FREQUENCY OF INTERSPECIFIC CROSSING UTILIZING CYTOPLASMIC MALE STERILE LINES OF <u>SORGHUM VULGARE</u> EXPOSED TO POLLEN FROM <u>SORGHUM HALEPENSE</u>

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INTRODUCTION

Hybrid grain sorghums with superior yielding abilities are becoming a very important part of the agricultural economy in the United States. With the development of cytoplasmic male sterile lines, hybrids are being produced that are adapted to specific growing conditions, thus increasing production in the less favorable regions of the country. With this increasing importance of hybrid grain sorghums, many new problems confront the plant breeders. One of these problems facing the breeders is the occurance of tall, off-type plants in the hybrid grain sorghum fields. Plants such as these have been checked and found to include tall mutants, haploids, triploids, and outcrosses to other grain sorghum, Sudangrass, and Johnsongrass. Information was needed to determine if a serious problem existed with the crossing of Johnsongrass and grain sorghum.

Considerable work has been done studying different characteristics of hybrids of Johnsongrass and grain sorghum, but very little work has been done concerning the frequency of such crosses. Many workers have expressed the need for investigations of this nature. From the results of studies relating to the nature and characteristics of these hybrids, researchers feel that, if the frequency of such interspecific crossing is very high, a serious problem can exist both to

farmers and plant breeders. If a problem does exist, more strict regulations will have to be imposed on the producers of hybrid seed to maintain purity.

The primary objective of this research was to test the relative frequency of interspecific crossing by exposing male sterile lines of grain sorghum, (<u>Sorghum vulgare</u>), to Johnsongrass, (<u>Sorghum halepense</u>), pollen under isolation. In addition, gibberellic acid was used on male sterile plants in an attempt to obtain seed without pollination.

LITERATURE REVIEW

Hadley (10) \angle 1 obtained 54 F₁ hybrids between nine lines of <u>Sorghum vulgare</u> and six lines of <u>Sorghum halepense</u> and observed them for chromosome number, fertility and rhizome expression. Evidence obtained showed that the 30-chromosome F₁ hybrids could become a weed threat by vegetative reproduction, and if Johnsongrass was available as a male parent, the hybrids could be a source of troublesome seedlings. The self-fertile 40-chromosome hybrids were so weakly rhizomatous they did not constitute a weed threat directly, but could be a source of troublesome seedlings. The male-sterile 40-chromosome hybrids constituted a problem as a source of troublesome seedlings if Johnsongrass was available.

Hybrids obtained from crosses between <u>S</u>. <u>vulgare</u> and <u>S</u>. <u>halepense</u> are usually highly sterile. Karper and Chisholm (14) reported work done on an F_1 hybrid between <u>S</u>. <u>vulgare</u> and <u>S</u>. <u>halepense</u> that had 30 somatic chromosomes. In meiosis of this triploid hybrid, univalent, bivalent, and quadrivalent chromosome associations were found. The average length of the chromosomes in <u>S</u>. <u>vulgare</u> in prepared

 $\angle 1$ Figures in parenthesis refer to Literature Cited.

root tips was 2.24 microns compared to 1.98 microns for <u>S. halepense</u>.

Although <u>S</u>. <u>vulgare</u> and <u>S</u>. <u>halepense</u> hybridize under natural conditions, the frequency of such crossing is very low. Vinall (22) obtained only 3 seeds in numerous attempts at crossing <u>S</u>. <u>halepense</u> with <u>S</u>. <u>vulgare</u>. Karper and Chisholm (14) reported only 1 seed out of 386 emasculated florets in an attempt to cross <u>S</u>. <u>vulgare</u> and <u>S</u>. <u>halepense</u>.

According to Jones and Brown (13), <u>S</u>. <u>halepense</u> sheds most of its pollen from 10:30 a.m. to 1:30 p.m., while the peak of pollen shedding for <u>S</u>. <u>vulgare</u> is from 4:00 a.m. to 10:00 a.m. These periods are greatly influenced by cloudiness and reduced temperatures which delay the time of maximum shedding.

Successful crosses between various cultivated annual sorghums and Johnsongrass were made by Mikhailovskii (21) when the sorghums were used as the female parent. Most of the hybrids obtained had rhizomes and grain intermediate in size and type.

