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INHIBITIONAL EFFECTS OF *Digitaria sanguinalis* AND POSSIBLE ROLE IN OLD-FIELD SUCCESSION

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INHIBITIONAL EFFECTS OF *Digitaria sanguinalis* AND POSSIBLE ROLE IN OLD-FIELD SUCCESSION

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INHIBITIONAL EFFECTS OF *Digitaria sanguinalis* AND POSSIBLE ROLE IN OLD-FIELD SUCCESSION

CHAPTER I

INTRODUCTION

Booth (1941) reported that succession in abandoned fields in central Oklahoma and southeastern Kansas includes four stages: (1) weed, (2) annual grass, (3) perennial bunchgrass, and (4) the climax prairie. The weed stage lasts 2 to 3 years, and the annual grass stage lasts from 9 to 13 years, dominated by *Aristida oligantha*.[1] The perennial bunchgrass stage dominated by *Andropogon scoparius* is of long duration, so the time required for the return of the true prairie is very great. Savage and Runyon (1937) reported that after 40 years abandoned fields in the Southern Great Plains do not possess a cover comparable with the climax of the region. The climax composition in an abandoned field in central Kansas had not been attained after 33 years (Tomanek, Albertson, and Riegel 1955).

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

Several species of the weed stage and several phenolic compounds produced by these species have been found to be inhibitory to seedlings of many species of Stage 1 but usually not to Aristida oligantha, the dominant of Stage 2 (Abdul-Wahab and Rice 1967, Brown 1968, Floyd and Rice 1967, Olmsted 1967, Wilson 1968). This differential inhibition may be the reason for the rapid disappearance of Stage 1 and the invasion by A. oligantha.

Digitaria sanguinalis (crabgrass) is prominent early in the first stage of succession, occurring sometimes in almost pure stands. Smith (1940) pointed out that it is one of the first species of the weed stage to be lost, however. Rice (1964) found that crabgrass is very inhibitory to several nitrogen-fixing and nitrifying bacteria.

I hypothesized, therefore, that crabgrass might have a direct inhibitory effect on its own seedlings and seedlings of other higher plants in addition to inhibiting the nitrogen-fixing and nitrifying bacteria. Such inhibition might help explain its own rapid disappearance from old-fields. This project was undertaken to obtain evidence concerning the ability of crabgrass to inhibit certain species of plants associated with it in abandoned fields, and to identify some of the inhibitory compounds produced.

CHAPTER II

HISTORICAL BACKGROUND

Growth inhibitors produced by plants have often been the center of much controversy. According to Garb (1961), De Candolle first raised the question of the existence of natural growth-inhibitors that limited the growth of other plants. For some time little was done to determine whether plant inhibitors existed. Loehwing, as late as 1937, concluded that the existence of naturally occurring differential plant growth-inhibitors had not yet been proven.

Went (1942) observed that certain annual plants did not grow under the desert shrub Encelia farinosa Gray. Gray and Bonner (1948) isolated 3-acetyl-6-methoxybenzaldehyde from the leaves of E. farinosa and found it to be inhibitory to the germination of seeds of species which normally do not grow under Encelia.

Bonner and Galston (1944) observed that the edge rows in guayule plantings in California had much larger plants than the center rows and differences could not be attributed to competition for water or minerals.

Keever (1950) working on old-field succession in

North Carolina reported that one of the dominant species of the first stage, Erigeron canadensis L., loses its dominance after the first year due to phytotoxic products from its own decaying roots.

Muller and Muller (1964), Muller (1965), and Muller and del Moral (1966) found that certain shrubs produce volatile inhibitors (terpenes) which enable them to invade grasslands in parts of California.

Rice, Penfound, and Rohrbaugh (1960) found that the order in which species invade abandoned fields in Oklahoma starting with Stage 2 was the same as the order based on increasing requirements for nitrogen and phosphorus. Recent studies by Rice (1964, 1965b, 1965c) suggest that the rate of plant succession in abandoned fields is dependent, in part, on the production by the pioneer species, of substances inhibitory to the nitrogen-fixing and nitrifying bacteria.

Thus, the ecological importance of plant inhibitors appears to be well documented.

CHAPTER III

MATERIALS

Crabgrass plants were collected from open areas around Norman, Oklahoma, or grown from seeds collected in these same areas. Tomato seedlings were used in the initial tests to determine whether or not crabgrass was inhibitory to higher plants. After preliminary experiments indicated inhibitory activity by crabgrass, various species were used as test plants because of their association with crabgrass in the field.

Extracts were prepared by grinding 10 g fresh weight of crabgrass plant material in a Waring Blendor with distilled water for 10 minutes, allowing to stand for 30 minutes, and filtering through Whatman No. 1 paper with a Buchner funnel. The volume of the extract was made up to 100 ml with distilled water. Entire crabgrass plants were collected at various stages of development, and used in making the extracts.

