A STUDY OF CERTAIN MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF JOHNSONGRASS

by

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INTRODUCTION

Johnsongrass (Sorghum halepense L. Pers.) has become a serious weed problem in Kansas. Although an important forage crop in some areas of the South, it is considered a noxious weed in many states. In Kansas, it has been declared a noxious weed by 80 counties under provisions of the Kansas Weed Law. Efforts are being made to control johnsongrass by cultural practices and applications of different herbicides. The increasing importance of this weed has necessitated study of its physiological and morphological characteristics.

The objectives of this study were multipurpose: (1) to determine the longevity of rhizomes, (2) to investigate the effect of different temperatures on rhizomes, (3) to determine the soil depth from which shoots will emerge, (4) to study the effect of rhizome extract upon germination of crop seeds, and (5) to compare the HCN content of johnsongrass with sudangrass (Sorghum vulgare var. sudanense (Piper) Hitchc.) under normal conditions and conditions of low and high temperatures.

Johnsongrass spreads vegetatively by vigorous rhizome development. It is known that rhizomes are one of the important modes of its dissemination. Rhizomes have been classified according to age by Cates and Spillman (4) and Johnson (18). It is desirable to have accurate information with respect to their longevity. In this study the longevity of rhizomes has been investigated.

Climatic conditions where johnsongrass is a problem are variable. Low temperatures in the northern states prevent johnsongrass from overwintering there. A knowledge of rhizome response to temperature variations is of value in a control program.

Root extracts of some plants have been shown to contain germination and growth inhibitors. Certain plants contain substances which spread slowly into the soil and inhibit the germination of seeds or growth of seedlings. A study was made to determine the possible occurrence of inhibitors in the extract obtained from johnsongrass rhizomes.

Several cases have been reported where cattle have been poisoned from grazing certain plants, and two of the commonly reported plants include johnsongrass and sudangrass. Various methods are available that make it possible to determine the HCN content of these grasses. In this study, attempts were made to compare HCN content of these grasses at various stages that were grown under similar conditions.

REVIEW OF LITERATURE

Longevity of Rhizomes

The first work reported on rhizome longevity in johnsongrass was by Cates and Spillman (4) early in the 20th century. They classified rhizomes as primary, secondary, and tertiary. They distinguished primary rhizomes as those that survive the winter and resume growth the following season; secondary rhizomes as those originating from primary rhizomes, then growing

upward to produce new crowns; and tertiary rhizomes as those which originate from new crowns. They found that primary and secondary rhizomes decompose each year leaving only the tertiary rhizomes which overwinter as living structures. Johnson (18) classified the overwintering rhizomes as primary root stocks, the shallow lateral root stocks as secondary, and the deep, vertical root stocks as tertiary. He observed that secondary and tertiary root stocks developed one year to become the primary root stocks the following year. Pollock (34) found that root stocks produced in a given year gave rise to new plants the succeeding year.

Temperature and Growth of Rhizomes

In the early 20th century it was known that frost had a detrimental effect on johnsongrass rhizomes. Winter fallow was a common practice used to kill rhizomes by exposure. Ball (1) found that ordinarily two plowings were sufficient for a satisfactory kill. Johnson (18) observed that top growth was killed back by frost, and in sufficiently cold climates, many of the shallow lateral rhizomes were also killed.

Ingle and Rogers (17) found that when isolated segments of johnsongrass rhizomes were cultured on sterile, moistened vermiculite, bud response to environmental factors could be observed. During their preliminary study they found that diurnal variations in temperature promoted sprouting, and that increases in temperature up to 90°F increased bud growth.

Extract of Johnsongrass Rhizome

Factors involved in weed and crop competition for moisture, light, and mineral elements are well known. This competition may not provide all the answers as to why weeds can be highly competitive to crops. Prior to 1900 agricultural workers proposed a partial explanation for the stimulating and/or depressing effects of one plant on the other as due to harmful substances excreted by roots or to materials liberated when roots or tops decayed. Helgeson and Konzak (13) in their preliminary studies found that aqueous extracts of field bindweed (Convolvulus arvense L.) and Canada thistle (Cirsium arvense Scop.) inhibited seedling growth and germination of wheat (Triticum aestivum L.), flax (Linumusitatissimum L.), alfalfa (Medicago sativa L.), and oats (Avena sativa L.). The increased concentration of phytotoxic solution resulted in a progressive decrease in germination and growth of roots and shoots. The inhibitory action of weed extracts tested varied with plant parts. They also found that variation in germination temperature affected the inhibitory action of the phytotoxin on both germination and top growth of all crop seeds included in the study. In studying the phytotoxic effects of aqueous extracts of leafy spurge (Euphorbia esula L.), LeTourneau (23) observed that aqueous extracts of stems and leaves of dried, leafy spurge contained some substances which inhibited radicle growth of germinating peas (Pisum sativum), wheat, and tomatoes (Lycopersicum esculentum Mill) and

