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ALLELOPATHIC EFFECTS OF *Sporobolus pyramidatus* ON VEGETATIONAL PATTERNING

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ALLELOPATHIC EFFECTS OF *Sporobolus pyramidatus*

ON VEGETATIONAL PATTERNING

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DISSERTATION COMMITTEE

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ALLELOPATHIC EFFECTS OF *Sporobolus pyramidatus* ON VEGETATIONAL PATTERNING

CHAPTER I

INTRODUCTION

Although central Oklahoma is well within the reported range of *Sporobolus pyramidatus*[1] (Hitchcock, 1951), it has always been a minor species in the vegetation of this area. Dr. Elroy Rice (personal communication) has observed that in the last five years *S. pyramidatus* has become increasingly more common in the Norman, Oklahoma area, especially where *Cynodon dactylon* is the dominant. This has obviously been true on the University-owned golf course where today large stands of *S. pyramidatus* can be found. Dr. Rice has personally observed *S. pyramidatus* expanding from a small stand of just a few plants to a stand that now covers approximately one-half acre.

Interest in *S. pyramidatus* in natural areas led to the finding of similar appearing stands of *Sporobolus* in the Wichita Mountains Wildlife Refuge in southwestern Oklahoma.

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

This time S. pyramidatus was associated with Buchlöe dactyloides. No observations were available as to the expansion of Sporobolus in this area.

The rapid encroachment of S. pyramidatus on C. dactylon and possibly on B. dactyloides presented an interesting problem in relation to the vegetational pattern of the area. Preliminary observation eliminated shading as a factor because all three species offer similar shading and all are kept mowed or grazed to the same height. Also, S. pyramidatus forms clumps that are widely separated, especially in the center of the stand. Allelopathy was suspected because Sporobolus occurs in virtually pure stands and appears to be less vigorous in vegetative growth and inflorescence production in the center of the stands than around the margin.

Many workers have reported that grasses inhibit other higher plants and microorganisms (Pickering, 1917, 1919; Benedict, 1941; Hamilton and Buchholtz, 1955; Rice, 1964, 1968; Parenti and Rice, 1969; Abdul-Wahab and Rice, 1967). Munroe (1966) showed that extracts from the roots of S. pyramidatus were inhibitory to ammonia-oxidizers and nitrate-oxidizers.

Therefore, this project was undertaken to test the hypothesis that the rapid spread of S. pyramidatus in certain areas is due to chemical inhibitors produced by Sporobolus, and the resulting vegetational pattern is primarily a result of an allelopathic effect.

CHAPTER II

LOCATION AND DESCRIPTION OF STUDY SITES

Two sites were chosen for plot location. Since primary interest was centered around the effects shown by *Sporobolus* on *Cynodon dactylon*, care was taken in choosing a site in the Oklahoma University Golf Course so soil could be removed from the area. Careful selection was necessary to avoid possible fertilizer run-off from greens, even though the same general effect of *S. pyramidatus* could be seen in these areas. Also, this site was selected to avoid drainage-erosion areas, eliminating this as a causal factor in the spread of *S. pyramidatus*. The other site for study was located just south of the animal viewing pasture in the Wichita Mountains Wildlife Refuge (Comanche County) in southwestern Oklahoma. This site was used primarily for obtaining test species associated with *S. pyramidatus* in a natural area. The chosen area is heavily grazed by both buffalo and cattle.

A reduction of cover within *S. pyramidatus* stands was quite evident at both sites. To quantify cover within, and one to five meters outside the *S. pyramidatus* stands, the point contact method was used (Crockett, 1964). Five

hundred points were taken systematically within and outside the S. pyramidatus stand at both sites in June and August. Cynodon dactylon and B. dactyloides dominate the area in their respective sites outside the S. pyramidatus stand (Tables 1, 2). It can be readily seen that little C. dactylon or B. dactyloides grow in their respective Sporobolus stands. Bare area is prominent within the S. pyramidatus stands in both June and August at both sites. Hordeum pusillum and Bromus catharticus are both winter annuals and were not found in the August sampling (Table 1).

A definite "edge-effect" could be seen on the perimeter of the S. pyramidatus stands at both sites (Figure 1). This was due to an actively growing dense cover of Sporobolus near the edge of the stand as opposed to the widely separated plants toward the center of the stand.

Table 1. Sampling data from Oklahoma University Golf Course plots.

Inside *Sporobolus* plot		Outside *Sporobolus* plot	
June, 1969		June, 1969	
Species	% Basal Cover	Species	% Basal Cover
Sporobolus pyramidatus	27.8	*Cynodon dactylon*	78.0
Bromus catharticus	8.6	Bare area	20.0
Hordeum pusillum	13.4	Other species	2.0
Cynodon dactylon	9.4		
Bare area	35.2		
Other species	6.0		
August, 1969		August, 1969	
Sporobolus pyramidatus	30.4	*Cynodon dactylon*	65.2
Cynodon dactylon	9.6	Bare area	31.5
Bare area	58.0	Other species	3.3
Other species	2.0		

Table 2. Sampling data from Wichita Mountains Wildlife Refuge plots.

