

## **Multistate Project NE-1010: Breeding and Genetics of Forage Crops to Improve Productivity, Quality, and Industrial Uses.**

**Duration: October 1, 2002 to September 30, 2017**

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### **Statement Of Issues And Justification:**

The economics of farming continues to be one of the major agricultural problems in the U.S. One important component of the solution is to increase profitability by crop improvement through plant breeding. Improved forage cultivars provide economic opportunities for livestock and crop farming operations and promote a more stable, sustainable agriculture. Farmers benefit by greater animal product per unit of land or forage produced, and the consumer benefits by maintenance of low cost food. Improved seed production capacity also ensures a satisfactory product for seed producers and a low seed price to the forage grower. All Americans benefit as genetic improvement is the least costly and most stable way to maintain international agricultural competitiveness, decrease the amount of land lost to agricultural production, and protect the environment by decreasing the use of pesticides, herbicides, and fertilizers.

Multistate cooperative research by NE-144 impacts directly upon all five national goals of the "Agricultural Research, Extension, and Education Reform Act of 1998." These goals are: (1) an agricultural system that is highly competitive in the global economy, (2) a safe and secure food and fiber system, (3) a healthy, well-nourished population, (4) an agricultural system which protects natural resources and the environment, and (5) enhanced economic opportunity and quality of life for Americans. What follows is a description of the problems, the general role of forage species in helping to solve these problems, and the specific role of forage breeders in researching these problem areas.

Forage crops constitute the foundation of livestock and dairy enterprises in the U.S. and Canada while also serving vital environmental functions. In order for livestock farms to be profitable, for environmental goals of reduced pollution and sustainability to be met, and for consumers to have meat and milk products available at low prices, forage cultivars with improved persistence, quality, and productivity need to be developed. As budget reductions have resulted in fewer forage breeding positions, the need for cooperative research to develop germplasm and cultivars with new traits is essential. The paucity of forage breeding belies the value of forage crops: measured solely as the value of hay, forages rank 4th behind corn, soybean, and wheat in cash receipts among all U.S. field crops. Yet, the combined number of scientist-years (SY) devoted to forage breeding at all state agriculture experiment stations is fewer than one for each species, with the exception of alfalfa, which had 15 SY's in 1994. For comparison, corn, soybean, and wheat had 32, 45, and 65 SY's respectively. Furthermore, half of all US plant breeding efforts, public and private, are devoted to corn, soybean, cotton, and wheat. Among all forage crops, only a few (alfalfa, perennial ryegrass) receive any investment from private breeding companies.

Those companies are disappointed that public institutions do not devote enough research on new traits and on forage species that companies do not breed.

Furthermore, all perennial forage species must be broadly adapted to market the volume of seed required to justify its production and marketing. Evaluation of genotypes, germplasm, and new cultivars must be done at multiple locations. A system currently is in place to accomplish this extensive evaluation among NE-144 cooperators. In addition, molecular marker systems have shown their potential usefulness for forage improvement, but prohibitive costs prevent all breeders from having laboratory facilities. The current NE-144 committee fosters the interactions necessary to accomplish more breeding with diminishing resources without unnecessary duplication of breeding efforts. Thus, enhancing our knowledge of forage crop breeding and genetics and developing improved germplasm and cultivars can only be done through collaboration among public scientists.

Plant breeders can improve pest resistance, persistence, and quality of forage crops without reducing forage yield potential (Anderson et al., 1988; Eichorn et al., 1986). These improvements can be made without passing increased seed costs to the farmer and without the need for increased management or input costs (Vogel et al., 1981). Thus, the farmer benefits by producing more animal product per unit of land or forage, and the consumer benefits through low cost food. Improved seed production capacity, or even maintenance at accepted levels, also ensures a satisfactory profit for seed producers and an acceptable seed price to forage growers.

The cooperative research with new traits, breeding methodologies, and molecular markers ultimately will result in new germplasms and cultivars that will enhance more economical production of forages and production of livestock products. Therefore, we will help foster a more diversified agricultural system that relies less on commodity grain production and improves agricultural sustainability.

Agriculture is the largest nonpoint source of water pollution in the U.S., accounting for 50% of all water pollution (Chesters and Schierow, 1985; Myers et al., 1985). Agricultural runoff carries nutrients, pesticides, and sediment into surface waters. Approximately 20% of U.S. cropland is subject to serious damage by erosion (Clark et al., 1985; USDA, 1987). Perennial forage crops are the most effective source of cropland vegetation in reducing water runoff and topsoil removal (Reganold et al., 1987; Wadleigh et al., 1974; Wischmeier and Smith, 1978). Their high level of ground cover and extensive root systems reduce soil erosion, increase water percolation, and reduce nutrient loading of both surface and ground water. Their perenniality also reduces the frequency of heavy tillage operations required in crop rotations. Reducing frequency of tillage reduces runoff and pesticide loading of surface waters (Glenn and Angle, 1987). In addition, tilled fields without cover crops are more susceptible to erosion and leaching of applied chemicals than are fields with a high level of ground cover (Hoyt and Hargrove, 1986; Reganold et al., 1987). A reduction in the use of forage crops has made control of soil erosion difficult in some regions (NRC, 1989).

Legume-based crop rotations can sustain high levels of grain production without the use of nitrogen fertilizer (Power, 1987). Legume cultivars with greater persistence, N<sub>2</sub> fixing ability, disease and insect resistance, and greater resistance to environmental stresses will be extremely

useful in the development and implementation of environmentally sound crop production systems.

Changes in crop production strategies toward reduced nitrogen fertilization and greater use of forage mixtures, will require changes in breeding strategies for continued development of improved cultivars and germplasm. Development of superior perennial grass cultivars for use in low applied nitrogen crop production systems will require breeding efforts under similar conditions. In perennial grasses, cultivars with the highest yield and quality under high nitrogen rates are not always the cultivars with the best performance under low nitrogen rates (Vose, 1963). Different nitrogen levels can also cause different genotypes to be selected from breeding nurseries. Grasses adapted to use in grass-legume mixtures may be more productive and stable than in monocultures (Chamblee and Collins, 1988) and have fewer weed problems (Drolsom and Smith, 1976). Breeding and testing under monoculture conditions does not allow selection of genotypes or cultivars with maximum performance in mixtures (Casler and Drolsom, 1984).

Cool-season forage species are extremely versatile with broad collective adaptation to virtually any cultivated lands in the northern U.S. and Canada. Therefore, they are often relegated to poorer soils and sites less conducive to row-crop production, such as waterlogged soils, hard pans, droughty sites, etc. Species such as alfalfa are highly productive on good soils but have severe production problems on heavy, wet, or acid soils. Other forage species are well adapted to each of these conditions and provide excellent alternatives under those circumstances. In addition, selection procedures can be used to genetically modify species for adaptation to stressful soil conditions (Charles, 1972; Snaydon, 1978). Breeding cool-season forage crops for resistance or tolerance to various environmental and biological stresses is an important component of research designed to maximize the efficiency of animal production on various types of marginal lands. Unfortunately, little support for these breeding programs exists outside of publicly funded institutions.

