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Effects of IAA (indole-3-acetic acid) and kinetin (6-furfurylamino-purine) on the synthetic lichen *Cladonia cristatella* and its isolated symbionts.

Selina B. Remmer, V. Ahmadjian and T.P. Livdahl

Department of Biology, Clark University, Worcester, Massachusetts 01610, USA.

Summary.- Isolated symbionts of the lichen *Cladonia cristatella* were grown in the presence of two concentrations of the hormones IAA and kinetin (0.1 mg/l and 1.0 mg/l of each). Each concentration of hormone was used separately and in combination. Controls were grown without hormones. Algal growth was stimulated and fungal growth inhibited at 0.1 mg/l IAA while at 1.0 mg/l IAA algal growth was not significantly affected but fungal growth was inhibited. Kinetin moderated the inhibitory action of IAA on fungal growth. A combination of 0.1 mg/l IAA and 0.1 mg/l kinetin altered fungal morphology and physiology and resulted in an increase in aplanospore formation, clumped growth form and gelatinous sheath formation.

Hormone treatments identical to those used on the symbionts were used during lichen synthesis. Kinetin at a concentration of 0.1 mg/l and in combination with 1.0 mg/l IAA stimulated the development of synthetic squamules, juvenile podetia, and apothecia.

Possible sources of hormones within the lichen symbiosis are discussed and a hypothesis of hormonal regulation of lichen development is proposed.

Key words: auxin, *Cladonia cristatella*, cytokinin, IAA, kinetin, *Trebouxia erici*.

Introduction.

The role of plant hormones in mutualistic associations has been of interest to investigators who wish to understand the control mechanisms which regulate interactions between symbionts. Most of the attention in this respect has focused on auxin and cytokinins. Following is a review of our current knowledge of hormones in some symbiotic systems.

1. Auxin.- Mycorrhizae produce auxin (Harley, 1971; Slankis, 1973; Harley and Smith, 1983; Jackson and Mason, 1984) the function of which is not clear. Slankis (1973) proposed that auxin may stimulate mycorrhizal infection as well as growth a development of the host, and alter various physiological and biochemical processes in mycorrhizal roots.

He suggested that, while auxin precursors are generally produced by the host, under certain nutritional conditions these precursors may originate from the fungus. Growth substances derived from the fungus may be involved in the development of mycorrhizal structures and gibberellins, kinins, and auxins may arise from ectomycorrhizal fungi under certain conditions (Harley, 1971). Jackson and Mason (1984) suggest that rizosphere soil organisms such as Azotobacter and Pseudomonas stimulate mycorrhizal formation by producing auxin.

Root nodules of legumes which contain the nitrogen-fixing bacterium Rhizobium have higher levels of auxin than uninfected roots (Chen, 1938; Fortin and Thibault, 1972; Kefford et al., 1960, Slankis, 1973; Thimann, 1936, 1952). Kefford et al. (1960) determined that nodule bacteria used tryptophan, exuded by legume roots, to produce auxin and Slankis (1973) reported that auxin could induce nodules on legume roots. Several plant pathogens appear to exert their influence by means of plant hormones. Verticillium albo-atrum, a fungus which infects tomatoes, and Taphrina deformans and T. cerasi, fungal pathogens of fruit trees, cause increased levels of auxin in infected tissues (Letham, 1978). Agrobacterium tumefaciens, the causal agent of crown gall disease, transfers some of its DNA (T--DNA) to the host cells. Genes on the T-DNA code for the synthesis of plant hormones such as auxin and cytokinins which stimulate the plant cells to form a tumor-like growth (Chilton et al., 1977).

Only a few researchers have studied hormonal relationships in lichens. Zehnder (1949) tested the effect of auxin on three genera of lichen algae, i.e. Chlorella, Coccomyxa, and Trebouxia (= Cystococcus) and found that, in general, low concentrations of the hormone stimulated growth while higher concentrations inhibited growth. Similar studies on lichen fungi gave mixed results depending on the species.

DiBenedetto and Furnari (1962) found that auxin increased the growth of two algae, i.e. Trebouxia albules-

found in the free-living state. Giles (1970) found that both red light and IAA stimulate the production of aplanospores in liquid cultures of the lichen phycobiont Trebouxia erici. Although aplanospores are the only means of reproduction of the alga within the lichen thallus, zoospores are normally found in liquid cultures. Giles concluded that aplanospore formation is stimulated when factors tending to raise the concentration of IAA within algal cells are present.

Fortin and Thibault (1972), who searched for auxin in Cladonia alpestris, stressed that lichens should be studied using methods of comparative physiology since the formation and maintenance of a symbiotic equilibrium may be similar in different systems. These investigators grew three lichen mycobionts in axenic culture, with and without tryptophan. In the media with tryptophan all of the mycobionts produced auxin while auxin was not produced in media without tryptophan. In addition, they found auxin in the natural thallus of the lichen and concluded that the algae produce tryptophan which is transformed into auxin by the fungus. Fortin and Thibault hypothesized that the principal role of auxin in lichens is either to control glycolysis in the algae, as it does in tobacco callus tissue (Skoog and Robinson, 1950), or to increase the permeability of the algal cell membrane (Glasziou et al., 1960).

