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PLANT INHIBITION BY JOHNSON GRASS AND ITS POSSIBLE SIGNIFICANCE IN PLANT SUCCESSION

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PLANT INHIBITION BY JOHNSON GRASS AND ITS POSSIBLE SIGNIFICANCE IN PLANT SUCCESSION

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CHAPTER I

INTRODUCTION

Booth (1941) reported that succession in abandoned fields in Oklahoma and Kansas includes four stages: (1) weed, (2) annual grass dominated by Aristida oligantha Michx., (3) perennial bunchgrass dominated by Andropogon scoparius Michx., and (4) the climax prairie.

Under certain conditions when Sorghum halepense (L.) Pers. (Johnson grass) is prominent in the field before abandonment, it remains an important species in the early stages of succession. Johnson grass is, of course, perennial and it is an extremely good competitor against many other plants. It often occurs in almost pure stands for rather protracted periods, suggesting that perhaps something more than an excellent ability to compete for light, minerals, and water might be involved.

I hypothesized that Johnson grass might produce

chemical inhibitors which accentuate its competitive ability (Abdul-Wahab 1964). Subsequent work indicated that extracts of the rhizomes of Johnson grass and the soil in the rhizosphere of that species were inhibitory to the growth of the primary root of rice plants.

The present project was undertaken to obtain more evidence concerning the ability of Johnson grass to inhibit certain species of plants with which it is associated in abandoned fields, and to identify the inhibitory compounds produced. Such information should help clarify some of the problems concerning succession in abandoned fields in Oklahoma.

CHAPTER II

HISTORICAL BACKGROUND

Many papers have been published concerning the production by plants of chemical inhibitors of other plants. Only a few will be reviewed briefly to give a bit of historical background to the present project.

Schreiner and Reed (1907, 1908), Schreiner and Shorey (1909), and Schreiner and Lathrop (1911) reported that soil extracts and diffusate of wheat roots inhibited seedlings of wheat, corn, cowpeas, and oats. Fletcher (1912) found that Sesamum indicum L. was inhibited by exudates from roots of Sorghum vulgare Pers. Bedford and Pickering (1914) and Pickering (1917, 1919) reported that different species of grass were fatal to the growth of apple seedlings and trees. Cook (1921) and Massey (1925) reported that tomatoes, potatoes, and alfalfa were inhibited by Juglans nigra L. Davis (1928) extracted the inhibitory substance juglone (5-hydroxynaphthoquinone) from mature J. nigra hulls and roots. Varma (1938) found that the leachates from Brassica alba L., and

Brassica oleracea L. inhibited the growth of these species of Brassica as well as other species. Benedict (1941) reported that Bromus inermis Leyss, produces a substance or substances inhibitory to its own growth.

Went (1942) reported that annual plants did not grow under the branches of the desert shrub Encelia farinosa Gray unless the shrub was dead. He suggested that the shrub probably produces chemical inhibitors. Gray and Bonner (1948) identified the inhibitor, 3-acetyl-6-methoxybenzaldehyde in E. farinosa leaves. Bonner and Galston (1944) found a potent growth inhibitor, trans-cinnamic acid, in water in which guayule roots were allowed to soak briefly. Osvald (1947) discovered that Brassica napus L. and Brassica rapa L. were inhibited by Agropyron repens (L.) Beauv. Goodwin and Taves (1950) found that several coumarin derivatives occurred naturally and had inhibitory properties, and Eberhardt in 1954 isolated scopoletin (7-hydroxy-6-methoxycoumarin) from oat and pea roots (Börner 1960). Audus and Thresh (1956) reported that sunflower shoots and pea roots contain plant inhibitors. Mergen (1959) found that Ailanthus altissima (Mill.) Swingle contained a substance in the leaves and stems which was toxic to many species of plants. Varga and Köves (1959) identified several growth-inhibitory substances (phenolics) from fruits of 24 species of plants. Some of these compounds

were tannic acid, caffeic acid, ferulic acid, chlorogenic acid, p-coumaric acid, and salicylic acid. Börner (1960) isolated ferulic acid, p-coumaric acid, and p-hydroxybenzoic acid from flax seeds. Grümmer and Beyer (1960), and Grümmer (1961) extracted vanillic acid and p-hydroxybenzoic acid from leaves of Camelina Crantz, a genus which is very inhibitory to flax plants. Muller and Muller (1964), Muller (1965), and Muller and del Moral (1966) found that the rapid invasion of some California grasslands by certain shrub species was due at least in part to the production by those shrubs of volatile inhibitors (terpenes).

CHAPTER III

MATERIALS

Johnson grass plants were collected from an open area in Norman, Oklahoma, or grown from seeds collected in the same area. Nine species of plants (Table 3, 4), most of which are important in succession in abandoned fields in Oklahoma, were chosen as test plants for determining the inhibitory activity of Johnson grass.

