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EFFECTS OF CERTAIN PLANTS OF OLD-FIELD SUCCESSION

ON THE GROWTH OF BLUE-GREEN ALGAE

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CHAPTER I

INTRODUCTION

Booth (1941a) reported that succession in abandoned
fields in Oklahoma and Kansas includes four stages: (1) weed
stage lasting for two or three years, (2) annual grass stage
of nine to thirteen years dominated by Aristida oligantha,[1]
(3) perennial bunchgrass stage persisting for thirty or more
years dominated by Andropogon scoparius, and (4) the climax
prairie.

Considerable evidence has accumulated indicating that
several plants of the first stage eliminate plants of that
stage through the production of chemical inhibitors, and
that Aristida oligantha invades because it is not inhibited
by the same toxins and is able to survive under the condi-
tions existing at the time (Abdul-Wahab and Rice 1967,
Brown 1968, Olmsted 1967, Wilson 1968). Muller (1966) and
Rice (1967) gave excellent reviews of investigations con-
cerning the roles of inhibitors in succession.

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[1]Nomenclature of vascular plants follows Waterfall
(1966) unless authority is given.

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Rice, Penfound and Rohrbaugh (1960) found that the order in which certain plants invade abandoned fields from Stage 2 to the climax is related to the increasing nitrogen and phosphate requirements of those plants. The third successional stage apparently invades only after soil phosphate and nitrogen have increased to adequate levels (Rice et al. 1960). In this respect nitrogen-fixation would be one very important factor affecting the rate of succession in abandoned fields.

Stewart (1966) indicated that several groups of micro-organisms have been shown to be capable of nitrogen-fixation. Biological sources of soil nitrogen in abandoned fields in Oklahoma include nitrogen-fixation by free-living bacteria, fungi, and blue-green algae, and nitrogen-fixation by the symbiotic Rhizobium-legume complex.

Rice (1964, 1965b, 1965c) found several low nitrogen requiring pioneer plant species of revegetating old-fields which inhibit nitrogen-fixing bacteria such as Azotobacter and Rhizobium. In addition he found that root exudates from three grass and three forb species from the first two stages of old-field succession inhibited nodulation of legumes heavily inoculated with Rhizobium (Rice 1968). Decaying material (1g in 454g of soil) from the same forbs was shown also to be inhibitory to the nodulation of legumes. Rice and co-workers isolated and identified several growth-inhibitory compounds such as p-hydroxybenzaldehyde,

chlorogenic acid, isochlorogenic acid, gallic acid, gallo-
tannic acid, p-coumaric acid, etc. from inhibitory seed
plants of the first and second successional stages. Most
of these compounds in very dilute solutions were shown to
be inhibitory to the growth of nitrogen-fixing bacteria.

Because of the information presented above, I sus-
pected that the low nitrogen requiring plants of the first
and second stages might inhibit the nitrogen-fixing soil
algae, thereby increasing the time in which the two first
successional stages could exist in abandoned fields.

Shields and Durrell (1964) reviewed the literature on
soil algae and indicated the importance and high frequency
of blue-green algae on prairie and desert soils of Oklahoma
and the southwestern United States. Booth (1941b) reported
that blue-green algae, primarily Nostoc which is an impor-
tant nitrogen-fixing genus, may form a 32% cover between
bunches of grass in the third stage of succession.

Many papers have been published concerning the allelo-
pathic effects of seed plants on other seed plants, but
there are few papers on the allelopathic effects of seed
plants on soil algae. Livingston (1905) observed the
effect of bog waters on the growth of Stigeoclonium sp.,
and he may have been the first to note allelopathic effects
of seed plants on algal growth. Katznelson (1946) observed
that Beta vulgaris L. (mangel) had a stimulatory effect on
the growth of soil algae rather than an allelopathic effect;

other workers noted this stimulatory effect for several
crop plants (De and Sulaiman 1950, Shtina 1957, 1960).
Shtina (1960) noted that the rhizosphere of diseased plants
had fewer algae than soil distant from the roots. Gonzalves
and Yalavigi (1959) working with cotton, sorghum, and wheat
reported: (1) the number of algal species was greater in
rhizospheres than in control soils, (2) the "rhizosphere
effect" varied with the crop plant, i.e., in addition to
the permanent algal inhabitants of the soil, different
species of algae were associated with each crop plant, and
(3) the plant seemed to be more important than the degree
of fertility of the soil in determining the number of algal
species in the rhizosphere. The data from Gonzalves and
Yalavigi seem to indicate that the plant is creating a
unique and very localized environment which acts selec-
tively upon the growth of soil algae. If such selective
growth occurs in the rhizosphere of a particular native
species, that species when present as a dominant would have
an important ecological role in succession. Finally,
Burges (1965) and Katznelson (1965) indicated that very
little is understood concerning the distribution and eco-
logical role of soil algae.