2. Cytokinins.- Several researchers have considered the role of cytokinins in mycorrhizal fungi. Laloue and Hall (1973) noted that the symbiotic fungus Rhizopogon roseolus excreted several natural cytokinins in axenic culture. These workers thought that the cytokinins represented a means of communication between the fungus and its host, which would explain the growth stimulating properties of the fungus. The production and release of cytokinins by many symbiotic fungi has been reported (Slakin, 1973; Letham, 1978; Horgan, 1984). Letham (1978) suggested that infections by symbiotic fungi could result in mutually beneficial associations due to cytokinin production. The cytokinin-induced mobilization of nutrients to the infection site would benefit the fungus while increased synthesis of phenolic compounds, also under

condary infections. According to Letham, cytokinins increase plant resistance to secondary infection in cucumber plants infected with powdery mildew and in wheat infected with rust fungus. Further evidence of the ability of cytokinins to affect host resistance by stimulating phenol production was shown by the decreased resistance of tomato plants infected with the root nematode Meloidogyne incognita. The nematode caused increased cytokinin levels in the host roots but reduced levels in the rest of the plant.

Interestingly, lichens, which are exceptionally long-lived, produce large auounts of phenolic compounds but their symbionts when cultured separately generally do not (Culberson and Ahmadjian, 1980).

Legume root nodules caused by Rhizobium contain high levels of cytokinin as well as other hormones (Letham, 1978). The bacteria can also produce cytokinin in vitro (Phillips and Torrey, 1970). Kefford et al. (1960) suggested that nodules result from the division of root cells which begins when the IAA contained in rhizobial infection threads interacts with an optimal concentration of cytokinin in the host cells. Letham (1978) observed that cytokinin alone could induce polyploidy.

As with auxin, cytokinins are involved in a number of pathogenic asociations. Tissues from crown galls caused by A. tumefaciens contain large quantities of cytokinins (Horgan, 1984). Swaminathan and Bock (1977) reported that tRNA from A. tumefaciens and Pseudomonas aeruginosa contained cytokinin which closely resembled a plant type hormome rather than the expected bacterial type. Since other plant pathogens such as Corynebacterium fascians, the causal agent of "witches broom", and Rhizopogon roseolus also contain plant type cytokinins (Swaminathan et al., 1977), it is possible that in earlier times there was a transfer of genetic material between a plant host and its associated bacteria. Other pathogenic organisms which produce cytokinins include the rusts Uromyces phaseoli and U. fabae, a powdery mildew, Erysiphe graminus and certain Verticillium fungi (Letham, 1978; Bearder, 1980, Slankis, 1973; Horgan, 1984;

There has been much speculation as to whether or not cytokinins produced by plant pathogens are involved in the physiology of the infection. Until more is known about the biochemical control of plant cell division, senescence and directed transport, such questions will remain unanswered (Morgan, 1984).

The purpose of our research was to determine how exogenous plant hormones affected the development of lichen thalli. The presence of hormones in natural lichens and hormonal involvement in other symbiotic systems suggested that hormones may also have a role in the lichen symbiosis.

Material and Methods.

1. Cultures.- Axenic, clonal cultures of *Trebouxia erici* and of the mycobiont of *Cladonia cristatella* were obtained from the collection of lichen symbionts at Clark University.

2. Media.- The alga was grown on Bold's Basal Medium with 3X $NaNO_3$ (BBM3N) (Deason and Bold, 1960). Liquid medium was used in the yield and rate experiments. For synthesis experiments the alga was taken from agar slants. The fungus was grown in liquid Lilly and Barnett medium (Lilly and Barnett 1951) for the yield and growth experiments and in liquid malt-yeast extract medium (Ahmadjian, 1967) for the synthesis experiments.

3. Hormones.- Indole-3-acetic acid (IAA) and 6-furfurylamino purine (kinetin) were obtained from the U.S. Biochemical Corporation. Sterile water was used in all of the stock solutions and all hormones were sterilized at the time of application by delivery through Gelman Acrodisc disposable filter assemblies (pore size 0.2 µm) on 5 ml lock tip disposable syringes.

4. Inoculation of yield and rate experiments.- Two week old cultures of *T. erici* grown on agar slants were added to 150 ml distilled water in a blender and mixed at high speed for 10 s in order to make uniform suspensions. Six week old cultures of the mycobiont grown in liquid malt-yeast extract

5. Yield experiments.- For each experimental procedure, 47 ml of the appropiate medium, 1 ml of the required stock hormone solution(s) and 1 ml of algal or fungal suspension were added to 125 ml Erlenmeyer flasks. Sterile technique was used throughout these procedures. In order to standardize dilution, 2 ml of distilled water was added to the flasks receiving only one hormone. Both 1.0 mg/l and 0.1 mg/l concentrations of each hormone were used alone and in all possible combinations (see Table 1). Each treatment was replicated five times. Flasks were plugged with foam stoppers, sealed with aluminium foil, and incubated at 18-20°C for seven weeks. Light for the algae was supplied by fluorescent tubes equivalent to 3,200 lux. During incubation, all flasks were rotated randomly about the incubator shelf in order to minimize differences caused by flasks position. Algal growth was removed from the flasks (repeated scraping and rinsing were necessary in order to dislodge material adhering to the flask walls and bases) and centrifuged for 15 min at 3,015 g to form a loose pellet. Filtration, using a suction filtration apparatus, was through 0.45 µm pore size Millipore membrane filters. The filter discs with the algae were placed in tared petri dishes and dried in an oven for 1 h at 80°C before weighing. Fungal cultures, which did not have to be centrifuged because of their normal clumped growth form, were filtered using 1.2 µm pore size Millipore membrane filters. The filter discs with the fungi were dried and weighed as for the algae. Two-way analyses of variance were conducted on the dry weight data, with a criterion for significance of $p < 0.05$.

