

Report on the Status of *Medicago* Germplasm in the United States

**Alfalfa Crop
Germplasm Committee**

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Alfalfa Crop Germplasm Committee

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Summary Status of the *Medicago* Germplasm in the United States

Production Trends and Value of Alfalfa

Alfalfa acreage is at 23.6 million acres and is predicted to maintain or drop slightly in acreage primarily due to the increase productivity of each acre.

Alfalfa is the best animal feed for ruminant livestock and horses and it ranks 4th among all U.S. agricultural crops grown with an annual production value of \$8.1 billion . When the value of animal products is added to the value of alfalfa hay, the dollar value of alfalfa reaches \$145 billion annually. Thus far exceeds the combined value of all other high value crops.

Annual medics have potential uses in sustainable agriculture systems, however, additional research is needed to discover their niche in the U.S.

Crop Vulnerability

The general impression has been that crop genetic vulnerability was not a large concern due to the autotetraploid nature of *Medicago sativa* subsp. *sativa*, the high level of outcrossing, and the diversity of germplasm sources utilized in the development of alfalfa cultivars. However, a survey of the cultivars released over the past 14 years has shown a narrowing of the germplasm utilized.

Germplasm Activities

Development of alfalfa cultivars has shifted from the public sector to private companies over the past 60 years. Today, virtually all commercial alfalfa cultivars are developed by private companies.

The USDA-ARS breeding effort has shifted towards germplasm collection, evaluation, enhancement and development as well as fundamental classical and molecular studies on alfalfa and related species.

The University's have also shifted towards germplasm evaluation, enhancement and development, however, a few public breeders continue to develop special use cultivars of alfalfa. The University's have also been involved in fundamental studies of alfalfa particularly in the area of molecular biology.

Who's Involved?

Organizations involved in germplasm development include federal, state, and private companies. The North American Alfalfa Improvement Conference continues to be the conduit through which scientific information is exchanged between public organizations and private companies for the benefit of the alfalfa crop.

Overview Summary of Recommendations

Prioritized recommendations

1. Collection maintenance
2. Germplasm collection
3. Germplasm evaluation
4. Germplasm enhancement

1. Collection Maintenance

Although the seed regeneration of almost all the collection has been completed, the need remains to a) regenerate accessions requiring special care/handling, b) obtain baseline seed viability data, c) store original seed and regeneration seed lots at -18 C, d) deal with backlog of "W6" and NSL accessions (ie. should they go into collection?) and duplicate accessions, and e) verify our regeneration procedures are adequate. Without the continued vigilance in maintaining our current collection, future collections, evaluations and enhancements would be of little value.

2. Germplasm collection

The greatest needs for the collection are for the wild perennial species in the secondary and tertiary germplasm pools, especially those species which are considered threatened.

Countries where future collections should occur include Georgia, Crimea and Southern Caucasus mountain regions of the former Soviet Union, the Himalayan region of India, south and north east regions of China, Sudan, Egypt and the Arabian Peninsula, South Africa, Northern Kazakhstan, and adjacent Central Asian countries (i.e. Kyrgistan, Uzbekistan, Afghanistan), Iran and Iraq.

3. Germplasm Evaluation

A set of descriptors for all accessions, especially all alfalfa accessions needs to be completed. The currently established core subsets should be reevaluated once descriptor data has been obtained on the entire collection. Accession classification based on molecular markers needs to be accomplished and molecular data compared to classifications based on morphological and ecogeographic descriptors. In addition, evaluations which can broaden the use of alfalfa need to be carried out.

4. Germplasm Enhancement

National and regional germplasm pools should be developed.

A Compilation of the Specific Recommendations is Located on Page 51.

Alfalfa Crop Germplasm Committee Report

Status of the Medicago Germplasm in the United States

Chapter 1 - The Genus *Medicago*

Introduction

The genus *Medicago* is a member of the Legume family. According to Small and Jompe (1989) the genus contains 30 perennial species and 60 annual species (Appendix 1). About 15 species are cultivated for various uses (Table 1.):

- C Animal food
- C Soil improvement
- C Cover crops
- C Human nutrition
- C Ornamental

Alfalfa (*Medicago sativa* L. sensu lato) is a perennial species and the most important forage legume in North America. The annual species, referred to as medics, serve as important components in pastures in regions with Mediterranean climates.

