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ACHIEVEMENTS AND PERSPECTIVES IN THE BREEDING OF TEMPERATE GRASSES AND LEGUMES

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Introduction

This paper will focus on a historical perspective on cool season forage production, plant breeding methods for cool season forages, major cool season forage selection criteria, some examples of significant achievements, and a future perspective. Topics similar to ours have been discussed at recent previous meeting of this Congress (Humphreys, 1997; Van Wijk et al., 1993); however, we will strive to avoid “plowing the same ground twice”. In an attempt to prevent duplication of content with other sections of this Congress, only limited attention will be given to genetic resource acquisition and conservation. Additionally, alfalfa (*Medicago sativa* L.), one of the primary temperate forage legumes, will generally not be discussed, since a full paper by Dr. Bouton will be presented in another session.

Temperate grasses and legumes form the basis for both extensive and intensive animal agriculture systems throughout the world. Producers generally view the grass component as the “foundation” for their production system, while legumes provide dietary diversity, with generally enhanced protein and mineral content. In most circumstances, the legume will be grown in association with grasses for grazed forage, although pure legume swards may be utilized for hay. Evaluation of “goodness” then becomes complicated by genotype by environment interactions of clipping vs. grazing defoliation and pure stand vs. mixed sward competition, issues that forage breeders continuously struggle to manage.

The important temperate grass species utilized for improved pastures around the world include the genera *Bromus*, *Dactylis*, *Festuca*, *Lolium*, *Phlaris*, *Phleum*, and *Poa*, with fewer important temperate legume genera including *Lotus*, *Medicago*, *Melilotus*, and *Trifolium*. In some of these genera multiple species are of agronomic importance and other species in other genera may fill critical roles in selected ecosystems. Thus, unlike many of the world’s major food plants, the focus on genetic improvement becomes diluted across many species. Although most of the important species are cross pollinated, variability in pollination vectors (*e.g.* wind vs. insect) also adds complications for the forage breeder. Finally, several of these species are polyploids adding complexity to genetic segregation and selection schemes.

Nevertheless, major strides were made in the improvement of temperate forage grasses and legumes in the past century. Breeding methodologies were evaluated and improved; disease and pest resistance mechanisms of resistance were better understood; techniques for mechanical, laboratory, and animal evaluation were defined; and germplasm variability was assembled and exploited. As we move forward, the challenge to forage breeders remains to, “make . . . two blades of grass grow upon a spot of ground where only one grew before,” and thus improve the feed and food supply for all humankind.

Improvement Of Cool Season Forages Prior To “Plant Breeders”

Domestication, Natural Selection, and Unconscious Selection

Darwin (1875) described three types of selective forces that may act to modify populations of organisms. Natural selection is the process whereby those individuals best fitted to a particular environment, a range of fluctuating environments, or a human-imposed management system have the greatest survival rate or contribute the greatest number of viable progeny to succeeding generations. Secondly, unconscious selection is the process by which humans save the phenotypically most valuable or desirable individuals, or their seed, and destroy or ignore the less valuable or desirable individuals. Unconscious selection is an integral part of crop domestication. Finally, methodical selection comprises the forces that are applied by humans in their systematic attempts to create predetermined changes to populations. All three of these types of forces have acted and continue to act in various ways, sometimes in concert, sometimes in disharmony, to create the world's pool of cool-season forage germplasm.

Humans have been selecting forage crops for thousands of years, often without explicit knowledge of having done so. The effects of domestication are easily seen in grain crops, for which wild and weedy relatives have dramatically different phenotypes from the domesticated form. While many annual grain crops are used as forage and fodder crops in many parts of the world, most traditional forage crops are not domesticated. Most forage crops have changed little in phenotype from their wild states, still possessing many traits that allow them to survive (and thrive) in natural or wild conditions. Plants and seeds collected on plant exploration trips to the most remote and untrammled parts of the world are usually phenotypically similar to representatives of the same species growing on dairy farms in similar climates. Indeed, many breeding programs rely almost exclusively on natural collections as the basis for all genetic variability (e.g. Charmet et al., 1990; Vogel and Pedersen, 1993).

Early agriculturists likely had little reason to collect seeds of grasses and legumes used principally as animal feed. Forage crops occur naturally in meadows and open areas that contain high levels of intra- and interspecific variation. Population buffering and mild natural selection provided a relatively stable and reliable feed source for thousands of years, likely until human population pressures began to dictate changes in animal husbandry and feeding. Until animal agriculture evolved into frequent use of hay harvesting and storage, evolutionary forces causing phenotypic changes in forage crops were likely due to climate, herbivory, and diseases. The practice of cutting hay is probably little more than 1000 years old (Casler et al., 1996).

Italian ryegrass (*Lolium multiflorum* Lam.) and perennial ryegrass (*L. perenne* L.) may represent the only examples of partially domesticated forage crops. These species appear to be derived by opposite selection pressures from a “huge hybrid swarm” that may be the ancestor of European ryegrass populations (Tyler et al., 1987). Italian ryegrass apparently evolved in the Lombardy and Piedmont plains of Italy during the 12th century, as an adaptation to hay harvesting and occasional reseeding (Beddows, 1953). Conversely, perennial ryegrass appears to have evolved its lower growth habit, higher tiller density, and greater longevity in response to centuries of grazing pressure. As animal agriculture spread and intensified throughout Europe, meadows and leys were subjected to increasing pressure to survive and reproduce in the presence of grazing animals. Perennial ryegrass is now rarely found in the wild, except in association with grazing animals (Charmet et al., 1990). Turf-type perennial ryegrass, with its reduced sub-crown internode length,

dwarf habit, and increased tiller density, represents a more extreme adaptation to mechanical defoliation. Apart from a few examples of improved seed retention or shattering resistance (Casler et al., 1996; Oram et al., 1985), there are no other examples of cool-season forage grasses or legumes that have been domesticated.