inhibited the germination of wheat and flax. Seeds which did not germinate in the presence of these extracts regained their ability to germinate if removed from the extract. Extracts had no effects when applied to beans (Phaseolus vulgaris L.) and tomatoes growing in the greenhouse. Helgeson (10), while studying the phytotoxic materials in several weeds, concluded that in general Canada thistle extracts were the most toxic, followed by field bindweed, willow (Salix), and leafy spurge. LeTourneau et al. (24) have reported the presence of growth and germination inhibitors in various plants. They found that aqueous extracts of dried plant material representing 23 species inhibited wheat germination and growth of wheat and pea seedlings. Hamilton and Buchholtz (9) observed that there usually was a delay of about one month in the maximum emergence period of weedy species on the plots having live quackgrass (Agropyron repens L.) rhizomes as compared to their emergence on plots with no living rhizomes. Helgeson and Green (12) concluded that the whole seed extract of wild oats (Avena fatua L.) had little or no effect on germination of wheat but did reduce the growth of roots at all concentrations used. Seedling shoots were less sensitive to the inhibitor than were roots. With further dilutions there was some stimulation of shoot growth. Leaf extracts reduced germination at 1:10 dilution and were extremely toxic to tops and roots. At 1:30 dilution top growth was stimulated, but roots were adversely affected. Stem extracts (upper node to base of first panicle branch) reduced seed germination by about half, and nearly prevented growth of seedlings at a 1:10 dilution.

At dilutions up to 1:30 germination was slightly reduced, and seedling growth was definitely inhibited.

Several workers have attempted to determine the toxic effects of quackgrass on germination of seeds and growth of seedlings. Kommedahl et al. (21) found that in field tests both stands and dry weights of alfalfa were half as great when it was seeded in soil previously occupied by quackgrass as when seeded in quackgrass-free soil. In the greenhouse, the addition to soil of dried rhizomes from quackgrass resulted in severe stunting and chlorosis of alfalfa seedlings, reduction in stand of alfalfa seedlings, and a delay in germination of alfalfa seeds. Furthermore, Kommedahl et al. (20) found that in all tests the differences were apparent after a week, and stands in all pots were virtually the same except in pots of non-sterile soil. They concluded that the only noticeable effect was a delay in germination. LeTourneau and Heggeness (25) observed that an aqueous extract of quackgrass rhizomes inhibited root growth of pea and wheat seedlings. Increasing the amount of rhizome material increased the inhibition. Higher concentrations inhibited wheat coleptile growth and germination of wheat kernels. Helgeson (11), while studying the effect of quackgrass root extract on the germination of several crop seeds, found that extract generally had little effect on the germination percentage. The more concentrated extract (1:10) had little or no effect on growth of roots, but the more dilute extract showed a definite stimulation of growth with a tendency to a greater stimulation as the extract became more dilute.

HCN Content of Johnsongrass and Sudangrass

The fact that sorghums may be toxic to animals has been recognized since 1838. Pister (33) observed rapidly growing new leaves, badly trampled plants, or wilted cuttings to be most dangerous for animals. Klosterman et al. (19) concluded that danger from poisoning is high immediately after a frost. Although freezing does not increase HCN content as such, new growth following injury generally is more dangerous. Sautter and Spurrell (35) found lethal doses to be 0.5 gram of cyanide in five pounds of johnsongrass. Huffman et al. (16) found that the concentrations in sorghums are toxic under certain conditions. Sorghums in the mature stage do not contain any appreciable amount of potential HCN, but the young plant or suckers of mature sorghums may be high in hydrocyanic acid.

Because of dangers to livestock due to this toxicity, a considerable amount of research has been done on methods of determining the amount of HCN in various parts of the plants. Nowosad and MacVicar (30) concluded that determination is based on the evolution of hydrocyanic acid. According to them, the reaction of acid will occur when it comes in contact with filter paper treated with an alkaline picrate solution. Hogg and Ahlgren (14) later modified the above method which made the determination more rapid and simple. They found that more accurate results could be obtained by using a colorimeter with suitable dilution series as checks. The test has been found sufficiently accurate quantitatively for the selection of plants low in HCN acid.

Several workers have attempted to determine the amount of HCN in johnsongrass and sudangrass. Martin and Couch (27) determined the HCN content of different parts of sorghum plants, and found that the HCN content of sorghum leaves was three to 25 times greater than that of the corresponding stalks of plants that had reached the boot stage. Heads and leaf sheaths were low in HCN content; upper leaves contained more HCN than lower leaves.

Franzke et al. (8) observed that sorghum in the early succulent stages of growth had a relatively high content of HCN, whereas plants harvested at more mature stages were likely to have a lower HCN content. They found that the shooting stage produced an average of 8,970 ppm., while the same strains in the late dough stage were found to contain an average of 2,490 ppm. of HCN. Norris and Valentine (29) found that young leaves and stems contain the greatest concentration of the poison. Swanson (36) reported that practically all the HCN was confined to the leaves; he did not find HCN in well developed stems. More HCN was found in younger plants than in the more mature ones. It was also observed that a high concentration of HCN is present when the plant is in vigorous growing condition. Casady (3) found the amount of HCN is greatest in young plants, and decreases as the plant becomes older. The amount of HCN content declined sharply when the plants were 20 to 25 days old, after 35 days HCN was very low. Patel (31) observed that the highest concentration of HCN was obtained in 20-day-old plants irrespective of soil type.

Several workers have investigated the effect of different environmental factors on HCN. Boyd (2) did not obtain any increase in the HCN content of sudangrass subject to either drought or frost. Swanson (36) and Patel (32) showed a reduction in the HCN content of frosted plants. Franzke and Hume (7) found that increase in soil moisture levels produced a decreased HCN content. They observed the greatest decrease in HCN content of sorghum plants where the application of manure and increase in soil moisture were combined.

