



**AgEcon** SEARCH  
RESEARCH IN AGRICULTURAL & APPLIED ECONOMICS

*The World's Largest Open Access Agricultural & Applied Economics Digital Library*

**This document is discoverable and free to researchers across the globe due to the work of AgEcon Search.**

**Help ensure our sustainability.**

Give to AgEcon Search

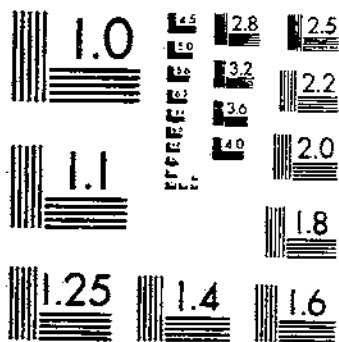
AgEcon Search  
<http://ageconsearch.umn.edu>  
[aesearch@umn.edu](mailto:aesearch@umn.edu)

*Papers downloaded from **AgEcon Search** may be used for non-commercial purposes and personal study only. No other use, including posting to another Internet site, is permitted without permission from the copyright owner (not AgEcon Search), or as allowed under the provisions of Fair Use, U.S. Copyright Act, Title 17 U.S.C.*

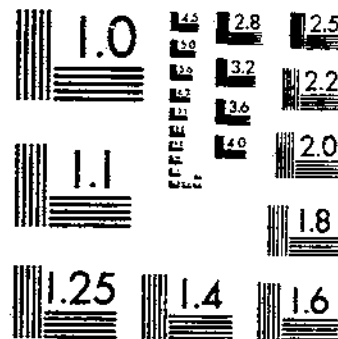
° TB 1145 (1956) USDA TECHNICAL BULLETINS  
BREEDING PERENNIAL FORAGE GRASSES  
HANSON, A. A. CARNAHAN, H. L.

UPDATA  
1 OF 2

# START



MICROCOPY RESOLUTION TEST CHART  
NATIONAL BUREAU OF STANDARDS-1963-A



MICROCOPY RESOLUTION TEST CHART  
NATIONAL BUREAU OF STANDARDS-1963-A

# CONTENTS

Introduction .....	1	Multiplication and varietal release .....	67
Problems and concepts .....	1	Experimental techniques .....	71
Adaptation and natural selection .....	3	Propagation of breeding materials .....	71
The role of cytogenetics .....	6	Vegetative propagation ..	71
Apomixis .....	8	Propagation from seed ...	71
Polyembryony .....	9	Planting .....	73
Polyploidy .....	11	Induction of flowering in the greenhouse .....	74
Induction of polyploidy .....	12	Isolation and hybridization ..	76
Heritability .....	14	Spatial isolation .....	76
Combining ability .....	16	Isolation under bags .....	77
Breeding objectives .....	17	Emasculation .....	79
Yield and vigor .....	19	Hand emasculation .....	81
Growth cycle .....	20	Bulk emasculation .....	82
Disease resistance .....	20	Pollination .....	83
Insect resistance .....	23	Mutual pollination .....	83
Winter-hardiness .....	24	Pollen transfer .....	83
High-temperature tolerance ..	26	Harvesting seed .....	85
Drought tolerance .....	27	Threshing and processing seed ..	86
Tolerance of soil conditions ...	28	Note-taking systems .....	87
Time of maturity .....	29	Maintaining records .....	89
Seed habit .....	30	Plot technique .....	90
Lodging .....	31	Soil selection and treatment .....	90
Root development .....	31	Experimental design and plot layout .....	90
Aggressiveness .....	32	Seeding methods and species mixtures .....	91
Growth habit .....	34	Establishing and marking plantings .....	92
Chemical composition .....	35	Clipping .....	93
Acceptance by livestock .....	37	Harvesting and determining yields .....	95
Breeding procedures .....	40	Botanical analysis .....	95
Selection of species .....	43	Hand separations and visual estimates .....	95
Source nurseries .....	44	Mechanical aids .....	96
Clonal evaluation .....	45	Conclusion .....	97
Ecotype selection .....	47	Literature cited .....	100
Mass selection .....	48		
Maternal-line selection .....	48		
Controlled hybridization .....	55		
Inbreeding and selection .....	56		
Interspecific and intergeneric hybridization .....	61		
Synthetic varieties .....	64		

# BREEDING PERENNIAL FORAGE GRASSES<sup>1</sup>

By A. A. HANSON and H. L. CARNAHAN,<sup>2</sup> *research agronomists,  
Field Crops Research Branch, Agricultural Research Service*

## INTRODUCTION

The current interest in grassland farming and the increased use of forage crops in agricultural systems have focused attention on the possibilities of developing new and better varieties of the major forage grass species. This publication is an effort to appraise the problems confronting the forage grass breeder and to summarize the techniques and methods that are at his disposal. Opinions vary widely with respect to the significance of particular objectives in grass breeding and the best procedures for attaining them. Until definite answers are available, diverse opinions should be considered in planning and executing forage grass breeding programs. Some of the theories presented here are tentative and may not be substantiated by future studies. A useful purpose will be served if this presentation contributes to the planning of research that will advance our knowledge of forage grass breeding.

Specific information on the improvement of a number of perennial forage grasses is available in reviews prepared by Atwood (9)<sup>3</sup> and Burton (37).

## PROBLEMS AND CONCEPTS

Forage grasses, in addition to serving as feed for livestock, stabilize soils and maintain soil fertility. In general, however, the value of grass on cultivated or range land must be considered primarily on the basis of utilization by livestock. Fortunately, most of the grasses that have been widely accepted and used for soil conservation are acceptable as feed to some class of livestock. Grasses unpalatable to livestock are sometimes used on sites especially liable to soil erosion, such as dune areas, where the first objective is to establish and maintain a good vegetative cover.

Quality tests for the forage grasses have not been so well defined as those for certain cereal and fiber grasses. Evaluation of improved forage grasses is complicated by wide differences between and within various classes of livestock in forage prefer-

<sup>1</sup> Submitted for publication November 16, 1955.

<sup>2</sup> The authors wish to acknowledge the cooperation of R. J. Garber, director, and Helen D. Hill, research geneticist, United States Regional Pasture Research Laboratory, who made valuable suggestions.

<sup>3</sup> Italic numbers in parentheses refer to Literature Cited, p. 100.

ences and grazing behavior. In addition, perennial grasses must be capable of persisting over wide variations in environmental conditions, both between and within years, whereas annual farm crops are exposed to only a portion of the environmental hazards of a single year.

Some grass species are well adapted for producing high yields of good-quality hay as a consequence of their leafiness, time of maturity, and compatibility with associated legumes but are incapable of sustained herbage production because of poor recovery or lack of tolerance of trampling and frequent defoliation. Species characterized by a creeping habit of growth and a high proportion of basal leaves often persist better under intensive continuous grazing than erect growing bunchgrasses, but their maintenance of herbage yields may be restricted by lack of tolerance of high temperatures or drought or by lack of disease resistance. Vigorous high yielding species that possess some drought resistance and recover well after grazing may be so competitive that the stand of associated legumes is reduced.

Kentucky bluegrass (*Poa pratensis* L.) offers a good example of the grass breeders' problems. It is an excellent pasture grass that thrives in association with white clover and tolerates trampling and frequent defoliation; but sustained production from bluegrass pastures is restricted by this species' low tolerance of drought and high temperatures, which result in poor herbage production during midsummer (27). In the Northeastern and North Central Regions considerable work has been devoted to efforts to isolate strains of this species producing consistently higher yields of forage at critical periods. These breeding programs have been relatively successful in developing strains that produced higher and better distributed yields than commercial lots (5, 108, 210, 286). The gains realized, however, have not been sufficient to meet midsummer grazing needs. It has generally been conceded that breeding Kentucky bluegrass to meet these needs is an unprofitable undertaking, because of the limited variation in heat and drought tolerance that exists within the species. Breeding Kentucky bluegrass for greater total forage production may, nevertheless, be justifiable, depending on the environment and the degree of improvement sought.

Sound objectives are of paramount importance in any breeding endeavor. To a great extent, the objectives determine the ultimate success of the program. Often the selection of forage grass breeding objectives has been influenced by conflicting or confused philosophies. The breeder has a choice between general and specific objectives, and if he chooses to adopt specific objectives he has a choice of trying to improve desirable characteristics or trying to reduce undesirable characteristics.

The general objective in forage grass improvement can be described as a search for a timothy or a brome grass, for example, that is superior in all respects. Improvement is sought on a broad front with that somewhat fanciful concept an "ideal grass" in mind. So far as possible, the features that contribute to the value of a species should be recognized and maintained, and

within this framework specific objectives of improvement should be sought. A specific objective should not be chosen on its own merits alone. Usually, for example, increased disease resistance or seed production should not be sought at the expense of other desirable characters. Under those circumstances additional time and effort would be required to incorporate good agronomic characters into the strain bred specifically for disease resistance or seed production.

The diversity of the forage grasses, each characterized by certain outstanding features, affords wide opportunity to select for study species that already possess many of the characteristics desired. The question then arises, should the breeder concentrate on strengthening the desirable characters of the selected species or on correcting its weaknesses? Let us assume that the grass species showing the most promise for meeting the needs of a certain region or subregion have been selected, and that one of them is outstanding because of its winter-hardiness, drought resistance, and high feeding value. It would be very shortsighted to breed this species for greater drought resistance if poor seed habits were limiting its widespread use. Breeding for increased seed production while maintaining drought resistance, winter-hardiness, and feeding value would appear to be a logical and desirable course. If serious limiting factors can be neutralized through either management or selection, then the breeder can profitably concentrate on one or more of the outstanding characters for which the species was originally selected. The emphasis placed on correcting inherent weaknesses in a species will, of course, depend on the genetic possibilities for improvement.

## ADAPTATION AND NATURAL SELECTION

A majority of the forage grasses are cross-fertilized and largely self-sterile. As a consequence, individual species are extremely variable, exhibiting wide ranges in phenotypic expression of most characters. Excellent examples of the variation encountered within species are provided by the early investigations conducted with timothy (*Phleum pratense* L.) by Clark (51) and Webber (309). Interspecific and intergeneric hybridization would appear to have contributed to the heterogeneity of many species. This characteristic feature of the Gramineae, in conjunction with polyploidy, has made it possible for man to accelerate the natural processes of distribution by exchanging valuable species between regions and continents, either deliberately or by chance.

The ecotype concept as developed by Turesson (293, 294), Gregor and Sansome (98), Gregor (97), and, more recently, Clausen, Keck, and Hiesey (56, 57) has proved invaluable to the grass breeder in clarifying the species concept and defining potential variations within species. The classification applied by Clausen and coworkers to variations found within genera and species is shown in figure 1.

Degree of separation Internal External	Hybrids fertile, second genera- tion vigorous.	Hybrids partially sterile, second generation weak.	Hybrids sterile or none.
In different environments.	Distinct <i>subspecies</i> ECOTYPES	Distinct <i>species</i> ECOSPECIES	Distinct <i>species complexes</i>
In the same environment.	<i>Local variations</i> of one species BIOTYPES	Species overlap- ping in common territory (with hybrid swarms).	CENOSPECIES

Figure 1.—Concept of species presented by Clausen, Keck, and Hiesey (56).

Turesson defined an ecotype as a population that has become differentiated in response to the conditions of a particular habitat. Ecotypes may differ in growth habit, maturity, and other characteristics such as pubescence and flower color. Ecotypes of a given species have the same chromosome number and hybridize readily. Gregor (97) stressed that an ecotype is not necessarily genetically uniform over its entire range, that populations can seldom be clearly delineated with respect to ecotypic characters, and that identification of an ecotype depends to a large degree on the proportional representation of "indicator" types. Gregor concluded that an ecotype's value in plant improvement is more likely to be associated with use as source material than with use as a commercial strain.

A population of a grass species invariably consists of a mixture of many different subgroups (biotypes), which vary in fitness for survival in a given environment. Environmental factors bring about a sorting of the biotypes, with an increase of the better adapted ones at the expense of the others. Thus, a species may respond to the challenge of diversified habitats by developing local races. The nature of the response depends on the range of biotypes encompassed within the population and the particular combination of environmental conditions to which the population is exposed. Mutations may play an important part in the process of adaptation. The effectiveness of current mutations is much greater in diploid than in polyploid members of the Gramineae.

Clausen, Keck, and Hiesey (57) considered several plant species and found no observable correlations between degree of polyploidy and environment. They found no evidence that the direct influence of environment produces heritable changes in species. Major environmental changes do, however, provide new habitats or refuges for the products of variation within and among species. Stebbins (268) suggests that polyploids may be well adapted to the colonization of newly available areas. Löve and Löve (180) concluded that the abundance of polyploids at northern latitudes and higher altitudes cannot be explained at present according to any simple and general physiological rule. Experiments have shown, however, that the genera of the higher



plants of Europe react to severe environments by reduction in number of species. In most genera the diploid species disappear first and consequently the proportion of polyploids increases in severe environments.

Harian, Snyder, and Celarier (113) found the diploid forms ( $2n = 20$ ) of blue grama (*Bouteloua gracilis* (H. B. K.) Lag.) adapted to a wider range of ecological conditions than the polyploid forms in the same region. The polyploid forms ( $2n = 40$  and  $2n = 60$ ) were apparently the more abundant at higher elevations. Data collected by Nielsen (217) suggest that polyploidy in switchgrass (*Panicum virgatum* L.) is not directly associated with winter-hardiness or the ability to grow in a more rigorous environment than that of the diploid forms. In an earlier study Nielsen (216) found a polyploid series of 18, 36, 54, 72, 90, and 108 somatic chromosomes in switchgrass but did not find evidence of any "regional" segregation of races on the basis of chromosome number.

In nature, the principal selective influences governing the production of ecotypes are latitude, altitude, moisture, temperature, and—under some circumstances—plant pathogens and insects. Man has superimposed on these selective factors seed production, which tends to eliminate infertile plants and, in accordance with time of harvest, either late or early flowering types; severe grazing, which favors low growing biotypes; and other management practices that may make plants more liable to severe drought damage or winterkilling.

The cool-season grasses make their maximum vegetative growth during the spring and fall. These grasses begin active growth quickly after the ground thaws in the spring, and under favorable moisture conditions they continue growing until late in the fall. Some cool-season species provide winter grazing in the southern United States. Most of the important cultivated grasses belonging to this group have a relatively wide range of adaptation. Well differentiated local strains of cool-season grasses are infrequent, probably because domestic seed production is concentrated in a few areas and substantial amounts of foreign-grown seed are commonly imported. An example of strains adapted to specific areas or regions is provided by the northern and southern types of smooth brome grass (*Bromus inermis* Leyss.). The southern brome grass strains are thought to have originated from Hungarian introductions, while the northern types apparently trace to Siberian sources (214). Within the two groups variation similar to that found in other cross-pollinated crops occurs from farm to farm and from locality to locality. Such variation may have resulted from selective factors operating both before and after the introduction of this grass into the United States. There is no definite evidence to show whether differentiation between the northern and southern strains took place chiefly in this country. Tests conducted by Newell and Keim (214) in Nebraska have shown that under their conditions the southern brome grass strains, as compared with the northern strains, produced seedlings having greater

vigor in fall and early spring. The southern strains were also more tolerant of drought and heat, and possessed more vegetative vigor and greater productivity.

The warm-season grasses start growth in late spring or early summer and attain their maximum vegetative development during midsummer. Ecotypes are remarkably well differentiated within the native warm-season grass species in the Great Plains; numerous geographical strains have been isolated there within the bluestems (*Andropogon* spp.), buffalograss (*Buchloë dactyloides* (Nutt.) Engelm.), the grama grasses (*Bouteloua* spp.), switchgrass, indiangrass (*Sorghastrum nutans* (L.) Nash), and others. At southern locations the northern ecotypes of a warm-season species are generally earlier, smaller, and less leafy than the southern. Working in Kansas with big bluestem (*Andropogon gerardi* Vitman), Law and Anderson (175) found that plants from Nebraska headed 21 days earlier and plants from Oklahoma headed 47 days later than local plants. The variation between ecotypes was always greater than that within ecotypes. Apparently only local strains of the warm-season grasses are sufficiently well adapted within a particular locality to be useful under cultivation or for reseeding native range. Results from strain tests indicate that seed of the native grasses should not be moved either north or south in excess of 300 miles (15).

## THE ROLE OF CYTOGENETICS

Cytological and genetic investigations of the grasses have two primary purposes—(1) to elucidate the taxonomy and phylogeny of the Gramineae and (2) to contribute fundamental information that can be applied to the improvement of grasses through breeding (207). It is important to the investigator to have sound taxonomic information on the particular species he is studying and also data on the degree of variation (morphological and cytological) encompassed within the limits of the species. Cytological and genetic studies contribute fundamental information on chromosome numbers, the nature of polyploidy, and the existence of aneuploids and chromosome series that is useful in breeding. A knowledge of chromosome numbers can be applied, for example, in deciding which of several crosses among species might succeed and may also influence the direction in which certain crosses are attempted.

Low fertility is associated with extreme meiotic irregularity, but in polyploid species considerable meiotic dislocation can occur without much reduction in seed set. In a study of interstrain hybrids in *Elymus glaucus* Buckl., Snyder (253) found some plants with 94-percent pollen abortion, but only a small part of this abortion could be attributed to cytological disturbances. Absence of correlation between production of abnormal pollen and degree of self-fertility has been noted in certain herbage grasses (221). In clones of orchardgrass (*Dactylis glomerata* L.) the frequency of micronuclei at the quartet stage was found

by Weiss et al. (312) not to be closely associated with forage yield, leafiness, or selfed or open-pollinated seed set.

In cross-pollinated long-lived perennial species capable of asexual increase, according to Elliott and Love (71), natural selection may favor plants that are vegetatively aggressive, some of which can survive in spite of irregular bivalent formation and low fertility.

There have been numerous reports of interspecific and intergeneric hybrids in the Gramineae; Myers (207) lists over 200 such hybrids. Interspecific and intergeneric hybrids have been produced with *Festuca* and *Lolium* species, and several interspecific hybrids have been found or produced in each of the genera *Agropyron*, *Elymus*, *Dactylis*, *Phleum*, *Poa*, and *Bromus*. Most of the controlled-hybridization studies have not led to production of new forms that could be utilized directly in crop production; they have been begun only recently, and their current results have not yet been thoroughly evaluated. There is every reason to believe that interspecific hybridization will serve the purpose of extending the range of variation beyond that existing in well-established species.

Only preliminary irradiation studies have been conducted. More information is needed on the usefulness of X-rays and thermal neutrons in producing useful variants, either for genetic experiments or for utilization in selection programs.

So far as possible, different lots of material for studying meiotic behavior should be collected under comparable environmental conditions. Maximum temperatures preceding collection and other environmental conditions may influence the degree of meiotic irregularity (312).<sup>4</sup> In many cool-season grasses meiotic divisions appear to reach their peak at or near midday. Heads that have emerged about halfway from their sheaths generally provide opportunity for study of all stages from early prophase through pollen-grain development. Material of some species must be collected somewhat earlier—e.g., when the head is just beginning to emerge from the sheath. Variation with respect to the optimum stage of development for collection of cytological material has been noted within species.

Smith (252) has reviewed the aceto-carminic smear technique; Hill and Myers (127) have reported a schedule that facilitates counts of somatic chromosomes; and Hanson and Oldemeyer (106) have described an aceto-carminic smear technique that gave satisfactory results with several grass species.

Plant-breeding methods and procedures are largely dependent on modes of reproduction. Observations on flowering together with data from selfing studies and progeny tests establish the dominant method of reproduction. Cytological examination has helped to explain many types of anomalous behavior characterizing certain grass species. Although most of the economic grasses are largely cross-pollinated, apomixis is fairly common and several species exhibit varying degrees of cleistogamy.

<sup>4</sup> OLDEMEYER, D. L. METHODS OF EVALUATING COMBINING ABILITY IN ORCHARD-GRASS, *DACTYLIS GLOMERATA* L. 105 pp., illus. 1952. [Unpublished doctor's thesis. Copy on file Library, Pa. State Univ., at State College.]

## APOMIXIS

Apomixis, broadly defined, includes any reproductive process that does not involve the typical union of egg and pollen nuclei. Uniformity with respect to maternal characters is the outstanding feature of apomictic progenies. Warmke (305) divides apomixis into two categories, apomixis with seed formation (agamospermy) and vegetative apomixis. The latter classification applies where vegetative reproduction substitutes for sexual reproduction as a consequence of sexual sterility. Vegetative apomixis is a characteristic of certain species of *Poa*, *Agrostis*, *Deschampsia*, *Festuca*, *Bromus*, *Phleum*, *Avena*, and *Panicum*. In some of these species the florets may be replaced wholly or in part by vegetative proliferations or bulbils.

Apomixis with seed formation in the grasses was subdivided and discussed by Warmke (305) in the following manner (references to publications by some other authors are interspersed):

Diplospory.—Meiosis initiated in macrospore mother cells. Chromosome number not reduced, usually owing to incomplete synapsis.

Apospory.—Embryo sac develops by mitotic divisions from unreduced cell.

Reduced macrospores are formed in *Poa pratensis*, but these degenerate and are replaced by an unreduced embryo sac formed by mitotic divisions of one or more cells of the nucellus (287). In *P. alpina* L. and *P. palustris* L. the embryo sac is formed through mitotic divisions of the archesporial cell.

Diplospory or apospory substitutes for normal meiosis in producing unreduced embryo sacs, while seed formation through processes defined as parthenogenesis and pseudogamy occurs in the absence of normal fertilization.

Parthenogenesis.—Egg cell divides and develops into an embryo in the absence of pollination. Parthenogenesis apparently occurs in *Calamagrostis* species, *Poa nervosa* (Hook.) Vasey, *Pennisetum villosum* R. Br., and *P. ruppellii* Steud. (272).

Pseudogamy.—Pollination required for seed formation. The initiation of endosperm divisions depends on the union of a male nucleus with the polar nuclei of the embryo sac. In *Poa pratensis* a proembryo may be formed prior to anthesis but no seed is formed in the absence of pollination.

The occurrence of uniform progenies has suggested apomictic reproduction in certain strains of *Poa alpigena* Lindm., *P. glauca* Vahl, *P. compressa* L., *Bouteloua curtipendula* (Michx.) Torr., *Paspalum dilatatum* Poir., *P. notatum* Flüge, *P. urvillei* Steud., and *P. malacophyllum* Trin. The usual method of reproduction in *Panicum maximum* Jacq. (305), *Pennisetum ciliare* (L.) Link., and *Cenchrus setigerus* Vahl (86) appears to be apospory followed by pseudogamy.

This abridged presentation of Warmke's discussion does not include all possible types of apomictic processes. Apomixis has been discussed in detail by Stebbins (271).

Brittingham (25) examined 10,000 plants grown from seed

of 115 selections of *Poa pratensis* and recorded data on morphological characters, pollen grain size, and chromosome numbers of aberrant plants from both twin and single seedlings. These data indicated that aberrant plants arose from apomictic development of reduced eggs, fertilization of reduced eggs by reduced pollen, and fertilization of unreduced eggs by reduced pollen. A highly significant negative correlation was reported for variability and survival. The percentage of variability (aberrants) for all offspring was 14.6, but for various families it ranged from 0 to 65.5. Smith and Nielsen (249) found that percentage of normals in progenies from normal and aberrant plants of *P. pratensis* varied widely. Normal plants tended to produce more uniform progenies than aberrants, but selection for normal types did not invariably increase the constancy of the progenies. In general, the most constant families maintained a high level of normals in three successive generations: examples are 98, 91, and 100 percent; 98, 79, and 88 percent; and 100, 83, and 75 percent.

### POLYEMBRYONY

Occurrence of multiple embryos and twins has been reported for several grass genera. Study of twin plants has contributed valuable information on reproductive processes, the possible origin of species, and interrelationships of species within genera. Nielsen (218) examined the cytological behavior, morphology, and breeding behavior of 28 sets of twin plants of *Bromus inermis*. Earlier work cited by Nielsen indicated that in this species twin plants were produced by about 1 seed out of 550. Individual brome grass plants produced as many as 8 percent twin-embryoed seed. None of the 28 sets of twins was found to be monozygotic. Three different chromosome numbers—28, 56, and 70—occurred in the material studied. Meiosis in the 1 set having 28 chromosomes was irregular, indicating that these plants do not represent stable tetraploid members of a regular polyploid series. A polyhaploid smooth brome grass plant reported by Elliott and Wilsie (72) was regular, with most of the chromosomes associated as bivalents.

Müntzing (194) isolated 6 "triploids" ( $2n=63$ ) and 3 "haploids" ( $2n=21$ ) from 446 twin plants of *Phleum pratense*. Müntzing and Prakken (196) found that "triploid" members of twin pairs had good vigor and fertility. The "triploids" were more robust than corresponding "diploids," having longer, thicker culms and leaves, larger spikelets, and larger pollen grains.

Müntzing (194) found 18 "triploids," 2 "haploids," and 5 other aberrants in a sample of 270 twin plants of *Poa pratensis*. He found (195) that in  $2n-3n$  pairs the twin with the higher chromosome number was more productive and had broader, thicker leaves than the other. The high-chromosome plants exhibited better pollen fertility and were somewhat more sexual than the "diploid" members of the same sets. Brittingham (25) found 7-percent polyembryony in a population of 10,000 plants

of *P. pratensis*. Aberrants among plants from twin embryos amounted to 16.9 percent, a proportion significantly higher than that found among plants from seeds with a single embryo. Åkerberg (1) determined the chromosome numbers of twin plants of several species, with results some of which are summarized in table 1.

TABLE 1.—*Chromosomal variation in twin plants of several grasses as determined by Åkerberg (1)*

Species	Twin plants examined				
	Total	Triploid	Haploid	Otherwise aberrant	Total aberrant
	Number	Number	Number	Number	Percent
<i>Dactylis glomerata</i> .....	198		2		1
<i>Festuca pratensis</i> .....	34	1			3
<i>Festuca rubra</i> .....	32	1			3
<i>Festuca ovina</i> .....	6				0
<i>Lolium perenne</i> .....	105	4			4
<i>Agrostis stolonifera</i> .....	31	1			3
<i>Cynosurus cristatus</i> .....	17				0
<i>Poa pratensis</i> (Müntz.)..	270	18	2	5	9
<i>Poa pratensis</i> (Åkerb.)..	63		8		13

More than two-thirds of the species included in Myers' review (207) were polyploid or had one or more polyploid races. Myers lists 99 species with chromosome races, exclusive of species in which apomixis is contributing to the persistence of aneuploid forms.

In species that contain chromosome races, the breeder should take note of this and ascertain to what extent phenotypic variations may be associated with variation in chromosome number. Hanson and Hill (104) determined chromosome numbers of seedlings grown from open-pollinated seed of hexaploid orchard-grass that was not isolated from normal tetraploid nurseries. In their study 14.6 percent of the sample differed from the hexaploid complement, 11.2 percent of the seedlings having 35 or fewer chromosomes; evidently considerable crossing between  $6\times$  and  $4\times$  plants had occurred. This points to the necessity of isolation unless one is interested in developing aneuploid stocks. Kramer (166) found that spreading rate and mildew susceptibility were positively correlated with chromosome number in *Poa pratensis*. Absence of other significant associations indicated that chromosome numbers could vary over a considerable range without causing appreciable variation of morphological characters or agronomic behavior.

## POLYPLOIDY

Stebbins (269) has reviewed polyploid nomenclature and the significance of the various forms of ploidy. He recognizes three general types of polyploids: Autopolyploids, segmental allopolyploids, and true allopolyploids.

Autopolyploids are derived either from essentially homozygous diploids or from hybrid progenies of strains or subspecies. The component genomes are similar, but genetic differences may exist between wild autopolyploids and their nearest diploid relatives. An autopolyploid species is distinguished by a high frequency of multivalent chromosome configurations at meiosis and the presence of tetrasomic ratios.

The genomes of segmental allopolyploids have a significant frequency of their chromosome segments in common. Segmental allopolyploids resemble autopolyploids to a greater or lesser degree in that they exhibit some multivalent chromosome associations and tetrasomic ratios, but both these features are less common in them than in autopolyploids.

True allopolyploids are derived from hybrids between species, and the chromosomes contributed by the respective parents are largely nonhomologous. In general, allopolyploids resemble diploids in their cytogenetic behavior and rarely exhibit multivalent chromosome configurations or tetrasomic ratios. They differ from diploids, however, in that they tolerate to a greater extent deficiencies in chromosomal material.

The characteristics of autopolyploidy and allopolyploidy may be combined in higher polyploids, at the hexaploid level or beyond.

The multiplicity of forms that may be encompassed in the range between autopolyploidy and allopolyploidy accounts for the divergence of opinion regarding the proper classification of many of our economic grasses. Müntzing (193) and Myers and Hill (211) have suggested, on the basis of cytological information, that orchardgrass is an autopolyploid. Limited data available from the segregation of chlorophyll-deficient seedlings indicate tetrasomic inheritance (202), but other observations suggest that two closely related diploid species were probably involved in the origin of tetraploid orchardgrass (269). Several workers (147, 241, 267) have shown that the inbreeding depression in this species approaches the degree of depression expected in a diploid species. This situation may be resolved by assuming that orchardgrass is a segmental allopolyploid and as such may exhibit a varying frequency of multivalent associations and some tetrasomic segregation. In such a species it might be assumed that some of the genes governing the expression of a given character may behave according to disomic expectations while others exhibit tetrasomic inheritance. A parallel situation may exist in many grass species.

The genetic consequences of the chromosomal behavior of orchardgrass have been reported by Myers (203). It has been calculated by Bartlett and Haldane (13) and by Haldane (103) that the rate of approach to homozygosis in selfing of auto-

tetraploids is slightly slower than that in brother-sister matings of diploids. Since most cross-pollinated forage grasses are relatively self-sterile, it is of interest to compare the rates:

Type of mating and ploidy	Generations required to halve heterozygosis	
	Number	
Selfing:		
Diploid	1	
Autotetraploid	3.80	
Brother-sister:		
Diploid	3.26	
Autotetraploid	8.72	

Ratios of dominants to recessives when a given heterozygous autopolyploid type is crossed with the homozygous recessive or selfed (assuming chromosome segregation) are as follows:

Level of ploidy	Genotype	Test-cross ratio	F <sub>2</sub> ratio
Diploid	A <sup>a</sup>	1:1	3:1
Autotetraploid	A <sup>2</sup> a <sup>2</sup>	5:1	35:1
Autohexaploid	A <sup>3</sup> a <sup>3</sup>	19:1	399:1
Autooctaploid	A <sup>4</sup> a <sup>4</sup>	69:1	4,899:1
Autodecaploid	A <sup>5</sup> a <sup>5</sup>	251:1	63,503:1
Autododecaploid	A <sup>6</sup> a <sup>6</sup>	923:1	853,775:1

It is quite clear from the above F<sub>2</sub> ratios that in most instances it would not be practical to attempt to obtain pure recessive plants in the F<sub>2</sub> from anything higher than an autohexaploid. Where two unlinked dominant-factor pairs are involved and chromosome segregation occurs, the corresponding ratios at several levels of autopolyploidy are as follows:

Level of ploidy	F <sub>2</sub> ratio
Diploid	9 : 3 : 3 : 1
Autotetraploid	1,225 : 35 : 35 : 1
Autohexaploid	159,201 : 399 : 399 : 1

Therefore, in practice, the breeder would probably not even attempt to obtain a<sup>4</sup>b<sup>4</sup> in the F<sub>2</sub> from A<sup>4</sup>b<sup>4</sup> × A<sup>4</sup>B<sup>4</sup>. More logically, he would self A<sup>4</sup>B<sup>-</sup> and A<sup>-</sup>b<sup>4</sup> individuals in the F<sub>2</sub> in order to obtain some double recessives in the F<sub>3</sub>.

### INDUCTION OF POLYPLOIDY

The use of colchicine to effect chromosome doubling has led to considerable enthusiasm and speculation regarding the usefulness of autopolyploids in crop production. Investigations have indicated, however, that artificial autopolyploids cannot be utilized directly to any appreciable extent in developing new grass varieties. This may be accounted for by (1) the fact that many of the important polyploid forage grasses are at or near the maximum chromosome number for optimum development, (2) the meiotic instability and reduced fertility of artificial autopolyploids, and (3) the difficulty of maintaining pure autopolyploids in the Gramineae.

The effect of chromosome reduplication in many comparisons of diploid and tetraploid material has been confounded by gene differences in the materials compared. Hill and Myers (126)



overcame this difficulty by isolating pure  $2n$  and  $4n$  clones from several mixoploid clones of perennial ryegrass (*Lolium perenne* L.) that arose from colchicine-treated seed. Morphologically the autotetraploids were distinguishable from the corresponding diploids in that they had somewhat wider, thicker, and longer leaves, thicker but fewer tillers, longer leaf sheaths, and larger florets, spikelets, pollen grains, and seeds. Their stomata were slightly larger, but  $2n$  and  $4n$  types could not be distinguished on this basis. In some pairs morphological differences were slight, in others they were striking. Myers (207) later observed that  $4\times$  *L. perenne* exhibited a greater sensitivity to winter injury than the corresponding diploids. Contrary to the usual effect of chromosome doubling, Myers (207) found no differences between related  $2\times$  and  $4\times$  clones of *L. perenne* in maturity or in rate of growth except that the  $2\times$  material produced more new tillers.

Stebbins (270) produced autopolyploids of 20 grass species and tried to establish 14 of them in natural habitats. He succeeded in establishing only 1. The only autopolyploids in his study that approached the vigor and fertility of their undoubled progenitors were those of the species *Bromus stamineus* E. Desv. and *Ehrharta crecta* Lam. The reduced vigor of autopolyploids in his study was not related to the original chromosome number of the species.

Colchicine and other agents for doubling chromosome numbers will probably have their greatest application in obtaining artificial amphidiploids from the sterile products of interspecific or intergeneric hybridization. In addition to fixing, or rendering fertile, hybrid combinations, chromosome doubling can be used to facilitate transfer of gene complexes from one species to another (59). Either of these approaches would be far more profitable, according to Stebbins (269), than chromosome doubling and intensive selection within a single strain. Stebbins has concluded that the major role of polyploidy in evolution has been in fixing and spreading hybrid combinations.

Chromosome doubling can be induced by applying colchicine to either germinating seeds or tillers. In general, tillers require higher concentrations and longer treatments than germinating seeds. Seeds are placed on colchicine-soaked blotting paper, and roots and crowns of tillers are immersed in colchicine solutions. Growing points can be treated by wrapping roots in moist blotting paper and inverting tillers in colchicine solutions so that the crowns and growing points are completely immersed. The concentration needed varies with species and with duration of treatment, ranging approximately from 0.02 to 0.50 percent for seeds and from 0.10 to 1.00 percent for tillers. Exposure ranges from about 6 to about 24 hours. Seeds and tillers should be thoroughly rinsed with water before they are planted.

Hill and Myers (126) describe a technique by which they isolated diploid and tetraploid clones from mixoploid ryegrass plants arising from seeds treated while germinating with 0.2- and 0.4- percent colchicine (201). Mixoploid plants were divided

into single tillers and the chromosome numbers of root tips were determined in successive vegetative generations. In some plants mixtures of  $2\times$  and  $4\times$  tissue persisted through 11 vegetative generations, indicating that an intimate mixture of  $2\times$  and  $4\times$  cells followed colchicine treatment and that tiller primordia were produced by the differentiation of groups of cells. Neither diploid nor tetraploid tissue apparently tended to eliminate the other, and pure tetraploid material was not noticeably harder to obtain than pure diploid material.

## HERITABILITY

Knowledge of the heritability of economic characters is essential for efficient selection. Genetic investigations on perennial forage grasses have been limited, however, by several factors over and beyond lack of research support. These include the absence of clearly defined qualitative characters and the complex nature of inheritance. Study of the inheritance of seedling characters would appear most profitable, since easily recognizable seedling mutants can be isolated in many species and large seedling populations can be grown in greenhouse flats. Most seedling mutants represent various degrees and patterns of chlorophyll deficiency. The major economic characters are quantitatively inherited, and strict limitations are imposed by population size, the possibility of more than one mode of inheritance, and the somewhat artificial assumptions that are frequently required for a genic interpretation.

Burton (38) estimated the minimum number of genes controlling the expression of characters in diploid pearl millet (*Pennisetum glaucum* (L.) R. Br.) by use of the formula, suggested by Sewell Wright,

$$n = \frac{0.25(0.75 - h + h^2)D^2}{\sigma^2 F_2 - \sigma^2 F_1}$$

in which

$$h = \frac{\bar{F}_1 - \bar{P}_1}{\bar{P}_2 - \bar{P}_1}$$

$$\bar{D} = \bar{P}_2 - \bar{P}_1$$

$\bar{P}_1$  = the mean of the smaller parent

$\bar{P}_2$  = the mean of the larger parent

$\bar{F}_1$  = the mean of the  $F_1$  population

$\bar{F}_2$  = the mean of the  $F_2$  population

This formula provides an unbiased estimate of gene numbers under these conditions: (1) No linkage between pertinent genes; (2) one parent supplies only plus factors and the other parent only minus factors among those in which they differ; (3) all genes equally important; (4) degree of dominance of all plus factors similar; (5) no interaction between pertinent nonallelic genes. Burton (39) considers that estimates based on this or

other formulae are of value to the plant breeder provided their inherent limitations are appreciated.

Most of the information available on parent-progeny relationships in the grasses has been expressed as correlation coefficients. This can be attributed to the need for data of this type and the relative ease with which they can be accumulated. The degree of parent-progeny correlation reported in any one study reflects several variables including method of planting, amount of replication, environment in which the material was grown, type of progeny, and parental clones. In a limited number of studies heritability estimates have been calculated in an effort to arrive at some approximation of the progress to be expected in practicing selection in segregating populations. The term "heritability" has been used in these studies in a broad sense, as signifying the proportion of the total variance due to genetic effects. Kalton, Smit, and Leffel (147) used plant-to-plant variances to estimate the extent of genetic variation in inbred progenies of orchardgrass. They considered the variance of the parental clones to be environmental and that of the first inbred generation to be partly environmental and partly genetic. The

heritability percentage is expressed as  $\frac{V_{e1} - V_{e0}}{V_{e1}} \times 100$ ,  $V_{e1}$  and

$V_{e0}$  signifying the variance of the first inbred generation and that of the parental clones, respectively. Genetic variances calculated in this manner are estimates of the sum of (1) total genetic variance, which includes (a) additive genetic variance, (b) variance due to dominance deviations from the additive scheme, and (c) variance resulting from interaction of nonallelic genes, and (2) variance attributable to genotype-environment interaction.

Burton (38) calculated heritability values for pearl millet by subtracting the variance of the  $F_1$  population from that of the  $F_2$  population and dividing the difference by the  $F_2$  variance. Warner (306) has suggested using the first backcross and  $F_2$  populations in estimating heritability in maize. He believes that inbred lines do not provide a satisfactory measure of nonheritable variance. The total variance of inbred lines measures the nonheritable variation comparable to that of normal populations plus the nonheritable variation characteristic of nonvigorous populations.

Burton and DeVane (41) used variance components from analyses of variance to estimate heritabilities in a replicated spaced planting of tall fescue (*Festuca arundinacea* Schreb.) clones. High heritability values were obtained for all the characters studied. The investigators suggest, however, that the actual gain may be lower than that calculated from data on spaced plantings. Grasses in solid stands generally exhibit less variation than comparable material in spaced plantings, at least if the solid stands are dense. Also, the additive portion of the genetic variance for some of the characters studied may be small, in which case simple selection would prove inadequate

and other breeding methods will have to be relied on for producing superior varieties.