6. Rate experiments.- Flasks were prepared as for the yield experiments except that a total of 10 flasks was used for each treatment and for the controls. Treatments used for the algae were 0.1 mg/l IAA and 0.1 mg/l kinetin combined and 0.1 mg/l IAA alone. For the fungi a combination of 0.1 mg/l IAA and 0.1 mg/l kinetin was used. Weight determinations, using two flasks each for the treatments and controls were made every seven days for the algae and every three

Table 1. Experimental desing for hormone treatments used in yield and synthesis experiments. Symbionts were treatedwith IAA and kinetin in the concentrations shown. Each treatment was replicated 5 times. Hormone treatment are expressed in mg/l with the concentrations of IAA expressed by the first figure and the concentrations of kinetin by the second. Where 0/0 appears treatment consisted only of sterile distilled water.

Concentration IAA mg/l	Concentration kinetin None	0.1	1.0
None	0/0 (controls)	0/0.1	0/0.1
0.1	0.1/0	0.1/0.1	0.1/0.1
1.0	1.0/0	1.0/0.1	1.0/1.0

Table 2. 2-way analysis of variance for algal yield (from (BMDP7D 1982). df = degree of freedom; SS = sum of squares; MS = mean square.

Source	df	SS	MS	F	P
IAA	2	0.0004	0.0002	6.67	< 0.01
Kinetin	2	0.0006	0.0003	11.62	< 0.001
IAA x Kinetin	4	0.0003	0.0001	2.86	< 0.05
Error	36	0.0010	0.0000		
Total	44	0.0023			

hour each time in order to minimize error. Methods for filtering, drying, and weighing were the same as those in yield experiments. Results were analyzed statistically using the Sokal and Rohlf (1981) test for differences among regression coefficients. Morphological observations were made on the algae and fungi during the experiment in order to determine if hormone treatments affected the growth form.

7. Synthesis experiments.- Six week old cultures of the mycobiont were selected. Excess malt-yeast extract medium was poured off and the cultures were washed in distilled water. After washing, each culture was centrifuged at 12,062 g for 20 min and the supernatant poured off. Each fungal pellet was placed in a petri dish and excess water was blotted with absorbent pads. Two week old cultures of the alga were scraped off agar slants with a spatula and a thin layer of the alga was spread over the flattened fungal mycelium. Stock hormone solutions were diluted to the correct concentrations by adding to distilled water and dropped directly onto the algal/fungal mats through Acrodisc filters of pore size 0.2 μm attached to 5 ml syringes. Controls were treated with distilled water in the same way. Both 1.0 mg/l and 0.1 mg/l concentrations of each hormone were used alone and in all possible combinations (see Table 1). Each treated mat was cut into five roughly equal sections. Flasks were prepared by pouring 30 ml plain agar into each and adding a thin strip of newly cleaved mica which had been previously soaked in BBM3N solution. Flasks*were then autoclaved and cooled. One algal/fungal section was pressed firmly onto each mica strip and the flasks were plugged with non-absorbent cotton and sealed with aluminium foil. Flasks were incubated at 18--20°C and 3,230 lux with the mica strip oriented toward the light source. The positions of the flasks were changed randomly. After 5, 9 and 15 weeks, flasks were examined under a dissecting microscope to determine the stage of differentiation of the culture.

7. Thin-layer chromatography.- TLC methods were those described by Culberson (1972).

hormones on the dry weight of algae. Maximum yield was obtained using 0.1 mg/l IAA which resulted in a 47% increase over the controls. A combination of 0.1 mg/l IAA with 0.1 mg/l kinetin also increased yield considerably giving a 30% increase over controls. IAA at 1.0 mg/l generally inhibited growth of the algae but the inhibition effect was moderated by the addition of 0.1 mg/l kinetin. Kinetin at 1.0 mg/l inhibited growth and the inhibition was unaltered by the addition of either 0.1 mg/l or 1.0 mg/l IAA. Analysis of variance for algal yield (Table 2) detected significant effects for each hormone and a significant IAA x kinetin interaction.

2. Yield experiments: Fungus.- Fig. 2 shows the effects of hormones on the dry weight of the fungus. All hormone treatments inhibited growth but interactive effects were again evident. IAA at 0.1 mg/l decreased yield by 37% compared to controls while 0.1 mg/l IAA combined with 0.1 mg/l kinetin decreased growth by only 7%. Thus, as with the alga, kinetin moderated the inhibitory action of IAA. IAA at 1.0 mg/l alone or in combination with kinetin was most inhibitory to fungal growth, reducing yield to as low as 21% of the controls. Analysis of variance for fungal yield (Table 3) detected significant effects due to IAA and IAA x kinetin interaction but no significant differences due to kinetin alone.

Table 3. 2-way analysis of variance for fungal yield (from BMDP7D 1982). df = degree of freedom; SS = sum of squares; MS = mean square.

Source	df	SS	MS	F	P
IAA	2	0.0180	0.0090	126.01	< 0.001
Kinetin	2	0.0004	0.0002	2.62	> 0.05
IAA x Kinetin	4	0.0023	0.0006	8.14	< 0.001
Error	33	0.0024	0.0001		
Total	41	0.0231			

