THE NATURE AND EXTENT OF VARIATION IN KENTUCKY BLUEGRASS AS CRITERIA FOR TYPE OF SEED-FORMATION AND ITS PRACTICAL IMPLICATIONS

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TABLE OF CONTENTS

TABLE OF CONTENTS (CONCLUDED)

LIST OF TABLES

TABLE

LIST OF FIGURES

FIGURE

LIST OF FIGURES (CONCLUDED)

FIGURE

THE NATURE AND EXTENT OF VARIATION IN KENTUCKY BLUEGRASS AS CRITERIA FOR TYPE OF SEED-FORMATION AND ITS PRACTICAL IMPLICATIONS

INTRODUCTION

Investigations conducted with both European and American biotypes of Kentucky Bluegrass have established the fact that seed formation is predominantly apomictic; that is, the embryo develops solely from maternal tissue without previous fertilization of the egg nucleus by a sperm from the pollen. The offspring may thus resemble the mother plant in morphological and physiological characters since they are, essentially, clonal increases of it.

Quite apart from its purely scientific interest, the method of seed formation in Poa pratensis has important practical implications. Is improvement in this species to be limited solely to selection of desirable types which already are available in Nature? Or do forms exist which allow the breeder to choose from among the various inbreeding and hybridization techniques one particularly adaptable to Poa?

In such a polymorphic species as Poa pratensis the previous experimental results have been so diversified and contradictory as to provide inadequate or unsatisfactory answers to many of the questions arising from a consideration of the problems involved in a breeding program.

The present study was devised to determine the extent and nature of the apomictic and sexual methods of seed production in a representative sample of Kentucky Bluegrass obtained from pasture and commercial seed sources in the United States and Canada. It was thought that by detailed statistical and descriptive analyses of the material and its progenies from both self-pollination and open-pollination some broad generalizations might be reached which could ultimately be applied to practical breeding methods in the improvement of Kentucky Bluegrass. The experimental results have been so diversified that it has been found necessary to present considerable detailed data in order that the subject be treated adequately.

It is hoped, however, that the results presented will indicate more clearly than hitherto (1) the extent of apomixis and sexuality in a representative group of Kentucky Bluegrass, (2) the nature of the aberrant forms which are produced by sexual phenomena, and (3) the practical application of the results to breeding techniques in Poa.

Practically all of the plant material for this study was grown and most of the data was collected while the writer was a member of the staff of the U. S. Regional Pasture Research Laboratory.

TERMINOLOGY OF APOMIXIS

Apomixis is a general term and, as originally proposed by Winkler, included all forms of asexual reproduction among plants. Apomictic phenomena among the Angiosperms have been discussed extensively in several recent publications (Gustafsson (14) and (15), Stebbins and Jenkins (34), Fagerling (10), and Gentcheff and Gustafsson (12). The reader is referred to them for admirable treatments of the history, descriptions, and terminology of apomictic processes in the higher plants.

The classification of apomictic processes which follows is based largely on the system proposed by Gustafsson (15), and Gentcheff and Gustafsson (12), and will find general acceptance among the workers in this field.

I. Amphimixis - sexual reproduction, the essential criterion being the fusion of female and male gametes.

II. Apomixis - propagation without fertilization.

A. Vegetative Reproduction

B. Agamospermy (Täckholm, 1922) - Seed production without fertilization.

1. Reduction division occurs.

a. Haplospory - A functional megaspore produces an embryo sac having a reduced number of chromosomes. Apomictic development results in the origin of haploids. Segregation occurs and the maternal type is not reproduced.

(1) Reduced Parthenogenesis (Winkler, 1908) - formation of the embryo from a reduced egg cell.

(2) Reduced Apogamety (Renner, 1916)- formation of the embryo from a reduced antipodal, synergid, or polar nucleus; that is, from a cell other than the egg.

2. No reduction division occurs. A new indi-

vidual will arise having the unreduced or somatic number of chromosomes and will conform to the maternal type. Comprises the phenomena of *Diplospory*, *Aspopory*, and *Adventitious* (*Nucellar*) *Embryony*.

a. *Diplospory* (Edman 1931) - formation of an embryo sac with the unreduced chromosome number from an egg mother cell in which meiosis is suppressed. The first division of the E. M. C. may be either (1) meiotic in character, followed by the organization of a restitution nucleus, or (2) strongly mitotic in character, in which the univalents divide lengthwise and separate at Division I.

(1) Unreduced Parthenogenesis

(2) Unreduced Apogamety

b. *Apospory* (Bower 1885) - formation of an embryo sac with the unreduced chromosome number from vegetative cells by mitotic divisions. The aposporous cell is usually derived from the nucellus.

(1) Unreduced Pathenogenesis

(2) Unreduced Apogamety

c. *Adventitious* (*Nucellar*) *Embryony* - formation of an embryo with the unreduced chromosome number directly from a vegetative cell without the origin of an embryo sac. The adventitious cell is usually derived from the nucellus.

In current literature, the terms apomixis and agamospermy are frequently used synonymously in referring to seed production without fertilization. In view of this, the writer proposes to use the simpler and more generally understood term, apomixis, when describing seed production in Kentucky Bluegrass. There has arisen the implication in discussing apomictic origins that the offspring will resemble the maternal parent. This is true only if offspring have arisen by diplospory, apospory, or adventitious embryony, since only these processes can result in the establishment of the original chromosome complement of the parent. Haplospory, while an apomictic process, may produce a wide range of morphological types in the offspring. As used in this paper, however, apomictically reproduced plants are those showing complete conformity to the mother plant.

In describing the manner of origin and the chromosomal complements of plants of Kentucky Bluegrass the expressions "haploid", "diploid", and "triploid" are employed. This is obviously a loose use of the terms when applied to a collection of biotypes whose chromosome numbers range from $2n = 36$ (Müntzing 1940) to $2n = \pm 110$, and whose basic number is $x = 7$. These terms are, however, convenient and will be employed with the following implications:

a. "Haploid" - a plant having approximately half the number of chromosomes found in the mother plant. Haploids arise by Haplosporic Parthenogenesis.

b. "Diploid by Apomixis" - a plant having the same chromosome number as the parent and conforming to it in morphological and physiological characters. It may arise by either Diplosporic Parthenogenesis or Aposporic Pathenogenesis.

c. "Diploid by Amphimixis" - a plant whose chromosome number is approximately the same as that found in its parent, and whose morphological, physiological, and cytological features suggest that it arose by the union of a reduced egg and a reduced sperm; that is, by normal sexual reproduction.

d. "Triploid" - a plant having approximately 50% more chromosomes than exist in the mother plant. It is generally believed that triploids arise through the fertilization by reduced pollen of an unreduced egg derived by either Diplospory or Apospory. The possibility may not be wholly eliminated, however, that in Poa certain triploids arise through the fertilization of a reduced egg by an unreduced pollen grain (Müntzing (22)).

REVIEW OF LITERATURE

The first suggestion that seed production in Kentucky Bluegrass was predominantly apomictic was made by Müntzing (19) who studied 8 Swedish biotypes of Poa pratensis. Apomictic seed production was also reported in Swedish forms of Poa alpina. He proposed the following criteria for apomixis, (1) an aneuploid chromosome number which is constant for the biotype, (2) morphological constancy within the biotype, and (3) good seed production even in plants with great chromosome irregularities at meiosis. Apomictic seed formation in Poa pratensis has been confirmed by Akerberg (1), (2), (3), (4), Engelbert (9), Müntzing (22), Nilsson (24), (26), Rancken (29), Tinney (36), and Tinney and Aamodt (37), and may be inferred from the morphological constancy observed by Armstrong (6) and Kemp (17). Several other species of Poa have been described as containing apomictic biotypes: P. pauluatris by Kiellander (18); P. arctica, P. alpigena, and P. glauca of northern Europe by Flovik (11); P. artica, P. alpina, and P. alpigena of Canada by Engelbert (9); and P. compressa by the writer (7).

Poa pratensis is a remarkably diversified species both in its polymorphism and in its wide range of chromosome numbers. Reports have been made by Akerberg (1)(2)(4), Armstrong (6), Avdulow (cited in (8)), Brown (8), Müntzing (19)(20)(21)(22), Rancken (29), Skovsted (31), and Tinney (36). The lowest somatic number reported was 2n = 28 by Avdulow (cited from (8)), the highest has been 2n = 110 by Akerberg (4). Brown (8) has compiled all the reports of chromosome numbers in Kentucky Bluegrass and finds a very steep mode at 2n = 56, indicating that most plants are octaploid. Meiosis in Poa pratensis has been reported by Müntzing (19)(22), Rancken (29), Armstrong (6), and Tinney (36). In general, the division is an irregular one, many univalents may occur which divide lengthwise at I and lag at II. Trivalents and quadrivalents may also occur. Chromosomes are often lost in the cytoplasm and pollen grains with different numbers undoubtedly arise. Tinney described meiosis in the megaspore mother cell, finding mostly bivalents but a considerable number of univalents and trivalents. A regular meiosis has been reported by Müntzing (22), as occurring in a 36 chromosome Poa pratensis "haploid". In 76% of the cells studied, perfect pairing occurred and 18 bivalents were observed. This plant was completely sexual in its method of reproduction.

The origins of the embryo sac and embryo have been described for *Poa* by Kiellander (18), Müntzing (22), Anderson (5), Armstrong (6), Akerberg (4) and Tinney (36). According to Kiellander, in the apomictic biotypes of *Poa palustris*, the megaspore mother cell does not, as a rule, divide reductionally but mitotically to form ultimately an embryo sac having the unreduced number of chromosomes, the egg then developing parthenogenetically into the embryo. Infrequently, however, the E. M. C. may divide somewhat typically; that is, meiosis may take place giving functional eggs with the reduced number. These may either develop without fertilization into haploids, or with fertilization into diploid forms. In *Poa alpina*, Müntzing observed essentially the same manner of origin of the embryo sac. In a highly apomictic *alpina* biotype with a somatic chromosome number of 33, the embryo sac, without exception, developed directly from a megaspore mother cell which failed to undergo meiosis.

In *Poa pratensis*, and *P. compressa*, Anderson (5) described the origin of the embryo sac as proceeding from the normal development of one of the four megaspores formed in the usual manner by two divisions of the E. M. C., the first division being meiotic. She found no evidence that embryos arise from cells of the nucellus, in contrast to early work by Nishimura (27) who described an embryo arising from a nucellar bud. Anderson did not observe fertilization although her slides were examined critically for this detail. She observed polyembryony, and stated that it arises from the development of more than one embryo sac within the same nucellus. The embryo sacs develop simultaneously if situated equally near the vascular strands, but if one is located nearer the micropyler end and hence nearer the source of nutrients, it will develop more rapidly. In the latter case, the two embryo sacs are not equally developed at maturity; in extreme cases, the poorly situated one may be only half developed. She considered that twin embryo sacs arise from the development of two sister megaspores. Armstrong (6) described the origin of the embryo sac in P. pratensis essentially as outlined by Anderson. He interpreted his observations as indicating sexual reproduction even in those biotypes with aneuploid chromosome numbers and morphological constancy of offspring. He attempted to explain these features by assuming selective elimination of chromosomes in the formation of the gametes, basing his theory on irregularities at meiosis, the origin of the embryo sac from a reduced megaspore, and the occurrence of pollen tubes in the stigma. He did not observe fertilization. His theory of heterogamy finds little support in experimental results.

The physical basis of apomixis in Poa pratensis was first described by Akerberg (4). In an apomictic biotype with ± 80 chromosomes, he indicated that the embryo sac arose from a cell of the nucellus, producing an egg with the unreduced number of chromosomes which then developed parthenogenetically. Tinney (36) reported the same series of phenomena in Kentucky Bluegrass from Wisconsin sources. His account is much more detailed and complete. The megaspore mother cell undergoes meiosis and haploid megaspores are formed, usually 3 since the micropylar dyad cell does not complete the second division. In all instances observed by Tinney, the megaspores degenerate and an embryo sac is formed from an enlarged cell of the nucellus. The unreduced egg then develops parthenogenetically. The process is one of apospory. In many instances, the embryo is well formed before flowering occurs, and it was suggested that the development of the embryo is not pseudogamous, but that, since the endosperm does not develop until later, the stimulus of pollination may be necessary for endosperm formation. The origin of twin embryos was described as originating from the simultaneous development of two nucellar cells. Usually one embryo sac is slower than the other in its development and may not have formed an embryo before flowering. This suggested that triploids might arise from the fertilization of these slowly developed egg cells. Also, while never observing functional reduced embryo sacs arising from megaspores, Tinney mentioned that haploids undoubtedly must arise this way. Akerberg (4) reported embryological studies on a sexual biotype of Kentucky Bluegrass and found complete absence of aposporous development.

Self- and cross-fertility in Poa pratensis has been analysed by Nilsson (24) (25) (26). He has shown that plants of Kentucky Bluegrass differ widely in their abilities to set seed under bag. Fertility has ranged from 0.0% to 78.9%. In 52 plants, the average seed set under bag was 20.7%; 5 were completely sterile, 14 showed less than 10% set, and 33 were fairly fertile. Three of the self-sterile or nearly self-sterile plants had defective floral organs. Nilsson has shown that the physiological influence of the bag on seed set may be considerable under some conditions, decreasing seed set in the field about 30%, but only about 10% in the greenhouse. The conclusion is reached by a statistical analysis that true differences in self-sterility and self-fertility exist among plants of Kentucky Bluegrass and that genotypical differences are responsible. On conditions of open pollination in the field, seed set in 13 plants ranged from 2.6% to 78.1%, with an average value of 55.1%.

There is little doubt that pollination is necessary for seed production in Poa pratensis. All experimental evidence points in this direction. Nilsson (26), working with sterile apomictic types, found that regardless of the type of pratensis pollen applied to the stigma, offspring was matroclinous. Akerberg (1) and (2), observed essentially the same phenomenon, using pollen of Poa alpina. In rare instances, hybrids were obtained, one from fertilization of an unreduced egg, another from fertilization of a reduced egg, but the vast majority of offspring resembled the pratensis mother and had the same chromosome number. Results of the writer (7) obtained by applying pratensis pollen on heat-emasculated florets of Poa compressa indicate similar pseudogamous development, but that occasional fertilization can occur. Akerberg (4) reported no seed formation in hand-emasculated florets of Poa pratensis unless pollen was applied. Pollen of alpina and compressa was as effective in initiating seed development as pollen of pratensis. More recently, Engelbert (9) reported hand-emasculation studies conducted over a period of three years, using the species Poa arctica, alpina, alpigena, and pratensis. No seed developed in any species without previous pollination. Seed production was good both upon self-pollination and upon outcrossing. Engelbert reported high germination of pollen on the stigmas whether from the same or different species and that, as a rule, the pollen tubes remained short but one pollen tube was found in Poa alpina which had reached ovarian tissue.

Numerous references have been made to the high frequency of polyembryony in Kentucky Bluegrass. Nashimura (27) first called attention to it and later Anderson (4), Armstrong (5), and Tinney (36) described the embryology of twin seedlings. It was in 1937 that Müntzing (20) presented an analysis of the morphology and chromosome complements of twin seedlings found in several genera of plants, mostly Gramineae. He found, in Poa pratensis, that while the great majority of twins are morphologically identical and have the same chromosome number as the mother plant, a significant proportion of them are dissimilar and that the atypical plant is generally a triploid and that it, without exception, starts out as the weaker seedling. He found 18% of the plants from twin seedlings deviating in chromosome number from their Matroclinous twin partner. One member of a set of triples was found to be triploid, the other two plants identical with the parent and having the same diploid number. In a later report (21) he reported the origin of haploids from twin seedlings. The percentage of plants different (triploids, haploids, and unclassified) was 9%. Müntzing (22) recently has given a fully detailed account of twin seedlings in *Poa pratensis*, describing plant weight, morphology, chemical data, fertility, and chromosome variation in progenies from twins. He finds, as a rule, that the triploid members need more time to mature and usually are not as productive as the diploid members, that their most reliable morphological criteria are thicker culms, wider leaves, thicker leaves, and heavier seed, that they have better pollen fertility, and that they give progenies with a great diversity of chromosome numbers, indicating an increased degree of sexuality.

Akerberg (4) reported on the occurrence of polyembryony in his experimental material. He found it a highly variable feature among his plants, stated the average frequency as about 10% and suggested that the sexual types show a significantly lower rate of polyembryony than do the predominantly apomictic types. Akerberg reported 12.7% aberrancy in plants from twin seedling sources, as compared with 6.9% in plants from single seedling sources. This difference is not statistically significant.

Skovsted (31) made an extensive survey of twin seedlings and reported cytological data from 16 species, 8 of them Gramineae. In Poa pratensis, in 31 twin pairs selected for study, 16 pairs had identical chromosome numbers, 14 aberrant plants were triploid, and 1 aberrant plant was haploid. In several instances, the triploid plant was a smaller plant compared with its diploid partner, and Skovsted accounts for this on the basis of poor development of the embryo while competing in the nucellus with the more advanced apomictic embryo.

Webber (38) has prepared a review of the subject of polyembryony in the higher plants.

Morphological variation in progenies of Kentucky Bluegrass and the chromosome complements of the aberrant plants have been investigated by Akerberg (4). Of 44 families grown from seed collected from wild Poa pratensis biotypes of northern Europe, 7 showed great variation and are considered basically sexual, 37 were rather uniform, showing collectively a variability of 5.9%. Four biotypes used in previous work (1) and (2) were investigated intensively. Selfed progenies showed a variability of 12.1%; artificially crossed progenies 13.4%. Fifty-eight of the plants of the offspring were investigated cytologically, 43 were of maternal types and had the same chromosome numbers as the parents, and 6 had chromosome numbers suggesting origin by triploidy. Many of the aberrant F_1 plants gave highly variable F_2 progenies, and the suggestion is made that the aberrant plants were more sexual than the parental type.

Tinney and Aamodt (37) have published the results of 102 progeny tests conducted on Kentucky Bluegrass material collected from sources in North America and Europe. Collections from pastures were included. The progenies from 48 of the selected plants were uniform and the two highest values obtained for morphological variability were 12.06% and 21.92%. The entire nursery showed an average value for variability of 1.59%; 31 progenies from Wisconsin pasture sources gave a value of 1.65%. The authors suggest that the variant plants have arisen by either gametic union or mutation.

Techniques which facilitate the identification of plants with aberrant chromosome numbers have been described. Müntzing (4) has presented evidence for the high positive correlation between chromosome number and diameter of pollen grains in *Poa pratensis*. Nissen (28) similarly showed a positive correlation between chromosome number and size of stomata in this species. Both of these correlations have been shown by Müntzing to exist also in *Poa alpina*.

Morphological constancy in the progenies of Kentucky Bluegrass is recognized by all investigators in this field as a reliable criterion of apomictic processes. Conversely, aberrant plants which arise are considered as visible proof of sexual reproductive processes. These criteria of reproduction were recognized first by Müntzing (19), followed by Akerberg (4) and Tinney and Aamodt (37), the latter suggesting the progeny test as a practical means of evaluating types of seed development.

MATERIALS AND METHODS

Source. The foundation stock from which selections were made was drawn from widely diversified sources. Detailed information is given in Table I. Seed for the original Kentucky Bluegrass nursery of approximately 10,000 individually spaced plants, established at State College, Pennsylvania in the spring of 1937, came from four sources: (1) seed collections from permanent pastures, 118 pedigrees from which 28 parental plants were drawn; (2) seed from commercial seed sources, 38 pedigrees from which 71 parental plants were selected; (3) seed of numbered strains and selections, 6 pedigrees from which were chosen 9 parental plants; and (4) seed of B. P. I. introductions, 5 pedigrees, from which 7 parental plants were selected.

A total of 115 parental plants was selected from among the 10,000 in the nursery. An effort was made to have these plants representative of the range of variation in plant type and response found in the original nursery material.

Procedure. Each of the selected plants of Kentucky Bluegrass was bagged in the spring of 1938 to determine the extent of sterility under bag existing in the material, and to provide seed for the study of inbred progenies. Open pollinated seed was also collected.