Favero (6) in his studies showed that HCN content decreased with later stages of development. He found considerable variation in HCN content in plants in an advanced stage of development which appeared to be related more to climatic conditions than to the actual planting. It was also observed that following a period of low rainfall and high temperature, the HCN content in plants at the boot stage to the end of the flowering stage was relatively high. Patel (32) in his study of the effect of fertility and soil types on the production of HCN concluded that varieties of sudangrass and sorghums inherently low or high in their content of HCN retained their relative position with respect to this constituent under any given set of conditions.

Hogg and Ahlgren (15) concluded that there was a positive correlation between temperature and HCN. On the contrary, Patel (32) later reported more HCN in sorghum and sudangrass plants grown at low temperatures than at high temperatures.

Several attempts have been made to establish new varieties of sudangrass low in HCN. Coleman and Robertson (5), in their progeny (S_3 and S_4 lines) test indicated that the differential ability to produce HCN may be inherited in inbred lines of sudangrass. They also reported that soil and seasonal differences seem to influence the production of HCN in inbred lines of sudangrass. Muckenhirn and Powers (28) found the new variety of sudangrass (Piper) low in HCN. Tesar (37) tested six varieties of sudangrass and found differences existed between them.

MATERIALS AND METHODS

Longevity of Rhizomes and Depth Relation to Shoot Emergence

Three rhizomes having 15, 16, and 19 nodes were selected from a single johnsongrass plant for this study. Plastic-covered wires were loosely wrapped around the internodes, and on January 18, 1958, the rhizomes were planted separately in three tubs containing soil. When the plants reached maturity, they were removed from the tubs, and the soil was carefully washed out of the roots and rhizomes (Plate I). The rhizome segments marked by wire loops were removed, examined, and again planted in a tub of soil (Plate II). After a three-month period the rhizomes were again removed from the soil and observed (Plate III).

Depth in Soil in Relation to Shoot Emergence

Rhizomes were obtained from dormant plants in the field. They were placed in containers of soil compacted at depths of

EXPLANATION OF PLATE I

Rhizomes and root system developed from a single, year-old segment of johnsongrass rhizome.

PLATE I

EXPLANATION OF PLATE II

Original rhizome segments after removal of newly developed rhizomes and roots.

PLATE II

EXPLANATION OF PLATE III

Condition of rhizomes pictured in Plate II three months after replanting.

PLATE III

2, 4, 6, 8, 10, 12, 14, and 16 inches in an attempt to determine the maximum depth from which shoots could emerge. After a period of 42 days the rhizomes were removed, the soil washed out and observations of shoot development were made.

Temperature Effect on Rhizomes

This study was conducted in the greenhouse during the fall, spring, and summer of 1957 and 1958. Johnsongrass rhizomes of six nodes each were obtained from a field near Manhattan, Kansas.

An attempt was made to obtain rhizomes of uniform length, diameter, and weight. These were kept in a cold room for 24 hours at 16, 20, 24, 28, 32, and 36°F after which they were immediately embedded in vermiculite in metal pans. Six rhizomes were included as a single replication in each pan for each temperature used. Three replications were used for each treatment. Emerging shoots were counted at weekly intervals for a five-week period (Plate IV). The rhizomes exposed to the 16, 20, and 24°F temperatures were discarded early due to decay.

In an attempt to determine more accurately the range of effective killing temperature, a second series of rhizomes were exposed to temperatures of 31, 32, and 33°F. These rhizomes were then placed in vermiculite to observe shoot development. Observations were made over a five-week period (Plate V).

Rhizome Extract and Its Effect on Seedlings of Various Crops

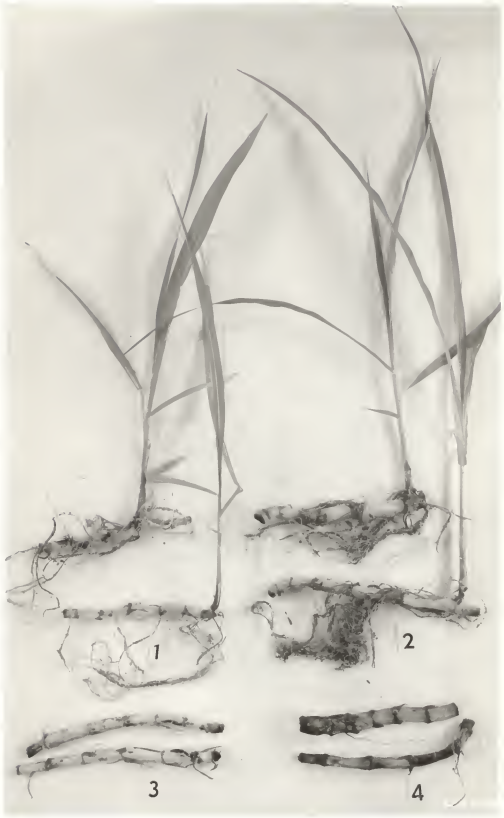
Alfalfa, corn (Zea mays L.), grain sorghum (Sorghum vulgare Pers.), oats, soybeans (Glycine max (L.) Merr.), wheat, and

EXPLANATION OF PLATE IV

Effect of different temperature treatments on winter dormant rhizomes, 35 days after planting:

Fig. 1. Control
Fig. 2. Exposed to 36°F
Fig. 3. Exposed to 32°F
Fig. 4. Exposed to 28°F

PLATE IV



EXPLANATION OF PLATE V

Effect of different temperature treatments on actively growing rhizomes obtained in summer, 35 days after planting:

Fig. 1. Control
Fig. 2. Exposed to 33°F
Fig. 3. Exposed to 32°F
Fig. 4. Exposed to 31°F.