Appropriate experiments were designed to test my
hypothesis, using eight species of plants which occur in
various stages of old-field succession. Care was taken to
eliminate factors of direct plant-plant competition as
completely as possible.

CHAPTER II

MATERIALS AND METHODS

Soil samples, minus litter, were collected immediately adjacent to eight species of seed plants (Table 1) in revegetating old-fields and undisturbed tall grass prairie near Norman, Oklahoma in May and September 1967. These species were selected to represent different stages of old-field succession and to test their possible inhibitory effect on the growth of blue-green algae. Several of the species were shown previously to be inhibitory to other seed plants, to nitrogen-fixing bacteria or to both. Ten soil samples were collected from the top one-half inch level and from sites where where each species of seed plant was growing in relatively pure stands.

After the soil samples were thoroughly mixed, one gram of soil was used to inoculate flasks which contained 10 ml of Modified Bristol's Solution (Bold 1949). Generally, a series of ten soil cultures was prepared for each soil sample. The cultures were incubated in a growth chamber and maintained during a sixteen hour photo-period at 27-28°C and 400-500 foot-candles light intensity and at 21°C during an eight hour dark period. The soil samples

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were incubated for two weeks after which each culture was carefully examined to determine the soil algae present. At the end of the third week a chlorophyll extraction procedure (Richards and Thompson 1952, Odum, McConnell and Abbott 1958) was used to quantify the algal growth in each culture. The procedure allows quantification of chlorophyll a by converting absorbance at 665 mµ (absorbance peak for chlorophyll a) into units of mg per liter. A Bausch and Lomb Spectronic 20 was used to determine absorbance values. Initially, chlorophyll and any other material in the soil which had an optical density near that of chlorophyll was extracted from one gram of each soil sample. These initial extractions served as blanks so that the change in absorbance after the three week period would essentially represent algal growth.

Plants of the test species were collected in June 1967 from the same fields in which soil samples were collected. These were dried and finely ground after being separated into roots, leaves, stems, etc. This dried material was inoculated into unialgal cultures to test its effect on growth of blue-green algae.

Axenic cultures of Lyngbya sp. (488) and Anabaena sp. (B380) were obtained from the Indiana Culture Collection. These were used in bioassays to determine the effects of decomposing plant parts, leachates, exudates, and certain phenolic compounds on their growth.

CHAPTER III

EXPERIMENTATION AND DISCUSSION

Effects of Rhizosphere Soils on Algal Growth

Soil samples were collected in May and September 1967 immediately adjacent to the stems of eight species of seed plants which occur in various stages of old-field succession. The soil samples were inoculated into 50 ml Erlenmeyer flasks and incubated for a two week period. At the end of this period each culture (10 cultures per seed plant soil sample) was analyzed for the algae present. Algal identification which follows Smith (1950) and Prescott (1964) was determined to the generic level (Table 1). Genera of the Cyanophyta were found to be more common in the cultures than genera of Chlorophyta or Chrysophyta. Several genera of possible nitrogen-fixers (Stewart 1966) were found growing in most of the cultures. Soil from around Erigeron canadensis L., Ambrosia psilostachya, Sorghum halepense, Helianthus annuus, Chenopodium album, and Rhus glabra (all members of the weed stage except R. glabra) and Aristida oligantha (the dominant of Stage 2) supported a relatively poor level of growth of blue-green algae. The rhizosphere sample of Andropogon

Table 1. Algal genera found in culture with rhizosphere (surface) soil after a two week period with principal species indicated by parentheses.