Krishnaswamy, et al. (16) found there was no irregularity in the meiotic divisions in an F_1 hybrid from a Johnsongrassgrain sorghum cross of <u>S</u>. <u>halepense</u> (2n=20) X <u>S</u>. <u>roxburghii</u> (2n=20). The S. halepense (2n=20) was obtained from diploid shoots in a normal tetraploid (2n=40) clump.

Bennett and Hogg (1) found an $F_1 \le halepense X \le$. <u>vulgare</u> hybrid in a field of sorghum and made a study of

the F_2 and F_3 generations. These investigators concluded from their preliminary studies that a rather close affinity exists between these two species notwithstanding the double chromosome numbers of Johnsongrass.

In their work with <u>Lycopersicon pimpinellifolium</u>, Cooper and Brink (5) stated that seed abortion due to endosperm breakdown is probably the greatest barrier to hybridization between a diploid and its autotetraploid. These investigators pointed out that the course of seed failure associated with matings between the diploid and its autotetraploid is very similar to that found following the interspecific cross.

Hadley and Mahan (11) studied the backcross progeny of 70 plants obtained by pollinating a 30-chromosome (Blackhull kafir X Johnsongrass) hybrid by Sweet Sudangrass, and found the fertility to be generally low and show great variation from plant to plant. Self-pollinated seeds from plants with segments of Johnsongrass chromosomes, particularly plants carrying genes for rhizome formation, germinated poorly and included many chlorophyll aberrants.

Krishnaswamy (15) reported that a perennial dual-purpose tetraploid type (2n=40) has been developed that is suitable for grain and fodder production. The breeding involved twice backcrossing hybrids of <u>S</u>. <u>halepense</u> (2n=40) X <u>S</u>. <u>durra</u> (2n=20) to desynaptic plants of <u>S</u>. <u>durra</u> (2n=20) as the female parent.

There has been considerable discussion concerning the origin of <u>S</u>. <u>halepense</u> and the basic number of its parents. Longley (19) concluded that <u>S</u>. <u>halepense</u> has been derived from annual ancestors having ten chromosomes.

By cytological observations of pollen mother cells from <u>S</u>. <u>vulgare</u> and <u>S</u>. <u>halepense</u> and their F_1 hybrids, Hadley (9) found that <u>S</u>. <u>halepense</u> behaved more like an autopolyploid than a strong allopolyploid and proposed a working hypothesis that <u>S</u>. <u>halepense</u> arose as a cross between two 20-chromosome (2n) species whose chromosome complements were similar but not identical.

Casady and Anderson (3) reported that the meiotic chromosome behavior of the F_1 progenies of autotetraploid Sudangrass X (Johnsongrass X 4n Sudangrass) was typically autotetraploid. The regularity of the distribution of the chromosomes in the F_1 progenies indicated that all the <u>S</u>. <u>halepense</u> chromosomes were capable of synapsing with <u>S</u>. <u>vulgare</u> chromosomes, and advanced the hypothesis that <u>S</u>. <u>halepense</u> is probably an autotetraploid of some variety of <u>S</u>. <u>vulgare</u>.

Endrizzi (7) concluded from his data that <u>S</u>. <u>halepense</u> arose as a segmental allopolyploid with only two genomes, and that its chromosomal complement now consists predominantly of one genome, which is similar to that of <u>S</u>. <u>vul</u>-<u>gare</u>.

Daura's (6) data indicated S. halepense originated

as a segmental allopolyploid containing two genomes derived from <u>S</u>. <u>vulgare</u> and two genomes derived from some related species with chromosomes partly homologous to those of <u>S</u>. <u>vulgare</u>.

From a cytological study of the genus <u>Sorghum</u>, Huskins and Smith (12) observed that in <u>S</u>. <u>halepense</u> there were most commonly 10-14 bivalents, and the remainder of the 40 chromosomes were in quadrivalent or higher associations. Of the diploid forms of <u>Sorghum</u> examined ten bivalents were the most commonly formed, but quadrivalent associations were also common and sexivalents were found occasionally. Considering all their data, these investigators have suggested that the basic number for the genus <u>Sorghum</u> may be seven instead of ten.