The use of pastures is widely practiced in the northern U.S. and Canada. It is an alternative means of producing high quality feed without the need for input costs associated with harvesting, processing, and storing cured feeds. Technological advances of the mid-20th century and the results of dairy production experiments (Larsen and Johannes, 1965) led to the widespread practice of hay and silage production from forage crops. Forage breeders, in their attempts to provide the best products for accepted management systems, followed this trend. Forage crop cultivars well adapted to hay and silage management systems tend to be more upright in growth habit, less densely-tillering, and earlier in maturity than those adapted to pasture systems (Stapledon, 1928). Consequently, cool-season forage crop cultivars are relatively unadapted to the frequent, intensive, rotational grazing systems (Voisin, 1988) now being adopted by many farmers.

Collaborative regional research is an essential component of an effective North American forage breeding research and cultivar development system. Forage breeders are trained at widely divergent institutions, which have differing specialties, resources, and philosophies. Most breeders have a secondary area of specialization, typically plant pathology, entomology, plant physiology, biometry, quantitative genetics, molecular genetics, or cytogenetics. Collaboration

on specific shared objectives is an efficient means of combining resources and expertise into important researchable objectives.

Forage breeding programs are unique in that nearly all forage breeding/genetics project leaders conduct research on multiple species. Although individual effort is small for some forage species, the cumulative impact from cooperative efforts among forage breeders is significant. Due to diversity among forage production environments and livestock production systems, farmers in any state or province collectively use a large number of species to meet their forage needs. No single breeder can conduct a research/cultivar development program on all species of economic importance. Furthermore, there are no public forage breeding programs in many important forage producing regions of the Northeast and Northcentral U.S., and few private companies actively breed perennial forages. As a consequence of these factors, relatively little effort is placed on some species for which important researchable goals remain unmet.

The members of the NE-144 Multistate Research Committee represent nearly all public efforts aimed at cool-season forage breeding in the humid, temperate portions of the U.S. and eastern Canada. The exceptions are a few alfalfa breeders and geneticists. Thus, research efforts described in this document do not represent duplication of any efforts underway in other institutions of the U.S. or Canada. A recent search of the CRIS system has further verified the validity of this conclusion.

Because of the long-term nature of research on perennial forage species, many of the collaborative efforts begun during the last NE-144 five-year period will continue into the next. In addition, completion of some efforts has led to new research projects that justify continued collaborative research in the proposed 15-year period. Furthermore, the high degree of collaboration among the NE-144 scientists will no doubt foster the initiation of additional collaborative efforts not yet envisioned.

Funding for these collaborative efforts will be only partially covered through the NE-144 project. Scientists currently supplement these cooperative research efforts with funding from other sources, such as from seed industry, royalties from seed of cultivars, and various sources of public funding (state and federal) for molecular and other types of research. USDA and Ag Canada participants fund their efforts from federal sources as well as from competitive grants. In addition, we anticipate that NE-144 funding will serve as seed or matching funds leading to more opportunities for research funding from other sources, as it has in the past.

### **Related, Current, And Previous Work:**

Formal forage breeding efforts began just over 100 years ago (Hays, 1892; Smith, 1948; Beddows, 1953). Early cultivars were developed by intentional (Hays, 1892; Jenkin, 1943) or unintentional (Levy, 1932; Evans, 1937) selection for morphological or sexual reproductive traits. Over the years, breeding became more sophisticated as more complex traits such as quality and resistances to biotic and abiotic stresses needed to be incorporated into cultivars.

Alfalfa is considered by many ranchers to be a highly desirable component of semiarid pastures and rangelands (Lorenz, 1982; Ries, 1982). Interseeding alfalfa into rangeland and depleted

pastures has been one approach to improve forage production and quality and animal performance (Lorenz, 1982). Berdahl et al. (1989) demonstrated that alfalfa populations with a high proportion of *M. sativa* subsp. *falcata* parentage had much higher survival, DM yield, and vegetative spread than *M. sativa* subsp. *varia* seven years after interseeding into rangeland near Mandan, ND. They also showed that populations of alfalfa that had persisted for over 50 years in association with rangeland grasses in western ND and SD survived grazing by sheep better than cultivars developed primarily for grazing in semiarid regions of the northern U.S. and southern Canada (Berdahl et al., 1986). Rumbaugh (1982) observed natural reseeding of alfalfa in 25-year-old pastures in southern UT, and more recently Smith (1999) described widespread recruitment of a population with predominantly *falcata* phenotypes on his ranch in northwestern SD. The parentage of that population traces back to plant introductions of *falcata* brought to western SD by N.E. Hansen during the early 20th century (Rumbaugh, 1979; Smith, 1999).

Lorenz (1982) suggested that introducing alfalfa to arid and semiarid rangelands was an efficient means of increasing animal output with a minimum input of fossil fuel energy. He also pointed out that to do that successfully would require the development of new cultivars that would be adapted to long-term productivity and persistence in an introduced legume-native grassland complex. Ries (1982) pointed out that for alfalfa to maintain itself in pastures or rangelands, it would have to have the capacity for recruitment since periodical reseeding would not be practical.

Evaluation of breeding material at multiple locations throughout a geographic region is the best means to develop cultivars with wide adaptation. Evaluation over a wide range of sites within or between regions is also useful in identifying the most appropriate sites for future tests (Barker et al., 1981). Additionally, some environments may be more conducive to selection progress than others (Snaydon; 1978; Casler and Walgenbach, 1990). However, many forage cultivars currently bred in Europe and North America are products of selection and breeding conducted at a narrow range of locations. The ultimate release and recommendation of a cultivar are often based on the results of a regional testing program, but are also frequently based on educated guesses and extrapolation on the part of the breeder. ‘Saratoga’ smooth brome grass, perhaps the most broadly-adapted cultivar of this species based on high yield in many diverse environments (Casler and Ehlke, 1986) is a product of regional testing prior to synthesis and testing of the cultivar *per se*. Without region-wide interaction among breeders, the coordination necessary to develop broadly adapted cultivars cannot occur.