Genera	Andropogon scoparius	Erigeron canadensis L.	Aristida oligantha	Ambrosia psilostachya	Sorghum halepense	Helianthus annuus	Chenopodium album	Rhus glabra
Oscillatoria	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Lyngbya	X	X	X	X	X	X	(X)	X
Chroococcus	X	X	X	X	X	X		
Gloeocapsa	X	X	X		X	X		
*Anabaena	X	(X)	X	X		X		
*Nostoc	(X)	(X)	(X)	(X)	(X)	(X)	X	X
*Tolypothrix	X	X		X	X			
*Scytonema	(X)	X	(X)	X	X	X		
*Cylindrospermum	X			X	X	X		
*Schizothrix	(X)	X	(X)	X	X	X	X	
Microchaete		X	X	X	X	X		
Hydrocoleum	X	X	X	X				
Gloeotheca						X		
Chlorella-like	X	(X)	X	X	X	X	(X)	(X)
Chlorococcum-like	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Chlamydomonas-like	X	(X)	X	X	X	(X)	X	(X)
Hormidium		X	X	X	X	X	X	(X)
Ulothrix	X	X	X			X	X		X
Zygnema		X				X		
Monocilia	X	X	X	X	X			X
Botrydium		X		X		X	X	X
diatoms	X	X	X	X	X	X	(X)	X

* Possible nitrogen-fixers (Stewart 1966).

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scoparius, a member of the climax prairie, supported the greatest growth of blue-green algae and nitrogen-fixing genera. The extremely reduced number of algal species which occurred in soil obtained near C. album and R. glabra was especially striking. In contrast, it appeared that growth of members of the Chlorophyta was unaffected or perhaps even stimulated by the soils taken near C. album and R. glabra. Virtually the same qualitative results were obtained in May and September.

A chlorophyll extraction technique was used to obtain estimates of quantitative algal growth during the culture period (Richards and Thompson 1952, Odum et al. 1958). The algal cultures were filtered from the nutrient solution with No. 2 Whatman filter paper, ground with mortar and pestle, and the filter paper and residue were placed in a 90% acetone solution and refrigerated for 24 hours. The solutions were then centrifuged for three minutes after which the optical density or absorbance was determined at 665 mμ (absorption peak for chlorophyll a). Optical density was converted to mg/liter by multiplying absorbance values by a factor of 13.4 (Odum et al. 1958). No other conversions were necessary for statistical purposes since concentration of chlorophyll a, at least within the range concerned here, is a linear function of its absorbance (Odum et al. 1958, McConnell and Sigler 1959). Several concentrations of chlorophyll-acetone solutions were

checked and the linear relationship was consistent.

Soil samples contained not only algae and plant litter, but also organic and inorganic compounds which result in slight optical densities at 665 mμ. Therefore, optical densities at 665 mμ were determined for duplicate soil samples at the beginning of incubation. At the end of the three week period the same cultures, for which algal identification was determined, were run through the chlorophyll extraction process. The absorbance values for the initial soil samples were subtracted from the absorbance values of the three week cultures. The difference represented the change in chlorophyll a content and, therefore, the total algal growth over a three week period. Ten cultures were grown for each seed plant soil sample, so that the data could be subjected to statistical analysis.

The soil taken near C. album in the spring had a significantly lower growth of algae than any of the other soil samples (Table 2). Also, values for H. annuus and A. psilostachya were significantly lower than the value for A. oligantha. The greatest algal growth was found in soil near A. oligantha, and the least in soil near C. album.

The same experiment was repeated in September 1967 to determine if the effect of soil on the growth of algae had changed through the summer season. There were no statistically significant differences in the values of algal growth for the fall soil samples. However, there were statistically

Table 2. Effects of rhizosphere soil on algal growth as
          determined by changes in chlorophyll a content.

Source of Rhizosphere Soil	Change in chlorophyll a, mg/l	
	Spring	Fall
Aristida oligantha	15.33	8.23*
Rhus glabra	12.76	8.65
Sorghum halepense	12.62	12.29
Andropogon scoparius	12.40	10.85
Erigeron canadensis L.	12.03	11.42
Helianthus annuus	9.41	10.72
Ambrosia psilostachya	8.11	9.97
Chenopodium album	4.67	10.62*

* Difference from spring value significant below 0.05
  level (Anova test).

significant differences in algal growth between spring and fall samples taken near C. album and A. oligantha. Samples from the vicinity of C. album demonstrated a significant increase in algal growth, while soil from the vicinity of A. oligantha showed a significant decrease. Aristida oligantha forms a distinct, relatively long-lived successional stage, and therefore the pronounced effect of its rhizosphere on the growth of soil algae is probably very important in slowing the rate of succession.