Seed was germinated in the fall of 1938 six weeks after harvest. One hundred seeds were placed on blotting paper in Petri dishes. The rest period of the seed was broken successfully by exposing the moistened seed to a temperature of 8° C

TABLE I

Sources of Material from which Kentucky Bluegrass Selections were made

Pedigree	Source	
37-KB 1 to 114	seed collection	West Virginia pasture
118	" "	New Jersey pasture
120	" "	New Hampshire pasture
127	" "	New York pasture
181	sod plug collection	West Virginia pasture
37-KB 128 to 140	commercial seed	Kentucky
142 to 146	" "	Missouri
152	" "	Missouri
161 to 165	" "	Missouri
147	" "	Kansas
151 to 156	" "	Minnesota
153	" "	South Dakota
154	" "	Iowa
155	" "	Nebraska
(CB)134(470) *	" "	Canada
135(119)(156)(234) *	" "	Canada
138(525) *	" "	Canada
(Asp) 19(1) **	" "	New York
37-KB 170	strain	Ottawa 959; Minnesota P-35
171	"	Ottawa 995; Minnesota P-36
172	"	Ottawa 994; Minnesota P-37
175	"	Ontario Agri. College 1
176	"	Ontario Agri. College 2
177	"	Ontario Agri. College 3
37-KB 173	introduction	F.P.I. 114272
174	"	F.P.I. 73165
186	"	F.P.I. 115314 Russia
187	"	F.P.I. 115405
(Psp) 1 ***	"	F.P.I. 95581 Canada

* Kentucky Bluegrass plants found in Canada Bluegrass progenies
** Kentucky Bluegrass plant found in *Agrostis* progeny
*** Introduced as an unnamed species of *Poa*

for a period of 10-14 days, followed by germination at room temperature; a procedure found effective by Sprague (33). On germination, the single seedlings and twin and triple seedlings were separated and handled separately. The nursery consisted entirely of plants from seeds giving rise to single seedlings. The use of the Petri dish method of germination provides a means of obtaining a representative sample of the products of germination of the selected Kentucky Bluegrass plants since all seedlings have equal chances for survival. Throughout this work an effort was made to keep the sample representative. The writer wishes to stress this point strongly for he feels that entirely different results could be obtained should the worker fail to obtain a representative progeny, either through certain cultural procedures which eliminate the weaker seedlings or conscious selection which chooses the stronger seedlings.

The single seedlings were transplanted from the Petri dishes to paper bands in flats, and allowed to reach sufficient size for transplanting to the field. They were removed to the field in October, 1938 during a favorable spell of weather which allowed them to become well established. Progenies were arranged in compact blocks to minimize environmental influences on the plants. With each progeny were planted clonal increases of the parental plant for comparisons of type and vigor.

Many of the plants flowered during the summer of 1939 and differences in morphological type within progenies were evident. It was believed, however, that the nursery was too young for the plants to have developed their true expression. Accordingly, all data presented in this paper were obtained during the spring and summer of 1940, at which time all plants had reached maturity.

The double and triple seedlings were also transplanted to paper bands and allowed to reach a size best suited for their separation. Each seedling was transplanted to a 3" pot. As the plants developed to the point where notes could be taken, those twins and triplets which seemed identical were discarded. Of those which remained, a representative sample was taken for transplanting to the field; 69 pairs of twins and 4 sets of triplets were set out.

EXPERIMENTAL RESULTS

Seed Set Under Bag. The 115 plants which had been selected for progeny tests were brought into the greenhouse in the fall of 1938 where 59, or approximately half of them, flowered. As nearly as could be judged, the flowering was normal. Four to eight panicles of each plant were placed in a parchment bag. Seed set was classified on the basis of percentage, and four identifiable classes were established: (1) No set; (2) Less than 30% set; (3) 30%-60% Seed set; and (4) More than 60% set.

All 115 plants flowered in the field in 1938. Bagging operations were repeated in the field the following year. This time the parchment bags were in duplicate. Table II presents these data. The figures in parentheses refer to the 59 selected plants which flowered previously in the greenhouse. Utilizing the replicated data, the column headed "Composite Data" was set up as the best available appraisal of seed set under bag.

It is perhaps significant that consistent values throughout the series are obtained by combining the classes "No set" and "Less than 30%" and the classes "30%-60% Set" and "More than 60% Set". Environmental influences apparently modify greatly the behaviours of plants in groups (1) and (2) and in groups (3) and (4), but insignificantly the behaviors between groups (1 and 2) and groups (3 and 4). In this connection, it may be pointed out that greenhouse conditions apparently provide a more exact environment for the determination of sterility than field conditions since higher percentages of plants set no seed and set good seed in the greenhouse than elsewhere.

To summarize this information, we may say that bagging tests carried out on 115 plants of Kentucky Bluegrass indicate that 34.8% set seed poorly (less than 30%) or not at all under bag, and that 65.2% set seed well under bag (more than 30%). There is no difference in inherent ability to set seed under bag between the sample of plants which showed ability to flower in the greenhouse and the entire group of 115 plants.

Analysis of Self-Pollinated and Open-Pollinated Progenies. It is of interest in connection with this study to determine if any significant differences exist between progenies from seed produced under bag and progenies from seed produced under conditions of open pollination. Both self-pollinated and

TABLE II

Seed Set under Bag in 115 Selected Kentucky Bluegrass Plants

	GREENHOUSE		FIELD 1958		FIELD 1959		COMPOSITE DATA	
	No.	%	Number	Percent	Number	Percent	Number	Percent
1. No Set	12	20.3	(9)* 16	(15.3)* 13.9	(2)* 9	(3.4)* 7.8	(5)* 10	(8.5)* 8.7
2. Less than 30% Set	7	11.7	(12) 23	(20.3) 20.0	(17) 35	(28.8) 30.4	(15) 30	(25.4) 26.1
Subtotal	19	32.2	(21) 39	(35.6) 33.9	(19) 44	(32.2) 38.2	(20) 40	(33.9) 34.8
3. 30% - 60% Seed Set	4	6.8	(12) 37	(20.3) 32.2	(7) 24	(11.7) 20.9	(7) 30	(11.7) 26.1
4. More than 60% Set	36	61.0	(26) 39	(44.1) 33.9	(33) 47	(55.9) 40.7	(32) 45	(54.2) 39.1
Subtotal	40	67.8	(38) 76	(64.4) 66.1	(40) 71	(67.8) 61.6	(39) 75	(66.1) 65.2
TOTALS	59	100.0	(59) 115	100.0	(59) 115	100.0	(59) 115	100.0

* Field Data from the 59 plants which flowered under greenhouse conditions

open-pollinated progenies are available from 87 parental plants. Data from this analysis are summarized in Table III.

a. Germination In the self-pollinated progenies the average percent germination was 82.4 ± 1.6, in the open-pollinated progenies 80.4 ± 1.3. The 2.0% difference is not significant (t = 1.02). There is a significant correlation between the germination of seed from self-pollination and the germination of seed from open-pollination (r = + .278; t = 2.67) for the paired progenies. (Figure 1).

b. Polyembryony The following values for polyembryony are based on the number of germinated seeds which showed evidence of containing more than one embryo. These were almost entirely twin seedlings, although triple seedlings appeared in some progenies in significant quantities. Invariably, the appearance of triple seedlings was associated with a high incidence of polyembryony. The average percent polyembryony in progenies from self-pollination was 6.9 ± 0.7, in progenies from open-pollination 8.1 ± 0.7. The 1.2% difference is not significant (t = 1.23). The correlation between the values for polyembryony in self- and open-pollinated progenies is very high (r = + .733; t = 9.93). (Figure 2).

The distributions of the values for polyembryony in 87 self- and 87 open-pollinated progenies are shown in Figure 5-A. In order to determine if significance could be ascribed to any differences existing between paired progenies from each parental plant, Chi Square determinations were made and plotted against the theoretical distribution for N = 87.

TABLE III

Statistical Data from Self-pollinated and Open-pollinated Progenies of 87 Plants

	CORRELATION self:open	MEAN	SIGNIFICANCE OF DIFFERENCES	LOWEST VALUE	HIGHEST VALUE	STANDARD DEVIATION
Germination (Percent)						
Self-pollination	+ .278 **	82.4 ± 1.6	2.0 ± 2.0	37.4	100.0	14.5
Open-pollination		80.4 ± 1.3	t = 1.00	28.0	98.0	11.8
Polyembryony (Percent)						
Self-pollination	+ .753 **	6.9 ± 0.7	1.2 ± 1.0	0.0	28.7	6.1
Open-pollination		8.1 ± 0.7	t = 1.23	0.0	36.1	7.0
Survival (Percent)						
Self-pollination	+ .610 **	84.9 ± 1.5	1.6 ± 1.9	22.0	100.0	13.6
Open-pollination		86.5 ± 1.2	t = 0.83	34.0	100.0	11.1
Variability (Percent)						
Self-pollination	+ .614 **	10.9 ± 1.4	3.8 ± 2.2	0.0	64.6	14.2
Open-pollination		14.8 ± 1.5	t = 1.71	0.0	74.6	15.3
Elimination of 3 widely divergent pairs:						
Self-pollination N=84	+ .818 **	10.5 ± 1.4	3.1 ± 2.1	0.0	64.6	12.8
Open-pollination		13.6 ± 1.5	t = 1.49	0.0	70.6	13.7
Size of Progeny (Number)	TOTALS					
Self-pollination	4260 plants	49.0 ± 1.2		12	60	10.8
Open-pollination	4437 plants	51.0 ± 0.8		17	60	7.4
	* P.05 = .208 ** P.01 = .270		* P.05 = 1.96 ** P.01 = 2.58			

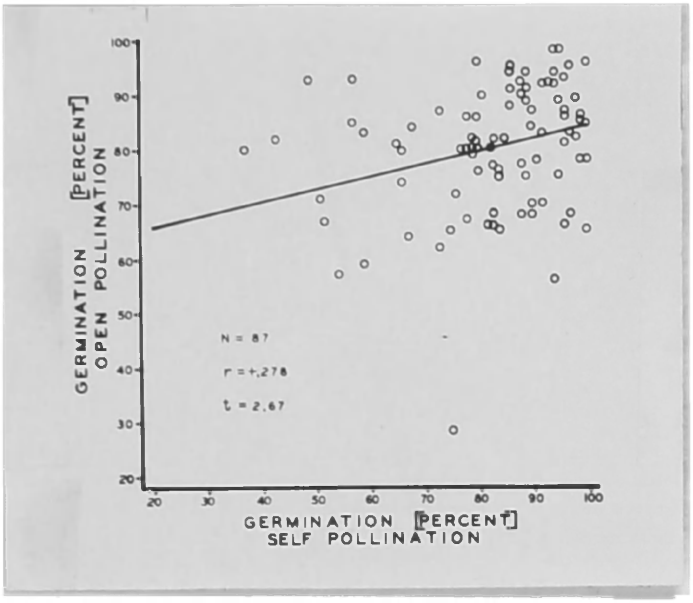

FIGURE 1 - Correlation of Germination in Progenies from Self-pollination with Germination in Progenies from Open-pollination.

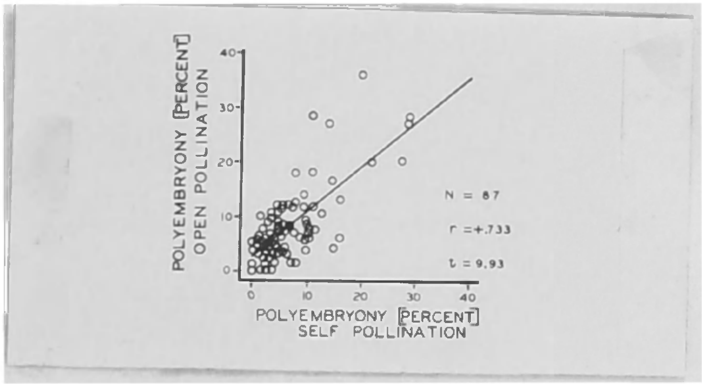

FIGURE 2 - Correlation of Polyembryony in Progenies from Self-pollination with Polyembryony in Progenies from Open-pollination.

This is shown in Figure 6. There is remarkably close agreement, indicating that while plants of Kentucky Bluegrass may show highly significant different rates of polyembryony, yet the rate for a particular plant is independent of the nature of pollination. No significance can be ascribed to the five plants showing Chi Square values beyond the 5% point because 4.35 values out of a sample of 87 are expected to show Chi Square values beyond the 5% point.

c. Survival The experimental nursery was planned so that ultimately each progeny would consist of 60 plants in the field. With few exceptions, sufficient seed germinated to supply this number. The studies of the mature plants in the nursery revealed the fact that certain progenies had lost a considerable number of plants and that others had lost none or very few. It was thought advisable to analyse the material to discover if the loss of plants was distributed at random throughout the progenies, and to discover, should the loss in plants be the expression of some inherent character of the parent plant, if any difference existed between self- and open-pollinated progenies. The results are expressed in percent survival and include those plants which survived transplanting to flats, transplanting to the field, and those which persisted in the field for two years.

The high correlation (Figure 3) between percent survival in self-pollinated progenies and percent survival in open-pollinated progenies ($r = \pm .610$; $t = 7.10$) establishes the fact that survival is not distributed at random through the nursery but is definitely associated with paired progenies

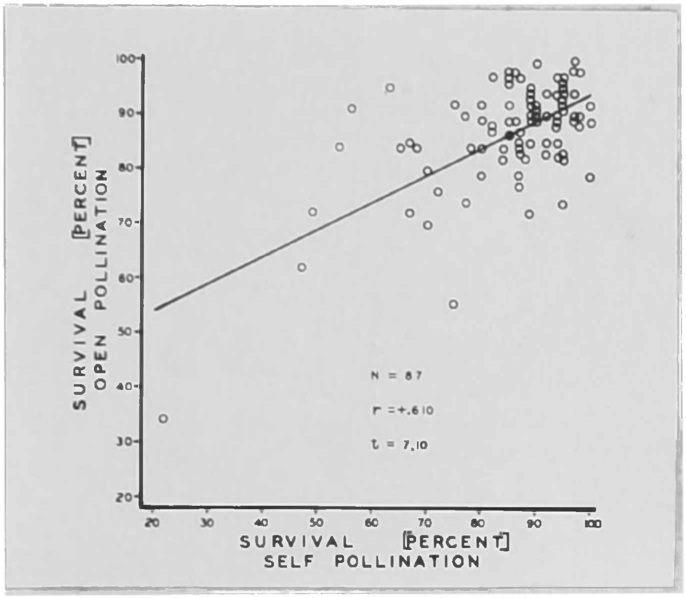

FIGURE 3 - Correlation of Survival in Progenies from Self-pollination with Survival in Progenies from Open-pollination.

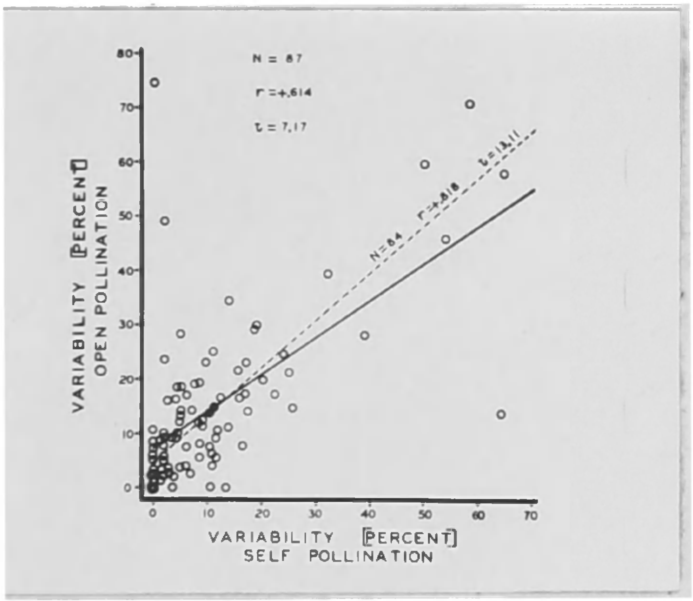

FIGURE 4 - Correlation of Variability in Progenies from Self-pollination with Variability in Progenies from Open-pollination.

of a given parental plant. In progenies from self-pollination the percent survival was 84.9 ± 1.5, in progenies from open-pollination the 86.5 ± 1.2. The difference of 1.6% is not significant (t = 0.83).

d. Variability As used in this study, the term variability refers to the morphological variations from the parental type found among the two-year old plants available for study. The aberrant plants include almost every conceivable departure from the parental type. Many were more vigorous and larger in all plant structures; many were much reduced in vigor and smaller in size. The plants varied greatly in rhizome development, degree of spread, height, leafiness, and length, width, and color of leaf. It was found that panicle characters were good criteria of morphological variability. Panicles showed great variation in over-all size and shape, size of spikelets, number of spikelets, and color.

All of the morphological variations mentioned above are grouped under variability, a term which the writer and other workers in this field assume to be an expression of sexuality existing in parental plants. Conservatism was exercised in classifying the plants in the categories of variant (sexual) and apomictic. No plant was classified as variant unless all evidence indicated the variability to be due unmistakably to genetic causes.

The total number of plants from both self-pollination and open-pollination of 87 parental plants was 8697, of which 4260 were in self-pollinated progenies and 4437 in open-pollinated progenies. The averages were 49.0 ± 1.2 plants per progeny and 51.0 ± 0.8 plants per progeny, respectively.

The correlation between the percent variability in self-pollinated progenies and in open-pollinated is highly significant ($r = + .614$; $t = 7.17$), indicating a genetic behavior inherent in any given parental plant. This correlation is greatly increased by the removal of three widely divergent pairs of observations. The correlation becomes + .818. (Figure 4). The observations which are divergent may possibly indicate plants with significantly different behaviors from those in the main body which show good fit with the regression line.

The average percent variability in the 87 self-pollinated progenies was 10.9 ± 1.4, in the 87 open-pollinated progenies 14.8 ± 1.5. The difference of 3.8% is not significant, although the $t = 1.71$ is the highest value yet obtained in this analysis of self- and open-pollinated progenies. Figure 5-B presents the frequency distribution in this material. The Chi Square analysis of each pair of progenies is presented in Figure 6. Comparison between the actual Chi Square distribution and the theoretical shows a discernible drift toward the region of high values. There are 12 significant values in the actual Chi Square distribution. 4.35 values beyond the 5% point are theoretically expected by chance alone. The question arises as to whether the increase over expectation represents plants which give self-pollinated progenies significantly different in variability than the open-pollinated progenies, or whether the increase might result from chance. The Chi Square analysis of the

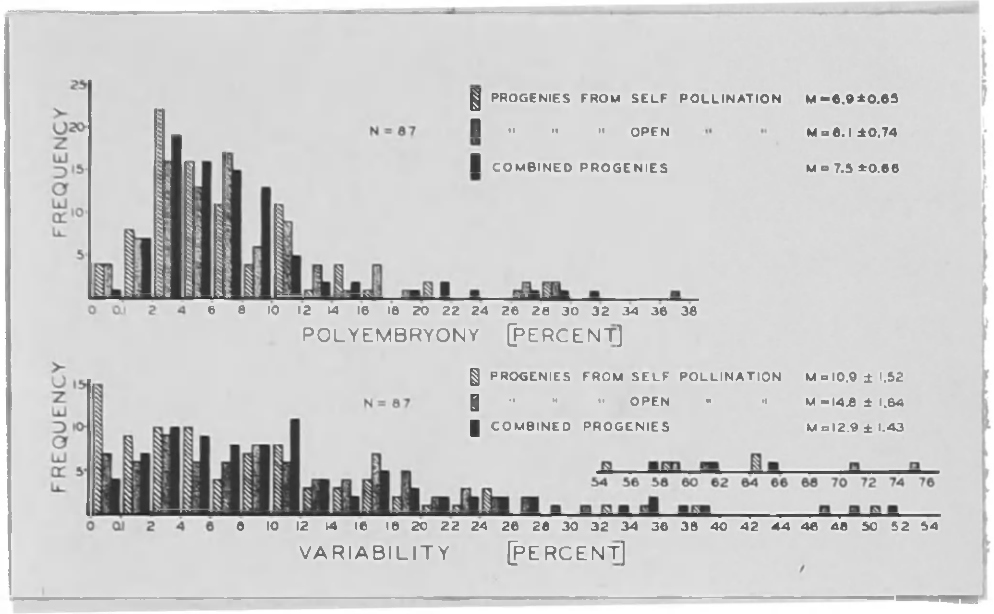

FIGURE 5 - Distribution of Values for Polyembryony(Part A) and Variability(Part B) in Self-pollinated and Open-pollinated Progenies of 87 Parental Plants.

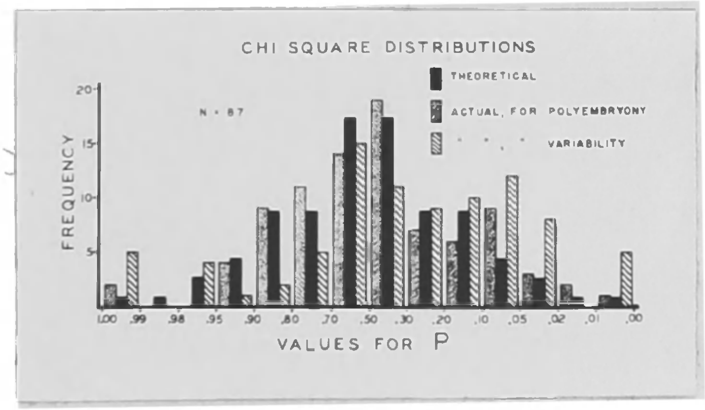

FIGURE 6 - Comparison of the Theoretical Chi Square Distribution with the Values for Differences between Polyembryony and Differences between Variability in Self- and Open-pollinated Progenies.

actual and theoretical distributions gives a value of $X^2 =$ 3.06. The conclusion is reached therefore, that, in general, the results show no statistically significant difference between the variabilities found in self- and open-pollinated progenies. However, the statistical values approach so closely the limit of significance that the possibility may not be eliminated that certain plants may give self- and open-pollinated progenies differing significantly in variability.