Garber (8) maintained that it is more probable for the basic number to be five, since no multivalents were observed in <u>S</u>. <u>vulgare</u> or <u>S</u>. <u>sudanense</u> (n=10), and no hexivalents were found in <u>S</u>. <u>halepense</u> (n=20), but univalents, bivalents, trivalents, and tetravalents were found.

From his investigations, Celarier (4) concluded that <u>S. halepense</u> arose from doubling the chromosomes of a hybrid between two 2n=20 species. He proposed <u>S. propinquum</u> as one of the tetraploid parents since it was the only 2n=20species definitely known to have rhizomes.

In 1926 a Formosan phytopathologist, Kurasawa (17), discovered gibberellin when he became curious about a fungus

disease (<u>Gibberella fujikuroi</u>) that affected rice. By making sterile culture filtrates of this fungus and spraying it on rice seedlings, he found it produced a tremendous overgrowth. However, not until 1956-57 did gibberellins attract much attention in the United States. Since that time many workers (2, 18, 20, 24,) have studied the various effects of gibberellins on plants.

Wittwer, et al. (23) reported parthenocarpic fruit development in the tomato. These workers found the response to gibberellin to be similar to that of indole-3-acetic acid, but much lower concentrations of gibberellic acid were needed to obtain the effects.

Other workers have attempted work related to parthenocarpic seed development, but little has been reported.

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MATERIALS AND METHODS

A study to determine the relative frequency of interspecific crossing between Johnsongrass and grain sorghum was conducted on the Oklahoma State University Agronomy Research Station, Blackwell lake area, in the summer of 1959. A location was chosen that offered as near complete isolation as possible to insure freedom from pollen coming from other sources.

Planting seed of six cytoplasmic male sterile lines were obtained from six different research stations. These stations and the varieties supplied were: (1) Kansas State University----Redlan, Martin, and Westland; (2) Nebraska Agricultural Experiment Station----Combine Kafir-60; (3) Oklahoma State University----Dwarf Early Redlan, and Redlan; (4) Texas Agricultural Experiment Station, Chillicothe Substation----Redlan, Martin, Combine Kafir-60, and Wheatland; (5) U. S. Southern Great Plains Field Station, Woodward, Oklahoma----Wheatland; and (6) Texas Agricultural Experiment Station, Lubbuck Substation----Combine Kafir-60.

A split-plot design was used for the study using four replications for each treatment. The male-sterile lines were the main treatments with the methods of pollination the sub-treatments. Johnsongrass was sprigged in

three rows 40 inches apart on each end of the area and between each replication. These rows ran the entire width of the field. The grain sorghum was seeded in rows 40 inches apart running perpendicular to the Johnsongrass. Each plot included two rows 40 feet long. To allow for cultivation, 20-foot allies were included on each side of the Johnsongrass plots.

The field was surrounded by trees on the north and south, Blackwell lake and trees on the east, and a hill on the west. The distances from other sorghum plots are shown in Figure 1.

The Johnsongrass was sprigged on two dates, April 18, and May 14. Also, two different sources of roots were used to insure plants free of disease. The Johnsongrass was sprigged early enough to allow for sufficient growth so an abundance of pollen would be available during the sorghum's blooming period. The Johnsongrass was cut back on two occasions as an added precaution to insure sufficient pollen.

In an adjoining field other 40-chromosome sorghum species to be used were seeded on June 2. These included <u>Sorghum almum</u>, Perennial Sweet Sudan, Johnsongrass, and ISJ X Kansas 40-Chromosome Sudan. Due to low germination, only a few plants from these species were obtained, therefore, pollen was scarce.

The grain sorghum was planted on June 8 and three weeks later thinned to two plants per foot to allow for normal