Effects on Algal Growth of Surface Soil Obtained Near Helianthus annuus

Soil samples were collected at intervals of one foot for a distance of three feet on the north and south sides of H. annuus stems which were growing on bare areas of a recently plowed field. As in the preceding experiment, cultures of the soil samples and blanks of the soil were quantified by the chlorophyll extraction technique. After incubation for two weeks the algal genera were determined (Table 3). The nitrogen-fixing genera, Anabaena, Nostoc, and Schizothrix increased with increase in distance from the plants. Non-nitrogen-fixing genera of the Cyanophyta and representatives of the Chlorophyta did not show this distributional pattern.

Chlorophyll extraction was performed after three weeks of incubation and it indicated that there was virtually no difference in algal growth on the north and south

Table 3. Algal genera found in surface soil samples taken at various distances from Helianthus annuus stems with principal species indicated by parentheses.

Genera	0'	1'	2'	3'
Oscillatoria	(X)	(X)	(X)	(X)
Lyngbya	(X)	X	(X)	X̄
Gloeotheca	X	X	X	X
Gloeocapsa	X	X	X	X
*Anabaena	X	X	X	X
*Nostoc	X	X	(X)	(X)
*Schizothrix		X	X	X
Chlorella-like	(X)	(X)	(X)	(X)
Chlorococcum-like	(X)	(X)	(X)	(X)
Chlamydomonas-like	X	X	X	X
Hormidium	X	X	X	X
Ulothrix	X	X	X	X
diatoms	X	X	X	X
Botrydium	X	X	X	X

* Possible nitrogen-fixers (Stewart 1966).

sides of the plant (Table 4). There was an increase of algal growth with each increase in distance from the stems of H. annuus. Thus, the evidence indicated that H. annuus inhibited algal growth, especially of the nitrogen-fixing algae.

Effects of Decomposing Plant Material

Two-tenths of a gram of ground plant material was placed into 15 ml of Modified Bristol's Solution. This mixture was steamed in an autoclave for five minutes at 100°C. This precaution against aerial algal contamination was carried out in all experiments which involved unialgal cultures. A 0.5 ml aliquot of a well dispersed unialgal culture of Lyngbya sp. (Indiana Culture Coll. No. 488) was inoculated into each of the test solutions. Ten control cultures which contained no plant material were prepared. The cultures were incubated for ten days after which they were harvested and analyzed for growth by the chlorophyll extraction procedure. Blanks for the dried plant material were obtained and subtracted from the total chlorophyll a content of the harvested material. Some plant parts significantly stimulated growth of Lyngbya, others significantly inhibited growth, and others had no effect (Table 5). All test plants except Andropogon scoparius, a member of the climax prairie, possessed at least one plant part which was inhibitory to algal growth.

Table 4. Effects of soil samples taken at various distances
from Helianthus annuus on growth of soil algae as
determined by change in chlorophyll a content.

Distance from	Change in chlorophyll a, mg/l		
H. annuus, ft.	North	South	Average
0	9.84	11.51	10.54*
1	15.30	20.62	17.96
2	18.65	19.48	19.07
3	22.24	23.21	22.73
Average	16.44	18.71	

* Differences from amounts at other distances significant
below 0.05 level (Anova test).

Table 5. Effects of 0.2 gram dried plant parts of various seed plants on growth of Lyngbya sp. (Indiana Culture Collection #488).*

Helianthus annuus	Rhus glabra	Erigeron canadensis	Ambrosia psilostachya	Sorghum halepense	Chenopodium album	Aristida oligantha	Andropogon scoparius
				inflores. roots	leaves roots	leaves & stems	roots
control	control	control stems	stems control	control	control	control	control leaves & stems
leaves roots stems	leaves stems roots & rhizomes	leaves roots	leaves roots & rhizomes	leaves rhizomes stems	stems	roots	

* Plant parts listed above upper line significantly stimulated growth compared with the control; those below the bottom dashed line significantly reduced growth (Anova test).