## COMBINING ABILITY

Combining ability is the relative ability of a biotype to transmit desirable characteristics to its crosses. The combining ability of a selection may be measured in terms of any one of several characters. Much of the progeny testing in cross-fertilized forage grasses is to determine combining ability as measured by yield.

The terms used in maize breeding to define combining ability have been summarized by Johnson (141) as follows: General combining ability—relative performance when selections are crossed with a wide source of heterozygous germ plasm (e.g., an open-pollinated variety), due primarily to additive gene action; specific combining ability—relative performance when selections are crossed with a narrow source of homozygous germ plasm (e.g., an inbred line), due primarily to deviations from the additive scheme; average combining ability—mean relative performance when selections are crossed with several homozygous testers. If the number of homozygous testers is fairly large, the values for average and general combining ability may be much the same. Definitions based exclusively on maize breeding cannot be applied directly to forage grass investigations, as most crosses in forage grasses involve heterozygous lines or clones. However, the broader definition provided by Sprague and Tatum (255) is acceptable in relation to forage grasses. These writers state that "specific combining ability" designates those cases in which certain combinations do relatively better or worse than would be expected on the basis of the average performance of the crosses of the lines involved.

Studies of combining ability in maize have shown the value of incorporating diverse germ plasm into breeding programs (62), and this observation applies equally to the breeding of forage grasses. In maize, Sprague and Tatum (255) and Federer and Sprague (84) have shown, the variance for specific combining ability is greater than the variance for general combining ability if the comparison involves lines selected on the basis of high general combining ability. In relatively unselected material, the component of variance for general combining ability may exceed that for specific combining ability. Exceptions must be expected, depending in part on the species, the degree of previous selection, the number of selections investigated, and the method of evaluating progenies. Knowles (163) found that the variance due to specific combining ability was much greater than that due to general combining ability for noninbred bromegrass clones, but that it was less than that due to general combining ability for noninbred clones of crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.).

## BREEDING OBJECTIVES

Grass breeding objectives, of necessity, vary with the species, the region, and the intended use of improved varieties. In brome-grass, for example, increased resistance to foliar diseases may be considered universally significant, but in some regions superior seedling vigor or superior recovery after grazing may well transcend increased disease resistance as a major objective. Uniform production, a possible consequence of increasing disease resistance, is especially desirable in areas where brome-grass is expected to contribute aftermath for midsummer grazing. The practical significance of realizing improvement in one or more characters depends on the increase in efficiency of production thus obtained.

Several investigators have listed the objectives that they consider should be sought in developing improved grass strains. In some instances, these objectives have been adapted to fit the intended use of the grass or the area where it was expected to be grown. Thus, in 1919 Witte (327) outlined the characteristics he sought in developing improved timothy varieties for 2-year hay and for perennial pasture. Greater emphasis was placed on longevity and aftermath in selecting the pasture strain. Later, Nilsson-Leissner and Nilsson (225) emphasized that the objectives sought in developing timothy varieties for various regions in Sweden were as follows: South—vigorous type with high nutritive value and the best possible aftermath; east—drought resistance and relatively early type; central—hardy strain with late development for growing with red clover; north—hardiness, disease resistance, tolerance of acid soils, and persistence.

A common feature of most published statements of grass-breeding objectives is the rather large number of characters in which improvement is contemplated. This is not necessarily objectionable, but the impression is sometimes created that a large number of objectives are all held to be equally important and will receive comparable emphasis in the program. Where this situation exists in practice, major objectives could be lost in a maze of detail that delays progress or limits it. Major objectives should be clearly delineated; others may be outlined with the clear understanding that they may have to be sacrificed in part.

Stapledon (262, 263, 264, 265) discussed in detail the characters that in his opinion determined the economic value of grasses: Nutritive value and palatability; leaf-to-stem ratio; production of tillers and resistance to repeated defoliation; and persistence and aggressiveness. According to Garber et al. (92), prime requirements of a new variety are high yield and better distribution of yield through the growing season. The sustained-yield characteristic is influenced by winter-hardiness, resistance to diseases affecting forage yield and persistence, longevity, tolerance of drought and flooding, and ability to tolerate occasional periods of overgrazing and undergrazing. Other important objectives listed by these writers include nutrient value, compati-

bility in mixtures, ease of harvesting and management, and adaptability to various management systems. Davies (66), in listing various characters that influence the value of pasture plants in England, included winter greenness and accessibility (the ease with which livestock can graze the plant).

At least five and sometimes six major objectives must receive some attention in the development of a superior forage grass variety. The five are yield of digestible nutrients, distribution of yield, persistence, palatability, and ease of reproduction, and the sixth is ease of management.

Yield of digestible nutrients can be divided into the two characteristics total dry weight and quality of herbage produced under given environmental conditions (such as latitude, soil type, drainage, and management). Total yield is a resultant of factors including heterosis, resistance to disease and insect attack, tolerance of drought and of temperature extremes, time of maturity, growth habit, aggressiveness, soil fertility, drainage, and management. Photoperiod and day and night temperatures have a profound effect on total yield. Thus, Hiesey (125) found that a subarctic form of *Poa pratensis* required relatively warm nights for successful growth, while a form from the Sierra Nevada, in California, produced maximum yields where nights were relatively cold. Nutritive value reflects not only the amounts of digestible nutrients, vitamins, and minerals produced under favorable environmental conditions but also factors such as resistance to disease and insect attack, maturity, lodging, and management.

Distribution of yield through the growing season depends on environmental factors, the growth cycle of the species, its habit of growth, the location of reserve carbohydrates, and management. Management practices influencing the seasonal distribution of herbage yields include time of fertilizer application (26) and height and frequency of clipping (245, 261).

Persistence is an expression of such characters as winter-hardiness, growth habit, aggressiveness, resistance to disease and insect attack, and tolerance of drought, temperature extremes, and flooding.

Palatability is not necessarily associated with nutritive worth, but it has real significance in relation to selective grazing, ease of management, and intake of digestible nutrients. Optimum palatability represents the culmination of numerous plant and animal variables that are difficult to define in simple terms.

The factors associated with reproduction are relatively easy to establish. Abundance of seed and ease of vegetative propagation are influenced by such variables as fertility, resistance of seed to shattering, growth habit, resistance to disease and to insect attack, temperature, moisture, day length, and soil productivity.

The adaptability of a strain to diverse kinds of management depends on growth habit, mode of reproduction, aggressiveness, location of reserve carbohydrates, and tendency to lodge.

Specific objectives include yield and vigor, growth cycle, disease resistance, insect resistance, winter-hardiness, high-tempera-

ture tolerance, drought tolerance, tolerance of soil conditions, time of maturity, seed habit, ease of harvesting, root development, aggressiveness (including seedling vigor), growth habit, chemical composition, and animal acceptance.

The objectives sought by Burton (33) in breeding bermuda-grass (*Cynodon dactylon* (L.) Pers.) illustrate the manner in which major objectives can be defined in terms of specific characteristics. Burton's major objective was development of more productive strains capable of providing highly nutritious and palatable forage during a greater part of the growing season. Specific objectives were (1) resistance to pathogens (*Helminthosporium* spp.), drought, and frost; (2) ability to grow in association with legumes; (3) tall-growing types for hay production; and (4) either strains that would not produce seed or non-stoloniferous seed-producing strains.

### YIELD AND VIGOR

Results obtained in experiments with several of the perennial forage grasses show that the heritability of forage yield is low. In brome-grass, the correlation coefficients for the forage yields of seed progenies and their parental clones have been positive for the most part but the magnitude and variability of the coefficients indicate a low predictive value (116, 163, 188, 292). Weiss et al. (312) found no appreciable association between the forage yield of selected orchardgrass clones and that of their open-pollination progenies. Similar results were obtained when single-cross yields were compared with the mean yields of the parental clones. McDonald et al. (188) studied plant-to-plant variations in 40 brome-grass families and found significant differences in yield, spread, and height both among families and between inbred and open-pollination progenies. Heritability estimates obtained for yield and spread were low or negative, indicating that much of the total variability among plants in  $S_1$  progenies could be attributed to environmental variation. Kalton et al. (147) arrived at essentially the same conclusion after estimating the genetic variation in inbred orchardgrass progenies. Attention has been drawn to the ineffectiveness of selecting for yield on the basis of superior phenotypes in space-planted nurseries of Kentucky bluegrass (5) and timothy (315).

Some form of progeny testing is essential to isolating genotypes capable of transmitting a high yield potential. Testing of progenies in solid seedings has not played a significant role in the development of most varieties of forage grasses that are currently available. The difficulties encountered in increasing yield cannot be attributed solely to inadequate progeny testing. The techniques adhered to in evaluating progenies and in grouping selections for experimental strains may impose certain restrictions on the magnitude of the gains realized under farm conditions. In addition, the performance of a new strain varies widely as a result of differences in environmental conditions including soil fertility and management. In spite of the difficulties en-

countered in breeding for increased yield this objective is extremely important, if for no other reason than the necessity of maintaining a reasonably high yield in varieties bred for other desirable characters. Yield attains its true significance as an objective when sufficient emphasis is placed on yield of digestible nutrients as contrasted with yield of dry matter per acre.

### GROWTH CYCLE

The developmental cycles of most grass species are intimately associated with the conditions under which the grasses are grown, including day length, temperature, and moisture. In a given environment, early varieties may produce more aftermath than later maturing varieties. At northern latitudes, a large proportion of such a difference in aftermath production is associated with more favorable temperature and moisture conditions prevailing in the spring and early summer and the fact that varieties harvested early have more favorable conditions for aftermath growth. Within-species differences in aftermath yield exist that do not appear to reflect time of maturity but may be due to one or more factors such as heat tolerance, drought tolerance, and temperature. If time of maturity is an objective in a grass-breeding program, consideration should be given to the possible relation between this factor and aftermath yield.

Since most forage grass species are utilized in several different ways—as silage, hay, and pasture—the distribution of their yield has major proportions as an objective. Considerable progress can be realized by selecting for different growth patterns within species. It is highly desirable from the standpoint of efficiency of selection to accumulate information on the specific factors influencing the phenotypic expression of the growth-pattern characteristic. For example, aftermath yields sometimes can be altered through rate and time of fertilizer application and, particularly, through frequency and time of defoliation.

### DISEASE RESISTANCE

Forage grasses are attacked by a large number of pathogens. The importance of their diseases varies according to the grass species, the environment in which it is grown, and the manner in which it is utilized. Leaf diseases, including rusts (stem, leaf, stripe, and crown rust), are among the most widespread. Root and crown rots are serious in many areas. Head and leaf smuts damage grasses in some years. Seed disorders such as ergot, those caused by nematodes, and the blind seed disease sometimes result in appreciable financial loss to seed producers. Pre-emergence rots, on an average, destroy an estimated 25 percent of all forage grass seed planted (256).

The leaf diseases have a marked effect on quality and quantity of forage, and plants severely weakened by them are less likely to survive extreme conditions of heat, drought, or low temperature. Some species are susceptible to a number of leaf diseases



that result in weathered, poor-quality forage and premature leaf loss. For example, in the Northern States smooth brome grass is subject to leaf scald (caused by *Rhynchosporium secalis*), brown spot (caused by *Pyrenophora bromi*) leaf spot (caused by *Selenophoma bromigena*), brown stripe (caused by *Scolecotrichum graminis*), septoria leaf spot (caused by *Septoria bromi*), and the bacterial disease caused by *Pseudomonas coronafaciens* var. *atropurpurea* (169). Experimental evidence is lacking on the extent to which different amounts of foliar infection reduce forage quality; but observations substantiated by limited chemical analyses indicate that the losses may assume major proportions. Burton (40) has reported a decrease in percentage of protein and fat and a substantial increase in percentage of lignin in leaves of sudangrass (*Sorghum vulgare* var. *sudanense* (Piper) Hitchc.) affected with anthracnose, caused by *Colletotrichum graminicola*. Results of an investigation by Bird (22) suggest that rust can have a marked effect on both yield and vigor of individual plants and strains of timothy.

Screening of selections for disease resistance under controlled conditions has been limited by the lack of satisfactory inoculation techniques. Inoculation work has been most extensive in connection with selecting for rust resistance. The usual way of inoculating with rust is to transfer the spores to seedlings or tillers when new plantings are being established in the field, either by rubbing spores on the leaves or by injecting a spore suspension. In breeding nurseries, susceptible checks may be interplanted and the development of rust further encouraged by spraying selections with an aqueous suspension of rust spores. Natural field infection has been supplemented through these procedures in order to select for rust resistance in timothy, orchardgrass, and Italian ryegrass (*Lolium multiflorum* Lam.). The effectiveness of selecting for increased rust resistance has been demonstrated in timothy (12, 22, 77).

Management methods (fertilizer application, clipping and grazing practices, etc.) have an important effect on the development of many foliar diseases. Carter and Ahlgren (47) made comparisons between inoculated and uninoculated plots of brome grass, using the pathogen *Pseudomonas coronafaciens* var. *atropurpurea*. Their results indicated that when brome grass plants were dispersed among alfalfa plants the accumulation of inoculum and the rapidity of dissemination were less than in pure brome grass stands, especially at the hay stage. Comparisons made during periods favorable to disease development showed that forage harvested at the pasture stage was infected more severely than that harvested at the hay stage. Application of nitrogen was not observed to influence disease development. The effect of blighted leaves upon palatability was not determined. Dickson (68) has pointed out that foliar diseases of *Bromus inermis* tend to be more of a problem in nurseries and seed fields than in meadows.

Carter and Dickson (48) have studied the development of two foliar diseases of brome grass, brown leaf spot (caused by

*Pyrenophora bromi*) and bacterial blight (caused by *Pseudomonas coronafaciens* var. *atropurpurea*), in different environments. Plants were inoculated with blight bacteria grown in nutrient broth in shaker flasks or with a suspension of conidia and macerated mycelium of the fungus. Blight inoculation of plants that had some open stomata or had been subjected to a 24-hour pre-inoculation moist-chamber treatment, followed by 24 hours in the moist chamber, resulted in adequate and uniform infection. Wounding followed by blight inoculation resulted in lesions on both susceptible and resistant lines. Apparently resistance in the lines studied was not completely physiological. Infection with *Pyrenophora bromi* succeeded if humidity was high during the period necessary for spore germination and penetration. In comparing the reactions of resistant and susceptible lines at four temperatures, rating by lesion size and number gave results similar to those of rating by disease index. It was concluded that rating by disease index was more efficient. Observations made on space-planted clones in the field showed that development of these two diseases was not influenced appreciably by plant type, spacing, or nitrogen applications.

Kreitlow (168) reports that leaves of *Dactylis glomerata* attacked by the leaf spot fungus (*Stagonospora maculata*) were dried at relatively low temperature and stored at 0° to 5° C. for 9 to 18 months with no apparent lessening of viability or virulence of the fungus. After the leaves were taken out of storage, spore suspensions were prepared by macerating some of them in distilled water in a Waring blender. Orchardgrass seedlings sprayed with the spore suspension developed lesions typical for the disease. Epiphytotics of *Helminthosporium turcicum* in field plantings of sudangrass can be created by spraying plants with aqueous solutions of macerated agar cultures of the fungus.

Inheritance of disease resistance has been demonstrated in a number of experiments such as one made by Tsiang (292) in which the resistance of 1-year selfed brome grass progenies to the leaf spot pathogen *Selenophoma bromigena* was correlated to a highly significant degree with that of the parental clones.

Corkill (61) reported differences among ryegrass strains in susceptibility to blind seed disease (caused by *Phiala temulenta*). Breeding for escape, rather than immunity, is suggested as the best procedure for controlling this disease. Because a study made in Oregon (110) has shown that this disease can be controlled very effectively by agronomic methods (deep and early plowing, seed inspection, thorough harvesting, destruction of infested screenings, stubble burning), little attention is being devoted in the United States to breeding strains that will escape or resist it.

Burton et al. (43) found within a sample of 147 bermudagrass selections plants that were highly resistant and others that were highly susceptible to root knot nematode. Kobe lespedeza plants taken from the resistant bermudagrass plots were practically free of root knot, while those from susceptible bermudagrass

plots were heavily infected. These results suggested the possibility that Kobe lespedeza, although susceptible to root knot nematode, can be grown successfully in association with highly resistant bermudagrass on the sandy soils of the Georgia coastal plain.

In the absence of satisfactory inoculation procedures many investigators have relied almost exclusively on natural inoculations in the field, scattered infected material over space-planted nurseries, or planted highly susceptible check varieties. Selections obtained through controlled tests should be grown under field conditions to determine the effectiveness of the selection program. Under certain conditions the correlation between results of controlled greenhouse tests and field response may not be significant, and the possibility that greenhouse selections are lacking in certain agronomic characters should not be overlooked. Jarvis (131) stressed the development of disease-resistant varieties on a foundation of locally resistant strains. In efforts to produce varieties with wide regional adaptation, investigators should recognize the relative nature of the resistance of a plant to a pathogen and the fact that resistance may be largely local, resulting from a specific interrelationship of host, environment, and pathogen.

Breeding for disease resistance is frequently hindered by the absence of resistant material or the narrow range in plant reaction exhibited under severe epiphytotics. For example, little if any resistance to the common root rots has been found. Active introduction programs are useful in locating disease-resistant plants, and in the absence of an economic level of resistance some consideration should be given to incorporating resistance from related species. This approach has been used successfully in isolating ergot-resistant lines of dallisgrass (*Paspalum dilatatum* Poir.), a species that is extremely susceptible to ergot. Resistant and immune lines have been developed by crossing *P. malacophyllum* Trin. with *P. dilatatum* (21).

Disease resistance may well be the most important breeding objective in much of the humid eastern and southeastern United States.

### INSECT RESISTANCE

Susceptibility to insect attack sometimes restricts the usefulness of an otherwise desirable forage grass. Rhodesgrass scale is a limiting factor in the production of rhodesgrass (*Chloris gayana* Kunth) in southeastern Texas (234). Before 1940 rhodesgrass stands could be maintained for 6 to 10 years, but owing to scale infestation present stands cannot be expected to last more than 3 years. Damage by the spittlebug reduces forage yields in humid areas, especially in the Northeastern States, and insects such as the timothy mite lower seed yields of a number of the forage grasses grown in the Pacific Northwest. Infestations of thrips, chinch bugs, grasshoppers, white

grubs, and other insects sometimes cause considerable forage losses in certain areas.

Heinrichs (123) observed that some lines of intermediate wheatgrass (*Agropyron intermedium* (Host) Beauv.) were damaged by grasshoppers more than others, and that lines palatable to grasshoppers suffered most winter injury. Differences in amount of damage by thrips have been observed by Kneebone<sup>5</sup> among selected lines of blue grama. For the most part, however, very little information, observational or otherwise, is available on differences in insect tolerance within grass species.

Chemical dusts and sprays have been used successfully in controlling spittlebugs and the timothy mite. The parasite *Anagyrus antoninae* offers some promise in controlling rhodesgrass scale (234). In the presence of effective methods of control through management or use of insecticides, insect resistance has not been considered a major objective of breeding. If satisfactory control measures are lacking and damage reaches economic importance, it can be recommended that selections be screened for insect tolerance or resistance. The necessity for timeliness in applying chemicals and the cost of chemical control are other reasons for efforts to find insect-resistant strains.

#### WINTER-HARDINESS

The adaptation of grass species over a large part of the United States is closely associated with their ability to survive very low temperatures, hazards associated with alternate freezing and thawing, desiccation, and long duration of low temperatures. Increased winter-hardiness is frequently listed as an objective in breeding forage grass varieties adapted to the northern tier of States, and it may be equally important at southern locations where low temperatures of relatively short duration sometimes reduce the stand of potentially valuable species. Marked differences in winter-hardiness exist between and within species and varieties.

Field tests conducted by Schultz (241) showed that winter-hardy orchardgrass selections tended to produce winter-hardy inbred progenies. In artificial-freezing tests highly significant differences in regard to this characteristic were found among  $I_2$  clones even though all the parental plants were relatively winter-hardy in the field. The field hardiness of inbred clones was not correlated with their reaction to artificial freezing.

Myers and Chilton (209) found statistically significant differences in winter injury among 59 clones of orchardgrass and among 60 clones of timothy. The correlation coefficients of the mean winter injury of clones and that of their inbred progenies were 0.91 and 0.85, respectively, in orchardgrass and timothy. Segregation occurred within inbred progenies, suggesting that it is possible to select for resistance to winter injury not only between progenies but within them. Severity of stem rust was

<sup>5</sup> Unpublished data of W. R. Kneebone.

correlated with degree of winter injury in parental clones of timothy, but a similar correlation was not found in orchardgrass.

Rogler (237) studied the responses of geographical strains of grasses to low temperatures in North Dakota. He grew seedlings in flats under 14-hour days, hardened them for 7 days at 2° to 4° C., subjected them to freezing temperatures, allowed them to thaw for 48 hours at 2° to 4°, and returned them to the greenhouse. Warm-season grasses of northern origin tended to survive in greater proportion than those of southern origin. Among the cool-season grasses, *Agropyron smithii* Rydb. seedlings from southern locations were less resistant to low temperatures than those from northern locations. *A. cristatum* survived better than *A. smithii*, and *A. smithii* survived better than *Bromus inermis*. In the field, the average survival of the warm-season grasses tested varied inversely with the southerliness of their origin, but there was no injury to any of the cool-season grasses.

Wit (320) describes the use of freezing chambers, in Holland, in screening grasses and legumes for cold resistance. Seed is sown in September in open frames, which are covered only when snow or heavy frost is threatening. Seedlings are dug in winter, washed, tied in bundles of from 25 to 40, and cooled in a freezing chamber for 1 day at 0° C. and at -2°. Then the temperature is lowered gradually "to the point thought best adapted to the hardiness of the material (between -12° and -16°)." After some hours the temperature is gradually raised to 0°. The seedlings are permitted to thaw, then are planted in moist peat moss in the greenhouse. After 2 to 3 weeks, hardiness scores are assigned to the various entries. Results obtained by Wit from different trials were fairly comparable and were fairly consistent with his results from earlier field tests. Wit mentions the necessity of compromise in attaining desired levels of winter-hardiness and early spring growth in ryegrass, the cold resistance and spring vigor of which are negatively correlated.

Close fall grazing or clipping reduces the reserves in some of the tall-growing grasses and increases their susceptibility to winter injury. The possible significance of reserve carbohydrates and of disease should be considered in selecting for improved winter-hardiness.

Spaced plantings generally provide a more critical measure of winter-hardiness than do solid seedings, because spaced plants do not offer so much mutual protection as plants in solid seedings. The more plentiful soil moisture in spaced plantings, however, and the consequent greater plant vigor may tend to offset this lack.

Selection for increased winter-hardiness has proved to be profitable in a number of the economic grasses. Winter-hardy strains of timothy, orchardgrass, bermudagrass, and other species have been developed (112).<sup>6 7</sup> The inheritance of winter-

<sup>6</sup> Unpublished data of E. L. Nielsen.

<sup>7</sup> Unpublished data of G. A. Rogler.

hardiness is admittedly complex, but survivors selected after severe winters have generally been observed to transmit some degree of this characteristic to their progenies.

### HIGH-TEMPERATURE TOLERANCE

Wide differences exist among forage grass species in ability to tolerate high temperatures at various stages of growth. A limited number of observations indicate considerable variation in this regard within species, also. Certain recent introductions of *Bromus inermis*, *Agropyron cristatum*, and *Dactylis glomerata* exhibit superior heat tolerance in comparison with domestic strains—and in some cases superior drought tolerance, also.

Laude et al. (174) investigated the effect on grass emergence of high soil temperatures during the pre-emergence period. Of the species studied, *Phalaris tuberosa* L., *Festuca arundinacea*, and *Lolium perenne* were the most sensitive to high soil temperatures, *Bromus catharticus* Vahl and *Oryzopsis miliacea* (L.) Benth. & Hook. were intermediate in their reactions, and *Stipa cernua* Stebbins was the most tolerant. A reduction in emergence was associated with delay in emergence of seedlings surviving the heat treatment, and the seedlings that emerged tardily were also considerably shorter than corresponding controls. Both the delay and the height reduction would place such seedlings at a decided disadvantage in competing with seedlings of species not so affected. In further studies, Laude and Chaugule (173) investigated heat tolerance in the brome grasses according to stage of seedling development. Significant differences in heat tolerance were found among mountain brome (*Bromus marginatus* Nees), prairie brome (*B. catharticus*), and Harlan brome (*B. stamineus*), prairie brome exhibiting the highest degree of tolerance. Differential injury was obtained by subjecting seedlings of different ages to 130° F. and 30- to 35-percent relative humidity for 4¾ hours. Among unhardened plants subjected to uniform heat stress at stages from emergence to 70 days after planting, those tested 7 and 8 days after planting (immediately after emergence) demonstrated the greatest degree of heat tolerance. Low tolerance was demonstrated from about the thirteenth to the twenty-eighth day after planting. From that stage onward, heat tolerance gradually increased with age. Thus, severe heat damage to new stands is most likely to occur during a 2-week period beginning about 5 or 6 days after emergence. It was demonstrated that plants of the species studied could be hardened to heat by brief periodic exposures to high temperatures.

Brome grass clones studied by Atwood and McDonald (10) exhibited differences in recovery at high temperatures after removal of the first crop. The plants were grown at 70° F. until the first cut, then at 80° until the second and the third cut, and at 85° thereafter.

Temperature is an important selective agent, and undoubtedly natural selection for tolerance to high temperatures has had an

important part in the development of certain forage grass ecotypes. In some environments, screening grass selections for their ability to become established and produce aftermath under high temperatures should be a profitable undertaking.

### DROUGHT TOLERANCE

Field observations indicate that forage grass species and strains differ in their ability to withstand drought, both during and after establishment (277). A machine for producing atmospheric drought conditions was developed by Aamodt (3) and used by him in evaluating spring wheat varieties for tolerance of this type of drought (4). Schultz and Hayes (242) tested 14 species of grasses, 4 clovers, and alfalfa for reaction to drought in Shirley's drought machine (244). Plants were treated as 30- and 60-day-old seedlings and as sod material grown in 4-inch pots. The treatment was essentially one of atmospheric drought, in which the plants were exposed for periods of 10, 16, and 20 hours to a temperature of 43° C., a relative humidity of 17 percent, and an air velocity of 5 miles per hour. The 16-hour treatment was the most effective in differentiating among the species and strains tested. The injury resulting from treatment was found to be proportional to the drought injury suffered by these species and strains under field conditions. Several turf grasses subjected by Carroll (46) to atmospheric drought under controlled conditions as potted sod plugs exhibited differential survival.

Mueller and Weaver (192) studied the resistance of seedlings of 14 prairie grass species to soil and atmospheric drought under greenhouse conditions, effecting soil drought by withholding water from the plants and atmospheric drought by passing a current of heated air over them for varying lengths of time. The short, or upland, prairie grasses blue grama, hairy grama (*Bouteloua hirsuta* Lag.), and buffalograss seemed to have greater resistance than others to soil drought. Although the results from the atmospheric-drought tests were not so clear cut, the short prairie grasses appeared to be more resistant to hot dry wind than the tall prairie grasses.

McAlister (187) studied the resistance of grass seedlings to soil drought by growing plants in galvanized iron flats for 3 weeks to 2 months under optimum conditions and then holding them in a drought chamber for 6 to 9 days. Plants tested usually were from 9 to 18 inches in height and had from 2 to 6 tillers, depending on the species. After exposure to drought the plants were removed to a greenhouse, the soil was saturated with water, and the dry leaves and stems were clipped 1 inch above the soil. Survival was recorded after the plants had been kept under optimum conditions for 2 to 3 weeks. The drought chamber used in these experiments was designed to provide these environmental conditions: Temperature, 80° F.; light intensity, 175 foot-candles; relative humidity, 30 to 35 percent; and air velocity, one-half mile per hour. Although field survival

data were available for only a small number of the strains tested, the greenhouse survival data were considered to agree satisfactorily with them. McAlister emphasized the limitations in characterizing drought resistance of a species from observations on a single strain or seed source.

The differences exhibited by strains within species suggest the possibility of selecting individual plants that are outstandingly tolerant of both atmospheric and soil drought. Artificial screening alone may be inadequate, but if coupled with field tests it might facilitate the isolation of superior genotypes.

Efficiency of water utilization is important in some areas. Keller (155) found a wide variation in the relative water requirements of 16 genotypes of orchardgrass. In his greenhouse tests, genotypes high in herbage yield were generally low in water requirement, and vice versa.

### TOLERANCE OF SOIL CONDITIONS

Grass strains for hay and pasture production should usually be bred to give the maximum response on soils of medium to high fertility. On the other hand, where a species or new strains of a species will be used on marginal land, or where fertilizer application is not practical, it may be advisable to direct the breeding program toward isolating material that will grow well on soil of relatively low fertility. A high requirement for some specific element, e.g., phosphorus, may in time place an improved strain at a disadvantage. Harlan<sup>8</sup> has isolated selections of bermudagrass that are capable of giving better yields on soils of low fertility than the more aggressive, high-producing strains developed for fertile soils or soils adequately treated with commercial fertilizers. In any breeding program, the soil conditions under which strains are developed should be taken into account in evaluating their usefulness. The immediate and the potential value of an improved strain may be intimately associated with its efficiency in utilizing soil nutrients.

Many questions regarding the soil requirements of grass species and strains remain unanswered. Although strains that are superior when grown on highly fertile soil are not necessarily superior to commercial strains when compared on soil of low fertility, even there some of the characteristics that distinguish them on highly fertile soil (e.g., improved seedling vigor) may give them an initial advantage in establishment and yield.

Varietal differences in efficiency of nitrogen utilization and growth on soils of high fertility have been demonstrated in timothy and orchardgrass (225), bermudagrass (42), and smooth brome grass (87).

Tall wheatgrass (*Agropyron elongatum* (Host) Beauv.), Russian wildrye (*Elymus junceus* Fisch.), and some other forage grasses exhibit considerable tolerance of saline conditions, but information is lacking on the ranges of salt tolerance that may exist within species.

<sup>8</sup> Unpublished data of J. R. Harlan.



## TIME OF MATURITY

Differences in time of maturity within species are determined to a large degree by the reactions of plants to the combined effects of photoperiod and temperature. In general, northern ecotypes require a much greater day length in order to become reproductive than southern ecotypes of the same species. In general, also, northern ecotypes can start growing at temperatures too low for growth of some southern ecotypes. Certain races of *Poa ampla* Merr. and *P. compressa* will not flower when night temperatures go no lower than 17° C. but will flower freely when temperatures go as low as 6°, other factors remaining the same (125). It appears that the maturity of a species and of plants within the species is governed by a multiplicity of interactions involving genotype, photoperiod, and temperature, which depend on the combination of environmental conditions prevailing in the native habitat of the species or ecotype.

Evans et al. (80) examined the responses of 13 timothy strains grown in 3 localities at different north latitudes—Washington, D.C., 38° 54'; North Ridgeville, Ohio, 41° 23'; and Guelph, Ontario, 43° 33'. The earliest strain began to bloom at the southern station 24 days sooner than at the northernmost station. In selections progressively less early, the differences in time of heading and time of blooming between strains grown at the northernmost and the southern station, respectively, diminished. Medium selections bloomed at nearly the same time at the 3 locations, and for the 3 latest strains the progress of heading and blooming was from north to south rather than from south to north. The investigators concluded that day length at the southern station became sufficient for the beginning of head development before temperatures at the northern stations did so. In other experiments, Evans (78) found that at a northern station both early and late selections produced relatively high yields, but that at a southern station the late selections were lower yielding than the early ones.

Olmsted (228) found considerable interspecific and intra-specific variation in the photoperiodic response of seven native grasses. Sideoats grama (*Bouteloua curtipendula* (Michx.) Torr.) from the northern Great Plains was of a long-day type, while a Texas strain was of an intermediate or short-day type. Species cannot be classed as short-day, intermediate, or long-day on the basis of limited sampling.

Species exhibiting wide ranges in time of heading and time of flowering are amenable to the development of strains that will mature at different times. Observations and experimental data on varieties representative of many species suggest that the heritability of time of flowering is relatively high. In orchard-grass, Kalton et al. (147) reported significant correlation coefficients for maturities of parents and inbred progenies that ranged from 0.35 to 0.42. Hanson<sup>9</sup> found evidence that in

<sup>9</sup> Unpublished data of A. A. Hanson.

orchardgrass date of heading is controlled by a relatively small number of major genes. This indication is substantiated by the fact that grass breeders have experienced at least as much success in modifying time of maturity as in modifying any other character. Generally, the problem is one of incorporating other desirable characteristics into a strain bred for a specific time of flowering—for example, increasing the vegetative vigor, after-math production, or total yield of a late-flowering strain of timothy or orchardgrass. The nature of this problem has been outlined under the heading "Yield and Vigor."

### SEED HABIT

Because seeding is the cheapest method of establishing most forage grass species, fairly high seed yield should be a characteristic of new varieties of these species. For species that can be propagated vegetatively on a field scale, seed sterility or near sterility may be of little consequence or actually desirable.

In space-planted tests, seed yields of progenies often are not closely correlated with those of the parents (123).

Because high seed yields are not always compatible with high forage yields, a decision must sometimes be made on the extent to which seed yields can be sacrificed in favor of better forage yields. Limited observations on many species indicate, however, that the problem of "seed vs. forage" may have been overstressed. Selections have been isolated in *Bromus inermis*, *Dactylis glomerata*, and *Agropyron cristatum* that are outstanding with respect to both seed and forage production.

At northern locations the seed production of southern ecotypes may be inferior to that of local strains even though differences in forage yield are negligible (165).

The irregular meiotic behavior that characterizes *Bromus inermis* (70, 162) does not appear to have any appreciable effect on seed set of the principal strains. The poor seed set of certain selections can, however, be attributed to meiotic disturbances, some of which are not completely understood (219). Lamp (171) stated that while degree of fertility of brome grass is in part determined genetically, the position of individual tillers within the clump and the associated favorable environmental conditions affecting growth appear to have an important effect upon reproduction in brome grass clones tending to be sterile.

It is possible that in dallisgrass poor seed set and high incidence of ergot are associated with meiotic irregularities.

Factors limiting the usefulness of forage grass species to at least as great an extent as low seed yield include tendency of seed to shatter, difficulties in harvesting, dormancy, and other objectionable characters like persistent awns. Observations made on several species suggest that selections superior in resistance to shattering could be developed into varieties, but little progress has been made in this direction. Much of the variation in shattering in reed canarygrass (*Phalaris arundinacea* L.) and tall oatgrass (*Arrhenatherum elatius* (L.) Presl) can be attributed

to differences in maturity between selections or strains rather than to inherent differences in ability to hold seeds after maturity. In certain species, it appears, the only way to incorporate resistance to shattering would be to transfer genes from other species or genera.

Green stipagrass (*Stipa viridula* Trin.) plants the seed of which remain dormant for less than the typical period have been isolated by Rogler.<sup>10</sup> This work points to a possibility of reducing seed dormancy in other species.

Considerable progress has been made in developing forage grass strains the seed of which are easier to harvest. Thus, Harlan<sup>11</sup> and Newell<sup>12</sup> have been able to increase the ease of harvesting buffalograss burs by selecting plants with longer culms.

Improving seed quality in several native grasses, including the bluestems and gramas, that are characterized by low-purity, chaffy seed would be a significant contribution.

### LODGING

Tendency to lodge may be a major problem in grass seed production, particularly when grasses are grown in cultivated rows. Excessive lodging at the hay stage makes harvesting difficult and, by causing loss of leaves, reduces hay quality. Rogler<sup>13</sup> has demonstrated, in work with Russian wildrye, that selection for lodging resistance on a space-planted basis is feasible. Care must be exercised, however, to avoid sacrificing quality to prevent lodging.

Before undertaking selection for lodging resistance, the breeder should consider the possibility of utilizing later maturing strains that can be cut for hay under more favorable weather conditions and also the likelihood that grasses of otherwise desirable species tending to lodge can be given effective support by growing other grasses or legumes in mixture with them.

### ROOT DEVELOPMENT

Extensiveness of root development has an important bearing on the ability of perennial forage grasses to become established and to tolerate low-moisture conditions. The dry weight of the root system produced by such a grass may exceed that of the top (11).

Studies of several range grasses (232) have indicated a relation between total root development prior to summer drought and initial success or failure. Cook (60) has shown that the extent of the root systems produced by eight smooth brome grass strains during the seedling year was directly related to ability to resist drought. In the absence of severe drought or in humid areas, deeper rooted species generally are more productive and

<sup>10</sup> Unpublished data of G. A. Rogler.

<sup>11</sup> Unpublished data of J. R. Harlan.

<sup>12</sup> Unpublished data of L. C. Newell.

<sup>13</sup> Unpublished data of G. A. Rogler.

continue to grow when shallow-rooted species have exhausted the available moisture. The root system of a given species is not always indicative of the species' ability to survive drought; in arid and semiarid regions some shallow-rooted species, by virtue of dormancy, can survive long dry periods that may eliminate deeper rooted species.

Seedling root development as reflected by seedling vigor is discussed in the next section, under the heading "Aggressiveness."

Aside from their primary role of absorbing moisture and anchoring the plants, the roots and rhizomes of grasses frequently accumulate an important share of the carbohydrate reserves, which influence the rate and degree of recovery after defoliation. Characteristics of the root system determine to some extent the reactions of plants to competition from associated plants of other species and the usefulness of a species in erosion control. Tolerance of flooding and poor drainage, also, are related to root characteristics.

The neglect of the root system in forage grass breeding efforts can be explained by the tediousness of the excavation methods available for studying root development (30, 89, 308) and the difficulty of relating controlled tests of roots (made in greenhouse pots, tanks, etc.) to field conditions.

In studying root development of species and strains under controlled conditions, it should be remembered that root distribution may be more significant than quantity of roots produced.

Preliminary studies (42) suggest that radioactive tracers may be useful for studying root activity and distribution. One such study has demonstrated that the roots of bermudagrass varieties differ in total weight, in distribution, and in efficiency of absorption. It remains to be seen whether it is practicable and advisable to use this procedure for screening large numbers of individual plants.

In general, the nature of the root system of a grass is reflected in the development of the aboveground parts. Selecting for superior plant and progeny performance, especially if the testing is conducted over a wide area and continued for several years, should lead to the development of new strains each of which has the kind of root system that is best for conditions under which the strain will be utilized.

### AGGRESSIVENESS

Aggressiveness of a grass species or strain can logically be divided into two phases—(1) seedling vigor as related to ease of establishment and (2) capacity of the well-established plants to compete with an associated grass, with an associated legume, or with weeds.

Soil germination tests of grasses conducted in the field and greenhouse have shown wide differences among strains, seed sources, and individual plants as to rate of seedling establishment. The importance of these differences lies in the fact that vigorous seedlings are better adapted to survive under unfavor-

able environmental conditions. It has been suggested by Hawk and Welch (115) that seed quality, especially when combined with varietal adaptation, may be an important factor in the resistance of brome grass strains to the root rot fungus *Pythium graminicola*.

In general, it appears, the forage grass species having the heaviest seed tend to produce the most vigorous seedlings (199, 232). Emergence is closely associated with depth of planting (199, 240), and within species heavy seed has better emergence percentages than lighter seed, especially for the greater seeding depths (124). Rogler (240) found a high degree of correlation between weight of seed and emergence in crested wheatgrass planted at depths of 2, 2½, and 3 inches. In his experiment, two types of emergence were observed in 1½-inch and deeper plantings. In one type both the first internode and the coleoptile elongated, while in the other type elongation was confined to the coleoptile. No relation between this difference and total emergence has been established. Nordan crested wheatgrass, characterized by larger seed and improved seedling vigor, has been released in North Dakota (239).