PLATE V

winter barley (Hordeum vulgare L.) were planted in four-inch pots. For each crop 12 such pots were used. Twelve kernels of corn and grain sorghum, and 12 seeds of soybeans, 18 seeds of oats, wheat, and winter barley, and 24 seeds of alfalfa were planted separately in each pot on January 24, 1958. An extract was obtained from dormant johnsongrass rhizomes which were crushed and diluted with distilled water. The diluted extract was applied to the soil in nine pots of each crop at a rate of 35 ml. per pot on January 27, 1958. Three pots were left untreated. Records were kept of emergence and seedling growth for each pot.

A similar study was initiated on June 7 when extract from actively growing rhizomes was applied to soil in pots planted with the previously mentioned crops. In addition, seeds of each crop were soaked in the extract for 24 hours after which they were planted. Extract was also applied to other pots when the first seedlings were visible. Data were recorded from June 11 through July 1, 1958.

Comparison of HCN content of Johnsongrass and Sudangrass

Johnsongrass and sudangrass plants to be used in this study were grown in pots in the greenhouse. The HCN content for each species was determined at weekly intervals in the youngest leaf of each plant. This procedure continued throughout the period when plants were three- to 12-weeks-old. The reagents and standards were prepared as follows. Determinations were made by using the picric acid test. The alkaline picrate solution

was prepared by dissolving 25 grams of Na_2CO_3 and five grams of picric acid in 1000 ml. of distilled water, and the KCN solution was prepared by dissolving 0.241 gram in 1000 ml. of distilled water. Five ml. of alkaline picrate solution and five ml. of KCN solution were placed in a test tube and the following amounts of KCN alkaline picrate solution were added to five test tubes to prepare a standard curve:

Tube number	Ml. solution
1	0.0
2	0.05
3	0.10
4	0.15
5	0.20

The volume of each tube was brought up to 10 ml. by addition of distilled water and test tubes were set in boiling water for five minutes. The readings were taken on a pH Coleman Junior spectophotometer.

In determining HCN content 0.15 gram of tissue obtained from the youngest leaf was cut into small pieces with a pair of scissors. The material was placed in a test tube and crushed with a glass rod. Three to four drops of U. S. P. grade chloroform were added and a 10 x 0.5 mm. filter paper strip saturated in alkaline picrate solution was placed in the test tube. Each test tube was sealed with a rubber stopper and kept at room temperature for 24 hours. The strip was then removed and placed in a second test tube in 10 ml. of distilled water. The resulting color was read on a pH Coleman Junior spectophotometer and the corresponding amount of HCN was determined from the standard curve.

Four- to six-week-old johnsongrass and sudangrass plants were exposed for 12-hour periods to temperatures of 31° and 115°F respectively. Following these exposures to abnormal growing temperatures the HCN content was determined as described above.

EXPERIMENTAL RESULTS

Results of the longevity study of johnsongrass rhizomes are illustrated by Plates II and III. When detached rhizomes were placed in the soil, shoots were produced that developed into plants which later matured seed. When the original detached rhizomes were again placed in soil, no shoots were emerged after a three-month period. When removed from the soil, the rhizomes were found badly decayed.

From Table 1 it appears there is little relationship between rhizome depth and ability to emerge. Shoots from the depth of four and 10 inches emerged on the same day. No shoot emerged from the depth of 12 inches, but shoots emerged from the depths of 14 and 16 inches. This tends to indicate that shoots from rhizomes may even emerge from below the 16-inch depth. The results are given in Table 1. The maximum depth from which shoots can emerge was not determined in the study.

Results of the influence of temperature on johnsongrass rhizomes appear in a series of illustrations in Plates IV and V. It is evident that there is no growth from the rhizomes exposed to 28°F or below, but it was observed that only two nodes out of 108 nodes produced shoots from rhizomes exposed

to 32°F. Rhizomes treated at 36°F appeared to produce more shoots than the control. The results of temperature effects on dormant rhizomes obtained during the winter are given in Table 2.

Table 1. Effect of depth on the emergence of shoots from johnsongrass rhizomes.

Date of emergence	Depth in inches below which rhizomes were placed							
	2"	4"	6"	8"	10"	12"	14"	16"
4-16-58	Emerged							
4-18-58		Emerged			Emerged			
4-20-58			Emerged					
4-21-58				Emerged				
4-26-58								Emerged
5-6-58						No Emergence	Emerged	

Table 2. Number of shoots developed from winter dormant johnsongrass rhizomes after exposure to varying temperatures for 24 hours.

Replicates	Control	Temperatures at which rhizomes exposed				
		36°F	32°F	28°F	24°F	20°F
1	7	27	1	0	0	0
2	4	24	1	0	0	0
3	3	14	0	0	0	0
Total	14	65	2	0	0	0

Table 2 (concl.).

Analysis of variance

Source of variation	D.f.	M.S.	F (calculated)
Temperature	6	195.20	33.95**
Replication	2	12.00	2.0869 N.S.
Error	12	5.75	

**Significant at 5% level.
L. S. D. for totals at 1% level: 5.87.

To determine the influence of different temperatures on actively growing rhizomes obtained in the summer, a second study was made involving temperatures ranging between 31° and 33°F. Data obtained in the study are included in Table 3.