A similar experiment was conducted involving Anabaena sp.
(Indiana Culture Coll. No. B380), a nitrogen-fixing genus.
Results were somewhat similar to those with Lyngbya (Table 6).
At least one plant part of all species significantly inhibited
growth of Anabaena, including A. scorarius. The inhibition
of this nitrogen-fixing alga by the roots of A. scoparius
was especially interesting, and may help explain in part
why the bunch grass stage of old-field succession dominated
by Andropogon scoparius remains for such a long time.

The experiment with Anabaena was repeated using 0.1
gram plant material in the test solutions. The 50% reduc-
tion in amount of plant material caused many plant parts to
lose their inhibitory activity (Table 7). However, the
leaves of H. annuus and A. psilostachya, the stems of
C. album, and all plant parts of R. glabra were still
inhibitory to growth of Anabaena. The remaining plant
parts, except the leaves of E. canadensis L., were stimula-
tory.

Inhibitory activity as found in field conditions
correlated very well with the striking effect of dried
material of Rhus glabra and Chenopodium album on the growth
of Anabaena.

Effects of Leaf Leachates

Seeds or rhizomes of the test plants which were used
in the above experiments were obtained from fields around
Norman, Oklahoma and were planted in pots filled with washed

Table 6.   Effects of 0.2 gram dried plant parts of various seed plants on growth of
          Anabaena sp. (Indiana Culture Collection #B380).*

Helianthus annuus	Rhus glabra	Erigeron canadensis	Ambrosia psilostachya	Sorghum halepense	Chenopodium album	Aristida oligantha	Andropogon scoparius
stems		stems	stems	inflores. roots leaves	leaves	leaves & stems	
control	control	control	control	control	control roots	control	control stems & leaves
leaves roots	leaves stems roots & rhizomes	leaves roots	leaves roots & rhizomes	stems rhizomes	stems	roots	roots

* Plant parts listed above upper line significantly stimulated growth compared with
the control; those below the bottom dashed line significantly reduced growth
(Anova test).

Table 7. Effects of 0.1 gram dried plant parts of various seed plants on growth of
Anabaena sp. (B380).*

Helianthus annuus	Rhus glabra	Erigeron canadensis	Ambrosia psilostachya	Sorghum halepense	Chenopodium album	Aristida oligantha	Andropogon scoparius
stems roots		stems roots	stems roots	inflores. rhizomes leaves stems roots	leaves roots	leaves & stems roots	leaves & stems roots
control	control	leaves control	control	control	control	control	control
leaves	leaves roots stems		leaves		stems		

* Plant parts listed above upper line significantly stimulated growth compared with
the control; those below the bottom dashed line significantly reduced growth
(Anova test).

quartz sand. The seed plants were allowed to grow under greenhouse conditions temporarily, after which they were kept in a growth chamber. The growth chamber was kept on a 16 hour photoperiod at 30°C. with an intensity of approximately 1300 foot-candles. The temperature during the eight hour dark period was 20°C. After the plants were kept in the growth chamber for one month, an artificial rain consisting of a 50% Modified Bristol's Solution, was sprayed over the leaves by means of an atomizer. The artificial rain was collected on a polyethylene surface as it dropped from the leaves and was allowed to drain into a collecting bottle. Leaf leachate was collected from each test species and placed in a series of ten flasks, 15 ml per flask. Each flask was inoculated with a 0.5 ml aliquot of a well dispersed suspension of Lyngbya. Controls were set up with 15 ml each of 50% Modified Bristol's Solution and the same amount of inoculum. The cultures were incubated for seven days after which they were quantified by the chlorophyll extraction technique. Only the leachate of E. canadensis L. inhibited the growth of Lyngbya (Table 8). The leachates of C. album, S. halepense, H. annuus, and Aristida oligantha were stimulatory to the growth of this alga.

A similar experiment was conducted with Anabaena except harvesting was done ten days after inoculation. The leaf leachate of Andropogon scoparius was the only one which inhibited growth of Anabaena significantly (Table 8).

Table 8.   Effects of leaf leachates on growth of two
species of algae.