On the basis of present information it would seem advisable to investigate the possibility of developing strains having greater seedling vigor within forage grass species that are deficient in this important characteristic. Information should be accumulated not only on variation in seedling vigor but also on possible relations to seedling vigor of such factors as presence of pathogens, low soil moisture, temperature extremes, and low light intensity. Information of this type for any given species would indicate what factors have the greatest limiting effect on establishment, and this would serve as a useful guide in selection.

Aggressiveness at mature stages of development depends on prevailing environmental conditions and the expression of such plant characteristics as time of blooming, height, rate of spread, and rate of recovery. Thus the aggressive tendencies of a species can sometimes be modified by management or by selection for any one of several characteristics. In selection work, first consideration is frequently given to rate of spread or vegetative increase. Knowles (163), studying brome grass clones and their open-pollination progenies, found a correlation of 0.87 for spread. Tsiang (292) reported a low correlation for this character between parental clones and their  $I_1$  progenies. MacDonald et al. (188) concluded that most of the variation in spread within inbred progenies was environmental.

It has been suggested that less vigor may be highly desirable in some of the more aggressive forage grass species, because it would mean greater compatibility with associated legumes. The fact that low yield, poor distribution of yield, and low seedling vigor are frequently associated with reduced aggressiveness makes this suggestion seem one of dubious value. Management factors—especially, time of fertilizer application, amount of fertilizer applied, and time of grazing or mowing—can be used to reduce the competition offered by a grass or an associated legume

and should be relied on to the greatest possible extent in solving the problem of "overaggressive" species (259). Interrelations among species may be complicated by other management factors such as irrigation (235).

If increasing the efficiency of production is recognized as one of the outstanding objectives in forage grass breeding, then the breeder cannot penalize himself by selecting for reduction in productivity. Truly outstanding varieties of the forage grasses, as of other farm crops, can and should be promoted in terms of management practices that will give the highest yield of total digestible nutrients per acre and produce this yield most economically.

In much of the Great Plains and of the western intermountain region, forage grasses are seeded alone or in combination with others except in irrigated pastures and meadows. Some work has been done toward developing nonspreading or weakly spreading varieties in certain species—e.g., intermediate wheatgrass—on the assumption that pure stands of such varieties would not become sodbound and decline in productivity so rapidly as those of more aggressive strains. Under most circumstances, however, aggressive strains are more valuable, by virtue of their greater persistence and ability to compete with weedy invaders.

### GROWTH HABIT

Practically every forage grass species has a wide range in growth habit. Strains may vary widely in degree of erectness, height, abundance of basal leaves, leafiness, number of tillers, size of rhizomes, and rate of spread. In general, selection for plant type has proved very effective (266). Travin (290) has explained the efficacy of type selection on the grounds that advantage is being taken of hereditary group variation, the basic heredity common to all plants of the same form. Stapledon (266) classified orchardgrass collections into a dozen main growth forms and, as a result of further studies, into the three main types hay, pasture, and dual-purpose. Jenkin (137) stated the opinion that persistence under severe grazing could be obtained by selecting the right type of plant from the right habitat.

Certain species by virtue of growth habit are very tolerant of frequent defoliation, trampling, and overuse. The characters distinguishing such species are generally abundant basal leaves, some form of vegetative propagation, and the capacity for rapid storage and buildup of reserve carbohydrates. In several characteristically erect species, such as orchardgrass, prostrate strains have been selected with the purpose of increasing adaptation to pasture use. Because most orchardgrass plants are characterized by considerable basal leaf development, however, selection of plants having different growth habits may not be especially profitable in this species. Tall, erect types or strains of orchardgrass persist and produce high yields in properly managed pastures, i.e., under rotation or ration grazing.

Information on growth habit should be accumulated at an early stage in the program. Clones representing a wide range in growth habit can be increased vegetatively and planted to simulate solid seedlings in replicated small-plot tests. Differential management treatments (different intensities of grazing or clipping and different fertilizer levels) assist in determining the relative merits of specific growth forms—their persistence, leafiness under use, etc. In general, the necessity of selection for different growth habits is determined by the species and the conditions under which new strains will be used.

### CHEMICAL COMPOSITION

It is well established that various forage grass species differ in nutritive value (37, 248, 281). Environmental and management variables exert a marked influence on forage quality and may accentuate or reduce the differences in this regard that separate species or genotypes (23, 45). In most species, individual plants exhibit a wide variation in morphological characters that may be correlated with variation in nutritive value. Such correlation has been substantiated for some species by chemical analyses, which have shown that plants selected under specified sampling conditions varied in chemical composition (23, 53, 231, 292). Literature on the chemical composition of pasture plants has been reviewed by Sullivan and Garber (281). These writers emphasize that precise comparisons among and within grasses are often complicated by the difficulty of sampling plants at comparable stages of growth.

The nutritive advantages of leaf vs. stem, namely, higher percentages of minerals, protein, and vitamins and lower percentages of fiber, have been brought out in several studies (23, 82, 83, 263, 266). Plants with short internodes have a larger proportion of leaves than plants with long internodes. In addition, some individual plants develop larger leaves at the tops of the culms than others. Genotypes have been selected that are capable of retaining their leaves for longer periods, and considerable variation has been observed as to the interval during which leaves remain green (81). Both these characters may reflect to some extent the susceptibility of plants to foliar pathogens.

Selection for broad leaves has been effective. Studies have indicated fairly high parent-progeny correlation for this characteristic in certain grasses (123, 147, 312). Observations on broad-leaved selections or lines of some species show, however, that selection for this character does not necessarily add substantially to the total leaf area or value of new strains. Yield was not found to be correlated with width of leaf in intermediate wheatgrass (123). If broader leaves are combined with short internodes, however, it should be possible to increase substantially the leaf-stem ratio.

Some forage grass investigators have developed lines and strains much leafier than commercial strains (175). Quantitative data demonstrating the effect of a slight increase in leafi-

ness on yield of nutrients are very meager, but observations and limited analyses suggest that the gain in quality may be appreciable (40).

While parent-progeny correlations for leafiness are low and inconsistent (292, 312), leafiness appears to be conditioned in part by genetic factors (147). There is a possibility that leafiness may be increased through selection, and that progress in the increase may be enhanced by practicing selection for characters such as disease resistance, maturity, growth cycle, and growth habit. Late plants, for example, tend to be somewhat leafier than early plants in *Andropogon scoparius* Michx. (6). Sampling progeny tests and varietal trials to obtain quantitative data on leaf-stem ratios can be recommended.

Variations in chemical composition between and within species do not necessarily reflect differences in feeding value (248), because of the many variables influencing digestibility and animal acceptance. Chemical analyses do, however, provide an approach to establishing nutritional differences among genotypes. They may be valuable, also, in determining chemical constituents that are limiting the growth or value of a species.

The variability of crude protein and carotene in smooth bromegrass was investigated by Pickett (231) in an experiment involving spaced plants of 175 lines from 25 unrelated first-generation inbred families. Top-growth characteristics were studied during the second and third years after transplanting, principally at the "early pasture" stage. Carotene content at the early-pasture stage varied significantly among plants in 6 of 17 families analyzed in the third year. It was positively correlated with color and protein content ( $r=0.29$  and  $r=0.49$ , respectively) at the same stage. Highly significant differences in carotene and protein content were found among families. Protein content was related more closely to degree of green color at the early stage and at anthesis than to any other character studied. It was concluded that morphological characters could not be used as indicators of high protein content. Direct testing for protein content during early stages of growth in advanced trials of bromegrass lines was proposed. Heinrichs (123) found the correlation between protein content and morphological characters in intermediate wheatgrass too low to be of predictive value.

Tsiang (292) provided evidence of heritable variation in the carotene content of bromegrass selections. He found a significant positive correlation ( $r=0.76$ ) between the beta-carotene content of parental clones and that of their 1-year selfed progenies. Clarke (54) obtained evidence of heritable variation in the carotene and crude protein content of orchardgrass. He found significant differences in content of carotene and crude protein for both parental selections and the  $F_2$  clones derived from them. Clarke concluded that the significant interaction of genotype  $\times$  management stressed the need for accurately defining sampling period when making comparisons among plant selections. He considered the vegetative stage (6- to 8-inch height



of spring growth) most satisfactory for characterizing plants as to relative content of carotene and of crude protein. Johnson and Miller (142), studying selections of Fairway crested wheatgrass and Parkland brome grass, found highly significant differences in percentages of total carotenoid pigments, beta carotene, and chlorophyll. Sullivan and Garber (280) found that in Kentucky bluegrass late flowering plants, as compared with individuals flowering a few days earlier, had a greater nitrogen content (1) during the flowering period, (2) several months later in the aftermath stage, and (3) when clones were grown in the greenhouse.

In general, actively growing forage grasses have more than enough protein and carotene to satisfy the dietary needs of grazing livestock; but this is not always true for many grasses native to the Tropics or for grasses grown on infertile soils. Specific situations may justify efforts to develop strains higher in protein and carotene content—for example, varieties that could be utilized more efficiently in the production of dehydrated leaf meal.

Considerable evidence has been accumulated on the nutritional importance of carbohydrates and lignin as forage plant constituents. Tsiang (292) studied 36 clones of smooth brome grass with reference to content of calcium, copper, iron, manganese, magnesium, and potassium and found highly significant differences in content of magnesium and of potassium. The proportions of these two minerals, respectively, varied from 263 to 758 p. p. m. and from 164 to 1,712 p. p. m. Sullivan and Myers (282) studied the chemical composition of tetraploid and diploid plants of *Lolium perenne* derived from the same mixoploid parent. The tetraploid plants were higher in reducing sugars, total sugars, sucrose, and soluble dry matter and were essentially similar to the diploids in content of soluble and insoluble nitrogen. Additional comparisons of diploid and tetraploid clones of *L. perenne* indicated that greater percentages of moisture and soluble constituents were associated with the higher chromosome number (279).

More information is needed on the variation in chemical composition among and within grasses grown under different environmental conditions. Conceivably the carbohydrate fraction or some other constituent may prove to be far more important in the development of improved strains than protein or carotene.

Smith (248) points out that although better quality may be a promising breeding objective there is danger that one desirable component may be increased at the expense of another.

#### ACCEPTANCE BY LIVESTOCK

For maximum economical production of livestock products through the use of grass, not only must the herbage have high nutritive value but it must be readily acceptable to the stock. Acceptance of a grass by livestock depends on the class of livestock, the palatability of the grass, its accessibility, and the

proportion of it in the forage mixture. According to Stoddart and Smith (278) the term "palatability" has been applied very widely in the literature to the percentage of the plant consumed under proper grazing, rather than to the avidity with which an animal ate the plant. These writers suggest that the term "palatability" be discarded and the term "preference" applied instead to the taste an animal displays for any plant.

Studies of factors affecting palatability have been made and reported by several investigators. Tribe (291) has emphasized that palatability of forage cannot be determined apart from observation of acceptance by livestock. White et al. (316) found that, in general, applications of nitrate of soda and lime improved the palatability of seven grasses tested at Ithaca, N. Y. Bender (19) and Beaumont et al. (16) also found that applications of nitrogen increased palatability. Beaumont and his associates attempted to determine the relation of toughness (breaking strength) of leaves to palatability. In their experiment one of the least palatable grasses, reed canarygrass, was found to have the least tough leaves. They stressed the relationship between palatability and stage of growth. From experiments to determine the palatability of grasses to sheep, Davies (65) concluded that succulence was the chief factor involved and, hence, that palatability was largely dependent on time of year and intensity of grazing. He found that leaves were preferred to stems and that palatability was lower in species characterized by harsh or hairy foliage. Thatcher et al. (285) reported that of 3 sudangrass varieties planted in strips in a 2-acre pasture, the common variety was the least palatable. This was attributed to prevalence of *Helminthosporium* leaf blight and anthracnose among the plants of the common variety. The preference of cows for certain grasses as determined by Archibald et al. (7) was in order of vitamin A content and, with one exception, in order of succulence. In general, high preference was associated with low fiber content and relatively high percentages of ether extract, soluble ash, and magnesium. Little correlation was found between the palatability of grasses and their nitrogen content.

In grazing studies conducted in the northern Great Plains, Rogler (238) found evidence that would suggest that taste is very important in determining palatability. Within species, he found, palatability generally declines as maturity advances; under some circumstances, however, steers may prefer grass of coarse-appearing species near maturity to other grass that is young and succulent. Factors related to palatability are listed by Rogler as follows: (1) Maturity of forage—new growth generally more palatable than old mature forage; (2) intensity of grazing—heavy intensities eventually lead to a situation in which previously ignored species are eaten readily; (3) rate of recovery—species recovering rapidly after defoliation are preferred; (4) proportion in mixture with associated species—a species that is poorly utilized if it constitutes a high percentage of the mixture may be utilized very fully if it constitutes a small percentage of a stand in which more palatable species predom-

inate; (5) drought resistance—under drought conditions, species that remain green are preferred; (6) previous feed or grazing activity—grasses previously eaten may be preferred to unfamiliar grasses, but animals that have been on a one-grass diet may prefer a change rather than continuance of the same grass; (7) individual differences in animals—e.g., certain steers readily eat mature crested wheatgrass and make good gains on it, but other steers consume very little of such forage and do not gain on it; (8) fertilizer—e.g., where soil nitrogen is deficient, grass on nitrogen-treated plots is generally more acceptable than that on plots not so treated; (9) kind of stock—cattle, horses, sheep, and goats have different preferences; (10) local conditions—environmental factors such as soil fertility, moisture, and available sunlight.

Differences in palatability have been observed within species. According to Buckner (28) dairy heifers exhibit consistent preferences for certain selections of tall fescue. Significant differences in palatability have been observed among individual plants of Kentucky bluegrass (299)—a species in which palatability is not generally recognized as a problem.

Before an investigator begins breeding work with any forage grass, he should learn something about the acceptance of that grass by livestock. If information based on field observations is lacking or limited, acceptance tests are in order. A strain and species test established in solid-seeded plots, replicated 4 to 6 times, can be fenced and grazed 3 or 4 times during the growing season. Each grazing should be limited to a few hours, enough livestock being used so that all species and strains are subjected to some grazing and the most palatable ones are completely utilized. After each grazing, the plots should be clipped and fertilizer applied. The amount of herbage consumed can be determined by removing samples from each plot before and after grazing. Data from these samples can be supplemented with observations on the order in which the species and strains are grazed. Less precise information can be obtained by seeding strips of various grass species and strains in well-managed pastures. The influence of slight differences in growth rate and maturity may be magnified in cafeteria trials, in which animals have access to several species and strains of grass, and may or may not be of practical significance when animals are confined to one species.

The relationship of palatability to nutritive value has not been fully established. Animals are capable of making good gains on some relatively unpalatable forage species, e.g., weeping lovegrass (*Eragrostis curvula* (Schrud.) Nees). The possibility should not be overlooked, also, that gains in palatability may be obtained at the expense of some other valuable character such as persistence. The plant breeder must concern himself with palatability, however, insofar as it complicates pasture management problems and influences the consumption of herbage and the yield of animal products. If an otherwise valuable species is not accepted very well, the feasibility of using animals in screening selections and in evaluating experimental strains should be in-

vestigated. Similarly if the growth habit, or type, of a new strain deviates widely from that of the original species, observations should be obtained in the testing program not only on animal acceptance but also on the influence of grazing on persistence.

## BREEDING PROCEDURES

The method of breeding used in improving a forage grass species depends in part on the species' mode of reproduction (table 2). Numerous summaries and outlines of procedures used or suggested for use in grass improvement have been published (33, 44, 77, 121, 134, 189, 190, 212, 266, 276, 321). A complete review of available literature would entail much duplication; therefore the discussion here has been limited to a selected group of references.

TABLE 2.—*Normal modes of pollination and somatic chromosome numbers of some important forage grasses*

Scientific name	Common name	Mode of pollination <sup>1</sup>	Chromosome number
<i>Agropyron</i>			
<i>cristatum</i> (L.) Gaertn. ....	Fairway wheatgrass.....	1	14
<i>dasystachyum</i> (Hook.) Scribn. ....	Thickspike wheatgrass.....	1	28
<i>desertorum</i> (Fisch.) Schult. ....	Crested wheatgrass.....	1	28
<i>elongatum</i> (Host) Beauv. ....	Tall wheatgrass.....	1	14, 56, 70
<i>inermis</i> (Scribn. & Smith) Rydb. ....	Beardless wheatgrass.....	1	28
<i>intermedium</i> (Host) Beauv. ....	Intermediate wheatgrass.....	1	28, 42
<i>repens</i> (L.) Beauv. ....	Quackgrass.....	1	28, 42
<i>sibiricum</i> (Willd.) Beauv. ....	Siberian wheatgrass.....	1	28
<i>smithii</i> Rydb. ....	Western wheatgrass.....	1	42, 56
<i>spicatum</i> (Pursh) Scribn. & Smith. ....	Bluebunch wheatgrass.....	1	14, 28
<i>trachycarum</i> (Link) Malte. ....	Slender wheatgrass.....	2	28
<i>trichophorum</i> (Link) Richt. ....	Stiffhair wheatgrass.....	1	42
<i>Agrostis</i>			
<i>alba</i> L. ....	Red top.....	1	28, 42
<i>palustris</i> Huds. ....	Creeping bentgrass.....	1	28, 56
<i>tenuis</i> Sibth. ....	Colonial bentgrass.....	1	28
<i>Alopecurus pratensis</i> L. ....	Meadow foxtail.....	1	28
<i>Andropogon</i>			
<i>caucasicus</i> Trin. ....	Caucasian bluestem.....	3	40
<i>gerardi</i> Vitman. ....	Big bluestem.....	1	40, 60, 70
<i>hathi</i> Hack. ....	Sand bluestem.....	1	60, 70, 100
<i>ischaemum</i> L. ....	Yellow bluestem.....	3	40, 50, 60 (38 to 47)
<i>nodosus</i> (Willem.) Nash. ....	Angletongrass.....	1	40, 60
<i>scoparius</i> Michx. ....	Little bluestem.....	1	40, 56
<i>Arrhenatherum</i>			
<i>elatus</i> (L.) Presl. ....	Tall oatgrass.....	1	28
<i>Bouteloua</i>			
<i>curtipendula</i> (Michx.) Torr. ....	Sideoats grama.....	1, 3	28 to 101
<i>eriopoda</i> (Torr.) Torr. ....	Black grama.....	1	21, 28
<i>gracilis</i> (H. B. K.) Lag. ....	Blue grama.....	1	20, 40, 42, 60 (21 to 84)

See footnotes at end of table.

TABLE 2.—Normal modes of pollination and somatic chromosome numbers of some important forage grasses—Continued

Scientific name	Common name	Mode of pollination <sup>1</sup>	Chromosome number
<i>Bromus</i>			
<i>arvensis</i> L.	Field brome grass	2	14
<i>carinatus</i> Hook. & Arn.	Mountain brome grass	2	56
<i>catharticus</i> Vahl.	Rescue grass	2	28, 42
<i>erectus</i> Huds.	Meadow brome grass	1	42, 56, 70
<i>inermis</i> Leyss.	Smooth brome grass	1	28, 42, 56, 70
<i>Bucklœ</i>			
<i>dactyloides</i> (Nutt.) Engelm.	Buffalograss	4	56, 60
<i>Chloris gayana</i> Kunth	Rhodes grass	1	20, 40
<i>Cynodon dactylon</i> (L.) Pers.	Bermudagrass	1	30, 36, 40
<i>Dactylis glomerata</i> L.	Orchard grass	1	28
<i>Digitaria decumbens</i> Stent.	Pangolagrass	5	30
<i>Elymus</i>			
<i>canadensis</i> L.	Canada wildrye	2	28, 42
<i>condensatus</i> Presl.	Giant wildrye	1	28, 56
<i>giganteus</i> Vahl.	Siberian wildrye	1	28
<i>glauca</i> Buckl.	Blue wildrye	2	28
<i>juncus</i> Fisch.	Russian wildrye	1	14
<i>Eragrostis</i>			
<i>chlorometas</i> Steud.	Boer lovegrass	2	
<i>curvula</i> (Schrud.) Nees	Weeping lovegrass	2	40
<i>trichodes</i> (Nutt.) Wood	Sand lovegrass	2	
<i>Festuca</i>			
<i>arundinacea</i> Schrob.	Tall fescue	1	42
<i>elatior</i> L.	Meadow fescue	1	14, 28, 42, 70
<i>ovina</i> L.	Sheep fescue	1	14, 42, 56, 70
<i>rubra</i> L.	Red fescue	1	14, 42, 56, 70
<i>Hordeum bulbosum</i> L.	Bulbous barley	1	28
<i>Lolium</i>			
<i>multiflorum</i> Lam.	Italian ryegrass	1	14
<i>perenne</i> L.	Perennial ryegrass	1	14
<i>Oryzopsis</i>			
<i>hymenoides</i> (Roem. & Schult.) Ricker	Indian ricegrass	1	48
<i>miliacea</i> (L.) Benth. & Hook.	Smilgrass	1	24
<i>Panicum</i>			
<i>antidotale</i> Retz.	Blue panicgrass	—	18
<i>maximum</i> Jacq.	Guineagrass	3	32, 48
<i>miliaceum</i> L.	Proso	2	36, 40, 42, 72
<i>obtusum</i> H. B. K.	Vine-mesquite	—	20, 36, 40
<i>purpurascens</i> Raddi	Paragrass	3	36
<i>virgatum</i> L.	Switchgrass	1	18, 36, 54, 72 70, 108
<i>Paspalum</i>			
<i>dilatatum</i> Poir.	Dallisgrass	3	40, 50
<i>notatum</i> Flügge	Bahiagrass	1, 3	20, 40
<i>urvillei</i> Steud.	Vaseygrass	1, 3	40, 60
<i>Pennisetum</i>			
<i>ciliare</i> (L.) Link.	Buffelgrass	3	26, 32, 40, 54
<i>glauca</i> (L.) R. Br.	Pearl millet	1	14
<i>purpureum</i> Schumach.	Napiagrass	1	28
<i>Phalaris</i>			
<i>arundinacea</i> L.	Reed canarygrass	1	14, 28
<i>tuberosa</i> L.	Hardinggrass	1	28
<i>Phleum pratense</i> L.	Timothy	1	14, 42

See footnotes at end of table.

TABLE 2.—Normal modes of pollination and somatic chromosome numbers of some important forage grasses—Continued

Scientific name	Common name	Mode of pollination <sup>1</sup>	Chromosome number
<i>Poa</i>			
<i>ampla</i> Merr.	Big bluegrass	3	62, 64
<i>arachnifera</i> Torr.	Texas bluegrass	4	42
<i>compressa</i> L.	Canada bluegrass	1, 3	35, 42, 49, 56
<i>pratensis</i> L.	Kentucky bluegrass	1, 3	28, 56, 70 (18 to 91)
<i>secunda</i> Presl	Sandberg bluegrass	—	82, ca. 84, 86
<i>trivialis</i> L.	Roughstalk bluegrass	1	14
<i>Sorghastrum nutans</i> (L.) Nash	Indiangrass	1	40
<i>Sorghum</i>			
<i>halapense</i> (L.) Pers.	Johnsongrass	1	40
<i>vulgare</i> var. <i>sudanense</i> (Piper) Hitchc.	Sudangrass	1, 2	20
<i>Sporobolus</i>			
<i>airoides</i> (Torr.) Torr.	Alkali saculou	—	126
<i>cryptandrus</i> (Torr.) A. Gray	Sand dropseed	—	18, 36
<i>Stipa</i>			
<i>comata</i> Trin. & Rupr.	Needle-and-thread	2	46 (42 to 46)
<i>viridula</i> Trin.	Green needlegrass	2	82

<sup>1</sup> 1=largely cross-pollinated; 2=cross-pollination usually less than 5 percent, but in some species greater than that under certain conditions; 3=largely apomictic; 4=largely dioecious; 5=sterile.

Breeding methods for forage grasses can be grouped into three main categories:

1. Methods designed for normally self-fertilized species. With these plants, as with wheat and most other small grains, the most practical breeding procedures are controlled hybridization, backcrossing, and selection. Varieties are developed by increasing superior individual plants selected from advanced inbred generations, commonly the  $F_5$ ,  $F_6$ , and  $F_7$ .

2. Methods designed for improving naturally cross-fertilized species that are normally propagated from seed. These methods include individual plant selection, inbreeding, hybridization, and recurrent selection. Varieties are developed by crossing, compositing, or interplanting two or more strains or clones. Breeding procedures for species of this group have been the object of several recent studies (88, 128, 313, 319).

3. Methods designed for species that are normally apomictic or that are male- and female-sterile. These methods, essentially the same as those applied to the normally self-fertilized species, are hybridization followed by individual plant selection where some degree of sexuality exists and, in the absence of sexuality, individual plant selection within wide collections followed by comparison of clonal lines. (This procedure can be applied wherever it is economical to multiply a strain vegetatively, regardless of the strain's seed-setting capabilities.)

Methods of selecting and evaluating individual plants and

evaluating experimental strains are identical for the three categories of breeding methods listed above. Problems encountered in progeny evaluation are similar for categories 1 and 2.

### SELECTION OF SPECIES

Comparative tests should be conducted to select species that may be of value at a particular location. Each species included in the tests should be represented by several strains or seed sources. Preliminary information on persistence, winter-hardiness, disease susceptibility, leafiness, and other agronomic characters can be obtained from observational plantings, established as either replicated drilled rows or solid-seeded plots. In making observational tests careful attention should be given to soil fertility and management. The success of a grass improvement project may well depend on the thoroughness of the preliminary evaluation at the species level. Burton (37) suggests that tests of this type can be greatly improved by conducting them at two or more levels of soil fertility and under two or more systems of management (e.g., hay and pasture management as simulated by clipping). Dallisgrass, which is a valuable species in Georgia, dies out if left unclipped, evidently from the combined effect of foliage diseases and the heading process, which weakens the plant. Conversely, vaseygrass makes an excellent showing if left unmowed but soon disappears under real or simulated close grazing.

Field observations on the needs of the area in question plus experimental comparisons should enable the investigator to make a sound choice of species to receive major emphasis in the breeding program. In selecting species for further improvement the following questions should be answered: What species are best adapted to meeting specific local hazards or specific local needs such as good distribution of herbage yields, ease of establishment, winter survival, or drought resistance? (Emphasis on high total dry-matter yields at this stage in the program may result in elimination of species having other desirable characteristics that are of the utmost importance in meeting the needs of the area.) Which of the species best adapted to the area would be most amenable to modification and, hence, most promising for development of new and improved varieties?

Preliminary tests will help to provide an answer to the first question; but more intensive studies are required before any conclusion can be reached with respect to the second. An exhaustive collection of foreign and domestic strains and ecotypes should be assembled in order to determine the range of variation within any species considered. If sufficient seed is available, these strains and ecotypes can profitably be established in plots to obtain quantitative data on their desirability. If a companion legume is commonly grown with the grass, a legume should be included in preliminary plot tests in order to evaluate the competitive ability of plants from the various sources. At the conclusion of or during the preliminary testing stage, source nurseries of one or more species are established.

## SOURCE NURSERIES

A wide gene base is essential in source nurseries. Usually sources of material include domestic collections of seed and clones (from waste places and old seedings), foreign introductions, named varieties, and experimental strains. The contribution of material collected in waste places and fields is frequently hard to assess, but it seems evident that plants should be collected under conditions that at least approximate the environment in which improved strains will be used. Extensive local collections are of the utmost importance for developing strains at locations outside or on the fringe of a species' area of greatest forage adaptation. One or several factors such as disease or temperature may be limiting persistence and forage production, and investigators should make every effort to capitalize on any beneficial effects of natural selection (156).

Smith and Nielsen (251) compared clones of *Poa pratensis* from good and poor pastures. In their study approximately 500 plants from each pasture were grown in space-planted nurseries. The clonal isolations were made from approximately 50 sod samples from each of 10 pastures. Vigorous types occurred in clonal progenies derived from poor pastures but were somewhat more frequent among progenies from good pastures. Intensity of grazing appeared to exert little influence on the relative proportion of high yielding biotypes. Significant differences occurred among the lots from individual pastures in the proportion of plants resistant to powdery mildew and leaf rust, but these differences did not appear to be closely associated with differences in soil type, available moisture, or management. The clonal isolations suggested rather clearly that though weak biotypes may contribute little to the forage production of a given area, they are nevertheless maintained among the components of the sward.

Plant material from various collections is space planted to obtain observations on variability among and within sources. Seedlings are established in flats and transplanted to the field in the spring or fall at intervals of about 2 to 4 feet within rows about 2 to 4 feet apart. Spacings of 2 feet or less are satisfactory for some bunchgrasses. Species that spread very rapidly, e.g., bermudagrass, should be planted on 8- or 10-foot centers. Absolute limits cannot be set as to population size, but for most grasses the initial nursery should include at least about 5,000 individuals and could well include 10,000 or more, according to the diversity of the collections. Management should be in accordance with the objectives of the program.

Selection of desirable plants of nonaggressive species may be aided by overseeding the source nursery with a legume. Species may be screened as to relative palatability by making the nursery accessible to livestock and recording their preferences. If legume compatibility and animal acceptance are major objectives, these two measures should be taken at all stages of the program. A "controlled competition" technique suggested by Keller (152) may be useful for screening individual plants in source nurseries or for evaluating space-planted progenies. This



technique consists in interplanting spaced plants of various grasses and legumes in such an arrangement that each plant is subject to approximately the same amount of competition from other selections of the same species and from each associated species. This procedure might permit breeding simultaneously all the species included in the planting. Controlled competition could be obtained also by drilling a single row of a legume between each pair of space-planted grass rows, thereby enclosing each grass selection within a square of the associated legume.

Selection should be directed toward isolating groups, or classes, of fairly comparable plants. The number and size of such groups will reflect the number and nature of the breeding objectives. Individuals that appear outstanding with regard to any characteristic sought should be retained. The heritability of the specific character, if known, should govern the size of the selected sample.

### CLONAL EVALUATION

The best phenotypes selected from source nurseries are subjected to varying degrees of clonal evaluation, either in conjunction with the production of seed for progeny tests or in special clonal tests. Selections are generally increased vegetatively and established (1) as space-planted clones—1 to 10 or more space-planted propagules per replicate; (2) as tiller plots or beds—plants divided into tillers, and tillers planted on narrow centers to simulate solid seedlings; or (3) as tiller rows—tillers planted rather densely in a single row. The first of these methods is relatively inexpensive and provides an opportunity to observe selections in several different habitats. The second permits study of the plant under conditions approximating those of ordinary sward. Tiller rows can be replicated with less expense than a tiller plot. The use of tiller rows and plots is restricted by the expense of vegetative multiplication.

The value of clonal tests depends on the degree of correlation between the responses of parental clones and those of their progenies. Fairly high correlations have been obtained between individual plant selections and their progenies with regard to maturity, leaf width, disease resistance, and habit of growth. Parental characters such as leafiness, seed yield, height, vigor, yield, and recovery of individual plants may not be strongly correlated with progeny performance. As mentioned previously, however, parent-progeny associations are relative, and characters such as height may exhibit 50-percent heritability in replicated nurseries (138). It is well established that the phenotypic characters of selected plants can be measured more accurately in replicated clonal nurseries than on individual plants in a source nursery. Likewise, overseeding clonal nurseries with a legume or grazing livestock in such nurseries provides more precise information than the same procedure applied to source nurseries. Calder (44) used clonal rows to compare orchardgrass selections under sheep grazing. Clonal evaluation is not a substitute for adequate progeny testing, but it does provide for further selec-

tion and thus reduce the size and expense of subsequent progeny tests.

Adequate clonal testing is the only procedure required for species that can be propagated vegetatively on a commercial scale. Burton (33) has outlined procedures that might be used in such a breeding program in his discussion of the development of the Coastal variety of bermudagrass. The Tift and common varieties of bermudagrass and two tall growing South African strains of this species were interplanted, and 5,000 seedlings were grown from the open-pollinated seed. The following year records were obtained on vigor, spread, stem length, leaf length, heading date, and resistance to *Helminthosporium*, and observations were made on head abundance and percentage of florets setting seed. Spring growth was recorded in the second year. From the 5,000 seedlings 128 were selected, and these were planted in 4-inch clay pots in triplicate and subsequently clipped at 3-week intervals. Sprigs from the potted plants and additional selections made in the field were planted in the centers of 4-by-24-foot plots established in triplicate. Cultivated alleys were maintained between plots, and the plots were fertilized each year from 1939 to 1946. In November of 1941 and 1942 crimson clover was seeded over the plots at the rate of 30 pounds per acre, and in February of 1944, 1945, and 1946 Kobe lespedeza was seeded at the rate of 40 pounds per acre on the same half of each plot. A total of 50 observations were made in the interval between 1939 and 1946 on the vegetatively established plots. Visual observations were recorded on rate of spread, sod density, head abundance, frost resistance, disease resistance, vigor, color, percentage of weeds, and percentage cover. Hay yields were recorded, and the plots were sampled for seed production. Separations were made on fresh samples to determine the percentage of clover, and data were obtained on the lespedeza-grass mixtures and on nematode resistance.

In March 1941 the five best bermudagrass selections were planted in duplicate 1/10-acre plots at 3-by-3- and 6-by-6-foot spacings. Five fertilizer treatments were applied at random on each plot. In July 1941, nine selections were planted in duplicate 30-by-60-foot plots in a pasture. These plots were subsequently grazed, and observations were made on palatability and the amount of forage removed from each plot. Palatability ratings were based on the distribution of 20 or more animals on the plots at 5-minute intervals. In June 1943 the nine selections were planted in 6-by-18-foot plots, in quadruplicate, and the next year these plots were clipped at frequent intervals to simulate close grazing.

Evidence appeared that yield ratings made the first year on spaced seedlings would be of some value in eliminating low yielding individuals. Morphological characteristics considered to be important factors in palatability did not provide a reliable guide to palatability as measured by grazing. The finest stemmed clone was very unpalatable, and the coarsest selection included in the comparisons, the Coastal, was highly palatable. Burton

suggests that hybridization of material from diverse sources may well precede selection when the end product sought in breeding is a single superior plant that may be propagated vegetatively. Under this procedure, thousands of hybrids should be produced and screened in order to isolate the best possible phenotype.

The methods used in breeding apomictic species differ from those outlined for vegetatively increased species principally in that promising selections are progeny tested to establish the frequency of aberrant plants. Smith and Nielsen (250) compared progenies from enclosed and open-pollinated panicles of *Poa pratensis* and found that bagging had some effect on seed formation but none on the frequency of aberrants. Classification errors are not unusual, and Myers (206) has suggested using second-generation progeny tests to verify the classification of individual plants as normal or aberrant. After this has been done highly apomictic selections are evaluated in plots established either from seed or from tillers.

Apomixis presents certain breeding advantages and definite limitations that should be recognized by the breeder. Plants exhibiting a high degree of apomixis need not be isolated in breeding nurseries. Vigorous heterozygous genotypes can be maintained without significant change as seed is increased from breeder to certified. The possibility of isolating a genetic mechanism conditioning apomictic reproduction in otherwise desirable sterile interspecific hybrids should not be overlooked.

Apomixis, in general, restricts the recombination of characters. Fortunately, many apomictic species exhibit some degree of sexuality. The direction in which facultative apomicts are crossed, whether within or between species, may be extremely important. For example, selection of species A may have many of the desirable characteristics but lack one of them while selection of species B is generally undesirable but possesses the characteristic in which A is deficient. Sexuality in facultative apomicts often results from the fertilization of an unreduced egg by a reduced pollen grain. In that situation, the cross  $A \times B$  results in some plants with  $2n$  from A and  $n$  from the undesirable B but the cross  $B \times A$  could give  $2n$  from the undesirable B and only  $n$  from the desirable A.

### ECOTYPE SELECTION

Ecotypes have played an important role in grass improvement (224). Local grass strains that have given rise to such varieties as Achenbach bromegrass, Fischer bromegrass, and Kentucky 31 tall fescue provide excellent examples of the value of ecotypes. Ecotypic strains provide good sources of seed for use while new varieties are being developed. Not only domestic but foreign plant collections may contain ecotypes capable of filling an important need in the agriculture of a region or subregion.

The procedures in selecting ecotypes are essentially the same as those outlined in the discussion of selection of species. Under many circumstances the number of entries or the limited supply

of seed necessitates using solid-row or spaced plantings with frequent checks of standard varieties in lieu of replication. After the preliminary studies, promising entries are increased under isolation for more comprehensive testing.

### MASS SELECTION

Many of the forage grass varieties available at the present time were developed by mass selection, and several of the current improvement programs depend, at least in part, on this procedure.

Environmental conditions are relied upon to eliminate unadapted types from space-planted source nurseries representing extensive seed collections. Such a nursery includes at least about 5,000 individuals and is fertilized and clipped to promote good growth. Seed harvested from surviving plants serve to repeat the cycle. Breeders may well supplement natural selection by cutting back undesirable plants or roguing them from the nursery before pollination.

An alternative procedure involves the use of solid-seeded plots. Seed from different sources are sown broadcast on plots, and the plots are clipped or grazed for one or more seasons before seed is harvested. If one desires to keep the sources separate or to maintain the original plots, seed for the next cycle can be produced from vegetative clumps transplanted to isolated crossing blocks. Mass seedings are easier to maintain than space-planted nurseries, their populations are generally larger, and their management is more comparable to that of fields. Spaced plantings are preferred, however, where they are more conducive to natural selection. Spacing is essential to selection for characters that may not be closely associated with persistence, e.g., resistance to some foliar diseases.

Mass selection has several distinct advantages, particularly in the early stages of a selection program. Not only is it relatively inexpensive and simple, but under certain conditions it is also remarkably efficient (266). The success of the program invariably depends on maintenance of a high level of selection pressure either by environmental factors or by the plant breeder. As practiced on cross-pollinated crops it has two primary disadvantages: Selection is based upon the phenotype of an individual plant, and generally it is restricted to half the total inheritance because the male parentage is not considered.

It is reasonable to expect that the possibility of making major advances in winter-hardiness, disease resistance, and productivity will be greater at locations outside or not far inside the periphery of the area where the species is best adapted.

### MATERNAL-LINE SELECTION

"Maternal-line selection" applies to any one of several breeding procedures based on screening plants according to the performance of their progenies. The one common feature of these methods is maintenance of the identity of the maternal clone.

The progenies may be of any one of three main types: Open-pollination (no restriction as to pollen source); topcross (selections interplanted with a common pollen source); and polycross (pollen source restricted to plants that have certain characters in common). Topcross and polycross nurseries must be isolated from other plantings of the same species.

Open-pollinated seed may be collected from selected plants growing in nonreplicated source nurseries or similar plantings in which no attempt is made to restrict pollen source. Tests of open-pollination progenies provide information on the general combining ability of individual plants at an early stage in the improvement program, a distinct advantage when increased yield is a primary objective. Confounded pollen source could result in some errors in classification for general combining ability. This would increase the difficulty of separating average from superior genotypes, but it should still be possible to discard at least some of the inferior genotypes. It may be advisable to practice some degree of selection in the source nursery by cutting back undesirable phenotypes before anthesis.

The large number of selections included in open-pollination progeny tests and the limited amount of seed of each selection available for planting necessitate the use of rather simple designs. Knowles (163) has suggested unreplicated row plots interplanted frequently with check varieties.

Because yield data from row plots may not be comparable with those from solid-seeded plots (167, 311), the time-saving practice of seeding in rows may involve loss in thoroughness of evaluation. Whether it does so depends in part on experimental design, particularly on replication. On the basis of results of replicated tests Murphy (198) suggests that various types of progenies and planting methods can probably be used with a fair expectation of success in isolating plants having high yield potential.