Extracts were prepared by grinding 10 g fresh weight of Johnson grass leaves or rhizomes in a Waring blendor with distilled water for 10 min, allowing to stand for 30 min 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.

The tops and rhizomes of Johnson grass were collected to a depth of approximately 6 and 2/3 inches (the depth of plowing) in ten 0.25 sq. m quadrats. The air dry weight was found to be 3.65 tons of leaves and culms per acre, and 2.4 tons of rhizomes per acre. This amounted to 1.85 g of leaves

and culms per 454 g of soil, and 1.2 g of rhizomes per 454 g of soil to the depth of plowing. These data were used in determining the effects of decaying Johnson grass plant materials on germination and growth of Johnson grass and six species of plants often associated with Johnson grass.

CHAPTER IV

EXPERIMENTATION AND DISCUSSION

Germination of Johnson grass seeds -- Preliminary tests indicated that Johnson grass seeds (fruits) would not germinate without some treatment to break dormancy. The following treatments were tested to determine their effectiveness in breaking dormancy: (1) seeds were soaked in water for 20 hrs., (2) seeds were leached with water for 20 hrs, (3) seeds were leached with water and aerated for 20 hrs, (4) seeds were soaked in water under vacuum for 20 hrs, (5) seeds were scarified by filing, (6) seeds were scarified by filing and germinated in an aqueous extract of the rest of the spikelets, (7) seeds were scarified with concentrated H_2SO_4 for 10 min, (8) seeds were fed to a cow and collected from the manure, and (9) seeds were stratified in moist sand at 2-5° C for varying periods of times. Tests of germination were all made at 28° C temperature.

The highest percentage of germination was obtained when the seeds were scarified by filing and germinated in

water which indicates that the pericarp acts as a barrier for moisture and/or oxygen, or the expansion of the embryo (Table 1). When the scarified seeds were germinated in the aqueous extract of the rest of the spikelets, a very low percentage of germination was obtained, which indicates the presence of a chemical inhibitor of seed germination in the rest of the spikelets. These results agreed with Harrington's findings (1923) who reported that an increase in germination as a result of removing the scales was especially noteworthy at the warm constant temperatures of 30^{o}, and 35^{o} C.

When seeds were fed to a cow, their percentage of germination was higher than for those scarified with H_2SO_4. Other treatments had no significant effect on germination.

Breaking of dormancy under field conditions was studied by burying seeds in small sacs made of nylon stockings, and testing the germination of groups of seeds every 2 weeks for 28 weeks starting on November 12, 1965. Soil temperatures were measured with a recording thermograph during the study period. Germination tests on all lots of seeds were run under similar laboratory conditions. The highest percentage of germination was reached after a period when there was an alternation of soil temperatures between 18.33^{o} and 28.08^{o} C (Table 2), which agreed with Harrington's findings (1923). The germination percentages were very low

Table 1. Effects of various treatments on the germination of Johnson grass seeds

Treatment	Percent of Germination at 28° C.
Control	0
Seeds soaked in water for 20 hrs	2
Seeds leached with water for 20 hrs	2
Seeds leached and aerated for 20 hrs	0
Seeds soaked in water under vacuum for 20 hrs	2
Seeds scarified by filing	58
Seeds scarified by filing and germinated in aqueous extract of the rest of the spikelets	7
Seeds scarified with H_2SO_4 for 10 min	26
Seeds stratified at 2-5° C.	0
Seeds fed to a cow	36

Table 2. Germination of Johnson grass seeds under field conditions.

	Time in Weeks	Avg. soil temp. °C at 5 cm depth Max.	Min.	Percent Germination
Nov. 12, 1965	0	-----	-----	0
Nov. 26, 1965	2	19.14	15.10	14
Dec. 10, 1965	4	14.44	12.11	10
Dec. 24, 1965	6	11.31	9.64	3
Jan. 7, 1966	8	10.20	7.98	1
Jan. 21, 1966	10	8.58	6.56	1 [a]
Feb. 6, 1966	12	4.54	3.22	0
Feb. 20, 1966	14	10.30	7.25	6
Mar. 6, 1966	16	8.53	6.31	8
Mar. 20, 1966	18	15.30	11.45	0
Apr. 3, 1966	20	17.78	13.43	0
Apr. 10, 1966	21	17.67	13.78	5
Apr. 17, 1966	22	20.00	15.90	10
May 1, 1966	24	18.78	15.44	15 [b]
May 15, 1966	26	27.11	16.67	28
May 29, 1966	28	28.08	18.33	37

a = January 20-28, 1966, 7.5 cm of snow accumulated and snow covered the soil for 4 weeks.

b = April 29, 1966, 10-13 cm of rain.

after periods of low temperatures. Apparently dormancy conditions change very rapidly in these seeds, and high temperatures (or at least moderately high temperatures) are required to break dormancy under natural conditions.