Germplasm collection and incorporation into breeding programs remains an important objective of most forage breeding programs (Breese and Tyler, 1986; Rumbaugh et al., 1988; Casler, 1991). Because forage breeding is a relatively young discipline, forage crops are generally not as domesticated as most cereal crops (Harlan, 1975). Thus, collections of naturalized populations in many species have similar phenotypic characteristics as cultivated germplasm (Breese and Tyler, 1986; Rumbaugh et al., 1988; Casler, 1991). Because introduced germplasm is adapted to very specific environments, in the case of natural collections (Snaydon, 1978; Rumbaugh et al., 1988), or diverse production environments and management systems, in the case of cultivated germplasm from other countries, identification of superior germplasm for use in breeding programs requires evaluation in a wide range of target environments. Germplasm evaluations in a wide range of environments can be extremely useful in identifying the breadth of

adaptation of individual accessions as well as environmental factors responsible for differences in performance or adaptation (Snaydon, 1978).

Genetic improvement of forage yield is extremely difficult and slow, due to low heritability and high genotype x environment interaction (GEI) (Barker and Kalton, 1989). Thus, many breeders have developed new selection criteria for indirect forage yield improvement, e.g., leaf area expansion rate (LAER) in tall fescue (Sleper and Nelson, 1989), specific leaf weight in reed canarygrass (Carlson, 1990), and leaf blade length in perennial ryegrass (Rhodes, 1969).

In addition to forage uses, perennial herbaceous crops can be grown for other reasons, such as biomass for energy. Conversion of plant biomass to fuel, either through fermentation to ethanol (Lynd et al., 1991) or via direct burning to generate electricity (McLaughlin, 1993), has a number of desirable attributes, including a reduced dependence on foreign fossil fuels and stabilizing greenhouse gasses in the atmosphere through carbon and nitrogen cycling. Other uses of these crops include paper pulp, hardboard for building construction, and pellets for use in home heating (Thons and Prufer, 1991; A. Teel, pers. comm.). Unfortunately, little effort has been directed toward the genetic characterization and improvement of most grasses for these uses.

The most promising cool-season grass for biofuel production is reed canarygrass. Because the most important restriction on cropland use in the Midwest and Northeast, after erosion, is wet soils (USDA, 1987), reed canarygrass appears to be an ideal species. Reed canarygrass grows extremely well in wet soils, even withstanding inundation for long periods (Carlson et al., 1996). Its wet soil tolerance often overshadows its excellent drought tolerance, which makes it relatively more productive in the summer relative to other cool-season species (Carlson et al., 1996). Biomass productivity of reed canarygrass exceeded that of switchgrass in northern OH (Wright, 1988) and occasionally in southern IA (Anderson et al., 1991). Numerous other studies also have indicated that reed canarygrass produces excellent yields of total biomass (e.g. Smith et al., 1984; Cherney et al., 1986; Marten et al., 1980).

Until recently, all breeding research on reed canarygrass have focused on forage traits--palatability, seed retention, disease resistance, persistence, leafiness, etc. (Carlson et al., 1996). Maximum biomass per se is being evaluated on germplasm from the National Plant Germplasm System (Brummer et al., 2001).

For successful pasture production, cultivars must be tested for grazing tolerance (Brummer and Moore, 2000). The best method to select and evaluate for grazing tolerance is by directly using animals. Mechanical mowers have been used by some researchers, but they "select" passively, i.e., they harvest everything above the height at which they are set and do not contribute damage from hooves and mouths. Animals, on the other hand, harvest selectively (Woledge et al., 1992). Cattle are less selective and less detrimental to white clover than are sheep (Evans et al., 1992) and rotational stocking is less harmful than continuous stocking (Sheath and Hodgson, 1989). Thus, it is possible to select the level of defoliation pressure by manipulating the animals and management used. Bouton et al. (1991) showed that grazing-tolerant alfalfa cultivars could be selected by intense grazing by cattle. Morphological and physiological traits are often radically different for high-performing pasture cultivars vs. high-performing hay-type cultivars

(Stapledon, 1928; Jenkin, 1943; Breese and Tyler, 1986). Further studies showed that several morphological and physiological traits were associated with alfalfa grazing tolerance (Brummer and Bouton, 1991, 1992). Further research on traits and selection methodology is needed for other species. Regional collaboration will be an essential requirement for the evaluation and development of cultivars adapted to relatively new management systems, such as intensive, rotational grazing.

Persistence of forage stands for more than 2 or 3 years is a major problem limiting use of several productive, high quality species, such as red clover (Smith and Kretschmer, 1989), birdsfoot trefoil (Miller et al., 1983), and several ryegrass species (Casler, 1988; Casler and Walgenbach, 1990). The ability of a plant or stand to persist for many years is governed by its resistance or tolerance to a multitude of stresses, both biotic and abiotic. Sometimes, improvements in resistance or tolerance to one stress factor can result in significant improvement in stand longevity (see review by Smith and Kretschmer, 1989). Persistence of some species has been improved by selection for survival in harsh environments (Casler, 1988; Consigli, 1991). However, more work needs to be conducted to determine the specific traits responsible for genetic improvement of persistence (Casler, 1988). For some species and for some stresses, research is still required to identify appropriate selection criteria and methodologies.

In the private forage breeding sector of the U.S., selection for increased disease resistance is a major component of alfalfa cultivar development programs, but very limited work on diseases is conducted on other forage crops (Brummer and Moutray, 1996). In birdsfoot trefoil, *Fusarium* wilt has been implicated in stand losses in NY and MN (Gotlieb and Dorski, 1983; Murphy et al., 1985; Tillapaugh, 1995; Ehlke and Samac, pers. comm). Although Hill and Zeiders (1987) improved resistance to *Fusarium* wilt in a birdsfoot trefoil population, no resistant cultivars have been released until recently when 'Pardee' trefoil was released as a result of NE-144 research. *Mycoleptodiscus terrestris*, first identified by Gerdemann (1953), has been isolated from forage legumes in MO (Pettit et al., 1966, 1969), eastern USA (Carroll and Whittington, 1991), and WI (C.R. Grau and R R. Smith, pers. comm., 1994). The root-lesion nematode (*Pratylenchus penetrans*) causes serious damage to birdsfoot trefoil in Nova Scotia and other regions of North America (Willis et al., 1976). Papadopoulos et al. (1994) demonstrated significant variability among trefoil populations for resistance. A host of disease problems exist on the forage grasses as well (Braverman, 1986). In order to adequately develop widely adapted cultivars of forages that incorporate resistances to a range of pathogens, collaboration among public sector researchers is clearly needed.

Lack of winterhardiness is a particular problem in the northcentral United States and Canada. If the cultivated germplasm of a given species does not have sufficient cold tolerance or winterhardiness, wild or unimproved germplasm often does. *Medicago sativa* subsp. *falcata*, yellow-flowered alfalfa from Siberia, and Russian collections of orchardgrass are sources of winterhardiness for these species (Barnes et al., 1972; Kalton, pers. comm.). Because selection for cold hardiness requires harsh winters, the intensity of selection will vary year to year at any location. For material from areas where cold winters are not common, selecting in more northerly areas may result in greater gains in winterhardiness.