Most phenotypic variation present in forage crops has arisen as a result of natural selection for adaptation. Grasses and legumes have evolved tolerance to a wide range of environmental stresses (including drought, soil acidity, salinity, ozone, heavy metals, low fertility, and temperature extremes) and biotic stresses (including pathogens, insects, and large herbivores). Variation in the magnitude and combination of these stresses has resulted in a plethora of land race populations of all forage crops. Many of these populations have evolved peculiar traits that make them specifically adapted to micro-sites or climates (Casler et al., 1996). Early agriculturists were aware that local populations were generally superior to exotic populations (Harlan, 1992).

Plant Breeding Methods For Cool-Season Forages

Use of Plant Introductions as Cultivars

Exploitation of any “new” crop in a region generally begins with the introduction of germplasm from other areas. For example, in the 18th, 19th, and early 20th century cool season forage legumes and grasses were introduced into the United States from various European and Asian sources (Barnes et al., 1988; Smith et al., 1985; Papadopoulos and Kelman, 1999; Sleper and Buckner, 1995). Through farmer selection and recognition of desirable attributes, many of these introductions became cornerstone “landrace” cultivars for their species. Most cultivars developed in the United States prior to 1940 were of this type (Fergus and Hollowell, 1960). Some examples include, ‘KY-31’ tall fescue (*Festuca arundinacea* Schreb.), ‘Grimm’ alfalfa, and ‘Ladino’ white clover (*Trifolium repens* L.). As will be discussed in later sections, considerable natural and farmer directed selection occurred over time to improve regional adaptation of the “raw” plant introductions. In rare circumstances, *e.g.* Ladino white clover, improved germplasm from another region will be sufficiently well adapted to a region to have immediate impact on use and production. Many of the early smooth brome grass (*Bromus inermis* Leyss.) cultivars in North America were seed increases of direct plant introductions from Eastern Europe and Asia (Casler et al., 2000b).

Mass Selected Cultivars

As desirable traits are identified in crop improvement programs, the next step in breeding programs is usually the development of mass selected cultivars. Frequently such cultivars are derived by selection within or among some of the cornerstone landrace cultivars for resistance to a specific pest or disease. Such breeding programs are relatively inexpensive to conduct since maintenance of large clonal nurseries is avoided and no pedigree bookkeeping is required. Cultivars developed from such schemes are usually broadly adapted since large germplasm populations are more easily handled than with some other schemes.

An example of a successful mass selected cultivar is ‘Vernal’ alfalfa, released in 1953. This broadly adapted cultivar dominated plantings in the north central USA for almost 25 years, principally because of its good winter hardiness and bacterial wilt resistance (Melton et al., 1988). ‘Florida 77’ alfalfa, selected for persistence and pest resistance under Florida conditions over a 25 year period was

also developed using mass selection (Horner and Ruelke, 1980). This cultivar dominated the southeastern alfalfa market for about 15 years, and was later shown to have high levels of root-knot nematode resistance (Baltensperger et al., 1985). Examples of highly successful mass selected clover cultivars include 'Kenland' (Hollowell, 1951) and 'Lakeland' (Fergus and Hollowell, 1960) red clover (*T. pratense* L.), each of which has good levels of resistance to the major diseases common in their regions of selection and adaptation. Both of these cultivars were developed by recurrent mass selection using several adapted landrace cultivars as the original source populations. The development of both included some greenhouse or field inoculation of plants with fungal diseases prevalent in their production zones and mass selection of surviving plants to enhance disease resistance. These cultivars dominated the eastern red clover production belt for about 20 years. 'Potomac' orchardgrass (*Dactylis glomerata* L.), historically one of the most widely grown cultivars in North America, was developed by mass selection for vigor, persistence, and disease resistance (Alderson and Sharp, 1994).

Synthetic Cultivar Development

Apart from the few annual cereals that are used as forage crops and a few naturally self-pollinated forage crops, synthetic cultivars represent the vast majority of cultivated forage germplasm. Synthetic cultivars are populations derived by making all possible crosses among a group of selected plants or clones and multiplying seed under isolated random-mating for a limited number of generations (Breese and Hayward, 1972). The initial crosses are often made in a replicated polycross block in which each clone has been replicated and randomized with respect to the others. Strain crosses represent a special type of synthetic in which a small number of parents are intercrossed, each parent being represented by a seed population rather than a single clone or plant.

Most synthetic cultivars are generated by one of two methods: clonal breeding or recurrent selection. Clonal breeding, the historically older method, involves establishment of huge spaced-plant nurseries in which groups of phenotypically similar and desirable plants are identified. Plants within each phenotypic group are transplanted into isolated crossing blocks for Syn-1 seed production. Morphological distinctness of each phenotypic group is encouraged to ensure that each candidate cultivar has the opportunity for approval under Plant Breeders' Rights or Patent Laws. The largest commercial breeding programs may produce up to 30 synthetics of a single species each year. During the processes of seed multiplication and evaluation of candidate cultivars in replicated field trials, most synthetics are discarded due to poor performance.

While clonal breeding places considerable selection pressure on morphological traits, vigor, maturity, and pest resistance within populations, there is relatively little direct selection pressure on forage yield. Post-synthesis selection provides the only opportunity to select for yield performance under proper environmental and management conditions (Casler, 1998a). This approach is largely responsible for the average 3.5 % decade⁻¹ gains in forage yield due to breeding during the latter half of the 20th century (Humphreys, 1999). Despite these gains, Gallais (1992) has shown that recurrent selection for a particular trait or group of traits is more efficient at generating long-term gains than post-synthesis selection for the same selection criteria. For example, six cycles of phenotypic recurrent selection in Pensacola bahiagrass (*Paspalum notatum* var. *saure* Parodi) increased forage yield by 6.3% year⁻¹ (Burton, 1982), far surpassing the average gains for both forage and grain crops reported by Humphreys (1999).

Phenotypic recurrent selection involves evaluating large numbers of plants for a single trait

or multiple traits, selecting and intercrossing the best plants, and repeating the cycle. Three key elements of efficient recurrent selection programs are: (1) high selection intensity, (2) short cycle time with rapid generation turnover, and (3) clean, efficient, and repeatable evaluations of the selection criteria. Phenotypic recurrent selection has been used with great success for traits that are readily evaluated on single plants, such as pest resistances (Casler and Pederson, 1996), many stress tolerances (Casler et al., 1996), forage quality traits (Casler and Vogel, 1999), and even the ability to regenerate from tissue culture (Quesenberry and Smith, 1993). Heritability as low as 20% can be sufficient to generate measurable and economically significant gains often without serious changes in other traits of the population.