CHAPTER IV

EXPERIMENTATION AND DISCUSSION

Seedling inhibition by extracts of crabgrass. Seedlings of six species of plants were grown in quartz sand for 2 weeks in a complete nutrient solution. They were then transferred to vials containing a 1:5 ratio of nutrient solution of plant extract and were allowed to grow for 10 to 12 days in a photoperiod of 16 hours at 27°C, and a night temperature of 18°C. Controls were run using a 1:5 ratio of nutrient solution to distilled water under the same conditions. The plant extract significantly reduced the oven-dry weight of the seedlings of all species tested (Table 1). This indicated that crabgrass contains inhibitory materials, so further experiments were devised which would have more ecological meaning.

Inhibition of seed germination. Four hundred seeds of each of six species of plants were germinated in extracts of crabgrass at 27°C in a 1:5 ratio of nutrient solution (Hoagland and Arnon,1950) to plant extract. Controls were germinated in a 1:5 ratio of nutrient solution to distilled water. There was a reduction in percentage of

Table 1. Effects of aqueous whole plant extracts of crabgrass on 12-day old seedlings.

Plant Name	Expt. No.	Mean Oven-Dry Weight, mg with Standard Error	
		Control	Test[a]
Amaranthus retroflexus	1	53±3.3	4±0.0
	2	54±6.6	5±4.1
Ambrosia elatior	1	71±2.0	59±2.6
	2	72±2.8	39±3.7
Aristida oligantha	1	37±7.1	11±9.5
	2	34±5.2	9±1.5
Bromus japonicus	1	29±1.4	13±1.4
	2	30±1.8	12±2.1
Digitaria sanguinalis	1	101±9.5	19±0.9
	2	100±3.1	18±2.1
Helianthus annuus	1	155±7.5	110±4.8
	2	151±6.6	107±4.1

[a]All test weights significantly different from appropriate controls at the 0.01 level or below.

germination in Amaranthus retroflexus, Digitaria sanguinalis, Helianthus annuus, Plantago ovata Forsk., and initially in Aristida oligantha and Bromus japonicus (Table 2). Final germination percentages of A. oligantha and B. japonicus were not affected.

Seedling inhibition by decaying crabgrass. Thick stands of crabgrass in the field contain sufficient cover to average over 1 g of air-dry weight per pound (454 g) of soil to the depth of plowing (top 6 2/3 in.). In order to ascertain the possible inhibitory effects of decaying crabgrass, seeds of this species and other species often associated with it were germinated in pots containing soil mixed with 1 g of air dried whole plant material per 454 g of soil, or 1 g of washed air dried peat moss per 454 g of soil for controls. The percentage of germination was determined after ten days, the plants were thinned to three per pot, and the oven-dry weights of the seedlings were taken after four weeks. This experiment was repeated after the crabgrass and peat moss were allowed to stand for several months in soil which was watered periodically. The percentage of germination was determined after ten days, and the oven-dry weights of the seedlings were taken after four weeks.

Decaying crabgrass did not exert any inhibitory activity on seed germination or seedling growth in either experiment (Tables 3, 4). This was also observed by Rice

Table 2. Effects of aqueous extract of Digitaria sanguinalis on rate and per cent germination of selected seeds.

Test Species		Number of Seeds Germinated at Indicated Time, hr.							Total % Germination
		24	36	48	60	72	84	108	
Amaranthus retroflexus	Test	-	68	86	88	90	94	94	24
	Control	-	94	168	180	214	228	228	57
Aristida oligantha	Test	-	0	8	170	304	336	382	96
	Control	-	0	166	304	344	360	388	97
Bromus japonicus	Test	-	188	302	340	352	360	360	90
	Control	-	364	364	364	364	364	364	91
Digitaria sanguinalis	Test	-	0	4	30	52	60	66	17
	Control	-	0	14	56	102	116	134	34
Helianthus annuus	Test	-	22	30	36	40	42	44	11
	Control	-	62	76	80	84	88	92	23
Plantago ovata	Test	0	2	8	20	38	40	44	11
	Control	6	38	88	152	226	304	396	99

Table 3. Effects of decaying crabgrass on seed germination.

Plant Name	Expt. No.	% Germination	
		Control	Test
Amaranthus retroflexus	1	60	57
	2[a]	58	55
Aristida oligantha	1	95	92
	2[a]	90	94
Bromus japonicus	1	87	91
	2[a]	93	92
Digitaria sanguinalis	1	41	47
	2[a]	33	30
Helianthus annuus	1	21	19
	2[a]	24	22

[a]Plant material mixed with soil and left to be decayed for 6 months.

Table 4. Effects of decaying crabgrass on seedling growth.