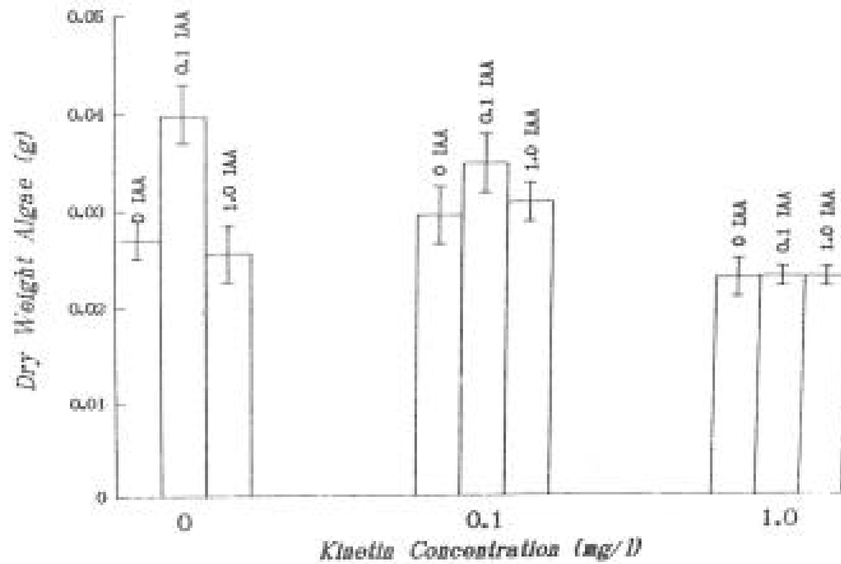

Fig. 1. Yield experiment: algae. Mean dry weight (± S.E.) of 5 replicates grown in different levels of IAA and kinetin (mg/l).

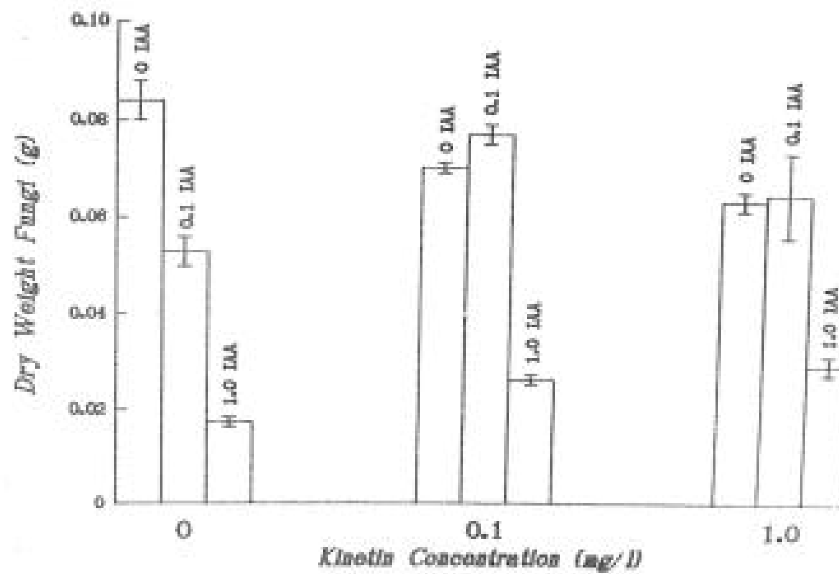

Fig. 2. Yield experiment: fungi. Mean dry weight (± S.E.) of 5 replicates grown in different levels of IAA and

3. Rate experiment: alga.- The results of the rate experiment on the growth rate of alga are given in Fig. 3. The regression coefficient (Table 4) are significantly heterogeneous. Examination of the slopes and standard errors (Table 4) suggests that the differences arise primarily from the contrast between de dual treatment of 0.1 mg/l IAA with 0.1 mg/l kinetin and the other two treatments.

Table 4. Rate experiment: algae. Summary of regressions for weight vs. time under three treatment regimes. Data for the dependent and independent variables were transformed to natural logarithms to achieve linearity. The F statistic summarizes a test for differences among regression coefficients (Sokal and Rohlf, 1981). S.E. = standard error of the slope; r^2 = coefficient of determination. Hormone treatments are expressed as concentrations of mg/l with IAA represented by the first figure and kinetin by the second.

Hormone treatment	Slope	$\pm$ S.E.	r^2
0/0 (controls)	1.65	0.19	0.90
0.1/0	1.51	0.19	0.89
0.1/0.1	2.16	0.16	0,96
	$F_{2,27} = 3.82$, $P < 0.025$		

4. Rate experiments: fungus.- The results of the rate experiments on growth rate of the fungus are given in Fig. 4. Examination of the regression coefficients and standard errors (Table 5) indicates that treatment with a combination of 0.1 mg/l IAA and 0.1 mg/l kinetin resulted in a significant increase in the growth rate of the fungus.

5. Morphological observations.- During the course of the rate experiments, algal cells and fungal hyphae were examined

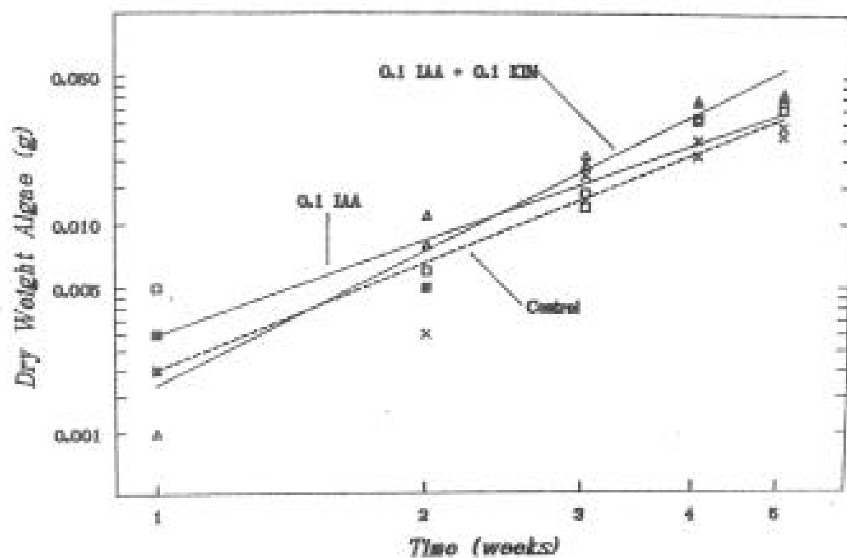

Fig. 3. Rate experiment: algae. Effect of IAA and kinetin on growth rate of algae. Weight and time shown on a logarithmic scale. x = control; = 0.1 mg/l IAA; = 0.1 mg/l IAA and 0.1 mg/l kinetin.