Inhibition of seed germination. -- Two hundred seeds each of seven species of plants were germinated in rhizome or leaf extracts of Johnson grass at room temperature for five days in the dark in a 1:5 ratio of nutrient solution (Hoagland and Arnon 1950) to plant extract. Controls were run with a 1:5 ratio of nutrient solution to distilled water. Both rhizome and leaf extracts caused a statistically significant reduction in percentage of germination in Amaranthus retroflexus L., Digitaria sanguinalis (L.) Scop., Setaria viridis (L.) Beauv., and Lycopersicon esculentum Mill. (tomato) (Table 3). Germination of seeds of Aristida oligantha Michx. and Bromus japonicus Thunb. was slightly inhibited, while seeds of Bromus tectorum L. were not affected. Both types of extracts had virtually the same inhibitory effect on germination and seedling growth.

Seedling inhibition. -- Seedlings of eight 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 to plant extract and were

Table 3. Percent germination of seeds grown in rhizome or leaf extracts of Johnson grass for 5 days.

Plants	Control	Rhizome Extract	Leaf Extract
Amaranthus retroflexus	78.5	52.0	58.0
Aristida oligantha	62.0	68.0	54.0
Bromus japonicus	95.0	86.0	87.0
B. tectorum	99.0	96.0	95.0
Digitaria sanguinalis	28.0	7.0	0.5
Setaria viridis	11.0	1.0	1.0
Lycopersicon esculentum	14.0	3.5	3.5

allowed to grow for 10 days in a photoperiod of 16 hours at 27° C, and a night temperature of 20° C. Controls were run with a 1:5 ratio of nutrient solution to distilled water under the same condition. Both rhizome and leaf extracts significantly reduced the oven-dry weight of the seedlings of all species except Chenopodium album L. (Table 4). This indicates that seedlings of C. album may survive and grow in a stand of Johnson grass much better than seedlings of the other species tested. The significant inhibition of B. tectorum and B. japonicus seedlings indicated that, although their seed germination was not inhibited by these extracts, these plants may not become established within a stand of Johnson grass. A. oligantha and D. sanguinalis were least inhibited in growth, with the exception of C. album. These results agreed with the observed situation as it occurs in the field.

Seedling inhibition by decaying Johnson grass. -- Seeds of Johnson grass and six species of plants often associated with Johnson grass were germinated in pots containing soil mixed with either 1.85 g of air dried leaves per 454 g of soil, 1.2 g of air dried rhizomes per 454 g of soil, or 1.85 g of washed, air dried peat moss per 454 g of soil for controls. The percentage of germination was determined for one week,

Table 4. Effect of rhizome or leaf extracts on seedling growth.

Plant name	Exp. No.	Oven-dry weight, g			F_s
		Control	Rhizome Extract	Leaf Extract	
Amaranthus	1	0.0321	0.0010[ab]	0.0021[ab]	1038.00
retroflexus	2	0.0325	0.0013[ab]	0.0022[ab]	788.50
Aristida	1	0.0432	0.0270[ab]	0.0172[ab]	90.74
oligantha	2	0.0427	0.0270[ab]	0.0166[ab]	22.42
Bromus	1	0.0430	0.0070[ab]	0.0150[ab]	170.19
japonicus	2	0.0440	0.0078[ab]	0.0160[ab]	189.63
Bromus	1	0.0380	0.0070[ab]	0.0140[ab]	189.18
tectorum	2	0.0385	0.0065[ab]	0.0145[ab]	115.58
Chenopodium	1	0.0100	0.0092	0.0097	2.10
album	2	0.0110	0.0096	0.0098	1.17
Digitaria	1	0.0365	0.0108[a]	0.0106[a]	39.63
sanguinalis	2	0.0373	0.0115[a]	0.0111[a]	23.72
Helianthus	1	0.1195	0.0205[ab]	0.0460[ab]	191.47
annuus	2	0.1205	0.0220[ab]	0.0465[ab]	361.75
Setaria	1	0.0225	0.0053[a]	0.0047[a]	12.77
viridis	2	0.0228	0.0056[a]	0.0049[a]	29.31

a = Dry weight significantly different from that of control.

b = Significant difference between rhizome extract and leaf extract treatments.

and the oven-dry weights of the seedlings were taken after three-weeks. In another experiment, pots containing either Johnson grass plant materials or peat moss were allowed to stand for six months, after which the same species used in the other two experiments were planted. The percentage of germination was determined for one week, and the oven-dry weights of the seedlings were taken after four weeks.