Analysis of all progenies - 115 parental plants. In addition to the 87 plants discussed in the foregoing section, there were 28 parental plants which, due to failure of seed set under bag, gave only open-pollinated progenies. Since the previous analysis has established that no statistically significant differences exist between self- and open-pollinated progenies, these from each parent plant have been combined, giving an average population of 100.0 ± 1.6 for each plant. There were 1369 plants in the field from the 28 plants which provided only open-pollinated progenies, and the average number of plants in a progeny was 48.9 ± 2.0. The total number of plants in the experimental nursery upon which this study is based was 10,066. This nursery represents offspring of 115 selected parental plants of Kentucky Bluegrass, which were chosen as representing the morphological range of types found in the species.

Table IV presents the data obtained from these plants.

a. Germination. The average percent germination in all plants was 80.3 ± 1.1. Germination in the 28 plants

TABLE IV

Statistical Data from Progenies of 115 Parental Plants

	MEAN	SIGNIFICANCE OF DIFFERENCES	LOWEST VALUE	HIGHEST VALUE	STANDARD DEVIATION
Germination (Percent)					
Combined progenies - 87 plants	81.4 ± 1.1	4.5 ± 3.1	56.0	98.0	10.5
Open-pollination - 28 plants	76.9 ± 2.9	t = 1.45	32.0	99.0	15.2
All progenies - 115 plants	80.3 ± 1.1		32.0	99.0	12.0
Polyembryony (Percent)					
Combined progenies - 87 plants	7.5 ± 0.7	1.9 ± 1.2	0.0	31.6	6.2
Open-pollination - 28 plants	5.6 ± 1.0	t = 1.58	0.0	21.3	5.2
All progenies - 115 plants	7.0 ± 0.7		0.0	31.6	7.0
Survival (Percent)					
Combined progenies - 87 plants	85.9 ± 1.1	1.6 ± 2.5	27.5	98.3	10.6
Open-pollination - 28 plants	87.5 ± 2.2	t = 0.64	53.5	100.0	11.8
All progenies - 115 plants	86.3 ± 1.0		27.5	100.0	10.4
Variability (Percent)					
Combined progenies - 87 plants	12.9 ± 1.4	7.6 ± 3.04	0.0	65.5	13.3
Open-pollination - 28 plants	20.5 ± 2.7	t = 2.50 *	3.4	54.2	14.5
All progenies - 115 plants	14.8 ± 1.3		0.0	65.5	13.9
Size of Progeny (Number of Plants)		TOTALS			
Combined progenies - 87 plants	100.0 ± 1.6	8,697 plants	29	118	15.2
Open-pollination - 28 plants	48.9 ± 2.0	1,369 plants	32	60	10.8
All progenies - 115 plants	Number in nursery	10,066 plants			

which did not set seed under bag was somewhat lower than that in the 87 plants giving paired progenies, 76.9% as compared with 81.4%, but the difference is not significant (t = 1.45). The lowest value obtained in germination was 32%, the highest 99%.

b. Polyembryony. The average percent of occurrence of polyembryony in the seeds which germinated was 7.0 ± 0.7. The incidence of polyembryony in the 28 plants which were sterile under bag was lower than that found in the plants which set seed under bag, 5.6% as compared with the 7.5%, but here again the difference is not statistically significant (t = 1.56). Figure 7 gives the distributions of percent polyembryony which occurred in the 115 progenies. Only four plants failed to give twin seedlings. The highest value obtained was 31.6%. In the histogram shown in Figure 7, the solid black squares indicate those progenies in which polyembryony was represented by only twin seedlings, the cross-hatched squares those progenies in which triple seedlings appeared in addition to twin seedlings. The highest value obtained for the frequency of triple seedlings was 4.3% and occurred in a progeny giving a value of 27.8% for polyembryony (twins plus triples). As mentioned previously, the appearance of triple seedlings was invariably associated with a high incidence of polyembryony.

c. Survival. The average value obtained for the percent survival of plants in the field at the end of the second year was 86.3 ± 1.0. There was only a slight difference in survival value between the 28 progenies from plants

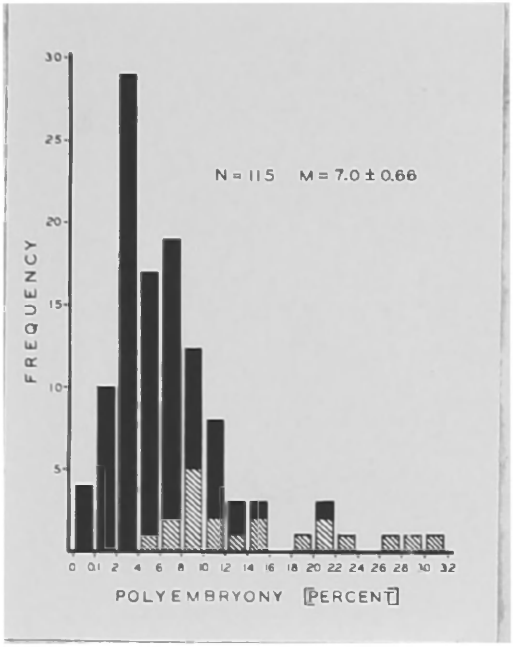

FIGURE 7 - Distribution of Values for Polyembryony in Progenies from 115 Plants of Kentucky Bluegrass.

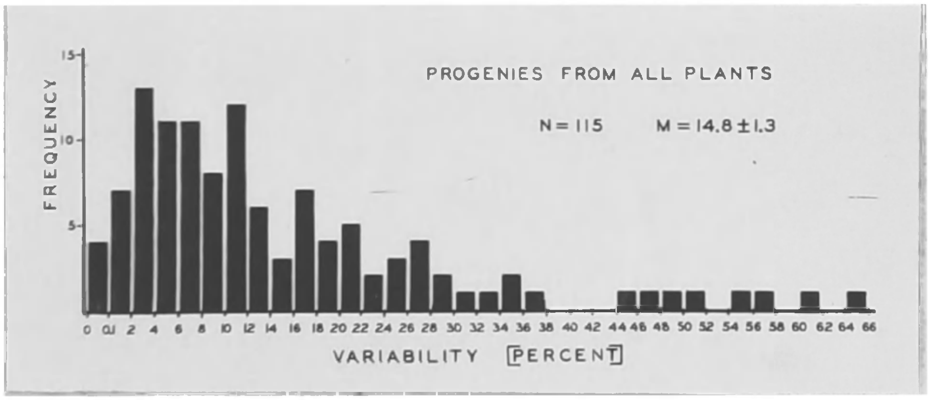

FIGURE 8 - Distribution of Values for Variability in Progenies from 115 Plants of Kentucky Bluegrass.

sterile under bag and the 87 progenies from plants fertile under bag. The lowest value for survival was 27.5% of the total number started in flats, the highest value 100%.

d. Variability. All available information gives an average percent variability of 14.8 ± 1.3 for the progenies from the 115 selected parental plants of Kentucky Bluegrass. The lowest value was 0.0%, that is, complete conformity to the parental type; the highest value was 65.5%. The distribution of values is given in Figure 8 . Only 4 of the 115 plants gave progenies showing complete uniformity. The majority of the progenies, 62, had variabilities between 0.1% and 12.0%. A smooth curve to fit the distribution should be expected to show a modal value of approximately 7.0%.

The average percent variability in the progenies from those 87 parental plants giving both self- and open-pollinated progenies was 12.9 ± 1.4. The lowest value was 0.0%, the highest 65.5%. In the 28 open-pollinated progenies from plants which set no seed under bag the average percent variability was 20.5 ± 2.7. The lowest value obtained was 3.4%, the highest 54.2%. The difference of 7.6% between these two means is significant (t = 2.50, P<.05). This would indicate a tendency for plants which were sterile under bag to be more sexual than other more fertile plants in their mode of reproduction.

Correlation Studies. In this study, an attempt has been made to determine if significant relationships exist between morphological variability in the offspring of a plant of Kentucky Bluegrass and any one of several more easily and quickly determined criteria from the same plant. The analysis centers around variability, for this is the feature in which the plant breeder is ultimately most interested.

a. Correlation between Source of Material and Variability. Figure 9 shows the distributions of variabilities found in the progenies of 115 parental plants of Kentucky Bluegrass arranged on the basis of source of the parental stock. Graph A contains the progenies of 16 plants from introductions and numbered strains, graph B the progenies of 28 plants from pasture sources, and graph C the progenies of 71 plants from commercial seed sources. The percent variabilities are 18.6 ± 3.8, 16.2 ± 2.9, and 13.3 ± 1.6, respectively. None of the differences between the means is statistically significant. ($t_{AB} = 0.50$, $t_{BC} = 0.88$, $t_{AC} = 1.29$). There is, therefore, no evidence from this material that plants of Kentucky Bluegrass from pasture sources and plants from commercial seed sources differ significantly in apomictic behavior. Nor do plants of selected strains show very pronounced apomictic behavior in comparison with plants of other sources.

b. Correlation between Seed Set under Bag and Variability. As mentioned previously in the discussion of seed set under bag in the 115 selected parental plants, the value of 30% apparently measures an inherent ability of a plant to set seed under bag with little distortion of results by environmental forces. Figure 10 shows the distributions of percent variability in the progenies of plants separated on the basis of

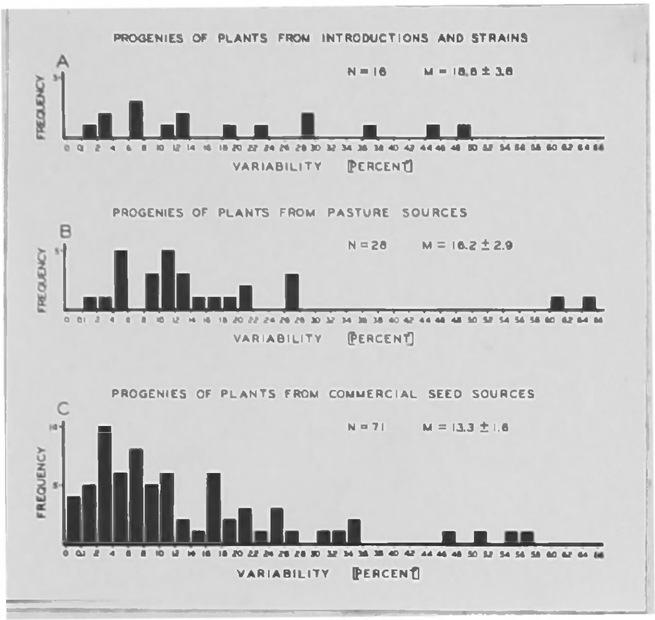

FIGURE 9 - Distribution of Values for Variability in Progenies from (A) Introductions and Strains (B) Pasture Sources (C) Commercial Seed Sources.

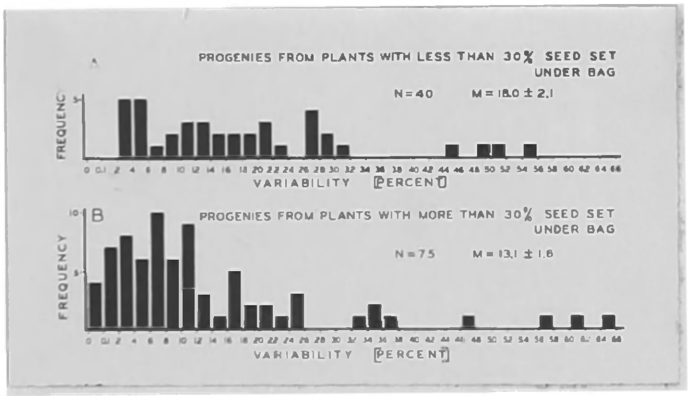

FIGURE 10 - Distribution of Values for Variability in Progenies from (A) Plants with less than 30% Seed Set under Bag (B) Plants with more than 30% Seed Set under Bag.

seed set under bag. Graph A contains the progenies of plants which set less than 30% under bag, graph B the progenies of plants which set more than 30% under bag. The percent variabilities are 18.0 ± 2.1 and 13.1 ± 1.6, respectively. The difference of 4.9% is not statistically significant (t = 1.88).

Table V contains the tabulations of correlations between (1) germination : polyembryony, (2) germination : variability, (3) variability : polyembryony, and (4) variability : survival. Both the simple correlations and the second degree partial correlations are given. Column A contains the correlations which existed in 87 self-pollinated progenies, column B those in 87 open-pollinated progenies, and column C contains the correlations found after combining self- and open-pollinated progenies. Column D, headed "Corrected for Attenuation", contains the correlation values calculated by the procedure suggested by Kemp (16) which makes use of paired values to eliminate systematic sampling errors. The values obtained by this procedure indicate the maximum degree of correlation existing in the material. Column E contains the correlation values obtained using all available data from the 115 selected parental plants of Kentucky Bluegrass. In the discussion which follows reference will be made only to Column E. Table V may be consulted for the other values.

c. <u>Correlation between Germination and Polyembryony in progenies of 115 parental plants</u> (Figure 11) There is a significant positive correlation of + .222 ($P < .05$) between these two measurements. The partial correlation between germination and polyembryony, holding survival and variability

TABLE V

Correlations of Variability with Germination, Polyembryony, and Survival in Progenies of Kentucky Bluegrass

		A SELF-POLLI. PROGENIES OF 87 plants	B OPEN-POLLI. PROGENIES OF 87 plants	C COMBINED PROGENIES OF 87 plants	D CORRECTED FOR ATTENUATION 87 plants	E ALL AVAILABLE DATA 115 plants
Germination – G	r_{GP}	+ .202	+ .150	+ .210 *	+ .310 **	+ .222 *
Polyembryony – P	$r_{GP \cdot SV}$	+ .206	+ .140	+ .205	+ .390 **	+ .202 *
Germination – G	r_{GV}	– .225 *	– .099	– .214 *	– .386 **	– .235 **
Variability – V	$r_{GV \cdot SP}$	– .052	+ .007	– .072	+ .068	– .153
Polyembryony – P	r_{PV}	– .167	– .168	– .210 *	– .260 *	– .205 *
Variability – V	$r_{PV \cdot SG}$	– .175	– .177	– .235 *	– .391 **	– .218 *
Survival – S	r_{SV}	– .432 **	– .265 *	– .444 **	– .645 **	– .380 **
Variability – V	$r_{SV \cdot PG}$	– .404 **	– .260 *	– .432 **	– .640 **	– .378 **

N = 87
* P.05 = .208
** P.01 = .270

N = 115
* P.05 = .180
** P.01 = .235

constant, is also significant, + .202.

d. Correlation between Germination and Variability in progenies of 115 parental plants. (Figure 12) The simple correlation existing between germination and variability shows a highly significant negative value of - .235 (P = .01). However, the partial correlation obtained by holding survival and polyembryony constant drops to the insignificant value of - .133. The introduction into the calculation of the value for survival accounts for the drop to insignificance.

e. Correlation between Polyembryony and Variability in progenies of 115 parental plants (Figure 13) There is a significant negative association between variability and polyembryony. The simple correlation is - .205 ($P < .05$). The value for the partial correlation, holding germination and survival constant, rises somewhat to - .218. In general, it may be said, therefore, that there is a tendency for the higher percentages of morphological variability in the progeny of a plant to be associated with a low percentage of polyembryony, and vice versa. This relationship, however, cannot be stated positively because of the barely significant values obtained for the correlation. A study of the character of the scatter diagram in Figure 13 suggests the possibility that two groups may be present; one group of plants whose progenies show a very high negative correlation between variability and polyembryony, and another group whose progenies show no association between these two characteristics. These selections of Kentucky Bluegrass appear to be sufficiently diversified and heterogeneous as to permit this supposition.

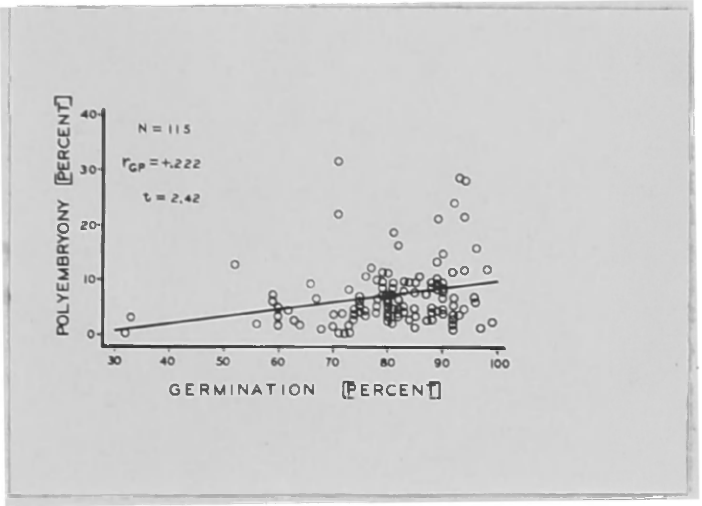

FIGURE 11 - Correlation of Germination with Polyembryony in Progenies of 115 Parental Plants.

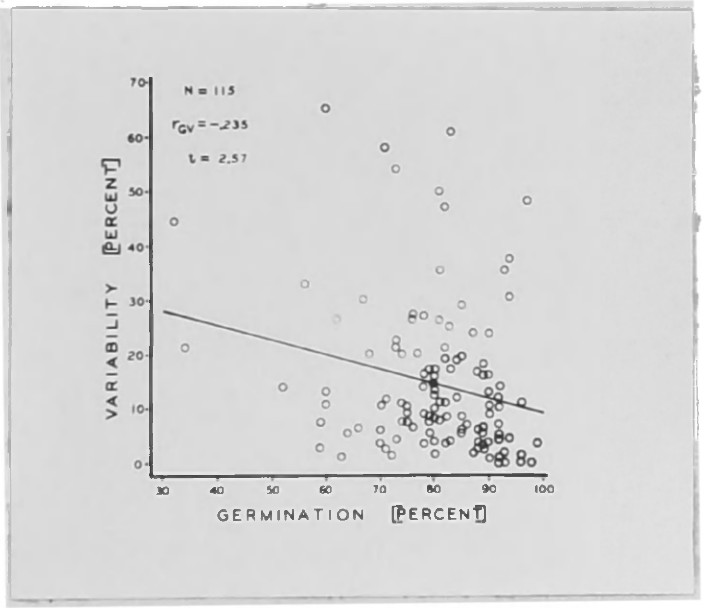

FIGURE 12 - Correlation of Germination with Variability in Progenies of 115 Parental Plants.

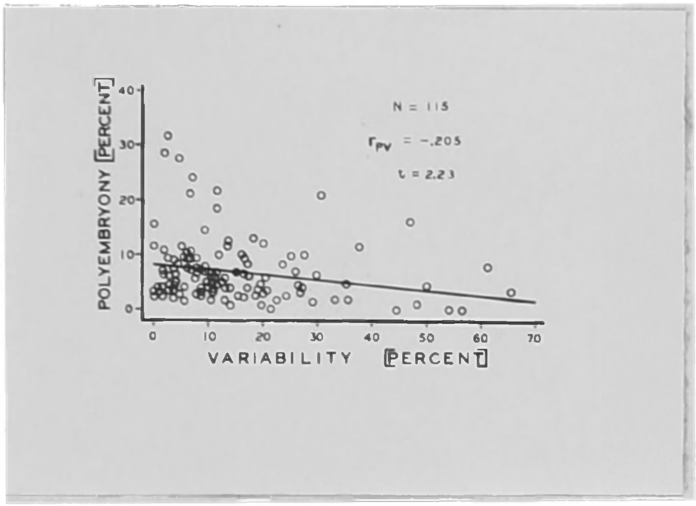

FIGURE 13 - Correlation of Variability with Polyembryony in Progenies of 115 Parental Plants.

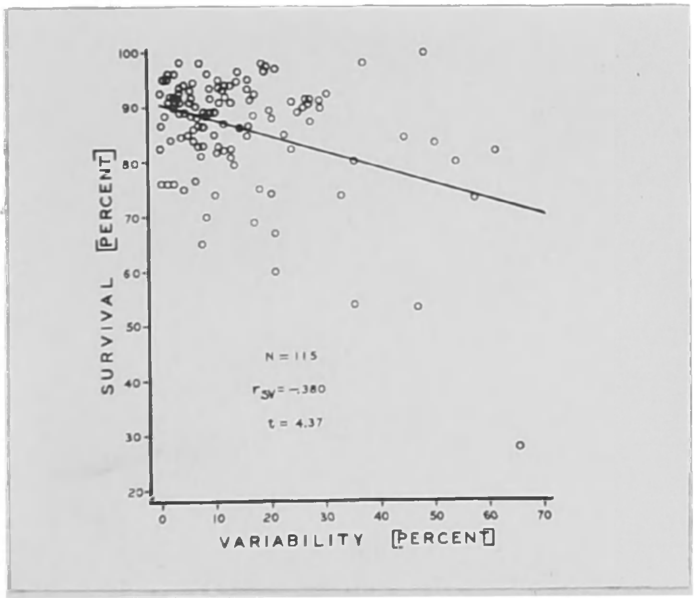

FIGURE 14 - Correlation of Variability with Survival in Progenies of 115 Parental Plants.

f. Correlation between Survival and Variability in progenies of 115 parental plants (Figure 14) The fact that the loss of plants was unusually high in some progenies has already been mentioned. This raised the question as to whether practically all of the plants which failed to survive were not actually weak aberrant forms with no survival value under field conditions. The highly significant correlation of - .380 between survival and variability suggests this strongly. Holding germination and polyembryony constant, the value for the partial correlation is - .378 ($P < .01$).