In Murphy's experiment vegetative pieces, polycross seedlings, and self seedlings were used to evaluate selected plants of orchardgrass, smooth brome grass, and red fescue. The progenies were planted in a split-plot design with six replications in which the main plots were species and subplots were planting methods, types of progeny, and, lastly, progenies of individual selected plants. All the average correlation coefficients reported by Murphy were highly significant (table 3). However, the correlations were subject to considerable variation indicating that row plots are not necessarily equivalent to solid seedlings as a means of evaluating progenies with regard to yield.

Considerable savings in time and expense could be realized if it were found possible to use seedling progenies to measure combining ability (116).<sup>14</sup> The relation between seedling response in the greenhouse and plot yields is extremely variable, but some encouraging results have been obtained.<sup>14</sup> Additional experiments involving comparisons among various methods of growing and measuring seedlings should be made before this use of seedling progenies is written off as impossible.

<sup>14</sup> Unpublished data of D. L. Oldemeyer.

TABLE 3.—Average and range of simple correlation coefficients for forage yield between methods of planting, between progeny types, and between progeny types and methods of planting<sup>1</sup>

Factors correlated	Correlation coefficient	
	Average <sup>2</sup>	Range
Same progeny and different methods of planting:		
Spaced vegetative vs. drilled vegetative.....	+0.88	+0.50 to +0.98
Spaced polycross vs. drilled polycross.....	+ .77	— .01 to + .88
Spaced polycross vs. broadcast polycross.....	+ .47	— .93 to + .95
Drilled polycross vs. broadcast polycross.....	+ .51	— .63 to + .89
Different progenies and same method of planting:		
Spaced vegetative vs. spaced polycross.....	+ .74	— .11 to + .96
Spaced vegetative vs. spaced self.....	+ .78	— .23 to + .99
Spaced polycross vs. spaced self.....	+ .78	+ .36 to + .99
Drilled vegetative vs. drilled polycross.....	+ .60	— .81 to + .93
Different progenies and different methods of planting:		
Spaced vegetative vs. drilled polycross.....	+ .60	— .22 to + .96
Spaced vegetative vs. broadcast polycross.....	+ .50	— .40 to + .93
Spaced self vs. drilled vegetative.....	+ .69	— .85 to + .99
Spaced self vs. drilled polycross.....	+ .63	— .03 to + .84
Spaced self vs. broadcast polycross.....	+ .36	— .81 to + .98
Spaced polycross vs. drilled vegetative.....	+ .69	— .51 to + .99
Drilled vegetative vs. broadcast polycross.....	+ .55	— .50 to + .91

<sup>1</sup> Data from Murphy (198).<sup>2</sup> All average correlation coefficients are highly significant.

Topcrossed seed can be obtained by interplanting spaced clones with a standard variety or an experimental synthetic strain. The tester is planted in a manner ensuring that a large proportion of the pollen will be supplied by it—e. g., in alternate solid-seeded rows. Several border rows of the tester should be planted around the topcross nursery. If the tester is seeded in alternate rows of a space-planted source nursery, the seed supplies obtained are not adequate for a progeny test more extensive than space-planted plots or nonreplicated drill rows with frequent checks such as Knowles (163) suggests for open-pollination progenies. Topcross nurseries in which selections are propagated vegetatively to provide five or more single-plant replicates each provide sufficient seed for planting replicated solid-seeded plots. They also provide an opportunity to study replicated clones and can be established at somewhat less cost than comparable polycross nurseries. Kalton<sup>15</sup> has suggested using replicated topcross nurseries to avoid excessive nonrandom pollination such as may occur in large polycross nurseries that have not been replicated sufficiently. There is considerable merit in this procedure provided the tester and the selected clones bloom at or near the same time. In some programs individual plants are

<sup>15</sup> Unpublished memorandum of R. R. Kalton.

topcrossed with a series of superior phenotypes. Kraft or parchment bags or sleeves are used to isolate maternal parents, and pollen collected from a group of paternal parents is applied with a pollen gun.

The polycross technique as defined by Frandsen (88) and by Tysdal et al. (296) is based on the principle of random pollination—that is, each of the original selections included in the nursery has equal opportunity of pollinating, or being pollinated by, any of the others. In general, each clone is randomized within complete blocks, 1 or more propagules to each block, and the blocks are replicated from 10 to 30 times, depending on the number of selections. The effectiveness of the procedure is greater if all the selections included in a nursery flower at approximately the same time—i.e., in most species, within 3 or 4 days of the time when anthers are extruded on the earliest clone.

Hittle (128) and Wit (319) have investigated the amount of variation that can occur in a polycross nursery as a consequence of limited replication and nonrandom pollination. Hittle (128) compared the performance of 10 polycross progenies from as many different replications of each of 20 bromegrass clones. Progenies were compared on the basis of height, green weight, vigor, aftermath, spread, and reaction to *Pyrenophora bromi*. In 12 of the 20 comparisons, the polycross progenies from plants of the same clone in different replications varied significantly in the expression of 1 or more characters. The variation was attributed to nonrandom pollination. Significant differences in variances among polycross progenies within clones provided further evidence of differences in male parentage. Wit (319) used a simple dominant marker, roughness of culms and upper leaf sheaths, to study cross-pollination in *Lolium perenne* in both unreplicated and polycross nurseries. Heterozygous rough clones (*Tt*) served as the source of contaminating pollen and homozygous recessive clones (*tt*) were used as testers. Cross-pollination decreased rapidly over the first 3 or 4 rows and only slowly beyond that. In spite of differences as great as 1 week in flowering time, clones were fertilized, on an average, 40 percent by the 2 adjacent clones on both sides and 74 percent by the 3 on both sides. Simultaneousness of flowering often had greater influence on the percentage of crossing than proximity. Clones were pollinated with genotypically different pollen mixtures in unreplicated clonal plantings, whereas in polycross nurseries genotypically homogeneous pollination was possible. The reliability of the polycross test was shown to depend on nearly simultaneous flowering and adequate replication.

Properly designed polycross nurseries have these three advantages: Random or near-random pollination among plants selected for common characteristics—maturity, disease resistance, seed habit, habit of growth, etc.; an opportunity to study replicated clones; and production of ample seed for progeny testing. Polycross blocks provide an excellent opportunity to practice recurrent selection within progenies. Normally, good

combining clones selected on the basis of topcross or open-pollination progeny performance are grouped in polycross blocks and the resulting progenies are available for reselections. Thus, if recurrent selection can be utilized, the polycross method would appear to be more efficient than either of the alternative procedures.

Recurrent selection, first described with reference to corn breeding (130), has been successfully applied in forage crop breeding—not always under that name (111, 140). Hull (130) applied the term to a system of corn breeding based on the recombination of first-generation inbred lines selected according to the test-cross performance of parental plants crossed on a homozygous line. The procedure was repeated in the bulked  $F_1$  population resulting from crosses among selected  $I_1$  lines. Sprague and Brimhall (254) have presented data to show that recurrent selection is more effective than inbreeding and selection in modifying the oil content of corn. Lonnquist (181) found that the frequency of favorable genes for yielding ability could be effectively increased in corn by practicing recurrent selection for general combining ability.

Differences among species, in facilities, in the nature of the improvement sought, and in the degree of selection pressure determine the number of selections and the size of the resulting polycross nurseries. The following discussion represents a generalized scheme:

In the first cycle of selection a large number of plants are ordinarily available—about 250 individuals, representing approximately 5 to 10 percent of the population of the original source nursery. In many species the selections represent a wide range in maturity and many different combinations of characters being sought in the breeding program. Maturity and sometimes other criteria are used to divide selected plants into several groups, each including 50 or so clones that could constitute a polycross nursery. Nurseries differing in average blooming date can be established adjacent to each other; otherwise, the nurseries should be spatially isolated. In general, individual nurseries are arranged in randomized-block designs with approximately 20 replications of single-plant plots each. Seed collected from all replications of individual clones (preferably an equal quantity of seed from each replication) is bulked and included in a solid-seeded plot test having at least 4 replications. The number of entries included in the plot test can be reduced on the basis of observations made on the replicated clones. The plot test can frequently be supplemented with space-planted progenies (approximately 4 replications of 10 plants each), on which observations can be made and from which reselections can be taken.

As the number of selections included in an individual polycross nursery increases, there should be a corresponding increase in the number of replications. If there is no logical basis for combining selections into relatively small groups, the physical



limitations imposed by replication requirements provide a strong argument for using the topcross method.

After data have been obtained from the progeny test for at least 3 years it should be feasible to discard from 75 to 85 percent of the original clones. At the conclusion of or during the testing program the better combining clones are increased vegetatively and established in isolated advanced polycross nurseries with from 10 to 20 replications.

One or several advanced polycross blocks are planted, depending again on differences in maturity, growth habit, seed production, and other characteristics that form a basis for separating selected clones. Seed is again collected from each replication of the individual clones and bulked for plot testing. Under some circumstances it is feasible to include the component lines and a mixture of all of them (using an equal amount of seed from each clone) in a variety test. In general, however, the number and size of the advanced polycross nurseries prohibit including each line in the regular testing program. Normally, bulked seed from each isolation block appear in a variety test and a separate progeny test is established to evaluate the component lines in comparison with bulked seed lots and check varieties.

Recurrent selection can be practiced within advanced polycross progenies that have demonstrated superiority in the solid-seeded yield test. A space-planted progeny test is established for this purpose.

Breeding by maternal-line selection should be a continuous operation in which new source materials (new introductions, collections, and reselections from within first-cycle progeny tests) are being evaluated in second-cycle polycross nurseries while progenies from advanced polycross nurseries are being tested. In practice, each new series of advanced polycross nurseries may contain approximately the same number of clones as were included in the first-cycle crossing blocks, representing the following classes: (1) Clones from the original source nursery, retained on the basis of their performance in two progeny tests; (2) clones included in the second-cycle source nursery and evaluated in one progeny test; (3) clones selected within superior advanced polycross progenies. One or more advanced polycross nurseries can be confined to selections made within outstanding polycross progenies in order to measure the effectiveness of recurrent selection.

The pedigrees of experimental strains vary according to the stage of the program at which plants are selected for intercrossing and the number of methods used in selecting and evaluating plants.

In the foregoing, procedures have been outlined on the premise that yield is one of the major objectives in breeding. Obviously, breeding objectives may affect not only the type of progeny utilized but the method of progeny testing. Progenies from plants selected for such characters as resistance to diseases influencing seed production, lack of awns, or seed size can be evaluated in space-planted nurseries. Progenies from plants selected for

superior palatability, seedling vigor, and lodging resistance can be tested in replicated single drilled rows. However, bulk seed from crossing blocks should be tested in solid-seeded plots to measure the effect of selection on yield, vigor, and persistence.

A difference in breeding objectives does not necessitate differences in the polycross method, but procedures can be modified in accordance with the requirements of the progeny test. Thus, open-pollinated and selfed seed can be collected in source nurseries and progenies evaluated in either replicated space-planted rows or single drilled rows with appropriate checks. Superior clones can then be grouped in polycross nurseries. In addition, selfed and open-pollinated seed can be collected within superior progenies to establish second-cycle progeny tests. Experimental strains may then be composed of clones traceable to plants of one or more of the following classes: (1) Selected from the original source nursery on the basis of selfed and open-pollination progeny performance; (2) selected within superior open-pollination progenies in the first-cycle progeny test; (3) selected on the basis of advanced polycross progeny performance; (4) selected within superior advanced polycross progenies; (5) selected on the basis of advanced inbred generations— $I_2$  or beyond.

Investigators have arrived at somewhat different conclusions regarding the effectiveness of certain grass-breeding procedures. For example, Knowles (163) and Heinrichs (123) concluded that open-pollination progenies served a very useful purpose in screening plants for general combining ability, while Harlan (111) stated that open-pollination progenies were seldom used in the Oklahoma program because of their variability and the lack of progress from this type of selection. It seems evident that the usefulness of any one procedure will vary according to the species, the objectives, and the area where the work is being conducted.

Several investigators have outlined methods that are applicable to the improvement of naturally cross-fertilized species. Outlines presented for timothy by Evans (76) and Frandsen (88) will serve as examples. The methods used by Evans are as follows: (1) Seed from single-plant selections is drilled in a seedbed or broadcast in a small plot; (2) growth of the resulting plants is observed and compared; (3) plants taken from superior plots are transplanted to cultivated row plots; (4) one or more selections are made from the row plot of each strain; (5) the selections are grouped and tested. Frandsen's program, which includes several refinements over Evans' open-pollination technique, is as follows: (1) Selection of from 50 to 100 individual plants (on the basis of observations over 2 or more years); (2) vegetative propagation into clones of not less than 100 propagules; (3) random planting of individual propagules in a "mixed" (polycross) nursery; (4) harvesting of seed from each clone; (5) selection of from 10 to 20 of the superior clones whose progenies have given the highest yield (these could be used for strain building by mixture and intercrossing); (6) self-pollination and diallel pair crossing, suggested for studying important charac-

teristics of families such as leafiness, tillering capacity, rust resistance, and cold resistance.

The significance of restricting pollen source to plants having similar characteristics is well illustrated by Harlan's (111) application of partial isolation to the breeding of sideoats grama. For each of 18 type classes, which represented a variety of growth forms, 14 plants were selected. These were transplanted to separate isolation blocks, approximately 25 feet square and 28 feet apart in each direction. The intervals between the blocks were planted to sorghum, which probably did not afford complete genetic isolation. Seed was harvested from each block in the fall, a population of approximately 180 plants was established from each lot, and reselections were made. The second-generation isolated populations included 12 plants each. Characters readily fixed in populations of sideoats grama were broad leaves, narrow leaves, fine stems, heavy stems, and late flowering. Types involving abundant leaf production showed the least progress toward fixation. Certain characters appeared to be closely associated in the sample populations—for example, broad leaves and late flowering, fine leaves and rust resistance, broad leaves and blue-green color, narrow leaves and yellow-green color.

### CONTROLLED HYBRIDIZATION

The emphasis placed on studying breeding behavior of perennial forage grasses through some form of maternal-line selection has been due largely to the self-incompatibility and inbreeding depression that characterize cross-fertilized grass species. An increase in the gene frequency for economic characters is generally sought by intercrossing selected plants and by practicing recurrent selection within maternal lines. In an effort to increase the effectiveness of these procedures, some workers have resorted to controlled hybridization. Various crossing schemes including diallel crosses (all combinations of single crosses) and cyclic crosses ( $1 \times 2$ ,  $2 \times 3$ ,  $3 \times 4$ , etc.) have been used to study the breeding behavior of selected plants. Diallel crossing is a sound procedure, although its expense and the limitations it imposes on population size constitute serious shortcomings. Single crosses can serve at least three purposes: To obtain desirable recombinations of characters or to transfer specific characters to otherwise desirable selections; to increase the gene frequency for desirable characters by crossing either inbred or noninbred clones (and practicing recurrent selection within the resulting  $F_1$  progenies); and to study the specific combining ability of selections constituting experimental strains.

If only a limited number of plants with the desired expression of a given character can be isolated, controlled hybridization is a logical approach to investigation of the possibility of maintaining, improving, or transferring this character. The value of certain disease-resistant ecotypes in grass improvement can be investigated by making single crosses and comparing the  $F_1$  progenies with the parental types. Differences in maturity among

ecotypes frequently necessitate making controlled crosses in the greenhouse.

Differences between progenies arising from reciprocal crosses have been observed on occasion (163). Probably most of the variation can be attributed to difference in degree of self-fertility and difference in the vigor of selfed offspring of individual parents. Zimmerman (323) obtained data on the performances of reciprocal single crosses of  $I_3$  lines of *Avena elatior* (*Arrhenatherum elatius*) and *Dactylis glomerata*. Coefficients of correlation between performances of reciprocal single crosses calculated from his data were +0.17 and +0.66 for 36 crosses of *A. elatior* and 22 crosses of *D. glomerata*, respectively.

In general, the limited quantity of single-cross seed restricts the testing of  $F_1$  progenies to replicated space-planted nurseries. Seed requirements for establishing plot tests can be met by bagging more plants or by arranging the clones in pairs in small isolated crossing blocks. Field crossing by either bagging or spatial isolation is applicable when clones flower at the same time—a situation commonly encountered in studying the specific combining ability of clones constituting synthetic strains.

Recombining specific plant characters is a difficult undertaking in species that behave genetically like atopolyploids. Nevertheless, controlled single crosses, recurrent selection, and backcrossing constitute an effective approach to the recombination of important quantitative characters. These procedures, especially backcrossing, can be expected to become more productive as the intensity of grass improvement increases.

### INBREEDING AND SELECTION

Although inbreeding has been used successfully in developing improved varieties of the naturally cross-pollinated forage grasses (2, 222), wide differences of opinion exist regarding the application of this technique to grass breeding. Several investigators (197, 212, 301, 302, 315) have reached the conclusion that self-pollination offers little promise in grass improvement.

Sufficient selfed seed can be obtained from many of the cross-pollinated grasses to permit establishment of inbred populations (120, 204, 223, 241). Cheng (49), studying the self-fertility of 3 grass species, obtained enough such seed for the production of progenies from 19 out of 29 clones of *Bromus inermis*, 3 out of 24 clones of *Agropyron cristatum*, and 23 out of 24 clones of *Alopecurus pratensis*. Most of the forage grass species exhibit a wide range in self-compatibility, from plants that are completely self-incompatible to those that are completely or almost completely self-fertile (17, 120, 218, 247). The interannual variation in self-fertility ratings is large (49, 205, 247). Data compiled by Smith (247) that illustrate the variation from year to year in seed set resulting from self-pollination and from open pollination are presented in table 4. Studying 10 plants each of orchardgrass and brome grass, Smith obtained correlation coefficients of 0.07 and -0.04, respectively, for percentages of seed

TABLE 4.—*Interannual variation of seed set of individual selected plants of Dactylis glomerata as a result of self-pollination and open pollination, respectively*<sup>1</sup>

Plant No.	Average seeds per floret resulting from—			
	Open pollination		Self-pollination	
	1938	1939	1938	1939
	Number	Number	Number	Number
11	0.56	0.42	0.000	0.030
29	.57	.67	.035	.070
31	.52	.69	.000	.030
38	.68	.49	.000	.030
51	.50	.49	.007	.020
63	.35	.53	.005	.080
65	.36	.39	.042	.050
72	.54	.31	.000	.004
77	.51	.51	.000	.010
93	.41	.46	.000	.090

<sup>1</sup> Data from Smith (247).

obtained by selfing the same plants in different years. Results obtained with several different species (49, 204, 218, 247, 318) indicate a positive relation between self-fertility (seed set under bags) and cross-fertility (seed set under open pollination). Inbreeding is generally accompanied by a reduction in average vigor (133, 134) and, at least in some species, by increase in meiotic irregularity and decrease in fertility (208).

Some evidence suggests the feasibility of selecting for self-fertility in brome grass (318). In orchardgrass, Myers (204) reported a significant parent-inbred progeny correlation ( $r = +0.62$ ) for seeds set per panicle under bag. Similarly, Jenkin (195) concluded that the self-fertility of perennial ryegrass plants has a profound effect on the self-fertility of their inbred progeny.

Waldron (304) compared inbred brome grass progenies with the parental clones over a 2-year period. Sibs from planted seed outyielded transplanted parental clones in the first year, but just about equaled them in yield in the second year. The leaf areas of parental clones were greater than those of their inbred progenies, but the range in leaf area was greater within the progenies. Hayes and Barker (118) and Hayes and Clarke (119) studied the effect of inbreeding on timothy and the possibility of improving this species by selection in self-fertilized lines. Data obtained by Hayes and Clarke (119) from lines that had been inbred for either 1 or 2 generations indicated to them that selection in selfed lines would be a practical method of improving timothy. Self-fertilization did not result in a great reduction in vigor; some selfed lines yielded less but others somewhat more than the commercial check. Self-fertilization permitted freeing

lines of undesirable recessive characters and isolating disease-resistant lines. Law and Anderson (175) observed a marked and progressive loss in vigor in most inbred lines of big bluestem. The response to inbreeding varied, however, and some inbred lines exhibited no less vigor than their open-pollination sibs. In a study by Anderson and Aldous (6) the vigor of *Andropogon scoparius* was not seriously affected by inbreeding. Schultz (241) found a wide range of self-fertility among and within self-progenies and among open-pollinated plants from eight collections of orchardgrass. The amount of self-seed produced varied greatly from year to year. Significant differences were observed within 2-year selfed material, within the open-pollinated group, and between the 2 groups for every character studied. In the 2-year self group a number of vigorous clones were found that were fully equal to the superior individuals in the open-pollinated group.

Hayes and Schmid (120) studied the feasibility of selection in self-pollinated lines of bromegrass, meadow fescue, and orchardgrass. Populations were studied in space-planted nurseries, and notes were taken on growth habit, disease resistance, and yield of individual plants. The procedure was to plant clonal progenies of 80 to 100 selected plants in replicated rows or beds; select 20 of the best clones; cut all others before pollination; and harvest open-pollinated seed from the 20 selections. The seed was mixed and increased in an isolated seed plot, and the desirability of the new strain was determined in field trials. Seed harvested from the isolated plot furnished the basis for second-cycle selection. For the selfing-and-selection test, vigorous plants of the same origin as those used for clonal trials were selected. Selfing and selection were continued for 5 years. After 2 or more years of selection in selfed lines, a few lines of each of the 3 species were as vigorous as the commercial check. Several crosses between  $I_1$  clones were made and the progenies studied. The yield of the  $F_1$  progenies of  $I_1$  clones ranged from 126.5 to 220.9 percent of that of the commercial check. The investigators deduced that a selfing-and-selection project, to be successful, must include selfing 2 or 3 times as many plants as will be used. They suggested that isolating clonal and selfed lines, testing them for combining ability, and combining them in crosses to produce synthetic varieties or to produce single- or double-cross seed may constitute a valuable approach in grass improvement.

Julén (146) concluded that inbreeding may improve the chance of obtaining positive results in grass improvement, by leading to the development of strains that are more or less homozygous for various characters. He obtained progenies by selfing in species exhibiting some degree of self-fertility and obtained satisfactory seed set in some of the plants after sib fertilization in the  $I_1$  and subsequent generations. It was assumed that through natural selection more or less cross-sterile plants would be eliminated and highly fertile plants would become increasingly predominant until full fertility was acquired. In spite of the

general occurrence of inbreeding depression among normally cross-fertilized grasses, Julén expressed the view that it should be feasible to isolate vigorous inbred lines in many species, if much material is examined. In addition, he suggested that inbred lines comparatively free of the ill effects of inbreeding may be the most valuable parents in crossbreeding. Nilsson-Leissner (222) found that  $I_1$  generation seed of *Dactylis glomerata* generally produced weaker stands than seed of later generations obtained from isolated fields of  $I_1$  lines. Evidently some of the weak, more or less abnormal segregates are suppressed in the dense plant rows of the seed fields, this is repeated in subsequent generations, and consequently the adaptation of the strain increases. Åkerman et al. (2) cite several examples of varieties derived from 1 or more advanced generations of the 1-year-selfed progeny of individual clones—Primus and Gloria timothy, Skandia II and Brage orchardgrass, Viking red fescue, and Viktoria perennial ryegrass.

Murphy and Atwood (200) suggest that more attention could well be given to the possibility of utilizing inbred lines in those species, such as brome grass, in which 1-year-selfed lines of excellent vigor and uniformity can be selected. They point out that  $I_1$  families may be used in at least three different ways: To progeny-test selected plants, as a source of new superior clones, and in the development of synthetic varieties through isolated seed increase of one or more families. They draw attention to the fact that several grass species may behave in part like autopolyploids. In addition, the use of  $I_1$  families for progeny-testing clones selected in source nurseries effects a considerable saving in time as compared with the production of polycross seed and subsequent progeny testing.

Zimmerman (323) developed  $I_1$ ,  $I_2$ , and  $I_3$  lines of *Avena elatior* (*Arrhenatherum elatius*) and *Dactylis glomerata* from local collections. The average yield of 22 crosses among  $I_3$  lines of orchardgrass was 93.5 percent of the average of the 3 commercial checks when all were grown as spaced plants. Two of the crosses yielded 135 and 140 percent, respectively, of the average of the checks. The results with *Avena elatior* were closely similar. The investigator concluded that crosses among inbred lines constitute a promising method for obtaining productive new varieties.

Stapledon (267) observed that in orchardgrass inbreeding was followed by a decline in vegetative vigor averaging about 50 percent, but that some of the  $I_1$  plants showed little or no decline in vigor. Valle (301) concluded that selfing could not lead to practical results in timothy. He believed that timothy improvement should not be dependent upon the rare appearance of constantly self-fertile and self-vital individuals. Travin (290) considered that intervarietal hybridization was far superior to selection of self-pollinated individuals. As one of several reasons for this he mentioned the advantage of obtaining, early in the program, large amounts of seed with which to conduct preliminary plot tests and varietal tests and to reproduce the variety rapidly if the results indicate that this is advisable. Generally, also, a variety

obtained by intervarietal hybridization has wider adaptation than a "pure line" and hence can be distributed through a region more readily, with a saving of many years of varietal testing in a number of locations.

Murphy (197) concluded that selection within selfed progenies of crested wheatgrass was not a very promising method of improvement, because of the difficulty experienced in obtaining selfed seed and because of the lack of uniformity and poor vigor observed in many of the  $I_1$  progenies. Greater self-fertility would have to be attained in order to make this procedure effective in crested wheatgrass breeding. Under the conditions of Murphy's experiments some  $I_1$  and  $I_2$  plants produced pollen distinctly smaller and less fertile than that of nonselfed plants, although others showed no appreciable inferiority with respect to this character. It was suggested that improvement over individual-plant selection can be made by selecting among superior plants after they have been tested in replicated clonal nurseries. Stevenson (276) has described procedures for breeding crested wheatgrass that involve aspects of inbreeding and also of maternal-line selection and diallel crossing. Although  $I_1$  progenies provided an opportunity to eliminate undesirable segregates, selection within selfed lines was not considered to be a profitable approach. Kalton et al. (147) concluded that inbreeding offered little promise in orchardgrass improvement.

A decision as to the probable usefulness of selection within inbred lines in grass improvement must be made with reference to the method that will be followed in constituting new varieties. Although vigorous inbred lines are apparently rare within most forage grass species, varieties can be and have been developed by the multiplication of individual plant selections. Evidence is lacking on which to base precise comparisons between the ranges of adaptation of varieties having a narrow gene base and varieties having a broad one. Limited observations indicate, however, that certain plant varieties developed through inbreeding are capable of producing reasonably good yields over a wide area (2). The difficulty of increasing or maintaining vigor in selfed lines must certainly be recognized, but regardless of this shortcoming it should be feasible to use selections from within several selfed lines in the development of synthetic varieties. This procedure could result in restoration of vigor and similarly contribute to greater uniformity in disease resistance and other economic characters. It is open to criticism because of the likelihood of selecting plants that are largely self-fertile and so would set considerable selfed seed when isolated for the production of synthetic varieties.

Data accumulated by Hanson et al. (105) on  $I_1$  orchardgrass lines suggest that general combining ability is not affected appreciably by inbreeding. In the development of these  $I_1$  lines, several lines were eliminated by very poor vigor or lack of seed, and bags that contained large amounts of seed were discarded because of possible contamination with foreign pollen. Thus, there was some selection against very low and probably some



against very high self-fertility. There was some indication that inbred lines within certain families were superior to their parental clones in general combining ability. Hawk and Wilsie (116) compared the yields of open-pollination progenies from I<sub>1</sub> and I<sub>2</sub> bromegrass selections and their parental clones. The yields of the progenies were not significantly different, demonstrating independence for level of inbreeding and general combining ability. Wilsie et al. (318) found little or no correlation between self-fertility and combining ability in bromegrass.

It would seem possible, at least in certain species, to use inbred lines in the production of synthetic varieties without danger of loss of vigor.

### INTERSPECIFIC AND INTERGENERIC HYBRIDIZATION

The possibility of utilizing interspecific and intergeneric hybridization in developing improved varieties of the forage grasses confronts the plant breeder with an interesting challenge. Love (182) and others (273, 297) have discussed the possible merits of this approach, and several workers have expressed the opinion that such hybridization offers the greatest promise of breaking the ceiling that exists or eventually will exist in the improvement of certain species by standard procedures. Many vexing problems are encountered in this type of investigation (incompatibility, sterility, identification of hybrids, and screening for agronomic value), and generally there is no absolute assurance that it will produce hybrids of practical importance or, in fact, any hybrids. Love (183) has stressed the value of accumulating fundamental data on diploid species and on phylogenetic relationships as a basic step in the application of these methods to forage-crop improvement. Stebbins (273) has concluded that there is no way of predicting the results to be expected from any particular hybridization. He is of the opinion that generalizing on the expected behavior of allopolyploids might restrict rather than guide future investigations. This view is based on the possibility that any particular hybrid combination desired by the practical breeder may prove to be an exception to the "established" rule.

Hybrids have been produced, naturally and artificially, between widely different species, and sometimes it is easier to cross species belonging to different genera than it is to cross species within a genus. Stebbins (273) suggests that this situation reflects the artificial character of presently accepted taxonomic systems. He cites several successful crosses, including *Festuca elatior* × *Lolium perenne* (136) and *Agropyron trachycaulum* × *Elymus glaucus* (274)—which are especially interesting because it is difficult or impossible to make crosses between certain *Festuca* species or between certain *Agropyron* species.

Ullmann (297) suggested that the following crosses might be very interesting from the standpoint of producing new strains and valuable breeding material: *Dactylis aschersoniana* Graebn. × *D. glomerata*, which might modify or eliminate, through the formation of short stolons, the undesirable turf-forming char-

acter of *D. glomerata*; *Festuca pratensis* (*F. elatior*)  $\times$  *F. arundinacea*, which might increase competitive ability in mixed stands, through increasing plant height; and *F. pratensis*  $\times$  *F. rubra*, which might be helpful in obtaining a high-yielding creeping type. He also mentioned the possible importance of hybridizing *Phalaris arundinacea* with *P. bulbosa* L. for alteration of time of flowering and time of ripening and for obtaining forms suitable for more arid areas. He suggested that annual *P. canariensis* L. might be hybridized with perennial *P. arundinacea* to produce more rapidly growing, tender-leaved hybrids with greater longevity and greater resistance to seed shattering.

Several of the hybrids suggested by Ullmann (297) have been produced. *Dactylis glomerata* has been hybridized with *D. aschersoniana* Graebn. and with *D. voronowii* Oucz. Although some of the hybrid plants look rather interesting, they are less vigorous than *D. glomerata*.<sup>16</sup> *Phalaris arundinacea*  $\times$  *P. tuberosa* hybrids show considerable promise, but there has been a notable tendency for the selected offspring to resemble one or the other parent, depending on conditions under which selections were made.<sup>17</sup>

Jenkin (186) and Crowder (64) have reported on inter-specific and intergeneric hybrids involving *Festuca* and *Lolium*. Crowder obtained reciprocal hybrids of *F. arundinacea*  $\times$  *F. elatior*, *F. arundinacea*  $\times$  *L. multiflorum*, and *F. arundinacea*  $\times$  *L. perenne*. He also obtained hybrids from the cross *L. multiflorum* var. *diminutum* Mutel  $\times$  *F. arundinacea*. He reported that several of the  $F_1$  derivatives were rather vigorous and that their foliage was not so harsh as that of the *F. arundinacea* parent. All the  $F_1$  hybrids were infertile.

Very interesting material has been obtained through inter-specific hybridization of *Paspalum* species, and although most of the hybrids are highly sterile it is possible that they may yet be utilized through vegetative reproduction or restored to fertility by doubling the chromosome number (37).<sup>18</sup> Bennett (21) has been successful in crossing *P. dilatatum* (dallisgrass) with *P. malacophyllum* and isolating fertile dallisgrass segregates that are immune or highly resistant to ergot.

Sweet johnsongrass segregates and perennial sorghum lines have been produced by crossing johnsongrass with other sorghums. Several of these lines look very promising for pasture and silage (21). Nielsen and Rogler (220) have reported on Mandan ricegrass, a fertile hybrid believed to have originated from a chance cross between *Stipa viridula* and *Oryzopsis hymenoides*. The adaptation and usefulness of Mandan ricegrass have not been definitely established, but observations suggest that it might be useful in certain localities in the northern Great Plains. Burton (32) found that perennial napiergrass crossed very readily with annual pearl millet. All the  $F_1$  plants were sterile, but a few looked very promising. Coastal bermudagrass is considered as

<sup>16</sup> Unpublished data of M. W. Myers and A. A. Hansen.

<sup>17</sup> Unpublished data of R. M. Love.

<sup>18</sup> Unpublished memorandum of I. Forbes.

having arisen from a chance interspecific hybrid (33). It is almost completely sterile and must be propagated vegetatively. Farmers have accepted this variety on the basis of its great superiority over common bermudagrass and many of the other warm-season grasses adapted to parts of the Southeastern States. Clausen et al. (55), at the Carnegie Institution of Washington, have used mutual pollination to obtain crosses among several *Poa* species. They have isolated a number of promising hybrids, but as yet the agronomic value of these has not been established.

Crosses between "species" or "genera" may be used in efforts to transfer a desirable character from an otherwise worthless species to a species that has demonstrated its economic worth. Backcrossing to the superior parent would probably be an essential feature of such a program. Hybridization also serves to produce new genetic combinations; at the present stage of grass improvement, this aspect has received more attention.

Hybrids can sometimes be produced between species that differ in chromosome number. The possibility of such a cross is frequently greater if the plant with the larger chromosome number is used as the paternal parent (64, 226). If the cross does not at first succeed, doubling the chromosome complement of the low-chromosome parent may facilitate it. If hybridization then succeeds, and if chromosome synapsis and disjunction are regular, the resulting  $F_1$  may be fertile. Complete autosyndesis tends to promote cytological stability. Consideration can be given, also, to the possibility of doubling the chromosome number of diploid grass species before crossing them. Successful crosses might result in immediate production of amphidiploid hybrids.

Induction of polyploidy is the most rapid method of obtaining fertile plants from sterile  $F_1$  hybrids. Selection for fertility in the  $F_2$  generation of partially fertile  $F_1$  hybrids may be effective in self-fertilized plants (273). The absence of chromosome pairing in undoubled  $F_1$  hybrids would indicate that the resulting allopolyploid might be fertile and retain its fertility in advanced generations. Conversely, a high degree of chromosome pairing in the undoubled  $F_1$  would suggest that the allopolyploid produced from the hybrid might not succeed (58). Stebbins (273) has found, however, that some sterile  $F_1$  hybrids with nearly regular chromosome pairing produced vigorous, fertile segmental allopolyploids.

Many controlled crosses have involved parents that were not specially selected for good agronomic characters, and many have been between species of limited economic importance. Information obtained from such crosses is important in establishing the phylogeny of species, but many of the resulting hybrids have little or no immediate economic value. One of the important principles established by Clausen and coworkers at Stanford University is that hybrids between types having widely different ecological preferences may be expected to exhibit broad adaptation. In general, both parental species should have a range of adaptation that approaches the requirements of the area where the new type is expected to be used (273).

Ullmann (297) has pointed out that the value of artificially produced hybrids depends on the presence of desired characters transferred from the parents. Certain desired characters may not be transmitted to the hybrid, and several undesirable characters may be retained. Hybrids between *Lolium perenne* and *Festuca elatior* appear highly resistant to crown rust, although both parents are highly susceptible.<sup>10</sup> It is presumed that the *Festuca* parent contributes resistance to the races of crown rust attacking *Lolium* and the *Lolium* parent contributes resistance to the races attacking *Festuca*.

Production of successful hybrids frequently depends on the extent of hybridization. Unquestionably, some negative results can be attributed to a restricted number of crosses involving relatively few genotypes. Smith (246) found that failure to produce certain crosses could not be considered as proof of complete incompatibility between the species in question. Interspecific or intergeneric hybridization is a long-term undertaking in which the parental species should be selected with care, crosses should be made on a scale commensurate with facilities, and doubled and undoubled hybrids should be examined thoroughly for adaptation and economic characteristics.

#### SYNTHETIC VARIETIES

"Synthetic variety," as defined by Tysdal and Crandall (295), means a variety developed by crossing, compositing, or interplanting two or more strains or clones (harvesting and replanting the bulk seed of successive generations). The definition has been enlarged on occasion to include "single plant" synthetics derived by selecting several plants within the inbred progeny of a superior clone.

The clones included in experimental synthetic strains should be randomized within replications and replicated sufficiently to avoid appreciable nonrandom pollination. Seed harvested from the component clones should be compared with bulk seed from the experimental strains and with seed of appropriate check varieties. Experimental synthetics should be carried to the Syn<sub>1</sub> or Syn<sub>2</sub> generation before they are subjected to advanced testing at the originating station or distributed for regional testing. Syn<sub>2</sub> seed is produced by planting, under isolation, equal amounts of Syn<sub>1</sub> seed from each clone. The Syn<sub>2</sub> generation is produced from bulked Syn<sub>2</sub> seed.

Lowe and Murphy (184) studied anthesis and open-pollinated seed set in bromegrass, and concluded that it might be advisable to make first-generation synthetics by compositing seed, produced in isolation, of all the various single crosses.

In contrast with single-plant strains, in which a certain degree of self-fertility is essential, Jenkin (134) is of the opinion that the selections constituting multiple-plant strains should produce ample pollen and at the same time be highly self-incompatible. The significance of a certain degree of self-fertilization when

<sup>10</sup> Unpublished data of H. L. Carnahan and J. H. Graham.

selected clones of normally cross-fertilized species are isolated for seed production has been the subject of considerable debate. The assumption has been widely accepted, however, that under most conditions self-fertilization does not have a major influence on the yield of the first synthetic generation. Yield tests show that the combining ability of selected clones is largely independent of their degree of self-fertility when they are provided with an ample supply of foreign pollen. The effect of selfing would be accentuated, however, in experimental synthetics whose component lines exhibited a relatively wide range in time of blooming. On the other hand, clones that contribute little to the yield of the synthetic variety would be eliminated by testing the progeny of individual clones.

In certain cases, competition within solid seedlings soon leads to the disappearance of inbred seedlings, or at least reduces their contribution to a point at which herbage yields are comparable to those of a pure hybrid planting. Burton (36) has presented some interesting data on the performances of various mixtures of hybrid and parent-inbred lines of the annual pearl millet. Seed mixtures containing 90, 80, 50, and 20 percent hybrid seed were compared with pure seed of the inbred parents and of hybrids. The forage production over a 6-year period of 90-, 80-, and 50-percent-hybrid seed mixtures did not differ significantly from that of pure hybrid seed. Under the conditions of this test, 3 to 3½ seedlings per inch of row seemed to be about the density required to give a yield performance from mixtures comparable to that from pure hybrid seed. Burton lists several factors affecting the production and utilization of hybrid seed: (1) Parent lines or clones should flower at about the same time, should be highly cross-fertilized, and should produce good seed yields; (2) hybrid seedlings should be vigorous; (3) the percentage of chance hybrids will be increased as the number of lines or clones is increased; and (4) seed of several parent lines is mixed in equal proportions for planting. All the seed produced should be harvested.

Results such as those obtained by Burton may be expected wherever a variable amount of selfing occurs and it is feasible to maintain inbred lines for direct natural crossing. The commercial seed is then a mixture of  $F_1$ 's and inbreds. The more common situation in forage grasses, however, is represented by advanced generations of multiple-heterozygous-clone synthetics. Such populations are not mixtures of  $F_1$  and inbred lines but are likely to be  $F_1$  and  $F_2$  crosses. Undoubtedly, although natural elimination of weaker genotypes occurs in synthetic varieties, many plants of an intermediate nature must become established. Obviously, there are insufficient data bearing directly on this subject.