Decaying Johnson grass plant materials appeared to exert at least some inhibitory activity on seed germination of most of the seven species (Table 5). Both decaying rhizomes and leaves exhibited considerable inhibitory activity against seed germination of A. retroflexus and S. halepense. In general, decaying rhizomes inhibited seed germination more than decaying leaves.

Both decaying rhizomes and leaves significantly inhibited the seedling growth in soil of all species except A. oligantha (Table 6). There was a significant difference also between the inhibitory effects of decaying rhizomes and the effects of decaying leaves against all species except B. japonicus and S. viridis. The decaying rhizomes were most inhibitory against about half the test species, whereas decaying leaves were most inhibitory against the other half. Decaying leaves were more inhibitory to Johnson grass seedlings themselves than were decaying rhizomes.

Table 5. Percent germination of seeds of different species of plants in decaying Johnson grass material.[a]

	Control			Rhizome			Leaf		
	Expt. 1	Expt. 2	Expt.[b] 3	Expt. 1	Expt. 2	Expt.[b] 3	Expt. 1	Expt. 2	Expt.[b] 3
Amaranthus retroflexus	90	63	56.6	60	33	16.6	57	59	50.0
Setaria viridis	53	40	--	53	33	--	40	40	--
Digitaria sanguinalis	60	56	--	60	36	--	47	53	--
Aristida oligantha	40	64	85.8	10	59	69.3	20	59	85.8
Bromus tectorum	90	86	90.2	83	64	75.9	90	86	75.9
Bromus japonicus	97	89	92.4	73	59	92.4	90	89	90.2
Sorghum halepense	60	53	56.1	53	33	29.7	43	33	36.3

a = Comparisons should be made between experiments which are numbered the same.

b = Plant material mixed with soil and left to be decayed for 6 months.

Table 6. Effect of decaying Johnson grass material on growth of species of plants often associated with Johnson grass.

Plant name	Expt. No.	Oven-dry weight, g			F_s
		Control	Rhizome	Leaf	
Amaranthus retroflexus	1	0.0862	0.0086[ab]	0.0517[ab]	17.36
	2	0.0850	0.0071[ab]	0.0480[ab]	53.38
	3[c]	0.2505	0.1100[ab]	0.1785[ab]	23.28
Aristida oligantha	1	0.0137	0.0126[b]	0.0074[ab]	6.88
	2	0.0140	0.0120	0.0085	1.60
	3[c]	0.0505	0.0230[ab]	0.0370[ab]	10.50
Bromus japonicus	1	0.0139	0.0085[a]	0.0078[a]	7.25
	2	0.0193	0.0103[a]	0.0100[a]	9.22
	3[c]	0.0555	0.0310[a]	0.0375[a]	10.06
Bromus tectorum	1	0.0107	0.0090[ab]	0.0073[ab]	4.14
	2	0.0225	0.0115[ab]	0.0160[ab]	4.65
	3[c]	0.0720	0.0435[a]	0.0405[a]	11.65
Digitaria sanguinalis	1	0.0583	0.0244[ab]	0.0067[ab]	41.02
	2	0.0600	0.0230[a]	0.0069[a]	45.49
	3[c]	0.4820	0.1900[a]	0.2770[a]	23.89
Setaria viridis	1	0.0420	0.0069[a]	0.0061[a]	324.46
	2	0.0747	0.0085[a]	0.0412[a]	9.43
	3[c]	0.3280	0.1240[ab]	0.2615[ab]	28.50
Sorghum halepense	1	0.0701	0.0462[ab]	0.0160[ab]	22.69
	2	0.1950	0.0705[a]	0.0661[a]	25.63
	3[c]	0.2720	0.1380[a]	0.1730[a]	17.07

a = Dry weight significantly different from the control.

b = Significant difference between rhizome and leaf treatments.

c = Plant material mixed with soil and left to be decayed for 6 months.

When Johnson grass plant materials were allowed to stand for 6 months, their inhibitory activity was more pronounced on seedling growth of all species including A. oligantha which was only slightly affected by these materials in the first two experiments.

Effects of root and rhizome exudate on seedling growth. -- Fourteen-day-old seedlings of plants to be tested were placed in glass vials through which culture solution was circulated (Fig. 1). The vials for the control plants were connected to a pot containing just quartz sand and the test vials were connected to a pot containing quartz sand in which Johnson grass was growing. A mineral solution was allowed to drip from a supply reservoir into the pot containing Johnson grass or into the control pot. These solutions were allowed to pass through the vials by gravitational force and then into collecting reservoirs. The solutions were then pumped back to the supply reservoirs so the cycle could continue over a 4-hour period each day.

The oven-dry weight of the plants was determined after 10 days growth (Table 7). The exudate caused a significant reduction in growth of A. retroflexus and S. viridis but not of B. japonicus.