Table 3. Number of shoots developed from actively growing johnsongrass rhizomes obtained in summer after exposure to varying temperatures for 24 hours.

Replicates	Control	Temperatures at which rhizomes exposed		
		33°F	32°F	31°F
1	8	12	8	9
2	6	5	11	9
3	8	11	9	10
Total	22	28	27	28

Analysis of variance

Source of variation	D.f.	M.S.	F (calculated)
Temperature	3	2.75	1.0658 N.S.
Replication	2	9.25	3.5852 N.S.
Error	6	2.58	

Nonsignificant.

The influence of rhizome extract on the growth of seedlings of different crops is summarized in Tables 4 and 5. The analysis of data is based on the rank test as described by Mann and Whitney (26) and Whitney (38). Upon analysis of the data it was noted that the extract of johnsongrass rhizomes delayed and inhibited the germination of alfalfa, soybeans, grain sorghum, winter barley, and oats. It was also found that rhizome extract had a much more detrimental effect on alfalfa and oat seed germination. Germination of corn and winter wheat was not affected by the rhizome extract.

Table 4. Effect of johnsongrass rhizome extract on germination of different crop seeds. The extract was applied after third day of planting at the rate of 35 ml. per pot. Number of seedlings per pot after 17 days of planting.

Crop seed	Number of seedlings per pot*												
	Check		Treated										
	1	2	3	1	2	3	4	5	6	7	8	9	
Alfalfa	11	12	7	2	1	6	5	4	2	4	1	5	**
Soybean	10	11	9	10	6	8	6	0	8	9	7	6	**
Corn	12	8	12	10	12	12	10	11	9	11	9	10	
Grain sorghum	6	8	12	5	3	7	7	7	6	4	3	4	**
Winter wheat	16	14	15	15	16	13	16	14	14	16	16	12	
Winter barley	18	17	17	7	12	13	7	11	12	14	11	12	**
Oats	18	18	16	13	16	10	15	16	13	15	16	14	**

*24 seeds of alfalfa, 12 of soybean, corn, grain sorghum, and 18 of winter wheat, winter barley, and oats per pot were planted.
**Significant at 5% level.

The differences within the treatment were determined by using the rank test.

Table 5. The effect of johnsongrass rhizome extract on germination of different crop seeds under different treatments. The extract applied at the rate of 30 ml. per pot. Number of seedlings per pot after 17 days of planting.

Crop seeds	Number of seedlings per pot* Treatments											
	Control			Seeds soaked 24 hours			Extract after 3rd day of planting			Extract on emergence of 1st seedling		
	1	2	3	1	2	3	1	2	3	1	2	3
Soybean	8	9	8	6	2	6	8	8	8	8	9	11
Corn	9	10	8	8	7	2	8	9	8	9	10	9
Grain sorghum	9	8	7	9	4	6	8	7	9	8	10	10
Winter wheat	17	15	17	9	1	4	14	12	10	8	16	15
Sudangrass	12	12	10	8	7	7	8	11	12	9	10	8

*12 seeds of soybean, grain sorghum, sudangrass, 10 seeds of corn, and 18 seeds of wheat per pot were planted.

The table of probability as suggested by Whitney (38) comparing control against treated is given below:

U	Probability of chance
0	.050
1	.100

The value of U above 0 does not show a significant difference.

In the analysis of data included in Table 6, X^2 and rank tests were used. It was found that soybean germination was decreased when beans were planted after being soaked 24 hours

in the rhizome extract. Application on the third day after planting and at emergence of first seedlings did not decrease the plant number significantly. The germination of corn was not affected.

Table 6. Ranks in one criterion variance analysis for the effect of johnsongrass rhizome extract on different crop seeds under different treatments in summer.

Crop seeds	Pot no.	Control		Seeds soaked for 24 hours		Extract after 3rd day of sowing		Extract after emergence of 1st seedling		X^2
		X	R	X	R	X	R	X	R	
Soybean	1	8	6.5	6	2.5	8	6.5	8	6.5	
	2	9	10.5	2	1	8	6.5	9	10.5	
	3	8	6.5	6	2.5	8	6.5	11	12	
	Ri		23.5		6		19.5		29	8.4827*
Corn	1	9	8.5	8	4.5	8	4.5	9	8.5	
	2	10	11.5	7	2	9	8.5	10	11.5	
	3	8	4.5	2	1	8	4.5	9	8.5	
	Ri		24.5		7.5		17.5		28.5	7.02
Grain sorghum	1	9	9	9	9	8	6	8	6	
	2	8	6	4	1	7	11.5	10	3.5	
	3	7	5.5	6	2	9	11.5	10	9	
	Ri		18.5		12		29		18.5	3.9175
Winter wheat	1	17	11.5	9	4	14	7	8	3	
	2	15	8.5	1	1	12	6	16	10	
	3	17	11.5	4	2	10	5	15	8.5	
	Ri		31.5		7		18		21.5	7.92*
Sudan-grass	1	12	11	8	4	8	6	9	4	
	2	12	11	7	1.5	11	7.5	10	9	
	3	10	7.5	7	1.5	12	4	8	11	
	Ri		29.5		7		17.5		24	7.49

X = Number of seedlings per pot.
R = Rank of that pot.

$$X^2 = \frac{\frac{12}{N(N+1)} \sum_{i=1}^{i} \frac{Ri^2}{ni} - 3(N+1)}{1 - \frac{T}{N(N^2-1)}}$$

N = Total number of observations = 12
Ri = Sum of the ranks in the ith treatment
Ni = Number of observations in the ith treatment.
T = (No. in given tie)^3 - (No. in the same tie).
*Significant at 5% level.
N.B. Reference to Kruskal and Wallis (22).