Multiple-plant strains are less obviously affected by inbreeding, but Jenkin (134) draws attention to the fact that the greater the number of plants used in developing a synthetic strain the greater is the danger of lack of uniformity. Lack of uniformity is not serious unless it includes nonuniformity in the character-

istics for which the strain was developed. The performance of a synthetic variety may be influenced to a greater degree by the general combining ability of the parent clones than by the number of clones used in any specific combination. Kinman and Sprague (158) have stressed the importance of using good combining inbred lines in formulating maize synthetics. Similarly, Tysdal and Crandall (295) observed that the highest yielding alfalfa synthetics can be expected from clones that are high in general combining ability. The yield rank of 8 synthetic alfalfa varieties consisting of 4 or 5 clones each was closely associated with the average polycross performance of the parental clones.

Graumann (95) compared the performances of 15 experimental alfalfa synthetics and found that the mean yields of one 3-clone, seven 4-clone, six 5-clone, and one 6-clone combination amounted to 112, 108, 103, and 105 percent of those of the check varieties. These data suggest the difficulty that might be encountered in producing high yielding synthetics by combining an increasing number of selections. In Graumann's experiment very few of the parental clones were common to various groups. Further comparison by Graumann and Kehr (96) indicated that in alfalfa 2-clone combinations exhibit a greater yield change in advanced generations than multiple-clone combinations. The  $Syn_1$  generation of 2-clone synthetics yielded considerably more forage than that of the multiple-clone synthetics, but there was little difference in yield performance of the two groups in the  $Syn_2$  and  $Syn_3$  generations. These results suggest that synthetic varieties should be advanced beyond the  $Syn_1$  generation before they are evaluated on an extensive scale.

Some grass breeders, in determining the number of clones or lines required in a synthetic variety to ensure satisfactory performance in advanced generations, have used as a guide the formula

$$S = F \left( \frac{F-1}{N} \right)$$

in which  $S$  is the yield of the synthetic in advanced generations,  $F$  the yield of the first generation,  $I$  the average yield of the inbred lines, and  $N$  the number of lines. Garber and Myers (91) point out that emphasis has been given to  $N$ ; i.e., that a large number of clones or lines have generally been used in constituting synthetic varieties. In most forage grasses it is doubtful that the relationship between inbreeding depression and restoration of vigor can be reduced to such simple terms. Grouping similar plant types in a restricted crossing block may result in yield depression not directly attributable to number of clones. Under these circumstances diverse germ plasm might be of greater significance in restoring vigor than an increase in the number of clones would be.

The minimum number of plants that should be used in developing a synthetic variety in order to prevent appreciable reduction in vigor in advanced generations may depend on the species and on the origin and pedigree of the clones. In the absence of specific information, the following approach would seem to warrant

consideration: Given 12 clones all of which look very promising in terms of a given progeny test but of which 3 or 4 are especially outstanding, include the limited number of outstanding clones in one experimental synthetic and all 12 in another. Then revise the composition of these synthetics in accordance with the performance of the Syn, and advanced synthetic generations.

## MULTIPLICATION AND VARIETAL RELEASE

Multiplication and release of the seed of a new variety frequently present problems that are among the most vexing encountered in forage-crop improvement. Who is responsible for testing new varieties? How long should a new variety be tested? To what extent should varieties be tested on a regional or national basis? Is wide adaptation a requisite of an improved variety? What characteristics should be stressed in evaluating varieties? To what extent should the performance of a new variety surpass those of commercial lots? What fiducial limits should be accepted in testing new varieties? These questions indicate some of the problems that must be considered in following breeding activities to their logical conclusion. The procedures for varietal release vary among individuals or agencies and may be either simplified or complicated by demand, competing species, or existing varieties.

Some of the questions regarding multiplication and release may be resolved as follows:

1. A high degree of uniformity is hard to attain in most cross-pollinated grass varieties. Uniformity is not essential, however, provided the variety breeds true with respect to the characteristics for which it was developed (236).

2. Initial comparative tests should be conducted by the breeder who developed the variety. If these tests are turned over to other workers, the breeder should follow them very closely. In the absence of effective cooperation the superior characteristics of new strains may be obscured by faulty management. Initial tests should be continued for 3 to 5 years if some of the experimental strains look promising. Experimental strains should be subjected to a comparative test after each complete cycle of the selection program. In species that develop slowly, strain tests should be maintained for at least 4 years in order to evaluate yield capacity, aggressiveness, and persistence (108).

3. Seed of interesting strains should be increased on a modest scale before the preliminary tests have been completed. If enough seed of the better experimental strains are available these strains should be subjected at an early date to more elaborate experiments (e.g., grazing tests) at the originating station and to comparative tests elsewhere. Small-scale seed increase does not ordinarily pose a serious problem for the plant breeder, except insofar as isolation requirements and facilities limit the number of strains that can be increased at any one time. On the other hand, certain species or strains may not set seed regularly

in the area of forage adaptation. In that case it is advisable to examine the possibility of having seed produced elsewhere.

4. An experimental strain should be subjected to comparative testing at several locations in the region where it was developed. If the testing program conducted at the originating station has been as comprehensive as it should be, however, not more than 2 or 3 new experimental strains need be included in any cycle of regional tests. It is usually desirable to have some one agency assemble and distribute, at regular intervals, seed of the strains to be included in uniform regional tests.

Varietal release based on comprehensive tests at a single location can be justified if the breeding program is directed toward developing strains to meet localized problems, especially if the variety in question is very different from available ones. Decision as to the advisability of releasing a variety is the prerogative of the originating station, but several arguments favor some degree of regional testing prior to release. Experiment stations in the region are asked for their recommendations as soon as the variety is released, but, because of the time involved in evaluating perennial species, several years may elapse before these stations can develop positive recommendations. This delay may lead to confusion that obscures the value of grass improvement.

Regional tests represent a logical effort to capitalize on grass-breeding activities to the fullest possible extent. Varieties that exhibit a wide range of adaptation help to simplify the problem of producing adequate supplies of certified seed. This does not mean that varieties should not be released unless they are adapted over wide areas, but the range of adaptation may determine which of two or more varieties should be released. In addition, an experimental strain may show more promise at other locations in the same region than it does at the originating station. The potential value of such a strain would never be realized if the strain were discarded on the basis of tests conducted over a narrow environmental range.

5. Testing and release of varieties within species are much more complicated than regional testing of new species and their eventual release for commercial seed production. Generally, differences at the species level are such that the potential value of a new species can be established in a relatively short time. Regional tests conducted for about 5 years are sufficient for release of a new species if information on such factors as animal acceptance and persistence under grazing has been obtained at one or more locations.

6. Quantitative data on varietal performance should be analyzed statistically. Some improved varieties are not appreciably different from commercial lots in dry-matter yield, but frequently distinct yield trends in favor of certain new varieties are encountered in individual tests. The differences are generally not significant if probabilities of less than 5 or 1 percent are accepted as indicating actual differences among varieties. On the other hand, if these differences are encountered in numerous tests in different years and at different locations it would appear that



they are real. Conceivably, wider fiducial limits can be accepted in interpreting varietal performance if the performance of some varieties sampled widely in terms of time and location is consistently superior. This would be equally true for quantitative or observational data obtained on any of the characters studied in the evaluation program.

7. Before a variety is released, breeder seed should be increased and, if possible, arrangements should be made to establish fields for the production of foundation seed. Generally, further seed increase of an improved variety through the registered and certified seed classes does not present any major problems if the seed can be produced economically in the area of adaptation for forage. If it cannot, this situation should be established prior to release and the necessary steps taken to ensure an adequate and continuing supply of certified seed from some other area or areas. Information on possible demand will be helpful in interesting other States in producing the seed. Interstate certification provides a basis for proper field inspection and labeling in the seed-producing areas. If environmental factors differ widely between the region of adaptation for forage and that of adaptation for seed production, some attention should be given to limiting the number of generations of seed increase outside the region of forage adaptation. The possibility of differential natural selection in the seed-producing region could prompt the originating station to specify that only 1 or 2 seed generations be produced outside the region of forage adaptation—i.e., only certified, only registered and certified, or only foundation and certified. Sylvén (284) has reported certain unfavorable changes in type in a northern strain of timothy that had been increased for 2 or 3 generations in middle Sweden. These changes were expressed in increased frost susceptibility and lower green-matter yields when the seed was used in northern Sweden.

The National Foundation Seed Project of the United States Department of Agriculture was established in 1948 to facilitate the multiplication of seed of improved forage-crop varieties. Its planning committee includes 2 representatives from each of the 4 regional forage crops technical committees, 2 from the International Crop Improvement Association, 2 from the American Seed Trade Association, and 4 from the Department of Agriculture. Varieties are recommended to the planning committee of the project by regional forage crops technical committees, each recommendation being supported by evidence of superiority. Breeder seed of varieties accepted by the committee is allocated to seed-producing States, in which foundation seed is grown under Government contract. Foundation-seed production is geared to meeting the demand for registered and certified seed. Production goals for a given variety are based on estimates of possible seed use in the region or regions where the variety is known to be adapted for forage. No perennial grass varieties are included in the National Foundation Seed Project at the present time. If lack of certified seed is limiting the usefulness of a new

forage variety, the possibility of having the variety accepted by the project's planning committee should be carefully considered.

8. Varieties should receive distinctive names. Consulting other grass breeders with regard to naming a variety is a commendable practice, since it keeps others informed of developments and may give them their first opportunity to test a variety. New varieties should be registered with the American Society of Agronomy.

9. The success of breeding work is measured by the extent to which the improved varieties are grown. Active extension work is necessary to the wide use of improved strains. Educational programs should emphasize the importance of purchasing certified seed, since there is no other guarantee that seed purchased will be true to type.

Frolik and Lewis (90) have reviewed the nature and importance of seed certification. The certification standards of the International Crop Improvement Association are available in published form. The seed classes recognized in these standards are breeder, foundation (including elite in Canada), registered, and certified. The grass standards include specific information on the increase of grasses according to whether they are or are not cross-pollinated, at least 80-percent apomictic, entirely apomictic, highly self-fertile, or vegetatively propagated. The minimum isolation standards approved by the I. C. I. A. are presented in table 5.

TABLE 5.—*Isolation standards for grass-seed certification by the International Crop Improvement Association, 1954*

Grasses	Minimum isolation distance for producing seed of indicated class		
	Foundation	Registered	Certified
	<i>Rods</i>	<i>Rods</i>	<i>Rods</i>
Cross-pollinated species	80	40	20
Strains at least 80-percent apomictic or highly self-fertile.	10	10	5

Data pertaining to isolation requirements for grass-seed production have been obtained by Bateman (14), Griffiths (99, 100), Hodgson (129), and Jones and Newell (144). Griffiths (100), in England, concluded that seed crops of cross-pollinated forage grasses can be expected to attain the standards of purity necessary for certification even if isolation distances are reduced to 50 yards, provided fields are properly managed. He points out that rigid regulations applied later in multiplication will be of no avail without adequate protection of improved strains during the first stages of increase. He proposes a minimum isolation distance of 200 yards for small plots of breeder seed.

## EXPERIMENTAL TECHNIQUES

### PROPAGATION OF BREEDING MATERIALS

In some naturally cross-pollinated species, potentially valuable germ plasm can be maintained either by inbreeding or by vegetative propagation. Because of its greater simplicity, vegetative increase has been preferred to inbreeding in most grass-breeding programs.

#### VEGETATIVE PROPAGATION

Plants selected for vegetative propagation are usually cloned in the fall or early spring, but this work can be done at any time of year if the plants are healthy and their nutrient reserves have not been depleted. Rooting of cool-season grasses is delayed or inhibited by high temperatures. Limited studies have failed to demonstrate a need for applying plant hormones to stimulate rooting in the grasses (227).

Plant fragments consisting of several tillers each are satisfactory material for vegetative propagation where a small number of propagules are needed and uniformity is not essential—e.g., in greenhouse crossing or in hold-over nurseries. The fragments may be planted in pots or flats or, under good moisture conditions, directly in the field. Individual tillers are preferable if uniformity is essential, large numbers of propagules are required, or the amount of plant material is limited. When a tiller has been separated from the plant its old leaf sheaths are removed and the roots are trimmed away from the cluster of nodes at its base. If the old sheaths and roots were left on the tillers, soft rots might develop. The trimmed tillers may be planted directly in soil, sand, or vermiculite. In order to hasten establishment and to reduce losses and the labor of replanting, it is preferable to root them first in water. Tillers from the same plant can be held together with a heavy elastic band and identified with a wooden pot label. The bundles can then be placed in water so that each tiller will be immersed at all times to a depth of  $\frac{1}{2}$  to 1 inch. Tillers may be rooted in beakers or tin cans if the water is changed 2 or 3 times a day. Use of a shallow galvanized pan equipped with an overflow outlet and supplied with running water reduces the labor involved in rooting them. Two chicken-wire screens, one placed at the bottom of the vessel and the other attached near the top, will serve to keep the bundles erect. To avoid excess root breakage, the tillers should be planted in pots or flats when the new roots are  $\frac{1}{4}$  to  $\frac{1}{2}$  inch long. Stoloniferous grasses may be increased by planting fragments or individual stolons either directly in the field or in pots or flats in the greenhouse.

#### PROPAGATION FROM SEED

Grass seed may be germinated on blotting paper or in sterilized sand, vermiculite, or soil. It is frequently advisable to plant small amounts of seed in finely sifted sterilized soil in petri dishes.

This permits considerable flexibility in subjecting the seed to various temperatures and in the period during which the seedlings should be transplanted. The young seedlings can be held in the petri dishes until they are ready to be transplanted into flats. This is done by gradually adding sifted soil to the petri dishes to support the seedlings and keeping the dishes covered with a cheesecloth canopy to prevent rapid loss of water. McAlister (186) germinated grass seed in shallow trays or pot saucers filled with saturated soil. He placed the seed on the surface of the soil and kept the saucers or trays in a closed chamber until sprouts appeared. Another method is to place the seed on the bottom of a water-soaked clay pot saucer and either cover the saucer with another or place it in a moist atmosphere. Additional water may not be needed. If needed, it can be supplied from time to time with an atomizer or any other device producing a fine spray. Germinated seeds can be transferred with forceps to water-saturated soil. Young seedlings can be separated with a pencil and planted by hand. McAlister (186) favored transferring the germinated seeds, because in his experience it was more rapid than pricking off seedlings and because it might decrease the chance of entrance of damping-off organisms via injured rootlets.

Freshly harvested seed frequently fails to germinate because of dormancy—an adaptation that prevents late-ripening seed from immediately germinating and producing seedlings, which would be liable to frost damage. Seed dormancy exists to some extent in most forage grass species. The native warm-season grasses of the Great Plains exhibit it to a marked degree. The seed of some of these species will not germinate satisfactorily until they have been kept in storage for several months. In order to break dormancy so as to obtain successive generations at short intervals in breeding projects, Sprague (257) suggests daily alternation of contrasting temperatures. He found that the best treatment for seed of *Dactylis glomerata*, *Poa pratensis*, *P. compressa*, *P. palustris* L., *P. alpina* L., and *P. arachnifera* was alternating 10° and 30° or 15° and 30° C., maintaining the lower temperature for from 16 to 18 hours and the higher for from 6 to 8 hours. He suggested that large quantities of field-grown grass seed may germinate satisfactorily if moistened and kept at 10° to 15° for 2 weeks. Kearns and Toole (148) used low-temperature treatments to break the dormancy of freshly harvested fescue seed, and got best results with temperatures approximately the same as those recommended by Sprague (257).

Fresh untreated seed of Indian ricegrass germinates best at 3° C. or if chilled at that temperature for at least 28 days before being placed at a higher temperature for germination (289). Scarification sometimes promotes germination of Indian ricegrass seed (289). In tests by Plummer and Frischknecht (233), the highest laboratory germination of this seed was obtained through scarification either by mechanical means or by treatment with 85-percent sulfuric acid for 60 minutes, and the highest field germination through treatment with 70-percent sulfuric acid for

60 minutes. Storage under moist conditions at temperatures of 3° to 5° for 4 to 10 weeks improved germination of both untreated and acid-treated seed, indicating that both embryo and seedcoat dormancy existed.

The effects of various germination techniques were studied by Dawson and Heinrichs (67) to determine their efficiency in overcoming dormancy in green needlegrass. Untreated seed tested 4 months after harvest gave a germination of only 2 percent over a 4-week period. Seed moistened and then subjected to chilling for 3 weeks at 4° C. before germination had a germination percentage of 42. The highest germination, 58 percent, was obtained after seed scarification in 95-percent sulfuric acid for 10 minutes. According to Laude (172), weakening of the seedcoat of *Danthonia californica* Boland. appeared essential to overcoming delay in germination. The seedcoat in this species did not prevent water absorption, but appeared to delay germination through mechanical restraint or restriction of gaseous exchange. In Laude's experiments field plantings benefited most from acid treatments of considerably shorter duration than those leading to the best results in the germinator. The seedcoat could also be weakened by cutting. Hulling without seedcoat injury was not beneficial.

Wenger (314) observed a marked increase in the germination percentage of mature buffalograss seed when the burs were soaked in water, and a still greater increase when the seed was presoaked for a comparable period in potassium nitrate. The germination of mature seed was increased with scarification or by puncturing the pericarp. The possibility of utilizing embryo culture to by-pass seed dormancy in buffalograss was investigated by Parkey.<sup>20</sup> The results of his experiment indicated that embryo culture would be a practical means of obtaining progenies. The vigor of the seedlings obtained from buffalograss embryos that were cultured at various stages of development varied proportionally with the age of the embryos. Seedlings from cultures of 10- and 15-day-old embryos were not so vigorous as those from cultures of embryos that were excised at more mature stages of development.

Considerable information on germination equipment and technique is available in published form (298). In the standardized regulations light is specified for all grasses except napiergrass, canarygrass, and certain fescues. The sensitiveness of seed—e.g., timothy seed—to temperature and light may change with age (94).

#### PLANTING

Under most circumstances 6-inch clay pots are satisfactory for growing forage grasses in greenhouses. Generally 2 to 4 tillers, seedlings, or germinated seeds are planted in each pot, and after the plants have become established any in excess of 2 per pot are removed. In using 4-inch clay or glazed pots, it is preferable to

<sup>20</sup> PARKEY, W. CERTAIN ASPECTS OF EMBRYO CULTURE AND PROGENY EVALUATION IN BUFFALOGRASS. 48 pp., illus. 1962. [Unpublished doctor's thesis. Copy on file Library, Univ. of Nebr., at Lincoln.]

leave only 1 plant per pot. Nitrogen or a complete fertilizer should be supplied as needed, the amount depending on the potting mixture and the growth of the plants. Insecticides such as nicotine and parathion can be used for controlling aphids and red spiders, respectively. Sprinkling sulfur on steam pipes reduces the incidence of rust and mildew. For late-spring and early-fall greenhouse plantings it is frequently advisable to reduce light intensity and temperatures by spraying the outside of the glass with whitewash or applying walnut dust on the inside. Walnut dust may be applied at any intensity as a spray, and a hose equipped with a nozzle attachment can be used to reduce or remove the covering.

Tillers or seedlings for transplanting to the field may be started in flats with or without plant bands. In the absence of plant bands a board with pegs set at any given interval (e.g., at  $1\frac{1}{2}$  by  $1\frac{1}{2}$  inches or 2 by 2 inches) can be used to mark flats for planting. When the plants are ready to be set out they are cut back to about  $2\frac{1}{2}$  inches and the soil is cut in both directions between them. At planting, the moist soil around the roots is firmed by hand.

McAlister (186) studied the usefulness of plant bands made from several kinds of paper. He tried No. 10 asphalt building paper; plain, oiled, and paraffined 50-pound kraft wrapping paper; and plain and paraffined newsprint. Bands  $\frac{3}{4}$  by  $\frac{3}{4}$  by  $3\frac{3}{4}$  inches of untreated newsprint proved satisfactory for grass seedlings. They were made by wrapping paper around a block,  $\frac{3}{4}$  by  $\frac{3}{4}$  by 5 inches, that had sharp metal edges for separating the paper. The plants were grown in bands for at least 8 weeks. By this technique, 9,000 seedlings were started in flats that occupied a space 4 by 12 feet on a greenhouse bench. Plant bands can be recommended when plants are to be held in flats for prolonged periods. Generally, bands are not removed at planting time. Under unfavorable planting conditions they may have an added advantage because they hold moist soil around the roots.

Horn-shaped, metal-tipped dibbles may be used to dig holes for planting, but this may tend to make planting too shallow. Short-handled hoes may be more satisfactory, particularly in heavy soils. McAlister (186) describes a dibble developed by J. W. Carlson that consists of an iron pipe  $5\frac{1}{2}$  feet long with a closed, bluntly pointed lower end and a 5-inch collar welded in position  $3\frac{1}{2}$  inches from the shoulder of the point. The collar serves to regulate the depth of the hole. Planting implements that remove a small soil core have been devised.

### INDUCTION OF FLOWERING IN THE GREENHOUSE

Heading and flowering of perennial forage grasses under greenhouse conditions during the winter months are dependent upon three major environmental factors: Day length, temperature, and soil fertility. Peterson and Loomis (230) studied the response of Kentucky bluegrass to different photoperiods and temperatures. Development of shoots was increased by short

days; carbohydrate accumulation was greatest when temperatures were low and photoperiods short. The investigators concluded that the induction of flowering conditions in bluegrass occurs during the fall season at normal cool temperatures and short day lengths. Flower development, after the induction period, was favored by longer photoperiods and moderately low temperatures.

Sprague (258) investigated the length-of-day requirement for perennial ryegrass, orchardgrass, timothy, meadow fescue, smooth brome grass, Canada bluegrass, and Kentucky bluegrass. He took field-grown material into the greenhouse in September or early October and established individual tillers at temperatures of 68° to 72° F. under normal day lengths—9½ to 10½ hours. Plants of each clone were subjected to a series of low-temperature treatments during the month of December. Best heading was obtained by subjecting plants to a 16-hour day or to a 10-hour day plus 1 or 2 hours of light during the middle of the night. Daylight was supplemented by using Mazda lamps. Little, if any, increase in number of panicles per clone was obtained by using light intensities above 75 foot-candles. Low-temperature treatments prior to the long-day treatment did not generally increase the number of clones flowering or shorten the time required for flowering. Adequate soil fertility proved to be an important factor in producing satisfactory heading.

Evans and Aillard (79) state that the later a forage grass strain flowers under natural conditions, the greater is the day length the plants require for normal development. Evans and Wilsie (73) found that an early maturing brome grass clone produced approximately the same number of panicles per plant when grown under 15- and 18-hour day lengths at temperatures of 65° and 75° F., respectively. The data suggest that northern brome grass strains will flower under a fairly wide range of temperature and soil fertility when exposed to a day length of 18 hours. The southern strain included in this experiment required higher fertility and higher temperatures for optimum flowering. Newell (213) concluded that brome grass plants require a period of growth under short-day and cool-temperature conditions before being subjected to a long photoperiod, if they are to produce panicles. The supply of available nitrogen during the short-day period influences the rapidity of growth and the abundance of tillering.

Hanson and Sprague (107) investigated the heading of orchardgrass, meadow fescue, brome grass, reed canarygrass, and timothy under greenhouse conditions. Plants were brought into the greenhouse in early September, and individual tillers were rooted and planted in pots. Exposure to a short photoperiod, at a temperature of 55° F., of plants that were subsequently exposed to a long photoperiod had a marked effect on time of initiation of floral primordia, heading, and flowering except in timothy. The results indicate the following procedure for handling several long-day species in the same greenhouse section: (1) Bring plants into the greenhouse in early fall for multiplication; (2) supply

adequate fertility to promote good vegetative development at normal temperatures; (3) subject plants to continuous low temperature, approximately 55°, for 4 or 5 weeks; (4) increase day length to 15 or 16 hours and raise temperature to 70°; (5) apply moderate amounts of supplementary nitrogen at intervals indicated by plant growth.

Knight and Bennett (161) have investigated the effect of photoperiod and temperature on the flowering and growth of five southern grasses. Seedlings and cuttings of each grass were transplanted into half-gallon glazed pots and grown in them for 2 months at temperatures of 55° to 65° F. before being subjected to the long-day treatment. Johnsongrass clones and seedlings were in the boot stage when cut back and placed under light. In relation to earliness of flowering, the hours-of-light treatments ranked in this order: 8, 10½ (normal), 12, 14, 16. The 16-hour day length inhibited seed-head formation in johnsongrass seedlings. Some flowering occurred in carpetgrass (*Axonopus affinis* Chase) under all day lengths. The 12- and 14-hour day lengths were the only ones under which all plants flowered. Dallisgrass plants grown under the 14-hour day flowered earlier and produced more panicles than those grown under the 16-hour day. In general, the plants grown under the 14-hour day produced a higher percentage of florets containing caryopses than those subjected to the 16-hour day. Bahiagrass flowering was confined to plants grown under the 14- and 16-hour day lengths. Bermudagrass failed to flower under the photoperiods provided, perhaps because of growing conditions other than photoperiod. Night temperatures below 55° tended to inhibit flowering in all 5 species. The vegetative characteristics of plants grown at low night temperatures resembled those of plants grown under normal night temperatures and an 8-hour day.

In order to facilitate crossing under greenhouse conditions, it is advisable to have each clone represented in more than one light series. With a given long-day species, 3 light series separated by 10 to 14 days in the time when the extended photoperiod is initiated are useful in obtaining crosses between plants that do not flower simultaneously under one long-day treatment. Light series are readily separated with black sateen curtains. Photoperiods are extended effectively and economically by subjecting plants to 1 hour of supplementary light in the middle of the night.

## ISOLATION AND HYBRIDIZATION

### SPATIAL ISOLATION

In forage grass breeding projects distance serves to isolate selections of most species that are naturally cross-fertilized, and it can be used very successfully for isolation purposes in special studies that require large amounts of inbred or single-cross seed. Distance is the principal means of isolation, also, in producing synthetic strains and ensuring the varietal purity of strains that have been released. It is hard, however, to obtain sufficient isola-



tion for a large number of lines that are to be either selfed or crossed.

Jones and Newell (144) studied pollen dispersal in brome grass, crested wheatgrass, intermediate wheatgrass, buffalograss, switchgrass, rye, and maize, using microscope slides covered with glycerin to catch pollen at various distances and in various directions from grass plots. They found striking differences in the amounts of pollen produced by the various grasses. Rye and brome grass produced the larger amounts. A somewhat larger part of the pollen of these 2 grasses was disseminated to distances of 5 to 15 rods, but the amount of pollen caught at 60 rods as related to that caught in the center of the field was about the same for each of the grasses. Pollen dispersal varied considerably according to the direction of prevailing winds. The data obtained suggest that the chance of maintaining genetic identity is much greater when seed is produced under an isolation of 60 rods or more than when the isolation distance is only 25 or 40 rods.

Griffiths (99) studied pollen dispersal in *Lolium perenne* by using non-red-stemmed plants (*c c R R*) for markers. Contamination decreased rapidly up to a distance of 300 yards, except that beyond 200 yards the effect of additional isolation was negligible when 30 or more plants were grown together. Griffiths suggests an isolation distance of 200 yards (36 rods) for this species. Apparently fertilization was effected chiefly by pollen from neighboring plants. In the same species, Wit (319) used the presence or absence of small downward directed teeth on the culms and upper leaf sheaths in progenies as an indicator of pollination habits. In his experiment simultaneousness of flowering often had a greater effect on the percentage of crossing than nearness of the plants. Grunder and Dermanis (102) studied the effect of a limited number of pollinator plants on seed set in a relatively self-sterile strain of orchardgrass, plants of which were set 18 inches apart in 3-foot rows. Plants within  $11\frac{1}{2}$  yards of the pollinators averaged 62.4 seeds per panicle, while those at greater distances up to  $11\frac{1}{2}$  yards averaged 27.2 seeds per panicle.

Obviously, the correct isolation distance depends largely on the species, on maturity differences among strains, and on the number of plants in each isolation block. It is affected also by the direction of prevailing winds and by the crops separating crossing blocks.

#### ISOLATION UNDER BAGS

Various types of cloth cages, bags, or tents and various types of paper bags have been used to isolate grasses for controlled pollination. Jenkin (184) concluded that cotton fabrics were unsatisfactory under the conditions of his experiments. At present, most investigators use either parchment or kraft paper bags or sleeves. Bags and sleeves to be used under field conditions should be assembled with waterproof glue. Keller (151) concluded that for smooth brome grass paper bags of 35-pound parchment were more satisfactory than those of 50-pound bleached kraft,

13-pound parchment, or 40-pound brown kraft. Seeds produced under 35-pound parchment and 50-pound bleached kraft were heavier than those produced under 40-pound brown kraft and 13-pound parchment. A relationship was found between weight of seeds and their ability to germinate. Measurement of penetration of light and air through the different papers and of evaporation of water from vials enclosed in the bags failed to reveal any characteristics that might account for the results obtained. Myers (205) found that vegetable-parchment bags 2 by 4 by 12 inches were satisfactory for selfing and crossing orchardgrass. Keller (150) used 3- by 25-inch bags. There has been little standardization in the size of bags, because of the range in inflorescence size found in the grasses.

Wire or bamboo stakes are used to support bags, in the field or the greenhouse; 1½- by 1½-inch wooden stakes are used by some workers to support parchment sleeves. Myers (205) describes a method for applying vegetable-parchment bags. In an upper corner of each bag eyelets are placed, and a string is passed through these to secure the bags to bamboo stakes. After the heads are inserted in the bag the bottom is closed around the culms and fastened to the stake by means of a copper-wired wooden tree label or, in the greenhouse, a paper label. Keller (150) supported bags in the field with stakes of No. 9 galvanized wire. The wire stakes had a 1¾-inch loop at the top, which was inserted into the bag with the panicles. The bottom corner of the bag was folded over and fastened with a paper clip.

Some workers have inserted cotton pads at the base of the bag to prevent damage to the stems and to improve air circulation. Jenkin (132) has used such pads in attaching parchment sleeves to plants. Inflorescences to be selfed are fitted with a cotton-wool pad, and the pad on the culms and that on a bamboo stake are then tied together with bast. The sleeve is slipped over the stake and the heads and is then made secure at the top and over the cotton-wool pads at the bottom by means of four firm string ties. The sleeve is tied to one of the nodes, to prevent slipping and sagging. Myers (205) found that the use of cotton to protect culms had no appreciable effect on seed set in orchardgrass.

Removal of the flag leaf from culms that are to be enclosed in isolation bags may help to keep down relative humidity.

Gregor and Sansome (98) concluded that use of isolation bags did not cause sterility, because plants found to be self-sterile under bags were likewise self-sterile when isolated by time of flowering. Cheng (49) found a high correlation between seed set under bag and seed set in isolation. Nilsson (221) concluded that the bag had only a small influence on seed set. Myers (205) found that selfed seed set in orchardgrass did not vary significantly according to whether 1, 2, or 4 panicles were enclosed in each bag, but that it was less if 8 panicles were enclosed in each bag. Under the conditions of Myers' experiments, the early panicles set a significantly larger average number of seed than the late ones. Myers remarked that factors contributing to variation in

seed set under bags could include variation in panicle size (and, hence, in number of florets per panicle), injury to culms during bagging, and differences in time of flowering among panicles of the same plant. He suggested that critical comparisons should not be based on a few determinations.

The type of isolation to be used depends on the results desired and the number of plants or panicles involved. Where absolute isolation is desired, vegetable-parchment bags or sleeves have proved to be most satisfactory. The choice between sleeves and bags depends in part on the species, the amount of seed desired, and local conditions (particularly, wind and hail).

In either selfing or crossing, plants should be bagged before any anthers have extruded. This generally entails bagging panicles 2 or 3 days before anthesis. As parchment bags tear very easily, it is advisable to prepare them for use by soaking each bag in water to a third of its length from the open end. This enables the operator to tie the bag firmly around the culms, with a wire label in the field or with a paper label in the greenhouse. Soaking is not necessary when the bags are to be secured by folding over the bottom and applying a paper clip. Plants should be checked periodically after bagging to prevent the culms from growing through the top of the bag or forcing the bag off the heads. This difficulty can be prevented by using larger bags or sleeves.

Stapledon (266) has suggested the use of small greenhouses as a substitute for bagging in crossing large numbers of grass plants. The houses are kept tight during pollination, and the panicles are agitated by means of cords that are pulled from the outside.

#### EMASCULATION

Although a large majority of the forage grasses have perfect flowers, a few species are dioecious, e.g., *Buchloë dactyloides* and *Poa arachnifera*. The male flower contains from 1 to 6 stamens (usually 3), each bearing 2-celled anthers on slender filaments. The pistil has 1 cell with 1 ovule. There are usually 2 styles and 2 feathery stigmas (occasionally 1 or 3). The florets are arranged principally in spikelets on the rachilla and are enclosed by the lemma (flowering glume) and palea. The two lowest bracts of the spikelet are glumes if present; one or both may be absent. Different species flower and shed pollen at different times of day; e.g., *Dactylis glomerata* and *Phleum pratense* bloom in the early morning and *Bromus inermis* normally blooms in the late afternoon. It is self-evident that the grass breeder should familiarize himself with the blooming habits of the species in which he is interested.

Blooming begins near the apex of the inflorescence and progresses toward the base. The basal florets of a spikelet open first and are followed in regular order by those above. Temperature, humidity (to a lesser extent), and other environmental conditions influence the time of day of blooming, the period of pollen dispersal, and the amount of pollen shed. Individual plants

and inflorescences of different species normally shed pollen over different lengths of time. Several workers have reported on the time required for all the flowers in a single inflorescence to bloom (17, 75, 143, 144, 322). Some of the results obtained in Nebraska by Jones and Newell (144) and a few obtained by other investigators are presented in table 6.

TABLE 6.—Duration of pollen-dispersal period and time of day of blooming and pollen shedding for 34 forage grasses<sup>1</sup>

Species	Average pollen dispersal period per inflorescence	Time of day of blooming and pollen shedding
<i>Days</i>		
Cool-season grasses:		
<i>Agropyron cristatum</i> (L.) Gaertn.	8	2 to 6 p. m. <sup>2</sup>
<i>A. elongatum</i> (Host) Beauv.	7	2 to 6 p. m.
<i>A. intermedium</i> (Host) Beauv.	10	2 to 6 p. m. <sup>2</sup>
<i>A. repens</i> (L.) Beauv.	7	2 to 6 p. m.
<i>A. smithii</i> Rydb.	7	2 to 6 p. m. <sup>2</sup>
<i>Alopecurus carolinianus</i> Walt.	4	5 to 8 p. m.
<i>Arrhenatherum elatius</i> (L.) Presl	7	3 to 7 p. m.
<i>Bromus inermis</i> Leyss.	10	2 to 7 p. m. <sup>2</sup>
<i>B. inermis</i> Leyss. <sup>3</sup>	7.2	
<i>Dactylis glomerata</i> L. <sup>4</sup>	6.7	
<i>Elymus junceus</i> Fisch.	7	3 to 7 p. m.
<i>Festuca arundinacea</i> Schreb.	9	1 to 6 p. m. <sup>2</sup>
<i>F. elatior</i> L.	7	3 to 8 p. m.
<i>F. ovina</i> L.	6	4 to 8 p. m.
<i>Koeleria cristata</i> (L.) Pers.	7	3 to 7 p. m.
<i>Phleum pratense</i> L.	10	4 to 9 a. m.
<i>Poa pratensis</i> L.	8	3 to 8 a. m. <sup>2</sup>
<i>Secale cereale</i> L.	8	3 to 11 a. m. <sup>2</sup>
Warm-season grasses:		
<i>Andropogon gerardi</i> Vitman.	7	4 to 7 a. m. <sup>4</sup>
<i>A. hallii</i> Hack.	8	4 to 9 a. m.
<i>Bouteloua curtipendula</i> (Michx.) Torr.	8	4 to 9 a. m. <sup>2</sup>
<i>B. curtipendula</i> (Michx.) Torr. <sup>4</sup>	8.1	
<i>B. gracilis</i> (H. B. K.) Lag.	6	3 to 9 a. m. <sup>2</sup>
<i>Buchloe dactyloides</i> (Nutt.) Engelm.	6.4	6 to 11 a. m. <sup>2</sup>
<i>Cynodon dactylon</i> (L.) Pers. <sup>3</sup>	3.1	
<i>Eragrostis curvula</i> (Schrad.) Nees <sup>2</sup>	10.5	12 to 8 a. m.
<i>B. trichoides</i> (Nutt.) Wood	7	7 to 11 a. m.
<i>Panicum virgatum</i> L.	12	10 a. m. to 2 p. m. <sup>2</sup>
<i>Phragmites communis</i> Trin.	10	6 to 10 a. m.
<i>Sorghastrum nutans</i> (L.) Nash	8	6 to 10 a. m.
<i>Sorghum halepense</i> (L.) Pers. <sup>2</sup>	9.5	9 a. m. to 3 p. m.
<i>S. vulgare</i> Pers.	10	4 to 10 a. m.
<i>S. vulgare</i> var. <i>sudanense</i> (Piper) Hitchc.	10	6 to 12 a. m.
<i>Spartina pectinula</i> Link.	10	7 a. m. to 4 p. m. <sup>4</sup>

<sup>1</sup> Table based on observations by Jones and Newell (144) in Nebraska in 1946 except as otherwise indicated.

<sup>2</sup> Observations confirmed by data from slides exposed to pollen.

<sup>3</sup> Observations by Jones and Brown at Stillwater, Okla. (149).

<sup>4</sup> Observations by Wolfe at Blacksburg, Va. (322).

<sup>5</sup> Indeterminate.

Emasculation is unnecessary if plants can be isolated that are either completely male-sterile or completely self-incompatible. Male-sterile plants have been observed in several of the perennial forage grasses (34, 146, 226, 300). Such plants are generally characterized by small yellow anthers that do not dehisce. Variation in the expression of male-sterility is encountered in certain plants, and a few late-maturing heads may shed pollen in plants that are otherwise fully male-sterile. The expression of partial male-sterility can be varied within limits by various combinations of temperature and humidity.<sup>21</sup> Nordenskiöld (226) has pointed out that male-sterile plants may be more or less pollen-sterile according to external conditions. She induced male-sterility in male-fertile plants of *Phleum commutatum* Gaud. by subjecting them to temperatures of 0° to 5° C. in a dark room just before they flowered.

#### Hand Emasculation

Hand emasculation is a slow, tedious process, generally employed only if one is working with a limited number of panicles or if pollen competition and selfing must be reduced to a minimum. It can be used very effectively in making interspecific and intergeneric crosses.

Compact inflorescences require thinning before emasculation is attempted. This leaves the remaining florets more accessible and reduces the chance of overlooking florets. In addition, greater uniformity in maturity can be obtained by removing the topmost and lowest spikelets of the inflorescences and the terminal florets of individual spikelets. The heads should be worked from the tip in a systematic manner in order to reduce handling of emasculated flowers and avoid further injury to them. To remove anthers, forceps are inserted between the lemma and the palea. Under no circumstances should the points of the forceps be sharp, since anthers are easily punctured. Plants having large anthers can be emasculated without use of a magnifying glass, but for many species binocular loops or horizontal-arm binoculars are indispensable in this operation. Commonly, the anthers should be removed at least 1 or 2 days before anthesis would ordinarily begin; the longer one waits, the easier is removal of the anthers but the greater is the danger of contamination.