Ideas relating to improved forage quality have changed as animal nutritionists have learned more about the complexities of nutrition. Protein is considered one of the major contributions of most legumes to animal nutrition if the forage is managed and stored correctly. Protein concentration is highly heritable and can be improved readily by breeding (Clements, 1969; Phillips et al., 1982; Coors et al., 1986). Perhaps a more important objective is to alter the proteins in forage legumes to make them more resistant to rumenal degradation (i.e., increasing 'bypass protein') so that nitrogen is less likely to be lost as ammonia (Broderick and Buxton, 1991). Skinner et al. (1994) found variability among alfalfa germplasm sources for protein degradability. Alternatively, plant protein utilization could be improved by increasing the amount of rapidly degraded carbohydrate in the forage so that energy would not be limiting to rumen microbes (Stokes et al., 1991a, b). Pectic polysaccharides represent a potential source of rapidly degraded carbohydrates (Gradel and Dehority, 1972; Van Soest, 1982, 1995), and a significant portion of the total structural polysaccharides in forage legume cell walls are pectic materials (Titgemeyer et al., 1991; Hatfield, 1992). Hatfield and Smith (1995) measured the concentration of uronosyls, pectic sugars and pectin in leaf and stem walls of alfalfa, red clover, and birdsfoot trefoil and found significant differences among and within species. Another means to increase available carbohydrates is to increase digestibility of fiber by breeding for higher ratio of digestible to indigestible fiber. Higher fiber digestibility could maintain total fiber concentration while increasing the availability of carbohydrates in forage. Jung (1996) reported that just small shifts toward more digestible fiber in alfalfa resulted in significant increases in milk production by dairy cows.

Breeding for improved forage quality (small changes in digestibility) in three grass species had a significant positive impact on animal performance and consequently economic returns (Eichhorn et al., 1986; Anderson et al., 1988, Moore et al., 1995). Continued research on divergent selection for forage quality traits in other species, followed by laboratory evaluation and animal performance trials, will be necessary to confirm the animal response to increased forage quality. Buxton and Casler (1993) and Rhodes (1985) concluded there is evidence that lignin and associated phenolics are involved in providing plant resistance to diseases, insects, cold temperatures, and other environmental stresses in various plant species. However, in smooth brome grass and switchgrass, increased digestibility did not result in changes in forage yield or lodging potential (Vogel et al., 1981; Casler and Ehlke, 1986), despite large reductions in lignin concentration for smooth brome grass. Fonseca et al. (1999b) found no significant correlations between disease resistances and quality constituents, including lignin concentration, in alfalfa. Further research into the structural and nonstructural roles of lignin is warranted. Although increased disease resistance increases forage quality in the presence of the pathogen (Catherall, 1987; Karn et al., 1989), the effects of genetically improved forage quality on pest resistance and stress tolerance are not clearly delineated.

The concept of progeny testing as a selection tool in forage crop breeding has been in existence since the 1890s (Frandsen, 1991). It was refined and put to use by considerable research at numerous breeding stations during the 1950s (see review by Hanson and Carnahan, 1956). Both polycross and testcross procedures have been proposed and compared, on a theoretical basis, with phenotypic selection, which is selection based on individual plant performance per se (Empig et al., 1972; Hill and Haag, 1974). Although empirical comparisons have been made among selection methods for disease resistance in alfalfa (Haag and Hill, 1974; Hill and Leath,

1979), they have not been made for other traits or of other species. Contrary to expectations for traits controlled by some dominant genes, high-performance testers did not differ from low-performance in empirical evaluations of the testcross procedure (Voigt, 1968; Frandsen, 1991). Further research is needed to define the limitations and practical uses of both phenotypic and progeny test selection methods. We also need to use both existing theory and empirical selection studies to improve the efficiency of breeding systems, thereby increasing the rate of genetic progress.

Improved performance by selection within inbred lines has been demonstrated several times in alfalfa. Hybrids or synthetics derived from inbred lines were found to out yield hybrids or synthetics of the respective noninbred parents (Hill, 1975; Rotili and Zannone, 1974; Rotili, 1976). Evidence has been reported that the most efficient way to improve quality of the genes and linkages is the use of selfing assisted by selection (Rotili et al. 1996) and the optimum point of efficacy seems to occur in the second generation of selfing (Rotili, 1992). Pfeiffer and Bingham (1983) reported improvement by selection during inbreeding in tetraploid alfalfa populations. Their best explanation for the genetic improvement was recombination that permitted accumulation of alleles with favourable additive effects over several sexual generations of selection. Early work in the US and Italy indicated that one or two generations of selfing prior to selection improved gain.

In the past decade, molecular markers have become widely used for genetic mapping and breeding methodology studies of agronomic crops (Phillips and Vasil, 2001). Molecular marker work on forages has been scattered among numerous species with little in-depth work on any species other than alfalfa and tall fescue (Phillips and Vasil, 2001; Casler et al. 1996). Molecular markers could be used to augment breeding efforts in two ways: (1) through genetic mapping and marker-assisted selection or (2) through diversity analyses for parental selection or varietal typing. Genetic linkage maps provide a framework for gene localization and open the potential of marker-assisted breeding of both qualitative and quantitative traits. Linkage maps have been constructed in alfalfa (Brummer et al., 1993; Kiss et al., 1993; Echt et al., 1994; Tavoletti et al. 1995), tall fescue (Xu et al., 1995), and eastern gamagrass (Blakey, 1993). Marker facilitated selection, which could be practiced in the seedling year, could greatly speed the forage breeding process. Other molecular marker work has focussed on assessing variability within and among populations (Brummer et al., 1991; Yu and Pauls, 1993; Huff et al., 1993; Kidwell et al., 1994; and Xu et al., 1994) and on varietal identification (Huff, 1996; Barker, 1996). Markers may potentially be useful for the selection of parental plants having alleles that maximize heterosis (Brummer et al., 1994), similar to work on heterotic groups in maize (e.g. Lee et al., 1989) and oilseed rape (Diers et al., 1996). Obviously more work remains to be done to assess the utility of molecular markers to forage breeding projects. Collaborative agreements are essential to enable the wide area testing required to associate markers with phenotypes across several environments. Region-wide cooperation also allows breeders without access to laboratory facilities to interact with others who have molecular marker capabilities.

## **Objectives:**

- 1) Evaluate new plant characters and develop germplasm and cultivars with these characters to improve perennial forage species as livestock feed and biofuel uses to enhance rural vitality and promote more secure energy sources.
- 2) Build on previous research to evaluate additional breeding methods for improving yield and persistence of alfalfa, red clover, orchardgrass, and other forage species to make production agriculture more economical and sustainable.
- 3) Evaluate new experimental populations and cultivars of perennial forage species for characteristics necessary for breeders, seed companies, seed and forage producers, and crop consultants to make decisions on commercial use over large regions.