Soaking had a marked influence on wheat and sudangrass in decreasing the germination percentage, but application of extract on the third day after planting and at emergence of first seedlings had no significant influence.

Table 7. Probability of chance for different crops under different treatments of Table 6 as compared with control.

Crop seeds	Treatment	U ranks	Probability of chance
Soybean	Soaked	0*	0.050
	3rd day	3	0.100
	Emergence	6.5	0.100
Corn	Soaked	0.5	0.100
	3rd day	2.5	0.100
	Emergence	5.5	0.100
Grain	Soaked	2.5	0.100
	3rd day	4.5	0.100
	Emergence	7.5	0.100
Winter wheat	Soaked	0*	0.050
	3rd day	0*	0.050
	Emergence	1.5	0.100
Sudangrass	Soaked	0*	0.050
	3rd day	3	0.100
	Emergence	0.5	0.100

*Significant at 5% level.

In general it was found that seeds soaked in rhizome extract for a 24-hour period did not germinate well, but extract applied to emerging seedlings had no inhibiting effect.

In comparing the data in Table 8, relative to the HCN content of the youngest leaves of johnsongrass and sudangrass, a highly significant difference was observed. It is also evident that the youngest leaves of the two- and three-week-old

johnsongrass plants contained more HCN than those of sudangrass seedlings of the same age.

Table 8. Paired comparison of HCN content in youngest leaf of johnsongrass and sudangrass at week intervals.

Week	Average HCN in milligrams per .15 gram youngest leaf	
	Johnsongrass	Sudangrass
2nd week	221	55
3rd week	251	75
4th week	25	106
5th week	56	124
6th week	67	117
7th week	14	61
8th week	157	23
9th week	8	7
10th week	17	11
11th week	6	9
12th week	83	1

t = 3.9403**
L. S. D. at .05% = 58.2648

Analysis of dats (Table 9) relative to low temperature treatment indicated significant differences in HCN content of johnsongrass and sudangrass. When the seedlings were subjected to high temperatures (Table 10), there was no significant difference in HCN content of johnsongrass and sudangrass seedlings of the same age. The HCN content of johnsongrass under normal, low, and high temperature exposures are given in Table 11. Upon analysis of the data it was found that there was an increase in HCN content when plants were exposed to low temperatures, but no significant difference in HCN content was found when plants were exposed to a temperature of 115°F.

Table 9. Paired comparison of HCN content in johnsongrass and sudangrass seedlings exposed to 31°F for 12 hours at week intervals. HCN in milligrams per .15 gram of youngest leaf.

Weeks	Johnsongrass Replicates			Sudangrass Replicates			Total
4th week	278	288	258	64	64	38	990
5th week	98	278	248	9	9	54	696
6th week	68	128	288	59	24	19	586
Total	444	694	794	132	97	111	2272

t = 6.801*
* Significant at 5% level.
L. S. D. at 5% level 112.52.

Table 10. Paired comparison of HCN content in johnsongrass and sudangrass seedlings exposed to 115°F for 12 hours at week intervals.

Replicates	HCN in milligrams per .15 gram of youngest leaf: Johnsongrass Replicates			Sudangrass Replicates			Total
	I	II	III	I	II	III	
4th week	79	159	268	168	88	38	800
5th week	256	116	64	49	19	44	548
6th week	79	34	0	24	29	49	215
Total	414	304	332	241	136	131	1563

t = 2.0408 N.S.

Table 11. Amount of HCN content in milligrams per .15 gram youngest leaf of johnsongrass under normal low and high temperatures.

Week	HCN in milligrams			
	Normal	Low	High	Total
4th week	25	275	169	469
5th week	56	208	145	409
6th week	67	161	38	266
Total	148	644	352	1144

Analysis of variance

Source of variation	D.F.	M.S.	F (calculated)
Temperature	2	20716.44	8.26*
Weeks	2	3608.94	1.43 N.S.
Error	4	2506.27	

*Significant at the 5% level.
L. S. D. for totals: 245.6.

Results of low and high temperatures on sudangrass are recorded in Table 12. Upon analysis of the data, it was found that a significant decrease in HCN content of sudangrass occurred when these plants were exposed to low or high temperatures.

In comparing the HCN content in sudangrass it was found that plants two, three, four, five, and six weeks old had a significantly higher content than plants from eight to twelve weeks in age. There was an increase in HCN as the age of plant advanced up to the fifth week, after which there was a rapid

decrease. It was found that the greatest amount of HCN was present in two- and three-week-old seedlings of johnsongrass. After a three-week period, there was a rapid decrease in HCN content. The HCN content of an eight-week-old seedling was also high. This increase might be due to variation in environmental conditions. With one exception there was no significant differences in HCN content in plants from four to 12 weeks of age.

Table 12. Amount of HCN in milligrams per .15 gram young leaf of sudangrass under normal, low, and high temperatures.

Week	Temperature			Total
	Normal	Low	High	
4th week	106	55	98	259
5th week	124	24	37	185
6th week	117	31	34	182
Total	347	110	169	626

Analysis of variance

Source of variation	D.f.	M.S.	F (calculated)
Temperature	2	5064.12	9.92*
Weeks	2	624.12	1.21 N.S.
Error	4	513.44	

*Significant at the 5% level.
L. S. D. for totals at 5%: 111.00.