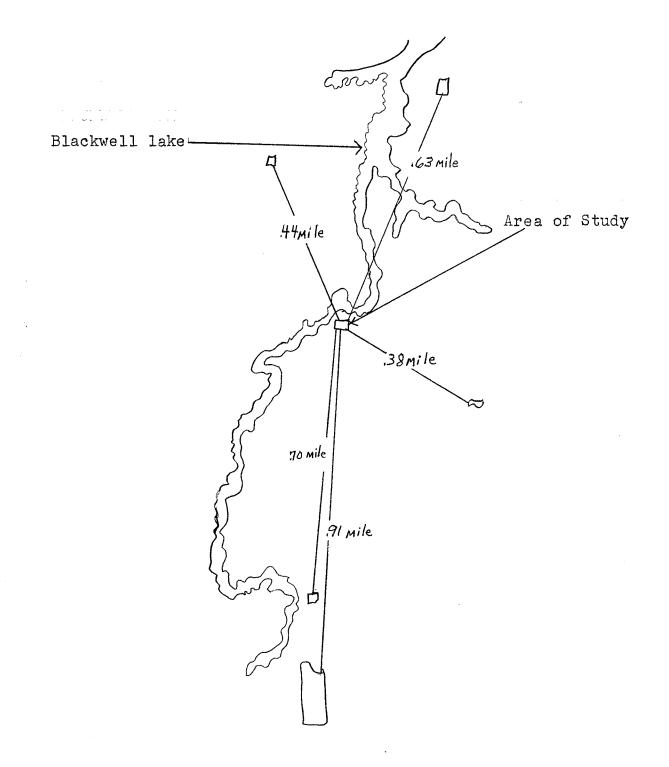


Figure 1. The sketch of Blackwell lake and surrounding areas illustrates the isolation of this study from other fields. Scale--4inches equal 1 mile.

growth. Irrigation was not possible, however, it was not needed due to the favorable rainfall during the growing season. Paper bags were placed on the sorghum heads for protection as soon as they emerged from the boot. The entire field was checked daily for pollen shedders. If a plant appeared to be a possible shedder it was removed from the field. Westland from Kansas was completely destroyed after anthers were checked and found to contain as high as 50% viable pollen. Only occasional shedders were found in the other lines.

The earliest male sterile lines initiated blooming on July 29, and the pollinations were begun. The various methods of pollination and other treatments used were: (1) remove bags for one complete day at full bloom; (2) remove bags for four complete days at full bloom; (3) remove bags for complete season; (4) leave bags on all season; (5) hand pollinate with <u>S</u>. <u>halepense</u> at full bloom; (6) hand pollinate with <u>S</u>. <u>halepense</u> four days after full bloom; (7) hand pollinate with <u>Sorghum</u> <u>almum</u> at full bloom; (8) hand pollinate with other 40-chromosome derivities at full bloom; (9) treat with gibberellic acid at boot stage; (10) treat with gibberellic acid at full bloom stage; and (11) treat with gibberellic acid at full bloom stage and hand pollinate with <u>S</u>. <u>halepense</u> 24 hours later.

Three heads per plot were used for each of the first six treatments, but due to a lack of pollen, fewer heads were used for treatments seven and eight. Also due to a lack of time, labor and material, fewer heads were used for

treatments nine, ten, and eleven.

The plots were harvested the first week of October. Each head was threshed separately by hand in an effort to obtain every seed.

On January 6 a random sample of 132 seed was drawn from the 400 seed obtained and planted in the greenhouse for observation. The remaining seed were kept in storage for use in future morphological and cytological studies.

RESULTS AND DISCUSSION

The field results of this study are presented in Tables I and II. Only 400 seed were obtained from all the treatments, with a majority coming from those treatments requiring open pollinations. Difficulties were experienced in obtaining sufficient pollen to make the hand pollinations. This could account for the reduced number of seed obtained from these treatments.

It was noted that for treatment four, which consisted of not removing the bag for the entire season, only three seed were obtained, obviously indicating a very low frequency of selfing. However, despite the attempt to isolate this study, under open conditions as for treatments one, two, and three, sorghum pollen could have drifted into the area from other sources and pollinated some of the florets.

The lower number of seed obtained from treatment three as compared to treatments one and two may have been the result of a slight webworm and sorghum midge infestation. The bags used in the experiment were Aldrin treated, which may have reduced the damage on treatments one and two.