Progeny-test recurrent selection schemes require the formation of progeny families, such as full-sib, half-sib, or selfed families. They are useful in forage crops because they can be easily structured to use seeded sward-plots, which can provide moderately heritable forage yield measurements. However, because selection among families squanders 75% of the additive genetic variance in the population, some form of within-family selection must be applied to make such a recurrent scheme efficient (Vogel and Pedersen, 1993). Furthermore, progeny-test selection in forage crops often requires a minimum of 3 years per cycle, reducing the potential for recombination in a long-term program. There are no reports of long-term applications of within-family or between-and-within-family selection in forage crops.

Hybrid Cultivar Development

Humphreys (1999) cited our lack of exploitation of heterosis as one of the chief reasons that forage yield gains from breeding have lagged far behind yield gains in grain crops. Although forage breeders have been interested in exploiting heterosis, and known of its existence in forage crops, for over 60 years, no commercially viable hybrid cultivars have been produced in the perennial, outcrossing forage crops. Significant high-parent heterosis for forage yield can be found in many forage crops, including grasses and legumes (Brummer, 1999). Of particular significance, high-parent heterosis can be found in crosses between individual plants and between populations, indicating that inbred lines are not a prerequisite for capturing heterosis in forage crop cultivars (Brummer, 1999).

Brummer (1999) has proposed a model where germplasm sources are selected and improved independently of existing breeding populations. Rather than mixing all germplasm sources together into a homogeneous pool, diverse populations, which may represent heterotic groups, are preserved for later hybridization. Heterotic groups are the foundation of the maize (*Zea mays* L.) hybrid seed industry. Plants, populations, and lines within a heterotic group will share many linkage blocks, but these linkage blocks will be complementary to those generally found in plants, populations, and lines of different heterotic groups. Although there are no known heterotic groups in forage crops, there are many intraspecific groups that have evolved separately through geographic isolation reproductive isolation, differential selection, or breeding (Brummer, 1999). Some examples include semidormant vs. nondormant alfalfa, steppe vs. meadow smooth brome grass, upland vs. lowland switchgrass (*Panicum virgatum* L.), Mediterranean vs. Northern European orchardgrass, and perennial vs. Italian ryegrass.

Once heterotic plants, populations, or lines are identified, semihybrids can be produced by mutual pollination, creating a population with 50% inter-group hybrids and 50% intra-group hybrids (Brummer, 1999). The ability to harvest seed on both parents will keep seed production costs at a

reasonable level, reducing the need for severe increases in seed prices. Semihybrids of perennial ryegrass have shown up to 128% high-parent heterosis, with little effort to identify heterotic groups. Self-incompatibility systems exist within most cool-season forage grasses, typically controlled by the two-locus SZ system. Hayward (1988) proposed a system to produce 83% hybrid seed, utilizing paired inbred populations that are internally self-incompatible, but externally cross-compatible, based on their SZ alleles. The system has yet to be tested, partly due to the great time and expense of developing the appropriate inbred populations and testing them for combining ability and seed production potential.

Major Cool-Season Forage Selection Criteria

Regional Adaptation

One of the most significant contributions of cool-season forage breeding has been selection for adaptation of species to a new area of production. As plants were brought into new regions from other areas where they had been historically utilized, they were subjected to new and unique abiotic and biotic stresses. The next section will deal with issues related to biotic stresses. Primary abiotic stresses affecting regional adaptation are soil moisture, temperatures, and day length differences. Variability for all these traits can likely be identified in cool-season forage germplasm, but inheritance is usually polygenic. Nevertheless, recurrent mass selection with some restrictions to enhance genetic gain can dramatically improve population adaptation.

Cool-season forages are quite variable in their response to drought or flooding (Kendall and Stringer, 1985). By selecting appropriate species mixes, producers can establish cool-season pastures on sites ranging from wet, poorly drained, clay and clay loam soils to excessively well drained upland sands. Kendall and Stringer (1985) cite several examples of the effects of soil types on depth of rooting in clovers, suggesting that over time natural selection would alter regional adaptation to a given soil type. Little research has been conducted to select for within species variability for drought or flooding tolerance in either cool-season forage grasses or legumes. Recent field research using a water application gradient over three white clover cultivars and three germplasms concluded that there was no difference in drought tolerance among the entries, or that drought tolerance does not impact white clover growth in the presence of normal pasture stresses (Brink and Pederson, 1998). Numerous selection criterion have been used in attempts to improve the drought tolerance of perennial grasses, including emergence from deep planting, increased stomatal resistance, shallow leaf ridging, low leaf water conductance, low osmotic potential, low carbon isotope ratios, and natural selection (Casler et al., 1996).

Considerable research has been conducted on variability and selection for root, stolon, and rhizome morphology in the cool season forages. One of the best documented examples is in alfalfa where evaluation of over 1067 plant introductions and 110 North American cultivars showed strong positive correlations of taproot diameter and lateral root diameter with increasing (Johnson et al., 1998). This research also showed that recent plant breeding strategies for semi-dormant alfalfa have resulted in alterations of the relationships between root morphology and dormancy class. In red clover, evaluation research showed that recently developed cultivars with higher persistence also had a higher percentage of plants with more fibrous roots (Smith, 1989). This research also concluded that bidirectional recurrent phenotypic selection for root type in the cultivar Arlington was effective, although shift toward a high percentage of plants with a strong taproot was

more rapid than shift toward a high percentage of fibrous rooted plants.

Regional adaptation can be dramatically impacted by day length induced dormancy responses. Research at Florida has demonstrated that one of the primary traits needed for adaptation of red clover to the lower coastal plains of the USA is enhanced spring non-dormancy. The major yield advantage of 'Cherokee' red clover (Quesenberry et al., 1993) comes at the first spring harvest where it often has yields of 2x or greater than cultivars developed at more northern latitudes. Research at Florida has also shown that when long-day flowering, cool-season forage germplasm is grown at lower latitudes, differences in spring day length response to flowering are more pronounced because the rate of increase in day length per day is less than at higher latitudes. This allows for identification of smaller differences in day length response and selection for superior regional adaptation.