Inside *Sporobolus* plot		Outside *Sporobolus* plot	
June, 1969		June, 1969	
Species	% Basal Cover	Species	% Basal Cover
Sporobolus pyramidatus	30.4	*Buchlöe dactyloides*	67.0
Buchlöe dactyloides	2.6	Bare area	31.0
Bare area	67.0	Other species	2.0
August, 1969		August, 1969	
Sporobolus pyramidatus	24.1	*Buchlöe dactyloides*	58.0
Buchlöe dactyloides	1.9	Bare area	42.0
Bare area	73.9		

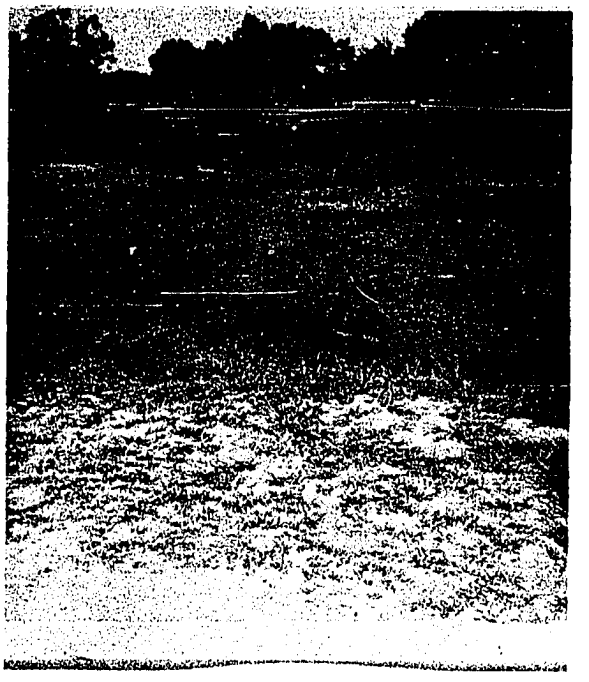

Figure 1. *Sporobolus* stand associated with *C. dactylon* showing "edge-effect." *Sporobolus* in foreground.

CHAPTER III

EXPERIMENTATION AND RESULTS

Species Selection

As a result of vegetative sampling and other considerations, the species chosen to be tested for possible allelopathic reaction to S. pyramidatus were C. dactylon, B. dactyloides, S. pyramidatus, Bromus japonicus, Amaranthus retroflexus, H. pusillum, and Aristida purpurea. Cynodon dactylon, B. dactyloides, S. pyramidatus, and H. pusillum were obvious choices. Bromus japonicus and A. retroflexus were chosen because they are commonly used indicator species of allelopathy in our area. Also, good seed sources of both species occur here. Although A. purpurea was not sampled, its seeds were found in great quantity in the Sporobolus stands at the Wichita Mountains Wildlife Refuge site, and plants grew profusely in the areas surrounding B. dactyloides.

Physical and Mineral Properties of Soils

To determine if S. pyramidatus was responsible for any changes in soil properties that could account for the pattern seen in the field, soil analyses were made. Ten

soil samples were collected systematically in July, 1969 to a depth of 30 cm both within and 1 to 5 meters outside a S. pyramidatus stand. The soil was air-dried, passed through a 2 mm sieve, and analyzed for pH by the glass electrode method of Piper (1942). The soils were then ground to pass through a 0.5 mm sieve and analyzed for organic carbon by the chromic acid digestion method of Piper (1942), total phosphorus by the method of Shelton and Harper (1941), and total nitrogen by the macro-Kjeldahl method (Bremner, 1965). Sporobolus pyramidatus caused no significant differences in these important soil factors (Table 3).

Soil Moisture

The greatest moisture stress in this area is generally in August, so moisture content of the soil was determined at that time. Ten soil samples were taken to a depth of 30 cm within a Sporobolus stand and ten outside the stand. The soils were compared for percent moisture on an oven-dry weight basis, and there was no significant difference in the means (Table 3).

Although the above results showed no significant differences in the soils tested, not all soil factors were examined, and possible differential uptake of ions by Sporobolus was not eliminated by these studies. An additional experiment was done to exclude these deficiencies in experimental design.

Table 3. Comparison of soil factors within a Sporobolus stand and outside it.[a]

	Moisture %	pH	Organic Carbon[b] %	Total Nitrogen %	Total Phosphorus %
In	11.4±0.6	8.0	0.89±0.05	0.076±0.003	0.030±0.039
Out	11.4±0.8	8.0	0.99±0.05	0.077±0.002	0.032±0.027

[a]No values were significantly different at 0.05 level.

[b]Walkley and Black organic carbon values.

Effects of Field Soils on Germination and Seedling Growth

To eliminate competition resulting from differential uptake of ions and to determine if phytotoxins exist and are stable in the soil under field conditions, soil minus litter was taken from within a *Sporobolus* stand by use of a sharp-nosed shovel and placed in eight 5-inch plastic pots. Soil was similarly removed from under *C. dactylon* as a control. Collections were made July 1, 1969, and January 21, 1970; and each collection was treated as a separate experiment. Thirty seeds of all test species except *Sporobolus* were planted in their respective pots. Germination was counted on the second day after it began and again 2 weeks after planting. The earliest determination of germination was done to see the initial effects on seed germination that may be of great consequence to plants exposed to the competitive mechanisms of their natural environment. Two weeks after planting, the plants were thinned to the 5 largest per pot and allowed to grow an additional 2 weeks. *Sporobolus* did not germinate well in soil even after pretreatment, so it was allowed to germinate and grow in sand for one week and then transplanted to the soil, where it was allowed to grow an additional 3 weeks. All species were compared on an oven-dry weight basis. This experiment and all others mentioned hereafter in this

paper were done in a Percival growth chamber on a 16 hour photoperiod at 28°C and a night temperature of 21°C.