Another point noted in this problem was the apparent swelling or filling of a majority of the florets following the period of normal pollination. The florets remained

TABLE I

>

						Tre	atmen	ts			
ms line	I	II	III	IV	V	VI	TIA	VIII	IX	X	XI
Martin (Chill.) D. Red.(Okla.) Comb-60(Chill.) Martin (Kan.) Wheat. (Wood.) Wheat. (Chill.) Redlan (Chill.) Comb-60(Lub.) Comb-60(Neb.) Redlan (Kan.) Redlan (Okla.)	267 1710 57980 2	6 6 7 10 16 29 5 35 5 35 5	89155653057	0 0 0 0 0 0 0 0 0 0 0 0	10121000762	1 0 0 0 0 0 0 0 0 0 2 0	0 0 0 0 0 0 0 0 0 0 1 0	000000000000000000000000000000000000000	000000000000000000000000000000000000000	000000000000000000000000000000000000000	0000014000
Total	87	214	64	3	20	3	l	0	0	3	5

TOTAL SEED OBTAINED PER TREATMENT FOR EACH MALE STERILE LINE

TABLE II

TOTAL SEED OBTAINED PER REPLICATION FOR EACH MALE STERILE LINE

ms line	Ī	ĪI	Rep. III	IV	Total
Martin (Chill.) D. Red.(Okla.) Comb-60(Chill.) Martin (Kan.) Wheat. (Wood.) Wheat. (Chill.) Redlan (Chill.) Comb-60(Lub.) Comb-60(Neb.) Redlan (Kan.) Redlan (Okla.) Total	7 6 13 5 12 17 5 12 18 11 16	52 396 104 17 27 97	2 7 35 32 11 7 9 19 19 87	4 2 6 6 6 6 6 12 18 15 9 4	18 21 25 27 325 67 400

turgid for approximately two weeks at which time they gradually collapsed. This was possibly a result of seed abortion due to endosperm breakdown, as described by Cooper and Brink (5) in their work with <u>Lycopersicon pimpinelli-</u> <u>forium</u>. Other workers have suggested that Johnsongrass pollen stimulated the floret to start developing with or without fertilization.

As shown in Tables I and II, only eight seed were obtained from the use of gibberellic acid, indicating a very low frequency of seed development.

Twelve seed of those obtained from each male sterile line were drawn at random and planted in the greenhouse. From these 132 seed, 98 germinated and produced plants that grew to the heading stage. A morphological study of these plants was made using the following characteristics: (1) awned versus awnless; (2) white, dry midrib versus dull, juicy midrib; (3) rhizome development versus no rhizome development; and (4) fertility versus sterility. The original male sterile lines were awnless, non-rhizomatous, and had dull, juicy midribs. The Johnsongrass was awned, rhizomatous, and had white, dry midribs. From these 98 plants, three definite types were observed: (1) tall grassy plants with open heads, white midribs, rhizome development, awned, and sterile; (2) grain sorghum-type plants with closed head, dull midrib, no rhizome development, awnless, and fertile; and (3) grain sorghum-type plants with closed head, dull midrib, no rhizome development, awnless, and sterile.

TABLE III

	· · · · · · · · · · · · · · · · · · ·	· · ·					
FEMALE PARENT	Treăt. No.	Rhizome Express.	Fert- ility	Midrib Color	Head Char.	Days Bloom	Ht. in.
Martin (Chill.)	I I I I I I I I I I I I I I I I I I I	_* x x - x - - - - -	- - - - - - - - - - - - - - - - - - -			82 76 86 86 86 79 86 79 86 79 86	404020887695 344327695
D. Red. (Okla.)	I I II III III III III	x - - - - - - - -		x - x - x - x -	x - - - - - - - - - - - - - - - - - - -	690 780 722 96 94 96 94	43444336168 134443331
Comb-60 (Chill.)	I I II II V V	- - - - - -		- - - - x	- - - - x	92 85 76 82 87 73	22 32 29 29 29 29 29 29 29 29 29 29 29 29 29
Martin (Kan.)	I I I I I I I I I I I I I I I I I I I	x x x x x x x x x x x x x x x x x		x x x x x x x x x x x x x x x x	x x x x x x x x x x x x x x x x x x	6663452642221 76642221	750314008886 75745545343

