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Influence of Gibberellic Acid and Indole-3-Acetic Acid on the Morphology and Population Dynamics of *Eudorina elegans*

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Abstract. Assuming optical density to be a function of population density, colorimetric techniques were used to determine the effects of two growth regulator substances, indole-3-acetic acid and gibberellic acid, on the morphology and population dynamics of *Eudorina elegans*.. A tube of culture medium without organisms was used as a reference point to calibrate the colorimeter. Chemically-treated cultures and soil-water control cultures were compared to the reference reading and growth curves were plotted. Colony counts were made at the termination of the experiment and comparisons were made with the colorimeter readings.

Results demonstrated gibberellic acid to be a promoter of cell division and/or growth in concentrations below 75 ppm and to be inhibitory at concentrations of 75 ppm and above. Indole-3-acetic acid produced little effect on populations at low concentrations and was strongly inhibitory above concentrations of 5 ppm.

Went's discovery in 1926 (1) that plants produced within themselves diffusible chemicals which behave as growth regulators opened for investigation an unexplored field of biology. By 1933, Went's regulator substance, auxin, had been isolated. It is reasonably certain that native auxin is indole-3-acetic acid. Initial interest in auxin and auxin-like synthetic substitutes was largely confined to economic problems of agriculture and horticulture. More recently, studies have centered upon physiological problems such as the effects of growth substances on cells and tissues of vascular and non-vascular plants. This study is of the latter type. It attempts to determine the effects of two growth substances, gibberellic acid and indole-3-acetic acid, on the growth form of populations of *Eudorina elegans*.

Eudorina elegans is a colonial organism classified in the family Volvocaceae. It is composed of a transparent gelatinous sheath or matrix in which cells of the organism are embedded. The matrix is composed of two layers, an inner-layer surrounding the component cells and a thin peripheral outer-layer (Figure 1). The general shape of a colony varies from spherical to ellipsoidal and shows a distinct anteroposterior polarity. Each cell possesses two flagella which project through the matrix and extend out about 15 microns. Concerted actions of the flagella enable the organism to move rapidly through a liquid medium.

Cell number per colony, the principal means by which preliminary investigators assigned species status, is highly variable. Clon-

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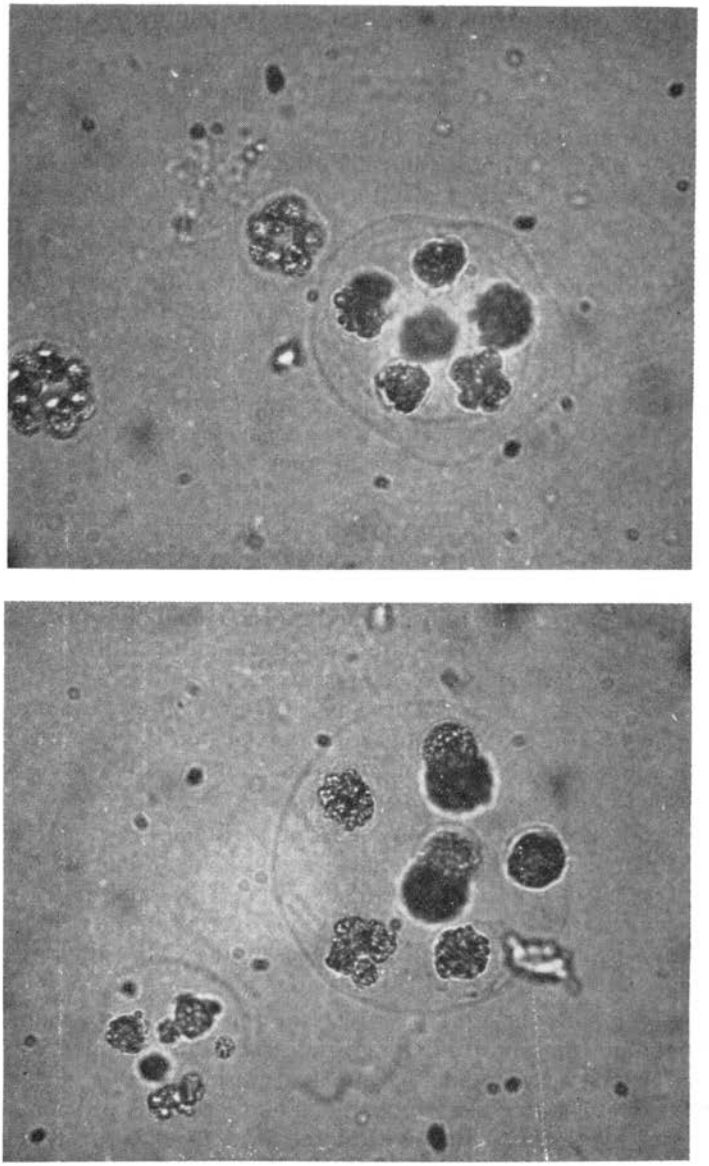