Beddows and Davis (18) have suggested that separation of the palea be started with a slight lateral pressure of the thumbnail and completed with forceps and that if there is any risk of damage to the anthers the floret should be discarded or its ovary taken out. They also suggest that the spike be bent over so that it lies without strain along the first finger of the left hand, and that the forceps be held pencil-fashion, almost perpendicular to the spike and partly supported by the outstretched middle finger of the left hand.

Jenkin (134) found that the very small florets of *Phalaris* could be emasculated by removing anthers as they were extruded

<sup>21</sup> Unpublished observations of V. G. Sprague and A. A. Hanson.

during anthesis. This technique is based on the observation that the anthers of certain species exhibit delayed dehiscence. Keller (154) mentions that artificial fogs have been used to retard dehiscence and thereby afford time to remove anthers as they are extruded. Continuous emasculation is an interesting technique, but considerable contamination may result from broken anthers or faulty timing.

#### Bulk Emasculation

Bulk emasculation with hot water, a procedure developed by Stephens and Quimby (275) for sorghum, has been applied successfully to rice (138) and many forage grasses (154). The technique is based on the fact that the thermal death point of pollen is slightly lower than that of the ovary. It is especially useful where large numbers of emasculated panicles are desired for hybridization. Domingo (69) reported that when applied to bromegrass it gave more consistent results than hot-air treatments. The success of emasculation in his study was measured by the difference in seed set between treated panicles that were selfed and those that were exposed to atmospheric pollen. The most effective temperatures for bromegrass appeared to be 46° and 47° C. Tsiang (292) found that bulk emasculation of bromegrass was effected rather satisfactorily by hot-water treatment of 47° for 3 minutes or 48° for 1 minute. Knowles and Horner (164) suggest a treatment of 48° for 1 minute to emasculate crested wheatgrass.

Clark (52) applied hot-water treatments of 45°, 46°, and 47° C. for 5 minutes at 1-hour intervals from 6 a. m. to 6 p. m. His results showed, for the grasses studied, that treatments applied at midday were less injurious to the plants than those applied in early morning or late afternoon. At 6 a. m., 45° appeared as effective for emasculation as 47° was at noon. Five methods of controlled pollination were tested after bulk emasculation of bromegrass, crested wheatgrass, and western wheatgrass. None of the methods used led to production of satisfactory seed yields: as similarly treated material gave satisfactory seed yields after exposure to continuous wind pollination, however, it was concluded that factors other than the method of emasculation were responsible for this.

Bulk emasculation can be conducted with simple equipment—a wide-mouthed thermos jug, an accurate thermometer, and a wire loop for stirring the water in the jug. A small gasoline stove for heating water and a supply of cold water for making rapid temperature changes are necessary for large-scale emasculation. Containers with thermostatically controlled heating coils are helpful (85). Treated panicles should be allowed to dry before being bagged.

Although the method is simple, several variables may contribute to erratic results. Satisfactory emasculation depends in part on the age of the florets treated; therefore, in some species in which florets on a single panicle vary widely in age the youngest and possibly some of the oldest florets should be removed. Time of day is important, likewise the compactness of the

panicle and the thickness of the glumes. Hot-water emasculation should not be attempted without careful preliminary tests.

#### POLLINATION

##### Mutual Pollination

Mutual pollination is the most simple and rapid technique for crossing plants. The practice is based on the observation that most grasses are highly self-incompatible. Available evidence indicates that differential fertilization exists in many grasses and that very little selfed seed is set even on highly self-compatible plants if an ample supply of foreign pollen is present. Reciprocal progenies resulting from mutual pollination are often uniform (219). Mutual pollination can be used in making crosses between species or genera, but selfing may be appreciable in the absence of compatible pollen.

Inflorescences at approximately the same stage of development are bagged together before anthesis. They should first be examined carefully to ensure that no florets have already bloomed. After anthesis, bags should be tapped occasionally for several days to scatter the pollen.

In general this method of crossing is more dependable under greenhouse conditions than in the field, for in the field slight differences in maturity can result in failure or at least contribute to an increase in proportion of selfed seed. Mutual pollination is a very satisfactory method of obtaining single-cross seed for tests in which a small amount of selfing would be of little consequence. Reciprocal crosses are obtained from each bag if the seed harvested from individual plants is kept separate.

Burton (35) dug rooted plants of Pensacola bahiagrass that had developed panicles and were due to flower the following day. The plants were labeled, the soil was washed from their roots, they were placed immediately in a bucket of water, and the inflorescences were bagged. Mutual pollination was relied on to effect crosses. The bagged plants were placed in a cool, moderately light location in the laboratory, where they remained until the seed was mature.

##### Pollen Transfer

Bag transference or pollen guns can be used for pollination of either emasculated or unemasculated florets. Domingo (69) and Knowles and Horner (164) used bag transfer to pollinate emasculated florets. The panicles of the male parent are bagged, and after anthesis the bags are shaken to collect pollen. The bags are then removed, placed over the female panicles, and fastened to the stakes that supported the original isolator bags. The pollinating bags are then agitated to spread the pollen inside. If sufficient material is available, emasculated florets can be pollinated very rapidly by enclosing entire panicles of the male parent in the bag protecting panicles of the maternal parent. The necessity of having the two parent plants side by side in order to pollinate either emasculated or unemasculated florets by

this method may be circumvented by techniques suggested by Keller (150) and Burton (35). Keller (150) achieved satisfactory results by cutting the male panicle, placing its stem in a test tube of water, attaching the test tube to the stake that supported a female panicle, and then enclosing both panicles in a bag.

The use of either entire heads still attached to the male plant or detached panicles as suggested by Keller is superior to most hand-pollination procedures. As Jenkin (132) has mentioned, however, this technique does not enable the hybridizer to detect unemasculated florets.

Pollination of emasculated inflorescences should be delayed until the stigmas have extruded.

Jenkin (132) warns that these precautions should be taken in pollinating grasses by hand: (1) All inflorescences that are not to be used should be kept cut back, so that no free pollen will be produced within the greenhouse; (2) pollination should not follow soon after flowering; (3) the greenhouse should be closed down for some time before the work of pollination begins; (4) the house should be kept as nearly draft proof as possible during pollination; (5) units, both male and female, should be exposed only for the briefest possible intervals.

It is advisable to use extreme caution in delaying pollination. The viability of the pollen may decline, with a corresponding decline in seed set. This problem may be overcome by pollinating emasculated florets in a section of the greenhouse where no grass is flowering.

Collected pollen can remain viable for several days if stored at low temperatures and high humidity. Jones and Newell (145) found that buffalograss pollen may remain viable for a week if stored at 40° F. and 90-percent relative humidity. If the pollen is left on the spike the time may be extended for a day or so. They found that some stigmas remained receptive up to 19 days but that maximum seed set was obtained by pollinating 5 days after anthesis began.

If pollen is plentiful, Jenkin suggests the following method of hand pollination: The bagged male panicle is held horizontally and shaken, the bag is then removed, the pollen is emptied onto a creased sheet of paper, and a soft brush is used to apply the pollen to the stigmas of the female parent. Soft brushes are recommended so that the pollen will not be violently scattered during pollination. Vinall and Hein (303) state that W. J. Sando prefers tweezers for applying pollen. When pollen is limited it may be collected from several panicles and brushed onto the stigmas with tweezers. A bouquet of male parent plants may be collected in the evening, placed in a vial of water, and set on a sheet of paper where it will be exposed to direct sunlight the following morning. In the morning, pollen is collected on the paper by gently tapping the panicles. This is stored in a vial and applied with a brush.<sup>22</sup>

Regardless of the method of applying pollen, it is advisable to

<sup>22</sup> Unpublished memorandum of J. R. Harlan.



make a second application in order to increase seed set. More than two pollinations are probably not justified by the additional amount of seed that would result.

### HARVESTING SEED

Seed of bagged plants is generally harvested when the culms have turned brown just below the head. For species the seed of which shatter very readily, harvest may have to be somewhat earlier. Selfed seed is harvested by clipping the culms and removing the bag from the stake. The heads can be left in the bags to dry or removed and placed in coin envelopes. Heads of plants that have been mutually pollinated should be harvested promptly, to avoid loss of identity through breaking of culms. Such heads should be placed individually in labeled envelopes at harvest, so that they can be kept identified. Special attention should be devoted to harvesting seed from emasculated heads, to avoid loss of seed.

In the field, seed from individual plants or replicated clones may be harvested by cutting the culms with a sickle just low enough to include all the heads. The heads can then be placed in a cotton bag, labeled, and allowed to dry in a sheltered place.

Some shattering from the apex of the earliest heads provides a satisfactory guide for the field harvest of bulk seed.

Data are available indicating that immature seed of forage grasses may justifiably be harvested under certain conditions. The viability and longevity of immature seed may vary among species. Gruber (101) noted that seed of most of the forage grasses he studied were viable if harvested 14 days after flowering. McAlister (185) investigated seed maturity of *Agropyron cristatum*, *A. smithii*, *A. trachycaulum*, *Bromus inermis*, *B. marginatus*, *B. polyanthus* Scribn., *Elymus glaucus*, and *Stipa viridula*. In 1937 he harvested seed at the premilk, milk, dough, and mature stages of development. He conducted soil germination tests in the greenhouse with seed that had been stored for 4, 9, 15, 22, 40, 51, and 58 months, and made field plantings in 1938, 1939, and 1940. In the greenhouse tests, seed of most of these species harvested at the premilk and milk stages proved inferior in most instances in both viability and longevity to seed harvested in either the mature or the dough stage. However, seed of *Bromus marginatus* collected in the milk stage and seed of *B. polyanthus* collected in the milk or even in the premilk stage gave as high germination during the entire storage period as did mature seed of these species. Dough-stage seed were similar in viability and longevity to mature seed in all species. Under field conditions, seedling emergence from immature seed was generally inferior to that from seed harvested at maturity. The only immature seed that gave as large a percentage of seedlings as mature seed during the 3 years following the seed collection were dough-stage seed of *B. marginatus*. No differences in size or relative survival could be detected by the end of the

seedling year between plants produced from mature and immature seed, respectively.

Hermann and Hermann (124) collected *Agropyron cristatum* seed 9 to 12 days after anther exsertion. None of the seed harvested at 9 days germinated, but seed harvested at 12 days germinated in low percentages. Bennett (20) found that the embryo of *Paspalum dilatatum* is mature 14 to 18 days after pollination and suggested harvesting seed earlier than usual in order to obtain seed of better quality. These examples suggest that early harvesting may well be resorted to with certain species in order to avoid loss of valuable seed from shattering during harvesting operations.

Keller (149) has reported that several forage grasses will mature viable seed if culms are detached before pollination, the cut ends are placed in tap water, and the inflorescences are exposed at anthesis to an appropriate pollen source. In his tests, seed developed on detached culms weighed about 40 to 83 percent as much as seed matured on intact culms. Germination of the seed was reasonably good for all species except *Bromus inermis*, for which it ranged from 25 to 35 percent.

### THRESHING AND PROCESSING SEED

Individual heads from selfed or crossed plants of forage grasses may be threshed by placing them on a sieve of appropriate size and rubbing them with a large rubber stopper. The sieve openings should allow seed to drop through to a finer sieve fastened below. When the lower sieve contains all the seed, it should be held over a revolving fan until the chaff has been blown out. A small seed lot can also be threshed very satisfactorily with a piece of corrugated rubber floor matting and a rubber stopper.

To reduce the amount of hand labor involved in threshing individual heads, several workers have adapted Waring blenders to thresh small seed lots by fitting the agitator with either rubber or leather flanges (63). A South Dakota blower can be used to clean the seed. Several modified seed blowers have been used in analyzing grass seed and cleaning small seed lots (29, 177). Excellent illustrations of the South Dakota and other seed blowers are available in United States Department of Agriculture Handbook 30 (298).

Seed from replicated clonal nurseries or isolation blocks may be threshed in a hammermill and cleaned with a small clipper. A modified hammermill suitable for threshing grass seed has been described by Kneebone and Brown (160). In modifying a hammermill for threshing seed, it is important to provide some mechanism for adjusting the speed of the machine. The mill described by Kneebone and Brown had a range in adjustment from 640 to 3,880 r. p. m.

Hammermills may be used also for removing awns and appendages from seed. For this purpose speeds of about 1,000 r. p. m. and slightly larger screen sizes are recommended.

Although seed may be processed very satisfactorily with a hammermill, it is possible that processing will have an adverse effect on the seed's capacity to germinate either immediately or after warehouse storage. Schwendiman and Mullen (243), investigating results of using a hammermill to remove appendages from seed of tall oatgrass, found that completely dehulled seed suffered a considerable loss in viability during the first 14 months as compared with seed not thus processed. Their results did not, however, indicate that moderate processing of the seed of tall oatgrass causes a rapid decline in its germination. Seed of this species milled twice immediately after harvest at controlled rates of speed and with the correct screen was safely stored until the second season following production without a substantial loss in viability.

The cottony filaments on rough seed of *Poa pratensis* may be removed by partly filling small containers with the seed, adding limestone fragments, and agitating the containers at a very moderate speed.

### NOTE-TAKING SYSTEMS

With the exceptions of heading and flowering dates, observations on grass characters are usually recorded as numerical ratings or as percentages. Actual measurements may be taken on characters such as height, spread, and leaf area. Although uniformity in note taking among grass-breeding programs is not essential, it gives certain advantages, especially when data from two or more stations are summarized with a view to characterizing regional responses of clones or selections. Newell and Tysdal (215) have suggested numerical expression of characters. Strong, vigorous growth, for example, could be recorded as 1, medium growth as 5, and weak growth as 9. Verbal equivalents of the numerical ratings with regard to any one plant character could be 1, excellent; 3, good; 5, medium; 7, fair; 9, poor. These investigators point out that it is not necessary or desirable to use the full scale of 1 through 9 for every particular set of readings. Various symbols and diagrams can be used to condense the expression of several characters in a single note.

In studying crown rust in *Festuca elatior*, Kreitlow and Myers (170) used a modification of Murphy's classification for infection types of crown rust of oats. They scored plants as follows: 1, immune (no macroscopic evidence of infection); 1, highly resistant (no uredia, or uredia few, small, always in necrotic areas, necrotic areas often produced without uredia); 2, moderately susceptible (uredia fairly abundant, small to midsize, always in necrotic or chlorotic areas); 3, susceptible (uredia abundant, midsize to large, with or without necrosis or chlorosis immediately surrounding the uredia).

Scores are frequently useful in classifying large numbers of plants for yield. Burton (31) found that some inexperienced individuals could satisfactorily score yield on a scale of 1 to 5. Individuals differed significantly not only in scoring ability but also in ability to improve their scoring under training. Scores

are very useful for characterizing plants with respect to retention of green color, leafiness, spring and fall vigor, and recovery after clipping.

Height may be recorded as the length of the longest culm from the surface of the soil to the tip of the head or as the distance from the surface of the soil to the level reached by the tips of about 50 percent of the heads.

The relative number of green leaves can be established visually or by sampling individual plants or plots. In either case, some method must be chosen for recording partly green leaves. A partly green leaf may be defined, for example, as one in which the tissue remains green across the entire width of the base of the blade but exhibits browning elsewhere.

Leaf area is a good measure of quality and yield of forage. Anderson and Aldous (6) calculated the leaf area of individual plants of *Andropogon scoparius* by multiplying average length of leaf by average width, by average number of leaves per culm, by total number of culms per plant. These computations gave values that slightly exceeded the actual leaf area but that were considered satisfactory for comparative purposes. In general, such detailed measurements are not practical.

The relative maturity of plants can be recorded by assigning dates for one or several of these developmental stages: (1) First head—at least 1 head completely emerged from the sheath; (2) heading—50 percent of the heads completely emerged from their sheaths; (3) first bloom—when first florets bloom on 1 or more heads; (4) early bloom—interval from first bloom to full bloom; (5) full bloom—when flowers are in bloom over at least two-thirds of the lengths of at least 25 percent of all heads and blooming of at least an additional 25 percent of the heads has begun; (6) late bloom—25 percent of all heads past full bloom; (7) very late bloom—all main-crop heads entirely past full bloom, and only a few late heads remaining in bloom. Under some circumstances seed maturity may have enough significance to justify recording it. In several species plants can be considered mature when all florets are straw colored on 25 percent of the heads and more than half the florets are straw colored on at least an additional 50 percent of the heads. Some of the categories listed above were developed by Evans (74), for recording maturity of timothy. Heading date is particularly valuable for preliminary separation of selections into maturity classes, especially with species having compact inflorescences. The variation encountered in assigning dates suggests that information should be obtained on date of blooming (probably of first bloom) before selections are placed in isolated crossing blocks.

Self-fertility is very commonly expressed as the ratio  $F/I$ , i.e., open-pollination seed set over isolated seed set (generally, under bags) (241). Good indices of self- and cross-fertility are provided by number of seeds per panicle (204), number of seeds per 100 spikelets (134), and average number of seeds per floret (247). Relative fertility has been reported also in terms of germination counts of the seed from individual panicles and

on the basis of examination with transmitted light (102). The germination method is fairly rapid and quite satisfactory for making comparisons. Keller (153) has discussed the use of frequency distributions in interpreting self-fertility data.

The time and labor involved in note taking may be reduced by using correlation coefficients to study the interrelations among plant characters. Fall and spring vigor, for example, are closely associated in smooth brome grass (188), and fall vigor is a good guide to winter-hardiness in intermediate wheatgrass (123).

### MAINTAINING RECORDS

Identification of individual plant selections necessitates a way of numbering that is both systematic and flexible. One simple method is to use numbers that indicate the year of planting, the row, and the individual plant. For example, 56-20(9) would signify plant number 9 in row 20 of a nursery planted in 1956. Selection numbers could be retained indefinitely by vegetatively propagated material. Newell and Tysdal (215) present a basic system of numbers that include year of seed production or plant selection, a treatment number or letter indicating the breeding procedure or the conditions under which the seed was produced, and the serial number of a selection. The year designation, which is the last figure of the calendar year, appears first and is followed by the number or letter indicating treatment, from which the serial number is separated by a dash. These writers present a system of treatment index numbers that would serve to identify pollination conditions or breeding procedure. The identification number 61-20, under their system, would be applied to selfed seed (index number 1) produced in 1956 by selected plant 20, and 69-20 would designate open-pollinated seed (index number 9) produced in the same year by a plant of the same clone.

Abbreviations may be used to designate different types of progenies in maintaining records—and also in publishing results. Acceptable abbreviations include the following: (1) Open-pollinated seed (seed obtained with no restriction on pollen source), OP; (2) plants from colchicine-treated seeds,  $C_1$  (subsequent seed generations,  $C_2$ ,  $C_3$ , etc.); (3) polycross seed (seed collected from individual clones in polycross nurseries), PC; (4) topcrossed seed (seed collected from clones or lines grown in association with a common pollinator or tester), TC; (5) seed produced by one sib pollination,  $Sib_1$  (subsequent generations so produced,  $Sib_2$ ,  $Sib_3$ , etc.); (6) seed produced by one self-fertilization,  $L_1$ ,  $I_1$ , or (the most frequent)  $S_1$  (additional generations of selfing,  $S_2$ ,  $S_3$ , etc.); (7) bulk seed of the first seed harvest produced by restricted polycrosses, in theory equal amounts of seed from all possible single-cross combinations (synthetic varieties including a limited number of clones but always more than two selections),  $Syn_1$  (advanced generations from successive plantings,  $Syn_2$ ,  $Syn_3$ , etc.); mechanical seed mixtures of different varieties or lines, blends (sometimes,  $Syn_0$ ); (8) seed derived by

a single cross of two clones (pollination controlled either by bags or by isolation),  $F_1$ . Use of the term " $F_1$ " seems justifiable even in the absence of emasculation, which may permit some degree of selfing.

Clones can be identified according to their source. For example,  $I_1C$  signifies vegetative propagation of a selection made in the first inbred generation, and OPC signifies vegetative propagation of a plant selected from a population produced with no restrictions on pollen source.

### PLOT TECHNIQUE

Evaluation of species, varieties, and experimental strains in small plots is an integral part of all grass-breeding endeavors. In many breeding projects plot tests are conducted at several different stages of the program.

### SOIL SELECTION AND TREATMENT

In general, species and strains should be compared on soil of optimum fertility, so that they can be differentiated on the basis of their efficiency in utilizing nutrients. On the other hand, relatively infertile soil of inferior moisture-holding capacity should be selected for tests intended to develop varieties especially well adapted to marginal land; in such varieties seedling vigor, drought resistance, and tolerance of low soil fertility may be the most important characters. Selection of a relatively homogeneous area is essential to the establishment of good plot tests.

The soil should be plowed well in advance of seeding, by mid-summer for fall plantings or by late fall for spring plantings. After being disked and harrowed, it should be cultipacked to provide a firm seedbed. A complete fertilizer should be applied if needed. In humid areas, pure grass seedings should be top-dressed annually with 50 to 100 pounds of nitrogen per acre, in addition to receiving adequate amounts of phosphorus and potash. An early spring application of 40 pounds of nitrogen per acre followed by application of 80 pounds after each clipping may be desirable. The amount of nitrogen needed varies with time of application, soil fertility, use of barnyard manure, and precipitation.

### EXPERIMENTAL DESIGN AND PLOT LAYOUT

When the site has been plowed and has been examined to determine the general plot layout and the location of alleys, a decision should be reached with respect to experimental design.

Lattice designs should be considered for progeny tests and similar trials, which commonly contain large numbers of entries. Both in these experiments and wherever soil heterogeneity is a problem, lattice designs are likely to be more efficient than randomized-block designs. The gain in precision obtained with them will not always compensate the added expense involved, however. Also, analysis of data from lattice-design studies in-

volving perennial species may be complicated by loss of some plots in later years. Randomized-block designs will serve for most varietal trials that contain a limited number of entries.

The size of plot to be used in testing forage grasses has been determined chiefly on the basis of experimental work done with other crops. Drilled and broadcast plots generally range in size from one-hundredth to one-thousandth of an acre. The areas of most plots are included in the range from 1 two-hundredth to 1 five-hundredth of an acre, and the harvested areas range approximately from 1 five-hundredth to 1 eight-hundredth of an acre. Standard dimensions are 5 by 20 feet with a harvested area of 3 by 18 feet. Plot width may be increased to 6 feet and the harvested sample to approximately 4 by 18 feet, depending on the mowing equipment available.

In Iowa, Wassom and Kalton (307) concluded that optimum plot size for smooth brome grass is  $3\frac{1}{2}$  by  $7\frac{1}{2}$  feet. Provision for adequate borders increases these measurements to  $5\frac{1}{2}$  by 10 feet. In discussing their findings these writers mention that plot size can vary appreciably from the optimum with little effect on efficiency.

Under most circumstances oblong plots are superior to square plots and should be laid out parallel to the direction of the fertility gradient, in blocks at right angles to it.

Four or more replications are used in most forage plot tests. Data from previous trials can be used in judging the advisability of a larger or smaller number of replications.

Border effect can be very pronounced next to roadways or cultivated alleys, or between species and varieties having diverse growth habits. Buffer plots should be used to separate experimental areas from roadways and from other such areas. Removal of borders at least 1 foot wide from the ends and sides of each plot is a sound precaution in all yield tests.

Frequently it is desirable to separate every double tier of plots with a seeded alley wide enough to permit easy access with mowing and weighing equipment.

#### SEEDING METHODS AND SPECIES MIXTURES

Pure stands established in either broadcast or multiple-drill plots are recommended for most experiments designed to compare the yield potentials of grasses. Drill seeding is recommended over broadcast seeding at locations where difficulty is experienced in obtaining good stands.

Plots commonly used in comparative testing of forage grasses include individual rows 15 to 25 feet long and  $2\frac{1}{2}$  to  $3\frac{1}{2}$  feet apart, multiple rows 7 to 12 inches apart, and broadcast seedings. Row plots are used commonly in seed-production studies, especially where row culture is a standard procedure in the production of seed. They are useful also in obtaining observational information on strains and progenies. The artificial nature of row plantings makes them inferior to solid seeding for forage yield comparisons. On the other hand, Patterson and Law (229) believe that much preliminary grass-strain testing could well

be carried out by the use of rows spaced 30 inches apart. Milton (191) reported that grass species performed differently in broadcast plots and 2-foot drill rows, respectively, but that the strains maintained the same relative performances within tests.

The fact that forage grasses are commonly sown in combination with legumes except in the semiarid and arid parts of the Great Plains and the western intermountain region makes it desirable that a new grass variety be subjected to some testing in association with an appropriate legume. Plot experiments have demonstrated differences among Kentucky bluegrass strains in ability to compete with white clover (210). Churchill (50) found that differences among brome grass varieties with respect to total yield were less pronounced when brome grass was grown with alfalfa than when it was grown alone. Wilsie (317), having studied brome grass varieties under various methods of planting, concluded that rows or broadcast plots of brome grass grown either alone or with legumes will serve for the preliminary evaluation of brome grass strains. He suggested that in final tests brome grass varieties should be grown in association with a commonly used legume.

Patterson and Law (229) found that many strain differences were masked in mixed plantings, and that such differences could be demonstrated most effectively by separating the forage into grass and legume fractions. Weiss and Mukerji (311), in comparing orchardgrass strains, used the following methods: (1) 3-foot drill rows; (2) 1-foot drill rows; (3) broadcast alone; (4) with alfalfa, alternate 1-foot drill rows; (5) with alfalfa, broadcast; and (6) with birdsfoot trefoil, broadcast. Under the conditions of their experiment, the mean performance of orchardgrass associated with methods 5 and 6 was considered indicative of the true strain potential and broadcast plots of the grass alone permitted a more accurate evaluation of the orchardgrass strains than 3- or 1-foot drill rows of the grass alone or 1-foot rows of grass and alfalfa alternated.

#### ESTABLISHING AND MARKING PLANTINGS

In preparing seed for planting, the entries are coded and are randomized within replications, and the seed is weighed into envelopes numbered according to the sequence in which their contents will be used in the field.

It is advisable to adjust seeding rates on the basis of germination tests, recognizing that sufficient viable seed must be sown to produce a uniform stand. Correction for germination in terms of viable seeds per unit weight of seed is preferable to correction for percent germination alone, as it takes into account variation in seed size. Adjusting seeding rate on the basis of emergence data, obtained by planting seed in greenhouse flats, can be recommended. Interplant competition tends to minimize population differences among plots, if the less dense populations are spaced uniformly enough to keep weeds from encroaching.

If it is necessary to use a companion grain crop, the seeding



rate of the grain crop should be not more than about 1 bushel per acre. A companion grain crop should be removed at the milk stage—or earlier, if drought or lodging becomes serious. In the absence of a companion crop, competition from weeds can be reduced by clipping and spraying with 2,4-D.

To ensure uniform distribution, usually seed for each individual plot is mixed with sand or soil in a shallow pan and equal amounts of the mixture are distributed over the plot in two directions. Keller<sup>23</sup> has pointed out that a mixture of seed and shorts or bran shows up better on the soil and therefore is likely to be distributed more evenly. Broadcast plots should be cultipacked or raked lightly to cover the seed. Drill seeding is done with small hand drills or belt seeders. Equipment capable of seeding multiple-drill row plots is still unavailable at most locations. Modified belt seeders have been adapted to sowing single-width plots, but the distribution of seed from many of these machines is comparable to broadcast seeding.

In broadcast plantings the boundaries of tiers can be marked with strings and individual plots clearly defined by placing pairs of strings across the tiers. On areas where seed are to be drilled, any one of various types of soil markers should be used. Temporary garden stakes can be used to identify plots during seeding, but they should be replaced with semipermanent wooden stakes, 1½ by 1½ by 10 inches, driven to ground level. If the stakes are placed at the ends of the plots they will serve as useful guides for centering the mower. Locating stakes can be simplified by attaching metal tabs to their tops or by removing vegetation around them with a soil sterilant. The practice of drilling a single row of alfalfa or a contrasting grass species between tiers and plots can be recommended.

#### CLIPPING

Cutting for hay or silage can be duplicated rather easily. Pasture or hay-plus-pasture management can be simulated with difficulty and not always without marked discrepancies.

Hein and Henson (122) compared the effects of clipping and grazing on the botanical composition of permanent pasture mixtures. Their results indicated that clipping was more severe in its effects than comparable grazing; thinner stands resulted under clipping. They concluded that increase in soil fertility of grazed land resulting from droppings contributed to the difference. Klapp (159), in considering the effects of clipping as compared with those of grazing, stressed the more complete removal of leaves by clipping as one of the principal differences. Defoliation by the grazing animal is largely selective (310). Other factors with which marked differences are associated include trampling, soil compaction, and soiling of the herbage.

It would be expected that pasture management involving rapid defoliation and a minimum of trampling, such as intensive rotational or strip grazing, would tend to reduce the difference

<sup>23</sup> Unpublished observations of W. Keller.

between the mower bar and the grazing animal in their effect on forage grasses. Under most conditions, however, it is advantageous to accumulate information on the persistence of new strains under grazing. Valuable information of this kind can be obtained from relatively simple experiments.

The proper heights and frequencies of clipping or grazing for grass species and strains differing in growth habit should be determined and used in evaluating experimental material. Greenhouse tests were used by Harrison and Hodgson (114) to compare the responses of several grasses when clipped weekly at three different heights. The amount of injury under close clipping varied considerably. From least to most injured, the grasses ranked as follows: Kentucky bluegrass, quackgrass, smooth brome grass, timothy, and orchardgrass (the last two were injured about equally). In general, the shorter a given grass was cut, the less was its subsequent top growth. Yield of underground parts (roots and rhizomes) varied inversely with severity of cutting treatment.

Correct height and frequency of clipping or grazing for given forage grasses depend in part on the location of carbohydrate reserves. Sprague et al. (260), having summarized studies of this factor, drew attention to the fact that while defoliation in itself does not remove carbohydrate reserves from legumes, clipping or grazing of certain grasses close to the soil surface removes a substantial proportion of the reserves that would normally serve in generating new leaves. The nature of reserve substances in the grasses has been studied (283, 310).

Low growing prostrate species such as Kentucky bluegrass, bermudagrass, and bentgrasses (*Agrostis* spp.) tolerate frequent close defoliation owing to their abundance of basal leaves and to the fact that defoliation does not remove a large proportion of the reserve carbohydrates they need for recovery. Rhizomatous and stoloniferous species are better adapted than bunchgrasses for spreading under close defoliation but might not be especially resistant to continuous defoliation without an appreciable development of basal leaves. Branson (24) observed that species whose growing points were far enough aboveground to be eaten by grazing stock did not tolerate heavy grazing so well as those whose growing points remained close to ground level, and that lower persistence under heavy grazing was associated with a high ratio of flowering stems to vegetative stems. The significance of both frequency and height of defoliation may be obscured by soil productivity, and the carry-over effect of severe defoliation may not be so conspicuous or serious under conditions of relatively low soil fertility (189).

Usually, all the entries in a comparative test are clipped at the same time if management is designed to simulate production of either silage or pasture. Cutting at more than one date can be recommended for hay treatments if the entries differ in time of maturity. Practical considerations frequently restrict differential clipping to either 2 or 3 cutting dates.

## HARVESTING AND DETERMINING YIELDS

In order to facilitate note taking, alleys can be mowed and the clippings removed a few days before harvesting. Generally, the end borders are mowed just before harvest and the borders separating plots are mowed immediately after it.

Common methods of harvesting include (1) raking the clippings, whether hay or pasture, with a wooden rake, and (2) using a galvanized pan or similar attachment on the mower to catch the clippings. It is frequently necessary to make some provision for sweeping the clippings back into the pan.

Green weight of the yield from each plot is determined either with stationary scales or with milk scales attached to an A-frame. Immediately after the green weight has been recorded, a random sample for determining dry weight should be taken, weighed, and transferred to the dryer in a labeled pan or cotton bag. (Pans or bags should be uniform in weight.) Such a sample from a small plot generally consists of about 2 pounds of green material for either chopped herbage or pasture clippings. Somewhat larger samples are preferred for hay- or silage-length clippings. Where yields are small, particularly in closely clipped pasture plots, it is feasible and advantageous to dry the entire plot yield. Dry-matter percentage may not vary appreciably under certain conditions, but dry-weight determinations are recommended in most plot work because of the numerous factors affecting this variable, such as growth habit, maturity, disease resistance, intraplot competition, time of day, and location in the field. The dry weight of forage samples can be established by successive weighings or, after checking the efficiency of the drying facilities, by leaving the samples in the dryer for sufficient time to provide a margin of safety and thus confining the work to a single weighing. Dry weights should be taken as soon as possible after the samples are removed from the dryer.

## BOTANICAL ANALYSIS

Botanical analyses are frequently used at various stages in grass-improvement projects either to evaluate grasses grown in mixtures in plots or to establish the contributions and importance of grass species under field use. Both qualitative and quantitative procedures are available for determining the botanical composition of swards. Quantitative procedures have the advantage of indicating relative abundance in addition to kind and number of species.

## HAND SEPARATIONS AND VISUAL ESTIMATES

Dry weight is the standard measure for establishing the percentage contribution of any one species or class of forage plants to total yield. Samples or entire yields from plots are hand separated, dried, and weighed in order to determine the relative dry-matter contributions of the component species. Because of the time required and the expense involved, it is not practical

as a rule to separate a sample from every plot. Visual estimates of the standing crop are commonly employed to establish the approximate dry-matter contribution of various species. The value of these estimates is enhanced if the observer periodically checks them against dry-weight values for hand-separated samples. Because of the errors to which visual estimates are subject, the use of hand separations for checking or adjusting them is strongly recommended. As it may be difficult to separate samples immediately after cutting, provision should be made for holding them in cold storage, if necessary, to prevent excessive wilting and respiration.

Estimates can be based on dried moisture samples and checked with occasional hand separations. This procedure has a certain advantage in that work based on dried material can be done at any time. It is better suited to simple mixtures than to complex mixtures, because of the difficulty in identifying component species.

Stands obtained by broadcasting are usually estimated visually. Stands in drilled plots can be determined with a high degree of accuracy by measuring vacant spaces within rows. A clearly marked measuring stick with a 4-foot handle attached at a 45° angle serves for measuring the vacant areas. Generally, only vacancies greater than 3 inches are recorded.

#### MECHANICAL AIDS

Mechanical aids widely used in making botanical analyses are the point quadrat and the grid quadrat.

The point quadrat, devised by Levy and Madden (179), consists of 2 pipes mounted in horizontal position on legs 12 inches high. Each pipe has 10 holes, at 2-inch intervals, through which 14-inch needles are moved up and down. Data are taken in two ways: 1. Each needle is pushed down until the point touches some plant part or bare ground, and the 10 hits at each station are recorded. 2. Each needle is pushed down to the ground and the number of times each species is hit during the downward thrust is recorded. The type of analysis used with the second method of recording data has been outlined by Levy (178) and by Tinney et al. (288). The percentage contribution of each species to the pasture sward according to the second method may be expressed as:

$$\frac{\text{Total number of times plants of that species are hit}}{\text{Total number of times vegetation is hit}} \times 100.$$

Hanson (109) found that results obtained with the point quadrat were in fair agreement with the percentages of the components in mixtures as determined on the basis of dry weights. On the other hand, his results indicate that use of this technique leads to overestimating the prevalence of species having a relatively large total leaf and stem surface for a given amount of dry weight. The "inclined point quadrat" method, in which the needles are pushed to the ground at an angle of 45°, tends to reduce this error but still may lead to underestimating or over-

TB 1145 (1956) — USDA TECHNICAL BULLETINS  
BREEDING PERENNIAL FORAGE GRASSES  
HANSON, A. A. ; CARNAHAN, H. L. — UPDATA  
2 OF 2

estimating certain species. Army and Schmid (8) suggest correction factors for use with the inclined-point-quadrat method. Leasure (176) reported that, while the point-quadrat method does not apparently introduce any bias into estimates of botanical composition, values obtained with it for a single sample area are likely to vary as much as 10 percent from the true percentage composition. The average of visual estimates seemed to be accurate within 10 percent, although individuals may tend to exhibit a bias toward overestimating or underestimating. Leasure suggests that visual estimation can be used in conjunction with the inclined-point-quadrat method to save time without impairing accuracy.

Grid quadrats are useful in closely grazed pastures or on clipped plots. These quadrats vary in size; the 50-by-50-centimeter frame divided by crosswires into twenty-five 10-centimeter squares has been used rather widely. The percentage of total area covered by each species and the percentage of bare ground are estimated for each of the 10-centimeter squares or for a lesser number of them—commonly, for 5 squares, 1 in each of the 4 corners and the other in the center of the quadrat. This procedure is subject to a large personal error, but it may be satisfactory if observers are well trained and especially if all readings are taken by one observer.

## CONCLUSION

Variation within species is indicative of the promise of grass breeding (93, 117, 157), but the potential may well remain only a promise if breeding projects are begun without due regard to existing problems. Sound objectives are at least as important as the methods of breeding used in efforts toward grass improvement. The rate of progress (and, hence, the efficiency) of a grass-breeding program depends on the thoroughness of the experimenters' knowledge of the species and on application of suitable breeding methods. The perennial forage grasses, by virtue of their cytogenetic behavior and modes of pollination, lend themselves to a variety of breeding procedures; and it is in choosing procedures that the plant breeder can make full use of his imagination, energy, and experience. The need for more data on the physiology and breeding behavior of the perennial forage grasses is obvious, and efforts should be made to accumulate such data either in separate experiments or in connection with breeding investigations.

The following points should be considered in either planning or reviewing grass-breeding work:

- (1) The outstanding importance of good source nurseries: As many important perennial forage grasses have been introduced into the United States, it is very probable that the introduced species are inadequately represented by the source nurseries now available. For some of these species, thorough foreign plant explorations would be necessary to provide adequate representation.

(2) The importance of evaluating widely diversified seed collections and plant material not only during the initial stages but also as a continuing, integral part of the work.

(3) The relationship of specific objectives to breeding progress.

(4) The necessity, at all stages in the program, of thorough evaluation of breeding materials. Emphasis should be given not only to large numbers of individuals but also to thorough screening. A wide source of germ plasm is extremely important, but mere numbers are not an adequate substitute for thorough testing.

(5) The fact that any one of several breeding methods may be satisfactory, depending on the species and objectives. Breeding methods are not mutually exclusive, and frequently more than one method can be used to advantage.

(6) The potential value of radical procedures in developing superior grass varieties. These include investigations of inter-specific and intergeneric hybridization, and studies on the utility of X-ray and thermal neutron irradiation.

(7) The possibility of capitalizing on heterosis, displayed by first-generation hybrids, either through distribution of clonal varieties or through field-scale use of  $F_1$  or  $Syn_1$  seed.

(8) The possibility of realizing gains in characteristics other than those selected for investigation. An example is provided by the nematode resistance obtained in breeding Coastal bermuda-grass.

A tentative breeding plan should be developed, indicating the main steps in the program. Typical outlines are presented here, with the warning that they will need to be adapted to specific conditions. The continuous nature of grass-breeding work is not clearly indicated in these outlines, but it is obvious that provision should be made for incorporating new source materials as they become available. Similarly, greenhouse tests, chemical analyses, and other procedures should be used in accordance with breeding objectives and facilities.

Essentially the same procedures are outlined for species reproduced vegetatively as for apomictic species.

#### BREEDING OUTLINES

Assumptions: Seed collections accumulated from local and other sources; available strains tested in nursery rows and solid-seeded plots to determine adaptation and productivity.

##### Group 1. Apomictic Species

##### *Year Procedure*

- 1 Establish space-planted source nursery with original seed collections of domestic and introduced material. Seed collected from nursery rows should be included on the chance that some hybridization has occurred. If some mutual pollination, under bags, between species has preceded the establishment of the nursery, the hybrid progenies can be included. Record observations.