	Chlorophyll a, mg/l	
	Lyngbya	Anabaena
Chenopodium album	2.002*	0.749
Sorghum halepense	1.532*	0.646
Helianthus annuus	1.442*	1.240*
Aristida oligantha	1.437*	0.563
Rhus glabra	1.258	0.927*
Andropogon scoparius	1.211	0.442*
Ambrosia psilostachya	1.208	0.871*
Erigeron canadensis L.	0.814*	0.559
Controls	1.179	0.563

* Difference from appropriate control significant below
0.05 level (Anova test).

In contrast, the leachates of H. annuus, R. glabra, and Ambrosia psilostachya stimulated growth. The only leachate which was stimulatory to both taxa of algae was that of Helianthus annuus.

Effects of Root Exudates

The same plants which were grown under growth chamber conditions were used to determine the effects of root exudates on algal growth. An experimental set-up designed by Abdul-Wahab (1967) was utilized for this experiment. It consisted of a train of nine vials which received root exudate for four hours each day during a three week period. A Modified Bristol's Solution was allowed to drop from a reservoir into a pot in which a test plant was rooted. The nutrient solution containing the exudate passed through the vials by gravity flow into a terminal reservoir where it was pumped back to the initial reservoir. Each day distilled water was added to bring the reservoir up to volume, except that nutrient solution was added every third day. The experiment was conducted under the same growth chamber conditions listed in the preceding section. Controls were run similarly using pots of sand devoid of any seed plants.

Two soil cultures which were used as inocula for the vials were maintained over the span of the experiment. The two soils represented in these cultures were obtained from a tall grass prairie plot and from a revegetating field. Each of the vials was inoculated with uniform

pieces of algal mats from each soil culture.

After three weeks the algae in the vials were identi-
fied and a chlorophyll a determination was made. The
following genera were found in most of the vials regard-
less of the source of the exudate: Nostoc, Anabaena,
Oscillatoria, Lyngbya, Chroococcus, Chlamydomonas-like spp.,
Chorella-like spp., and Chlorococcum-like spp. Schizothrix,
Cylindrospermum, Scytonema, Ulothrix, and Hormidium occurred
sporadically. A definite pattern of distribution with
regard to occurrence of genera was not apparent, but the
predominance of a particular genus over other genera was
striking in some cases. In addition, the ratio of green
algae to blue-green algae and the ratio of nitrogen-fixing
genera to total algal growth was of significance. Since
it was difficult to estimate growth of each genus, only
presence, absence, or relative prominence of the various
genera was noted. An arbitrary value of one was given to
the genus if it was present, and a value of two was given
if a genus was a principal one in the vial. These values
for the nine vials were averaged and percentage values
were established for total blue-green algae, green algae,
and nitrogen-fixing genera (Table 9). The exudate of
Andropogon scoparius was the only one which resulted in a
greater growth of blue-green algae than in the controls.
The other exudates caused only a slight decrease in growth
of the blue-green algae except for the exudate of R. glabra

Table 9. Effects of seed plant exudates on growth of
          algae from a soil inoculum over a three week
          growth period.

	% blue-greens	% N_2-fixers	Chlorophyll a, mg/l
Controls	63.7	13.7	6.27
Ambrosia psilostachya	62.6	21.4	5.65
Chenopodium album	62.2	17.9	8.74
Erigeron canadensis L.	60.9	23.0	6.93
Helianthus annuus	59.8	18.3	10.83*
Sorghum halepense	59.5	15.4	14.36*
Aristida oligantha	56.8	1.7	10.59*
Rhus glabra	38.3	0	25.72*
Andropogon scoparius	68.5	18.5	10.97*

* Difference from appropriate control significant below
  0.05 level (Anova test).

which was very inhibitory to their growth. In contrast, exudates of all test species stimulated growth of the nitrogen-fixing genera slightly, except for Aristida oligantha and R. glabra, which almost completely inhibited these organisms.

The vial cultures were analyzed for total algal growth by the chlorophyll extraction procedure, and the exudate of Ambrosia psilostachya was the only exudate to cause less total growth than the controls. The difference was not statistically significant (Table 9). The exudates of H. annuus, S. halepense, A. oligantha, R. glabra, and Andropogon scoparius caused statistically significant increases in total algal growth over that of the controls. It was especially interesting to note that R. glabra and Aristida oligantha were significantly stimulatory to the growth of green algae, although they were very inhibitory to blue-green nitrogen-fixing genera.