The July soils collected within Sporobolus stands significantly reduced the oven-dry weights of H. pusillum, B. japonicus, and A. purpurea but stimulated the growth of C. dactylon (Table 4). Sporobolus pyramidatus and B. dactyloides were also stimulated, but not to a statistically significant level. Germination was appreciably reduced in all species at the second day, but recovered considerably in B. japonicus, A. retroflexus, and B. dactyloides after 2 weeks.

Soils collected in January from Sporobolus stands significantly inhibited all test species except S. pyramidatus itself, and germination of all tested species was markedly affected (Table 4). Dry weight of Sporobolus was reduced in the test, but not significantly.

The above results indicate a phytotoxic effect of the soils closely associated with Sporobolus and eliminate any competitive mechanism associated with the presence of the Sporobolus plant.

Effects of Leaf Leachate on Germination and Seedling Growth

To explain the results obtained from field soil studies discussed previously, preliminary tests were conducted using extracts of Sporobolus to determine what parts of the plant had phytotoxic effects on the indicator

Table 4. Effects of field soils previously in contact with *Sporobolus* on germination and growth of test species.

Species	Date soil taken	Mean dry weight and standard error, mg		Germination % of control	
		Control	Test	2nd day	2 weeks
Cynodon dactylon	July	34± 4.3	73± 7.4[a]	10	46
	Jan.	21± 2.8	9± 0.1[a]	17	29
Hordeum pusillum	July	62± 3.5	46± 2.6[a]	16	77
	Jan.	21± 1.1	14± 1.8[a]	3	12
Bromus japonicus	July	189±10.0	114± 8.0[a]	53	92
	Jan.	55± 2.7	27± 2.7[a]	8	13
Amaranthus retroflexus	July	216±29.3	161±15.8	62	88
	Jan.	141±12.8	90±15.7[a]	15	26
Sporobolus pyramidatus	July	61± 9.6	84± 6.8	--	--
	Jan.	42± 3.2	37± 3.0	--	--
Buchlöe dactyloides	July	31± 3.4	40± 3.4	70	90
	Jan.	33± 1.6	24± 2.0[a]	65	60
Aristida purpurea	July	41± 3.4	14± 1.9[a]	10	50
	Jan.	14± 0.1	6± 0.5[a]	40	32

[a]Dry weight signifcantly different from control at 0.05 level or better.

species, A. retroflexus and B. japonicus. These studies indicated that the shoots were most inhibitory, although the root extracts showed some inhibitory activity. Morgan and Tukey (1964) and others have shown that a large number of compounds can be leached from leaves, so an experiment was designed to determine the effect of leachate from the shoots of Sporobolus on the growth of test species.

Artificial rain in the form of finely-sprayed cistern water was used to leach the shoots of Sporobolus. The leachate collected was used to water 8 pots of a soil mixture containing soil, sand, and peat moss (6:4:1) and 30 seeds of the test species. Eight control pots of each test species were watered with cistern water that had not passed over Sporobolus. Sporobolus seeds were germinated and grown in sand for one week and transplanted to the soil mixture. Germination was recorded the second day after it began and again at 2 weeks. After 2 weeks the plants were thinned to the 5 largest per pot, allowed to grow an additional 2 weeks, and compared on an oven-dry weight basis. Sporobolus seedlings, after transplanting, were grown an additional 3 weeks.

Bromus japonicus was inhibited significantly in both tests (Table 5), and A. retroflexus and S. pyramidatus were inhibited in the second test. Germination was not greatly reduced overall. With the exception of the effects

Table 5. Effects of Sporobolus leaf leachate on seed germination and seedling growth.

Species	Exp. No.	Mean dry weight and standard error, mg		Germination % of Control	
		Control	Test	2nd day	2 weeks
Cynodon	1	157± 9.1	143±10.4	73	86
dactylon	2	88± 5.0	106± 7.5	93	89
Hordeum	1	39± 2.7	38± 1.8	87	100
pusillum	2	22± 0.8	22± 1.0	71	98
Bromus	1	328±15.0	270±11.7[a]	70	87
japonicus	2	205±10.6	154± 7.3[a]	87	96
Amaranthus	1	440±46.3	379±52.0	52	72
retroflexus	2	380±39.2	234±26.0[a]	93	89
Sporobolus	1	133± 8.9	128± 7.7	--	--
pyramidatus	2	244±13.2	181±10.8[a]	--	--
Buchlöe	1	150± 8.1	140± 8.5	85	95
dactyloides	2	59± 2.8	64± 3.0	90	98
Aristida	1	22± 1.2	22± 1.2	55	78
purpurea	2	21± 0.9	25± 1.7	40	74

[a]Dry weight significantly different from control at 0.05 level or better.

on Sporobolus itself, little insight could be gained from these experiments concerning the patterns seen in C. dactylon and B. dactyloides dominated areas.

Effects of Decaying Shoots on Germination and Seedling Growth

It was noted from field observations that even in early June, Sporobolus leaves begin to die and are associated in large quantities with the living plant. As much as 8-10 g of dead material could be found on a single plant depending, of course, upon the size of the plant.

To determine the effect of decaying Sporobolus shoots on the test species, 1 g of shoots (air-dried 3 weeks) was added to each 454 g of a mixture consisting of 6 parts soil, 4 parts sand, and 1 part peat moss. A control series was run with 1 g of extra peat moss added per 454 g of the soil-sand mixture. The test and control series contained 8 pots each and all were watered equally with distilled water. Thirty seeds were planted and germination was determined 2 days after it had begun and at 2 weeks. Sporobolus seedlings were transplanted from sand after one week. The plants, except Sporobolus seedlings, were thinned to the 5 largest per pot after 2 weeks, allowed to grow an additional 2 weeks, and compared on an oven-dry weight basis. Sporobolus seedlings were allowed to grow 3 weeks after transplanting.