Figure 1. Above: Double layered structure of colony matrix. Below: Mitotic division within a colony.

ally-derived populations contained colonies varying from 8 to 128 cells. Reproduction in mature colonies occurrs by mitotic division (Figure 1) and by monoecious or dioecius sexual forms (2). The organism exhibits a strong positive phototactic response. If the

container of organisms was disturbed, the population reoriented itself toward the light in 5-15 minutes.

Materials and Methods

To minimize the effects of variable heredity, a female clonal culture number 1193, strain 56f of *Eudorina elegans* was purchased from the Indiana Culture Collection, Bloomington, Indiana. Since the species is dioecious, variables due to sexual reproduction were thus eliminated. The organisms were cultured in a sterile soil-water medium. Population growth-form was measured by colorimetry, with population increases indicated as increases in optical density of culture tubes. By comparing optical densities of tubes of sterile culture medium without organisms to identical culture tubes with organisms, an index of population growth was obtained. Cultures were necessarily maintained axenically, since populations of microorganisms would have also produced optical densities.

The stock-culture of organisms was obtained by transferring a loopful of *Eudorina elegans* from the purchased culture agar slant to culture tubes containing 10 ml of soil-water extract. The transfer was performed in a disinfected transfer chamber and a single loopful was used to inoculate each stock-culture tube. Cultures were incubated at room temperatures in white and Gro-lux fluorescent light at an intensity of 300 foot candles. Illumination was for 16 consecutive hours each day. In ten days, a sufficient population was obtained in each stock-culture tube for inoculation of the experimental tubes.

Stock solutions of growth-regulator substances were prepared by dissolving 150 mg of indole-3-acetic acid and of gibberellic acid in 500 ml of sterile distilled water. This provided a stock solution of 300 ppm. These solutions were filtered through a millipore filter to establish axenic conditions. The soil-water solutions were sterilized by autoclaving.

To compare the relative effects of the growth regulators on population growth of the organism, serial dilutions of gibberellic acid in soil-water, indole-3-acetic acid in soil-water, and distilled water in soil-water were prepared. Four replicates of each serial dilution were inoculated with 5 ml of stock-culture organisms. Serial dilutions of 1.75, 2.5, 7.5, 10, 15, 25, 40, 75, 125, 175 and 250 ppm were made. In addition, a tube of soil-water, distilled water and growth-regulator substance was prepared without organisms for calibration of the colorimeter at each experimental dilution. This tube was used for selecting the wave length of light which produced the maximum deflection of the colorimeter and for the necessary adjustments to register 100 per cent light transmission. Then tubes of appropriate concentration were measured

and compared to the corresponding blank reading. To provide an additional control, four tubes of undiluted soil-water extract were prepared to compare population patterns under identical laboratory conditions.