Another aspect of regional adaptation often ignored in forage selection programs is adaptation to persist under the typical grazing stresses imposed in a region. The grazing stress imposed on swards by sheep in a wet-dry Mediterranean climate may be quite different from those imposed by dairy cows in a humid temperate climate. Using livestock as defoliators at early stages in selection programs, can identify unique genotypes best adapted to persistence under conditions prevalent in a region (Quesenberry et al., 1977; Bouton et al., 1998). When grass or legume species have a history of poor pasture persistence in a region, cultivar development programs should include direct and early use of grazing animals to select the more persistent genotypes (Bouton et al., 1997).

Pest Resistance

Pest resistance characteristics of a cool-season forage are often the determining factors between success or failure. Numerous examples could be cited in both grasses and legumes. A review of the important diseases affecting persistence of the major clover species and birdsfoot trefoil concludes that it is usually not one pest, but a combination of several that ultimately impacts persistence of a species (Leath, 1989). Thus, multiple pest resistance selection programs are generally incorporated into long term breeding programs (Taylor and Smith, 1979). Evidence of the success of such breeding programs is the report documenting improved resistance to *Fusarium oxysporum* in newly developed cultivars and germplasms of red clover (Steiner et al., 1997).

In the sandy soils of the lower southeastern USA and in New Zealand, root-knot nematodes (RKN) (*Meloidogyne* spp.) have been shown to be significant pests, limiting production and persistence of clovers (Caradus and Williams 1989; Pederson, 1995; Quesenberry et al., 1989). Although variability for RKN resistance was limited in red and white clover plant introductions (Kouame et al., 1997, 1998; Quesenberry et al., 1986), recurrent phenotypic selection has been shown to be effective in improving RKN resistance in red (Quesenberry et al., 1989) and white clover (Pederson and Windham, 1992; Mercer et al., 2000). Additionally, the clover cyst nematode (CCN) (*Heterodera trifolii*) has been shown to a factor limiting yield of white clover in New Zealand, but here again recurrent phenotypic selection has been effective in improving CCN resistance (Mercer et al., 2000).

Cherokee red clover (Quesenberry et al., 1993) illustrates the power of recurrent selection for enhancement of limited variability for pest resistance. The original population from which Cherokee was developed rated about 4.5 for number of galls or egg masses (0 to 5 scale with 5 = >100 galls or egg masses per plant). After five cycles of field recurrent restricted phenotypic selection, Cherokee had a rating of 2.5 (approximately 20 galls or egg masses) (Quesenberry et al., 1989). Additional recurrent selection cycles under greenhouse conditions resulted in the development of FLMR6 red clover germplasm with even lower gall and egg mass ratings (Quesenberry et al.,

1997). Recent research with this population showed that the enhanced resistance was due to a dramatic reduction in numbers of nematodes reaching maturity and reduced fecundity of mature females (Call et al., 1996, 1997a, 1997b). Similar research in white clover in New Zealand has shown a decrease in cyst numbers averaging 7.3% per cycle over five cycles (Mercer et al., 2000).

Forage and Seed Yield

Long-term gains in forage yield from European forage breeding programs averaged about 3.5 % decade⁻¹ during the latter part of the 20th century (Humphreys, 1999). Increases in forage yield of alfalfa cultivars in the North-central USA during the 1970s and 1980s were approximately half this amount (Hill et al., 1988; Holland and Bingham, 1994). White clover forage yields and stand contributions both increased by 6 % decade⁻¹ during the latter half of the 20th century (Woodfield and Caradus, 1994). In North America, early-maturing orchardgrass forage yields improved by 2.5 % decade⁻¹ during the latter half of the 20th century, while there were no changes in yields of medium- and late-maturing orchardgrass cultivars (Casler et al., 2000a). North American smooth brome grass breeding began in the 1950s, with an initial 7.2 % increase over direct introduction from Eastern Europe, but showed no further increases in forage yield during the next 50 years of breeding (Casler et al., 2000b).

Historically, forage crop breeders have relied on three main approaches to improving forage and seed yield: post-synthesis selection, indirect selection, and recurrent selection (Casler et al., 1996). All three forms of selection have likely led to improvements in forage and seed yields. However, it is impossible to fully separate and estimate the effects of each method, because there have been no comprehensive studies designed to compare the methods and experiments designed to estimate long-term gains in forage crops have always measured the combined effects of all methods together. Holland and Bingham (1994) suggest that much of the forage yield gains in North-central USA alfalfa breeding programs was due to efforts to develop synthetic cultivars with increased levels of heterozygosity. Increases in forage yield from the 1940s/50s to the 1980s were not due to increases in the frequency of favorable alleles for forage yield (Holland and Bingham, 1994).

Indirect selection for morphological or physiological traits in grasses has led to numerous significant improvements in forage yield, although there are no reports of new cultivars from any of these efforts. Some examples include leaf length (Rhodes, 1971), specific leaf weight (Carlson, 1990), mesophyll cell size (Wilson and Cooper, 1970), dark respiration rate (Robson, 1982), leaf tensile strength (Wilson and Jones, 1982), and leaf area expansion rate (Sleper and Nelson, 1989). In many cases selection for modified plant morphology led to increases in forage yield under specifically defined management systems, such as frequent vs. infrequent clipping (Casler et al., 1996). In white clover, selection for increased leaf size and higher stolon growing point density has been hampered by high negative genetic correlations between the two traits, but intensive breeding has led to a large reduction in this correlation among recent cultivars (Caradus et al., 1996).

Many forage yield improvements in legumes have resulted from increases pest resistance and its effect on persistence. Casler and Pederson (1996) cite several examples in which disease resistance increases stand longevity, increasing forage yields in later years of the stand for red clover, or increasing forage yields per se in areas with heavy inoculum pressure from disease-causing organisms in alfalfa. Many abiotic stress tolerances are also responsible for indirect improvements in forage yield, created by extending the life of a stand in a stressful environment or extending the region of adaptation of a forage species (Casler, 1998b; Casler et al., 1996). For example, increases

in adaptation of white clover to low-P soils or acid soils (Caradus, 1994), low temperature (Rhodes et al., 1994), or grazing pressure (Evans et al., 1996) can all increase forage yield per se and stand persistence.