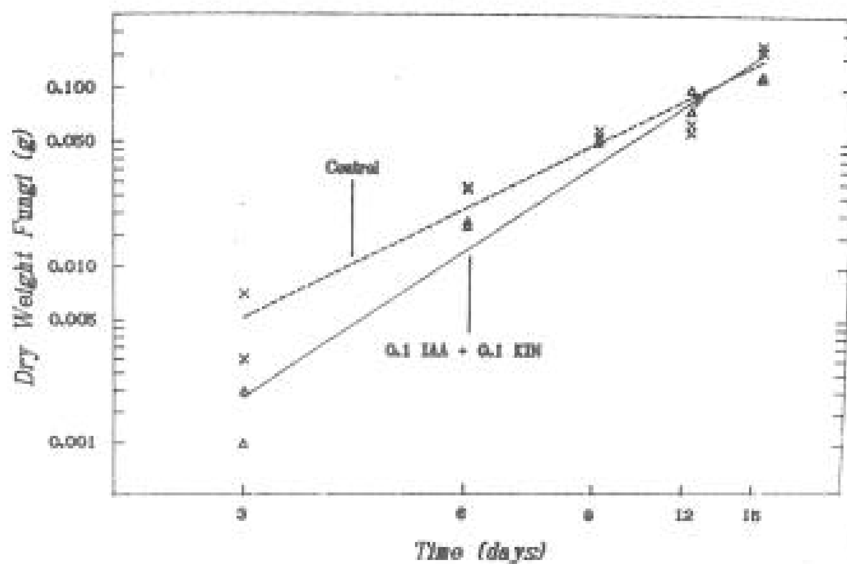

Fig. 4. Rate experiment: fungi. Effect of IAA and kinetin on growth rate of fungi. Weight and time shown on a lo-

of the flasks, differences in growth form of the alga were evident. The untreated alga, i.e. controls, grew in a diffuse suspension of separate cells. Treatment with 0.1 mg/l IAA caused some of the cells to stick together forming small clusters and treatment with a combination of 0.1 mg/l IAA and 0.1 mg/l kinetin caused all of the cells to cluster. The clumping effect in the dual treatment group was so complete

Table 5. Rate experiment: fungi. Summary of regressions for weight vs. time under two treatment regimes. Data for the dependent and independent variables were transformed to natural logarithms to achieve linearity. The F statistic summarizes a test for differences among regression coefficients (Sokal and Rohlf, 1981). S.E. = standatd error of the slope; r^2 = coefficient of determination. Hormone treatments are expressed as concentrations of mg/l with IAA represented by the first figure and kinetin by the second.

Hormone treatment	Slope	$\pm$ S.E.	r^2
0/0 (controls)	2.05	0.19	0.94
0.1/0.1	2.77	0.20	0.96
	$F_{1,18} = 7.75,\ P < 0.05$		

that after 5 weeks the medium appeared clear with the algae forming sheet-like mats of attached cells. In contrast, media of the control flasks appeared pale green due to the uniform suspension of single algal cells. Microscopic examination of the algal cells showed extensive mucilage coats around those treated with hormones with the widest coats found in the dual treatment flasks. In addition, aplanospore production in all treatment flasks was high while mostly zoospores were produced in the control flasks. No observable differences were apparent between the treated fungi and con-

7. Synthesis experiment.-

a) Five week old cultures.- Some variation could be seen between the different treatment groups (Table 6). The highest degree of differentiation occurred in the synthetic cultures treated with 1.0 mg/l kinetin and in those treated with a combination of 1.0 mg/l IAA and 1.0 mg/l kinetin. More than 50% of the fungal/algal mat surfaces were covered with presquamules showing the characteristic gray-green color typical of *C. cristatella*. All the other dual treatments resulted in presquamule formation in some of the flasks in each group, but approximately 25% only of the fungal/algal mat surface was involved. Control flasks and those given single treatments of 0.1 mg/l IAA, 1.0 mg/l IAA and 0.1 mg/l kinetin showed some presquamule development in some of the flasks, but differentiation was at an extremely low level.

Table 6. Presquamule formation by 5-week-old cultures of synthetic *C. cristatella*. Cultures were examined under a dissecting microscope and an estimate of surface area involvement was made. Hormone treatments are expressed as mg/l with IAA represented by the first figure and kinetin by the second. 5 replications were made for each treatment.

Hormone treatment	# with presquamules	Est. area involved
0/0 (controls)	3	<< 25%
0.1/0	3	<< 25%
1.0/0	3	<< 25%
0/0.1	2	<< 25%
0/1.0	5	> 50%
0.1/0.1	5	~ 25%
1.0/1.0	5	> 50%
0.1/1.0	4	25-50%
1.0/0.1	4	25-50%

tiation with well developed squamules and juvenile podetia and apothecia. All dual hormone treatments also produced good development of lich- zed structures in at least some of the flasks for each tr tment group. The remaining four groups, i.e., controls, 0. mg/l kinetin, 1.0 mg/l IAA and 0.1 mg/l IAA all showed li le differentiation (Table 7).