## **Methods:**

### Abbreviations

NGPRL=Northern Great Plains Res Lab.

PGRU=Plant Genetics Research Unit

PSWMRL=USDA/ARS Pasture Sys. & Watershed Man. Res Lab.

AFCCH=Agri-Food Canada, Charlottetown

AFCSF=Agri-Food Canada, St. Foy

AFCSK=Agri-Food Canada, Saskatoon

AFCL=Agri-Food Canada, Lethbridge, Alberta

FRRL=USDA/ARS Forage and Range Research Lab

## **1.0 Evaluate new plant characters and develop germplasm and cultivars with these characters to improve perennial forage species as livestock feed and biofuel uses to enhance rural vitality and promote more secure energy sources.**

### **1.1 Alfalfa**

#### **1.1.1 Identification of traits useful for improving *Medicago sativa* ssp. *falcata* germplasm.**

Lead: Brummer, IA

Cooperating locations: KS, AFCSF, NGPRL, SD.

We began a long-term project to evaluate and improve *falcata* germplasm in 1997 as part of the NE-144 project (Brummer et al., 1997b) and have recently completed an evaluation of 100 *falcata* accessions (manuscript in preparation), and a number of desirable accessions were identified. In this project, we will evaluate another 96 of the tetraploid, yellow-flowered accessions in spaced-plant nurseries at all locations. Data will be collected in 2003 and 2004. In 2005, superior plants will be selected as parents for improved populations.

#### **1.1.2 Genetics and improvement of naturalized alfalfa (*M. sativa* subsp. *falcata*).**

Lead: Boe, SD

Cooperating locations: IA, NGPRL.

Nurseries were established at SD, IA, and Brandon and Miami, Manitoba during 2001, with additional nurseries planned for ND and MT in 2002. Data will be collected from 2002-2006 on genetic variation for traits related to morphological adaptation to grazing, forage production, quality, seed production, and resistance to diseases and insects. Superior plants will be selected from each location for development of synthetics. In addition, synthetics will be developed based on family performance across environments. In 2008 seed produced from those experimental synthetics will be interseeded into depleted pastures/rangelands in replicated trials with several grazing- and hay-type cultivars for long-term (about 5 years) evaluations of productivity and persistence at several locations in North America. Superior synthetic cultivars will be released.

### **1.1.3 Breeding for resistance to alfalfa snout beetle in alfalfa.**

Lead: Viands, NY

Cooperating locations: AFCSF, SD, IA.

Alfalfa snout beetle (ASB, *Otiorhynchus ligustica*) causes severe productivity and stand losses on alfalfa in northern NY. Because this insect threatens to spread across northern North America, we initiated research to identify resistant/tolerant germplasm for developing resistant cultivars. All the perennial Medicago core collection and other germplasm in a field trial were susceptible; however, significant variation existed, suggesting that resistance genes may exist at a low level in a few populations. Therefore, in 2001, we initiated recurrent phenotypic selection for resistance in the most resistant populations. We plan to conduct at least four cycles of selection under controlled environment to develop resistant or tolerant germplasm. Ultimately, we may need to use a backcross program to transfer resistance into an adapted background. Cooperators in NE-144 will evaluate these populations for yield and persistence in replicated plot trials. NY also will evaluate them for yield, persistence, and root damage from ASB in an infested field.

### **1.1.4 Developing alfalfa germplasm with potato leafhopper resistance from three diverse genetic sources.**

Lead: Viands, NY

Cooperating locations: IA, SD, KS, WV.

Previous NE-144 research resulted in identification at SD of a *M. falcata* population with resistance to potato leafhopper. This resistance is a different mechanism than that from the glandular hairs in the most recent alfalfa cultivars in the USA (Hansen et al., 2001). We plan to create more stable resistance by developing alfalfa germplasm with all three types of resistance: *falcata*, glandular trichome, and non-glandular pubescent Peruvian germplasm.

After crossing followed by three cycles of selection at several locations, the level of resistance of this germplasm will be compared with those of current resistant and susceptible cultivars and with the original resistant populations at all participating locations. Leafhopper resistance will be evaluated in replicated spaced-plant nurseries. In addition, yield will be assessed in replicated plot trials.

### **1.1.5 Genetic mapping agronomic traits in alfalfa**

Lead: Brummer, IA

Cooperating locations: KS, NY, AFCSF.

A tetraploid alfalfa population, created by crossing an elite *M. sativa* subsp. *sativa* genotype with a semi-improved genotype of *M. sativa* subsp. *falcata*, is being genotyped using various types of molecular markers and phenotyped for yield and agronomic traits (Brummer, 2001), forage quality (Alarcon-Zuniga et al., 2000), and winter hardiness (Brummer et al., 2000) in IA in order to map quantitative trait loci (QTL). To extend the utility of the population and make inferences across broad geographic regions, we planted this population in NY and KS. Under the new NE-144 project, we will continue to evaluate this population for yield, winter survival, pest resistances and physiological traits. Other diploid and tetraploid alfalfa populations are being developed to continue our genetic dissection of these traits.

### **1.1.6 Aluminum tolerance in tetraploid alfalfa.**

Lead: St. Amand, KS

Cooperating locations: AFCCH, NY.

An estimated 40% of arable soils worldwide are acidic and contain phytotoxic levels of aluminum. Alfalfa is very sensitive to low pH and high aluminum soils. To develop acid-tolerant cultivars, widely adapted germplasm with acid-tolerance must be identified. Greenhouse and laboratory methods for evaluating acid-tolerance have been investigated (Bouton, 1996; Campbell et al., 1988), but evaluation in acidic soil was most effective for identifying tolerant plants (Bouton, 1996).

PIs most tolerant to low pH were crossed with several germplasms adapted to the Central Great Plains. Progeny of these crosses will be evaluated for yield, vigor, and adaptation in replicated plots of low pH native soil at diverse locations in the U.S. Recurrent phenotypic selection will be done to improve the population. Because progress is expected to be slow (Bouton and Sumner, 1983), 8 to 10 breeding cycles (years) will likely be needed to produce acceptable Al tolerant lines with wide adaptation.

## **1.2 Birdsfoot Trefoil**

### **Rhizomatous birdsfoot trefoil for yield improvement.**

Lead: Beuselinck, PGRU

Cooperating locations: AFCCH, NY, IA, SD.