Recurrent selection for forage yield per se is used to a lesser frequency than post-synthesis selection for yield or indirect selection for morphological or physiological traits. Because of low correlations between spaced-plant and sward-plot forage yield, many attempts to improve sward-plot forage yield by selecting on the basis of spaced plants have been unsuccessful (Casler et al., 1996).

Notable successes include one perennial: Pensacola bahiagrass (Burton, 1982) and two annuals: Italian ryegrass (Fujimoto and Suzuki, 1975, and annual rye (*Secale cereale* L.) (Bruckner et al., 1991). Despite these successes, gains made from selecting for increased forage yield of spaced plants are typically reduced by at least 50% when progeny are evaluated under sward-plot conditions.

Forage crops serve a dichotomous marketplace in that they belong to two distinct commodity groups - livestock producers and seed producers. Cultivars that represent superior products for livestock production will ultimately fail if they do not have adequate seed production traits. Conversely, for species such as orchardgrass, there are many cultivars on the marketplace that represent significant improvements in seed production traits, but offer no advantage to livestock producers.

Seed yield appears to be a moderate heritable trait, amenable to selection in many species (Casler et al., 1996). These authors provide examples of improved seed yield due to selection for fertility, shattering resistance, inflorescence type, seed retention, and seed yield per se. Furthermore, it has been documented that seed and forage yield can be simultaneously improved in several forage crops (Casler et al., 1996), but careful attention must be paid to the selection protocol. For example, orchardgrass seed and forage production in North America occur in extremely different and mutually exclusive locations. Multiple-location selection for combined forage and seed traits was effective at improving seed yield in a target environment that was not included among the selection environments (Barker et al., 1997). Conversely, selection at any one of four individual locations resulted in no improvements in seed yield at the target environment.

Forage Quality Traits

Development of an in vitro procedure for rapid, repeatable, and relatively inexpensive evaluation of forage digestibility (Tilley and Terry, 1963) is the single most important event in the evolution of cool-season forage grass selection criteria. Genetic variability exists for in vitro dry matter digestibility (IVDMD) or related traits within all economically-important cool-season forage species that have been studied. Despite this knowledge, reports of actual genetic progress in breeding for improved forage digestibility of cool-season forages are limited to alfalfa, orchardgrass, perennial ryegrass, smooth brome grass, and timothy (*Phleum pratense* L.) (Casler and Vogel, 1999). The rate of genetic progress in these species has ranged from 8 to 45 g kg⁻¹ cycle⁻¹ (1.3 to 12.1% cycle⁻¹ or 0.7 to 2.5 % year⁻¹). Divergent phenotypic selection for neutral detergent fiber (NDF) concentration, the single best laboratory predictor of intake potential (Van Soest, 1994), was successful in smooth brome grass (Casler, 1999) and reed canarygrass (*Phalaris arundinacea* L.) (Surprenant et al., 1988).

There are three principal reasons for this small number of studies and species with documented genetic improvements. First, while laboratory procedures are less expensive than actual animal evaluations, they are more expensive than most field-trait selection procedures. Limited funds or access to specialized laboratories prevent many breeders from using these selection criteria. Near-

infrared reflectance spectrophotometry is one avenue that can be used to reduce the cost and time spent in the laboratory and increase the efficiency of selection in some cases (Reich and Casler, 1985).

Second, negative correlations between forage yield and nutritive value have been frequently reported and may serve as a deterrent to forage nutritional value breeding efforts (Van Bogaert, 1977). However, genetic improvements in forage digestibility do not necessarily lead to reduced forage yield (Casler and Vogel, 1999). Third, there are still some breeders who, despite the overwhelming volume of evidence, still do not believe that genetic improvements in forage nutritional value traits are real (Van Wijk et al., 1993).

Forage quality traits, such as IVDMD and NDF, are far less sensitive to genotype x environment (GxE) interactions than most important agronomic traits, such as forage and seed yield.

Recent reviews cite numerous studies that document little or no change in ranking of genotypes or populations across locations, years, harvest managements, or plant spacings. This phenomenon seems to apply to most forage quality traits of most species (Buxton and Casler, 1993; Casler and Vogel, 1999; Vogel and Sleper, 1994). These and other studies cited by these authors indicate that most of the more important forage quality traits can be improved by evaluation of unreplicated spaced plants from one harvest. However, there may be some traits, such as Klason lignin concentration, that require environmental replication due to severe GxE interactions (Casler and Jung, 1999).

Generally, recurrent selection for increased IVDMD has not led to correlated responses for forage yield (Buxton and Casler, 1993; Casler et al., 1996; Casler, 1998b). Notable exceptions are forage quality mutants such as brown-midrib and dwarf genes which provide large and rapid increases in whole-plant IVDMD, but with large reductions in forage yield, suggesting pleiotropic effects of these genes (Casler, 1998b). Because recurrent selection slowly increases the frequency of favorable alleles for selected traits, there is considerable potential to apply concomitant selection pressure for forage yield and other important agronomic traits during the selection process. Without this concomitant selection pressure, selection for increased IVDMD can lead to reduced winter survival in marginally hardy species, such as orchardgrass and switchgrass, in harsh winter environments (Casler et al., 1998, unpublished data). Furthermore, selection for reduced NDF may also result in reduced forage yield, because the fiber is such an important component of dry matter yield (Casler, 1999).