Table 7. Morphological characteristics 9-week-old cultures of synthetic *C. cristatella*. Hormone treatments are expressed as mg/l with IAA represented by the first figure and kinetin by the second. Figures in column represent the number, out a total of 5, showing the characteristics noted. con. = contaminated; pre. = presquamules; sq. = squamules; pod. = podetia; apo. =apothecia.

Hormone treatment	con.	pre.	sq.	pod.	apo
0/0 (controls)	4	2	0	0	0
0.1/0	4	3	3	2	1
1.0/0	4	3	0	1	1
0/0.1	5	3	0	1	0
0/1.0	0	5	5	5	5
0.1/0.1	0	5	5	5	5
1.0/1.0	0	5	5	5	5
0.1/1.0	0	5	5	5	5
1.0/0.1	0	5	4	4	4

c) Fifteen week old cultures.- The results of our observations after 15 weeks are shown in Table 8. Development of lichens in the dual treatment flasks and in the 1.0 mg/l kinetin treatment group was generally very advanced. The squamules in these cultures resembled those of the natural lichen, i.e., they were attached by one end to the substrate and were generally erect, with a uniform gray-green color. Juvenile podetia and apothecia were present, the latter

their surface had a marbled appearance due probably to an incomplete cortical layer.

Table 8. Morphometric characteristics of natural and 15--week-old synthetic C. cristatella. Hormone treatments are expressed as mg/l with IAA represented by the first figure and kinetin by the second. Where 0/0 appears treatment consisted only of sterile distilled water. All measurements were taken from 5 cultures for each treatment and for natural and controls. con. = number contaminated; sq. = number with erect, foliose, lobed squamules; sq. sz. = average size squamules in mm (approximately 10 measurements per culture); pod. = average number podetia; ht. pod. = average height podetia in mm; apo. sz. = average size apothecia in mm.

Hormone treatment	con.	sq.	sq. sz.	pod.	ht. pod.	apo. sz.
None (natural)	-	5	1.3	10.2	2.8	0.9
0/0 (controls)	4	1	0.14	12.8	0.64	0.16
0.1/0	4	3	0.34	9.4	0.88	0.24
1.0/0	5	4	0.26	8.8	0.76	0.30
0/0.1	5	1	0.20	3.8	0.18	0.14
0/1.0	0	5	0.56	14.2	0.90	0.42
0.1/0.1	0	4	0.44	27.0	0.74	0.26
1.0/1.0	0	5	0.76	11.4	0.84	0.42
0.1/1.0	1	5	0.60	17.4	0.74	0.44
1.0/0.1	0	4	0.58	18.8	0.88	0.28

Single classification analyses of variance were performed on squamule size (Fig. 5), apothecium size (Fig. 6), podetium number (Fig. 7) and podetium height. Data from the natural lichen was included only in the analy-

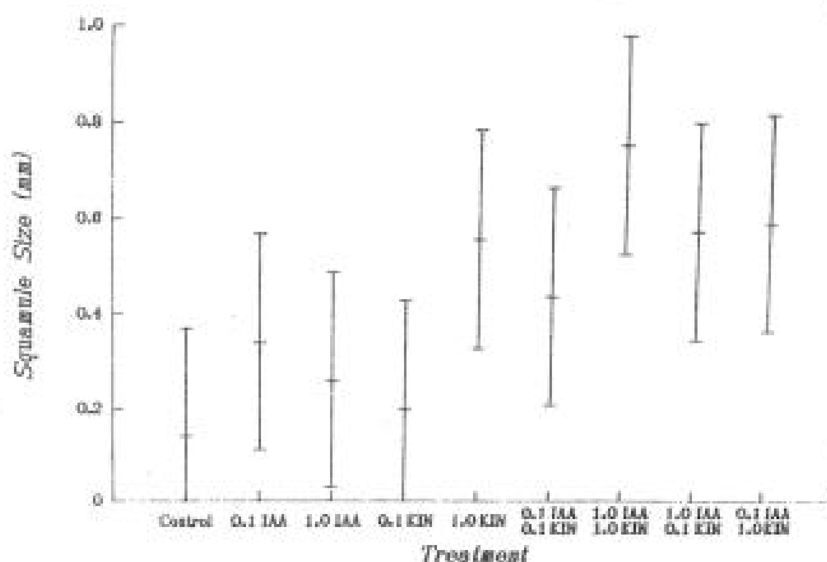

Fig. 5. 95% comparison intervals by the T-method for the means of squamule size data calculated according to methods in Sokal and Rohlf (1981). Means whose intervals do not overlap are significantly different. Treatments = mg/l concentrations of IAA and kinetin.

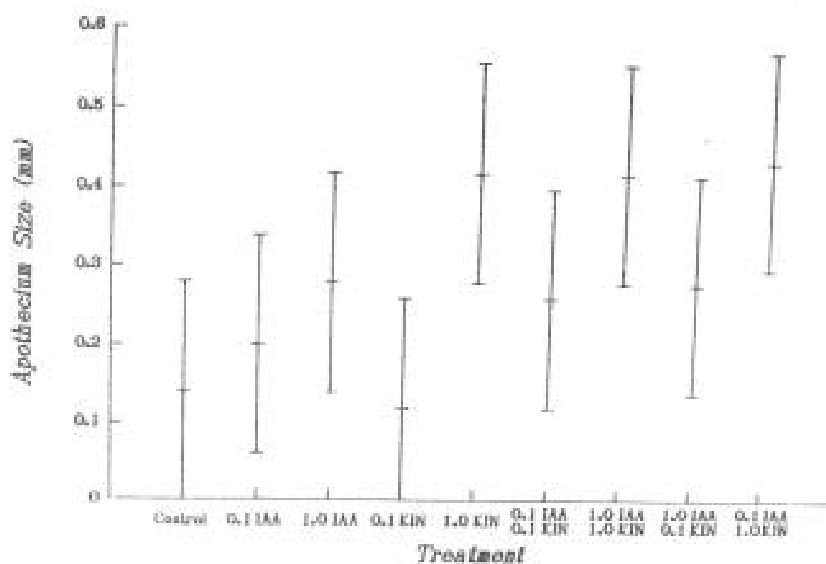

Fig. 6. 95% comparison intervals by the T-method for the means of apothecium size data calculted according to methods in Sokal and Rohlf (1981). Means whose intervals do not overlap are significantly different.