Identification of inhibitors. Procedures used for identi-



Figure 1. Set-up to test the effect of Johnson grass exudate: (a) pot containing quartz sand in which Johnson grass is grown,(b)pot containing just quartz sand (control),(c)vials containing test plants and circulating solution,(d)vials containing circulating mineral solution and control plants,(e & f) exit tubes leading to collecting reservoirs, (g & h) tubes through which solutions are pumped from collecting reservoirs,(i & j)reservoirs from which the solutions drip into pots.

Table 7. The effect of Johnson grass root and rhizome exudate on seedling growth

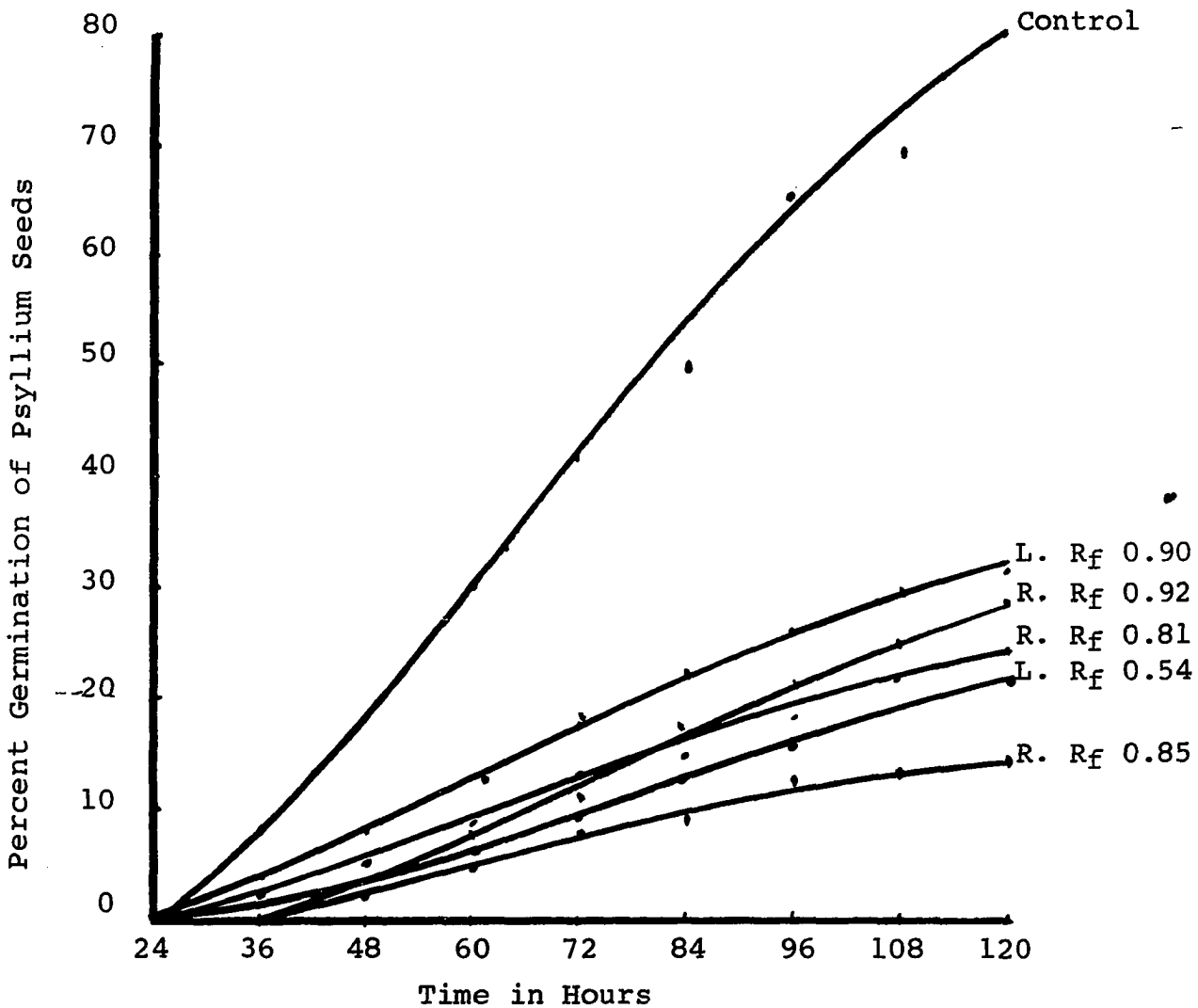
Treatment	Mean oven-dry weight, g		
	Amaranthus retroflexus	Setaria viridis	Bromus japonicus
Control	0.103	0.188	0.012
Exudate	0.070[a]	0.128[a]	0.008
F_s	8.670	7.080	1.500