Root exudates which were collected from the same species of seed plants grown in growth chamber conditions as described in the preceding experiment, were tested against growth of unialgal cultures of Lyngbya and Anabaena. After the plants had been watered for five days with distilled water, a 50% Modified Bristol's Solution was allowed to slowly flow by gravity through the root mass until an adequate supply of exudate was obtained from each species. The exudate of each seed plant was placed in a series of

ten flasks, 15 ml per flask. Each flask was inoculated
with a 0.5 ml aliquot of a well dispersed suspension of
Lyngbya. Controls were set up with 15 ml of 50% Modified
Bristol's Solution per flask and the same amount of in-
oculum. The cultures were incubated for an appropriate
amount of time after which they were harvested for chloro-
phyll extraction. The exudate of A. psilostachya, A. sco-
parius, and C. album inhibited the growth of Lyngbya
(Table 10). All other exudates were stimulatory to the
growth of this alga. The root exudates of C. album and
H. annuus were inhibitory to Anabaena, whereas all other
root exudates except E. canadensis L. were stimulatory to
growth of Anabaena (Table 10).

Effects of Certain Phenolic Compounds

Eight phenolic compounds, previously found to be pro-
duced by plants involved in this study and to be inhibitory
to seed plants and bacteria (Rice 1965a, 1965b, 1965c,
Abdul-Wahab and Rice 1967, Olmsted 1967, Wilson 1968), were
tested against pure cultures of Lyngbya and Anabaena.

Aqueous solutions of the phenolics were made up in
concentrations of 10^{-3}M, 10^{-5}M, 10^{-7}M, and 10^{-9}M. Test
solutions were made by mixing the phenolic solutions and
Modified Bristol's Solution in a ratio of 2:1(v/v). After
addition of the nutrient solution, the actual concentration
of each phenolic solution was 0.66 of the original value.

Table 10. Effects of root exudates on growth of two
          species of algae.

	Chlorophyll a, mg/l	
	Lyngbya	Anabaena
Erigeron canadensis L.	1.771*	1.219
Rhus glabra	1.705*	2.435*
Aristida oligantha	1.526*	2.880*
Helianthus annuus	0.806*	0.000*
Sorghum halepense	0.799*	1.822*
Andropogon scoparius	0.443*	2.710*
Chenopodium album	0.236*	0.000*
Ambrosia psilostachya	0.196*	1.431*
Controls	0.661	1.066

* Difference from appropriate control significant below
  0.05 level (Anova test).

The control solutions were made by mixing distilled water and the nutrient solution in a ratio of 2:1. Ten cultures of Anabaena and ten of Lyngbya were run in the 0.66×10^{-3}M and 0.66×10^{-5}M concentration of each phenol and in the control solution. A 0.5 ml aliquot of a well dispersed suspension of each genus was used for each inoculum. In each instance in which a 0.66×10^{-5}M concentration of a phenol caused a statistically significant inhibition in growth of a particular alga, a 0.66×10^{-7}M concentration was subsequently tested. If this concentration inhibited growth significantly, a 0.66×10^{-9}M concentration was tested.

The cultures were incubated for seven days after which they were analyzed by the chlorophyll extraction technique. All phenolic compounds tested were inhibitory to the growth of Anabaena and Lyngbya in concentrations of 0.66×10^{-3}M (Tables 11, 12). Gallotannic acid was the only compound inhibitory to Lyngbya in a concentration of 0.66×10^{-5}M whereas chlorogenic acid, p-coumaric acid, gallic acid, alpha-naphthol, and gallotannic acid were significantly inhibitory to the growth of Anabaena at this concentration. Gallotannic acid was inhibitory to Anabaena even at the 0.66×10^{-7}M concentration.

These experiments indicated that Anabaena sp., the nitrogen-fixing species, was much more sensitive to some of the known phenolic inhibitors than Lyngbya. This information obtained from experiments with known phenolic

Table 11.   Effects of selected phenolics on the growth of
            Lyngbya sp.

	Chlorophyll a, mg/l		
	0.66×10^{-3}	0.66×10^{-5}	0.66×10^{-7}
p-hydroxybenzaldehyde	0.081*	1.230	-
chlorogenic acid	0.010*	1.321	-
isochlorogenic acid	0.027*	1.372	-
p-coumaric acid	0.001*	1.401	-
gallic acid	0.003*	1.393	-
gallotannic acid	0.000*	0.726*	1.501
alpha-naphthol	0.048*	1.469	-
scopoletin	0.003*	1.351	-
controls	0.526	1.241	1.505

* Difference from appropriate control significant below
  0.05 level (Anova test).

