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Growth Responses of a Myxomycete to Treatment with Plant Growth Regulating Substances

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Growth Responses of a Myxomycete to Treatment with Plant Growth Regulating Substances[1]

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Abstract. Protoplasmodia of *Clastoderma debaryanum* multiply in culture to produce more individual plasmodia, each of which fruits into a single sporangium. After treatment of cultures with 1 ppm kinetin and with 1 ppm, 5 ppm, and 10 ppm aqueous solutions of 3-indoleacetic acid, the number of sporangia produced was consistently greater than in controls. Fewer sporangia, as compared to controls, were produced after treatment with kinetin at 5 ppm and 10 ppm, and no consistent results were obtained after treatment with gibberellic acid at the same concentrations.

Growth regulating substances have been found to influence all phases of plant growth and development: breaking of dormancy, seed germination, shoot inhibition, bud and internode elongation, root growth and inhibition, flower initiation, fruit setting. Both endogenous and externally applied substances have been studied. Those most intensively investigated have been the indole auxins, the gibberellins, and kinetin.

The studies of growth regulator effects on higher plants are very numerous, and effects on fungi have been studied. In the review on auxins and fungi by Gruen (1) there are no reports of studies on myxomycetes. However, in his review on the myxomycetes, Alexopoulos (2) mentions only that the plasmodium of *Didymium nigripes* was found to be positively chemotactic to low concentrations (0.5 mg/1) of indoleacetic acid (IAA) and negatively so to higher concentrations (5 mg/1).

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The present investigation was made for the purpose of determining whether externally applied growth regulating substances of the three best known types have any effect on growth in myxomycetes.

Materials and Methods

The myxomycete *Clastoderma debaryanum* has a plasmodium of the type that has been called "protoplasmodium." Each plasmodium is an individual, microscopic, coenocytic mass that fruits to produce one individual sporangium. When a plasmodium from a growing culture is transferred to a fresh substrate and fed, it divides and eventually many new plasmodia are produced in the new culture. Individual mature plasmodia and sporangia can easily be counted under a dissecting microscope. This species has recently been cultured on agar medium in our laboratory, and so provided a good subject for testing whether treatment of a culture, into which a plasmodium had recently been transferred, with a growth regulating substance would result in the production of more or fewer plasmodia and thus increase or decrease the resulting number of sporangia.

Twenty-nine experiments were carried out, in six series, in which the effect of treatment with 3-indoleacetic acid (IAA), kinetin (K), and gibberellic acid (GA) was tested. Cultures of the myxomycete plasmodia were grown on sterile 1.5% Difco corn meal agar in quadrant bottom culture dishes. Inoculation was made by transferring two well-developed plasmodia from an actively growing culture to each quarter of the dish. Five drops of dilute bark extract and 0.1 gm ground oatmeal were also added.

The first three series of experiments were carried out as follows. One week after inoculation, 5 drops of a 5 ppm aqueous solution of IAA were added to one quadrant of the culture dish, 5 drops of an aqueous solution of K (from Nutritional Biochemicals, Cleveland, Ohio) at 5 ppm were added to the second quadrant, 5 drops aqueous GA (Lilly) at 5 ppm to the third, and 5 drops of distilled water to the fourth. Thus an untreated control grew in the same dish with each culture under treatment. Cultures were kept moist with bark extract, and maintained at room temperature under normal autumnal daylight cycles. In the first two series of experiments, each treatment was replicated 4 times, and in the third series, three times.

In experimental series 4, 5, and 6, three concentrations of the growth substance were used: 1 ppm, 5 ppm, and 10 ppm. Treatments were begun one day after inoculation and repeated at 7-day intervals. Each treatment was replicated twice. Otherwise, the procedure was the same as in the first three series.

Results

Cultures fruited about one month after transfer of the vegetative plasmodia to new medium. After treatment with 5 ppm K, fewer sporangia were produced than in control cultures; after treatment with 5 ppm IAA, there were more sporangia than in controls; and there was no consistent stimulation or inhibition apparent after treatment with 5 ppm GA (Table 1).

Table 1. Mean numbers of sporangia produced after treatment with kinetin (K), gibberellic acid (GA), 3-indoleacetic acid (IAA), as compared with controls (C) (concentration: 5 ppm)

	C	K	GA	IAA
First Exptl. Series-Exp. #3	187.66	168	187	248.66
First 3 series of experiments	118.8	100	113	132.7
All (17) experiments	132.78	120.3	146.1	165.63

At both 1 ppm and 10 ppm, IAA again appeared to be stimulatory to growth (Table 2). At 1 ppm there may have been slight stimulation of growth by K, but at 10 ppm only about half as many sporangia were produced as were present in controls (Table 2). Again, GA did not produce consistent results.