A = Significant difference between control and exudate treatments.

fications were basically those of Rice (1965). -- Extracts of Johnson grass rhizomes or leaves were acidified to pH 2.5 with 1 N HCl and extracted with two half volumes of diethyl ether. The ether was evaporated and the residue was taken up in 10 ml of 95% ethanol. The aqueous fraction remaining after extraction with ether was evaporated to dryness in vacuo, and taken up in 10 ml of 50% aqueous methanol. The ether and water fractions were chromatographed on Whatman 3 MM paper in one dimension with n-butanol-acetic acid-water (63:10:27-called BAW). The chromatograms were examined with short (2537Å) and long (3360Å) ultraviolet light. Three distinctinve bands were present on the chromatograms of rhizome extracts and one band on the chromatograms of the leaf extracts. These bands were cut from the chromatograms and eluted with 50% aqueous methanol.

The biological activity of the eluates was determined by the chestnut brown psyllium seed bioassay (Lane 1965). All bands were found to be inhibitory (Fig. 2).

Eluates of equivalent bands were reduced to dryness, taken up in 5 ml 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 different



L. = Leaf
R. = Rhizome

Figure 2. Inhibitory Activity of Bands from Chromatograms

solvent systems, colors in U.V. light, and reactions with various reagents (Rice 1965) indicated that the inhibitory compounds were chlorogenic acid, p-hydroxybenzaldehyde, and p-coumaric acid (Table 8). The activity of these chemicals obtained from a commercial source was determined by the psyllium seed bioassay using a 2 mg/ml concentration of each. Germination expressed as percent of control germination was as follows: (1) chlorogenic acid, 7.1; (2) p-hydroxybenzaldehyde, 10.7; and (3) p-coumaric acid, 3.6.

The suspected p-hydroxybenzaldehyde was the most prominent inhibitor present and the most persistent at different seasons. Therefore it was eluted from the chromatograms with 85% ethanol and the absorption spectrum was determined with a Beckman Model DU spectrophotometer. The spectrum of known p-hydroxybenzaldehyde in 85% ethanol was similarly determined. Maximum absorption occurred at 283 mμ in both cases (Fig. 3).

The presence of p-hydroxybenzaldehyde in the extracts of leaves and rhizomes of Johnson grass at all sampling periods suggested the possibility that dhurrin, a cyanogenic glucoside of p-hydroxybenzaldehyde, might be present as previously reported for Sorghum vulgare by Dunstan and Henry (1902). This possibility was checked by use of the procedure suggested by Koukol, Miljanich, and Conn (1962).

Table 8. Chromatography of inhibitors from Johnson grass

Compound	R_f's on Whatman[a] 3 MM			Fluorescence[c]				p-nit	Reagent colors[b,c]		
				Long U.V.		Short U.V.			Sulfan. acid	$FeCl_3$ $K_3Fe(CN)_6$	Hoepfner reaction
	BAW	IBW	6%AA	$-NH_3$	$+NH_3$	$-NH_3$	$+NH_3$				
Chlorogenic acid	0.51	0.57	0.63	l bl	yel-gr	l bl	yel-gr	br	yel-tan	dk bl	+
Inhibitor 1	0.52	0.56	0.63	l bl	yel-gr	l bl	yel-gr	br	yel-tan	dk bl	+
p-Hydroxy-benzalde-hyde	0.89	0.94	0.77	-	dk pur abs	pur abs	dk pur abs	f red	yel-tan	f bl	-
Inhibitor 2	0.89	0.95	0.75	-	dk pur abs	pur abs	dk pur abs	f red	yel-tan	f bl	-
p-Coumaric acid	0.88	0.93	0.54	-	bl	pur abs	bl	dk gray-viol	red-br	dk bl	-
Inhibitor 3	0.87	0.92	0.56	-	bl	pur abs	bl	dk gray-viol	red-br	dk bl	-

a = See text for solvent systems. R_f's are averages of three 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; pur, purple, br, brown; viol, violet; dk, dark; abs, absorption; f, faint.