The dry weights of all test species except A.

purpurea were significantly decreased by Sporobolus shoot decay (Table 6). Cynodon dactylon and A. retroflexus were greatly reduced in dry-weight in the second test, and the latter never attained growth past the cotyledon stage in the month that it grew. Seed germination was appreciably reduced in all species at both check periods.

Effects of Decaying Roots on Germination and Seedling Growth

Since preliminary experiments indicated an inhibitory effect caused by root extracts, an experiment was designed to see if decaying roots could help account for the observed field patterning.

Eight pots containing 1 g of air-dried roots per 454 g of a soil mixture (6 soil, 4 sand, 1 peat moss) were used in a test series. The control series of 8 pots contained 1 g of additional peat moss per 454 g of soil mixture. Thirty seeds were placed in each pot and equal amounts of distilled water were added daily. Sporobolus seedlings were transplanted from sand after one week. Germination was determined after 2 days and after 2 weeks; the plants were thinned to the 5 largest per pot after 2 weeks, allowed to grow an additional 2 weeks, and compared on an oven-dry weight basis. Sporobolus seedlings were allowed to grow 3 weeks after transplanting.

Cynodon dactylon was significantly inhibited in dry weight in both tests and the germination reduction

Table 6. Effects of decaying Sporobolus shoots (air dried 3 weeks) on seed germination and seedling growth.

Species	Exp. No.	Mean dry weight and standard error, mg		Germination % of Control	
		Control	Test	2nd day	2 weeks
Cynodon	1	6±0.4	4±0.3[a]	22	43
dactylon	2	7±0.4	1±0.1[a]	20	47
Hordeum	1	13±0.8	11±0.7[a]	40	60
pusillum	2	14±0.8	12±0.5[a]	53	49
Bromus	1	26±1.3	13±1.0[a]	54	80
japonicus	2	25±1.4	10±0.4[a]	68	78
Amaranthus	1	75±5.7	37±4.7[a]	56	65
retroflexus	2	21±2.1	1±0.1[a]	40	62
Sporobolus	1	19±2.1	13±1.2[a]	--	--
pyramidatus	2	20±2.5	13±1.3[a]	--	--
Buchloe	1	26±1.1	21±1.0[a]	41	78
dactyloides	2	27±1.0	18±1.0[a]	55	70
Aristida	1	8±0.3	7±0.4	40	55
purpurea	2	8±0.4	7±0.4	42	51

[a]Dry weight significantly different from control at 0.05 level or better.

was quite pronounced (Table 7). Amartanthus retroflexus was stimulated in dry weight in one test and germination was also stimulated in this test.

Effects of Root Exudate on Germination and Seedling Growth

It is known that roots of plants are not closed systems and that many compounds exude from them (Rovira, 1956; Woods, 1960). An experiment was designed to determine if exudates from Sporobolus roots were inhibitory to the test species. The experimental design was modified from Parenti and Rice (1969) in that the staircase structure was used only as a method for collecting exudate. Distilled water was pumped from reservoirs at the bottom to the reservoirs at the top and allowed to drip down through the pots of Sporobolus growing in sand. The water was then re-cycled by pumps for 4 hours each day. It was collected each day and used to water a set of 8 pots containing a mixture of soil, sand, and peat moss (6:4:1). A control series of 8 pots was watered with distilled water that had not passed over Sporobolus roots. Thirty seeds were planted in each pot, and germination was determined after the second day it began and at 2 weeks. The plants were thinned 2 weeks after planting, allowed to grow an additional 2 weeks, and compared on an oven-dry weight basis. Sporobolus pyramidatus seedlings were transplanted after one week and allowed to grow an

Table 7. Effects of decaying Sporobolus roots (air dried 3 weeks) on seed germination and seedling growth.

Species	Exp. No.	Mean dry weight and standard error, mg		Germination % of Control	
		Control	Test	2nd day	2 weeks
Cynodon	1	8±0.4	5±0.3[a]	33	60
dactylon	2	35±2.2	30±1.5[a]	76	72
Hordeum	1	14±0.8	12±0.9	100	78
pusillum	2	16±1.0	17±0.8	100	89
Bromus	1	30±1.4	33±1.5	100	89
japonicus	2	39±2.1	39±1.5	80	79
Amaranthus	1	22±1.9	21±1.7	78	70
retroflexus	2	38±5.1	79±6.6[a]	125	159
Sporobolus	1	45±3.5	47±3.8	--	--
pyramidatus	2	43±2.3	41±3.1	--	--
Buchlöe	1	22±0.8	22±1.0	41	75
dactyloides	2	33±1.4	31±1.4	33	90
Aristida	1	11±0.8	11±1.0	45	95
purpurea	2	12±1.3	10±1.1	50	100

[a]Dry weight significantly different from control at 0.05 level or better.

additional 3 weeks.

Root exudates significantly reduced the dry weight of C. dactylon in both tests and markedly reduced its germination (Table 8). The dry weights of H. pusillum, B. japonicus, S. pyramidatus, and A. purpurea were significantly reduced in one test. Amaranthus retroflexus was significantly stimulated in one test and appreciably increased in the other. Germination of A. retroflexus was also stimulated in both tests. Buchlöe dactyloides was stimulated in one test; however, its germination was reduced in this test.