Table 1. List of cultivated *Medicago* species their common name, usage and growth cycle.

Cultivated species of <i>Medicago</i>	Common name	Use	Growth Cycle
<i>Medicago arabica</i> (L.) Huds.	Spotted bur clover, spotted medic	Animal forage	Annual
<i>Medicago arborea</i> L.	Tree alfalfa, tree medic	Animal forage, ornamental	Perennial
<i>Medicago italica</i> (Mill.) Fiori	Disc Medic	Animal forage, soil improvement, companion crop	Annual
<i>Medicago littoralis</i> Rohde ex Loisel.	Strand Medic	Animal forage, soil improvement, companion crop	Annual
<i>Medicago lesinsii</i>		Animal forage, soil improvement, companion crop	Annual
<i>Medicago lupulina</i> L.	Black medic	Animal forage, soil improvement, companion crop	Annual
<i>Medicago minima</i> (L.) Bartal.	Little bur clover	Animal forage	Annual
<i>Medicago murex</i> Willd.		Animal forage, soil improvement, companion crop	Annual
<i>Medicago orbicularis</i> (L.) Bartal.	Button clover	Animal forage, soil improvement, companion crop	Annual
<i>Medicago polymorpha</i> L.	Toothed bur-clover, Toothed medic, California bur-clover	Animal forage, soil improvement, companion crop	Annual, biennial, perennial
<i>Medicago rigidula</i> (L.) All.	Tifton bur-clover, Tifton medic	Animal forage, soil improvement, companion crop	Annual

<i>Medicago rugosa</i> Desr.	Gama medic	Animal forage, soil improvement, companion crop	Annual
<i>Medicago sativa</i> nothosubsp. <i>varia</i> (Martyn) Arcang.	Sand lucerne , variegated lucerne	Animal forage	Perennial
<i>Medicago sativa</i> subsp. <i>falcata</i> (L.) Arcang.	yellow-flower alfalfa, sickle alfalfa, yellow Lucerne	Animal forage	Perennial
<i>Medicago sativa</i> subsp. <i>sativa</i>	Alfalfa , Lucerne	Animal fodder and forage, soil improvement, human food-sprouts, medicinal-tea	Perennial
<i>Medicago scutellata</i> (L.) Mill.	Snail medic	Animal forage, soil improvement, companion crop	Annual
<i>Medicago truncatula</i> Gaertn.	barrel medic	Animal forage, soil improvement, companion crop	Annual

Alfalfa

Alfalfa, called the "Queen of the Forages," is the fourth most widely grown crop in the United States. Alfalfa hay is used primarily as animal feed for dairy cows but also for horses, beef cattle, sheep, chickens, turkeys and other farm animals.

Alfalfa is recognized as one of the most important crops to U.S. agriculture due to its ability to:

- adapt to a wide range of climates; only crop grown in all 50 states,
- fix up to 200 kg/acre (560 kg/ha) atmospheric nitrogen per year.
- yield large amounts of biomass per acre, the record yield of one acre of alfalfa is 10 tons/acre (22 Mg/ha) without irrigation and 24 tons/acre (54 Mg/ha) with irrigation.
- produce highly nutritious animal feed that contains between 15 and 22% crude protein, as well as an excellent source of vitamins and minerals.
- requires low energy input due to it's ability to grow for several years without reseeding and no nitrogen fertilizer requirements.
- attract bees due to it's sweet nectar for the production of honey during seed multiplication.
- act as a barrier to the spread of diseases and insects from other crops used in rotation.
- improve soil tilth by penetrating soils with large carrot-like tap roots.
- reduce soil erosion through it's perennial growth habit.

In addition to the traditional uses of alfalfa as an animal feed, alfalfa is beginning to be used as a bio-fuel for the production of electricity, bioremediation of soils with high levels of nitrogen, and as a plant factory for the production of industrial enzymes such as peroxidase, alpha-amylase, cellulase, and phytase. Alfalfa is also consumed directly by humans as alfalfa sprouts and alfalfa juice is found in some health food products. Plant molecular biologist use alfalfa as a model crop due to its ability to grow entire plants from single cells and its ability to be genetically transformed thus allowing improvements of the crop through gene insertion.