Ten grazing experiments comparing high-IVDMD cultivars with check or standard cultivars have allowed some cautious generalizations about the effects of breeding for increased IVDMD (Casler and Vogel, 1999). Some caution is warranted because inconclusive grazing experiments and/or those that do not show the expected improvement in animal gain associated with IVDMD may not have been published. Genetic improvement in IVDMD generally resulted in improved animal daily gains and the relationship was broadly positive, with a 3.2% increase in daily gains for each 1% increase in IVDMD. Genetic changes in IVDMD appeared to be negatively correlated with changes in forage availability (on this broad scale), but forage availability increased in nine of 10 cultivars, compared to their checks. Finally, the combination of increased animal daily gains and forage availability always led to increased animal production per ha. Augusta perennial ryegrass has a 3% advantage in in vitro organic matter digestibility and a 27% advantage in organic matter intake over 'RvP' perennial ryegrass (Munro and Walters, 1986). In a six-farm test, the advantage of Augusta over RvP ranged from 0.02 to 0.26 kg animal⁻¹ day⁻¹ (3.2 to 31.0%) for mean daily gains of beef cattle and from 0.11 to 1.47 kg ha⁻¹ day⁻¹ for mean liveweight production. Animal performance data, such as these, can be used to document the value of a breeding program to livestock agriculture and society (Casler and Vogel, 1999).

Some Significant Achievements

Perennial Ryegrass

Strain selection and preferential marketing of agronomically superior strains of perennial ryegrass have occurred for over 200 years (Beddows, 1953). Formal breeding of perennial ryegrass began in the early 20th century throughout most of northern and western Europe (Jung et al., 1996). Its high forage quality and superior grazing tolerance compared to most other perennial grasses have made perennial ryegrass the most preferred perennial forage grass in humid, temperate climates. Perennial ryegrass has received more breeding effort by more breeding programs in more countries than any other perennial forage grass, perhaps more than all other humid, temperate, perennial forage grasses combined.

Perennial ryegrass is thought to have spread from its center of origin in the Mediterranean region, largely with the evolution, development, and spread of livestock agriculture in Europe, Asia, and the Middle East (Breese and Tyler, 1986). Natural variability and natural selection were responsible for its ability to colonize new environments. Breeding for increased cold or freezing tolerance and broad-based resistance to an array of pathogens (Jung et al., 1996; Wilkins, 1991) has facilitated the continued spread of perennial ryegrass into new regions and climates. Increases in water-soluble carbohydrate concentration, achievable by conventional breeding methods, have the potential to increase both forage nutritional value and persistence (Humphreys, 1989; Wilkins, 1991). Finally, genetic variability within the worldwide germplasm pool of perennial ryegrass cultivars does not appear to have been eroded by breeding, most likely due to the diversity of breeding programs, objectives, and locations that have contributed to its development (Casler, 1995). Forage yields of perennial ryegrass cultivars increased by 0.3-0.5 % year⁻¹ during the latter half of the 20th century (Van Wijk and Reheul, 1991; Wilkins, 1991). Nevertheless, there have been no significant improvements in seed yield of perennial ryegrass during this time, likely due to lack of attention to seed yield and related traits (Elgersma, 1990). Most forage-type perennial ryegrass cultivars are considered to be relatively low in seed yield, largely due to infertility and seed shattering (Elgersma et al., 1988). Despite all the germplasm collection and breeding efforts on perennial ryegrass, there is no known source of seed shattering resistance. Abscission layers develop in the rachilla below each floret prior to spike emergence and begin breaking 4 to 5 weeks post-anthesis (Elgersma et al., 1988). Nevertheless, seed yield is heritable and genetic progress could be made with proper error control and a high selection intensity (Bugge, 1987).

Reed Canarygrass

Forage of reed canarygrass contains alkaloids which reduce palatability and average daily gains of grazing livestock. Up to nine different alkaloid compounds can be present in reed canarygrass: a phenol (hordenine), five indoles (gramine and four tryptamines), and three b-carbolines (Marten, 1973). The tryptamines and b-carbolines are responsible for high incidence of diarrhea, phalaris staggers, and bovine pulmonary emphysema in ruminants grazing reed canarygrass (Carlson et al., 1996). The presence of tryptamine and b-carboline alkaloids is controlled by two epistatic loci (Marum et al., 1979). Cultivars without tryptamine or b-carboline alkaloids had 91-94% reduced incidence of diarrhea among grazing ruminants (Duynisveld and Wittenberg, 1993; Marten et al.,

1981). In addition, a 68% reduction in gramine concentration was associated with an average of 185% greater lamb gains and 136% greater steer gains (Marten et al., 1976). Recent cultivars of reed canarygrass all have reduced gramine concentration, combined with no tryptamine or b-carboline alkaloids. These cultivars have excluded all others from the reed canarygrass pasture seed market.

Red Clover

On a world wide basis, red clover is the most widely grown of all the clovers (Taylor and Smith, 1995). The major type of red clover grown world wide is the early flowering, “medium” type, although some “mammoth” type may be grown in Europe, Asia, and Canada. Red clover demonstrates a high level of “plasticity” for a broad range of traits and progress in selecting for a trait is not necessarily dependant on a high initial level of variability for the trait (Taylor and Quesenberry, 1996; Taylor et al., 1990). Recurrent selection for multiple-parted flower heads is an example of this plasticity. Six cycles of selection increased the maximum number of parts per head in the largest head from 1.86 (1 to 2 is the norm in red clover) to 7.4 with a maximum of 11 (Taylor, et. al., 1985). Prior to initiation of this research the maximum number of head parts observed in any red clover was 4. This high level of plasticity has resulted in the development of red clover cultivars and germplasm adapted worldwide.

Swedish and Norwegian cultivars may be grown to near 60 N latitude, while non- dormant germplasm such as Cherokee is very successful below 30 N, and can be grown at higher altitudes in the tropics to the equator. Selection for various pest resistances in breeding programs in North American and Europe has developed cultivars with enhanced resistance to southern anthracnose (Taylor and Anderson, 1973), northern anthracnose (Taylor et al., 1990), fusarium root rots (Steiner et al., 1997), aphanomyces (Tofte et al., 1991), sclerotinia (Smith et al., 1994), root-knot nematodes (Quesenberry et al., 1997), and stem nematodes (Bingefors, 1985). High levels of research in central and eastern Europe hold promise for continued progress in development of superior red clover cultivars. Improvements in stand persistence now result in three to four year stand life compared to one to two in earlier cultivars.