After the final colorimeter reading was taken, at the end of 21 days, each tube was shaken and a 1 ml sample of solution removed from it. These samples were counted on a slide having 7 mm squares of mm paper mounted under a cover glass (Figure 2). Colonies located in the center square and in the 4 squares situated 1 mm from each corner of the measuring grid were counted. By averaging the counts obtained in the five squares for each sample, the number of colonies per area per concentration was calculated. Cell diameters of all colonies located in the center square were then measured using an ocular micrometer. Colony diameters of organisms located in the 4 squares adjacent to the center square were measured also (Tables 1-3) .

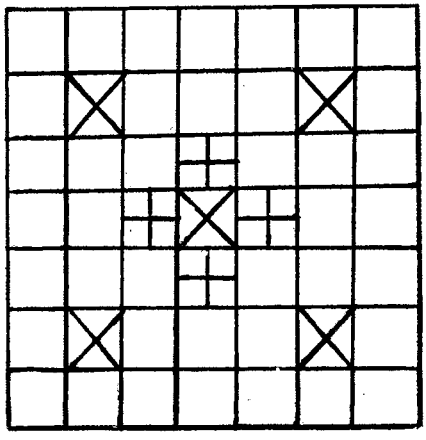

Figure 2. Grid for making colony counts.

Results

The effects of gibberellic acid-treated cultures compared to soil-water extract cultures, and to indole-3-acetic acid-treated cultures is summarized by the following:

1. Concentrations of 1.75 ppm of gibberellic acid in soil-water produced an increase in optical density of 42.5 per cent compared to 31.75 per cent for soil-water extract after 21 days. During the first 6 days of the study, no visible differences in population densities were noted and each showed typical exponential growth. The exponential growth continued for 4 more days in the gibberellic acid medium producing the higher population asymptote. Colorimeter readings were constant for the last 10 days of

Table 1. Mensural Data for Gibberellic Acid-treated Cultures

	Concentration in parts per million											
	1.75	2.5	5	7.5	10	15	25	40	75	125	175	250
Total area of colonies*	10105	6513	3840	6020	4952	9872	5659	6652	2763	0	0	291
Number of colonies counted	286	125	142	193	147	209	222	146	68	3	0	6
Number of colonies measured**	53	83	69	107	102	153	131	102	60	0	0	6
Number of cells measured	21	15	17	34	27	36	40	29	13	0	0	5
Total colony diameters	7450	3817	2680	4340	4100	5925	4550	4195	2150	0	0	225
Average colony diameter	34.75	46	38.8	40.5	40.2	38.8	34.7	41	36	0	0	37.5
Total cell diameters	356	148	123	292	245	348	344	220	91	0	0	48
Average cell diameter	6.75	9.86	7.25	8.6	9.6	9.7	8.6	7.6	7	0	0	9.6
Colonies per cm^2	475	208	237	317	245	349	370	243	114	5	0	10
Initial colorimeter reading	95.75	100	99	98.5	93.25	98.25	98	98.25	98.25	100	100	99.75
Final colorimeter reading	53.25	60.25	55.25	57.25	56	61.75	66.5	63.75	87.75	99	97	95.25
Percentage decrease in optical density	42.5	39.75	43.75	41.25	37.25	36.5	31.5	34.5	10.5	1	3	4.5

*Multiply by 100 to get actual area in square microns

**All measurements are in microns

Table 2. Mensural Data for Indole-3-Acetic Acid-Treated Cultures

	Concentration in parts per million							
	1.75	2.5	5	7.5	10	15	25	Pure soil extract
Total area of colonies*	4257	2928	50	50	187	193	237	5101
Number of colonies counted	105	57	7	3	6	3	7	26
Number of colonies measured**	73	44	1	1	2	3	4	30
Number of cells measured	21	8	1	1	2	2	2	5
Total colony diameters	2810	1905	40	40	110	135	170	2040
Average colony diameter	38.5	43.3	40	40	55	45	42.5	68
Total cell diameters	192	86	8	10	28	22	24	70
Average cell diameter	9.3	10.7	8	10	14	11	12	14
Colonies per. cm^2	175	95	12	5	10	5	12	48
Initial colorimeter reading	95.5	95.5	99	98	93	96.5	99.5	98.75
Final colorimeter reading	61	74.5	97.25	91	91.75	88.75	87.5	75.75
Percentage decrease in optical density	34.5	21	1.75	7	1.25	7.5	12	23