Production

Alfalfa is grown in all 50 states over a wide range of climatic conditions from fall dormant types in the Midwest and Northeast where winter survival is key, and non-dormant types in the Southwest U.S. where extensive growth is needed from October to March. There are 23.6 million acres of alfalfa cut for hay with an average yield of 3.5 tons per acre. Alfalfa is sometimes grown in mixtures with forage grasses and other legumes. Since the introduction of alfalfa into the U.S. the productivity of forages has dramatically increased (Figure 1). The acreage of all hay harvested including alfalfa is 60.8 million per year.

The production of alfalfa hay on a large scale began around 1919 in the U.S. and has grown from 14 million acres in 1940 to it's highest number of acres of nearly 30 million acres in 1965. Since 1965 the total number of acres of alfalfa has decreased slightly, however, the productivity of alfalfa fields has been steadily increasing (Figure 2). Over the past 10 years the total number of acres has decreased from 26.7 million acres in 1987 to 23.6 million acres in 1997, however, the production has increased from 2.59 tons per acre with a total of 69.3 million tons to 3.47 tons per acre with a total of 82 million tons. The increased productivity of alfalfa over the years can be attributed to better management practices, genetic improvement of cultivars, and the increased use of mechanization for harvesting alfalfa.

Table 2. Alfalfa and All-Hay production by states in 1998¹.

Region	States	Alfalfa Production Tons X 10⁶	Alfalfa Acreage Acres X 10⁶	All Hay Production Tons X 10⁶	All Hay Acreage Acres X 10⁶
East	CT, DE, MA, ME, MD, NC, NH, NJ, NY, PA, RI, VA, VT	4.5	1.7	12.6	6.1
South East	AL, FL, GA, LA, MS, SC	0.0	0.0	7.0	2.9
Central	AR, IL, IN, KY, MI, MO, OH, TN, WV	11.8	3.4	33.2	13.5
North Central	IA, MN, MT, NE, ND, SD, WI	29.9	11.6	41.3	19.6
Great Plains	CO, KS, OK, TX	9.4	2.2	27.0	11.0
Intermountain	ID, UT, WY	8.6	2.2	10.4	3.3
South West	AZ, NM, NV	4.1	0.7	4.8	1.1
North West	CA, OR, WA	11.5	1.9	15.3	3.3
Totals		79.8	23.7	151.6	60.8

¹ Statistics obtained from the 1998 USDA - National Agricultural Statistics Service.

Figure 1.