Table 2. Mean numbers of sporangia formed after treatment with kinetin (K), gibberellic acid (GA), and 3-indoleacetic acid (IAA) at concentrations of 1 ppm and 10 ppm, as compared with controls (C)

	C	K	GA	IAA
1 ppm	74.1	82.1	77.6	97.3
10 ppm	89.6	46.5	96.3	113.5

Table 3. Total numbers of sporangia produced in all experiments after treatment with kinetin (K), gibberellic acid (GA), and 3-indoleacetic acid (IAA) at concentrations of 1 ppm, 5 ppm, and 10 ppm, as compared with untreated controls (C)

	C	K	GA	IAA
Total Number of Sporangia	2678	2321	2679	3100

The overall figures (Table 3) are lower after treatment with K and higher after treatment with IAA than the total for the controls. Student's t test applied to the data showed that the differences with 5 ppm and 10 ppm were significant at the 0.1 level of confidence or better.

Discussion

The multiplicity of effects that are produced by growth regulating substances probably are explainable as the net result of stimulation or inhibition of the processes of cell enlargement and cell division. The complicated pattern of action is hard to follow, because each of the substances probably has multiple functions, and actions overlap. Furthermore, the substances interact, sometimes synergistically, sometimes in opposition.

Particular interest has centered on kinetin, which is the first chemically defined substance known to cause cell division. But

we have found that application of kinetin is sometimes followed by a decrease rather than an increase in mitotic activity in both plant tissue, such as onion root (3), and animal cells, such as *Paramecium* (4), and *Blepharisma* (5). Wittwer and Dedolph (6) recently found that dry matter accumulation in aerial parts of tomato, cucumber, and pea plants decreased after kinetin treatment. The root morphology of tomatoes showed marked changes, including formation of pseudonodules. Schaeffer *et. al.* (7) found that nontumorous tobacco tissue required kinetin for rapid growth in culture, but tissue with a tumorous genotype was not affected, and tissues whose growth had been speeded up by glutamine and inositol were inhibited.

Gibberellin was discovered as the product of a pathogenic fungus. A number of fungi produce pathogenic effects which are accompanied by either destruction of plant auxins or by an abnormal increase in their level. Increased auxin content of parasitized tissue might, of course, be due to synthesis by the host rather than to secretion by the fungus. In the very earliest studies of the indole auxins, growth was found to be promoted by agar on which *Rhizopus* had been growing.

Reports of effects of treating fungi with auxins are conflicting, and, as Gruen (1) remarks, "the available information is confusing." To our knowledge, there is no previous report of any effect of chemical growth regulating substances on the growth of myxomycetes.

The myxomycete *Clastoderma debaryanum* has a plasmodial or vegetative stage in which growth is somewhat comparable to growth by cell division in higher plants. Individual plasmodia never enlarge beyond microscope size; in growing cultures, they can be seen to divide into two or sometimes more smaller plasmodia. These grow and divide again, producing many individual plasmodia. Because each plasmodium fruits to produce a single sporangium at maturity, the number of sporangia produced is an indication of the preceding growth activity. Since this myxomycete can now be grown on artificial medium, it provided an excellent subject for the testing of the effects of growth regulating substances.

The results reported here seem to indicate that applied substances of the indole auxin and kinetin type can, at least to some extent, regulate growth in myxomycetes. Indoleacetic acid appeared to stimulate vegetative growth of the plasmodia at all concentrations tested (1, 5, and 10 ppm), and kinetin appeared to be slightly stimulatory at 1 ppm and inhibitory at 5 and 10 ppm. No conclusion can be drawn from the results with gibberellic acid.

All cultures were examined for obvious aberrations of morphology of plasmodium and fruiting body, but none were noted. Sporangial size, capillitial and spore morphology, and stalk characteristics remained within average range.

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The Effect of Wind on Transpiration and Evaporation Through Multiperforate Septa[1]

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Abstract: Diffusion of water vapor through single-pore membranes with pores 100 to 800 μ in diameter and multipore membranes with pores 2.5 to 20 μ in diameter was studied as a function of wind velocity. The results of these studies are compared with data obtained with transpiring leaves in wind. It was found that wind had relatively little effect on small pores as compared with large pores and free water surfaces. The primary response of stomates or small, isolated pores to wind was simply an increase in the diffusion gradient. In general, the wind-to-still-air diffusion ratios determined with the use of small pores, either isolated or as a part of a multipore system, were less than 2, while the ratio for open surface evaporation in 1,000 feet per minute wind exceeded 15. The relatively small response of transpiration to wind is tenable, considering the epidermis as a multiperforate septum.

Considerations of the effect of wind on transpiration have varied from the work of Hygen (1), which indicated that wind increased stomatal transpiration, to the work of Manzoni and Puppo (2), which indicated that wind had no average effect. Martin and Clements (3) concluded that wind increased the rate of transpiration at low velocities but had relatively little additional effect at higher velocities. Martin (4) found that the ratio of transpiration in wind to that in still air averaged just over 2 for *Helianthus annus* under a variety of conditions. Huber (5), studying the effect of wind on small pore diffusion, concluded that the smaller the pore the less effect wind would have

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