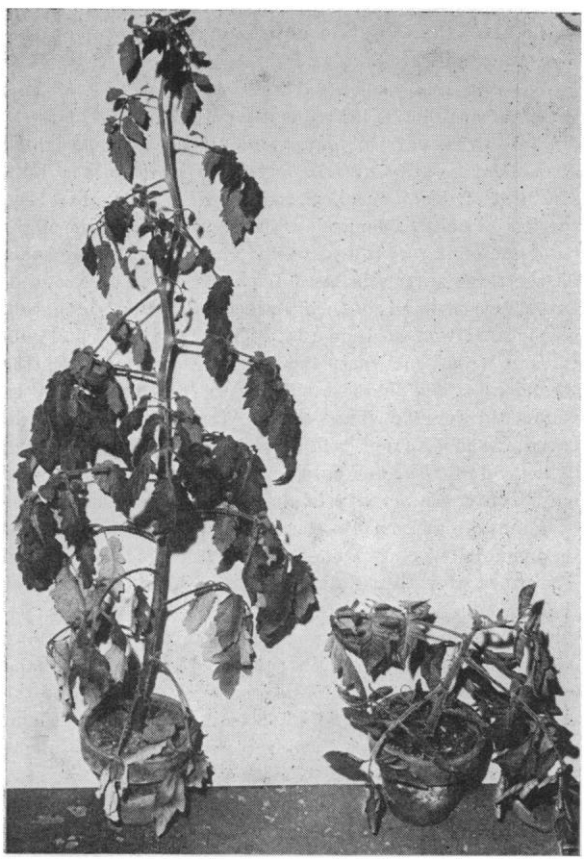


FIG. 1. Effect of maleic hydrazide on the growth of tomato plants. Plant at right was sprayed with 2000 ppm of maleic hydrazide one month before photograph was taken. Untreated control on left shows normal growth.

TABLE 1

EFFECT OF AMMONIUM SALT OF MALEIC HYDRAZIDE ON THE GROWTH OF TOMATO PLANTS

Concentration	8 Days		14 Days	
	Ave. increase in height*	Inhibition†	Ave. increase in height*	Inhibition†
ppm	cm	%	cm	%
0	11	0	25	0
2000	2.5	77	2.5	90
1000	3.0	73	4.0	84
500	5.0	55	7.0	72

* Original height, 7 cm.

† % Inhibition = $\frac{\text{Increase in height of control} - \text{increase in height of treated plant}}{\text{Increase in height of control}} \times 100$

In another experiment, similar results were obtained using the triethanolammonium and diethanolammonium salts of maleic hydrazide. These salts seemed to be somewhat more effective than the free compound when applied at equal concentrations of the active ingredient. This increase in activity may be due in part to better wetting of the leaf surface, since we have observed that maleic hydrazide is reduced in activity when the wetting agent is omitted.

Fig. 1 shows two tomato plants one month after the one on the right was sprayed with a solution containing 2000 ppm of maleic hydrazide. During this interval the control increased in height about sixfold. It may be noted that the stem of the treated plant gave the normal increase in diameter and that leaves already formed increased normally in size. There was, however, virtually no terminal growth.

Semiquantitative growth data have been obtained on tomato plants treated in duplicate with three different concentrations of the ammonium salt of maleic hydrazide. The spray included one drop of Emulphor EL per 100 mg of the ammonium salt. Growth data were taken after 8 days and after 14 days. The results are presented above in Table 1.

Turf was treated with 8, 4, 2, and 1 lb per acre of maleic hydrazide. The chemical with Emulphor EL as the dispersing agent was applied as a spray in about 200 gal of water per acre. The 1-lb rate had a slight retarding effect; the 2-lb rate inhibited growth for 1–2 weeks; the 4-lb rate for about 4 weeks and the 8-lb rate for over 2 months, when all growth was stopped by cold weather. After the quiescent period, growth at the

1-, 2-, and 4-lb rates appeared normal. Bluegrass, bentgrass, crabgrass, plantian, and chickweed were inhibited in this experiment. Observations will be continued to determine whether the treated plants will survive the winter. During the course of this study there were several weeks without rain, and the inhibition periods might be altered in a more normal growing season.