Plant Name	Expt. No.	Oven-Dry Weight, g	
		Control	Test
Amaranthus retroflexus	1	8.89	9.00
	2[a]	8.76	8.33
Aristida oligantha	1	5.10	4.90
	2[a]	4.39	4.82
Bromus japonicus	1	9.41	10.00
	2[a]	10.13	10.86
Digitaria sanguinalis	1	6.34	6.93
	2[a]	5.80	6.00
Helianthus annuus	1	9.75	8.80
	2[a]	8.90	9.10

[a]Plant material mixed with soil and left to be decayed for 6 months.

(personal-communication) in similar experiments utilizing decaying crabgrass mixed in soil.

Effects of root exudate of crabgrass on plant growth. Seedlings of plants to be tested were placed in 4 inch crocks filled with quartz sand through which culture solution was circulated. The experimental group consisted of alternating pots of crabgrass and test plants. The control group was composed of all test plants. Nutrient solution (Hoagland and Arnon 1950) was put in reservoirs at the bottom of a stairstep set up, and pumped to other reservoirs at the top of the apparatus. The solutions dripped from the upper reservoirs into pots containing the test and control plant combinations. These solutions were allowed to flow from pot to pot by gravitation into the reservoirs at the bottom of the set up. The solutions were then pumped back to the upper reservoirs to maintain a continuous circulation of solutions for a 4-hour period each day for six weeks.

The oven-dry weight of the plants was determined after 6 weeks growth. The exudate caused a significant reduction in growth of all species tested except Ambrosia elatior L. (Table 5).

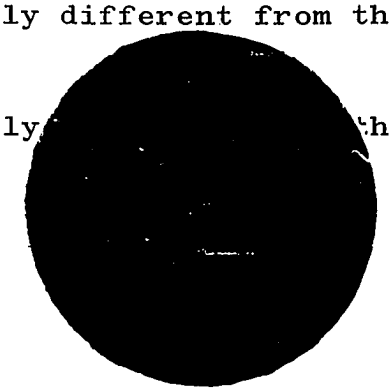
Identification of inhibitors. Procedures used for identification were essentially those of Rice (1965a) and Rice and Parenti (1967). Extracts of crabgrass were acidified

Table 5. Effects of crabgrass root exudate on 6-week old plants.

Plant Name	Expt. No.	Mean Oven-Dry Weight, g with Standard Error	
		Control	Test
Amaranthus retroflexus	1	9.49±0.96	5.45±0.56[a]
	2	9.45±0.84	5.32±0.51[a]
Ambrosia elatior	1	4.98±0.50	4.32±0.62
	2	4.83±0.27	4.33±0.45
Aristida oligantha	1	4.26±0.30	2.18±0.25[a]
	2	4.32±0.30	2.35±0.32[a]
Bromus japonicus	1	11.51±0.79	7.80±1.21[b]
	2	11.57±0.75	8.00±1.00[a]
Helianthus annuus	1	8.05±0.75	4.05±0.74[a]
	2	8.23±0.61	3.95±0.52[a]

[a]Dry weight significantly different from the control at the 0.01 level.

[b]Dry weight significantly [redacted] the control at the 0.05 level.



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		Control	Test
Amaranthus retroflexus	1	9.49±0.96	5.45±0.56[a]
	2	9.45±0.84	5.32±0.51[a]
Ambrosia elatior	1	4.98±0.50	4.32±0.62
	2	4.83±0.27	4.33±0.45
Aristida oligantha	1	4.26±0.30	2.18±0.25[a]
	2	4.32±0.30	2.35±0.32[a]
Bromus japonicus	1	11.51±0.79	7.80±1.21[b]
	2	11.57±0.75	8.00±1.00[a]
Helianthus annuus	1	8.05±0.75	4.05±0.74[a]
	2	8.23±0.61	3.95±0.52[a]

[a]Dry weight significantly different from the control at the 0.01 level.

[b]Dry weight significantly different from the control at the 0.05 level.

to pH 2.5 with 1N HCl and extracted with two half volumes of diethyl ether. The ether was evaporated and the residue dissolved in 10 ml of 95% ethanol. The remaining aqueous fraction was evaporated *in vacuo*, and dissolved in 10 ml of 50% aqueous methanol.

Chromatograms were prepared from the ether and water fractions on Whatman 3 MM paper and developed in n-butanol:acetic acid:water (63:10:27, called BAW). The chromatograms were examined with short (2537Å) and long (3360Å) ultraviolet light, and three distinctive bands were present. Two of these fluoresced blue with both long and short UV light, fluoresced a brilliant yellow-green when exposed to ammonia vapors, and gave a dark blue color with the ferric chloride--potassium ferricyanide reagent for phenols. The third band fluoresced lavender-blue with both long and short UV, and gave a faint lavender-blue when first dipped in ferric chloride-potassium ferricyanide. Subsequent dippings in other solutions caused the spot to disappear. These data plus R_f's suggested that the first two bands mentioned were chlorogenic acid and isochlorogenic acid and the third band was sulfosalicylic acid.