*Year Procedure*

- 2 Record observations on vigor, spread, disease resistance, seed characteristics, recovery, and other factors. Collect seed from interesting plants.
- 3 Establish replicated space-planted progeny test. Record observations.
- 4 Collect seed from superior lines that exhibit a high degree of uniformity.
- 5 Establish replicated space-planted progeny test to check degree of apomixis and to repeat observations. A preliminary plot test may be made with some "outstanding" selections.
- 6 Collect seed from superior lines.
- 7-9 Plant selections in solid-seeded plots. In humid areas, preferably use 6 replications, overseeding 3 of these with a legume.
- 10 Continue to record observations and measure yields on solid-seeded plots. Start to increase superior lines for regional testing.
- 11-14 Include superior lines in regional tests. Establish observational tests to check persistence under grazing.

*Group II, Species Reproduced Vegetatively*

- 1-2 Space-plant source nursery.
- 3 Establish selected plants in replicated clonal nursery. If a limited number of plants are selected, these plants can be established directly in replicated tiller plots.
- 4-5 Evaluate clones.
- 6-9 Establish replicated tiller plots. Overseed half the planting with an adapted legume. If selections are limited in number, plant them also on an area that can be grazed by livestock.
- 10 Continue to obtain data from tiller plots and observational grazing tests. Increase selected clones for regional testing.

*Group III, Species Largely Cross-Pollinated*

- 1-2 Space-plant source nursery.
- 3 Increase selected plants vegetatively and plant them in replicated clonal nursery.
  - (a) If a large number of plants exhibiting a relatively narrow range in maturity are selected, they may be planted in a replicated topcross nursery.
  - (b) Polycross nurseries are preferable if selected plants can be grouped into relatively small blocks on the basis of maturity or other specific characteristics. A few heads of each clone can be bagged to obtain self seed for further selection and progeny evaluation.
- 4 Make observations on replicated clones. Collect seed from each clone for progeny tests.
- 5-7 Establish replicated space-planted or row test to evaluate progenies for characters such as seed production, seed



*Year Procedure*

- size, disease resistance, and winter-hardiness. If progenies are to be evaluated for forage yields, establish solid-seeded plots.
- 8 Isolate superior clones in restricted polycross nurseries.
- 9 Evaluate replicated clones and collect seed from each clone.
- 10-12 (a) Establish solid-seeded plots with and without an adapted legume. Seed of each line should be used in the plot test together with bulk seed from the restricted polycross blocks and appropriate checks. Plots should be established with bulk seed on areas that can be grazed by livestock.
- (b) Establish space-planted nursery of polycrosses from clones included in restricted polycross nursery. Practice recurrent selection within lines and include new introductions in this nursery.
- 13 Continue observations on plot tests. Establish isolated plantings of selected clones for production of synthetic seed.
- 14 Collect seed for preliminary testing and for production of Syn<sub>2</sub> seed.

## LITERATURE CITED

- (1) ÅKERBERG, E.  
1940. THE APPLICATION OF CYTOLOGY TO HERBAGE PLANT BREEDING. Imp. Agr. Bur. Joint Pub. 3, pp. [52]-61.
- (2) ÅKERMAN, Å., TEDIN, O., FRÖIER, K., and WHYTE, R. O., eds.  
1948. SVALÖF 1886-1946, HISTORY AND PRESENT PROBLEMS. 389 pp., illus. Lund, [Sweden].
- (3) AAMODT, O. S.  
1935. A MACHINE FOR TESTING THE RESISTANCE OF PLANTS TO INJURY BY ATMOSPHERIC DROUGHT. Canad. Jour. Res. 12: 788-795, illus.
- (4) ——— and JOHNSTON, W. H.  
1936. STUDIES ON DROUGHT RESISTANCE IN SPRING WHEAT. Canad. Jour. Res., Sect. C, 14: 122-152, illus.
- (5) AHLGREN, H. L., SMITH, D. C., and NIELSEN, E. L.  
1945. BEHAVIOR OF VARIOUS SELECTIONS OF KENTUCKY BLUEGRASS, *POA PRATENSIS* L., WHEN GROWN AS SPACED PLANTS AND IN MASS SEEDINGS. Amer. Soc. Agron. Jour. 37: 268-281.
- (6) ANDERSON, K., and ALDOUS, A. E.  
1938. IMPROVEMENT OF *ANDROPOGON SCOPARIUS* MICHX. BY BREEDING AND SELECTION. Amer. Soc. Agron. Jour. 30: 862-869, illus.
- (7) ARCHIBALD, J. G., BENNETT, E., and RITCHIE, W. S.  
1943. THE COMPOSITION AND PALATABILITY OF SOME COMMON GRASSES. Jour. Agr. Res. 66: 341-347.
- (8) ARNY, A. C., and SCHMID, A. R.  
1942. A STUDY OF THE INCLINED POINT QUADRAT METHOD OF BOTANICAL ANALYSIS OF PASTURE MIXTURES. Amer. Soc. Agron. Jour. 34: 238-247.
- (9) ATWOOD, S. S.  
1947. CYTOGENETICS AND BREEDING OF FORAGE CROPS. In Demerze, M., ed., *Advances in Genetics* 1: 1-67.
- (10) ——— and MACDONALD, H. A.  
1946. SELECTING PLANTS OF BROMEGRASS FOR ABILITY TO GROW AT CONTROLLED HIGH TEMPERATURE. Amer. Soc. Agron. Jour. 38: 824-832, illus.
- (11) BAILEY, L. F.  
1940. SOME WATER RELATIONS OF THREE WESTERN GRASSES. I. THE TRANSPIRATION RATIO. Amer. Jour. Bot. 27: 122-128, illus.

- (12) BARKER, H. D., and HAYES, H. K.  
1924. RUST RESISTANCE IN TIMOTHY. *Phytopathology* 14: [363]-371, illus.
- (13) BARTLETT, M. S., and HALDANE, J. B. S.  
1934. THE THEORY OF INBREEDING IN AUTOTETRAPLOIDS. *Jour. Genet.* 29: [175]-180, illus.
- (14) BATEMAN, A. J.  
1947. CONTAMINATION OF SEED CROPS—II. WIND POLLINATION. *Heredity* 1: 235-246, illus.
- (15) BEARD, D. F., and HOLLOWELL, E. A.  
1952. THE EFFECT ON PERFORMANCE WHEN SEED OF FORAGE-CROP VARIETIES IS GROWN UNDER DIFFERENT ENVIRONMENTAL CONDITIONS. *Internatl. Grassland Cong. Proc.* 6: 860-866.
- (16) BEAUMONT, A. B., STITT, R. E., and SNELL, R. S.  
1933. SOME FACTORS AFFECTING THE PALATABILITY OF PASTURE PLANTS. *Amer. Soc. Agron. Jour.* 25: 123-128.
- (17) BEDDOWS, A. R.  
1931. SEED SETTING AND FLOWERING IN VARIOUS GRASSES. *Welsh Plant Breeding Sta., Aberystwyth, (Bul.), Ser. II, 12, pp. 5-99.*
- (18) ——— and DAVIS, A. G.  
1938. ILLUSTRATED NOTES ON THE TECHNIQUE OF GRASS-BREEDING AT ABERYSTWYTH. *Imp. Bur. Pastures and Forage Crops, Herbage Rev.* 6: 221-227, illus.
- (19) BENDER, C. B.  
1934. FIVE YEARS' RESULTS ON PASTURE FERTILIZATION AND ROTATION MANAGEMENT. *N. J. Agr. Expt. Sta. Bul.* 664, 8 pp., illus.
- (20) BENNETT, H. W.  
1944. EMBRYOLOGY OF PASPALUM DILATATUM. *Bot. Gaz.* 106: 40-45, illus.
- (21) ———  
[1953.] FORAGE AND PASTURE CROPS BREEDING. DALLISGRASS [HYBRIDS AND] JOHNSONGRASS SORGHUM HYBRIDS. *Miss. Agr. Expt. Sta. Ann. Rpt. (1952-53)* 66: 18-19. (See also *Miss. Agr. Expt. Sta. Ann. Rpt. 1947-48: 6-7, and 1948-49: 6.*)
- (22) BIRD, J. N.  
1934. INFLUENCE OF RUST INJURY ON VIGOUR AND YIELD OF TIMOTHY. *Sci. Agr.* 14: 550-559, illus.
- (23) ———  
1943. STAGE OF CUTTING STUDIES: I. GRASSES. *Amer. Soc. Agron. Jour.* 35: 845-861, illus.
- (24) BRANSON, F. A.  
1953. TWO NEW FACTORS AFFECTING RESISTANCE OF GRASSES TO GRAZING. *Jour. Range Mgmt.* 6: 165-171, illus.
- (25) BRITTINGHAM, W. H.  
1943. TYPE OF SEED FORMATION AS INDICATED BY THE NATURE AND EXTENT OF VARIATION IN KENTUCKY BLUEGRASS, AND ITS PRACTICAL IMPLICATIONS. *Jour. Agr. Res.* 67: 225-264, illus.
- (26) BROWN, B. A., and MUNSELL, R. I.  
1943. GRASSES FERTILIZED WITH NITROGEN COMPARED WITH LEGUMES FOR HAY AND PASTURE. *Amer. Soc. Agron. Jour.* 35: 811-816.
- (27) BROWN, E. M.  
1943. SEASONAL VARIATIONS IN THE GROWTH AND CHEMICAL COMPOSITION OF KENTUCKY BLUEGRASS. *Mo. Agr. Expt. Sta. Res. Bul.* 360, 56 pp., illus.
- (28) [BUCKNER, R. C.]  
[1953.] PREFERENTIAL GRAZING TRIALS WITH TALL FESCUE. *Ky. Agr. Expt. Sta. Ann. Rpt. (1952)* 65: 16.
- (29) BURTON, G. W.  
1938. A USEFUL SEED BLOWER FOR THE GRASS BREEDER. *Amer. Soc. Agron. Jour.* 30: 446-448, illus.
- (30) ———  
1943. A COMPARISON OF THE FIRST YEAR'S ROOT PRODUCTION OF SEVEN SOUTHERN GRASSES ESTABLISHED FROM SEED. *Amer. Soc. Agron. Jour.* 35: 192-196, illus.
- (31) ———  
1944. ESTIMATING INDIVIDUAL FORAGE PLANT YIELDS. *Amer. Soc. Agron. Jour.* 36: 709-712.

- (32) BURTON, G. W.  
1944. HYBRIDS BETWEEN NAPIER GRASS AND CATTAIL MILLET. *Jour. Hered.* 35: 227-232, illus.
- (33) ———  
1947. BREEDING BERMUDA GRASS FOR THE SOUTHEASTERN UNITED STATES. *Amer. Soc. Agron. Jour.* 39: 551-569, illus.
- (34) ———  
1948. THE METHOD OF REPRODUCTION IN COMMON BAHIA GRASS, *PASPALUM NOTATUM*. *Amer. Soc. Agron. Jour.* 40: 443-452, illus.
- (35) ———  
1948. A METHOD FOR PRODUCING CHANCE CROSSES AND POLYCROSSES OF PENSACOLA BAHIA GRASS, *PASPALUM NOTATUM*. *Amer. Soc. Agron. Jour.* 40: 470-472, illus.
- (36) ———  
1948. THE PERFORMANCE OF VARIOUS MIXTURES OF HYBRID AND PARENT INBRED PEARL MILLET, *PENNISETUM GLAUCUM* (L.) R. BR. *Amer. Soc. Agron. Jour.* 40: 908-915, illus.
- (37) ———  
1951. THE ADAPTABILITY AND BREEDING OF SUITABLE GRASSES FOR THE SOUTHEASTERN STATES. *In Advances in Agronomy* 3: 197-241.
- (38) ———  
1951. QUANTITATIVE INHERITANCE IN PEARL MILLET (*PENNISETUM GLAUCUM*). *Agron. Jour.* 43: 409-417.
- (39) ———  
1952. QUANTITATIVE INHERITANCE IN GRASSES. *Internat. Grassland Cong. Proc.* 6: 277-283.
- (40) ———  
1954. DOES DISEASE RESISTANCE AFFECT FORAGE QUALITY? *Agron. Jour.* 46: 99.
- (41) ——— and DeVANE, E. H.  
1953. ESTIMATING HERITABILITY IN TALL FESCUE (*FESTUCA ARUNDINACEA*) FROM REPLICATED CLONAL MATERIAL. *Agron. Jour.* 45: 478-481.
- (42) ———, DeVANE, E. H., and CARTER, R. L.  
1954. ROOT PENETRATION, DISTRIBUTION AND ACTIVITY IN SOUTHERN GRASSES MEASURED BY YIELDS, DROUGHT SYMPTOMS AND P<sup>32</sup> UPTAKE. *Agron. Jour.* 46: 229-233, illus.
- (43) ———, McBETH, C. W., and STEPHENS, J. L.  
1946. THE GROWTH OF KOBE LESPEDEZA AS INFLUENCED BY THE ROOT-KNOT NEMATODE RESISTANCE OF THE BERMUDA GRASS STRAIN WITH WHICH IT IS ASSOCIATED. *Amer. Soc. Agron. Jour.* 38: 651-656, illus.
- (44) CALDER, J. W.  
1933. METHODS EMPLOYED IN THE BREEDING OF PASTURE PLANTS. *Imp. Bur. Plant Genet., Herbage Plants, Bul.* 11, pp. [36]-39.
- (45) CAREY, V., MITCHELL, H. L., and ANDERSON, K.  
1952. EFFECT OF NITROGEN FERTILIZATION ON THE CHEMICAL COMPOSITION OF BROMEGRASS. *Agron. Jour.* 44: 467-469, illus.
- (46) CARROLL, J. C.  
1943. ATMOSPHERIC DROUGHT TESTS OF SOME PASTURE AND TURF GRASSES. *Amer. Soc. Agron. Jour.* 35: 77-79.
- (47) CARTER, J. F., and AHLGREN, H. L.  
1951. FORAGE YIELDS AND DISEASE DEVELOPMENT OF TWO VARIETIES OF SMOOTH BROMEGRASS, *BROMUS INERMIS* LEYSS., GROWN UNDER VARIOUS CONDITIONS IN THE FIELD. *Agron. Jour.* 43: 166-171, illus.
- (48) ——— and DICKSON, J. G.  
1952. THE INFLUENCE OF ENVIRONMENT ON THE DEVELOPMENT OF TWO FOLIAR DISEASES OF *BROMUS INERMIS* LEYSS. *Agron. Jour.* 44: 119-125, illus.
- (49) CHENG, C.-F.  
1946. SELF-FERTILITY STUDIES IN THREE SPECIES OF COMMERCIAL GRASSES. *Amer. Soc. Agron. Jour.* 38: 873-881.
- (50) CHURCHILL, B. R.  
1947. PRODUCTIVENESS OF BROMEGRASS STRAINS FROM DIFFERENT REGIONS WHEN GROWN IN PURE STANDS AND IN MIXTURE WITH ALFALFA IN MICHIGAN. *Amer. Soc. Agron. Jour.* 39: 750-761, illus.
- (51) CLARK, C. F.  
1910. VARIATION AND CORRELATION IN TIMOTHY. *N. Y. (Cornell) Agr. Expt. Sta. Bul.* 279, pp. [299]-350, illus.

- (52) CLARK, J. W.  
1944. THE EFFECT OF SOME ENVIRONMENTAL INFLUENCES IN BULK HYBRIDIZATION OF GRASS. Amer. Soc. Agron. Jour. 36: 132-140, illus.
- (53) CLARKE, M. F.  
1953. A STUDY OF THE CAROTENE AND CRUDE PROTEIN CONTENT OF ORCHARD-GRASS (*DACTYLIS GLOMERATA* L.). I. VARIATION DUE TO STAGE OF GROWTH, CUTTING MANAGEMENT AND CLONES. Canad. Jour. Agr. Sci. 33: 184-194.
- (54) ———  
1953. A STUDY OF THE CAROTENE AND CRUDE PROTEIN CONTENT OF ORCHARD-GRASS (*DACTYLIS GLOMERATA* L.). II. HERITABLE VARIATION. Canad. Jour. Agr. Sci. 33: 246-253.
- (55) CLAUSEN, J., HIESEY, W. M., and NOBS, M. A.  
1953. THE POA PROGRAM. Carnegie Inst. Wash., Yearbook (1952-53) 52: 170-173.
- (56) ———, KECK, D. D., and HIESEY, W. M.  
1939. THE CONCEPT OF SPECIES BASED ON EXPERIMENT. Amer. Jour. Bot. 26: 103-106.
- (57) ———, KECK, D. D., and HIESEY, W. M.  
1941. REGIONAL DIFFERENTIATION IN PLANT SPECIES. Amer. Nat. 75: 231-250, illus.
- (58) ———, KECK, D. D., and HIESEY, W. M.  
1945. EXPERIMENTAL STUDIES ON THE NATURE OF SPECIES. II. PLANT EVOLUTION THROUGH AMPHIPOIDY AND AUTOPOIDY, WITH EXAMPLES FROM THE MADINAE. Carnegie Inst. Wash., Pub. 564, 174 pp., illus.
- (59) CLAUSEN, R. E.  
1941. POLYPOIDY IN NICOTIANA. Amer. Nat. 75: 291-306.
- (60) COOK, C. W.  
1943. A STUDY OF THE ROOTS OF *BROMUS INERMIS* IN RELATION TO DROUGHT RESISTANCE. Ecology 24: 169-182, illus.
- (61) CORKILL, L.  
1952. BREEDING FOR RESISTANCE TO BLIND SEED DISEASE (*PHIALEA TEMULENTA*, PRILL & DELACR.) IN RYEGRASS (*LOLIUM* spp.). Internatl. Grassland Cong. Proc. 6: 1578-1584.
- (62) COWAN, J. R.  
1943. THE VALUE OF DOUBLE CROSS HYBRIDS INVOLVING INBREDS OF SIMILAR AND DIVERSE GENETIC ORIGIN. Sci. Agr. 23: 287-296.
- (63) ———  
1951. SOME METHODS FOR BREEDING TALL FESCUE. Amer. Soc. Agron., Abstracts, Ann. Meeting 43: 4-5. [Processed.]
- (64) CROWDER, L. V.  
1953. INTERSPECIFIC AND INTERGENERIC HYBRIDS OF *FESTUCA* AND *LOLIUM*. Jour. Hered. 44: 195-203, illus.
- (65) DAVIES, W.  
1925. THE RELATIVE PALATABILITY OF PASTURE PLANTS. [Qt. Brit.] Min. Agr. Jour. 32: 106-116.
- (66) ———  
1937. MODERN CONCEPTS OF GRASSLAND IMPROVEMENT. Imp. Bur. Plant Genet., Herbage Plants, Herbage Rev. 5: 194-199.
- (67) DAWSON, M. D., and HEINRICH, D. H.  
1952. THE EFFECTS OF VARIOUS GERMINATION TECHNIQUES TO OVERCOME DORMANCY IN GREEN STIPAGRASS SEED. Sci. Agr. 32: 266-271, illus.
- (68) DICKSON, J. G.  
1947. DISEASES OF FIELD CROPS. 429 pp., illus. New York and London.
- (69) DOMINGO, W. E.  
1941. BULK EMASCULATION AND POLLINATION OF SMOOTH BROMEGRASS, *BROMUS INERMIS*. Amer. Soc. Agron. Jour. 33: 993-1002.
- (70) ELLIOTT, F. C.  
1949. THE CYTOLOGY AND FERTILITY RELATIONS OF *BROMUS INERMIS* AND SOME OF ITS RELATIVES. Agron. Jour. 41: 298-303, illus.
- (71) ——— and LOVE, R. M.  
1948. THE SIGNIFICANCE OF MEIOTIC CHROMOSOME BEHAVIOR IN BREEDING SMOOTH BROMEGRASS, *BROMUS INERMIS* LEYSS. Amer. Soc. Agron. Jour. 40: 335-341, illus.

- (72) ELLIOTT, F. C., and WILSIE, C. P.  
1948. A FERTILE POLYHAPLOID IN *BROMUS INERMIS*. Jour. Hered. 39: 377-380, illus.
- (73) EVANS, M., and WILSIE, C. P.  
1946. FLOWERING OF BROMEGRASS, *BROMUS INERMIS*, IN THE GREENHOUSE AS INFLUENCED BY LENGTH OF DAY, TEMPERATURE, AND LEVEL OF FERTILITY. Amer. Soc. Agron. Jour. 38: 923-932, illus.
- (74) EVANS, M. W.  
1922. SOME METHODS OF RECORDING DATA IN TIMOTHY BREEDING. Amer. Soc. Agron. Jour. 14: 62-69, illus.
- (75) ———  
1934. THE PRODUCTION OF TIMOTHY POLLEN. Amer. Jour. Bot. 21: 34-41, illus.
- (76) ———  
1936. SELECTION OF OPEN-POLLINATED TIMOTHY. Amer. Soc. Agron. Jour. 28: 389-394, illus.
- (77) ———  
1937. IMPROVEMENT OF TIMOTHY. U. S. Dept. Agr. Yearbook 1937: 1103-1121, illus.
- (78) ———  
1939. RELATION OF LATITUDE TO CERTAIN PHASES OF THE GROWTH OF TIMOTHY. Amer. Jour. Bot. 26: 212-218, illus.
- (79) ——— and ALLARD, H. A.  
1934. RELATION OF LENGTH OF DAY TO GROWTH OF TIMOTHY. Jour. Agr. Res. 48: 571-586, illus.
- (80) ——— ALLARD, H. A., and McCONKEY, O.  
1935. TIME OF HEADING AND FLOWERING OF EARLY, MEDIUM, AND LATE TIMOTHY PLANTS AT DIFFERENT LATITUDES. Sci. Agr. 15: 573-579, illus.
- (81) ——— and ELY, J. E.  
1936. TIMOTHY SELECTION FOR IMPROVEMENT IN QUALITY OF HAY. Amer. Soc. Agron. Jour. 28: 941-947.
- (82) FAGAN, T. W.  
1929. THE NUTRITIVE VALUE OF PASTURE GRASS AS INFLUENCED BY MANAGEMENT. Welsh Jour. Agr. 5: [99]-109.
- (83) ——— and JONES, H. T.  
1924. THE NUTRITIVE VALUE OF GRASSES AS SHOWN BY THEIR CHEMICAL COMPOSITION. Welsh Plant Breeding Sta., Aberystwyth, [Bul.], Ser. H., 3, pp. 85-130, illus.
- (84) FEDERER, W. T., and SPRAGUE, G. F.  
1947. A COMPARISON OF VARIANCE COMPONENTS IN CORN YIELD TRIALS: I. ERROR, TESTER  $\times$  LINE, AND LINE COMPONENTS IN TOP-CROSS EXPERIMENTS. Amer. Soc. Agron. Jour. 39: 453-463.
- (85) FISHER, W. D.  
1949. THE CONTROLLED CROSS POLLINATION OF STIFFHAIR WHEATGRASS, *AGROPYRON TRICHOPHORUM*, FOLLOWING EMASCULATION WITH HOT WATER. Agron. Jour. 41: 421-424.
- (86) ——— BASHAW, E. C., and HOLT, E. C.  
1954. EVIDENCE FOR APOMIXIS IN *Pennisetum ciliare* AND *Cenchrus setigerus*. Agron. Jour. 46: 401-404, illus.
- (87) FORTMANN, H. R.  
1953. RESPONSES OF VARIETIES OF BROMEGRASS (*BROMUS INERMIS* LEYSS) TO NITROGEN FERTILIZATION AND CUTTING TREATMENTS. N. Y. (Cornell) Agr. Expt. Sta. Mem. 322, 67 pp., illus.
- (88) FRANKS, H. N.  
1940. SOME BREEDING EXPERIMENTS WITH TIMOTHY. Imp. Agr. Bur. Joint Pub. 3, pp. [80]-92.
- (89) FRIDOURG, H. A.  
1953. A RAPID METHOD FOR WASHING ROOTS. Agron. Jour. 45: 334-335, illus.
- (90) FROLIK, E. F., and LEWIS, R. D.  
1944. SEED CERTIFICATION IN THE UNITED STATES AND CANADA. Amer. Soc. Agron. Jour. 36: 183-193.
- (91) GARRER, R. J., and MYERS, W. M.  
1949. METHODS AND TECHNIQUES OF BREEDING AND MAINTAINING GRASS STRAINS. Internatl. Grassland Cong. Rpt. 5: 89-93. [German summary, pp. 92-93.]

- (92) GARBER, R. J., MYERS, W. M., and SPRAGUE, V. G.  
1946. PASTURES AND PASTURE PROBLEMS IN NORTHEASTERN STATES. Pa. Agr. Expt. Sta. Bul. 485, 44 pp., illus.
- (93) GERNERT, W. B.  
1937. VARIATION IN BUFFALO GRASS. Amer. Soc. Agron. Jour. 29: 242-246, illus.
- (94) GORDON, E. M.  
1951. LIGHT- AND TEMPERATURE-SENSITIVENESS IN GERMINATING SEED OF TIMOTHY (*PHLEUM PRATENSE* L.). Sci. Agr. 31: 71-84, illus.
- (95) GRAUMANN, H. O.  
1952. THE POLY-CROSS METHOD OF BREEDING IN RELATION TO SYNTHETIC VARIETIES AND RECURRENT SELECTION OF NEW CLONES. Internatl. Grassland Cong. Proc. 6: 314-319.
- (96) ——— and KEHR, W. R.  
1954. THE PERFORMANCE OF TWO-CLONE AND MULTIPLE-CLONE COMBINATIONS IN ADVANCED GENERATIONS. Alfalfa Impr. Conf. Rpt. 14: 37-39. [Processed.]
- (97) GREGOR, J. W.  
1938. REFLECTIONS CONCERNING NEW CROP VARIETIES. Imp. Bur. Pastures and Forage Crops, Herbage Rev. 6: 234-239.
- (98) ——— and SANSOME, F. W.  
1927. EXPERIMENTS ON THE GENETICS OF WILD POPULATIONS. PART I. GRASSES. Jour. Genet. 17: [349]-364, illus.
- (99) GRIFFITHS, D. J.  
1950. THE FERTILITY OF SEED CROPS OF PERENNIAL RYEGRASS (*LOLIUM PERENNE*) TO CONTAMINATION BY WIND-BORNE POLLEN. Jour. Agr. Sci. [England] 40: 19-38, illus.
- (100) ———  
1952. ISOLATION REQUIREMENTS FOR SEED PRODUCTION OF CROSS-POLLINATED FORAGE CROPS. Internatl. Grassland Cong. Proc. 6: 853-859.
- (101) GRUBER, F.  
1936. [GRASS SEED PRODUCTION AND GERMINABILITY.] Cukortépa 9: 43-46. (Abstract in English by R. Fleischmann, Hungary, in Imp. Bur. Plant Genet., Herbage Plants, Herbage Abs. 6: 370. 1936.)
- (102) GRUNDER, M. S., and DERMANIS, P.  
1952. THE EFFECT OF POLLINATOR PLANTS ON SEED SET IN VEGETATIVELY PROPAGATED ORCHARD GRASS. Agron. Jour. 44: 275-276.
- (103) HALDANE, J. B. S.  
1930. THEORETICAL GENETICS OF AUTOPOLYPLOIDS. Jour. Genet. 22: [359]-372.
- (104) HANSON, A. A., and HILL, H. D.  
1953. THE MEIOTIC BEHAVIOR OF HEXAPLOID ORCHARD GRASS (*DACTYLIS GLOMERATA* L.). Torrey Bot. Club Bul. 80: 113-122, illus.
- (105) ——— MYERS, W. M., and GARBER, R. J.  
1952. THE GENERAL COMBINING ABILITY OF ORCHARDGRASS SELECTIONS AND THEIR 1<sub>4</sub> PROGENIES. Agron. Jour. 44: 84-87.
- (106) ——— and OLDENMEYER, D. L.  
1951. STAINING ROOF-TIP SMEARS WITH ACETO-CARMINE. Stain Technol. 26: 241-242, illus.
- (107) ——— and SPRAGUE, V. G.  
1953. HEADING OF PERENNIAL GRASSES UNDER GREENHOUSE CONDITIONS. Agron. Jour. 45: 248-251.
- (108) ——— SPRAGUE, V. G., and MYERS, W. M.  
1952. EVALUATION OF KENTUCKY BLUEGRASS STRAINS GROWN IN ASSOCIATION WITH WHITE CLOVER. Agron. Jour. 44: 373-376.
- (109) HANSON, H. C.  
1934. A COMPARISON OF METHODS OF BOTANICAL ANALYSIS OF THE NATIVE PRAIRIE IN WESTERN NORTH DAKOTA. Jour. Agr. Res. 49: 815-842, illus.
- (110) HARDISON, J. R.  
1953. SEED DISORDERS OF FORAGE PLANTS. U. S. Dept. Agr. Yearbook 1953 (Plant Diseases): 272-276, illus.
- (111) HARLAN, J. R.  
1950. THE BREEDING BEHAVIOR OF SIDE-OATS GRAMA IN PARTIALLY ISOLATED POPULATIONS. Agron. Jour. 42: 20-24, illus.

- (112) HARLAN, J. R., BURTON, G. W., and ELDER, W. C.  
1954. MIDLAND BERMUDA GRASS. A NEW VARIETY FOR OKLAHOMA PASTURES. Okla. Agr. Expt. Sta. Bul. B-416, 10 pp., illus.
- (113) ——— SNYDER, L. A., and CELARIER, R. P.  
1952. CYTOLOGICAL STUDIES OF SOUTHERN GREAT PLAINS GRASSES. Internatl. Grassland Cong. Proc. 6: 228-232.
- (114) HARRISON, C. M., and HODGSON, C. W.  
1939. RESPONSE OF CERTAIN PERENNIAL GRASSES TO CUTTING TREATMENTS. Amer. Soc. Agron. Jour. 31: 418-430, illus.
- (115) HAWK, V. B., and WELCH, A. W.  
1948. TOLERANCE OF VARIETIES OF SMOOTH BROMEGRASS, *BROMUS INERMIS* LEYSS., TO INJURY BY ROOT ROT, *PYTHIUM GRAMINICOLA* SUBR. Amer. Soc. Agron. Jour. 40: 809-817, illus.
- (116) ——— and WILSIE, C. P.  
1952. PARENT-PROGENY YIELD RELATIONSHIPS IN BROMEGRASS, *BROMUS INERMIS* LEYSS. Agron. Jour. 44: 112-118, illus.
- (117) HAYES, H. K.  
1935. GREEN PASTURES FOR THE PLANT BREEDER. Amer. Soc. Agron. Jour. 27: 957-962.
- (118) ——— and BARKER, H. D.  
1922. THE EFFECTS OF SELF-FERTILIZATION IN TIMOTHY. Amer. Soc. Agron. Jour. 14: 289-293.
- (119) ——— and CLARKE, S. E.  
1925. SELECTION IN SELF-FERTILIZED LINES AS A MEANS OF IMPROVING TIMOTHY. Sci. Agr. 5: 313-317, illus.
- (120) ——— and SCHMID, A. R.  
1943. SELECTION IN SELF-POLLINATED LINES OF *BROMUS INERMIS* LEYSS., *FESTUCA ELATIOR* L. AND *DACTYLIS GLOMERATA* L. Amer. Soc. Agron. Jour. 35: 934-943.
- (121) HAYS, W. M.  
1892. IMPROVEMENT OF TIMOTHY. Minn. Agr. Expt. Sta. Bul. 20: [45]-46.
- (122) HEIN, M. A., and HENSON, P. R.  
1942. COMPARISON OF THE EFFECT OF CLIPPING AND GRAZING TREATMENTS ON THE BOTANICAL COMPOSITION OF PERMANENT PASTURE MIXTURES. Amer. Soc. Agron. Jour. 34: 566-573.
- (123) HEINRICH, D. H.  
1953. METHODS OF BREEDING AGROPYRON INTERMEDIUM. Canad. Jour. Agr. Sci. 33: 470-493, illus.
- (124) HERMANN, E. M., and HERMANN, W.  
1939. THE EFFECT OF MATURITY AT TIME OF HARVEST ON CERTAIN RESPONSES OF SEED OF CRESTED WHEATGRASS, *AGROPYRON CRISTATUM* (L.) GAERTN. Amer. Soc. Agron. Jour. 31: 876-885, illus.
- (125) HIESEY, W. M.  
1953. GROWTH AND DEVELOPMENT OF SPECIES AND HYBRIDS OF POA UNDER CONTROLLED TEMPERATURES. Amer. Jour. Bot. 40: 205-221, illus.
- (126) HILL, H. D., and MYERS, W. M.  
1944. ISOLATION OF DIPLOID AND TETRAPLOID CLONES FROM MIXOPLOID PLANTS OF RYEGRASS (*LOLIUM PERENNE* L.), PRODUCED BY TREATMENT OF GERMINATING SEEDS WITH COLCHICINE. Jour. Hered. 35: 359-361.
- (127) ——— and MYERS, W. M.  
1945. A SCHEDULE INCLUDING COLD TREATMENT TO FACILITATE SOMATIC CHROMOSOME COUNTS IN CERTAIN FORAGE GRASSES. Stain Technol. 20: 89-92, illus.
- (128) HITTLE, C. N.  
1954. A STUDY OF THE POLY-CROSS PROGENY TESTING TECHNIQUE AS USED IN THE BREEDING OF SMOOTH BROMEGRASS. Agron. Jour. 46: 521-523.
- (129) HODGSON, H. J.  
1949. FLOWERING HABITS AND POLLEN DISPERSAL IN *PENSACOLA BAHIA* GRASS, *PASPALUM NOTATUM* FLUGGE. Agron. Jour. 41: 337-343, illus.
- (130) HULL, F. H.  
1945. RECURRENT SELECTION FOR SPECIFIC COMBINING ABILITY IN CORN. Amer. Soc. Agron. Jour. 37: 134-145.
- (131) JARVIS, T. D.  
1932. THE ENVIRONMENTAL COINCIDENCE AS A FACTOR IN INCIDENCE AND CONTROL OF PLANT DISEASES. Sci. Agr. 13: 36-57.

- (132) JENKIN, T. J.  
1924. THE ARTIFICIAL HYBRIDISATION OF GRASSES. Welsh Plant Breeding Sta., Aberystwyth, [Bul.], Ser. H, 2, 18 pp.
- (133) ———  
1926. SELF AND CROSS-FERTILISATION IN *LOLIUM PERENNE* L. Jour. Genet. 17: [11]-17.
- (134) ———  
1931. THE METHOD AND TECHNIQUE OF SELECTION, BREEDING AND STRAIN-BUILDING IN GRASSES. Imp. Bur. Plant Genet., Herbage Plants, Bul. 3, pp. 5-34.
- (135) ———  
1931. SELF-FERTILITY IN PERENNIAL RYE-GRASS (*LOLIUM PERENNE* L.). Welsh Plant Breeding Sta., Aberystwyth, [Bul.], Ser. H, 12, pp. 100-119.
- (136) ———  
1933. INTERSPECIFIC AND INTERGENERIC HYBRIDS IN HERBAGE GRASSES. INITIAL CROSSES. Jour. Genet. 28: [205]-264.
- (137) ———  
1937. SOME ASPECTS OF STRAIN-BUILDING IN THE HERBAGE GRASSES. Internatl. Grassland Cong. Rpt. 4: 54-60. [German summary, pp. 59-60.]
- (138) JODON, N. E.  
1938. EXPERIMENTS ON ARTIFICIAL HYBRIDIZATION OF RICE. Amer. Soc. Agron. Jour. 30: 294-305, illus.
- (139) JOHNSON, A. A., and DEXTER, S. T.  
1939. THE RESPONSE OF QUACK GRASS TO VARIATIONS IN HEIGHT OF CUTTING AND RATES OF APPLICATION OF NITROGEN. Amer. Soc. Agron. Jour. 31: 67-76, illus.
- (140) JOHNSON, I. J.  
1952. EFFECTIVENESS OF RECURRENT SELECTION FOR GENERAL COMBINING ABILITY IN SWEETCLOVER, *MELILOTUS OFFICINALIS*. Agron. Jour. 44: 476-481, illus.
- (141) ———  
1952. EVALUATING BREEDING MATERIALS FOR COMBINING ABILITY. Internatl. Grassland Cong. Proc. 6: 327-334.
- (142) ——— and MILLER, E. S.  
1940. LEAF PIGMENT CONCENTRATION AND ITS RELATION TO YIELD IN FAIRWAY CRESTED WHEAT GRASS AND PARKLAND BROME GRASS. Amer. Soc. Agron. Jour. 32: 302-307.
- (143) JONES, M. D., and BROWN, J. G.  
1951. POLLINATION CYCLES OF SOME GRASSES IN OKLAHOMA. Agron. Jour. 43: 218-222, illus.
- (144) ——— and NEWELL, L. C.  
1946. POLLINATION CYCLES AND POLLEN DISPERSAL IN RELATION TO GRASS IMPROVEMENT. Nebr. Agr. Expt. Sta. Res. Bul. 148, [43] pp., illus.
- (145) ——— and NEWELL, L. C.  
1948. SIZE, VARIABILITY, AND IDENTIFICATION OF GRASS POLLEN. Amer. Soc. Agron. Jour. 40: 136-143.
- (146) JULÉN, G.  
1948. INBREEDING IN HERBAGE PLANTS. In Åkerman, Å., Tedin, O., Fröier, K., and Whyte, R. O., eds., Svalöf 1886-1946, History and Present Problems, pp. [211]-236, illus. Lund, [Sweden].
- (147) KALTON, R. R., SMIT, A. G., and LEFFEL, R. C.  
1952. PARENT-INBRED PROGENY RELATIONSHIPS OF SELECTED ORCHARD GRASS CLONES. Agron. Jour. 44: 481-486.
- (148) KEARNS, V., and TOOLB, E. H.  
1939. TEMPERATURE AND OTHER FACTORS AFFECTING THE GERMINATION OF FESCUE SEED. U. S. Dept. Agr. Tech. Bul. 638, 35 pp.
- (149) KELLER, W.  
1943. SEED PRODUCTION ON GRASS CULMS DETACHED PRIOR TO POLLINATION. Amer. Soc. Agron. Jour. 35: 617-624, illus.
- (150) ———  
1944. THE BULK HYBRIDIZATION OF SMOOTH BROMEGRASS. Jour. Hered. 35: 49-56, illus.