Persistence of birdsfoot trefoil in humid regions of North America is shortened by root- and crown-rotting diseases. *Lotus corniculatus* L. germplasm accessions from Morocco produce rhizomes that may increase persistence in cultivars. Rhizomes have been successfully transferred into cultivars of *L. corniculatus*, and the cultivar ARS-2620 was developed and released. Interspecific hybrids between *L. corniculatus* and *L. uliginosus* produce rhizomes and have potential for development into another forage crop.

ARS-2620 and the *L. corniculatus* X *L. uliginosus* hybrid will be evaluated and selected for adaptation to diverse environments. Selection will be on growth form (very prostrate or erect), winter hardiness, and rhizome expression.

### **1.3 Reed Canarygrass**

#### **Collection and evaluation of naturalized reed canarygrass populations for biofuel and forage traits.**

Lead: Brummer, IA

Cooperating locations: SD, AFCSF, KY, AFCSK, NGPRL, WI.

We have evaluated the PI collection of reed canarygrass and other populations and cultivars for biomass in IA and WI. A local ecotype ('Fraser') collected near Boone, IA had among the highest yields, which suggests that native and naturalized populations of reed canarygrass could provide substantial variation for biomass traits that is not currently in the USDA collection. We will evaluate up to 100 accessions collected throughout North America. In addition, we will make some targeted collections along the southern edge of reed canarygrass adaptation and in uncollected areas of the Northeast U.S., MN, and WI. Evaluation for maturity, plant height, biomass yield (two harvest system), and cell-wall constitution will be done in 2004 and 2005. The best accessions will be increased for further evaluation, selection, and possible release.

### **1.4 Tall Fescue**

#### **Interactions of nonstructural carbohydrates, seed yield, forage quality and yield components.**

Lead: Balasko, WV

Cooperating locations: KY.

Development of tall fescue populations with a range of total nonstructural carbohydrate (TNC) concentrations will be continued. Correlations among TNC, seed and forage yields, and forage quality components will be investigated. The studies will include related species and derivatives such as perennial ryegrass, meadow fescue, and festulolium. The role of TNC concentration in palatability, winter hardiness, and persistence also will be determined.

### **1.5 Multiple Species**

#### **1.5.1 Selection for fiber digestibility and cell wall pectin.**

Lead: Viands, NY

Cooperating locations: AFCSF, WI.

We are developing breeding objectives for improving forage quality in alfalfa and grasses, primarily by increasing carbohydrate concentration, which is essential for rumen microbes to convert forage protein into forms that dairy cows can use for milk production.

NY and USDFRC are using different assay techniques to improve pectin concentration in alfalfa. USDFRC has been developing populations with high and low leaf and stem pectin concentrations to evaluate the effect of altered cell wall pectin concentration on animal nutrition. NY has developed several alfalfa populations with various ratios of fiber constituents. Replicated

plot trials are being sampled for at least two production years to determine progress from selection and the effect on in vitro dry matter digestibility and bypass proteins.

At AFCSF, divergent selection was done in four timothy populations for various fiber ratios and other quality constituents. Populations will be evaluated in two field tests for forage yield, quality of stems and leaves, leaf:stem ratio, and stem diameter. Heritability based on individual plants and on half-sib progenies will be determined for the above traits.

At WI, selection for divergent lignin, etherified ferulic acid, and total fiber concentrations is being done on several forage grasses. Evaluation of these grass populations will be done at various locations.

### **1.5.2 Grass-grass and grass-forb mixtures for long-term sustainable biomass production.**

Lead: Boe, SD

Cooperating locations: IA, WV, PGRU, NY, MN.

Switchgrass (*Panicum virgatum* L.) has been identified by the U.S. Department of Energy as a potential herbaceous biomass/energy crop. Current research is focusing on developing high-yielding cultivars of switchgrass along with cultural practices that minimize inputs, promote sustainability, and maximize biomass production (Sanderson et al., 1996). Tilman et al. (1996, 2001) demonstrated a positive relationship between biomass productivity and species richness in seeded plots of native North American prairie plant species.

We plan to apply this ecological research to practical agronomic situations by comparing: (1) long-term productivity of seeded plant communities composed of 1, 2, 4, 6, or 8 native North American plant species with switchgrass as a common component of all five communities, and (2) biomass production in binary mixtures of switchgrass and big bluestem cultivars of similar or different latitudinal origins. Data from plot trials at various locations will be collected on total biomass production and biomass species composition over time.

### **1.5.3 Molecular fingerprinting in forage populations.**

Lead: St. Amand, KS

Cooperating locations: PSWMRL, AFCCH, PGRU.

Most forage cultivars are synthesized from multiple sources, creating maximum heterogeneity. Mixed genomes makes identification of populations difficult. Both RFLP and PCR-based methods generally have shown that high variability within populations obscure variation between populations (Skinner, 2000). A DNA fingerprint using hypervariable regions in the chloroplast genome can help differentiate alfalfa cultivars. Populations are compared based on the distribution of the fragment sizes using the Kolmogorov-Smirnov (KS) test (Wayne, 1990). Comparisons of the hypervariable cpDNA, RAPDs and AFLPs showed that the hypervariable cpDNA regions were the most efficient kind of marker for distinguishing populations (Kisha et al., 2001). Two hypervariable regions (HindIII and HaeII), successfully used by Skinner (2000) to evaluate relationships of alfalfa accessions, may be useful to distinguish individual alfalfa cultivars and to evaluate potential parents in a breeding program.

Our objective is to test the robustness, utility, and reproducibility of using cpDNA hypervariable regions to distinguish between populations of forages. Each location will provide three closely related alfalfa populations. A sample of 96 seedlings per population will be sprouted and cpDNA extracted individually. Marker profiles generated on each population from each location will be compared. The same methods will also be applied to other forage species.

## **2.0 Build on previous research to evaluate additional breeding methods for improving yield and persistence of alfalfa, red clover, orchardgrass, and other forage species to make production agriculture more economical and sustainable.**

### **2.1 Alfalfa**

#### **2.1.1 Comparison of mass, S1, and S2 selection in alfalfa.**

Lead: Michaud, AFCSF

Cooperating locations: IA, NY.

Selection began in 1997 to compare the effectiveness of two cycles of phenotypic selection made at the S1 and at the S2 level versus two cycles made at the non inbred level for improving yield potential of alfalfa. For each cycle, about 200 each of S1 and S2 families were evaluated for 2 years. The best plant within each of the 20 best S1 and the 20 best S2 families were selected and intercrossed within each group. Similarly, 2000 plants from the same base population were evaluated and after 2 years, and the best 100 plants were intercrossed to produce another population. The second cycle of selection was initiated in 2001. Each cooperator selecting in his own population. The base population and the Syn. 2 generation of the first and second cycles of selection, along with several check cultivars, will be established in replicated plot trails at multiple locations to determine selection progress.