DISCUSSION

Results of the rhizome longevity study are in agreement with observations reported by Cates and Spillman (4) and Johnson (18). Plate I illustrates the shoot and rhizome development of a mature plant that originated from a buried rhizome section. The condition of the primary rhizome from which shoots and secondary rhizome originated during the growing season is illustrated in Plate II. Since no shoots appeared when the detached primary rhizomes were placed in soil, it is evident that rhizomes cease to function after the second year (Plate III).

In attempting to determine the depth from which rhizome shoots will emerge, it appears deeply buried rhizomes have a potential similar to those located near the surface. The maximum rhizome depth from which shoots will emerge was not determined. It is obvious that a similar study conducted under field conditions would be more desirable.

Results of temperature effect on shoot development are illustrated in Tables 2 and 3 and by Plates IV and V. In exposing rhizomes to various temperatures, there was no shoot development from rhizomes exposed to 28°F or below. Only two shoots developed from 108 nodes when rhizomes were exposed to 32°F for 24 hours. On analysis of these data, it was found that development of these two shoots was due to chance. It was also noted there was significant difference in shoot development from control rhizomes and rhizomes exposed to 36°F. The

apparent stimulation of shoots from rhizomes exposed to 36°F may indicate some physiological changes in the rhizomes which affect the development of shoots.

In general these observations agree with the conclusions of Ball (1) and Johnson (18) that frost has a detrimental effect on shoot development of johnsongrass rhizomes, and this is why winter fallow was a common practice used to kill rhizomes by exposure.

In a second study using plant material obtained during the summer when rhizomes were in active growth, exposures to low temperatures gave different results. There was neither a detrimental nor a stimulating effect on the development of shoots whether rhizomes were exposed to temperatures either 1° above or 1° below freezing. These observations lead to the belief that the season has a great influence on the development of the shoot from rhizomes.

The results of the rhizome extract study are summarized in Tables 4 and 5. In this study the effect of johnsongrass rhizome extract on germination of alfalfa, soybeans, corn, grain sorghum, winter wheat, winter barley, oats, and sudangrass was studied. It was observed that diluted rhizome extract inhibited the germination of alfalfa, soybeans, grain sorghum, winter barley, and oats, but did not inhibit the germination of corn and winter wheat. From these results it appears that there are some substances present in johnsongrass extract which inhibit the germination of certain crop seeds. It also appears that alfalfa, winter barley, and oat seeds are

more susceptible to the rhizome extract than those of soybeans and grain sorghum.

The effect of rhizome extract under different treatments on the germination of soybeans, corn, grain sorghum, winter wheat, and sudangrass seeds was studied. It has been observed that soybeans, winter wheat, and sudangrass seeds soaked in rhizome extract are affected more by the inhibiting property of the extract, and corn and grain sorghum seeds are not influenced by the inhibiting action. The application of concentrated rhizome extract, on the third day of planting, and emergence of first seedlings has no effect of inhibition on soybeans, corn, grain sorghum, and sudangrass seeds; application on the third day after wheat planting has shown inhibiting influence on seedling emergence. There was no adverse effect of concentrated extract after the seeds had started germinating.

The results of this study reveal that concentrated extract and diluted extract have different effects on germination of the winter grains and soybean used, but the same effect on corn seeds. It was also observed that pure extract had no detrimental effect on soybeans, but diluted extract had an inhibitory effect. The reverse is true in the case of wheat where germination was retarded in the pure extract.

These results agree with the observations of Helgeson and Konzak (13) that the aqueous extract of field bindweed and Canada thistle retarded the germination of alfalfa and oats. These results agree where wheat was treated with the pure

extract, but not when the aqueous extract was applied. The results on wheat seed also agree with the results of LeTourneau and Heggeness (25).

It is evident from the study that corn is not affected by the phytotoxic properties of johnsongrass rhizome extract.

In comparing the HCN content of the youngest leaves of johnsongrass and sudangrass, it is evident that under conditions of this study johnsongrass contains more HCN than that of sudangrass. It is clear from Table 8 that the amount of HCN in both the grasses increases as the age of the seedlings advance up to the third and fifth weeks in johnsongrass and sudangrass respectively, but is followed by a sudden decrease in the amount of HCN present. These observations agree with the results of Franzke et al. (8), Norris and Valentine (29), Swanson (36), and Casady (3) that the amount of HCN is greatest in young plants and decreases as the plants become older. These results are similar to the observations of Patel (31) that the highest concentration of HCN is found in plants three weeks old. However, it does not agree with the observations of Patel (31) in the case of sudangrass. There was a considerable variation in HCN content in plants in an advanced stage of development. This, however, may be due to the variation in climatic conditions.

The results of the low and high temperature study on johnsongrass are given in Table 11. This indicates that there is an increase of HCN in johnsongrass when the seedlings were exposed to 31°F for 12 hours. There was no significant increase when seedlings were subjected to high temperatures.

From the above evidence it seems that when seedlings of johnsongrass are exposed to low temperatures there is a rapid change of intermediary unstable chemical products into HCN, but there is no rapid transformation of intermediary products into HCN when seedlings are subjected to high temperatures.