Rate of Spread of Sporobolus Associated with Cynodon dactylon

Five Sporobolus stands on the University of Oklahoma Golf Course were measured and stakes were placed around the perimeter of each stand in July of 1969 to determine the rate of spread. In June, 1970, measurements were taken and results show a very rapid spread of Sporobolus. Two of the five stands increased in size so rapidly that they integrated with adjacent stands and accurate measurements were not possible. However, approximately 12 ft separated the stands at the July, 1969, measurement date. Two other stands averaged an increase of 9 ft in diameter. The last stand measured 1 ft 6 in. in width and 2 ft 9 in. in length at the July, 1969, measurement. In June, 1970, the stand had increased to 4 ft 8 in.

Table 8. Effects of Sporobolus root exudate on seed germination and seedling growth.

Species	Exp. No.	Mean dry weight and standard error, mg		Germination % of Control	
		Control	Test	2nd day	2 weeks
Cynodon	1	9±0.6	4±0.4[a]	28	40
dactylon	2	14±0.7	10±0.7[a]	42	54
Hordeum	1	13±0.7	13±0.7	59	87
pusillum	2	20±1.1	18±0.8[a]	63	79
Bromus	1	29±1.8	24±1.2[a]	98	95
japonicus	2	47±1.8	44±1.8	96	95
Amaranthus	1	18±1.6	31±2.7[a]	150	111
retroflexus	2	41±4.8	55±4.6	260	132
Sporobolus	1	18±2.2	19±2.0	--	--
pyramidatus	2	42±2.3	35±2.2[a]	--	--
Buchlöe	1	25±1.3	24±1.0	116	123
dactyloides	2	42±1.2	48±1.9[a]	50	69
Aristida	1	9±0.4	7±0.3[a]	70	105
purpurea	2	10±0.9	11±1.0	122	90

[a]Dry weight significantly different from control at 0.05 level or better.

in width and 7 ft 8 in. in length.

Identification and Biological Activity of Inhibitors

Since much of the inhibitory activity of Sporobolus was associated with dead plant material, 20 g of air-dried (3 weeks) shoots were boiled in 100 ml of distilled water, allowed to stand for 30 minutes, and filtered. The extract was concentrated in vacuo and chromatograms were prepared on Whatman No. 3 MM paper and developed with n-butanol-acetic acid-water (63:10:27, v/v), BAW, followed by 6% aqueous acetic acid, 6% AA. The chromatograms were inspected with both short (2537 Å) and long (3360 Å) ultraviolet light, with and without the addition of ammonia. Three fluorescent compounds were detected (Table 9). These chromatograms were subjected to various reagent tests (Rice, 1965).

To determine the biological activity of these three fluorescent compounds, each was eluted from the chromatograms with 40% ethanol and evaporated to dryness in vacuo. A paper developed in BAW followed by 6% AA without the application of extract was eluted similarly as a control. Each residue was taken up in 2 ml of 0.05 M phosphate buffer (pH 5.6) and added to petri dishes containing 5 cm disks of acid-washed Whatman 3 MM paper and 100 seeds of Amaranthus palmeri. Amaranthus palmeri seeds germinate well and are commonly used for

Table 9. Inhibitory compounds from water extracts of air-dried Sporobolus shoots.

Compound	Rf's on Whatman 3 MM[a]		Fluorescence[b]		Reagent colors[c]		
	BAW	6%AA	Long and short U.V.		P-Nit	Sulfan. acid	FeCl $K_3Fe(CN)_6$
			$-NH_3$	$+NH_3$			
#1	0.82	0.40	br.bl.	br.bl.	none	none	none
#2	0.40	0.61	l.bl.	l.bl.	none	none	none
#3	0.31	0.86	bl.vio.	bl.vio.	none	none	none

[a]See text for solvent systems. Rf's are averages of 6 runs.

[b]br, bright; bl, blue; vio, violet; l, light.

[c]Diazotized p-nitraniline (Bray, et al., 1950), diazotized sulfanilic acid (Bray et al., 1950), and ferric chloride-potassium ferricyanide (Smith, 1960, p. 324).

bioassays in our laboratory. The seeds were kept in darkness at 25°C and germination was determined daily for 5 days. Final germination expressed as a percentage of the control was as follows: #1, 64%; #2, 2%; $3, 80%.

Since none of these compounds gave color reactions in the phenolic reagents (Table 9), it was suspected that these compounds were not free phenols. Acid and alkaline hydrolyses were run on each to determine if these compounds were complex molecules.

The compounds were eluted, evaporated to dryness in vacuo, and taken up in 10 ml of 0.5N HCl. These were refluxed for one hour, cooled, and extracted with two half volumes of diethyl ether. The ether fraction was evaporated and taken up in absolute ethanol. The water fraction was evaporated three times in vacuo to eliminate the HCl. The residue was then taken up in 40% ethanol. Chromatograms were prepared for each fraction and developed in BAW followed by 6% AA. No fluorescent spots were found and no colors were found with phenolic reagents. Apparently acid hydrolysis destroyed these compounds. This is not uncommon for some phenolic acids (Guenzi and McCalla, 1966). Additional chromatograms were prepared with only the water fractions, developed in BAW followed by isopropanol-butanol-water (140:20:40, v/v), IBW, and dipped in a benzidine sugar reagent (Smith, 1960, p. 25).

All three compounds yielded glucose and fructose upon acid hydrolysis (Table 10).