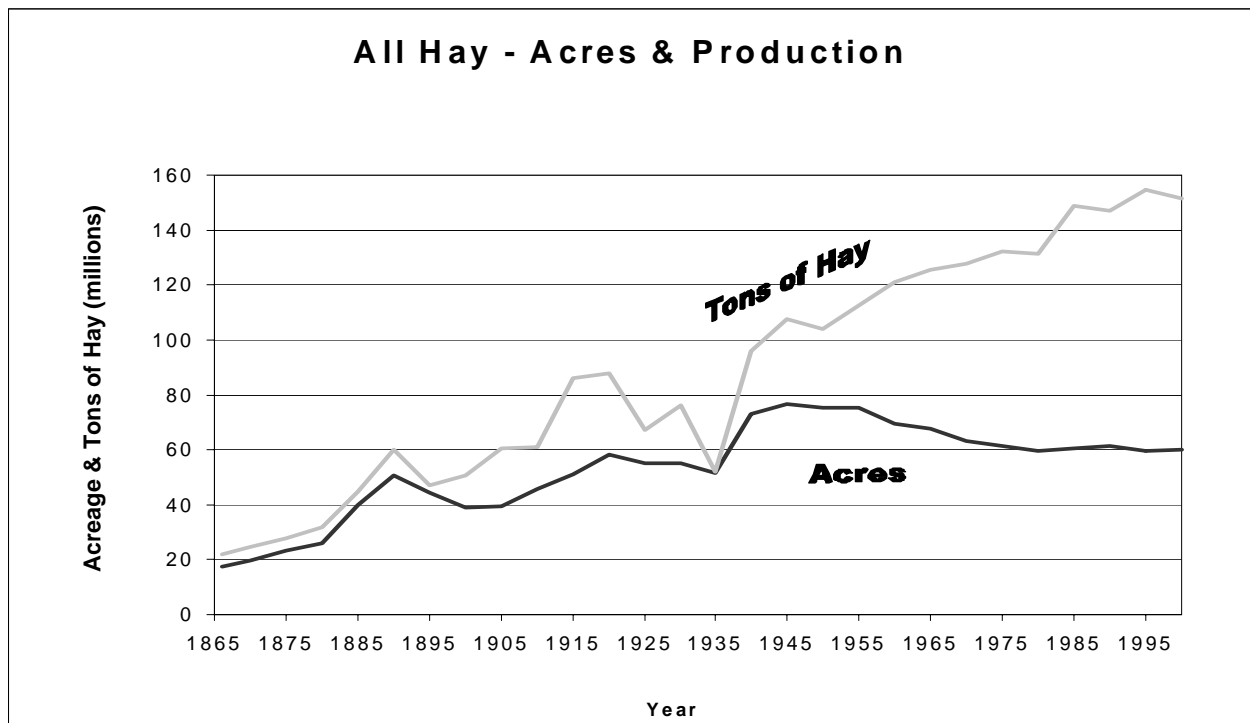
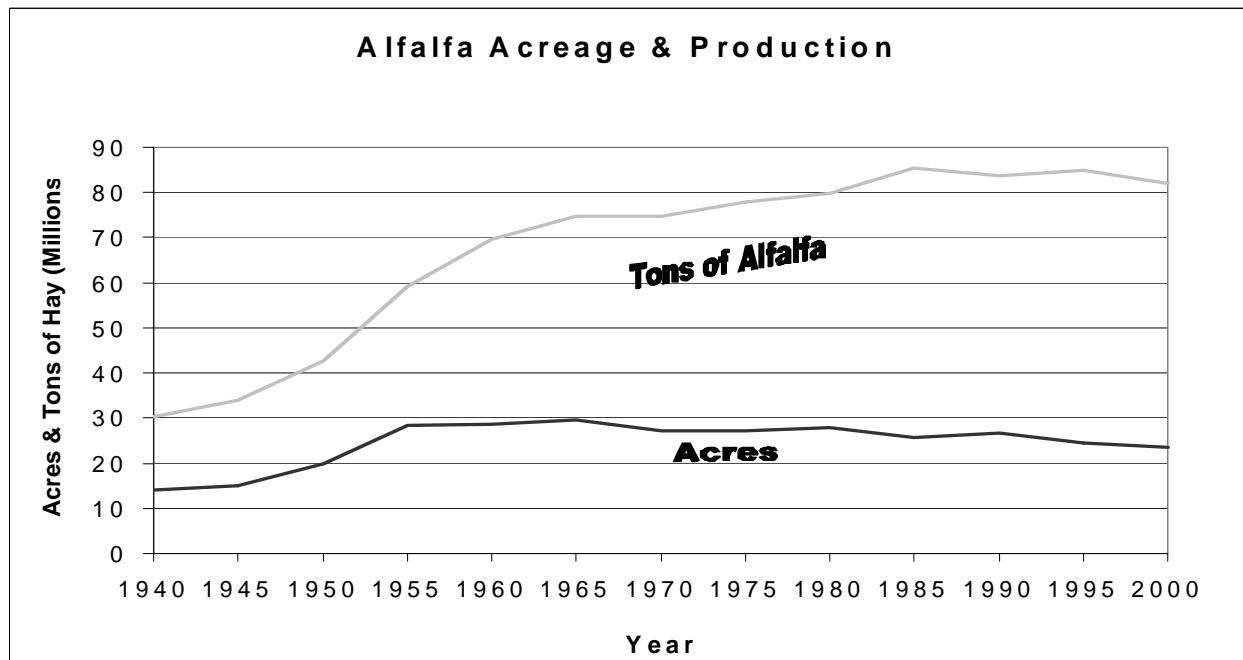


Figure 2.