Comparative Variabilities in Plants from Single Embryo Seed and in Plants from Poly-Embryo Seed. Facilities were not available for as complete an analysis of the twin and triple seedlings as was made of the single seedlings. It is, however, possible to obtain an average figure for the variability which was found in the polyembryo material. Table VI gives the complete analysis. Of the 1196 sets, representing 7.4% of the total germination, 39, or 0.24%, were triple seedlings, and 1157, or 7.2% were twin seedlings. Only 711 sets were available for study due to the failure of the weaker member of a set to survive. The percent survival was 80.1%. This is to be compared with the value of 86.9% for survival among the plants from single seedlings. The difference of 6.8% is not statistically significant; ($X^2 = 2.40$). 421 of the 711 sets of twin and triple seedlings were discarded in the greenhouse from 3" pots. These discards were those classified as consisting of identical plants. Of the remaining 290 sets which were classified as containing

TABLE VI

Comparative Variabilities in Plants from Seed with Single Embryos and in Plants from Polyembryonic Seed

Number of seed started 19,858 Number germinated 16,084 Germination = 81.0%

	POLYEMBRYO SEED	Triples	Twins	SINGLE-EMBRYO SEED
Number appearing	1,196 7.4%	39 0.24%	1,157 7.2%	14,888 92.6%
Number surviving for study	711	19	692	10,066
Percent Survival	80.1%	82.9%	80.0%	86.9%
Groups discarded as identical	421	15	406	
Groups dissimilar or questionable	290	4	286	
Number of Plants as Individuals: Total Number from Germination which Survived	1,441	57	1,384	10,066
Number of Aberrant Plants	290 - 244*	4	286 - 240*	1,314
PERCENT VARIABILITY	20.1% - 17.1%*	7.0%	20.7% - 17.3%*	13.1%

* a corrected value - explained in text

plants which differed morphologically, 73 sets, 69 twin seedling pairs and 4 triple seedling groups were put into the field and grown to maturity. At maturity, it was ascertained that 11 of the twin pairs consisted of identical plants. On the basis of this error in identification of aberrant pairs in the greenhouse, a correction factor of 84% was utilized. Thus, out of 1441 plants from seed containing more than one embryo, 290 to 244 (84% of 290) may be considered as aberrant. This gives the figure for percent variability in plants from twin and triple seedlings of 20.1% to 17.1%. The true value is in this range, with the greater likelihood of it being nearer the 17.1% value. This is to be compared with the percent variability of 13.1% found in plants from seeds with a single embryo. The Chi Square test indicates that great significance may be ascribed to this difference ($X^2 = 16.85$). It may be said therefore, that variability is significantly higher among plants derived from polyembryonic seeds than from plants obtained from single-embryo seeds. But while the extent of morphological variation is significantly different between these two populations, the nature of this variation is the same. In other words, plants of the same general morphological configurations may be found from either source. Merely the relative chances of obtaining them are increased by a study of populations from twin and triple seedlings. The nature of variation in morphological characters found in plants from seeds with single embryos has already been described. The same range of types was found in the aberrant plants which occurred in the groups of twin and triple seedlings.

In the instances where twin seedlings and triple seedlings were identical the type of plant invariably conformed to that of the parent. In the cases where triplets were dissimilar, one plant was larger and more vigorous and two plants were identical with the parent plant type (Plates II and 12, F_1, F_2, F_3). In the triple seedlings no aberrant plant was found which was smaller and less vigorous than the parent plant, but this can hardly be significant in view of the small number of triplets available for study (19 triplets). In the instances where twin seedlings were dissimilar, the aberrant plant almost invariably started out as the smaller and slower growing seedling although in the majority of instances it was to become the larger and more vigorous plant of the pair. It would seem, therefore, that the aberrant, or sexually produced, member of a dissimilar pair of seedlings usually comes from an embryo which, for some reason, is later in germination than the apomictically produced embryo associated with it.

Studies on aberrant plants in progenies Representative parental plants, apomictically reproduced offspring, and aberrant sexually produced offspring were analysed to determine the relative rate of polyembryony between parental plants and offspring and also the occurrence of albino seedlings, since a few of these had appeared in seed lots of the original material. Open-pollinated seed was germinated in Petri dishes in the manner described above in establishing the experimental nursery.

The probable chromosomal complements of many of the aberrant plants have been determined by an analysis of pollen grain size. These results are presented, and supplemented with actual chromosome counts in a number of instances to show the manner of origin of the aberrant plants.

a. Polyembryony The results of an analysis of relative rates of polyembryony are presented in Table VII. In each family, P represents the parental plant; A, the apomictically reproduced type; and the other letters refer to plants of the progeny which deviated from the parent type in morphological characters and which are presumed to have arisen by sexual processes. Determinations were made on seed from 68 plants, 10 parental and 58 offspring. It was found that the percent polyembryony in eleven offspring plants was significantly different from that in the parents; 2 had significantly higher rates and 9 significantly lower rates. In only one instance did the parent and the apomictic type differ in rates of polyembryony; germination in the latter was poor and sampling errors may account for observed difference. The value of $X^2 = 4.77$ is barely beyond the 5% point.

The points of interest in connection with these studies on relative rates of polyembryony are these:

(1) That apomictically reproduced plants show the same values for polyembryony as do the parental types from which they are derived. This is self-evident if one accepts the idea that the ability to produce polyembryonic seeds is related in some way to the genetic constitution of the plant. The statement is made however, to indicate that

evidence points to the fact that a plant has a characteristic and inherent tendency towards the production of polyembryonic seed, and that the environmental forces encountered in the prosecution of these studies change this basic rate little, if at all.

(2) That aberrant plants, that is, those which are not produced apomictically, fall into 3 classes on the basis of relative rates of polyembryony in comparison with the parental plant; (a) The aberrant plants may show no significantly different rate of polyembryony. This is by far the largest group and this behavior seems to have no association with plant type or manner of origin; (b) The aberrant plants may show a significantly lower rate of polyembryony. Nine plants comprise this class. Both more vigorous plants and weaker plants may be represented (Plate 1, F; Plate 6, I; Plate 10, C; and Plate 11, C_1, E_1; or (c) The aberrant plants may show a significantly higher rate of polyembryony. This must be regarded as exceptional since only two plants out of 58 tested fall into this group. One plant is shown in Plate 8, G; the other, the triploid member of a group of triple seedlings, in Plates 11 and 12, F_1.

b. <u>Albino seedlings</u> Albino seedlings have appeared in progenies of three plants.

1. <u>37-KB 1 (11)</u> This plant and its offspring are discussed in the Appendix and evidence indicates that it is perhaps the most sexually reproduced of the selected material (see Appendix). In 279 germinated seed of the plant shown in Plate 1, P, there appeared 1 albino single seedling

TABLE VII

Pollen Grain Size, Chromosome Numbers, Polyembryony, and Albino Seedlings in Selected Plants of Kentucky Bluegrass and their Progenies

	POLLEN GRAIN SIZE (MICRONS)	CHROMOSOME NUMBER	PERCENT POLYEMBRYONY		ALBINO SEEDLINGS
PLATE 1					
P 37-KB 1(11)		± 56	13-279[x]	4.7%	2 albino seedlings[σ]
A 38-KB 3(8) type		56	0- 99	0.0 *	2 albine seedlings
B 3(6)		± 48	0- 0	——	
C 3(9)		no material	0- 0	——	
D 4(4)		± 50	1- 30	3.3	
E 4(7)		± 56	6-234	2.6	4 albine seedlings
F 4(3)		± 58	0-252	0.0 **	
PLATES 2 and 3					
P 37-KB 38(12)		no material	3-139	2.2%	
A 38-KB 26(40) type		± 56	5-191	2.6	
B 25(22)		± 73	0- 38	0.0	
C 26(10)		± 60	2-118	1.7	
D 26(26)		± 52	———	——	
E 26(30)		± 56	6-259	2.3	
F 26(50)		± 76	0-132	0.0	
PLATES 11 and 12					
A_1 26(65)-1		± 50	1- 40	2.5	1 albino seedling
A_2 (65)-2 type		56	5-329	1.5	
PLATES 4 and 5					
P 37-KB 140(11)	25.9 ± 0.3 ref.	± 45	7-305	2.3%	
A 38-KB 130(4) type	25.2 ± 0.2	± 45	7-439	1.5	
B 130(8)	34.8 ± 0.6 **	± 62	2-207	0.9	
C 130(21)	33.4 ± 0.5 **	± 70	0-145	0.0	
D 130(13)	33.8 ± 0.4 **	± 65	5-229	2.1	
E 130(26)	32.2 ± 0.6 **	± 67	1-256	0.3	
F 130(1)	32.4 ± 0.6 **	± 60	7-199	3.5	

TABLE VII (continued)

	POLLEN GRAIN SIZE (MICRONS)	CHROMOSOME NUMBER	PERCENT POLYEMBRYONY		ALBINO SEEDLINGS
NO PLATES					
37-KB 172(14)		± 80	1-21- 90[xx]	24.4%	
38-KB 195(1) type			1-39-157	25.5	
195(21)		± 42	0- 12	?	
196(1) type			4-44-171	25.7	
196(5)		110-120	17-182	9.3 **	
PLATES 6 and 7					
P 37-KB 175(14)	29.0 ± 0.7 ref.	± 30	1-18-213	8.9%	1 albino seedling
A 38-KB 206(16) type			30-382	7.8	
B 206(1)		34	no seed		
C 206(11)		diploid	0- 43	0.0 *	
D 206(4)		diploid	3- 99	3.0	
E 206(8)	17.5 ± 0.5 **	no material	0- 2	———	
F 206(6)	30.0 ± 0.7	diploid	16-186	8.6	
G 206(28)		diploid	2-185	1.1 **	
H 205(6)	25.9 ± 0.6 **	diploid	2-22-173	13.9	
I 205(14)	30.5 ± 0.4	diploid	1-108	0.9 **	
PLATES 11 and 12					
B_1 206(62)-1	21.8 ± 0.4 **	± 48	no seed		
C_1 (63)-1	25.4 ± 0.5 **	no material	1-199	0.5 **	
C_2 (63)-2 type	30.7 ± 0.4	± 30	37-303	12.2	6 albino seedlings[σσ]
PLATES 8 and 9					
P 37-KB 175(46)	26.2 ± 0.6 ref.	± 36	0-201	0.0%	
A 38-KB 208(40) type			0-135	0.0	
B 208(7)	20.4 ± 0.6 **	diploid	0- 83	0.0	
C 208(18)	27.4 ± 0.6	diploid	0-139	0.0	
D 208(2)	26.6 ± 0.4	diploid	0-128	0.0	
E 208(6)	34.1 ± 0.9	triploid	0- 67	0.0	
F 208(54)		triploid	0- 77	0.0	
G 208(56)		diploid	4-121	3.3 **	
H 208(9)		diploid	0- 38	0.0	
I 208(30)		diploid	3-195	1.5	

TABLE VII (concluded)

	POLLEN GRAIN SIZE (MICRONS)	CHROMOSOME NUMBER	PERCENT POLYEMBRYONY		ALBINO SEEDLINGS
PLATE 10	PLATE 13				
P 37-KB 155(151)	P 29.5 ± 1.0 ref.	42	10-242	4.1%	
A 38-KB 118(40) type	28.1 ± 1.4	42	4-216	1.9	
B 118(47)		no material	15-195	7.7	
C 118(42)		± 42	1-172	0.6 *	
D 118(36)		± 45	0-170	0.0 *	
E 118(39)	A 22.3 ± 0.5 **	± 40	9-128	7.0	
F 118(46)	B 34.6 ± 0.5 **	± 75	2-165	1.2	
PLATES 11 and 12	PLATE 13				
37-KB 114(12)			21-183	11.5%	
D_1 38-KB 47(62)-1	C_1 18.2 ± 0.2 **	no material	5- 70	7.1	
D_2 (62)-2type	C_2 28.6 ± 0.4 ref.	no material	36-396	9.1	
37-KB 157(240)			12-184	6.5%	
E_1 38-KB 124(65)-1	D_1 34.1 ± 0.5 **	no material	3-243	1.2 **	
E_2 (65)-2type	D_2 27.4 ± 0.8 ref.	no material	3-102	3.0	
37-KB 152(145)			13-265	4.9%	
F_1 38-KB 160(91)-1	36.2 ± 1.0 **	± 65	1-13-159	8.8 *	
F_2 (91)-2type	32.1 ± 0.7 ref.	42	5-185	2.7	
F_3 (91)-3type	31.7 ± 0.6	± 42	1- 3- 55	7.3	
-(2+3)			1- 8-240	3.7	
68 plants; 58 are offspring					

x Last term total number seed, first term number twins.
xx Last term total number seed, middle term number twins, first term number triples.

o One albino single; 1 pair of twins, one member albino and other member green.
oo Three albino singles; 1 pair of twins, one member albino and other green; 1 pair of twins, both albino.

* P less than .05
** P less than .01 t test for significance of difference.

and 1 albino seedling associated with a green seedling from a twin embryo seed. Two progenies from plants of its offspring have contained albinos. One was the progeny from an apomictically reproduced member of the population (Plate 1, A) which gave 2 albino single seedlings in 99 germinated seed, the other was an aberrant plant from the same population (Plate 1, E) which gave 4 albino single seedlings in 234 germinated seed.

2. 37-KB 175 (14) This plant and its progenies also have been described (see Appendix). In 213 seedlings from the parent plant shown in Plate 6, P there appeared 1 albino seedling. An apomictically reproduced plant of its progeny has given a surprisingly large number of albinos. This plant is shown in Plate 11, C_2 and was the plant associated with C_1 in a pair from a twin embryo seed. In 303 germinated seed, there were 3 albino single seedlings, 1 albino seedling which occurred with a green seedling to comprise a pair of twins, and 2 albino seedlings which occurred together as twin seedlings. No albinos have appeared in seedlings from any aberrant plants obtained from this parent.

3. The other instance of albinism was found in the progeny grown from seed of the aberrant plant from a twin pair shown in Plate 11, A_1. In 40 seedlings, 1 was an albino. No albinos have appeared in seedlings from either the parental plant or any other aberrant plants in its progeny.

e. Pollen Measurements Mature pollen grains were mounted in acetocarmine and their diameters measured by means

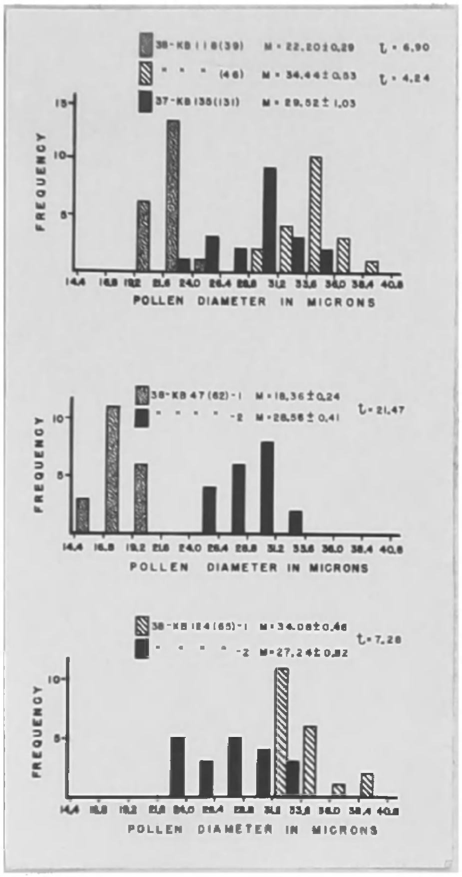

FIGURE 15 - Distribution of Values for Pollen Grain
Diameters in (A) Parental Plant and Two Aberrant Offspring
(B) Twin Seedlings with Aberrant Plant smaller
(C) Twin Seedlings with Aberrant Plant larger

of a filiar micrometer. The number of grains measured per plant varied between 20 and 25. This number of observations, while small, allowed many more determinations than otherwise could be made due to limitations of time and assistance. Actually, the small number seems entirely adequate, judging by the sizes of the standard errors which are decreased, on the average, only about 0.3 micron when N = 100.

The measurement of pollen grains is useful in determining gross chromosomal changes, since, as has been found in numerous plants, pollen grain size is proportional to chromosome number (plate 13). This has been applied to Poa by Müntzing (22) and the writer (7). The results are presented in Table VII and require little individual comment. The fact is perfectly clear that the pollen of many of the aberrant plants is significantly different in size and may be assumed to reflect the chromosomal complement of the plant. In no case, was the size of pollen significantly different between the parental plant and the apomictically reproduced progeny. However, the aberrant plants, on the basis of pollen grain size, may be placed in one of three catagories; (1) Pollen significantly smaller in size than the parent, indicating lower chromosome numbers; (2) Pollen significantly larger in size, indicating plants with a great increase in chromosome number, probably "triploids"; and (3) Pollen not significantly different in size, indicating chromosome numbers so near that of the parent that the pollen differences did not give statistically significant values; these may be called "diploids by amphimixis", as distinguished from "diploids by apomixis".

d. Chromosome Counts The somatic chromosome number was obtained from root-tip divisions. The material was killed and fixed in the modification of Navaschin Fluid proposed for *Poa* by Müntsing (19). Its constitution is as follows:

A.	Chromic Acid, 10% aq. sol.	10cc	B.	Formalin U.S.P.	30cc
	Acetic Acid, glacial	10cc		95% Ethyl Alcohol	10cc
	Water, distilled	80cc		Water, distilled	60cc

The material was dehydrated and embedded by the procedure devised by Randolph (30), was sectioned at 12 microns, and was stained by the modified crystal-violet-iodine technique proposed by Smith (32).

The extent and nature of chromosomal phenomena in *Poa pratensis* have been analysed in very detailed studies by Müntzing, Armstrong, and Akerberg. Therefore, it has not been thought necessary in this study to do more than to determine with certainty the manner of origin of the aberrant or sexually produced plants which have appeared in the writer's material. Some indications of their probable chromosomal complements have already been obtained from a study of morphological features and pollen grain size.

The results of the cytological study are tabulated in Table VII. It is seen that by far the greatest number of the aberrant plants are to be considered as arising through the fertilisation of a reduced egg cell by a reduced pollen grain since their chromosome numbers do not deviate markedly from the chromosome number found in the parent plant. Two "haploid" individuals have been identified, one of which is shown in Plate 6, B. "Triploid" aberrant plants have been identified with certainty in twelve instances. These plants are usually of increased vigor, but attention is directed to the plant figured in Plate 2, B which is an exception to this general statement. Another triploid of the same progeny, however, is decidedly more vigorous than the parent plant (Plate 2, F). This might indicate that chromosomal multiplication in Poa is subordinate to genic constitution in the determination of plant vigor. Of considerable interest is the progeny shown in Plate 4 where every aberrant plant studied has been demonstrated to be of triploid origin. On the other hand, in the progeny of the most highly sexual plant studied shown in Plate 1, all aberrant plants presumably have arisen by the union of reduced egg and sperm; that is, "diploid by amphimixis".

Only two pairs of twin seedlings have been studied cytologically. In each instance, the aberrant plant has been shown to be of probable diploid origin (Plate 11 - A_1, A_2, and B_1, B_2). The aberrant plant of the group of triple seedlings (Plate 11, F_1) has been shown to be a triploid.

To summarize the results of the cytological studies, we may enumerate the following points:

(1) That the aberrant plants which have appeared in the progenies of Kentucky Bluegrass may be classified in their manner of origin as haploids, sexually produced diploids, and triploids. The diploids have been by far the most prevalent aberrant type. The haploid individual is

either of infrequent origin or usually fails to survive for study.

(2) That pollen grain size is of value in identifying certain types of aberrant plants but must be used with caution when applied to the identification of others. Pollen size may be used with complete reliability, for example, in identifying plants of triploid origin and sexually produced diploids. Its use in presuming a plant to be of haploid origin if its pollen is significantly smaller than the parent plant is not reliable since many aberrant plants with extremely small pollen grains have been shown to have chromosome numbers not greatly different from the parental type. Presumably, if the aberrant plants are of low vigor, as many are, physiological factors, such as rate of growth and efficiency of nutrition, may cause the formation of smaller cells than should theoretically be produced on the basis of the plant's chromosomal complement.

(3) That similar chromosomal conditions are present in aberrant plants from either seed with multiple embryos or seed with a single embryo. There is thus no essential difference in their manner of origin.