Alkaline hydrolysis was done under nitrogen using 2N NaOH at 25°C for one hour. The resulting solution was passed through an Amberlite IR-120 (Hydrogen Form) ion exchange column to remove sodium ions and lower the pH to 2. Each hydrolysate was ether extracted with two one-half volumes of diethyl ether. The ether fraction was reduced to dryness and taken up in absolute ethanol. The water fraction after drying was dissolved in 40% ethanol. Chromatograms were prepared for each fraction and developed in two dimensions by BAW followed by 6% AA. Several fluorescent and two absorbing spots were found from each original compound, indicating a complex molecule connected by ester linkages. Final amounts of the aglycones resulting from hydrolysis were so small that the compounds were not identified. Chromatograms were prepared with the water fraction only and developed in BAW followed by IBW. These papers were then dipped in benzidine sugar reagent. These results indicated that fructose was present and connected by ester linkages in all of the original compounds and that glucose was held by ester linkages in all but compound #1 (Table 10).

It is known that complex compounds are broken down readily in the soil (Wang, Yang, and Chuang, 1967; Wilson and Rice, 1968), so a new technique modified from

Table 10. Compounds in water fractions after acid and alkaline hydrolysis of inhibitors from Sporobolus water extracts. Symbols as in Table 9.

Compound and Treatment	Rf's on Whatman 3 MM[a]		Benzidine sugar test
	BAW	IBW	
#1 acid hydrolysis			
a.	0.21	0.35	brown
b.	0.24	0.45	gold-brown
#1 alkaline hydrolysis			
a.	none	none	none
b.	0.26	0.43	gold-brown
#2 acid hydrolysis			
a.	0.21	0.34	brown
b.	0.26	0.40	gold-brown
#2 alkaline hydrolysis			
a.	0.20	0.36	brown
b.	0.24	0.43	gold-brown
#3 acid hydrolysis			
a.	0.20	0.36	brown
b.	0.25	0.43	gold-brown
#3 alkaline hydrolysis			
a.	0.20	0.38	brown
b.	0.25	0.45	gold-brown
Known Glucose	0.20	0.35	brown
Known Fructose	0.24	0.43	gold-brown

[a]See text for solvent systems.

Guenzi and McCalla (1966) was used to identify some of the chief breakdown products associated with alkaline hydrolysis. Ten grams of air-dried (3 weeks) Sporobolus shoots were ground to pass through a 10 mesh screen and hydrolyzed directly with 2N NaOH in an autoclave for 45 minutes. The suspension was filtered through cheesecloth and the resulting solution was acidified to pH 2 with HCl. Ether extraction was performed using two one-half volumes of diethyl ether. The ether extract was shaken with 5% $NaHCO_3$ and the ether portion discarded. The alkaline portion was again acidified to pH 2 and re-extracted with diethyl ether. The final ether extracts were taken to dryness and the residues dissolved in absolute ethanol.

The alkaline hydrolysate was chromatographed in two dimensions (BAW--6% AA) on Whatman 3 MM paper. Four fluorescent and two absorbing spots were observed under ultraviolet light. Two of these were in particularly large quantity and were suspected to be ferulic acid and para-coumaric acid. The suspected compounds were eluted from the paper with 40% ethanol and co-chromatographed in one dimension on Whatman No. 1 MM paper with the known compounds in four solvent systems: BAW; 6% AA; isopropanol-ammonia-water (200:10:20, v/v), IAW; and IBW. Similar eluates of 40% ethanol were used to determine the absorption spectra for both knowns and unknowns with a Beckman

Model DB-G spectrophotometer before and immediately after the addition of one drop of 2N NaOH to the spectrophotometer cuvettes.

The Rf's in various solvent systems, colors under ultraviolet light, and reagent colors (Rice, 1965) indicated that the suspected compounds were p-coumaric acid and ferulic acid (Table 11). The results of the spectral analyses were consistent with the other findings. The absorption maxima for both suspected and known p-coumaric acids were 283 mμ without and 333 mμ with NaOH. The known and suspected ferulic acids gave their maximum absorption at 312 mμ without and 343 mμ with NaOH.

The biological activity of suspected p-coumaric and ferulic acids was determined using a germination bioassay with A. palmeri. The results expressed as a percent of the control were as follows: suspected p-coumaric acid, 32%; suspected ferulic acid, 54%. Phytotoxin C (Table 11), which has not been identified, caused a reduction in germination similar to that of suspected p-coumaric acid.

In earlier work with the original aqueous extracts, chromatograms (Whatman 3 MM) developed in methylisobutyl ketone-formic acid-water (14:3:2, v/v), KFW, followed by IAW were found to have two broad yellow streaks when dipped in diazotized sulfanilic acid reagent (Bray et al., 1950). This was of particular interest because Dr. Elroy

Table 11. Chromatography of phytotoxins from alkaline hydrolyzed plant residues of Sporobolus air-dried shoots.

Compound	Rf's on Whatman 1 MM[a]				Fluorescence[c]		Reagent Colors[b,c]		
	BAW	6%AA	IAW	IBW	Long	Short	p-Nit	Sulfan. acid	FeCl $K_3Fe(CN)_6$
Phytotixin A	0.89	0.44	0.20	0.80	bl.	purple abs.	gray black	red bn.	dk.bl.
p-Coumaric acid	0.89	0.45	0.20	0.80	bl.	purple abs.	gray black	red bn.	dk.bl.
Phytotoxin B	0.89	0.38, 0.68	0.15	0.71	bl.vio.	bl.vio.	black bn.	tan	dk.bl.
Ferulic acid	0.89	0.39, 0.68	0.16	0.72	bl.vio.	bl.vio.	black bn.	tan	dk.bl.
Phytotoxin C	0.89	0.73	0.25	0.79	abs.	abs.	none	none	bl.