A SUMMARY OF MORPHOLOGICAL CHARACTERISTICS OF 98 F1 PLANTS

TABLE III

CONTINUED

Female Parent	Treat. No.	Rhizome Express.	Fert- ility	Midrib Color	Head Char.	Days Bloom	Ht. in.
Wheat. (Wood.)	I II II III III V	- x x x x x x		- x x x x x x x	x x x x x x	80 67 66 69 66 77 65	254 44 352 4 526
Wheat. (Chill.)	II II III	x	x - -	- - x	- - x	68 90 68	35 26 45
Redlan (Chill.)	I I I I I I I I I I I I I I I I I I I	- - - - - - - - - - - - - - - - - - -				66525729229 888887866	12107010200 45342435354
Comb-60 (Lub.) Comb-60	I I I I I I I I I I I I I I I I I I I	-	X - - - -			77 80 80 86 86 91 83	3274459591 32743219591
(Neb.)	V N N N N N N N N N N N				123 754 754 756 750 750 750 750 750	82 782 80 98 82 93	308 1 46024958 33334354

TABLE III

С	ON	\mathbf{T}	IN	UED
-	~ ~ ~ ~	-		

Female Parent	Treat. No.	Rhizome Express.	Fert- ility	Midrib Color	Head Char.	Days Bloom	Ht. in.
Redlan (Kan.) Redlan	I I II II II III III III III	X - - - - - - - - - -		X - - - - - - - - - - - - - - - - - - -	X 	73 80 80 86 86 86 72 73	4422064556
(Okla.)	I II II III III V	x - - - x x -		x	x - - - - - - - - - - - - - - - - - - -	689986655 998668	574260 3260 3500 400

* x Indicates rhizomatous, fertile, white midrib, and open head.

- Indicates non-rhizomatous, sterile, dull midrib, and closed head.

Table III summarizes this study of morphological characteristics.

Thirty-five plants were observed that exhibited characteristics of S. vulgare X S. halepense hybrids. Each of these developed definite rhizomes, had white midribs, and were awned and sterile. The height ranged from 36 to 60 inches. The male sterile plants and Johnsongrass used as checks in the morphological study averaged 31 and 53 inches respectively. Chromosome counts were not made from these hybrids, but it is assumed they all contained 30 chromosomes. Both 30 and 40-chromosome hybrids obtained by Hadley (10) from cytoplasmic male sterile lines were sterile; however, the 40chromosome types exhibited very limited rhizome development, while the 30-chromosome types had rhizome development, in many cases equal to or exceeding that of Johnsongrass. The hybrids obtained from this study developed very vigorous rhizomes. Figures 2, 3, and 4 illustrate the characteristics of those plants.

Six plants obtained were awnless, non-rhizomatous, had dull, juicy midribs and were fertile. The plants ranged in height from 28 to 44 inches, had closed, compact heads, and exhibited characteristics very similar to their male sterile parents. Since fertility was restored in these plants, evidently restorer genes were involved, therefore it is assumed pollen came from some other source.

The remaining 57 plants exhibited the same character-

istics as the six plants mentioned above with the exception that they were sterile. The height of these plants ranged from 18 to 51 inches. Basing an assumption on Hadley's (10) work, there is the possibility that these are 40-chromosome <u>S. vulgare X S. halepense</u> hybrids; however, it is more probable that they are a result of either selfing or crossing with some "B" line of <u>S. vulgare</u>. Since the exact nature of the cytoplasmic male sterility in sorghum has not been completely determined, it is not possible to definitely decide what type of gene action occurred to obtain these plants.

Another point noted in Table IV was the proportion of Johnsongrass-type plants to sorghum-type plants among the male sterile lines. From the plants studied of Combine Kafir-60 from all three sources, only one Johnsongrasstype plant was obtained, whereas from the 24 plants of Martin studied, 15 exhibited Johnsongrass characteristics. However, from small numbers such as these, there is a much greater possibility for error in sampling.