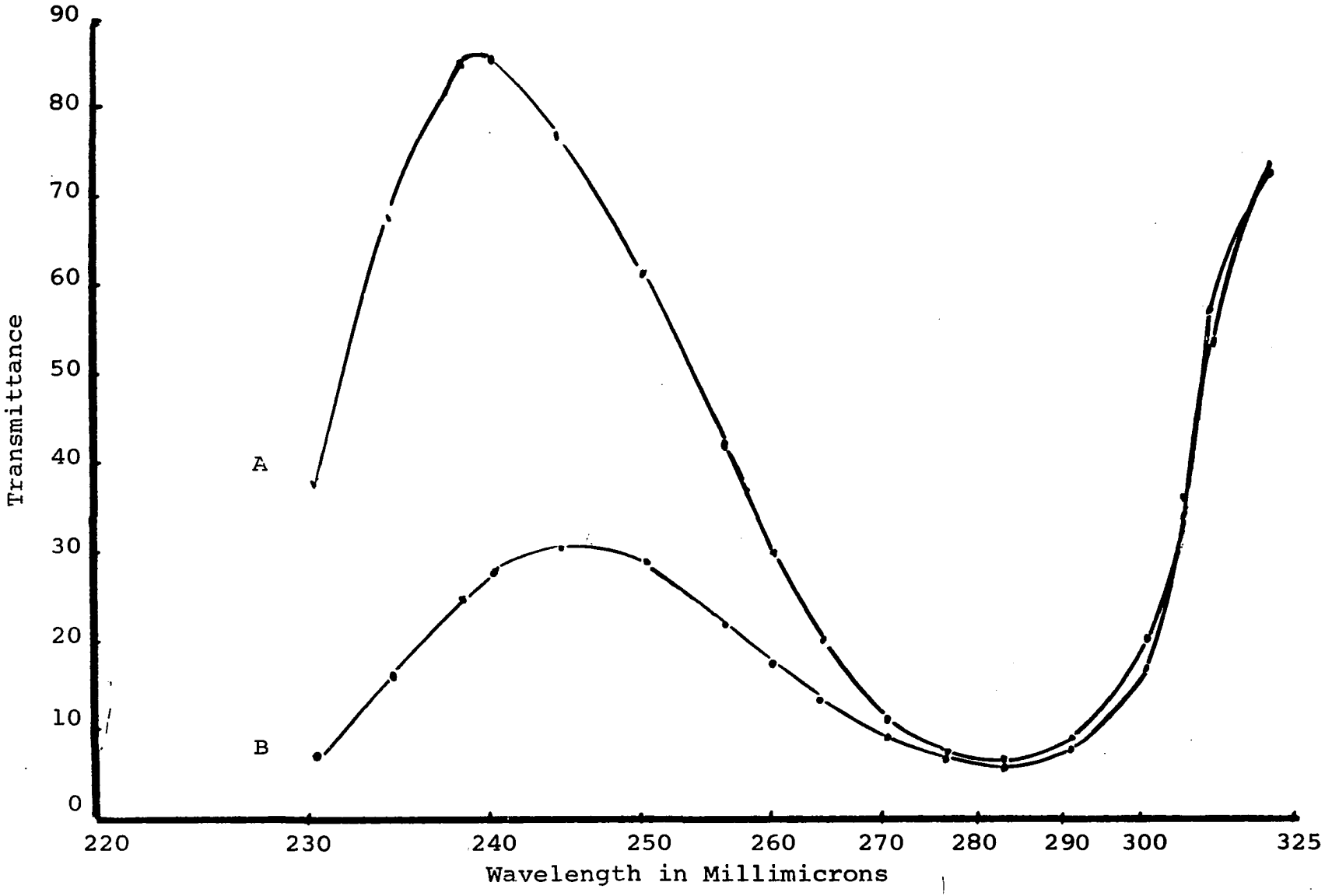


Figure 3. Absorption Spectra in 85% Ethanol: (A) p-hydroxybenzaldehyde, (B) suspected p-hydroxybenzaldehyde from Johnson grass rhizome.

Freshly ground rhizomes were extracted immediately in boiling 80% ethanol, the solution was filtered, and the filtrate was concentrated *in vacuo* to 10 ml. This solution was extracted with 10 ml of petroleum ether three times, mixed with 10% aqueous trichloroacetic acid (v/v), and centrifuged for 10 min to remove the proteins. This solution was reduced to dryness *in vacuo* and taken up in 5 ml of distilled water. The material was chromatographed on Whatman 3 MM paper in n-butanol-pyridine-water (6:4:3- called BPW), and the suspected dhurrin (R_f 0.79) was eluted with 40% aqueous methanol, and extracted with three half volumes of diethyl ether to remove any p-hydroxybenzaldehyde present. The methanol fraction was reduced to dryness *in vacuo*, the residue was taken up in 60% aqueous methanol, and aliquots were chromatographed on Whatman 3 MM paper with two different solvents, BAW and BPW. R_f's in different solvents, colors in U.V. light, and reactions with various reagents were determined (Table 9). The structural formula of dhurrin is given below.