The results of the low and high temperatures on sudangrass are given in Table 12. There was a significant difference in HCN content of treated and untreated plants, but there was no difference in HCN content of plants exposed to low and high temperatures. These results agree with the conclusions of Swanson (36) and Patel (32) that there was a reduction in HCN content in frosted plants.

It appears that the physiology of a sudangrass plant is somewhat different from johnsongrass. In sudangrass the HCN present in the plant is rapidly broken down by enzymes to some intermediary products and hence there is a decrease in HCN of sudangrass subjected to abnormal temperatures.

SUMMARY

The present work involves the study of certain morphological and physiological characteristics of johnsongrass and a comparison of HCN content in johnsongrass and sudangrass. Based on experimental results it has been found that;

Primary rhizomes of johnsongrass produced shoots the year after their formation, but decayed the following year.

Shoots will develop from the rhizomes at depths of 16 inches in the soil.

The maximum killing temperature for winter dormant rhizomes was found to be 32°F when exposed for 24 hours.

There appeared to be a growth-stimulating effect when rhizomes were exposed for 24 hours at 36°F.

The maximum killing temperature of winter dormant rhizomes was ineffective in killing actively growing rhizomes obtained during the summer.

Rhizomes of johnsongrass appear to contain certain substances which inhibit the germination of some crop seeds.

Diluted johnsongrass rhizome extract inhibits the germination of alfalfa, soybeans, grain sorghum, winter barley, and oats, but does not inhibit the growth of corn and winter wheat. Concentrated rhizome extract inhibited the germination of soybeans, winter wheat, and sudangrass, but did not affect the germination of corn and grain sorghum.

The inhibitory effects were more pronounced when seeds were soaked in concentrated extract for 24 hours.

The highest concentration of HCN was found in three-week-old plants; there was variation in HCN content in the advanced stages of development. In sudangrass the highest concentration of HCN was obtained in the five-week-old plants. The concentration decreased as the plants became older.

In the early stage of development johnsongrass has more HCN content than sudangrass.

There was a significant increase in HCN content in johnsongrass when the plants were subjected to low temperatures; the increase due to high temperatures was not significant.

When sudangrass was exposed to low and high temperatures, there was a significant decrease in HCN content.

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A STUDY OF CERTAIN MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF JOHNSONGRASS

by

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Johnsongrass (Sorghum halepense L. Pers.) was introduced as a forage crop, but has become a troublesome weed in many areas of the United States. It has been declared a noxious weed in 80 counties in Kansas. The objectives of this study were: (1) to determine the longevity of johnsongrass rhizomes, (2) to investigate the effect of different temperatures on rhizomes, (3) to determine the soil depth from which rhizome shoots will emerge, (4) to study the effect of rhizome extract upon germination of crop seeds, and (5) to compare the HCN content of johnsongrass with sudangrass (Sorghum vulgare var. sudanense (Piper) Hitchc.).

In the longevity study, marked rhizomes were buried in separate containers filled with soil where they remained until the emerged shoots had grown to maturity. Roots and new rhizomes were removed and the original rhizomes were replaced in the soil. It was observed that these rhizomes did not produce shoots a second time, but gradually decayed.

In investigating the effect of low temperatures, rhizomes were kept in a cold room at varying temperatures for 24 hours and then placed in plots containing vermiculite. Observations were made at weekly intervals to determine bud development on the exposed rhizomes. During this study it was found that winter dormant rhizomes were killed by a temperature of 32°F. This was not true for actively growing rhizomes obtained during the summer where lower temperatures were required. It was also observed that exposure to a temperature of 36°F had a stimulating effect on shoot growth.

In studying the depth and shoot emergence relationship, rhizomes were placed in the soil at depths varying from two to 16 inches. Shoot emergence was observed and dates recorded. It was found that deeply buried rhizomes had shoot emergence potential similar to that of rhizomes located near the surface.

The effect of diluted johnsongrass rhizome extract upon germination and seedling emergence of alfalfa (Medicago sativa L.), soybean (Glycine max (L.) Merr.), corn (Zea mays L.), grain sorghum (Sorghum vulgare Pers.), winter wheat (Triticum aestivum L.), winter barley (Hordeum vulgare L.), and oats seed (Avena sativa L.) was studied. In a second experiment involving rhizome extract, various crop seeds were soaked for a 24-hour period before planting, while the third study involved application of the extract at the time of seedling emergence. Counts were made daily for a 17-day period. Rhizome extract inhibited the germination of alfalfa, soybeans, grain sorghum, winter barley, and oats, but it did not inhibit the germination of corn and winter wheat. Extract-soaked seeds of soybeans, winter wheat, and sudangrass were significantly lower in germination than those of corn and grain sorghum. Application of extract on the third day after planting and at emergence of first seedlings did not show a toxic effect on soybeans, corn, grain sorghum, and sudangrass. Application on the third day after wheat planting had an inhibiting effect on germination.

HCN content in leaves of 2- to 12-week-old seedlings of johnsongrass and sudangrass was determined by the "picric acid test." In the same way HCN content of 4- to 6-week-old

seedlings was determined after subjecting the seedlings to 31°F and 115°F temperatures for 12 hours. Under normal conditions it was found that johnsongrass contained more HCN than sudangrass. There was an increase in HCN content in the early stages of development, but as the plants matured, a rapid decrease was observed. In studying the temperature effect, it was observed that low temperature caused an increase where high temperature had no significant effect in HCN content of johnsongrass. The opposite was found in the case of sudangrass where both low and high temperatures caused a decrease in HCN content.