Highlights of Alfalfa Production (Barnes, et. al, 1988)

- 1850 Introduction of non-dormant alfalfa's from South America into southwestern US (Chilean)
- 1901 First field trials of dormant alfalfa in Minnesota (Grimm)
- 1940 Shortage of winter-hardy seed affected acreage planted.
- 1942 First two bacterial wilt-resistant cultivars are released. (Ranger and Buffalo)
- 1943 Introduction of mechanized hay conditioners.
- 1948 Forced-air hay dryers introduced and wilted silage generally recommended.
- 1953 First high winter-hardy bacterial wilt resistant variety released. (Vernal)
- 1954 First variety of nematode and bacterial wilt resistant variety is released (Lahontan)
- 1955 Direct-cut silage increases in popularity associated with development of new equipment.
- 1957 Release of the first spotted aphid resistant varieties. (Moapa and Zia).
- 1958 Alfalfa seed industry initiates research on alfalfa breeding.
- 1963 Release of Flemish type alfalfa with bacterial wilt resistance. (Saranac)
- 1968 Release of a moderately resistant variety to the alfalfa weevil. (Team)
- 1968 First variety released with pea aphid resistance. (Kanza)
- 1970 Beginning of use of hay and silage preservatives.
- 1970 Release of a variety with resistance to four biotypes of the spotted alfalfa aphid. (Hayden)
- 1972 First variety released with Phytophthora root rot resistance. (Agate)
- 1973 First variety released with Anthracnose resistance (Arc)
- 1976 First variety released with blue aphid resistance (CUF-101)
- 1979 Cultivated alfalfa at the diploid level developed for basic genome studies (CADL)
- 1980 First release of multifoliolate leaf variety. (Multileaf)
- 1981 Release of five varieties with resistance to Verticillium wilt resistance. (Apollo II, DK-135, Trumpetor, WL 316 and Vernema)
- 1982 Ten-ton hay yield barrier broken without irrigation in East Lansing, Michigan
- 1983 USA hay quality standards developed for ADF, DDM, CP and DM..
- 1986 Release of a variety with increased N₂ capabilities for short-term crop rotation. (Nitro)
- 1989 First germplasm release of highly regenerable tissue culture type. (Regen S)
- 1991 Release of a grazing tolerant alfalfa. (Alfagraz)
- 1991 Release of the first high quality alfalfa (WL322HQ)
- 1997 First variety released with potato leafhopper resistance (Trailblazer)

Value

Alfalfa is primarily grown on the farm in which it is consumed, thus it is difficult to estimate the actual value of alfalfa. Alfalfa hay is used primarily as animal feed with an estimated value of \$ 8.1 billion. Alfalfa is also grown in mixtures with forage grasses and other legumes. The estimated value of all hay is \$13.4 billion. The average cost of alfalfa hay is \$102.50 per ton. Alfalfa meal and cubes are exported to other countries with a value of \$49.4 million to the U.S. economy. When the value of animal products estimated at \$132 billion from products such as milk, cheese, butter, meat, wool, etc. is added to the value of hay, the value of alfalfa reaches the \$145 billion level. This far exceeds the combined value of all other high value crops.

Alfalfa seed is primarily grown in the western areas of the U.S. primarily in the states of California, Idaho, Nevada, Oregon, Wyoming, and Washington. The total U.S. production of alfalfa seed in 1999 was 115 million pounds, with an average price of \$190 per 100 pounds of seed. Thus, the estimated value of alfalfa seed in the U.S. is \$218.5 million. A fringe benefit to the production of alfalfa seed is the production of honey from bees. In the U.S., \$147.7 million dollars worth of honey is produced each year.

Alfalfa is also directly consumed by humans in the form of alfalfa sprouts. According to the International Sprout Growers there are approximately \$250 million dollars worth of sprouts sold annually in North America. Alfalfa juice is used in some health food products with an undefined economic value.

Annual Medics

Annual *Medicago* species, referred to as medics, are excellent candidates for use in sustainable agriculture systems such as pastures and cover crops. Medics are native to semiarid areas around the Mediterranean Sea and have long been used in unimproved pastures in the region. Medics are now widely distributed throughout the world, largely in areas with mild, rainy winters and alkaline soils. Medics are among the principal legumes used in the Australian ley farming system, and constitute over 50 million hectares of the Australian agriculture zone (Crawford et al., 1989). Medics have been most successful in cereal-legume crop rotation systems in southern Australia where winter rainfall averages between 250 and 500 mm. The species most widely grown in Australia are: *M. littoralis* Rohde ex Lois., *M. polymorpha* L., *M. rugosa* Desr., *M. scutellata* (L.) Miller, *M. italica* (Miller) Fiori, and *M. truncatula* Gaerth.