- (151) KELLER, W.  
1945. AN EVALUATION OF KRAFT AND PARCHMENT PAPER BAGS FOR THE CONTROL OF POLLINATION IN GRASSES. Amer. Soc. Agron. Jour. 37: 902-909.
- (152) ———  
1946. DESIGNS AND TECHNIC FOR THE ADAPTATION OF CONTROLLED COMPETITION TO FORAGE PLANT BREEDING. Amer. Soc. Agron. Jour. 38: 580-588, illus.
- (153) ———  
1948. INTERPRETATION OF SELF-FERTILITY IN GRASSES BY FREQUENCY DISTRIBUTIONS. Amer. Soc. Agron. Jour. 40: 894-900, illus.
- (154) ———  
1952. EMASCULATION AND POLLINATION TECHNIQS. Internatl. Grassland Cong. Proc. 6: 1613-1619.
- (155) ———  
1953. WATER REQUIREMENT OF SELECTED GENOTYPES OF ORCHARDGRASS, DACTYLIS GLOMERATA L. Agron. Jour. 45: 622-625, illus.
- (156) KEMP, W. B.  
1937. NATURAL SELECTION WITHIN PLANT SPECIES. Jour. Hered. 28: 329-333, illus.
- (157) KETSER, A.  
1913. VARIATION STUDIES IN BROME GRASS (A PRELIMINARY REPORT). Colo. Agr. Expt. Sta. Bul. 190, 20 pp., illus.
- (158) KINMAN, M. L., and SPRAGUE, G. F.  
1945. RELATION BETWEEN NUMBER OF PARENTAL LINES AND THEORETICAL PERFORMANCE OF SYNTHETIC VARIETIES OF CORN. Amer. Soc. Agron. Jour. 37: 341-351, illus.
- (159) KLAPP, E.  
1938. PRINCIPLES GOVERNING THE VALUE OF HERBAGE PLANTS FOR HAY AND PASTURE USE. Imp. Bur. Pastures and Forage Crops, Herbage Rev. 6: [57]-63.
- (160) KNEEBONE, W. R., and BROWN, H. W.  
1953. A MODIFIED HAMMERMILL FOR PROCESSING GRASS SEED. Agron. Jour. 45: 389-391, illus.
- (161) KNIGHT, W. E., and BENNETT, H. W.  
1953. PRELIMINARY REPORT OF THE EFFECT OF PHOTOPERIOD AND TEMPERATURE ON THE FLOWERING AND GROWTH OF SEVERAL SOUTHERN GRASSES. Agron. Jour. 45: 268-269.
- (162) KNOBLOCH, I. W.  
1943. MORPHOLOGICAL VARIATION AND CYTOLOGY OF BROMUS INERMIS. Torrey Bot. Club. Bul. 70: 467-472, illus.
- (163) KNOWLES, R. P.  
1950. STUDIES OF COMBINING ABILITY IN BROMEGRASS AND CRESTED WHEATGRASS. Sci. Agr. 30: 275-302.
- (164) ——— and HORNER, W. H.  
1943. METHODS OF SELFING AND CROSSING CRESTED WHEAT GRASS, AGROPYRON CRISTATUM (L.) BEAUV. Sci. Agr. 23: 598-611.
- (165) ——— and WHITE, W. J.  
1949. THE PERFORMANCE OF SOUTHERN STRAINS OF BROME GRASS IN WESTERN CANADA. Sci. Agr. 29: 437-450, illus.
- (166) KRAMER, H. H.  
1947. MORPHOLOGIC AND AGRONOMIC VARIATION IN POA PRATENSIS L. IN RELATION TO CHROMOSOME NUMBERS. Amer. Soc. Agron. Jour. 39: 181-191.
- (167) ———  
1952. AGRONOMIC PROBLEMS OF STRAIN EVALUATION WITH FORAGE CROPS. Internatl. Grassland Cong. Proc. 6: 341-346.
- (168) KREITLOW, K. W.  
1952. INDUCED EPIPHYTICS. Internatl. Grassland Cong. Proc. 6: 1573-1577.
- (169) ——— GRAHAM, J. H., and GARBER, R. J.  
1953. DISEASES OF FORAGE GRASSES AND LEGUMES IN THE NORTHEASTERN STATES. Pa. Agr. Expt. Sta. Bul. 573, 42 pp., illus.
- (170) ——— and MYERS, W. M.  
1947. RESISTANCE TO CROWN RUST IN FESTUCA ELATION AND F. ELATION VAR. ARUNDINACEA. Phytopathology 37: 59-63, illus.

- (171) LAMP, H. F.  
1952. REPRODUCTIVE ACTIVITY IN *BROMUS INERMIS* IN RELATION TO PHASES OF TILLER DEVELOPMENT. *Bot. Gaz.* 113: 413-438.
- (172) LAUDE, H. M.  
1949. DELAYED GERMINATION OF CALIFORNIA OATGRASS, *DANTHONIA CALIFORNICA*. *Agron. Jour.* 41: 404-408.
- (173) ——— and CHAUGULE, B. A.  
1953. EFFECT OF STAGE OF SEEDLING DEVELOPMENT UPON HEAT TOLERANCE IN BROMEGRASSES. *Jour. Range Mangt.* 6: 320-324.
- (174) ——— SHRUM, J. E., Jr., and BIEHLER, W. E.  
1952. THE EFFECT OF HIGH SOIL TEMPERATURES ON THE SEEDLING EMERGENCE OF PERENNIAL GRASSES. *Agron. Jour.* 44: 110-112.
- (175) LAW, A. G., and ANDERSON, K. L.  
1940. THE EFFECT OF SELECTION AND INBREEDING ON THE GROWTH OF BIG BLUESTEM (*ANDROPOGON FURCATUS*, MUHL.). *Amer. Soc. Agron. Jour.* 32: 931-943, illus.
- (176) LEASURE, J. K.  
1949. DETERMINING THE SPECIES COMPOSITION OF SWARDS. *Agron. Jour.* 41: 204-206.
- (177) LEGGATT, C. W.  
1939. A NEW SEED BLOWER. *Assoc. Off. Seed Anal. No. Amer. Proc.* (1938) 30: 120-132, illus. [Processed.]
- (178) LEVY, E. B.  
1933. STRAIN TESTING AND STRAIN BUILDING. *Imp. Bur. Plant Genet., Herbage Plants, Bul.* 11, pp. 6-16, illus.
- (179) ——— and MADDEN, E. A.  
1933. THE POINT METHOD OF PASTURE ANALYSIS. *New Zeal. Dept. Agr. Jour.* 46: 267-279, illus.
- (180) LÖVE, A., and LÖVE, D.  
1952. THE GEO-BOTANICAL SIGNIFICANCE OF POLYPLOIDY. *Internatl. Grassland Cong. Proc.* 6: 240-246.
- (181) LONNQUIST, J. H.  
1951. RECURRENT SELECTION AS A MEANS OF MODIFYING COMBINING ABILITY IN CORN. *Agron. Jour.* 43: 311-315, illus.
- (182) LOVE, R. M.  
1947. INTERSPECIFIC AND INTERGENERIC HYBRIDIZATION IN FORAGE CROP IMPROVEMENT. *Amer. Soc. Agron. Jour.* 39: 41-46.
- (183) ———  
1952. THE VALUE OF INDUCED POLYPLOIDY IN BREEDING. *Internatl. Grassland Cong. Proc.* 6: 292-298.
- (184) LOWE, C. C., and MURPHY, R. P.  
1951. ANTHESIS AND OPEN-POLLINATED SEED SETTING IN BROMEGRASS. *Amer. Soc. Agron., Abstracts, Ann. Meeting* 43: 9. [Processed.]
- (185) McALISTER, D. F.  
1943. THE EFFECT OF MATURITY ON THE VIABILITY AND LONGEVITY OF THE SEEDS OF WESTERN RANGE AND PASTURE GRASSES. *Amer. Soc. Agron. Jour.* 35: 442-453, illus.
- (186) ———  
1943. A TECHNIC FOR GROWING SEEDLINGS OF GRASS AND OTHER PLANTS FOR FIELD TRANSPLANTING. *Amer. Soc. Agron. Jour.* 35: 836-840, illus.
- (187) ———  
1944. DETERMINATION OF SOIL DROUGHT RESISTANCE IN GRASS SEEDLINGS. *Amer. Soc. Agron. Jour.* 36: 324-336, illus.
- (188) McDONALD, E. D., KALTON, R. R., and WEISS, M. G.  
1952. INTERRELATIONSHIPS AND RELATIVE VARIABILITY AMONG  $S_1$  AND OPEN-POLLINATION PROGENIES OF SELECTED BROMEGRASS CLONES. *Agron. Jour.* 44: 20-25.
- (189) McMILLAN, J. R. A.  
1940. PASTURE PLANT BREEDING IN AUSTRALIA. *Imp. Bur. Pastures and Forage Crops, Bul.* 29, pp. 22-26.
- (190) MALTE, M. O.  
1921. BREEDING METHODS IN FORAGE PLANTS. *Sci. Agr.* 1: 25-29, illus.
- (191) MILTON, W. E. J.  
1938. A COMPARISON OF THE HERBAGE YIELDS FROM BROADCAST PLOTS VERSUS CULTIVATED BRILLS OF CERTAIN SPECIES AND STRAINS OF GRASS. *Welsh Jour. Agr.* 14: 203-212.

- (192) MUELLER, I. M., and WEAVER, J. E.  
1942. RELATIVE DROUGHT RESISTANCE OF SEEDLINGS OF DOMINANT PRAIRIE GRASSES. Ecology 23: 387-398, illus.
- (193) MÜNTZING, A.  
1937. THE EFFECTS OF CHROMOSOMAL VARIATION IN DACTYLIS. Hereditas 23: [113]-235, illus.
- (194) ———  
1938. NOTE ON HETEROPLOID TWIN PLANTS FROM ELEVEN GENERA. Hereditas 24: [487]-491.
- (195) ———  
1940. FURTHER STUDIES ON APOMIXIS AND SEXUALITY IN POA. Hereditas 26: [115]-190, illus.
- (196) ——— and PRAKKE, R.  
1940. THE MODE OF CHROMOSOME PAIRING IN PHLEUM TWINS WITH 63 CHROMOSOMES AND ITS CYTOGENETIC CONSEQUENCES. Hereditas 26: [463]-501, illus.
- (197) MURPHY, R. P.  
1942. METHODS OF BREEDING CRESTED WHEATGRASS, AGROPYRON CRISTATUM (L.) BEAUV. Amer. Soc. Agron. Jour. 34: 553-565.
- (198) ———  
1952. COMPARISON OF DIFFERENT TYPES OF PROGENY IN EVALUATING BREEDING BEHAVIOR. Internat. Grassland Cong. Proc. 6: 320-326.
- (199) ——— and ARNY, A. C.  
1939. THE EMERGENCE OF GRASS AND LEGUME SEEDLINGS PLANTED AT DIFFERENT DEPTHS IN FIVE SOIL TYPES. Amer. Soc. Agron. Jour. 31: 17-28.
- (200) ——— and ATWOOD, S. S.  
1953. THE USE OF 11 FAMILIES IN BREEDING SMOOTH BROMEGRASS. Agron. Jour. 45: 24-28.
- (201) MYERS, W. M.  
1939. COLCHICINE INDUCED TETRAPLOIDY IN PERENNIAL RYEGRASS. Jour. Hered. 30: 499-504, illus.
- (202) ———  
1940. TETRASOMIC INHERITANCE IN DACTYLIS GLOMERATA. (Abstract) Genetics 25: 126.
- (203) ———  
1941. GENETICAL CONSEQUENCES OF THE CHROMOSOMAL BEHAVIOR IN ORCHARD GRASS, DACTYLIS GLOMERATA L. Amer. Soc. Agron. Jour. 33: 893-900, illus.
- (204) ———  
1942. HERITABLE VARIATIONS IN SEED SET UNDER BAG AMONG PLANTS OF ORCHARD GRASS, DACTYLIS GLOMERATA L. Amer. Soc. Agron. Jour. 34: 1042-1051.
- (205) ———  
1942. ANALYSIS OF NONHERITABLE VARIATIONS IN SEED SET UNDER BAG AMONG PLANTS OF ORCHARD GRASS, DACTYLIS GLOMERATA L. Amer. Soc. Agron. Jour. 34: 1114-1124.
- (206) ———  
1943. SECOND GENERATION PROGENY TESTS OF THE METHOD OF REPRODUCTION IN KENTUCKY BLUEGRASS, POA PRATENSIS L. Amer. Soc. Agron. Jour. 35: 413-419.
- (207) ———  
1947. CYTOLOGY AND GENETICS OF FORAGE GRASSES. Bot. Rev. 13: 319-367, 369-421.
- (208) ———  
1948. INCREASED MEIOTIC IRREGULARITY AND DECREASED FERTILITY ACCOMPANYING INBREEDING IN DACTYLIS GLOMERATA. Amer. Soc. Agron. Jour. 40: 249-254.
- (209) ——— and CHILTON, S. J. P.  
1941. CORRELATED STUDIES OF WINTERHARDINESS AND RUST REACTION OF PARENTS AND INBRED PROGENIES OF ORCHARD GRASS AND TIMOTHY. Amer. Soc. Agron. Jour. 33: 215-220, illus.
- (210) ——— and GARBER, R. J.  
1942. THE EVALUATION OF INDIVIDUAL PLANTS OF PASTURE GRASSES IN ASSOCIATION WITH WHITE CLOVER. Amer. Soc. Agron. Jour. 34: 7-15, illus.

- (211) MYERS, W. M., and HILL, H. D.  
1942. VARIATIONS IN CHROMOSOMAL ASSOCIATION AND BEHAVIOR DURING MEIOSIS AMONG PLANTS FROM OPEN-POLLINATED POPULATIONS OF *DACTYLIS GLOMERATA*. Bot. Gaz. 104: 171-177.
- (212) NEW SOUTH WALES DEPARTMENT OF AGRICULTURE.  
1950. GRASSES. N. S. Wales Dept. Agr. Sci. Bul. 72, pp. 127-133, illus.
- (213) NEWELL, L. C.  
1951. CONTROLLED LIFE CYCLES OF BROMEGRASS, *BROMUS INERMIS* LEYSS., USED IN IMPROVEMENT. Agron. Jour. 43: 417-424, illus.
- (214) ——— and KEIM, F. D.  
1943. FIELD PERFORMANCE OF BROMEGRASS STRAINS FROM DIFFERENT REGIONAL SEED SOURCES. Agron. Jour. 35: 420-434, illus.
- (215) ——— and TYSDAL, H. M.  
1945. NUMBERING AND NOTE-TAKING SYSTEMS FOR USE IN THE IMPROVEMENT OF FORAGE CROPS. Amer. Soc. Agron. Jour. 37: 736-749.
- (216) NIELSEN, E. L.  
1944. ANALYSIS OF VARIATION IN *PANICUM VIRGATUM*. Jour. Agr. Res. 69: 327-353, illus.
- (217) ———  
1947. POLYPLOIDY AND WINTER SURVIVAL IN *PANICUM VIRGATUM* L. Amer. Soc. Agron. Jour. 39: 822-827.
- (218) ———  
1951. CYTOLOGY AND BREEDING BEHAVIOR OF TWIN PLANTS OF *BROMUS INERMIS*. Bot. Gaz. 113: 23-54, illus.
- (219) ———  
1952. CYTOLOGY IN RELATION TO BREEDING BEHAVIOR IN GRASSES. Internatl. Grassland Cong. Proc. 6: 233-239.
- (220) ——— and ROGGER, G. A.  
1952. AN AMPHIDIPLOID OF *XSTIPORYZOPSIS*. Amer. Jour. Bot. 39: 343-348, illus.
- (221) NILSSON, F.  
1934. STUDIES IN FERTILITY AND INBREEDING IN SOME HERBAGE GRASSES. Hereditas 19: [1]-162.
- (222) NILSSON-LEISSNER, G.  
1942. A CASE OF INCREASED VITALITY IN SUBPOLINATED LATER GENERATIONS OF SELF-FERTILIZED *DACTYLIS GLOMERATA* STRAINS. Hereditas 28: 222-224.
- (223) ———  
1942. ON THE POSSIBILITIES OF BREEDING NEW, IMPROVED STRAINS OF TIMOTHY BY MEANS OF SELFING. Hereditas 28: 500-502.
- (224) ———  
1948. NATURAL SELECTION AND THE BREEDING OF CROSS-FERTILIZING PLANTS. In Åkerman, Å., Tedin, O., Fröier, K., and Whyte, R. O., eds., Svalöf 1886-1946, History and Present Problems, pp. [198]-210, illus. Lund, [Sweden].
- (225) ——— and NILSSON, F.  
1940. HERBAGE PLANT BREEDING IN SWEDEN. Imp. Agr. Bur. Joint Pub. 3, pp. [15]-51.
- (226) NORDENSKIÖLD, H.  
1945. CYTO-GENETIC STUDIES IN THE GENUS *PHLEUM*. Acta Agr. Suecana 1: 1-[138], illus.
- (227) NOWOSAD, F. S.  
1939. PRELIMINARY TESTS WITH SOME PLANT HORMONES IN THE ROOTING OF CUTTINGS OF CERTAIN FORAGE PLANTS. Sci. Agr. 19: 494-503, illus.
- (228) OLMSTED, C. E.  
1952. PHOTOPERIODISM IN NATIVE RANGE GRASSES. Internatl. Grassland Cong. Proc. 6: 676-682.
- (229) PATTERSON, J. K., and LAW, A. G.  
1952. A TECHNIQUE STUDY IN GRASS STRAIN TESTING. Agron. Jour. 44: 520-524.
- (230) PETERSON, M. L., and LOOMIS, W. E.  
1949. EFFECTS OF PHOTOPERIOD AND TEMPERATURE ON GROWTH AND FLOWERING OF KENTUCKY BLUEGRASS. Plant Physiol. 24: 31-43, illus.

- (231) PICKETT, R. C.  
1950. VARIABILITY OF CRUDE PROTEIN AND CAROTENE CONTENTS AND THEIR RELATIONS WITH OTHER CHARACTERS IN BROMEGRASS, *BROMUS INERMIS* LEYSS. *Agron. Jour.* 42: 550-554.
- (232) PLUMMER, A. P.  
1943. THE GERMINATION AND EARLY SEEDLING DEVELOPMENT OF TWELVE RANGE GRASSES. *Amer. Soc. Agron. Jour.* 35: 19-34, illus.
- (233) ——— and FRISCHNECHT, N. C.  
1952. INCREASING FIELD STANDS OF INDIAN RICEGRASS. *Agron. Jour.* 44: 285-289, illus.
- (234) RIHERD, P. T., and CHADA, H. L.  
1952. SOME SCALE INSECTS ATTACKING GRASSES IN TEXAS. *Tex. Agr. Expt. Sta. Prog. Rpt.* 1461, 5 pp. [Processed.]
- (235) ROBINSON, R. R., and SPRAGUE, V. G.  
1952. RESPONSES OF ORCHARD GRASS—LADINO CLOVER TO IRRIGATION AND NITROGEN FERTILIZATION. *Agron. Jour.* 44: 244-247, illus.
- (236) ROGGER, G. A.  
1941. GRASS BREEDING AND IMPROVEMENT. *In* N. Dak. Agr. Expt. Sta. Bul. 300, pp. 93-98, illus.
- (237) ———  
1943. RESPONSE OF GEOGRAPHICAL STRAINS OF GRASSES TO LOW TEMPERATURES. *Amer. Soc. Agron. Jour.* 35: 547-559, illus.
- (238) ———  
1944. RELATIVE PALATABILITIES OF GRASSES UNDER CULTIVATION ON THE NORTHERN GREAT PLAINS. *Amer. Soc. Agron. Jour.* 36: 487-496.
- (239) ———  
1954. NORDAN CRESTED WHEATGRASS. *N. Dak. Agr. Expt. Sta. Bimonthly Bul.* 16: 150-152.
- (240) ———  
1954. SEED SIZE AND SEEDLING VIGOR IN CRESTED WHEATGRASS. *Agron. Jour.* 46: 216-220, illus.
- (241) SCHULTZ, H. K.  
1941. A STUDY OF METHODS OF BREEDING ORCHARD GRASS, *DACTYLIS GLOMERATA* L. *Amer. Soc. Agron. Jour.* 33: 546-558.
- (242) ——— and HAYES, H. K.  
1938. ARTIFICIAL DROUGHT TESTS OF SOME HAY AND PASTURE GRASSES AND LEGUMES IN SOIL AND SEEDLING STAGES OF GROWTH. *Amer. Soc. Agron. Jour.* 30: 676-682, illus.
- (243) SCHWENDIMAN, J. L., and MULLEN, L. A.  
1944. EFFECTS OF PROCESSING ON GERMINATIVE CAPACITY OF SEED OF TALL OATGRASS, *ARRHENATHERUM ELATIUS* (L.) MERT. AND KOCH. *Amer. Soc. Agron. Jour.* 36: 783-785.
- (244) SHIRLEY, H. L.  
1934. A METHOD FOR STUDYING DROUGHT RESISTANCE IN PLANTS. *Science* 79: 14-16, illus.
- (245) SMELOV, S. P.  
1937. THEORETICAL ASPECTS OF GRASSLAND FARMING. *Imp. Bur. Plant Genet., Herbage Plants, Herbage Rev.* 5: 132-145.
- (246) SMITH, D. C.  
1942. INTERGENERIC HYBRIDIZATION OF CEREALS AND OTHER GRASSES. *Jour. Agr. Res.* 64: 33-47, illus.
- (247) ———  
1944. POLLINATION AND SEED FORMATION IN GRASSES. *Jour. Agr. Res.* 68: 79-95.
- (248) ———  
1952. BREEDING FOR QUALITY. *Internatl. Grassland Cong. Proc.* 6: 1597-1606.
- (249) ——— and NIELSEN, E. L.  
1945. MORPHOLOGICAL VARIATION IN *POA PRATENSIS* L. AS RELATED TO SUBSEQUENT BREEDING BEHAVIOR. *Amer. Soc. Agron. Jour.* 37: 1033-1040, illus.
- (250) ——— and NIELSEN, E. L.  
1946. COMPARATIVE BREEDING BEHAVIOR OF PROGENIES FROM ENCLOSED AND OPEN-POLLINATED PANICLES OF *POA PRATENSIS* L. *Amer. Soc. Agron. Jour.* 38: 804-809.

- (251) SMITH, D. C., and NIELSEN, E. L.  
1951. COMPARISONS OF CLONAL ISOLATIONS OF *POA PRATENSIS* L. FROM GOOD AND POOR PASTURES FOR VIGOR, VARIABILITY, AND DISEASE REACTIONS. *Agron. Jour.* 43: 214-218.
- (252) SMITH, L.  
1947. THE ACETOCARMINE SMEAR TECHNIC. *Stain Technol.* 22: 17-31, illus.
- (253) SNYDER, L. A.  
1951. CYTOLOGY OF INTER-STRAIN HYBRIDS AND THE PROBABLE ORIGIN OF VARIABILITY IN *ELYMUS GLAUCUS*. *Amer. Jour. Bot.* 38: 195-202, illus.
- (254) SPRAGUE, G. F., and BRIMHALL, B.  
1950. RELATIVE EFFECTIVENESS OF TWO SYSTEMS OF SELECTION FOR OIL CONTENT OF THE CORN KERNEL. *Agron. Jour.* 42: 83-88, illus.
- (255) ——— and TATUM, L. A.  
1942. GENERAL VS. SPECIFIC COMBINING ABILITY IN SINGLE CROSSES OF CORN. *Amer. Soc. Agron. Jour.* 34: 923-932.
- (256) SPRAGUE, R.  
1953. ROOT AND CROWN ROTS OF THE GRASSES. U. S. Dept. Agr. Yearbook 1953 (Plant Diseases): 267-272.
- (257) SPRAGUE, V. G.  
1940. GERMINATION OF FRESHLY HARVESTED SEEDS OF SEVERAL *POA* SPECIES AND OF *DACTYLIS GLOMERATA*. *Amer. Soc. Agron. Jour.* 32: 715-721.
- (258) ———  
1948. THE RELATION OF SUPPLEMENTARY LIGHT AND SOIL FERTILITY TO HEADING IN THE GREENHOUSE OF SEVERAL PERENNIAL FORAGE GRASSES. *Amer. Soc. Agron. Jour.* 40: 144-154, illus.
- (259) ——— and GARBER, R. J.  
1950. EFFECT OF TIME AND HEIGHT OF CUTTING AND NITROGEN FERTILIZATION ON THE PERSISTENCE OF THE LEGUME AND PRODUCTION OF ORCHARD-GRASS-LADINO AND BROMEGRASS-LADINO ASSOCIATIONS. *Agron. Jour.* 42: 586-593, illus.
- (260) ——— ROBINSON, R. R., and GARBER, R. J.  
1952. MANAGEMENT OF GRASSLANDS IN THE NORTHEASTERN STATES. Pa. Agr. Expt. Sta. Bul. 554, 30 pp., illus.
- (261) ——— and SULLIVAN, J. T.  
1950. RESERVE CARBOHYDRATES IN ORCHARD GRASS CLIPPED PERIODICALLY. *Plant Physiol.* 25: 92-102, illus.
- (262) STAPLEDON, R. G.  
1927. CHARACTERS WHICH DETERMINE THE ECONOMIC VALUE OF GRASSES. I. NUTRITIVE VALUE AND PALATABILITY. [*Gt. Brit.*] *Min. Agr. Jour.* 33: 1083-1091.
- (263) ———  
1927. CHARACTERS WHICH DETERMINE THE ECONOMIC VALUE OF GRASSES. II. RATIO OF LEAF TO STEM. [*Gt. Brit.*] *Min. Agr. Jour.* 34: 11-19.
- (264) ———  
1927. CHARACTERS WHICH DETERMINE THE ECONOMIC VALUE OF GRASSES. III. TILLER PRODUCTION AND POWERS OF RESISTANCE TO REPEATED DEFOLIATION. [*Gt. Brit.*] *Min. Agr. Jour.* 34: 146-154.
- (265) ———  
1927. CHARACTERS WHICH DETERMINE THE ECONOMIC VALUE OF GRASSES. IV. PERSISTENCY AND AGGRESSIVENESS. [*Gt. Brit.*] *Min. Agr. Jour.* 34: 251-258.
- (266) ———  
1931. METHODS AS APPLIED TO COCKSFOOT GRASS (*DACTYLIS GLOMERATA* L.) AND REMARKS AS TO TECHNIQUE IN GENERAL. *Imp. Bur. Plant Genet., Herbage Plants, Bul.* 3, pp. 35-45, illus.
- (267) ———  
1931. SELF- AND CROSS-FERTILITY AND VIGOUR IN COCKSFOOT GRASS (*DACTYLIS GLOMERATA* L.). *Welsh Plant Breeding Sta., Aberystwyth, [Bul.]*, Ser. H, 12, pp. 161-180.
- (268) STEBBINS, G. L., Jr.  
1942. POLYPLOID COMPLEXES IN RELATION TO ECOLOGY AND THE HISTORY OF FLORAS. *Amer. Nat.* 76: 36-45, illus.
- (269) ———  
1947. TYPES OF POLYPLOIDS: THEIR CLASSIFICATION AND SIGNIFICANCE. In Demerec, M., ed., *Advances in Genetics* 1: 403-429.

- (270) STEBBINS, G. L., JR.  
1949. THE EVOLUTIONARY SIGNIFICANCE OF NATURAL AND ARTIFICIAL POLY-  
PLOIDS IN THE FAMILY GRAMINEAE. Internatl. Cong. Genet. Proc.  
(1948) 8: 461-485, illus. In *Hereditas*, Supplementary Volume  
1949.
- (271) ———  
1950. VARIATION AND EVOLUTION IN PLANTS. 643 pp., illus. New York.
- (272) ———  
1952. DISCUSSION [CONCERNING APOMIXIS]. Internatl. Grassland Cong.  
Proc. 6: 215.
- (273) ———  
1952. SPECIES HYBRIDS IN GRASSES. Internatl. Grassland Cong. Proc. 6:  
247-253.
- (274) ——— VALENCIA, J. I., and VALENCIA, R. M.  
1946. ARTIFICIAL AND NATURAL HYBRIDS IN THE GRAMINEAE, TRIBE HORDEAE  
I. ELYMUS, STANION, AND AGROPYRON. Amer. Jour. Bot. 33: 338-351,  
illus.
- (275) STEPHENS, J. C., and QUINBY, J. R.  
1933. BULK EMASCULATION OF SORGHUM FLOWERS. Amer. Soc. Agron. Jour.  
25: 233-234, illus.
- (276) STEVENSON, T. M.  
1939. IMPROVEMENT OF CROSS-FERTILIZED CROPS—STRAIN BUILDING. Sci.  
Agr. 19: 535-541.
- (277) STITE, R. E.  
1949. COMPARATIVE STANDS, AND FORAGE AND SEED YIELDS, OF BROMEGRASS  
STRAINS UNDER DRYLAND AND IRRIGATED CONDITIONS IN MONTANA.  
Agron. Jour. 41: 200-203, illus.
- (278) STODDART, L. A., and SMITH, A. D.  
1943. RANGE MANAGEMENT. 547 pp., illus. New York.
- (279) SULLIVAN, J. T.  
1944. FURTHER COMPARISONS OF PLANTS WITH DIFFERENT CHROMOSOME  
NUMBERS IN RESPECT TO CHEMICAL COMPOSITION. Amer. Soc. Agron.  
Jour. 36: 537-543.
- (280) ——— and GARBER, R. J.  
1941. THE NITROGEN CONTENT OF POA PRATENSIS: ITS RANGE AND RELATION  
TO FLOWERING DATE. Amer. Soc. Agron. Jour. 33: 933-937.
- (281) ——— and GARBER, R. J.  
1947. CHEMICAL COMPOSITION OF PASTURE PLANTS. Pa. Agr. Expt. Sta. Bul.  
489, 61 pp.
- (282) ——— and MYERS, W. M.  
1939. CHEMICAL COMPOSITION OF DIPLOID AND TETRAPLOID LOLIUM PERENNE  
L. Amer. Soc. Agron. Jour. 31: 869-871.
- (283) ——— and SPRAGUE, V. G.  
1943. COMPOSITION OF THE ROOTS AND STUBBLE OF PERENNIAL RYEGRASS  
FOLLOWING PARTIAL DEFOLIATION. Plant Physiol. 18: 656-670, illus.
- (284) SYLVÉN, N.  
1937. THE INFLUENCE OF CLIMATIC CONDITIONS ON TYPE COMPOSITION. Imp.  
Bur. Plant Genet., Herbage Plants, Bul. 21, 8 pp., illus.
- (285) THATCHER, L. E., MONROE, C. F., and DAVIDSON, R. S.  
1948. SUDANGRASS. 1947 WOOSTER TEST. Ohio Farm and Home Res. 33:  
136-141, illus.
- (286) THOMAS, H. L., and HAYES, H. K.  
1947. A SELECTION EXPERIMENT WITH KENTUCKY BLUEGRASS. Amer. Soc.  
Agron. Jour. 39: 192-197.
- (287) TINNEY, F. W.  
1940. CYTOLOGY OF PARTHENOGENESIS IN POA PRATENSIS. Jour. Agr. Res.  
60: 351-360, illus.
- (288) ——— AAMONT, O. S., and AHLGREN, H. L.  
1937. PRELIMINARY REPORT OF A STUDY ON METHODS USED IN BOTANICAL  
ANALYSES OF PASTURE SWARDS. Amer. Soc. Agron. Jour. 29: 835-840,  
illus.
- (289) TOOLE, V. K.  
1940. THE GERMINATION OF SEED OF ORYZOPSIS HYMENOIDES. Amer. Soc.  
Agron. Jour. 32: 33-41.

- (290) TRAVIN, I. S.  
1938. METHODS OF BREEDING HERBAGE PLANTS BASED ON GROUP VARIABILITY. Imp. Bur. Pastures and Forage Crops, Herbage Rev. 6: [1]-9. (An adapted and revised review of report delivered in 1932 at the USSR Institute of Fodders.)
- (291) TRIBE, D. E.  
1952. THE RELATION OF PALATABILITY TO NUTRITIVE VALUE AND ITS IMPORTANCE IN THE UTILIZATION OF HERBAGE BY GRAZING ANIMALS. Internatl. Grassland Cong. Proc. 6: 1265-1270.
- (292) TSIANG, Y. S.  
1944. VARIATION AND INHERITANCE OF CERTAIN CHARACTERS OF BROME GRASS, *BROMUS INERMIS* LEYSS. Amer. Soc. Agron. Jour. 36: 508-522.
- (293) TURESSON, G.  
1922. THE SPECIES AND THE VARIETY AS ECOLOGICAL UNITS. Hereditas 3: [100]-113, illus.
- (294) ———  
1925. THE PLANT SPECIES IN RELATION TO HABITAT AND CLIMATE. Hereditas 6: [147]-236, illus.
- (295) TYSDAL, H. M., and CRANDALL, B. H.  
1948. THE POLY-CROSS PROGENY PERFORMANCE AS AN INDEX OF THE COMBINING ABILITY OF ALFALFA CLONES. Amer. Soc. Agron. Jour. 40: 293-306.
- (296) ———, KIESSELBACH, T. A., and WESTOVER, H. L.  
1942. ALFALFA BREEDING. Nebr. Agr. Expt. Sta. Res. Bul. 124, 46 pp., illus.
- (297) ULLMANN, W.  
1936. NATURAL AND ARTIFICIAL HYBRIDIZATION OF GRASS SPECIES AND GENERA. Imp. Bur. Plant Genet., Herbage Plants, Herbage Rev. 4: [105]-142.
- (298) UNITED STATES PRODUCTION AND MARKETING ADMINISTRATION.  
1952. MANUAL FOR TESTING AGRICULTURAL AND VEGETABLE SEEDS. U. S. Dept. Agr. Handbook 30, 440 pp., illus.
- (299) UNITED STATES REGIONAL PASTURE RESEARCH LABORATORY.  
[1941.] EVALUATION OF PLANT TYPES. U. S. Region. Pasture Res. Lab. Ann. Rpt. (1940) 4: 18-19. [Processed.] (Div. Forage Crops and Dis., U. S. Bur. Plant Indus., and the experiment stations of 12 North-eastern States cooperating.)
- (300) ———  
[1946.] INHERITANCE OF MALE STERILITY IN *DACTYLIS GLOMERATA*. U. S. Region. Pasture Res. Lab. Ann. Rpt. (1945) 9: 35-36. [Processed.] (Div. Forage Crops and Dis., U. S. Bur. Plant Indus., Soils, and Agr. Engin., and the experiment stations of 12 Northeastern States co-operating.)
- (301) VALLE, O.  
1931. UNTERSUCHUNGEN ÜBER DIE SELBSTSTERILITÄT UND SELBSTFERTILITÄT DES TIMOTHES (*PHLEUM PRATENSE* L.) UND ÜBER DIE EINWIRKUNG DER SELBSTBEFRUCHTUNG AUF DIE NACHKOMMENSCHAFT. EINE ZÜCHTUNGSMETHODISCHE STUDIE. Acta Agr. Fenn. 24, [262] pp., illus. [In German. Finnish summary, pp. 258-261.] [INVESTIGATIONS ON SELF-STERILITY AND SELF-FERTILITY IN TIMOTHY (*PHLEUM PRATENSE* L.) AND THE EFFECT OF SELFING UPON THE DESCENDANTS. A STUDY OF BREEDING METHODS. English title and abstract in Imp. Bur. Plant genet., Herbage Plants, Herbage Abs. 2: 165. 1932.]
- (302) ———  
1932. ITSESHITOSMENETELMÄN KÄYTTÖ NURMMEINIEN JALOSTUKSESSA. Suomen Maataloustiet. Seura, Maataloustiet. Aikakausk. (Sci. Agr. Soc. Finland Jour.) 4: [1]-19, illus. [In Finnish. German summary, pp. 17-19.] [THE SELFING METHOD IN GRASS BREEDING. English title and abstract in Imp. Bur. Plant Genet., Herbage Plants, Herbage Abs. 2: 79. 1932.]
- (303) VINALL, H. N., and HEIN, M. A.  
1937. BREEDING MISCELLANEOUS GRASSES. U. S. Dept. Agr. Yearbook 1937: 1032-1102, illus.
- (304) WALDRON, L. R.  
1921. SOME PHYSICAL AND CHEMICAL STUDIES OF CERTAIN CLONES AND SIBS OF BROME-GRASS. N. Dak. Agr. Expt. Sta. Bul. 152, 28 pp., illus.



- (305) WARMKE, H. E.  
1952. APOMIXIS IN GRASSES, WITH SPECIAL REFERENCE TO PANICUM MAXIMUM. Internatl. Grassland Cong. Proc. 6: 209-215, illus.
- (306) WARNER, J. N.  
1952. A METHOD FOR ESTIMATING HERITABILITY. Agron. Jour. 44: 427-430.
- (307) WASSON, C. E., and KALTON, R. R.  
1953. ESTIMATIONS OF OPTIMUM PLOT SIZE USING DATA FROM BROMEGRASS UNIFORMITY TRIALS. Iowa Agr. Expt. Sta. Res. Bul. 396, pp. [293]-320, illus.
- (308) WEAVER, J. E.  
1920. ROOT DEVELOPMENT IN THE GRASSLAND FORMATION. A CORRELATION OF THE ROOT SYSTEMS OF NATIVE VEGETATION AND CROP PLANTS. Carnegie Inst. Wash., Pub. 292, 151 pp., illus.
- (309) WEBBER, H. J.  
1912. THE PRODUCTION OF NEW AND IMPROVED VARIETIES OF TIMOTHY. N. Y. (Cornell) Agr. Expt. Sta. Bul. 313, pp. [337]-392, illus.
- (310) WEINMANN, H.  
1952. CARBOHYDRATE RESERVES IN GRASSES. Internatl. Grassland Cong. Proc. 6: 655-660.
- (311) WEISS, M. G., and MUKERJI, S. K.  
1950. EFFECT OF PLANTING METHOD AND NITROGEN FERTILIZATION ON RELATIVE PERFORMANCE OF ORCHARDGRASS STRAINS. Agron. Jour. 42: 555-559.
- (312) ——— TAYLOR, L. H., and JOHNSON, I. J.  
1951. CORRELATIONS OF BREEDING BEHAVIOR WITH CLONAL PERFORMANCE OF ORCHARDGRASS PLANTS. Agron. Jour. 43: 594-602.
- (313) WELLENSEK, S. J.  
1952. THE THEORETICAL BASIS OF THE POLY-CROSS TEST. Euphytica 1: 15-19.
- (314) WENGER, L. E.  
1941. SOAKING BUFFALO GRASS (BUCHLOE DACTYLOIDES) SEED TO IMPROVE ITS GERMINATION. Amer. Soc. Agron. Jour. 33: 135-141, illus.
- (315) WEXELSEN, H.  
1940. SELECTION AND INBREEDING IN RED CLOVER AND TIMOTHY. Imp. Agr. Bur. Joint Pub. 3, pp. [93]-114.
- (316) WHITE, P. J., ROBERTS, I. P., and WARREN, G. F.  
1910. PASTURES IN NEW YORK. N. Y. (Cornell) Agr. Expt. Sta. Bul. 280, pp. [351]-[398], illus.
- (317) WILSIE, C. P.  
1949. EVALUATION OF GRASS-LEGUME ASSOCIATIONS, WITH EMPHASIS ON THE YIELDS OF BROMEGRASS VARIETIES. Agron. Jour. 41: 412-420.
- (318) ——— CHING, C. B., and HAWK, V. B.  
1952. SELF-FERTILITY AND PROGENY PERFORMANCE IN BROMUS INERMIS. Agron. Jour. 44: 605-609.
- (319) WIT, F.  
1952. THE POLLINATION OF PERENNIAL RYEGRASS (LOLIUM PERENNE L.) IN CLONAL PLANTATIONS AND POLY-CROSS FIELDS. Euphytica 1: 95-104.
- (320) ———  
1952. TECHNIQUES OF BREEDING COLD-RESISTANT GRASSES AND CLOVERS. Internatl. Grassland Cong. Proc. 6: 1607-1612.
- (321) WITTE, H.  
1919. BREEDING TIMOTHY AT SVALÖF. Jour. Hered. 10: 291-299, illus.
- (322) WOLFE, T. K.  
1925. OBSERVATIONS ON THE BLOOMING OF ORCHARD GRASS FLOWERS. Amer. Soc. Agron. Jour. 17: 605-618.
- (323) ZIMMERMANN, K.  
1954. MODERNE METHODEN IN DER GRÄSERZÜCHTUNG. Züchter 24: [33]-39, illus. [Abstract in Comm. Bur. Plant Breeding and Genet., Plant Breeding Abs. 24: 576. 1954.]

**END**