If the number of Johnsongrass-type plants is projected to the total number of seed obtained as in Table IV, a total of 116 such plants could be expected. Although this constitutes a very low percentage, considering the total number of florets that were exposed to Johnsongrass pollen, nevertheless it represents a number sufficient in size to be of great concern to plant breeders. Each of these 116 plants is a potential perennial weed threat due to its vigorous

TABLE IV

TOTAL NUMBER OF JOHNSONGRASS-TYPE PLANTS EXPECTED FROM 400 SEED OBTAINED

ms line	Total Seed Obtained	Number Seed Planted	Maturë Plants Obtained	Johnson- Grass Types	Number Expected From Total
Comb-60 (Lub.)	45	12	9	0	0
Comb-60 (Neb.)	50	12	10	0	0
Comb-60 (Chill.)	21	12	7	1	3
Martin (Chill.)	18	12	12	Ц.	6
Redlan (Kan.)	63	12	10	1	6
Wheat. (Chill.)	31	12	3	1	10
Redlan (Chill.)	32	12	11) ₄ .	12
D. Redlan (Okla.)	21	12	9	5	12
Whéat. (Wood.)	27	12	7	5	19
Martin (Kan.)	25	12	12	11	23
Redlan (Okla.)	67	12	8	3	25
Total	400	132	98	35	116

rhizomes as illustrated in Figure 4. If Johnsongrass were available as a pollen parent they could also be a source of troublesome seedlings.

Cytological examinations to determine the chromosome numbers of these hybrids were not made. The material could not be collected in time for these examinations, but future studies will be made on this material to determine chromosome numbers.



Figure 2. Relative size of F₁ hybrid as compared to both parents. A. the male sterile line. B. F₁ hybrid. C. Johnsongrass.

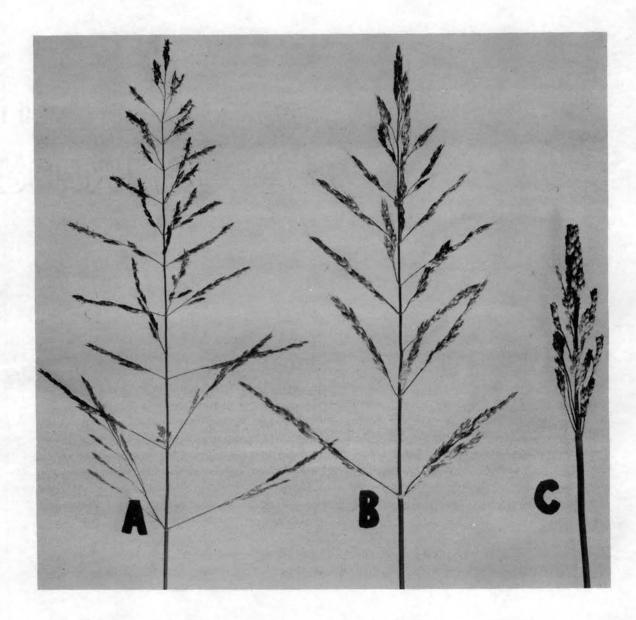


Figure 3. Head development typical of F₁ hybrid and its parents. A. Johnsongrass head. B. F₁ hybrid head. C. Sorghum male sterile head.



Figure 4. Typical rhizome development of the F1 hybrid. A. Sorghum plant. B. F1 hybrid. C. Johnsongrass.

SUMMARY AND CONCLUSIONS

The frequency of interspecific crossing between <u>Sorghum vulgare</u> and <u>Sorghum halepense</u> was studied on the Oklahoma Agronomy Research Station, Blackwell lake area, in 1959. Six male sterile lines of <u>S. vulgare</u> were pollinated with <u>S. halepense</u> by several different methods in an attempt to study this frequency.

Statistical tests were not made due to the low number of seed obtained. However, there appeared to be considerable variation among varieties in regard to seed set.

A total of 400 seed was obtained from which 132 were drawn and planted in the greenhouse for a study of morphological characteristics. From the 98 plants that reached anthesis, 35 exhibited definite Johnsongrass characteristics. By projecting this to the total number of seed harvested, approximately 116 <u>S. vulgare X S. halepense</u> hybrids could be expected. Combine Kafir-60 appeared to be least susceptible to crossing with only one Johnsongrass-type plant being observed.

The Johnsongrass-type plants observed exhibited very vigorous rhizome development. Although no information has been obtained as to the winterhardiness of these hybrids, these rhizomes appeared to be vigorous enough to withstand

considerable cold temperatures. If Johnsongrass is available as a pollen parent, these plants represent additional threats as a source of troublesome seedlings.

Since there is evidence these crosses do occur in high enough frequency to yield several hybrids per acre, extreme caution should be taken by the producers of hybrid seed to insure freedom from contamination.

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