These bands (referred to as bands 1, 2, and 3) were cut from the chromatograms and eluted from the paper. The elutions were reduced to near dryness, dissolved in absolute methanol, and chromatographed on Whatman 3 MM

paper in three different solvent systems: 6% aqueous acetic acid (called 6% AA), BAW, and isopropanol:n-butanol:water (70:10:20, called IBW). The R_f's in the different solvent systems, colors in long and short UV light, and reactions with various reagents were determined (Rice 1965a). These data confirmed the original identifications.

Chlorogenic, isochlorogenic and sulfosalicylic acids were spotted alternately with the suspected compounds on the same papers and developed in the three previously mentioned solvent systems. The three unknown substances gave R_f values comparable to those of the reference compounds (Table 6). Band 1, band 2, and band 3 were apparently the same as the commercial preparations of chlorogenic, isochlorogenic, and sulfosalicylic acids, respectively.

The biological activity of the compounds was determined with the chestnut brown psyllium seed, Plantago ovata, bioassay (Abdul-Wahab and Rice 1967). All compounds were found to be inhibitory.

Table 6. Chromatography of inhibitors from crabgrass.

Compound	R_f's on Whatman[a] 3MM			Fluorescence[c]					Reagent colors[b,c]		
				Long U.V.		Short U.V.					
	BAW	IBW	6%AA	$-NH_3$	$+NH_3$	$-NH_3$	$+NH_3$	p-Nit.	Sulfan. acid	$FeCl_3$-$K_3Fe(CN)_6$	Hoepfner Reaction
Chlorogenic acid	0.55	0.58	0.65	l bl	yel-gr	l bl	yel-gr	br	yel-tan	dk bl	+
Band 1	0.54	0.57	0.65	l bl	yel-gr	l bl	yel-gr	br	yel-tan	dk bl	+
Iso-chlorogenic acid	0.84	0.89	0.40	l bl	yel-gr	l bl	yel-gr	br	yel-tan	dk bl	+
Band 2	0.81	0.87	0.38	l bl	yel-gr	l bl	yel-gr	br	yel-tan	dk bl	+
Sulfo-salicylic acid	0.30	0.37	0.89	lav bl	n/c	lav	n/c	f red-BAW f yel-IBW none-6%AA	yel-BAW f yel-IBW none-6%AA	violet (fades)	-
Band 3	0.27	0.38	0.90	vf lav bl	n/c	vf lav	n/c	vf red-BAW vf yel-IBW none-6%AA	vf yel-BAW none-IBW none-6%AA	vf violet (fades)	-

[a]See text for solvent systems. R_f's are averages for five runs.

[b]Diazotized p-nitraniline (Bray et al. 1950), diazotized sulfanilic acid (Smith 1960, p. 296), ferric chloride-potassium ferricyanide (Smith 1960, p. 324), and Hoepfner reaction (Hoepfner 1932).

[c]l, light; yel, yellow; gr, green; bl, blue; br, brown; dk, dark; f, faint; vf, very faint; n/c, no change; lav, lavender.

CHAPTER V

CONCLUSIONS

Substances produced by crabgrass plants inhibited the germination and growth of that species as well as others often associated with it.

Seed germination and seedling growth of Amaranthus retroflexus, Ambrosia elatior, Aristida oligantha, Bromus japonicus, Digitaria sanguinalis, and Helianthus annuus were inhibited by whole plant extracts, except for seed germination of A. oligantha and B. japonicus. Decaying crabgrass had no effects on germination or growth of test plants.

Growth inhibitors were apparently released by crabgrass as root exudates and retarded the growth of most species tested.

These studies demonstrated the existence of three inhibitors, chlorogenic acid, isochlorogenic acid, and sulfosalicylic acid in the whole plant extracts. The quantities of chlorogenic acid and isochlorogenic acid appeared to be greater in extracts made from plants brought in from the field. Sulfosalicylic acid was found only in fresh extracts.

Crabgrass is inhibitory to many of the pioneer species of abandoned fields, including its own seedlings. Sorghum halepense (Abdul-Wahab and Rice 1967), Helianthus annuus (Wilson 1968), and several species of Euphorbia (Rice 1965a, 1965b; Floyd and Rice 1967) are very inhibitory to crabgrass seedlings also. These are all species which are important in the first stage of old-field succession. The rapid disappearance of crabgrass from the weed stage may be due to inhibition by several of the early invaders including crabgrass itself.

Crabgrass did not inhibit the germination of Aristida oligantha seeds, but unlike other species of the weed stage, it was inhibitory to the growth of A. oligantha seedlings. The rapid disappearance of crabgrass from the weed stage, however, would prevent this effect from having any bearing on the invasion of the old-field by A. oligantha.

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