DISCUSSION OF RESULTS

Embryology of Apomixis in Poa. The embryology of origins of the embryo sac and the apomictic development of the embryo in the Bluegrasses is by no means as complete as one might wish. Two reasons may be assigned for this and these will explain the limited knowledge we have, and the apparent contradictions which exist among the researches of the several workers. In the first place, the material is technically difficult to prepare and to obtain an adequate supply of critical stages; and secondly, we have not fully realized that in as variable a species as Poa pratensis it is entirely possible that biotypes may exist which differ materially in methods of embryo sac formation and the origin of the embryo. Both Anderson and Armstrong described haplosporic development of the embryo sac, Tinney and Akerberg aposporic development. There seems little question that, according to all available data, apomixis in Poa pratensis is by Apospory. It must not be overlooked, however, that predominantly sexual types unquestionably exist. Akerberg presented limited embryological data from such a biotype and showed that no aposporic development occurred but that functional reduced eggs arose by Haplospory. Nor should it be overlooked that Diplospory has been described for Poa palustris by Kiellander and for P. alpina by Müntzing. It is not improbable that, as embryological work is extended in Poa pratensis, some biotypes may show diplosporic embryo sac development. This may be found either in naturally occurring biotypes or may occur in hybrid types between pratensis and diplosporous forms, such as alpina and palustris. Pratensis-alpina hybrids have already been obtained by Akerberg (1) and (2) and Müntzing (22).

Sterility and Fertility Studies in Poa pratensis. It is unfortunate that the investigator usually must depend on seed set under bag as a measure of self-fertility. This may introduce physiological factors such as altered aeration, light, and moisture relationships which ultimately determine, not the plant's inherent fertility but merely its ability to set seed under conditions of bagging. A better measure of fertility would be that obtained by the use of pollen-tight cages, or ideally, by isolating the biotype.

However, with all the disadvantages attending the use of bags, Nilsson's work and the results of the writer show clearly that plants of Kentucky Bluegrass differ markedly in their ability to set seed under bag and that these observations undoubtedly indicate a genotypical basis for sterility and fertility. These conclusions, both by Nilsson and the writer, have been based on replicated observations, and these are essential. A single bagging operation has questionable value. Results to have significant value should be accumulated over several years and by the use of replicated bags. Even then, the results must be used with caution. There will be no _a priori_ reason for assuming that a plant which sets no seed under bag will also be sterile when unbagged but isolated from foreign pollen. This has important practical implications if the breeding program should center about such methods as hybridization and strain building.

Analysis of self- and open-pollinated progenies. The measurements made on germination and polyembryony have indicated that no statistical significance may be ascribed to differences between self- and open-pollinated progenies of the same plant. This is particularly striking with polyembryony. Plants vary tremendously in their inherent rates, but this rate is apparently little affected, if at all, by the type of pollination, or by environmental factors encountered in this study. This follows naturally if it be assumed that apomixis and its attendant phenomena are genic in character.

In the case of morphological variability, the relationship is less clear, but it may be said with a fair degree of certainty that most of the plants give self- and open-pollinated progenies which do not differ significantly in variability. Figures 4, 5-B, and 6 and Table III illustrate this similarity. However, there are some paired progenies which differ markedly in variability and this might reflect a fundamental difference in manner of origin of the plants belonging to self- and open-pollinated progenies. Such a difference is readily understandable if it be assumed that the plant's own pollen is incapable of fertilising its own eggs, but is capable of initiating pseudogamous development to form apomictic offspring. However, if foreign pollen were available it is logical to believe that pollen tube growth would be rapid enough to effect fertilization and produce aberrant forms. An instance of this behavior is strongly suggested by progenies from one plant shown in Figure 4. Variability in the self-pollinated progeny was zero, the 59 plants conforming perfectly to the parental type. On the other hand, in the open-pollinated progeny only 15 of 59 plants resembled the parent, while the aberrant forms showed the morphological features of triploids. It is possible to select a graded series of behaviors from this extreme case to paired progenies which show no differences.

Summarizing the data, we may say that plants of Kentucky Bluegrass may fall into one of three general classifications: (1) Variability is higher in the self-pollinated progeny than in the open pollinated progeny. (This is a theoretically possible class not demonstrated with certainty in the present material); (2) Variability is essentially the same in both self- and open-pollinated progenies. (By far the greatest number of plants studied fall into this group); or (3) Variability is lower in the self-pollinated progeny than in the open-pollinated progeny. (An explanation for this behavior has been presented).

The origin of offspring in Poa. The studies which have been conducted on the embryology and on morphological and cytological features of aberrant and matroclinous plants in species of Poa have furnished more or less satisfactory evidence, however indirect, of the manner of origin of offspring. In the following classification all theoretically possible methods are outlined:

I. Reduction Division - embryo sac arises by Haplospory.
    A. Non-fertilization
        1. Pathenogenesis - production of "haploids".
    B. Fertilization
        2. By reduced pollen - production of "diploids by amphimixis".
        3. By unreduced pollen - production of "triploids".
II. No Reduction Division - embryo sac arises by either Diplospory or Apospory.

A. Non-fertilization

4. Parthenogenesis - production of "diploids by apomixis", with maternal characters and chromosome complement.

B. Fertilization

5. By reduced pollen - production of "triploids".

6. By unreduced pollen - production of "tetraploids".

Four of the six types have been definitely proven in Poa pratensis, namely types 1, 2, 4, and 5. It has been possible in the present study to determine these types of origin by an analysis of chromosome numbers. Previous work by other investigators leaves little doubt that matroclinous plants in Poa arise regularly by type 4 and that aberrant plants arise by types 1, 2, or 5. Recently, Müntzing (22) has shown quite conclusively that functional unreduced pollen grains may form in Poa alpina. In this particular case, tetraploids were obtained in the progeny, thus establishing type 6 as an actual method of reproduction in Poa. It follows from this that type 3 is also no longer theoretical but may be considered an actual method of reproduction in Poa, since there is no reason to assume that unreduced pollen cannot fertilize a reduced egg. The wide range of pollen sizes found in some plants of Poa pratensis suggest the possibility that functional unreduced pollen may be formed in this species also (Plate 13,F).

To summarize, one can say that all of the six theoretically possible methods of reproduction are possible among species of Poa. Four have been clearly established for Poa pratensis, while the other two depend only on the demonstration of functional unreduced pollen grains in this species.

The appearance of albino seedlings is additional indirect evidence that reduced megaspores may develop into embryos. They may be considered as haploids, since it is unlikely that the high chromosome numbers and autopolyploid relationships found in Poa pratensis would permit such frequencies to arise by diploid methods of origin.

The study of polyembryony affords additional evidence of the manner of origin of offspring in Poa pratensis. Twin seedlings have been shown to be largely maternal, that is, diploid:diploid and have probably arisen by the simultaneous development of two nucellar cells. Triploid:diploid twins have arisen in considerable quantities and have been subjected to intensive study. Workers are in agreement that the triploid member has arisen through the fertilization of an aposporous embryo sac by reduced pollen and that the triploid member is invariably weaker and later in starting development. Tinney (36) observed the probable reason for this when he reported that one embryo sac nearer the supply of food generally develops at the expense of the weaker member. Anderson (5) reported the same observation. Haploid:diploid twins have been observed by Müntzing, Skovsted, and the writer, and have presumably arisen by the development of a reduced megaspore and an aposporous egg cell. Of considerable interest is the albino pair of twins observed in the writer's cultures. The most probable constitution of these plants is haploid:haploid and they must represent the simultaneous development of sister megaspores which have been derived from the same cell of a dyad after meiosis.

Triple seedlings appearing in the writer's cultures have been shown to be of two types, diploid:diploid:diploid and triploid:diploid:diploid. Triple seedlings have also been reported by Müntzing. Their origin seems to be no different from that of the corresponding twins. Presumably three nucellar cells develop instead of two.

Pseudogamous Seed Development. The complete agreement of pollination studies in showing that pollen must be applied to the stigmas of *Poa pratensis* for seed to be formed and the observations of Tinney that the embryo was frequently well developed before flowering occurred need to be reconciled. Tinney observed further that the endosperm was not well developed at the time of flowering and suggested that pollination may stimulate its development. This explanation finds support in the work of Gentcheff and Gustafsson (13). They report two biotypes of *Potentilla collina* which differ in response to pollination and fertilization. In one, strain C-B, apomictic embryos are formed independently of pollen stimulation, but the endosperm cannot develop without the stimulus of applied pollen. In the second strain, C-G, an actual fertilization of the endosperm nucleus is needed to initiate seed formation. The application of these results to *Poa* is obvious.

The Nature and Extent of Variability. The morphological variability found by the writer in progenies of Kentucky Bluegrass has been higher than that reported by other investigators. The writer reports an average value of 14.8%. Akerberg gives the values of 5.9% to 13.4% depending on the nature of his material, while Tinney and Aamodt give as the average percent variability in their material the low figure of 1.59%. The slight deviations between Akerberg's values and the values obtained by the present writer may well be explained by sampling within a highly polymorphic species. On the other hand, the discrepancies in the reports of variability presented by the writer and those presented by Tinney and Aamodt need to be accounted for, particularly since the parental material came, in part, from similar sources.

The writer believes that the most plausible explanation comes from the contrast in the methods employed in germinating the seed for the establishment of the respective nurseries. Tinney and Aamodt germinated seed in soil; the writer utilized Petri dishes for this purpose, thus permitting equal chances for survival of all the products of germination. Since many aberrant plants in progenies of Kentucky Bluegrass are weak types barely able to survive, it is readily apparent that this type of plant stands little chance of ever appearing if it must force its way through soil and grow in competition with more vigorous seedlings. The writer believes that the differences between his results and those of Tinney and Aamodt may well be accounted for by this explanation. A statistical treatment of data pertaining to survival of plants and variability of the remaining plants emphasizes this statement.

Loss of plants in the field has been shown to be associated with progenies from certain plants and not to be distributed at random throughout the population. The high correlation between survival in self-pollinated progenies and survival in open-pollinated progenies can mean only one thing: That loss of population is, in some way, related to an inherent characteristic of the parent. It is logical to relate this loss of plants to the fact that they were forms too weak to survive. If they were too weak to survive they would not have the genic constitution of the parent and hence should be classified as aberrant. By the proposed criteria for apomixis they must be presumed to have arisen sexually.

The high positive correlation between loss of plants and the morphological variation existing in the plants which remain makes the relation between loss and sexuality even more apparent. One cannot escape the conclusion that the two groups of plants should actually be considered as one and that the only difference between them is one of degree of vigor.

These results permit one to suggest that the values for sexuality in the parental plants of Kentucky Bluegrass may be higher than indicated by morphological variation in their progenies, since plants which would have been aberrant never appeared for study. By combining the number of plants which failed to survive and the number of the remaining plants showing morphological variations, we obtain the following values in percent: mean, 25.5 ± 1.5, lowest value 5.0%; highest value 90.5%; standard deviation 15.5%. The plant and its progeny which gave the highest value is shown in Plate 1; loss, 72.5%, variability in remaining progeny, 65.5%.

The criticism may be raised in connection with this treatment of loss of plants and variability: Why be concerned about plants which are lost even under the most exact cultural conditions since they will never appear and influence the character of the plant's progeny? This is true, but the study was designed to determine the extent of apomixis and sexuality of selected plants of Kentucky Bluegrass and no valid criterion of sexuality may be disregarded. Furthermore, by the proper breeding techniques these highly sexual plants may be utilized for hybridization, and, again, any criterion of value in their identification should not be ignored.

It has not been possible in these studies to establish with any degree of certainty correlations between apomixis, as measured by the morphological features of a plant's progeny, and certain other more easily and quickly determined measurements.

1. No correlation is observed between apomictic reproduction and source of material. Variability, however, is low in many progenies from plants grown from commercial seed. In fact, the most apomictic types were isolated from commercial seed. This is in agreement with the general opinion that apomictic types are more vigorous and more prolific

seeders and are thus better fitted to survive under conditions of natural selection. However, highly variable forms were obtained from this material, and the mean differences between plants from commercial sources, pasture sources, and strains are not statistically significant.

2. In regard to seed set under bag and variability, it was found, in general, that those plants completely sterile under bag gave open-pollinated progenies more variable than fertile plants. However, exceptions make this relationship difficult to apply to practical work. One thing is definite: That good seed set is no criterion for apomictic reproduction, as some of the most variable progenies came from plants with good seed production under bag (Plate 1).

3. The simple correlation between germination and variability has a significant negative value and one might infer that the apomictic types have a higher rate of germination. This correlation, however, is misleading, since the partial correlation drops to an insignificant value if percent survival of plants in the field is held constant. It seems likely, therefore, that sexually produced embryos are not significantly less viable than apomictically produced embryos in their ability to germinate, and that no practical significance may be ascribed to percent germination as a measure of apomictic reproduction. The significance of the simple correlation must, then, be caused by the widespread elimination of weak seedlings which would ordinarily go unmeasured in reckoning variability.

4. Embryological studies by Akerberg and Tinney

have indicated that polyembryony and apomictic development should be closely associated phenomena. As a result, one might reasonably expect a significant negative association between polyembryony and variability. Akerberg first suggested this relationship and presented rather sketchy data in its support. The families studied were merely classified as "sexual" and "apomictic" and no statistical treatments or further descriptions were presented. The present writer has found a correlation of - .205 between variability and polyembryony, a figure barely significant (P.05 = .180). It is difficult to infer from this value just what the relationship between polyembryony and apomixis might be. A study of Figure 13 suggests the probability of the occurrence of two fundamentally different groups of plants, (1) Those in which there is a high negative association between polyembryony and variability, and (2) Those in which there is little, if any association. Granting the probable existence of these two classes, it would seem that in Kentucky Bluegrass we have two inherently different types (1) "Obligate Apomicts", in which fertilization rarely occurs and the rate of polyembryony might be a reasonably sound measure of apomictic seed development, and (2) "Facultative Apomicts", in which fertilization may be readily effected under the proper conditions and in which the rate of polyembryony bears no inverse relation to sexual seed development.

The practical use of the rate of polyembryony as a measure of apomictic seed formation must, therefore, be held in abeyance until greater reliability can be demonstrated for this criterion.

To summarize, correlation studies in Kentucky Bluegrass between variability and source, seed set, germination, and polyembryony have indicated no statistically significant relationships. The investigator is, therefore, without any reliable criterion for apomictic seed formation in Kentucky Bluegrass, other than the actual progeny test. With our present knowledge, we are fully justified in adopting the progeny test as a reliable measure of apomictic or sexual seed formation in parental plants. It should be pointed out, however, that low values for variability will be obtained if cultural practices are employed which suppress the weaker plants.

The Cause of Apomixis in Poa. Observations on apomictic forms in all genera have established, in general, that apomixis is usually associated with (1) high chromosome numbers and meiotic irregularities, and (2) hybridization. The inference has been made, in lieu of experimental facts, that hybridization with its subsequent sterility and meiotic irregularity may be considered as contributing to apomictic seed development. Experimental results in several genera have indicated that, while apomixis and hybridization may be associated phenomena, it is a fallacy to consider hybridization, as such, the basic cause of apomixis. Rather, if apomictic seed development follows hybridization it may simply mean that a certain "constellation of genes" has been brought together which suppresses the usual sexual behaviors. Müntzing has advanced this theory and suggests that there may be genes which condition non-reduction and others which condition parthenogenetic development. To this list might well be added genes for the development of cells of the nucellus to form aposporous embryo sacs since the results of the present writer show a definite inherent ability of plants to produce a characteristic percentage of polyembryo seeds.

Müntzing (22) has obtained completely sexual F_1 hybrids from crossing a sexual biotype of Poa alpina with an apomictic biotype, indicating that apomixis in Poa is recessive. Furthermore, breeding tests have not disclosed any apomictic behaviors in the F_2 plants. The sample was sufficiently large to identify such plants if apomixis were inherited in a fairly simple manner. Preliminary evidence from both Akerberg (4) and Müntzing (22) indicates that, in Poa pratensis, aberrant plants, whether haploid or triploid, are less apomictic in their mode of reproduction than the mother plant. Müntzing interprets this as a suggestion that apomixis is determined by a balanced "constellation of genes" and that any disturbance involves a change from predominant apomixis to more or less sexuality. It remained to be demonstrated if this type of response is true for all plants of known hybridity in Poa, and more important, if the sexual types which arise remain as such, or whether it is possible for them to be stabilized eventually as apomictic biotypes.

Additional evidence that hybridization is not causal in its relation to apomixis is presented by Nannfeldt (23) after a study of naturally occurring apomictic and viviparous biotypes of Poa of northern Europe. Lidforss (cited in (22)) and Gustafsson (cited in (22)) have shown also for Rubus that apomixis is recessive to sexuality since artificially produced hybrids are sexual even though both parents are strongly apomictic in their method of reproduction. In Crepis, the conclusion is reached, (34) and (35), that hybridization in itself is not the cause of apomixis since many autopolyploid forms are as strongly apomictic as allopolyploids of the species.

In some forms, however, apomixis seems to be dominant over sexuality. In Hiercium, Mendel (cited in (22)) and Ostenfeld (cited in (22)) found somewhat variable behaviors but, in general, hybrids between sexual forms were apomictic. Also, Müntzing (22) finds that hybrids between sexual types of Potentilla are true-breeding.

If hybridization alone and hybridization with polyploidy are excluded as playing a causal role in reproductive phenomena, as experimental evidence seems to indicate, we may place apomixis and sexuality on a genetic basis . Hybridization, therefore, may serve only to bring together either genes for sexuality or apomixis and the reproductive processes of the hybrid will depend on its genic complement.

H. Concluding Remarks. The results presented in this report allow one to make the broad generalization that Poa pratensis is predominantly an apomictic species. There is, however, a gradual range of apomictic behaviors from complete uniformity in progenies to over 70% aberrancy, indicating, first of all, the genic nature of the reproductive phenomena, and secondly, the caution which must be used in applying any criterion to a species as highly polymorphic as Poa pratensis. This polymorphism is not confined to morphological types in the taxonomic sense, but is found in every character of the species the investigator chooses. The writer is strongly of the opinion that we know too little about the species to generalize on any one criterion, whether it be embryology, polyembryony, polyploidy, breeding behavior, or morphological and taxonomic relationships. A perfect analogy exists in the fable which tells of the four blind men who each received individually different impressions of what an elephant must look like depending on what portion of the beast's anatomy he explored.

PRACTICAL CONSIDERATIONS

The investigations reported in this paper were started with the ultimate purpose of applying the results of fundamental work in Poa pratensis to problems of practical breeding. Kentucky Bluegrass is important both as a forage grass and as a sod grass in lawns and golf fairways. Improvements are sought in (1) disease and drought resistance, (2) higher productivity, especially in the period between the spring flush and the secondary fall flush, (3) higher nutritive value, especially in the fall flush, (4) greater adaptability to soil and climate factors, (5) better association with other species, particularly legumes, (6) greater tolerance to close grazing and clipping, and (7) higher palatibility.

Available breeding procedures may be classified as follows:

A. Selection of apomictic biotypes which show desirable characteristics. This is the method which may be expected to give the quickest results and, considering the polymorphic nature of the species and the prevalence of highly apomictic biotypes, should prove fruitful. Indeed, this procedure has already been utilized in the creation of commercial strains by European breeders and is employed in Canada and the United States, (Tinney and Aamodt (37)). By taking advantage of the apomictic method of seed production, problems of isolation and the maintainance of purity of the selected types are non-existent. In this connection it is well to point out that while certain desirable biotypes may produce a significant proportion of sexual offspring these may be of no practical importance if they are of sufficiently low vigor. In practice, seeding is in excess of requirements and the aberrant plants would probably never appear in quantities large enough to alter the character of the stand. On the other hand, if the sexually produced aberrant forms are more vigorous and possess undesirable agronomic characters, as many of them do, the value of the selected biotype may be seriously impaired. As far as the writer is aware, selection of desirable apomictic biotypes is the only breeding procedure so far utilized in improving Kentucky Bluegrass. Its advantages are simplicity, ease of conducting the work and rapidity with which results may be achieved. It runs into difficulty if a high proportion of undesirable aberrant plants appear regularly in the progenies and, also, if the naturally occurring material does not provide the particular combination of plant characters needed. Nature and the agriculturist often work at cross purposes. This is particularly true in forage species where Nature favors those forms which send all their energy and nutrient materials into seed production but where the agriculturist develops forms which, while producing seed sufficient for commercial purposes, emphasize the vegetative phases of growth. One need only grow plants of Kentucky Bluegrass from commercial seed to see the predominance of the coarse, tough, stemmy types which are prolific seeders but are of little value for forage purposes.

These points brings us logically to a consideration of breeding methods theoretically feasible for the genus *Poa*.

B. Utilization of aberrant plants and inbreeding practices followed by selection of aberrant plants or homozygous lines. The writer has been impressed by the appearance of some of the aberrant plants which have appeared (see Plate 2-E, Plate 4-E, Plate 6-D, Plate 8-I, and Plate 10-E). Many of these possess characters of agronomic importance. They are often more disease resistant, more vigorous and more leafy types which suggest their utilization in pasture improvement. Some others are compact, leafy types, not quite so vigorous and suggest the types suited for lawn and fairway purposes. These aberrant plants, whether obtained from apomictic biotypes or obtained by the breeding of sexual forms, will provide more material for agronomic evaluation. Their dominant method of reproduction will assume primary importance. If, as Müntsing and Akerberg suggest, they are sexual, then their practical importance depends on their degree of homozygosis and the character of their progeny. The possibility must not be overlooked, however, that the sexual nature of their reproductive processes may be transitory and that they will later be stabilized as apomictic types. On the other hand, the experimental evidence is too incomplete regarding the sexuality of aberrant forms to exclude the possibility that some, from their time of origin, may be apomictic.