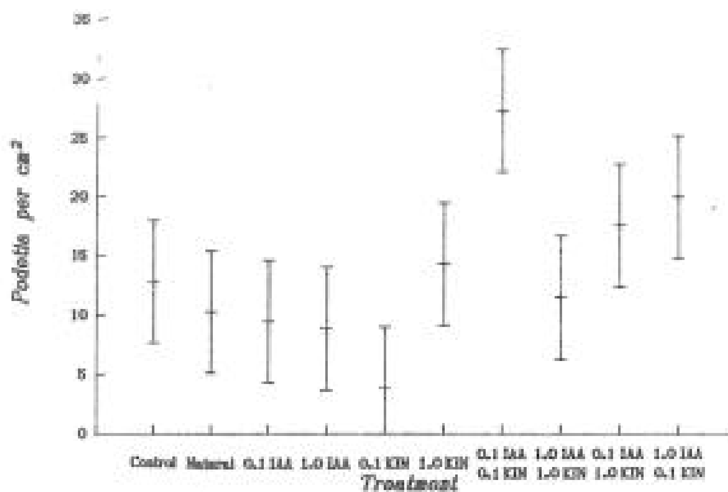

Fig. 7. 95% comparison intervals by the T-method for the means of podetium number data calculated according to methods in Sokal and Rohlf (1981). Means whose intervals do not overlap are significantly different. Treatments = mg/l concentrations of IAA and kinetin.

li that differences due to treatment alone would have been obscured.

Squamule size increased significantly ($F = 4.58$, $p < 0.01$) in lichens treated with a combination of 1.0 mg/l IAA with 1.0 mg/l kinetin and with a combination of 0.1 mg/l IAA with 1.0 mg/l kinetin. Apothecium diameter increased significantly ($F = 4.26$, $p < 0.01$) in lichens treated with a combination of 0.1 mg/l IAA with 1.0 mg/l kinetin. Podetium number increased significantly ($F = 4.55$, $p < 0.01$) in lichens treated with a combination of 0.1 mg/l IAA with 0.1 mg/l kinetin. No significant differences ($F = 1.36$, $p > 0.05$) were detected in podetium height. All comparisons were made to control.

8. Thin layer chromatography.- usnic acid was not detected

Discussion.

While our study has not proven the existence of hormones in lichens, it has, by demonstrating the effects of two hormones on the development of a synthetic lichen and on its symbionts, suggested possible roles for hormones within the lichen symbiosis.

Symbiotic systems, in order to be successful and to allow the partners to coexist, need to maintain a delicate balance between exploitation and destruction. Any long-lived association such as lichens, some of which have estimated ages of up to 1000 and 4500 years (Ahmadjian, 1967), must have evolved mechanisms to ensure their survival. Neither partner in a lichen benefits from rapid uncontrolled growth since this leads to overgrowth of one partner by the other and loss of the lichen habit. Similarly, inhibition of one partner in favor of the other leads to a loss of the lichen habit. Since lichens can withstand conditions that would be lethal to either partner alone, long term survival depends on the symbionts not responding individually to local conditions.

Several investigators have speculated on the presence of some regulatory process in lichens. Ahmadjian and Jacobs (1983) viewed lichens as a system of "controlled parasitism". Leonian (1936) felt that the algae produced "growth and reproduction-inducing" substances important in controlling the lichen associations and Giles (1970) postulated the existence of a system regulating growth of the symbionts of lichens.

Pearson (1970) felt that all of the control necessary to maintain the lichen habit, i.e. supression of each partner from time in order to prevent it from overgrowing and destroying the other partner and thus itself, could come from daily fluctuations in climate alone. He devised a set of experiments varying light, temperature and humidity and correlated the different conditions with changes in morphological appearance and physiological behavior of the lichen. Unfortunately, Pearson conducted his studies on natural lichens gathered from different areas over a period of several

months. The differences he observed, therefore, could have due to the presence of bacteria or other contaminant organisms in the lichen. Additionally, since the fungal and algal partners in natural lichens presumably are not clonal, his environmental regimes could have selected for different strains and the lichen at the end of his experiments might have been genetically different from the original specimens. To date, there is no evidence that lichen associations are maintained by environmental conditions alone.

The results of our research have shown that the phytohormones IAA and kinetin affect the growth and morphology of the fungal and algal partners of the lichen C. cristatella and also affect the success of lichen synthesis. The effects depend on the concentration of hormones used and whether the hormones are used alone or in combination. In general, the yield experiments showed that high concentrations of hormones inhibited while low concentrations stimulated algal growth. This agrees with results from several other researchers who studied the effects of hormones on a variety of algal, fungal, and plant species, e.g. Zehner (1949), Kefford and Goldacre (1961), Slankis (1973), Arendarchuk (1974) and Provasoli and Carlucci (1974).