Table 12. Effects of selected phenolics on the growth of
Anabaena sp.

	Chlorophyll a, mg/l		
	0.66×10^{-3}	0.66×10^{-5}	0.66×10^{-7}
p-hydroxybenzaldehyde	0.001*	0.659	-
chlorogenic acid	0.000*	0.526*	1.041
isochlorogenic acid	0.000*	0.551	-
p-coumaric acid	0.017*	0.565*	1.165
gallic acid	0.005*	0.458*	1.108
gallotannic acid	0.008*	0.001*	0.473*
alpha-naphthol	0.060*	0.475*	1.474
scopoletin	0.002*	0.561	-
controls	1.952	0.721	0.920

* Difference from appropriate control significant below
0.05 level (Anova test).

compounds corroborated very well data which has been

obtained by other workers concerning the inhibitory

activity of the seed plants involved in this study.

CHAPTER IV

CONCLUSIONS

The causative factors involved in the long term
existence in old-field succession in Oklahoma and Kansas
of the annual grass stage dominated by <u>Aristida oligantha</u>
and the bunch grass stage dominated by <u>Andropogon scoparius</u>
have long been sought. The present study was undertaken
to ascertain the effects on the growth of soil algae of
certain seed plants from various stages of old-field suc-
cession. Special emphasis was placed on nitrogen-fixing
blue-green algae, since succession in old-fields beginning
with Stage 2, the annual grass stage, has been shown to
be correlated with nitrogen levels in the soil.

Soils from rhizospheres of plants of Stages 1 and 2
supported poor growth of nitrogen-fixing blue-green algae,
and the effect was especially striking with soils obtained
near <u>Rhus glabra</u> and <u>Chenopodium album</u>. On the other hand,
soils obtained near <u>Andropogon scoparius</u> appeared to be
stimulatory to such algae. The inhibitory effect of
<u>Helianthus annuus</u> was found to decrease with an increase
in distance from the base of the plants. Decomposing plant
material of several species was found to be inhibitory to

the growth of <u>Lyngbya</u> and <u>Anabaena</u>, and the inhibitory

activity was especially prominent with decomposing mate-

rials from plants of Stage 1.

Certain phenolic compounds previously found to be

produced by plants involved in this study and to be in-

hibitory to seed plants and nitrogen-fixing bacteria were

found to be inhibitory to the growth of <u>Lyngbya</u> and

<u>Anabaena</u>. <u>Anabaena</u> sp., a nitrogen-fixing species, was

found to be more sensitive to these compounds than <u>Lyngbya</u>

sp., a non-nitrogen-fixing species.

Leaf leachate from <u>Erigeron</u> <u>canadensis</u> L. was found

to be inhibitory to the growth of <u>Lyngbya</u>, and leaf leachate

from <u>Andropogon</u> <u>scoparius</u> was inhibitory to <u>Anabaena</u>.

Root exudates from <u>Aristida</u> <u>oligantha</u>, <u>Chenopodium</u> <u>album</u>,

<u>Helianthus</u> <u>annuus</u>, and <u>Rhus</u> <u>glabra</u> were very inhibitory

to growth of nitrogen-fixing blue-green algae, but were

stimulatory to the growth of green algae. The results

indicate that there are several methods by which inhibitors

of the soil algae can get out of the plants which produce

them. These methods apparently vary from one species to

another.

Certain weeds from Stage 1 of old-field succession

and <u>Aristida</u> <u>oligantha</u> from Stage 2 were inhibitory in

several ways to the nitrogen-fixing soil algae, whereas

<u>Andropogon</u> <u>scoparius</u> from Stage 3 and the climax was not

inhibitory in most tests. These results compliment the

findings of Rice (1964, 1965b, 1965c, 1968) who reported similar inhibitions of nitrogen-fixing bacteria and the inhibition of effective nodulation of legumes by many of the same species. The combined effects may result in a slowing of the rate of addition of nitrogen to infertile old-fields and, thus, the slowing of succession. This could certainly explain why the intermediate stages remain so long.

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