#### **2.1.2 Replicated clonal line selection for improving forage yield of alfalfa.**

Lead: Viands, NY

Cooperating locations: AFCSF, IA, KS.

Research will be initiated to determine if replicated clonal selection improves alfalfa yield potential. Replicated clonal selection enables evaluation across environments, thus increasing heritability, while theoretically utilizing all the genetic variability of the plant population. Most types of progeny testing exploits only one-half or less of the additive genetic variability (Rowe and Hill, Jr., 1984; Rumbaugh et al., 1988).

Germplasm sources from each participant will be randomly intermated for two generations to create one genetically broad population. Each participant will transplant three ramets per replicate (3) of each of 200 genotypes to a field nursery. At each harvest, the number of plants and forage yield will be recorded for two production years. At the end of the second production year, the highest yielding 10% (20 genotypes) across locations will be intercrossed to produce Cycle 1 seed. The scientists also will produce their own populations using data from their own locations. After two cycles, selection progress will be determined with Syn. 2 seed in replicated plot trials at all the locations.

## **2.2 Orchardgrass**

### **Clonal selection in orchardgrass for broad adaptation.**

Lead: Tim Phillips, KY

Cooperating locations: IA, AFCSF, AFCSK, SD.

Similar to the alfalfa study described above, a broad-based orchardgrass population will undergo clonal selection. After two years' evaluation for winter hardiness, disease reactions, forage yield, seed yield, and tolerance to grazing, synthetics will be constructed based on performance across environments. Selection progress will be determined like above and compared with that of alfalfa.

## **2.4 Red Clover**

### **Selection for general adaptation in red clover**

Lead: Papadopoulos, AFCH

Cooperating locations: NY, AFCSF, AFCSK, WV, SD, MO, IA.

Poor persistence of red clover is attributed mainly to lack of winter hardiness and to susceptibility to root and crown diseases and root-feeding insects. Selecting specifically for these traits has not improved general adaptation and long-term persistence. Natural selection in the target regions may be most effective for improving persistence.

Broad-based germplasm will be established at each site. In the third year, plants selected from the sites will be crossed in a diallel design to produce all combinations of synthetic populations within and between sites. Syn. 2 seed will be used to establish yield trials at all sites to determine selection progress and to differentiate between general versus regional adaptation methodology for improving long-term persistence.

## **3.0 Evaluate new experimental populations and cultivars of perennial forage species for characteristics necessary for breeders, seed companies, seed and forage producers, and crop consultants to make decisions on commercial use over large regions.**

### **3.1 Alfalfa**

#### **Evaluation of new *M. sativa* subsp. *falcata* populations.**

Lead: Brummer, IA

Cooperating locations: KS, AFCSF, NGPRL, SD.

Each location involved in the *M. sativa* subsp. *falcata* evaluation project (1.1.1) has developed populations from the most desirable accessions. Seed quantities will be increased in 2003 and comparative hay trials will be planted along with check cultivars in 2004.

### **3.2 Black Medic**

#### **Evaluation of naturalized *Medicago lupulina* collections.**

Lead: Brummer, IA

Cooperating locations: NY, PGRU, SD.

Black medic is common in pastures, lawns, and roadsides throughout most of North America. Little selection for improved black medic cultivars has been conducted throughout the world, and only one cultivar, 'George', is available in the U.S. (Sims et al., 1985). Blaser and Stokes (1946) suggested that local ecotypes performed substantially better than other germplasm in Florida.

The objective of this research is to evaluate black medic populations that have been collected during the last 5 years throughout North America along with accessions from NPGS to determine the potential for improvement. Nearly pure lines, developed by single seed descent, will be planted in replicated field plots consisting of five plants each. Plots will be evaluated for growth habit, days to flowering, vigor, powdery mildew resistance, dry matter yield, regrowth rate, seed production, regrowth vigor, and perenniality during 2003-4. Seed of the best genotypes will be increased, planted at multiple locations, and evaluated for potential germplasm or cultivar release.

### **3.3 Multiple Species**

#### **Evaluation of cultivars and germplasm under grazing.**

Lead: Brummer, IA

Cooperating locations: NY, AFCSE, AFCCH, PSWMRL, KY, SD, PGRU, NGPRL, WI.

Grazing tolerance is a major requirement for many forage species destined for pasture production, yet evaluation under grazing stress often is not conducted, particularly in production regions. Material to be evaluated will be new grass and legume cultivars from Europe and New Zealand that are used in North America, and the NE-144 *M. sativa* subsp. *falcata* populations. Evaluation experiments will be conducted as germplasm is developed or as new cultivars are introduced. Grazing will be rotational and/or continuous by beef cattle or sheep (Brummer and Moore, 2000), depending on the intended goals of the specific experiment. Grazing tolerance will be assessed visually or by stand counts two weeks after terminating grazing in the autumn. Each test will last 3 years post establishment.

#### **Measurement of Progress and Results:**

##### **Outputs:**

Output 1: Release of improved germplasm and cultivars of the following:

- a. Alfalfa suitable for interseeding and becoming naturalized into depleted pastures and rangelands,
- b. Alfalfa resistant to the snout beetle,
- c. Yellow flowered alfalfa that could be used alone under pasture or range conditions or in crosses with purple flowered alfalfa to produce heterosis,
- d. Alfalfa with multiple mechanisms for resistance to the potato leafhopper,
- e. Alfalfa with improved tolerance to Al soils

- f. Birdsfoot trefoil with enhanced adaptation to the NE-144 members' regions and possessing rhizomes to aid in survival,
- g. Red clover with adaptability to various areas of north central and northeast US and contiguous areas in Canada,
- h. Orchardgrass with broad adaptation, high yield, and enhanced persistence,
- i. Reed canarygrass superior for use as a biofuel crop,
- j. Timothy with enhanced nutritive value,
- k. Black medic for use in mixed pastures.

These cultivars and germplasms will improve the profitability of forage crops and their development would be impossible without the widespread cooperation facilitated by NE-144.

Output 2: Development of improved selection methodologies by evaluating:

- a. Clonal selection for yield and persistence in alfalfa and orchardgrass,
- b. Selfed progeny selection for yield and persistence in alfalfa,
- c. Natural selection for adaptation in red clover,
- d. Recurrent phenotypic selection on cell wall components in several species.

Output 3: Increase in knowledge about the structure of alfalfa populations and the alfalfa genome, specifically,

- a. Mapping chromosomal regions in alfalfa associated with biomass yield, winter survival, and disease resistance,
- b. Evaluation of molecular marker based methods to differentiate among alfalfa cultivars.

Output 4: Elucidation of the role of biodiversity on long-term agricultural productivity, using biofuel production as a model system to evaluate grass-grass and grass-forb mixtures versus monocultures.

Output 5: Evaluation of new species and germplasms for their persistence under grazing stress to enable more useful recommendations to farmers.