Interactive effects of hormones also were discovered during the course of the yield experiment. We found that the addition of kinetin to either algal or fungal cultures growing in normally inhibitory levels of IAA led to a reduction of the inhibition. Skoog and Miller (1957) found the same results during their work with tobacco callus and concluded that such interactions represented a mechanism for the regulation of all types of growth from cell enlargement to organ formation. The significance of this possible regulatory mechanism in lichens could be considerable.

The most striking result from our studies on lichen symbionts was the effect of hormone treatments on algal growth habit. During the yield experiment we noticed that in some flasks the algae appeared to be clumped rhater than dispersed in the medium. Since this occurred only in a small percentage of the total number of flasks, we assumed it to

characteristics not present in the controls were found in the treatment groups: high aplanospore production, clumped sheet-like growth form, and a heavy gelatinous matrix around the algal cells. High aplanospore production agrees with the results of Giles (1970). Aplanospores, unlike zoospores, do not disperse, an obvious advantage in a lichen thallus where an intact algal layer results in maximum photosynthesis and possibly more efficient nutrient exchange.

The presence of mucilage around the hormone treated algal cells has significance to algal receptivity to lichen fungi. Ahmadjian and Jacobs (1983) stated that the gelatinous matrix around algal cells appeared to bind fungal hyphae in early interactions between the symbionts. Possibly, the more extensive the matrix the easier it would be for the fungus to maintain physical contact prior to actual lichenization. The heavy mucilage coat also may bind algal cells together, which would explain the clumped growth seen in our treatment flasks. Numerous aplanospores would further encourage clumping since they too would be held within the mucilage layers. In this experiment, hormone treatment mimicked the effects of lichenization since algal growth and morphology were more typical of the kind found within the lichen thallus than that found generally in liquid media cultures.

The most surprising result of this study was the high degree of differentiation found in many of the treated synthetic lichen cultures. Synthetic lichens which were treated with combinations of hormones were strikingly similar to natural lichens with respect to squamule morphology and the production of podetia and juvenile apothecia. One difference, however, was the lack of usnic acid in the synthetic lichens, a finding consistent with the earlier ones (Culberson et al., 1983).

The origin of hormones inside the thallus is not known. There are several possibilities, e.g. hormones may be produced by the alga, the fungus, or by bacteria within the thallus. With regard to auxin, it is possible that the alga produces tryptophan which fungus converts into auxin.

b. inhibition of fungal growth; c. stimulation or inhibition of algal growth; d. stimulation of the algae to produce an extracellular matrix; e. increased permeability of algal cells; f. stimulation of aplanospore formation.

It is possible that cytokinins also could affect natural lichens in a number of ways, e.g. a. enhancement of chloroplast development; b. regulation of nutrient translocation; c. reduction of the inhibitory effect of auxin; d. stimulation of algal division; e. delay of algal senescence.

The answer to the question regarding control mechanisms in lichens is complex. Lichens are ancient symbiosis and we feel that they may have evolved a complex set of adaptations. From a purely hypothetical point of view, lichens may have originated from pathogenic associations in which fungi and bacteria parasitized and killed algal cells. The chance production of auxin by some pathogens would give them an advantage over others since the auxin would weaken the algal cell wall and mobilize nutrients to the area of contact between parasite and host. This would make the exploitation of the food source faster and more efficient. Cytokinins, also produced by the pathogens, would stimulate chloroplast development, increase the life span of the algae and protect the algae from further infection by promoting the synthesis of phenolic compounds, i.e. "lichen acids". Thus, the pathogens would be simultaneously enhancing, maintaining, and protecting their food source. However, before the fungus and bacteria could take full advantage of this potentially limitless food source, the parasitic nature of the association would have to be controlled. Something was necessary to prevent the pathogens from killing the algae and here the dual interactive nature of the hormones would come into effect. Auxin, produced by both algae and bacteria could inhibit fungal development perhaps by limiting the number of haustoria penetrating each algal cell. The presence of cytokinins would keep the inhibition under control so that the fungal growth was not completely prevented. We feel that it is the ability of the algae to resist fungal attacks that leads to the lichen habit.

tion for the failure of synthetic lichens to develop into fully functional counterparts of natural lichens. Early interactions between symbionts in synthesis experiments seem relatively normal with the fungus and the alga responding to each other by first forming presquamules and then squamules. After this initial algal-fungal encounter, however, considerable differentiation has to occur, such as the formation of a complex thallus with fruits and often asexual propagules. It is at this stage of development that we feel the absence of bacteria might be significant. Research has shown that when hormones are used to mimic the effects of symbiosis in a single symbiont, for example, in plants forming mycorrhizal roots, the hormones must be present continuously in order to maintain the effects. In synthetic lichens, kinetin would initially be present from the residue of the malt--yeast extract medium in which the mycobiont is grown. Kinetin would most likely be depleted after several weeks and the requirement for the hormone would still be present. We believe that bacteria play a role in the production of hormones in lichen thalli and their absence in synthetic lichens would explain why the latter do not develop to maturity in axenic cultures.

Our research has shown that the regulation of and by lichen symbionts through hormone action is a reasonable hypothesis. Giles (1970) stated that the "morphology of lichen thallus is maintained by the balance between the growth rates of the two partners and any factor influencing the growth rate of either partner must be responded to by other in order to maintain the lichen habit". Thus, far the details of this complex system of communication remain unknown. If, as our results suggest, the rate of lichenization and/or the morphology of lichens can be altered by varying hormones and their concentrations, we may be closer to understanding the mechanisms which permit the formation and maintenance of the symbiotic equilibrium in lichens and perhaps, by analogy, in other symbiotic systems.

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