[a]See text for solvent system. Rf's are averages of 3 runs.

[b]Diazotized p-nitraline (Bray et al., 1950), diazotized sulfanilic (Bray et al., 1950), ferric chloride-potassium ferricyanide (Smith, 1960, p. 324).

[c]bl, blue; vio, violet; bn, brown; dk, dark; abs, absorption.

Rice (personal communication) had found streaks with similar Rf's from developed chromatograms of root exudate of sterile seedlings of Aristida oligantha. The compounds associated with these streaks are very inhibitory to the germination of radish seeds. Upon hydrolysis of the eluates of these two streaks with 2N HCl, compounds with similar Rf's in BAW followed by 95% ethanol-ammonium hydroxide-water (80:5:15 v/v), EAW, and colors in diazotized sulfanilic acid were found in both Sporobolus extracts and A. oligantha exudates. These compounds have been very hard to characterize and at present thin-layer chromatography techniques are being used to obtain better resolution.

CHAPTER IV

DISCUSSION

The presence of the single species stand has never been explained adequately on the basis of superior adaptability to a particular area (Garb, 1961). The occurrence of pure stands of S. pyramidatus in areas dominated by C. dactylon and B. dactyloides serves as an excellent example of this. The invasion and spread of Sporobolus is undoubtedly due in part to environmental factors in these areas. The fact that both study areas are frequented often by either man or grazing animals is very important to the overall picture. Experimentation with Sporobolus seed indicates that it has a light requirement, as well as an aging or chilling requirement for germination. Since both C. dactylon and B. dactyloides produce a dense cover, disturbed sites are necessary for germination of Sporobolus seeds and hence its spread. Also, effects of a given plant species on other plants are evident in some soils and climatic circumstances but not in others (Muller, 1969). The invasion of Sporobolus is apparently due to a combined effect of allelopathy and mechanical disturbance.

Decaying material and root exudate of S. pyramidatus and soil previously in contact with roots of that species significantly reduced the dry-weight and markedly reduced the germination of C. dactylon. The effect on seed germination is probably quite important even though C. dactylon is perennial and rhizomatous. Sporobolus stands are quite open, especially at the center, and could be reseeded by C. dactylon even though encroachment by rhizomes is seemingly eliminated. Germination is probably also reduced by the nature of the soil surface, which is hard due to packing and drying resulting from the lack of vegetation. The stimulatory effect of July (1969) field soil is probably a result of leaching by heavy rains received in June, 1969. It is known that dilute quantities of inhibitory compounds can be stimulatory (Olmsted, unpublished M.S. thesis, Univ. of Okla., 1967). Results of all experiments show that allelopathy is the major factor concerned in the invasion and spread of Sporobolus into stands of C. dactylon.

The effects of S. pyramidatus on B. dactyloides in relation to observed field patterning is not as clear. Shoot decay and January field soils inhibited growth and germination, but the overall effects were not as striking as with C. dactylon. Southwestern Oklahoma is severely affected by moisture stress throughout the summer season, and this could possibly be an important factor because

Muller (1969) believes that a weakening effect of slight inhibition interacting with deleterious physical factors constitutes a synergism more powerful than either alone.

To be able to survive the allelopathic effects on itself, Sporobolus must be able to move constantly into new areas. To do this it must be less inhibitory to itself than to the surrounding vegetation, and this is apparently true for field soils did not affect Sporobolus at either collection date. This probably means that the effective concentrations inhibitory to Sporobolus can be maintained only by continued association with its own decay material. Also, it is possible that active Sporobolus growth is necessary because in one experiment with both exudate and leachate the growth of Sporobolus seedlings was significantly inhibited. The differences in results seen with regard to leachate and exudate could be an effect of age on the materials released (Rovira, 1956; Rice, 1964; Tukey, 1966; Koeppe et al., 1970). The evidence indicates that the survival of Sporobolus in C. dactylon dominated areas is a result of the extreme allelopathic effect on C. dactylon allowing Sporobolus to invade the area before it eliminates itself.

Hordeum pusillum, although adversely affected by Sporobolus, seems to survive by evasion. It grows to maturity and flowers at a time when inhibitors associated with Sporobolus are at their lowest concentration in the soil.

Bromus japonicus and A. retroflexus are severely retarded by Sporobolus. All inhibitory mechanisms tested, except decaying roots, significantly reduced the dry-weight of B. japonicus. Even though A. retroflexus was stimulated in one experiment by both root exudate and root decay, its growth in association with Sporobolus is almost impossible because of the pronounced effect of the decaying shoot material.

The field patterning with regard to A. purpurea is probably not a result of allelopathy alone. Crocket (1964) has shown that soils associated with B. dactyloides, and thus Sporobolus, are poor, dry soils. These soil factors probably are more responsible for the elimination of A. purpurea than any allelopathic effects of Sporobolus.

Although the hypothesis for this study necessitated the removal of competitive mechanisms, competition in nature is very real and important. Any reduction of growth due to Sporobolus could greatly accentuate the effects of competition. A combination of allelopathic and competitive mechanisms could cause a synergistic effect far exceeding that of either factor alone. The only valid explanation for the pure stands of Sporobolus would have to include allelopathy, competition and disturbance.