In one experiment it took much more chemical to inhibit growth of tomato plants when the chemical was used as a soil drench than when it was used as a spray. Maleic hydrazide used as a pre-emergence treatment on a variety of different plants had no inhibiting effect when used at a rate of 8 lb per acre. When applied as a dust to corn seed before planting it had no effect on the amount or time of germination, but the seedlings suffered a marked retardation of growth. As with foliage, the amount of inhibition was directly proportional to the concentration applied.

Reference

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A Preliminary Report of the Successful Treatment of Amebiasis with Aureomycin[1]

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Amebiasis, or human infection by the protozoan parasite *Endamoeba histolytica*, is a world-wide and prevalent infection. Although a great deal of work has been done with amebicidal drugs, the treatment of this condition remains unsatisfactory. Under certain conditions, currently used amebicidal drugs, especially emetine, involve danger of toxic reactions.

In several cases of other infections treated with aureomycin hydrochloride, we observed an alteration in the gross character of the stool and a reduction in the fecal bacterial flora. This evidence of local intestinal effect, combined with its known systemic action, led us to investigate the use of aureomycin in amebiasis.

Three cases of infection with *E. histolytica* have been treated with aureomycin. While this is a small series and the patients have not yet been observed for an adequate period following therapy, we feel that the results are sufficiently encouraging to warrant a preliminary report so that investigation of this apparently useful drug in amebiasis might be furthered.

W. W., a 27-year-old white male complaining of flatulence, heartburn, diarrhea, and malaise was found to have trophozoites and cystic forms of *E. histolytica* in his stool. After the administration of 7.0 g of aureomycin by mouth in divided doses over a 3-day period, the stools became negative for *E. histolytica* and the patient became asymptomatic. At this time, the aureomycin blood level was 8 μg %. Therapy was continued until the patient had been given a total of 19 g. Six negative stools have been obtained since treatment.

No stool is reported as being negative until it has been studied by fresh saline and iodine preparations, by iron hematoxylin stains, and by culture.

B. C., a 63-year-old colored male was examined because of paraumbilical pain, abdominal fullness, and constipation. *E. histolytica*, in both the trophozoite and cystic forms, was demonstrated in the stool. After 6.75 g of oral aureomycin in divided doses over a 3-day period, the stools became negative. This patient received a total of 21.75 g of aureomycin. All of the gastrointestinal symptoms disappeared and 14 stool examinations over a period of 3 weeks have been negative for *E. histolytica*.

A. T., a 43-year-old colored female was found to have trophozoite and cystic forms of *E. histolytica* in her stools. She complained of rather severe indigestion, epigastric pain, and flatulence. In this case, the stools became negative after 15 g of aureomycin. The blood level of aureomycin at this time was 8 μg %. The patient received a total of 22.7 g of aureomycin. Seven negative stools have been observed since treatment.

In the first two cases there were no toxic reactions to aureomycin. The last patient complained of nausea for 48 hr after the initiation of treatment. No other reactions were observed.

While it was recognized that *in vitro* and *in vivo* action of antibiotics is frequently quite different, *in vitro* studies were carried out. Hewitt (*2*) demonstrated amebicidal activity of aureomycin *in vitro* on the NRS strain of *E. histolytica*. Since the NRS strain has been carried in culture for many years, we thought it might be of some interest to test *in vitro* activity of aureomycin on the three strains isolated from the above cases. Cultures were isolated and maintained in Nelson's egg yolk alcoholic extract medium (*3*). The first and second subcultures from one case, the third subculture from the second case, and the fifth subculture from the third case were used for the assay study. Inoculation of several tubes was made from each strain. These were examined at the end of 24 hr and only heavily positive cultures were used to test the activity of aureomycin. At the time of examination, aureomycin was introduced into the cultures in amounts varying from 0.2 to 3.2 mg/cc of overlay. The tubes were then examined at 6, 18, and 48 hr after the introduction of aureomycin. All ameba were destroyed at the end of 6 hr exposure in the tubes having 0.8 mg or more aureomycin per cc. Most of the ameba in tubes having 0.2 and 0.4 mg/cc were destroyed at the end of 18 hr. At the end of 48 hr no ameba were found in any of the tubes containing aureomycin, while all of the control cultures were heavily positive.

A much larger series of cases with adequate follow-up examinations after treatment will be necessary for an evaluation of aureomycin in the therapy of amebiasis. The work of Anderson, *et al.* (*1*) has shown that there is a sufficient number of cases in Memphis to allow us to accomplish this. The high blood levels obtained sug-

[1] Aureomycin furnished through the generosity of the Lederle Laboratories, Pearl River, New York.