What are Medics?

- They are true annuals, they flower, set seed and die within one growing season.
- They all have yellow flowers, are self-pollinating and therefore, bees are not required to produce seed.
- In Australia, the annual medics are used as a winter annual, they germinate in autumn, grow during the winter, set seed and die in spring.
- In the U. S., several species of annual *Medicago* have been naturalized and some occasionally are cultivated. If planted in the spring they would be called summer annuals. Most of the medics complete their life cycle in 65 to 100 days after planting.
- Medics grow rapidly, produce large amounts of biomass with many pods, and can be cut for hay with a single cut yielding as much as 3 tons/acre (5.7 Mg/ha) 60-70 days after planting.
- Medics tend to lodge, thus grazing is a suitable alternative. If grazed high with some stems remaining, regrowth will occur. Medics can cause bloat in ruminant livestock.
- Medics are equal to alfalfa in forage quality with crude protein (CP) ranging from 13 to 26%, neutral detergent fiber (NDF) ranging from 22 to 49%, and acid detergent fiber (ADF) ranging from 19 to 42%.
- Medics have been known to produce up to 200 kg/acre of nitrogen provided effective *Rhizobium* inoculant is used.
- The annual medics are not generally cold hardy, although research is on going to identify cold hardy lines.
- In dry climates, like Australia, southern California, and Arizona; annual medics have the potential to produce hard seed, which can regenerate and become a subsequent crop, thus allowing for good stand persistence from year-to-year. However, in other parts of the country such as the Midwest where there is adequate soil moisture a few hard seed will be produced that can germinate the next growing season.
- Medics have unique, distinct pods with seeds that are usually larger than those of alfalfa. Annual medics generally have greater seedling vigor than alfalfa; especially the large-seeded types.
- Medics appear to be best adapted to soils with a pH of 6 and above; however, some species are more tolerant than alfalfa to low pH soils.
- *Medicago scutellata* (snail medic) and *M. rugosa* (gama medic) have some natural resistance to the alfalfa weevil and potato leafhopper due to hairs on stems, leaves and pods.

Uses of annual medics

The agronomic potential of the medics has been investigated and several different systems have been developed. 1) The medics can be used as permanent pasture; 2) mmedics can be used as a green manure crop due to its ability to fix nitrogen and as a cover crop to prevent soil erosion; 3) annual medics can be used as a companion crop to control weeds, add organic matter, lower soil temperatures and provide fall residue after harvest. Various crops have been utilized including corn, soybeans, small grains, sunflower, squash and some herbs; 4) vineyards and fruit orchards have used annual medics in the alleyways to provide ground cover for soil erosion, an environment for beneficial insects, and a stable track for equipment; and 5) use of annual medics as wildlife habitat for deer, quail and water fowl have been initiated. Medics do have potential uses in sustainable agriculture systems, however, additional research is needed to discover their niche (Bauchan, 1999).

Chapter 2. Genetic Vulnerability in Alfalfa

Introduction

The general impression has been that crop genetic vulnerability was not a large concern due to the autotetraploid nature of *Medicago sativa* subsp. *sativa*, the high level outcrossing, and the diversity of germplasm sources utilized in the development of alfalfa cultivars between 1930 and 1977 (Barnes 1977). Two decades have past since this initial assessment was made, it seems prudent to reassess the present day status of the genetic vulnerability in alfalfa.

Origins of Alfalfa Cultivars (1850 -1977)

Alfalfa originated in Vavilov's "Near Eastern Center"--Asia Minor, Transcaucasia, Iran and Turkistan. In the wild, *M. sativa* and related perennial species are found throughout Eurasia and as far north as Siberia. Alfalfa spread from its center of origin into Europe, North Africa, Arabia and eventually South America with invading armies, explorers, and missionaries as feed for horses and other livestock. In 1736, European colonists brought alfalfa to the eastern U.S. where the crop was referred to by its European name, lucerne. These introductions generally were not successful, except for a few planted on well-drained limestone soils. Alfalfa was well suited to the dry climates and irrigated soils of the western U.S., where it was introduced from Mexico by Spanish missionaries as early as the 1830s. Alfalfa eventually spread eastward to the Intermountain region and the southern Great Plains. Movement into areas with severe winters was limited by the lack of winter hardiness in the primarily Spanish-derived (non-dormant) germplasm. The introduction of four winter-hardy (dormant) types (cv. Grimm, cv. Ontario Variegated, cv. Baltic, and cv. Cossack) from northern Europe to the North Central States between 1858 to 1910 allowed successful alfalfa culture in the colder and more humid areas of the Midwestern and Northeastern U.S.