Much of the increased longevity in newly released red clover cultivars is at least partially due to improved disease resistance. When cultivars released in the 1950s, 1960s, 1970s, and 1980s, were compared, there were no differences in yield in the second year of production. However, cultivars released in the 1970s and 1980s produced excellent third and fourth year yields, while earlier cultivars showed depleted stands and limited yields in the third and fourth years (Smith, 1997). This research showed that the two to three cycles of selection for northern anthracnose resistance which were conducted during each cycle of selection for persistence had resulted in linear increases of from 25% resistant plants in Lakeland (released in 1953) to over 60% resistant plants in Wisconsin Experimental (1997).

White Clover

White clover is probably the most widely distributed and recognized of the clovers on a world-wide basis. It can be found growing from the Arctic Circle to the equator and will usually be found in any park or public area where irrigation is supplied for turf maintenance. The plant’s stoloniferous growth habit makes it an ideal plant for use in combination with both cool- and warm-season grasses throughout the world wherever there is adequate moisture from rainfall or irrigation.

However, but it is rarely grown in pure stands, except for seed production. Major research programs in Europe, New Zealand, and North America have focused on white clover improvement for almost 100 years. Since 1930 more than 250 synthetic cultivars and ecotypes of white clover have been released (Caradus, 1986). There are typically high levels of phenotypic plasticity and genetic variability within and among white clover populations (Caradus et al., 1989).

A recent study evaluated improvements in white clover performance by comparing the performance of 110 cultivars and ecotypes released over the six decades from 1930 to 1990 (Woodfield and Caradus, 1994). Lines were evaluated as spaced plants both with and without grass competition. When decades of release were considered, statistically significant ($P < 0.01$) linear gains were achieved for both clover yield and percentage clover by weight. Cultivars released in the 1980s yielded 28% more dry matter than those released pre 1939 and 44% more than those released in the 1940s. Mean yield over the time period increased 1.44 gm m^{-2} , and when this is divided by the mean yield of all 100 lines this represents a 6% rate of genetic gain per decade. These results are similar to data from South Africa comparing 31 cultivars which showed a yield increase of 6.8% per decade (Rhind et al., 1979), and from Czechoslovakia comparing 24 cultivars showing a 4.6% gain per decade (Vacek and Zapletalova, 1982). The New Zealand study also showed a 6% increase per decade in clover content of grazed swards. The total sward yield did not increase, indicating that clover cultivars have become more competitive with increased clover yield and content occurring at the expense of grass yield. White clover yield improvements were greatest for small-leaved and large-leaved non-ladino types and from breeding programs located in cool climates, rather than cold or warm climates. These rates of gain are greater than the 1.8 to 2.2% gain per decade observed for alfalfa (Holland and Bingham, 1994) and similar to the 5% gain per decade observed of annual and perennial ryegrass (van Wijk and Reheul, 1991).

Two recent studies in the USA have shown that the large leafed ladino type white clover are not as competitive in grazed swards as the intermediate leaf types with higher stolon density (Bouton et al., 1998; Woodfield et al., 1998). Both of these studies suggest that white clover ecotypes which develop and persist under pasture grazing defoliation will have smaller leaves with higher stolon density. Additional research (Hoveland and Bouton, 2000) has confirmed that hybridization of these local ecotypes with Ladino or New Zealand intermediate types followed by additional selection under grazing has produced populations with superior persistence in both fescue and bermudagrass sods. These results suggest potential for additional gains in white clover production and utilization.

A Future Perspective

Molecular Markers and Genomics

Applications of molecular (DNA) marker analyses and technologies to forage crops have been slow relative to grain crops, largely due to the complex polyploid nature of most perennial forage crops (Osborn et al., 1998; Sleper and Chen, 1998). Ironically, certain aspects of polyploidy, including genome organization, origins, and evolution represent some of the most important riddles that will eventually be solved by use of molecular markers. The genus *Festuca* represents the most prominent example illustrating the power of this technology to answer complex genomic questions (Sleper and Chen, 1998).

Molecular markers are becoming increasingly important in forage crop germplasm research

(Brummer, 1998). They are useful in managing germplasm collections, by helping to identify novel and/or redundant accessions, quantifying genetic variability within and among accessions, measuring contamination during seed multiplication, or determining the geographic distribution of genes or gene sequences (Gunter et al., 1996; Gustine and Huff, 1999; Huff et al., 1998). Molecular data on germplasm collections can also be used to make decisions regarding the most useful germplasm to incorporate into breeding programs.

Molecular linkage maps and marker-assisted selection will eventually become useful in selection and breeding programs. Molecular markers have the advantage of high repeatability, low environmental noise, and, with the proper linkage map, the potential for genome-wide coverage. Two of their most important disadvantages are the cost of map development and the population or cross specificity that appears to be associated with each map. Markers associated with quantitative trait loci are highly inconsistent among different crosses or populations, reflecting differential linkage disequilibria among populations (Casler, 2000). Among forage crops, the phosphoglucose isomerase (*Pgi-2*) locus of perennial ryegrass potentially represents the single most useful molecular marker. While the *b*-allele of this locus confers increased water-soluble carbohydrate concentration, the most remarkable observation is the consistency in this relationship among diverse populations and crosses (Hayward et al., 1994; Humphreys, 1992; Smith et al., 1998). Because this enzyme is directly involved in carbohydrate metabolism, this marker may represent the actual quantitative trait locus as well (Casler, 2000). Finally, molecular markers will certainly become one of the most important tools for identifying parents of hybrid forage cultivars and for grouping potential hybrid parents into heterotic groups (Brummer, 1998).

Plant Transformation

Current plant transformation technology offers the opportunity to insert cloned DNA into nearly any forage species. Techniques for transformation of most important species have been reported for at least ten years (*e.g.* White and Voisey, 1994; Quesenberry et al., 1996). New enzymes or non-enzymatic proteins can be introduced to plants by transformation. For example, sunflower seed albumin (SSA), rich in both methionine and cysteine, is highly resistant to degradation during rumen fermentation (Tabe et al., 1993). Both alfalfa and subterranean clover (*Trifolium subterraneum* L.) were successfully transformed with a chimeric SSA gene that, in the case of subterranean clover, was stably expressed through one generation of sexual reproduction (Khan et al., 1996; Tabe et al., 1995). Transgenic plants with the highest levels of SSA expression had 0.1% and 0.75% of soluble leaf protein in the form SSA in alfalfa and subterranean clover, respectively (Khan et al., 1996; Tabe et al., 1995). For subterranean clover, this level meets the lower end of the range at which a wool-growth response would be expected, based on dietary supplementation research (Khan et al., 1996). Forage plants can also be made into factories that will synthesize foreign proteins useful for non-forage industrial or commercial purposes (Ziegelhoffer et al., 1999).