The evidence indicates that the bulk of the inhibitory effect of Sporobolus is exerted by the decomposition

of plant parts, especially the shoots. Many authors have shown plant residues to be inhibitory to both natural vegetation and crop plants (Abdul-Wahab and Rice, 1967; Wilson and Rice, 1968; Patrick, Toussoun, and Koch, 1964; McCalla and Haskins, 1964). Wang, Yang, and Chuang (1967) consider plant parts left in the soil to be one of the main sources of soil phenolic acids. Winter (1961) found that the inhibitory activity from plant residues was highest at mid-winter, and soils associated with Sporobolus in January were more inhibitory than soils collected in July. It is quite possible that mowing is a factor in the C. dactylon area because this process would tend to increase the dead material in contact with the soil.

The results of the chemical analyses show large quantities of p-coumaric acid and ferulic acid in the dead shoot material of Sporobolus. It was necessary to air-dry the shoots at least 3 weeks for inhibitory action to follow decomposition in the soil. This is undoubtedly due to some effect of senescence on the composition of the material. Guenzi and McCalla (1966) found both of these phenolic compounds in the residues of corn, wheat, sorghum and oats. They believe that under ideal conditions p-coumaric acid could be released in amounts large enough to affect plant growth.

Both p-coumaric and ferulic acids have been

extracted from soils with p-coumaric usually in larger quantities depending on the soil type (Wang, Yang and Chuang, 1967). Soil phenolic acids can increase markedly under certain soil conditions to a concentration where many plants would be seriously affected (Wang, Cheng, and Tung, 1967). Apparently colloidal materials in soil play a role in accumulating phytotoxins to a toxic level (Del Moral and Muller, 1970; Wilson and Rice, 1968).

Ferulic and p-coumaric acids both inhibited A. palmeri seed germination considerably in this study. These two acids have been well documented as inhibitors to both seed germination and overall growth. However, it is important to stress that plants have differential susceptibility to specific inhibitors. Abdul-Wahab and Rice (1967) found p-coumaric acid as one of the primary inhibitors in Sorghum halepense. At a concentration of 2 mg/ml psyllium seed germination was reduced to 3.6% of the control. Del Moral and Muller (1970) found both p-coumaric and ferulic acids in the leachate from the litter of Eucalyptus camaldulensis. At concentrations of 10^{-2}M these two compounds were most toxic to germination of the test seeds, but at 10^{-3}M they were still quite inhibitory. Olmsted (unpublished M.S. thesis, Univ. of Okla., 1967) found that p-coumaric acid at a concentration of 0.83×10^{-3}M was significantly inhibitory to the growth of 12 day old seedlings of A. retroflexus.

At a concentration of 50 ppm Wang, Yang, and Chuang (1967) found these two phenolic acids to significantly decrease the growth of sugar cane cuttings. Guenzi and McCalla (1966) observed that a concentration of 625 ppm of p-coumaric acid was comparable to the toxicity of 1250 ppm of ferulic acid on the growth of wheat seedlings. Hennequin and Juste (1967) found both compounds to be phytotoxic to germination. The phytotoxicity was even stronger on the growth of seedlings. Ten parts per million of p-coumaric acid caused a 50% decrease in the dry matter yield of maize cultivated in a nutrient solution up to ripeness.

The proposed mechanism for the effects of these two compounds concerns the indole-3-acetic acid (IAA) oxidase system. Gortner and Kent (1958) found p-coumaric acid to be the most effective activator for the IAA oxidase enzyme of pineapple and considered it a co-enzyme for this enzyme system. These authors also found that at low concentrations ferulic acid is an activator of IAA oxidase, but at high concentrations it inhibits the activated IAA oxidase system. Zenk and Müller (1963) found that both compounds decrease growth *in vivo* in etiolated peas by increasing IAA decarboxylation. They observed that phenolic acids taken up by plant tissue are converted to glucose esters which are still effective activators of IAA oxidase.

It is impossible to assess the accumulative effects of several inhibitors. However, the additive effect of a combination of inhibitors may be even more detrimental than each compound separately (Wilson and Rice, 1968).

CHAPTER V

SUMMARY

Field observations of stands of S. pyramidatus indicated a reduction of growth of Sporobolus in the center of the stands. Associated species were either reduced in growth or eliminated from these stands. Rapid encroachment of Sporobolus on C. dactylon was also observed. Experiments were designed to determine if the observed vegetational patterning was a result of an allelopathic effect. Early studies eliminated shading and soil factors as causal agents. Experiments with field soil taken from around Sporobolus plants indicated that phytotoxins were present and were relatively stable in the soil.

Decaying shoot material exerted the most phytotoxic effect on growth and germination of the test species; however, C. dactylon was adversely affected by all factors tested except leaf leachate. Buchlöe dactyloides was inhibited by field soils and by decaying shoots. The decaying shoots of Sporobolus inhibited the growth of its own seedlings. There is also an indication that the leaf leachate and root exudate of Sporobolus may be responsible for its own decline. Hordeum pusillum

was inhibited, but being a winter annual allows it to evade the allelopathic effects of Sporobolus. Both B. japonicus and A. retroflexus were severely inhibited in various tests. Soil factors were given as the primary reason that A. purpurea did not occur with Sporobolus.

Para-coumaric acid and ferulic acid were extracted from Sporobolus shoot residue in large quantities. Both compounds were very inhibitory to germination of A. palmeri seed.

The results of this study show that invasion and rapid encroachment of Sporobolus on C. dactylon and possibly B. dactyloides is primarily an allelopathic effect with competitive mechanisms and mechanical disturbance probably accentuating this effect. Evidence indicates that Sporobolus is more inhibitory to C. dactylon than to itself thus allowing Sporobolus to invade new areas before it eliminates itself.

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