*Multiply by 100 to get actual area in square microns
**All measurements are in microns

Table 3. Mensural Data for Soil-water cultures

	Concentration in parts per million											
	1.75	2.5	5	7.5	10	15	25	40	75	125	175	250
Total area of colonies*	5831	7280	6281	5003	7651	6968	3518	5172	5163	2318	2301	2078
Number of colonies counted	103	75	45	52	40	43	35	88	31	25	28	19
Number of colonies measured**	71	37	36	35	37	38	19	64	41	17	29	19
Number of cells measured	21	12	8	16	7	9	7	16	13	10	10	9
Total colony diameters	3335	2940	2465	2010	2900	2870	1330	2905	2320	1100	1405	940
Average colony diameter	40	79.5	68.6	57.5	76.5	75.5	70	45.5	57	64	49	49
Total cell diameters	240	150	107	165	50	132	104	156	133	119	124	94
Average cell diameter	11.4	12.5	13.4	10.3	7.2	14.6	14.8	9.8	10.2	11.9	12.4	10.4
Colonies per. cm^2	172	125	75	86.5	67	71.7	58	65	52	42	47	32
Initial colorimeter reading	96.75	100	99	98.5	94.5	98.25	99.5	98	95.5	100	98	97.25
Final colorimeter reading	65	76.5	68.75	71.25	71.25	74.5	76.75	62	72	78	79.75	86.5
Percentage decrease in optical density	31.75	23.5	31.75	27.25	23.25	23.75	22.75	36	23.5	22	19.75	10.75

*Multiply by 100 to get actual area in square microns

**All measurements are in microns

the study leading us to conclude that the carrying capacity of our environment had, in fact, been reached.

2. Concentrations of gibberellic acid of 2.5, 7.5 10, 15, and 25 ppm produced little variability from that described above for 1.75 ppm. Both the sigmoid pattern of population growth and the carrying capacities of the environments were similar.

3. At 40 ppm the gibberellic acid curve and the corresponding soil-water control curve were almost identical and remained so throughout the experimental period.

4. At concentrations of 75 ppm, curves of gibberellic acid-treated cultures separated from control cultures by the seventh day. The treated culture leveled off in an asymptote at that time, but the soil-water culture continued steady growth for 5 more days before leveling out. Treated cultures showed a 4 per cent decrease in optical density after the ninth day, an observation for which no explanation was found.

5. Gibberellic acid concentrations of 125 ppm and above were decisively limiting to population growth. A conclusion that gibberellic acid somehow interfered with mitotic and/or metabolic patterns seems warranted.

6. At indole-3-acetic acid concentrations of 1.75 and 2.5 ppm, population growth curves of treated cultures approximated those of untreated soil-water cultures.

7. At indole-3-acetic acid concentrations of 5, 7.5, 10, 15 and 25 ppm, normal growth curves did not exist. Apparently mitotic division or some other metabolic process was so severely inhibited at these concentrations that normal population growth patterns did not occur. Experimentation at levels above 25 ppm was discontinued.

Discussion

Indole-3-acetic acid has been used on a wide variety of experimental organisms and was found to be a growth promoter by Brannon and Sell (3), Pratt (4), Williams (5), and Davidson (6). In our study, indole-3-acetic acid produced only minor growth-promoter effects at low concentrations and severely inhibited growth at moderate and high concentrations. Results similar to those of this study were reported by Accorinti (7), Conrad (8), and Brannon and Bartsch (9).

Gibberellic acid was a growth stimulator in low concentrations and an inhibitor at high concentrations in studies of Goryachev (10), Zetsche (11), Conrad (8), Ermolaeva (12), and in this study.

One assumption of this investigation was that optical density was a function of population growth. Final colorimeter readings

in relation to sample colony counts made at the termination of the experiment confirmed the correctness of this assumption.

ACKNOWLEDGEMENT

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