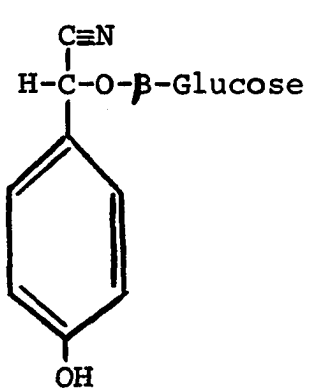


Table 9. Characteristics of suspected dhurrin

U.V. light				R_f's[a] 3 MM Paper		Visible $+NH_3$	$FeCl_3$-$K_3Fe(CN)_6$	Sulfan acid	Benzidine	p-Nit.
Short		Long								
$+NH_3$	$-NH_3$	$+NH_3$	$-NH_3$	BPW	BAW					
gr-br	pur-br	gr-br	None	0.79	0.80	gold-br	None	yel-tan	None	dk red

a = R_f's are averages of three runs.

The purified suspected dhurrin was hydrolyzed with 1 N HCl for one hour, the hydrolysate was ether extracted, and the ether fraction was chromatographed on Whatman 3 MM paper in BAW. A zone resulted with all the characteristics of p-hydroxybenzaldehyde listed in Table 7. The suspected p-hydroxybenzaldehyde was eluted with 85% ethanol and the absorption spectrum was determined on a Beckman DU spectrophotometer. Maximum absorption occurred at 283 mμ just as for known p-hydroxybenzaldehyde in 85% ethanol.

Some of the suspected dhurrin was hydrolysed for one hour at 30° C with a 2 mg/ml β-glucosidase solution in a citrate buffer at pH 4.9 (1 ml per ml of the suspected dhurrin solution). The hydrolysate was chromotographed on Whatman 3 MM paper with BAW in the first dimension, and BPW in the second dimension. The papers were dipped in a benzidine sugar reagent (Smith 1960, p. 250). Comparison with known glucose indicated that glucose was definitely produced by the hydrolysis (Table 10). Evidence at this stage certainly indicated that the suspected dhurrin was actually dhurrin because hydrolysis resulted in the production of p-hydroxybenzaldehyde and glucose.

As a further check, the method suggested by Akazawa, Miljanich, and Conn (1960), and the Liebig method described by Willard, Furman, and Bricker (1956) were used to test

Table 10. Chromatography of glucose and hydrolysate of suspected dhurrin

	R_f on 3 MM Paper[a] BAW	BPW	Benzidine reagent
Glucose	0.18	0.51	Brown
Glucose from suspected dhurrin	0.17	0.52	Brown

a = R_f's are averages of three runs.

for the production of HCN on hydrolysis. HCN was definitely found to be produced and the yield was 9.6×10^{-4} M of CN in 10 g of rhizomes. The method of Friedmann and Haugen (1943) was used to determine the presence of p-hydroxybenzaldehyde, and the result was positive. The U.V. light absorption curve of the hydrolysate resulting from hydrolysis with 0.1 N NaOH was determined also, because according to Akazawa, Miljanich, and Conn (1960), p-hydroxybenzaldehyde in 0.1 N NaOH strongly absorbs light at 330 mμ. Maximum absorption of known p-hydroxybenzaldehyde in 0.1 N NaOH and of the hydrolysate were found to occur at 330 mμ, thus indicating that p-hydroxybenzaldehyde was present in the hydrolysate.

The evidence from all procedures indicated, therefore, that dhurrin was definitely present in leaves and rhizomes of Johnson grass and in considerable quantities.

CHAPTER V

CONCLUSIONS

These studies demonstrated the existence of three plant inhibitors, chlorogenic acid, p-coumaric acid, and p-hydroxybenzaldehyde, in the rhizome and leaf extracts of Johnson grass. p-Hydroxybenzaldehyde was present in the extracts of leaves and rhizomes at all sampling periods, chlorogenic acid was more pronounced in the leaf extracts, and p-coumaric acid was present only during the early months of the growing season.

Dhurrin, the cyanogenic glucoside of p-hydroxybenzaldehyde, was found to be present in considerable quantities in the rhizomes, and probably serves as a source of p-hydroxybenzaldehyde.

According to the findings of Koukol et al. (1962) p-coumaric acid may be converted to dhurrin by a series of reactions not involving tyrosine. There may be a definite pathway involved, therefore, from p-coumaric acid to dhurrin to p-hydroxybenzaldehyde.

Seed germination and seedling growth of all species of plants chosen for investigation were inhibited by leaf or rhizome extracts and decaying leaves and rhizomes with the exception of A. oligantha which was inhibited in only a few instances and then mostly slightly. Exudates from roots and rhizomes were inhibitory to seedling growth of most species tested, also.

The fact that Johnson grass is very inhibitory to many early invaders of abandoned fields, including its own seedlings, and is much less inhibitory to A. oligantha may help to explain why the primary weed stage is replaced by A. oligantha which is the only dominant in the second stage of succession. The pronounced inhibition by Johnson grass of pioneer species which normally occur in our abandoned fields certainly explains why Johnson grass is able to persist as long as it does, and in almost pure stands in many cases.

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