Output 6: Understanding of the naturalization process of introduced species by studying the attributes of yellow flowered alfalfa that was introduced into South Dakota in the early 20<sup>th</sup> century.

Output 7: Journal papers, technical bulletins, state agricultural experiment station reports, extension bulletins, popular press releases, and web sites describing the research conducted and the enhancements of newly released cultivars and germplasms, and reporting yield data of new forage crop cultivars.

## **Measurement of Progress and Results:**

### **Outcomes or Projected Impacts:**

Outcome/Impact 1: Farmers and ranchers will have access to new cultivars that have improved yield, quality, persistence, grazing tolerance, disease resistance, and other traits designed to

enhance their usefulness to the farming enterprise, particularly that of small to medium sized farmers.

Outcome/Impact 2: Improved forages will improve farm profitability by providing a more nutritious, more stable feed supply to the dairy and livestock industries; this in turn will increase forage cultivation, lessen the need for grain in rations, and help diversify the farming economy.

Outcome/Impact 3: An increase in forage cultivation will have major positive impacts on environmental quality by increasing the proportion of land in perennial crop cover, thereby decreasing erosion, improving soil tilth, adding nitrogen to the cropping system, and improving water quality.

Outcome/Impact 4: As a result of this project, breeders will be able to develop new germplasm and cultivars more efficiently and more quickly using newly evaluated breeding methods and molecular markers to move particular chromosomal regions in a targeted manner, while avoiding genetic vulnerabilities that may be present in some germplasm.

Outcome/Impact 5: High producing biomass cultivars would be developed from this project that would be essential to make the nascent biofuel industry successful. Given the beneficial environmental characteristics of biofuels, having superior cultivars in hand when the industry is ready is a sensible goal.

Outcome/Impact 6: Producers will get practical information on which to base their selection of higher yielding and more stable cultivars for their pastures, which will improve grazing systems throughout the region, improving economic welfare for all growers and producers.

Outcome/Impact 7: Forage crops that have received limited attention in the past—such as birdsfoot trefoil, black medic, and reed canarygrass--will be evaluated and improved for adaptation (e.g., evaluation of naturalized germplasm) and persistence (e.g., the rhizome trait in trefoil).

Outcome/Impact 8: The utility of ecological experiments relating biodiversity and biomass productivity will be empirically tested using biofuel crops as a model system. The importance of species mixtures to ensure long-term productivity must be evaluated. This will be an important verification of the relevance of ecological experiments to applied agricultural situations.

Outcome/Impact 9: The characteristics associated with invasive or introduced species will be elucidated by close examination of an introduced alfalfa population that has been expanding its range in western South Dakota over the past decade. A better understanding of what makes species successful invaders will help manage inadvertent introductions more effectively.

Outcome/Impact 10: The utility of molecular markers to both develop and describe forage populations and germplasm will be tested. Though these technologies have significant potential, their usefulness in outcrossing, polyploid forage crops like alfalfa is not well tested. Application of these technologies to the ongoing breeding programs of NE-144 participants will help gauge the possible efficiencies they can provide to the development of improved cultivars.

## **Measurement of Progress and Results:**

### **Milestones:**

Germplasm and cultivars will be released and articles published periodically throughout duration of the project. In addition, yield data will be reported annually. Specific milestones are listed below.

2002: Milestones: Identification of an orchardgrass population with suitable broad adaptability. *L. corniculatus* X *L. uliginosus* population increased for seed availability. Cycle 2 selection at the S1 and S2 levels.

2003: Milestones: Establishment of orchardgrass clonal material at four locations. Development of a genetically diverse starting population of red clover. Seed of *L. corniculatus* X *L. uliginosus* and ARS-2622 are distributed to cooperators. Initial cross of potato leafhopper resistant alfalfa populations. Initial testing of Al tolerant alfalfa germplasms.

2004: Milestones: Identification of clones to use in constructing orchardgrass synthetics. Cycle 1 selection of potato leafhopper resistant alfalfa populations.

2005: Milestones: Production of an orchardgrass synthetic. Selection of adapted red clover clones. Initial evaluation of black medics. Initial evaluation of first set of falcatas. Cycle 2 selection of potato leafhopper resistant alfalfa populations. Produce Syn. 2 seed of populations derived from two cycles of selection for the breeding methods study.

2006: Milestones: Production of orchardgrass clones for a second cycle of selection. Evaluation and selection of *L. corniculatus* X *L. uliginosus*. Cycle 3 selection of potato leafhopper resistant alfalfa populations.

2007: Milestones: Development of red clover diallel crossing populations.

2008: Milestones: Release of black medic germplasms. Initial QTL maps of alfalfa made. Seed increase of *L. corniculatus* X *L. uliginosus* germplasm. Nursery testing of potato leafhopper resistant alfalfa populations.

2009: Milestones: Initial evaluation of second set of falcatas.

2010: Milestones: Identification of superior orchardgrass parents from the second cycle of selection. Evaluation of red clover populations at each site.

2011: Milestones: Yield testing of potato leafhopper resistant alfalfa populations.

2012: Milestones: If necessary, backcross potato leafhopper resistance into an adapted alfalfa background.

2013: Milestones: Possible release of alfalfa with multiple potato leafhopper resistant mechanisms.

2014: Milestones: Release of improved orchardgrass synthetic with broad adaptability.

2015: Milestones: Yield testing of AI tolerant alfalfa germplasm.

2016: Milestones: Release of improved falcata populations.

2017: Milestones: Release of improved AI tolerant alfalfa germplasm.

### **Outreach Plan:**

Performance results will be available in various forage variety trial results published by several states. Normal information transfer via the extension component of the land-grant institutions will be utilized. In cooperation with extension personnel, field days will be conducted to showcase the qualities of new germplasm and cultivars and to describe the various benefits. The primary goal of some evaluations is to provide information to producers about which cultivars and/or species are most useful to their hay and grazing systems. Information will be distributed through university extension bulletins, popular press articles, and field days within each participating state.

Research findings will be disseminated through refereed publications, non-refereed but peer reviewed publications, and abstracts or meetings. International conferences will also provide an outlet for research findings. Germplasms and cultivars will be released by cooperating institutions. Some states already have interactions with seed companies who produce and market seed of public cultivars. Forage breeders across North America and the world will be notified about germplasm releases via articles in Crop Science.

### **Organization and Governance:**

The recommended Standard Governance for multistate research activities will be used for this project. This project will have an elected Chair, a Chair-elect, and a Secretary. All officers are to be elected for at least two-year terms to provide continuity. Administrative guidance will be provided by an assigned Administrative Advisor and a CSREES Representative.

**Authorization: [Electronic Signature of the Administrative Advisor with the date of submission.]**

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