Alternatively, plant transformation technology can be used to reduce the activity of targeted enzymes by down-regulation with antisense RNA of the cloned enzyme. The past 5 years have seen an explosion of activity focused on genetic modification of lignin biosynthesis by plant transformation (Boudet and Grima-Pettenati, 1996; Casler, 2000; Jung and Ni, 1998). Much of this research has yet to be directly applied to forage crops, with the exception of a small number of transformed alfalfa plants. Nevertheless, plant transformation has proven an important tool in the advancement of scientific understanding of the pathway of lignin biosynthesis. For example, lignin is a highly plastic

polymer, synthesized by a metabolic grid with numerous pathways to the various monolignols that comprise the lignin polymer (Casler, 2000). Molecular polymorphisms for most of the enzymes appear to be responsible for genetic changes in lignin concentration and composition, cell wall modifications that have a direct and strong impact on forage-crop digestibility (Casler, 2000). Indeed, such novel genetic techniques have shown that natural genetic variability for lignin concentration and composition is as great or greater than that created by plant transformation, suggesting that many of these genetic polymorphisms already exist in natural populations (Casler, 2000). The challenge for plant breeders and physiologists is to find them.

Challenges for Cultivar Development

While plant transformation can be a boon to science and education, it has caused a backlash of public opinion toward plant breeding, causing even conventional plant breeding programs to be scrutinized and criticized as never before. Conferences such as this one are great for information exchange among scientists, but do nothing to alleviate the concerns of producers and the public. Scientists involved in potential commercialization of transgenic plants must become more sensitive to concerns of their stakeholders and more involved in the education process. While education should (and hopefully will) eventually soothe the concerns of a scientifically illiterate public, there are major obstacles to commercialization of transgenic plants, several of which are unique to forage crops.

Nearly all forage crops, including both annuals and perennials have wild relatives. As stated above and in several recent, more-detailed reviews, forage crops are not highly domesticated - phenotypically, they are still similar to wild species. As such, crossing barriers with their wild relatives are either nonexistent or, at best, weak. For most of these species, pollen can travel for many miles, either by wind or insect pollinators. Because many transformation programs are working to introduce adaptive traits (such as herbicide, stress, or pest tolerances) to forage crops, pollen from these transgenic plants *will be released, it will pollinate wild relatives, and it will help to create progeny that have the adaptive trait*. Plant scientists must acknowledge, despite all the pollen travel and movement research, that this will likely happen and that they will be ready to help producers, consumers, and the public deal with and solve the new problems that these transgenic plants may create.

Forage crops are also unique in the agricultural world in that they seldom represent a directly marketable product. They are largely used as animal feed, relegating them to just one of the costs/inputs for a livestock producer. As such, the marketplace has little tolerance for increased seed prices, despite the breeder's opinion of the value of a new cultivar with a new and/or unique trait. This attitude furthermore maintains extremely low profit margins for organizations involved in marketing of forage seeds. In the USA, royalties for forage cultivars are so small that cultivars developed by joint efforts of more than two organization, who each want a share of the royalties, will often fail simply because seed prices must be increased to cover the royalty split. Transgenic plants of forage crops will stretch the limits of this market structure, simply because there are too many organizations that will require a share of the royalty income (e.g. the owners or developers of: the DNA delivery system, the transgene itself, the transgene promoter, the selectable marker and its promoter, the tissue culture selection process, the germplasm to be transformed, and the final product of the breeding process). Some sort of reasonable expectation for royalties must be developed prior to commercialization, because profits from forage crop cultivars are simply too small to share among

all these organizations.

Some additional problems associated with transgenic technology, not necessarily specific to forage crops, provide additional impediments to use of this technology. Current methods available for genetic engineering of crop plants tend to be genotype specific, often requiring non-elite genotypes as the initial transformation targets. Thus, transgenic lines developed from non-elite genotypes must be backcrossed into elite germplasm, resulting in the need for additional crossing and selection. Because current methods of plant transformation can result in unstable expression or silencing of transgenes, any transgenic lines must undergo further assessment over several locations, years, and generations to confirm stability of transgene expression. Each transgenic event derives from a single cell of a single genotype. Thus, the genetic variability within heterogeneous crop varieties, such as most forage crops, is lost during the transformation process, unless large numbers of genotypes from the target variety are transformed and used to form the new variety. Loss of genetic variability within varieties may lead to reduced phenotypic stability and fitness in stressful environments. Furthermore, use of relatively few transformation events carries the additional risk that relatively inferior genotypes may become donors for transformation events, creating the need for additional selection.

Because of the above problems with implementing transgenic technology, applied forage breeding programs that use transgenic technology should focus on traits that are expected to have maximal economic impact for producers and minimal ecological, economic, or social impact. Examples of this include: commercial or industrial proteins that are otherwise benign, rumen-stable and/or specific amino acid-rich proteins, genes that facilitate hybridity or propagation of heterosis (e.g. reproductive regulatory genes such as self-incompatibility or apomixis genes), and disease resistance.

Conclusion

Progress in the improvement of cool season forages through plant breeding was significant in the 20th century, but much remains to be accomplished in the 21st century. Major areas of focus should include: 1) Development of genetically diverse synthetic cultivars through utilization of multiple recurrent selection methods with improved selection criteria, 2) Enhancement of regional adaptation of cultivars including selection for biotic and abiotic stress resistance, 3) Improvement of nutritional quality through use of laboratory *in vitro* procedures, 4) Selection for improved pasture persistence by including grazing animals in the early phases of selection programs, and 5) Utilization of appropriate molecular tools in the cultivar development process.

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