No knowledge of the effects of continued inbreeding in sexual biotypes is available for Kentucky Bluegrass. It may be supposed, however, that, in conformity with most other work on normally open-pollinated grasses, vigor and fertility will decline. Whether or not this decline will be so serious as to prevent the utilization of inbreeding procedure remains to be determined. If it is feasible, the specialized techniques in combining inbred lines may be extended to Poa. At any rate, it must not be overlooked that, for some uses, vigor in Kentucky Bluegrass is not a primary consideration. Inbreeding could conceivably isolate strains well suited for lawn and fairway purposes.

C. Intraspecific Hybridization and Strain Building

1. Intraspecific Hybridization In the previous section, aberrant plants which appeared spontaneously in cultures of Kentucky Bluegrass were discussed. Here it is

proposed to discuss aberrant plants which are produced consciously by controlled pollinations. By utilizing self- or partial-sterility or by employing emasculation techniques it is possible to produce intraspecific hybrids from parents, especially the pollen parent, of known agronomic value. Problems exist in adapting this technic to apomictic forms but the writer suggests that it is feasible. The case of a plant whose progeny from self-pollination was wholly apomictically derived but whose open-pollinated progeny showed 74.5% variability has already been mentioned. The variant plants in this progeny conformed, with very few exceptions, to one basic more vigorous type. This must mean that foreign pollen is active enough to effect fertilization of the aposporous egg cell before it produces the embryo apomictically. This suggests that even apomictic biotypes may be found whose reproductive behaviors adapt them for hybridizing purposes. Evidence for this same type of behavior was found in the progenies of several other plants, not to the extent indicated above, but sufficient to warrant consideration of the use of such plants in a breeding program. One such plant is shown in Plate 10-P, with the dominant type in its progeny shown in F.

2. Strain Building With the identification of sexual plants of Kentucky Bluegrass showing self- or pollen-sterility and of facultative apomictic plants in which fertilization occurs regularly if foreign pollen is applied, the utilization of strain building methods deserves consideration. Supplied with material of this kind adapted to

bulk hybridizing, the breeder may find a relatively simple method of obtaining new morphological and physiological types. These types if coming from apomictic parentage may prove highly apomictic themselves when isolated and thus give rise to a true-breeding biotype. If the new types prove to be sexual, it would mean that basic breeding stocks must be maintained on a scale sufficient for practical production, as is done with corn and some improved strains of rye grass, orchard grass, and clover.

D. Interspecific Hybridization in Poa Species hybrids of Poa have been obtained between arachnifera-pratensis (Oliver, and later by E. Marion Brown (cited in (39)), pratensis-alpina (Akerberg (2), Müntzing (22)), and compressa-pratensis by the writer (7). Interspecific hybridization has thus been shown to be possible and, further, that it might be applicable in practical improvement in Poa since Brown has reported increased vigor and drought resistance in some of the arachnifera - pratensis hybrids and the present writer describes characters of the hybrid between P. compressa and P. pratensis which may prove of practical value.

The techniques of species hybridization in Poa are essentially no different from those involved in hybridizing within the species Poa pratensis and the same types of breeding material previously mentioned could be utilized. Apparently, species of Poa are relatively easy to hybridize, judging by the list of known and supposed natural hybrids. One physiological factor may prove troublesome and that is differences in flowering dates, but proper handling of the plants in the greenhouse under differential conditions of light and temperature may be expected to overcome this handicap.

Of the breeding practices mentioned which are theoretically applicable to Poa, one, the selection of apomictic biotypes with desirable characters, has already proven of value. If this method will take us to our goal in the ultimate improvement of Poa pratensis well and good because it is by far the easiest line of approach and the one which will yield the quickest returns. On the other hand, if it should prove incapable of providing the agronomic types we need, as pure selection has proven with many of our plants, then hybridization in Poa is theoretically feasible and practical procedures for both intraspecific and interspecific hybridizing could undoubtedly be devised.

SUMMARY

1. From a nursery of 10,000 spaced plants of Kentucky Bluegrass grown from seed collected from pasture, commercial, and numbered strain sources, 115 parental plants were selected which represented a wide range of morphological and physiological types. The experimental nursery of 10,066 plants was established from seed produced by open-pollination and from seed, where available, which was produced under parchment bag. Both self-pollinated and open-pollinated progenies were available from 87 selected plants.

2. Studies on sterility and fertility under condition of bagging conducted in the greenhouse and in the field

show that about 35.0% of the plants regularly showed less than 30% seed set under bag. Behaviors varied from complete sterility under bag to well over 60% seed set. While the physiological effect of the bag may be a factor in seed set the results indicate that sterility and fertility in *Poa pratensis* is largely genic in nature.

3. There are significant positive correlations between self-pollinated progenies and open-pollinated progenies in germination, polyembryony, survival in the field, and variability, indicating an inherent nature of the parental plant which produces characteristic frequencies of these measurements regardless of type of pollination. Evidence is presented that variability may be significantly higher in open-pollinated progenies than self-pollinated progenies of some of the parental plants.

4. For the offspring of all 115 plants the following average values were obtained: germination, 80.3%; polyembryony, 7.0%; survival 86.3%; and variability, 14.8%. The highest value obtained for polyembryony was 31.6%, the highest for variability 65.5%. The lowest value for survival was 27.5% and was found in the progeny giving the highest value for variability among the plants which remained.

5. Variability among the plants from twin seedlings was 17.1% and was significantly higher than the value found in plants from seed with a single embryo. However, the same morphological and chromosomal aberrant plants appeared in each population. A study of morphological features, pollen

grain sizes, and chromosome counts have indicated that the aberrant plants from both twin seedling and single seedling sources arose from (1) apomictic development of reduced eggs, ("haploid") (2) fertilization of reduced eggs by reduced pollen, ("diploids by amphimixis") and (3) fertilization of unreduced eggs by reduced pollen, ("triploids"). The matroclinous plants are thought to have arisen from the apomictic development of an unreduced egg which was derived from a cell of the nucellus by apospory, ("diploid by apomixis").

6. No significant correlations were found between variability and source of material, seed set under bag, and germination. A barely significant negative correlation existed between polyembryony and variability. The data suggest that in some plants of Kentucky Bluegrass a high negative association between polyembryony and variability may be found, while in others no association exists. A highly significant correlation was found between survival and variability, indicating that variability was generally higher in those progenies which had lost the greater number of plants. This suggests that the plants lost were weak aberrant forms and should be considered in any measure of apomictic seed development in parental plants.

7. The results indicate that while Poa pratensis may be considered as a predominantly apomictic species, such extremes of types and behaviors are found that caution is necessary in generalizing on too few data. The progeny test does appear to be, however, the only practical means of de-

termining the type of seed development of Kentucky Bluegrass.

8. The breeding methods available for improvement of plant types in Kentucky Bluegrass are discussed. Selection of desirable apomictic biotypes provides the quickest and easiest method of improvement. If this reaches a limit of usefulness due to lack of naturally occurring desirable biotypes, the breeder has at his disposal the methods of inbreeding, intraspecific hybridization, strain building, and interspecific hybridization. The results of the study presented in this paper are not inconsistent with the conclusion that any of these methods are applicable to breeding problems in _Poa pratensis_.

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APPENDIX

Description of Representative Progenies

a. 37-KB 1 (11) (Plate 1) This plant was obtained in a progeny grown from seed collected in a West Virginia pasture. It was of moderate vigor and spread, fairly leafy, and not very coarse. It flowered profusely in the greenhouse where it set seed well under bag. Subsequent bagging in the field showed fertility to be quite high, well over 60% seed set under bag.

Selfpollinated progeny, 38-KB 3. Germination of seed, 64.0%; polyembryony, 3.1%. Survival of plants was the lowest encountered, 21.5%, only 12 of the original 56 plants persisting. Seven of these surviving plants differed from the parental type, a variability of 58.3%. Three of these were similar, being much larger, coarser plants and very stemmy (Plate 1-F). The other four aberrant plants were less vigorous than their parent. One (Figure 1-B) was an exceedingly small plant, having short, narrow, dark green leaves and few culms, one-third as tall as the parent and extremely small panicles; another (Figure 1-C) was very erect in its growth, had little spreading ability and its leaves were narrow, wiry and light green. The other two aberrant plants which were less vigorous than the parental type were not as extreme as the above two, but differed unmistakably from the parent.

Open-pollinated progeny, 38-KB 4. Germination, 56.0%; polyembryony, 3.8%. Survival was very low, 34.0%, only 17 remaining out of an original 50 plants. Fourteen of these plants differed from the parental type, a variability of 70.6%, the highest obtained. Eight of these were more vigorous; 7 were similar and correspond in type of plant to the group of 3 already described in the selfed progeny and shown in Plate 1-F. The other more vigorous plant was not as tall, but a vigorous spreader. Its leaves were more numerous and dark green (Plate 1-E). The other aberrant plants were smaller and less vigorous than the parent. One is shown in Plate 1-D.

No complete set of twin seedlings survived for study, and, in view of the high rates of loss of plants and variability and the high correlation between them, the conclusion may be reached that all twin pairs had dissimilar members. The aberrant plants were presumably too weak to survive.

These progenies of a plant obtained from a pasture source have been described in some detail because they present certain extremes in behavior and some rather interesting relationships. The points of interest are (1) good seed set under bag; (2) poor germination, 60%; (3) a low value for polyembryony, 3.3%; (4) the lowest value obtained for survival, 27.5%; and (5) the highest value obtained for morphological variation, 65.5%. It has been shown that aberrant plants in the progeny may show a wide range of types, that these may be either more vigorous or less vigorous than the parental type, and that similar aberrant plant types are obtained in self-pollinated and open-pollinated progenies.

b. 37-KB 38 (12) (Plates 2 and 3) This plant was selected from a progeny grown from seed also collected in a West Virginia pasture. It was a comparatively low-growing type, compact and leafy. The plant was not a prolific seeder and after flowering maintained its green color longer than do most plants of Kentucky Bluegrass.

This plant failed to flower under greenhouse conditions. When bagged in the field, it set seed poorly, less than 30%.

Self-pollinated progeny, 38-KB 25. Germination, 77.0%; polyembryony, 16.3%; survival in the field, 80.5%; variability, 20.7%. There were 6 aberrant plants in a population of 29.

Open-pollinated progeny, 38-KB 26. Germination, 80.0%; polyembryony, 6.3%; survival, 92.0%; variability, 34.5%. There were 19 aberrant plants in a population of 55. A wide variety of morphological types was represented.

The extremes of variability found in these progenies are shown in Plates 2 and 3, B, C, D, E, and F. The plant shown in C is typical of a group of eight, all of which showed the same general characters; leaves few, shorter, wider, and darker green, panicles larger and heavier.

Ten pairs of twin seedlings were available for study. Of these, 3 pairs were dissimilar, making the variability among plants from twin-embryo seed 15.0%. One of these pairs of twins is shown in Plate 11 and 12, A_1 and A_2.

These progenies are presented as showing a group of aberrant plants whose extremes differ widely from the parental type. One extreme is represented by the plant shown in Plates 2 and 3, E, erect, coarse, and stemmy type of growth, 50% taller than the parent plant; the other extreme by the plant shown in E, semi-prostrate leafy type of growth, leaves shorter and softer in texture; panicles smaller and finer, culms about half the height of those of the parental plant.

c. 37-KB 140 (11) (Plates 4 and 5) This plant was selected in a progeny from commercial seed grown in Kentucky. It was in no way representative of the plants from commercial seed but is presented as one of the most unusual plants of Kentucky Bluegrass in the selected material. Leaves were numerous, light green in color, and were the narrowest found in the nursery from which selections were made. The plant had a decidedly different type of growth. Rhizomes were unusually poorly developed and the plant assumed the appearance of a "bunch" grass. (Plate 4-P and A). Panicles were delicate in texture and spikelets extremely small. (Plates 5, P and A). This plant has never flowered in the greenhouse. It has been entirely sterile under conditions of bagging in the field. Seed set is poor on open pollination.

<u>Open-Pollinated progeny, 38-KB 130</u>. Germination, 76%; polyembryony, 3.6%; survival of plants in the field, 90.5%; variability, 27.1%. Thirteen of 48 plants were aberrant. These plants differed from most groups of aberrant plants in that none of them was smaller or less vigorous than the parental plant. No two were quite alike, but instead presented

a gradual range of types from the plant shown in Plates 4-B and 5-B which had a type of growth similar to the parent plant but differed in being darker green and having leaves somewhat wider, to the extreme plant shown in Plate 4-E and 5-E which had an entirely different type of growth and plant characters. It was a vigorous spreading type, leaves deep green, unusually broad and thick, panicles and spikelets much larger and heavier than those of the parent.

No twin seedlings of this plant were available for study.

d. 37-KB 172 (14). This plant was obtained in a uniform progeny grown from seed of the strain of Kentucky Bluegrass, Ottawa 994 (and also Minnesota P-37). It was a plant unusually high in vigor, good spread, leaves numerous, broad, and the longest found in the nursery.

No flowering occurred under greenhouse conditions. In the field, the plant was later in its blooming date than the bulk of Kentucky Bluegrass material, and produced comparatively few panicles. Seed set under bag was poor, less than 30%.

Self-pollinated progeny, 38-KB 195. Germination, 59%; polyembryony high, 20.3%; survival in the field, 68%; variability, 3.1%. The progeny was remarkably uniform, conforming exactly to the parental type plant. Only 1 aberrant plant out of 32 was found. This was a very small plant with no spread, leaves short, wiry, light green, culms reaching only one-third the height of the parent, panicles short and very small. Chromosome counts show this plant to be haploid.

<u>Open-pollinated progeny, 38-KB 196.</u> Germination, 83%; polyembryony the highest found, 36.1%, with a high incidence of triple seedlings, 2.4%; survival, 83.5%; variability, 2.4%. The open pollinated progeny was also uniform, except for 1 plant in the population of 41. This differed from the parental type in being more leafy, leaves wider and darker green, culms not as tall as in the parent, but the panicles significantly larger and heavier. Chromosome counts indicate this plant to be triploid.

Forty-two pairs of twin seedlings were obtained from 142 germinated seed of this plant. Of these, only 26 pairs survived for study, 4 of which were dissimilar. One aberrant plant showed greater vigor and larger foliage characters, the other 3 aberrant plants were smaller than the apomictic member of the pair. This represents a variability in the surviving plants from twin seedlings of 7.9%. No triple seedlings survived as intact groups of three available for study.

This parental plant and its progenies are presented to establish the following points of interest (1) poor seed set under bag, (2) comparatively poor germination, 71.0%, (3) an unusually high value for polyembryony, 31.6%, the highest obtained, and (4) practically complete conformity to the parental type in the progenies, variability, 2.7% (2 plants out of 73).

e. 37-KB 175 (14) (Plates 6 and 7). This plant was selected in a progeny grown from seed of a strain of Kentucky Bluegrass received from The Ontario Agricultural

College (O. A. C. No. 1). It was a bunchy, compact plant; leaves short, broad, and dark green; culms hardly taller than the leaves; panicles compact, thick, very broad in relation to their length, spikelets exceedingly large.

This selection failed to flower in the greenhouse. In the field, it set seed only moderately well under bag, about 50%. Plants of this type were the latest of any Kentucky Bluegrass to flower in the field, being at least a week to 10 days later than all other plants of the species.

<u>Self-pollinated progeny, 38-KB 205</u>. Germination, 86.0%; polyembryony, 10.5%; survival in the field, 90.0%; variability, 18.5%. Ten plants out of 54 were aberrant, the remaining conforming perfectly to the parental type. As a general rule, all plants obtained which did not conform to the parent type were smaller and less vigorous. A wide range of plant types was present. Four of the 10 aberrant plants were of the same type, being extremely small and weak, barely able to survive and producing only 2 or 3 culms (Plates 6 and 7, B and C).

<u>Open-pollinated progeny, 38-KB 206</u>. Germination high, 94.0%; polyembryony, 6.4%; survival, 91.5%; variability, 29.1%. Sixteen of the 55 plants were aberrant, the others resembled the parental type perfectly. As was the case in the self-pollinated progeny, all of the aberrant plants were less vigorous than the parent plant. Many morphological types were represented. It was possible, however, to group many of the variant plants into groups having more or less the same general growth characteristics. Thus, there were 5 plants

all of which were similar to the two shown in Plates 6 and 7, B and E; 3 plants similar to the one illustrated in C; and 5 plants of the same general type as that shown in G. The remaining aberrant plants conformed to none of the above groupings. Two interesting departures from the parental type are showin in Plates 6 and 7, D and H. The plant shown in D had shorter, narrower, lighter green leaves, the culms exceeded the leaves, and the panicle was much larger and of a more open type.

Ten of the 15 pairs of twin seedlings of this plant survived for study. Six pairs were dissimilar, giving a value for variability of 30.0% in the seedlings from polyembryo seeds. Without exception, these aberrant members of the pair were less vigorous than the apomictic type and differed strikingly from it. Two pairs of twin seedlings from this plant are figured in Plate 11, B_1, C_1 and B_2C_2, the latter plant being the apomictically reproduced member and identical in each pair of twin seedlings. Attention is called here to the demonstration that the same plant types may be found in both progenies from seed with a single embryo and progenies from polyembryo seed.

f. 37-KB 175 (46) (Plates 8 and 9). This parental plant and the one described in the preceding section came from the same progeny grown from O. A. C. strain No. 1. They are similar in morphological characters as shown by a comparison of Plates 6 and 8, P and A. Neither flowered in the greenhouse. The plant under discussion, 175 (46), differed, however, in failing to set seed under bag in the field.

Open-pollinated progeny, 38-KB 208. Germination very high, 97.0%; polyembryony low, 1.0%; survival in the field, 100.0%; variability high, 48.3%. Twenty-nine plants showed morphological deviations from the parental type in a population of 60. The aberrant plants were greatly diversified in type and vigor, and many showed a strong tendency to group themselves into distinct classes having similar morphological features. The plant shown in Plates 8 and 9, B is illustrative of a group of 4 smaller, less vigorous plants, the leaves of which became yellowed and brown even before flowering. Photograph H shows one of a group of 7 plants, having the general features of the group shown in B, but differing in that the leaves remained green. A representative plant of a group of 4 is shown in D, the plant not as large, about half the height of the parent, leaves narrower, softer in texture, and decidedly lighter green. Shown in F is one of a group of 5 similar plants. All had much broader, thicker, dark blue-green leaves. Photograph I shows one of a group of 4 plants which had similar morphological features. These plants were very striking. They were more vigorous than the parental type, more aggressive, more leafy, the leaves narrower and softer in texture, culms not as heavy, and spikelets smaller.

The remaining five plants of the progeny were different from the types described and differed among themselves. Three of the more interesting and striking plants are shown in Plates 8 and 9, C, E, and in Plate 8, G. The plant shown in C was more stemmy than the parent, much taller, leaves narrower and lighter green, and panicles very much larger and of an open type, spikelets much smaller.

This progeny has been described to show an association between (1) almost perfect germination of seed, 97.0% (2) a low rate of polyembryony, 1.0%, (3) perfect survival in the field, and (4) a high rate of variability, 48.5%, with an unmistakable tendency for the aberrant plants to fall into definite morphological groups.

g. 37-KB 135 (131) (Plate 10) This plant was chosen from a progeny grown from commercial seed from Kentucky. It was rather distinctive in vegetative characters. As it appeared in the nursery of spaced plants, it was one of the most leafy and aggressive types found, spreading extensively and forming a loose sod. Leaves were of moderate length and width and very soft. This plant flowered frofusely in the greenhouse. Set seed under bag was less than 30% under both greenhouse and field conditions.

<u>Self-pollinated progeny, 38-KB 117</u>. Germination, 79.0%; Polyembryony, 5.1%; survival under field conditions, 83.5%; variability, 54.0%. Of the population of 50 plants, only 23 resembled the parental type. (Plate 10, P and A). A surprising feature of the plants differing from the parent type was that 23 of the 27 aberrant plants showed unmistakably the same morphological features. This group bore no resemblance to the parent. The plants were quite erect, very coarse and stemmy; leaves were longer, wider, and not as soft as the

parental type. The plant illustrated in Plate 10, F shows the general morphological features of the group of 23 aberrant plants. Of the 4 remaining variant plants, one resembled the type described above but had significantly wider leaves, two were alike in general features, showing poor spreading qualities and few, narrow, light green leaves (Plate 10, B) and one was a small low-growing plant, with short, soft, wide, dark green leaves (Plate 10, E).

Open-pollinated progeny, 38-KB 118. Germination, 82.0%; polyembryony, 3.6%; survival 83.5%; variability, 46.0%. Twenty-seven of the 50 plants in the progeny resembled the parental plant. The aberrant plants showed a greater diversity of types than did the plants of the selfed progeny, but the greatest number of them showed the features already described for the group illustrated by the plant in Plate 10, F. There were 11 plants of this type. The remaining 12 aberrant plants were practically individual in their features. Four of the widely deviating plants are shown in Plate 10, B, C, D and E. The one shown in E was the most striking variation in the progeny. Leaves were numerous, very soft, short, narrow, and deep green; panicles were quite small and delicate.

All twin seedlings from this parental plant survived. Of seven pairs, 4 were dissimilar, giving a value for variability of 28.5% among plants from polyembryo seeds. All aberrant plants from twin sources were smaller and less vigorous than the apomictic member of the pair.

h. Twin and Triple Seedlings (Plates 11 and 12)

Representative sets of twin seedlings and a typical set of triple seedlings are shown.

1. 38-KB (65)-1 and -2 (A_1 and A_2) The parental plant, 37-KB 38 (12), has already been discussed. At transplanting, the pair of seedlings measured as follows: -1, 37mm.; -2, 65mm., a ratio of 1:1.76. Differences between the two plants were readily apparent in the seedling stage in 3" pots. In the field, -1 plant was very small and erect; leaves small, short, light green; culms half the height of the parental type; panicles few, small, and compact; -2 plant was the apomictic member of the pair and resembled the parent plant (Plates 2 and 3, P).

2. 38-KB 206 (62) -1 and -2; (63) -1 and -2. These two pairs of twin seedlings are shown in Plates 11 and 12, B_1, C_1, and B_2 C_2. They appeared in the progeny of 37-KB 175 (14), a plant already discussed. At transplanting, (62) -1 measured 26mm.; -2, 50mm., a ratio of 1:1.83; (63) -1 measured 35 mm.; -2, 53mm., a ratio of 1:1.52. In describing the two aberrant plants, it is sufficient to say that the same general types were to be found among the variant plants in the progeny from seeds containing a single embryo.

3. 38-KB 47 (62) -1 and -2 (Plates 11 and 12, D_1 and D_2) When the members of this pair were separated and transplanted -1 measured 40mm.; -2, 57mm.; a ratio of 1:1.42. Morphological differences between them were apparent even at a very early seedling stage. In the field, the -1 plant was very small but leafy, leaves narrow and short, culms one-half

as tall as the parent, panicles one fourth the length. This general type of plant was also present in the progeny from single embryo seed, as was the larger, more vigorous type of aberrant.

4. 38-KB 124 (65) -1 and -2 (Plates 11 and 12, F_1 and F_2). The members of this pair of twin seedlings at transplanting measured 98 mm. and 120 mm., respectively, a ratio of 1:1.22. It was possible to detect morphological differences in this early seedling stage; -1 had much wider and harsher leaves. In the field, -1 was the more aggressive and vigorous plant, leaves were coarser and wider, culms about 20% taller, panicles larger and heavier. In the progenies from single embryo seed from the same parental plant there were no fewer than six aberrant plants which subscribed to the general type described above for (65) -1. Attention is directed to this similarity of plant type irrespective of whether the plant comes from a single embryo seed or is an aberrant member of a pair of twin seedlings.

5. 38-KB 160 (91) -1 and -2 and -3 (plates 11 and 12, F_1, F_2, and F_3). This set of triple seedlings was one of four sets which contained an aberrant plant, and is representative in that the aberrant plant was larger, darker green, and more vigorous, and the other two plants were identical and conformed to the parental type. In the above set of triple seedlings, measurements at transplanting were: -1, 59mm; -2, 80mm; and -3, 82mm; or a ratio of 1:1.36:1.39. Morphological differences were clearly evident in the young

seedling stage. The leaves of (91) -1 were coarse, wider and much darker than those of (91) -2 and -3. In the field, the mature plant of -1 was larger and more vigorous, more leafy, and less stemmy, the leaves were wider and darker green, culms fewer but taller, panicles decidedly larger, longer, and heavier; flowering occurred later (F_1). The other two mature plants of the triple set were identical and showed complete conformity to the parental type (F_2 and F_3). The progeny grown from single embryo seed contained 4 plants having the same general features of the aberrant plant (91) -1 described above from a set of triple seedlings. One of these plants in particular was strikingly similar, indicating once more that there are no differences in the *types* of aberrant plants which appear in progenies from polyembryonic seeds. Results, in general, show only that the *frequency* of aberrant plants may be higher in plants obtained from polyembryonic seed.

LIST OF PLATES

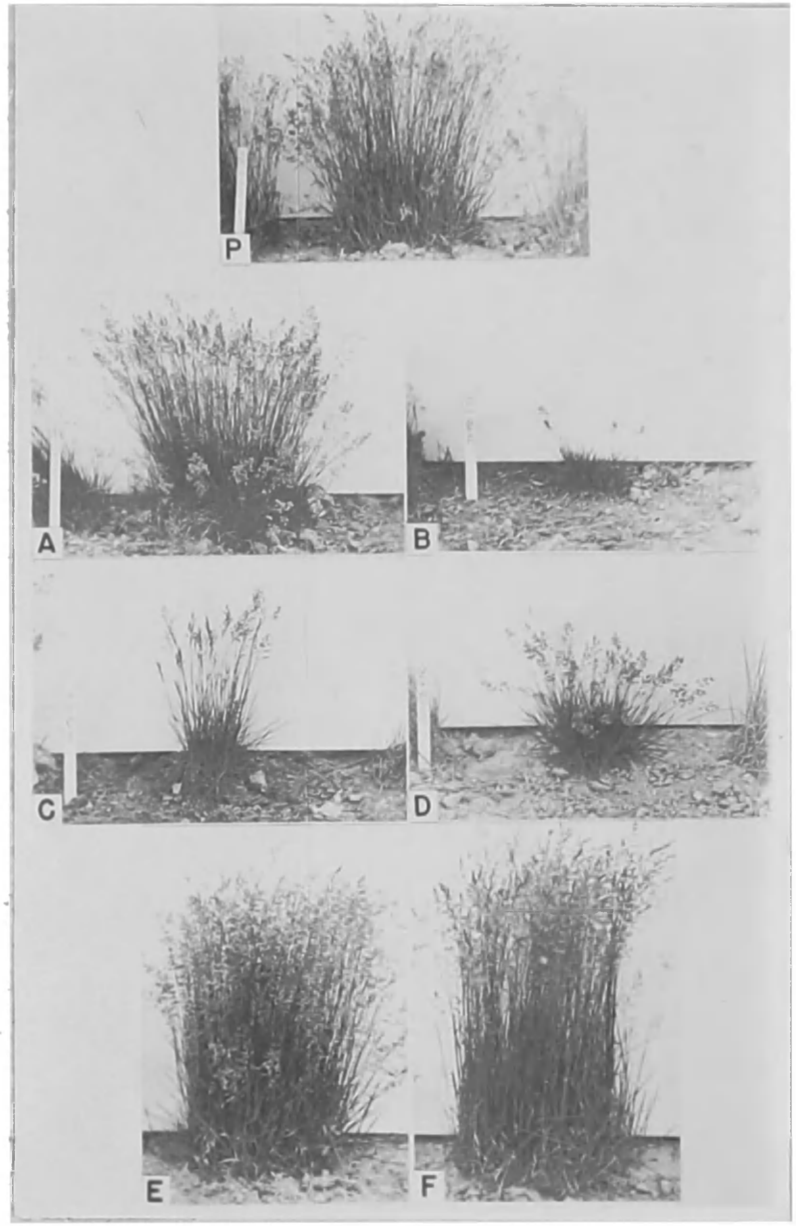
			Field Habit	Panicle Characters
			PLATE 1	
37-KB	1(11)	parent	P	
38-KB	3(8)	type	A	
	3(6)		B	
	3(9)		C	
	4(4)		D	
	4(7)		E	
	4(3)		F	
			PLATE 2	**PLATE 3**
37-KB	38(12)	parent	P	P
38-KB	26(40)	type	A	A
	25(22)		B	B
	26(10)		C	C
	26(26)		D	D
	26(30)		E	E
	26(50)		F	F
			PLATE 4	**PLATE 5**
37-KB	140(11)	parent	P	P
38-KB	130(4)	type	A	A
	130(8)		B	B
	130(21)		C	C
	130(13)		D	D
	130(26)		E	E
	130(1)		F	F
			PLATE 6	**PLATE 7**
37-KB	175(14)	parent	P	P
38-KB	206(16)	type	A	A
	206(1)		B	B
	206(11)		C	C
	206(4)		D	D
	206(8)		E	E
	206(6)		F	F
	206(28)		G	G
	205(6)		H	H
	205(14)		I	I

LIST OF PLATES(CONCLUDED)

			Field Habit	Panicle Characters	Pollen Photomicrographs
			PLATE 8	PLATE 9	
57-KB	175(46)	parent	P	P	
58-KB	208(40)	type	A	A	
	208(7)		B	B	
	208(18)		C	C	
	208(2)		D	D	
	208(6)		E	E	
	208(54)		F		
	208(56)		G		
	208(9)		H		
	208(50)		I		
			PLATE 10		PLATE 13
57-KB	155(151)	parent	P		P
58-KB	118(4[illegible])	type	A		
	118(47)		B		
	118(42)		C		
	118(56)		D		
	118(59)		E		A
	118(46)		F		B
			PLATE 11	PLATE 12	PLATE 13
58-KB	26(65) -1		A_1	A_1	
	(65) -2	type	A_2	A_2	
58-KB	206(62) -1		B_1	B_1	
	(63) -1		C_1	C_1	
	(63) -2	type	B_2C_2	B_2C_2	
58-KB	47(62) -1		D_1	D_1	C_1
	(62) -2	type	D_2	D_2	C_2
58-KB	124(65) -1		E_1	E_1	D_1
	(65) -2	type	E_2	E_2	D_2
58-KB	160(91) -1		F_1	F_1	
	(91) -2	type	F_2	F_2	
	(91) -3	type	F_3	F_3	

PLATE

1



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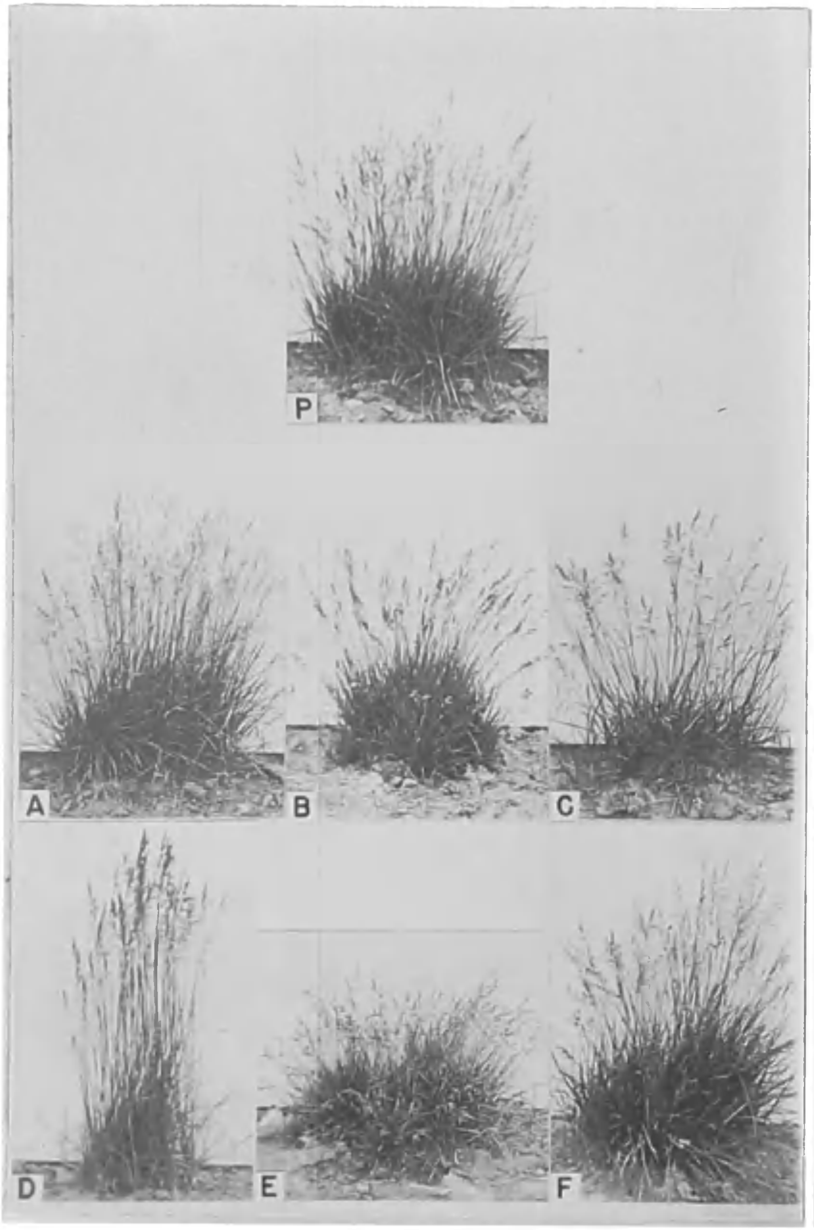

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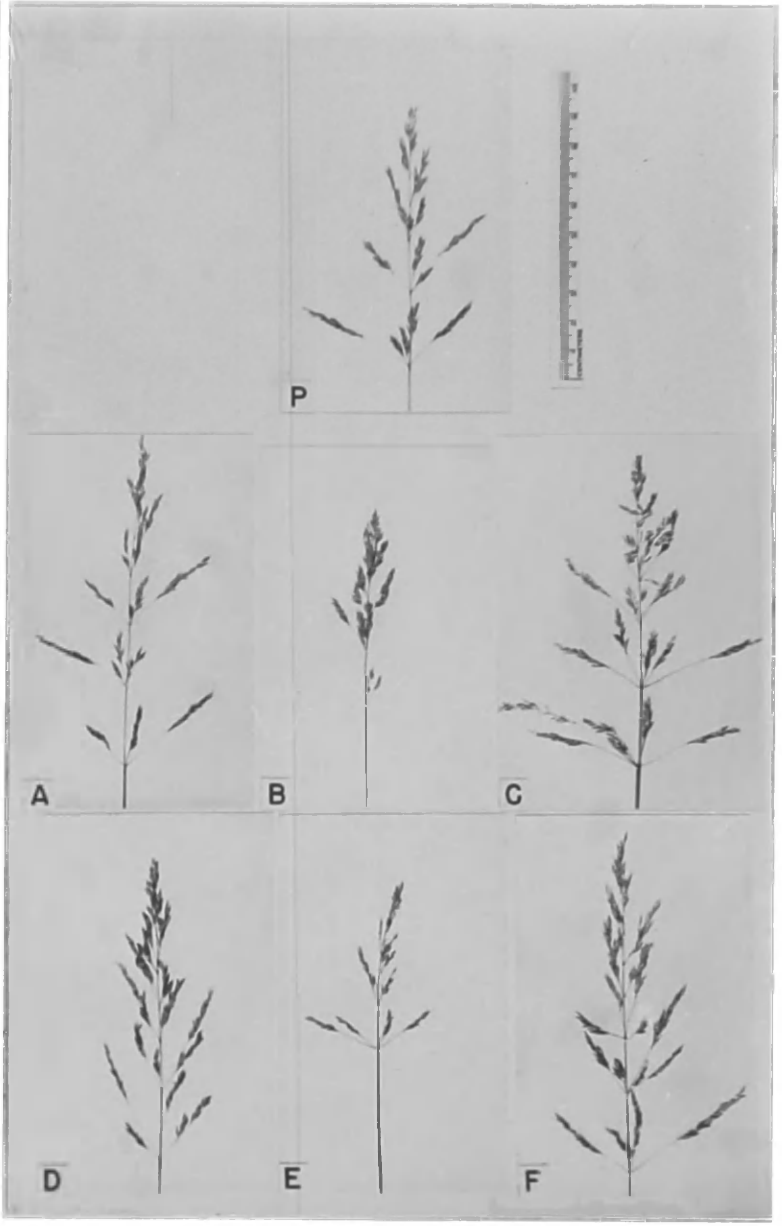

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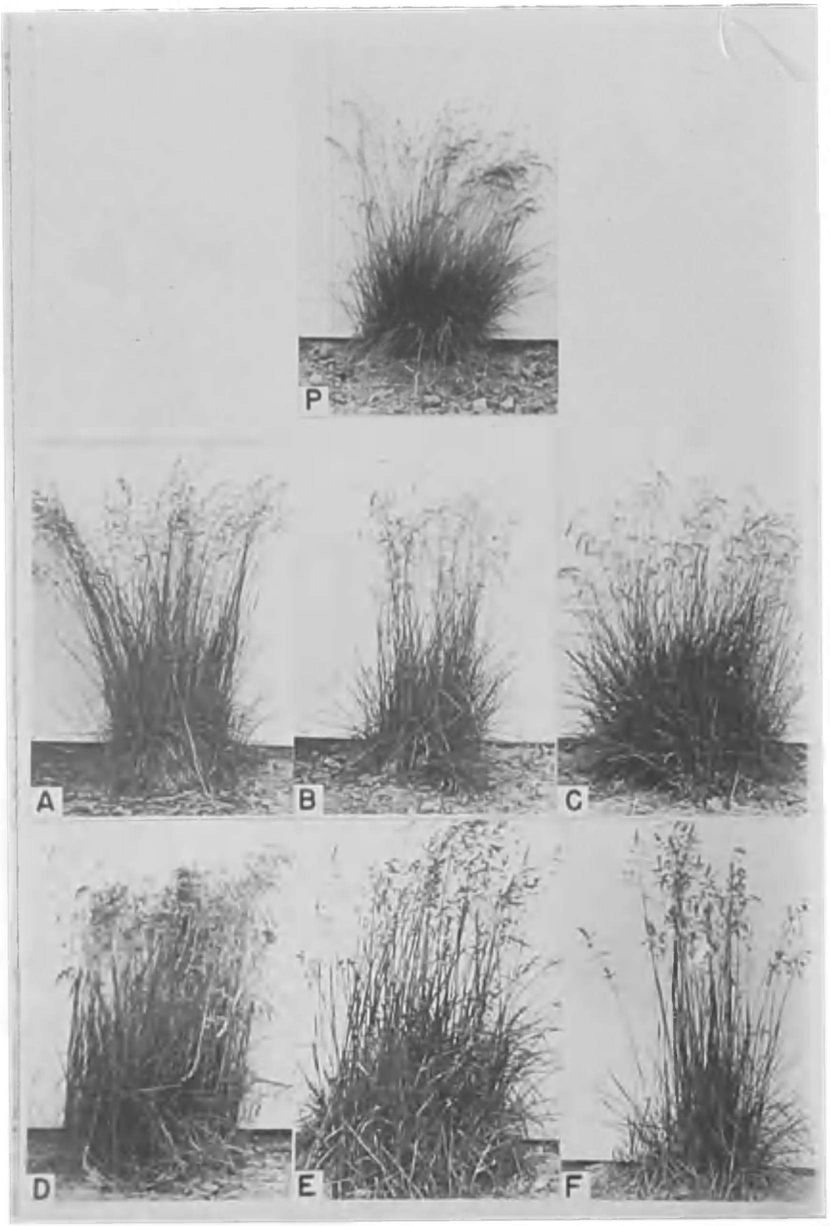

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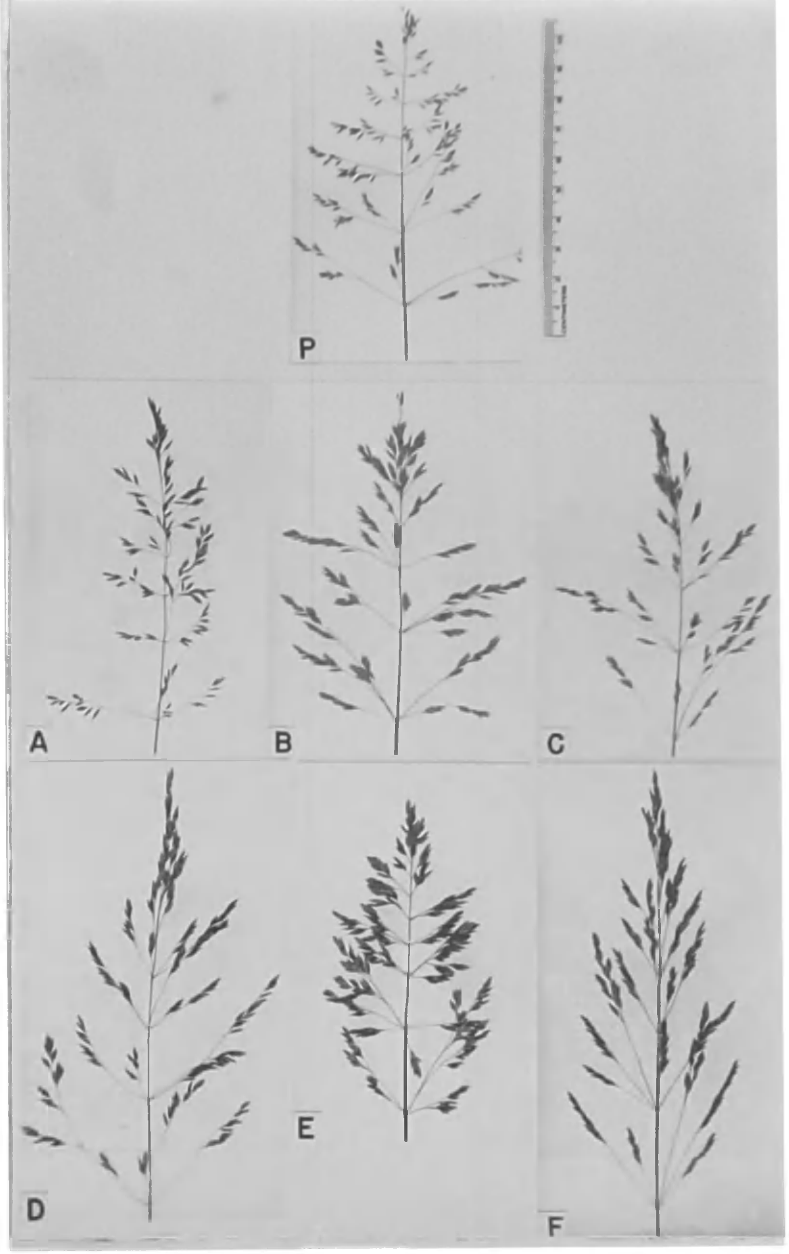

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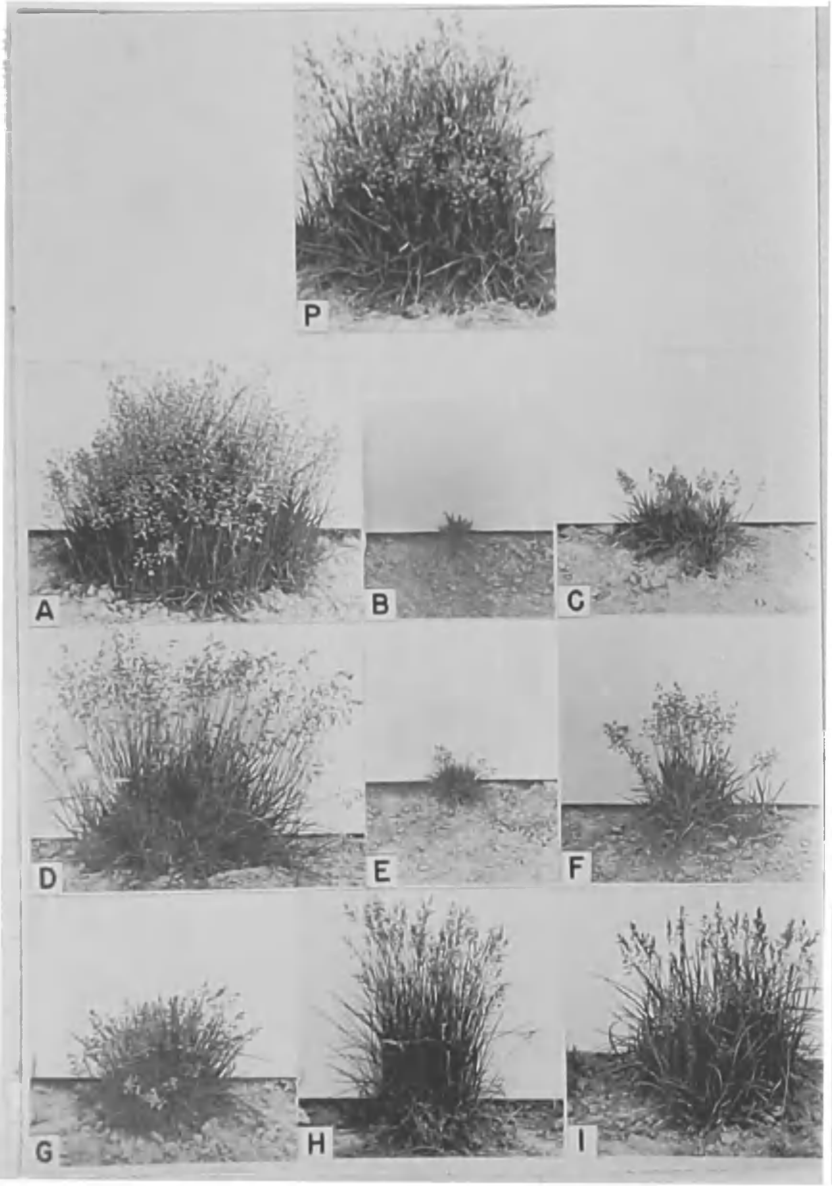

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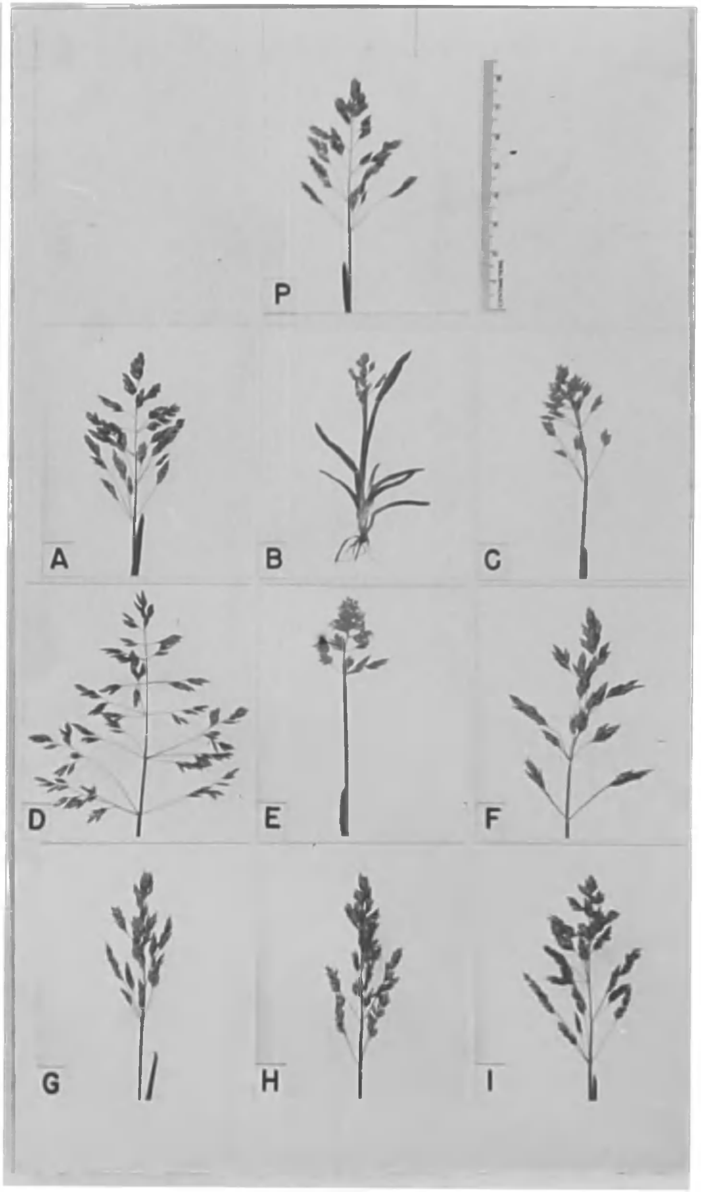

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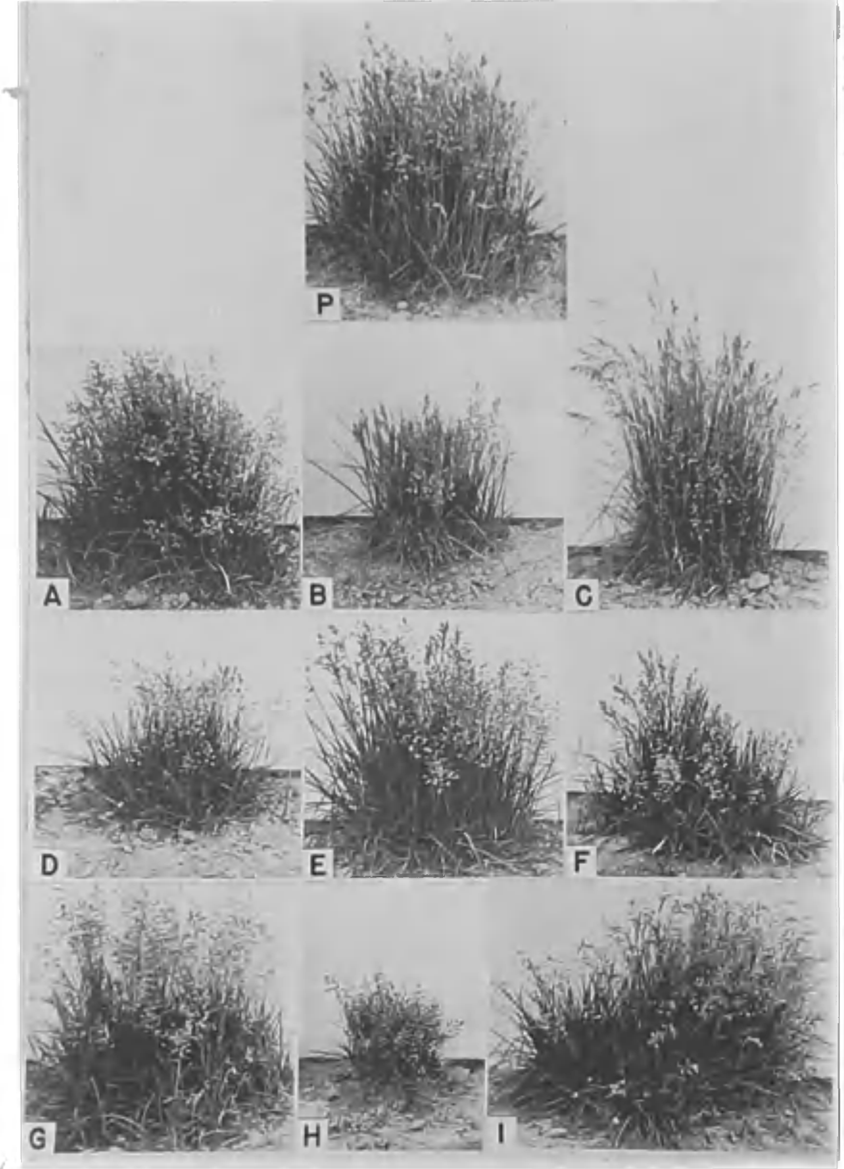

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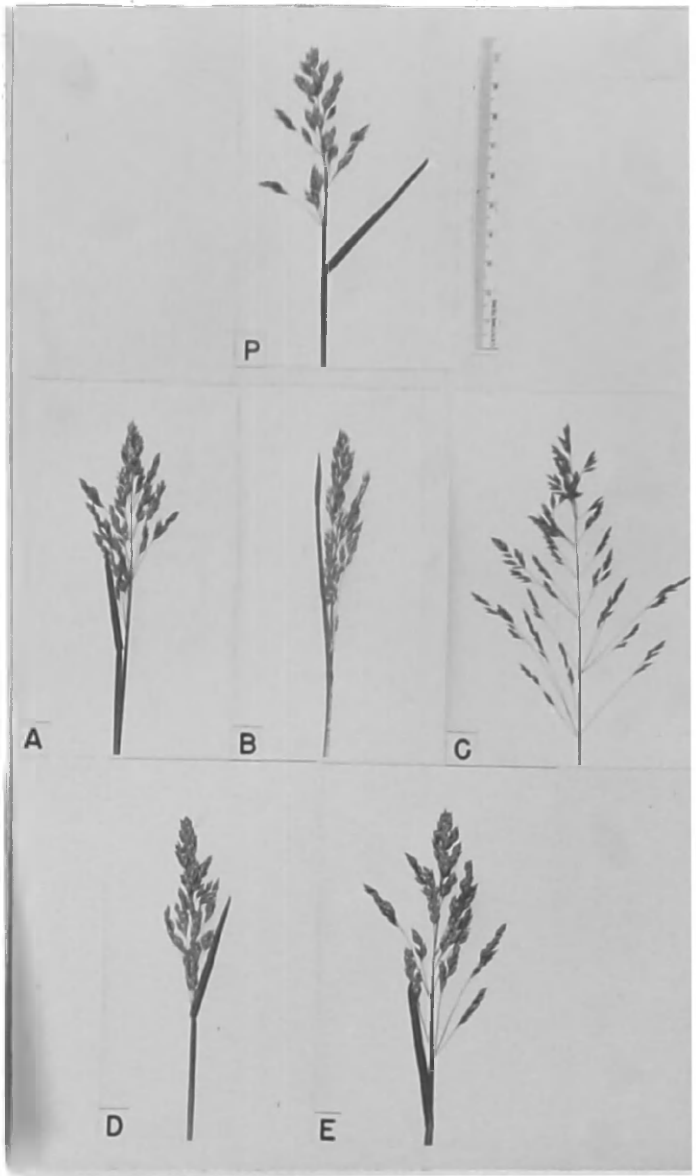

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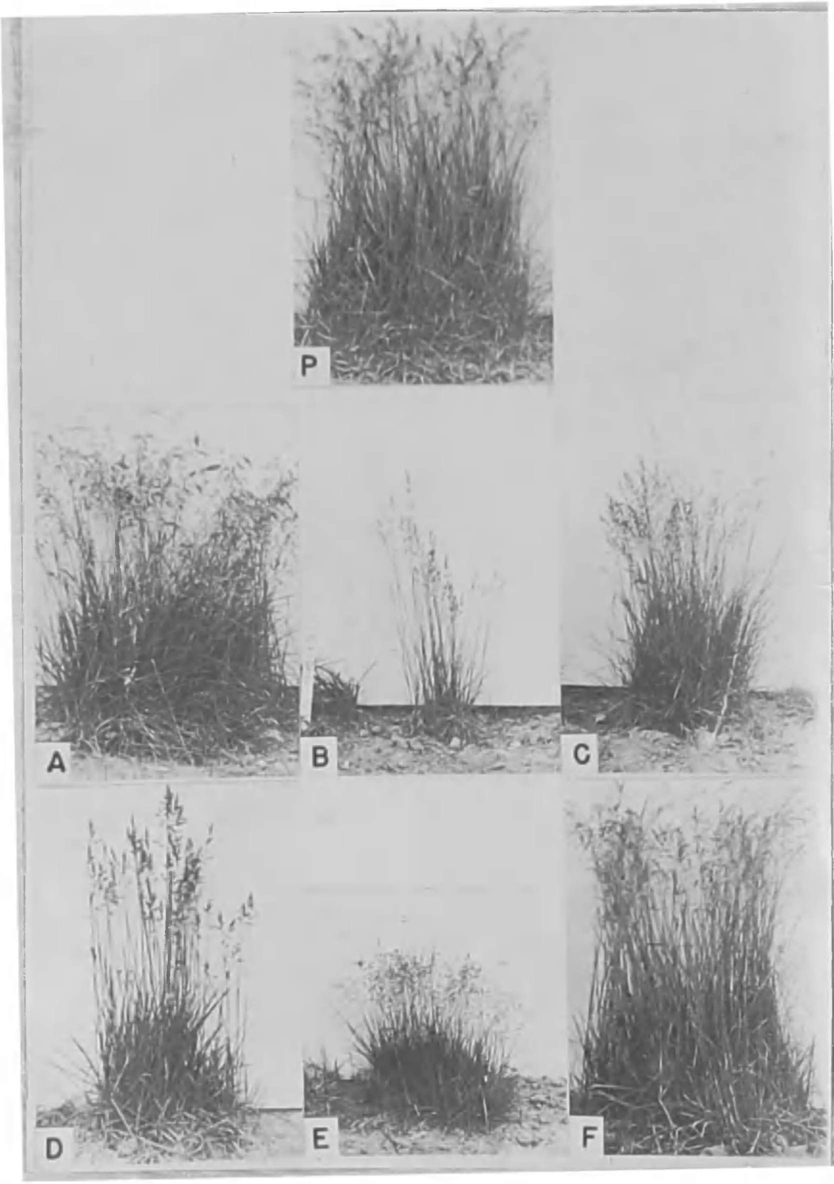

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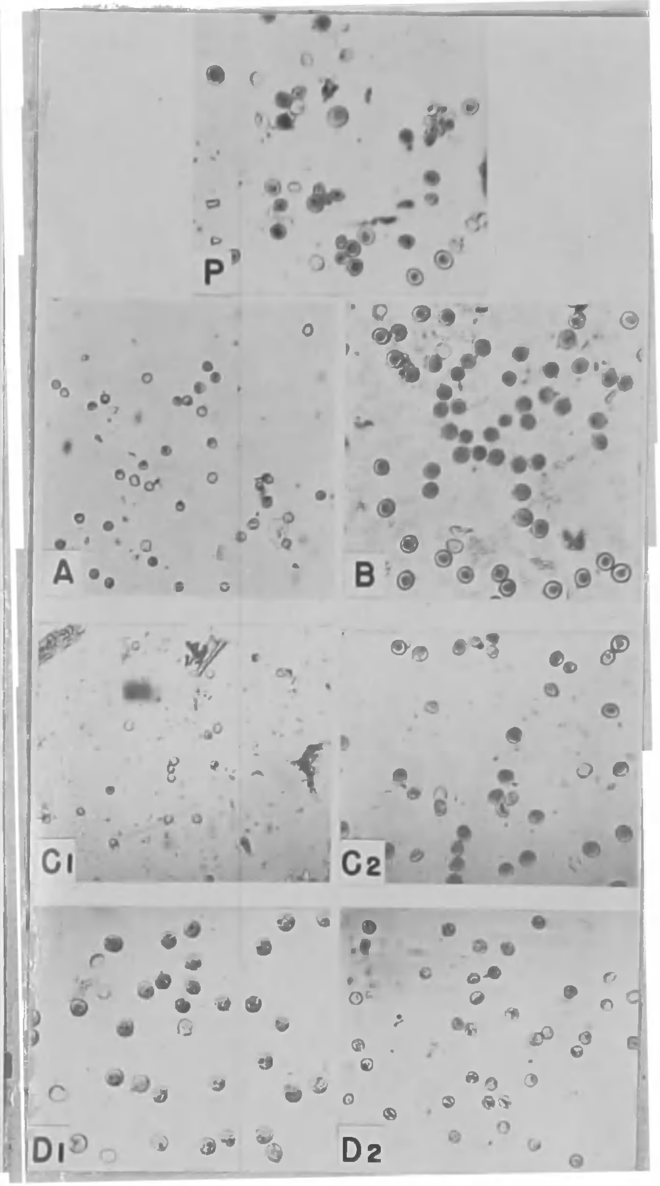

PLATE 11

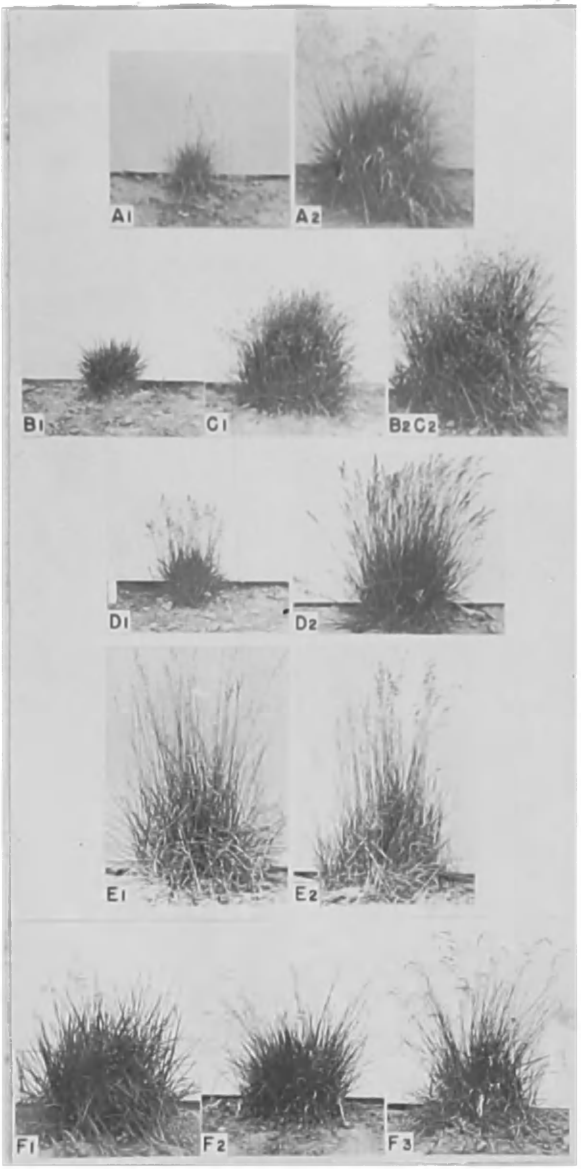

PLATE 12