Before 1925, most alfalfa breeding efforts in North America were directed toward selecting strains that were more winterhardy. During the next 30 years, emphasis was placed on developing cultivars that combined winterhardiness and resistance to bacterial wilt. During the late 1950s, the emphasis was placed on developing cultivars resistant to other diseases and several insect pests (Barnes et al., 1988). Beginning in the 1950's there was an emphasis on breeding alfalfa with multiple pest resistance through recurrent selection breeding schemes. Most modern varieties of commercial alfalfa have moderate to high levels of resistance to the major disease, insect and nematode pests. Scientists belonging to the North American Alfalfa Improvement Conference have developed standardized test procedures for screening alfalfa for the following diseases, insects and nematodes.

Diseases of Alfalfa

- Anthracnose
- Bacterial Wilt
- Downey Mildew
- Lepto Leaf Spot
- Rust
- Spring Black Stem & Crown Rot
- Stemphylium Leaf Spot
- Yellow Leaf Blotch
- Aphanomyces Root Rot
- Common Leaf Spot
- Fusarium wilt
- Phytophthora Root Rot
- Sclerotinia Crown & Root Rot
- Stagnospora Leaf & Crown Rot
- Verticillium Wilt
- Pythium Seed Rot & Damping-off

Insects of Alfalfa

- Alfalfa Weevil
- Potato Leafhopper
- Blue Alfalfa Aphid
- Pea Aphid
- Spotted Alfalfa Aphid

Nematodes of Alfalfa

- Alfalfa Stem Nematode
- Columbia Root-Knot Nematode
- Root-Knot Nematode
- Root-Lesion Nematode

Alfalfa Genetic Vulnerability

In assessing the genetic vulnerability of alfalfa in 1977, Barnes credited nine distinct germplasm sources which were introduced into the U.S. from different regions of the world, in the development of modern alfalfa varieties. They are described in descending order from the most winter hardy (most fall dormant) to the least winter hardy (least fall dormant): ‘Falcata’, ‘Varia’, ‘Turkistan’, ‘Flemish’, ‘Chilean’, ‘Peruvian’, ‘Indian’, and ‘African’ (Barnes et al. 1977). The 1977 report concluded that a trend toward increased genetic diversity in alfalfa cultivars, spurred largely by breeding efforts begun in the 1950s, and the inherent genetic heterogeneity of the species had made alfalfa less vulnerable to catastrophic loss than it was prior to the 1950’s. However, the report also stated that “the broad use of the recognized germplasm in most varieties is cause for concern about future yield improvements” (Barnes 1977).

Current Status of Alfalfa Genetic Vulnerability (1985-2000)

Over the past 15 years (1985-2000) a majority (76%) of the cultivars released have been fall dormant type alfalfas. There are three germplasm sources that have been used extensively, Flemish (31%), Varia (17%) and Turkistan (11%) (Figure 3). Within the dormant alfalfas, most of the selections have been made from two germplasm sources, Flemish (39%) and Varia (21%) (Figure 4). Whereas, within the non-dormant types of alfalfa most of the selections have been made from three germplasm sources, African (34%), Indian (17%), and Turkistan (14%) (Figure 5). Research begun in the 1980's increased the use of plant introductions which were wild perennial relatives of alfalfa. Three perennial species, *M. glandulosa*, *M. glutinosa*, and *M. prostrata* are recognized as the sources of glandular hairs which have been transferred to alfalfa to provide tolerance to the potato leafhopper.

Recommendations:

There is a need to broaden the germplasm base used in alfalfa cultivars released by the alfalfa seed industry. The introduction of wild perennial germplasm and very non-dormant types from the Saudi Arabian Peninsula are examples of the beneficial attributes available.

Enhanced support for germplasm evaluation and enhancement projects would encourage the use of a broader germplasm pool by plant breeders which would lead to more diverse alfalfa cultivars.

Figure 3.

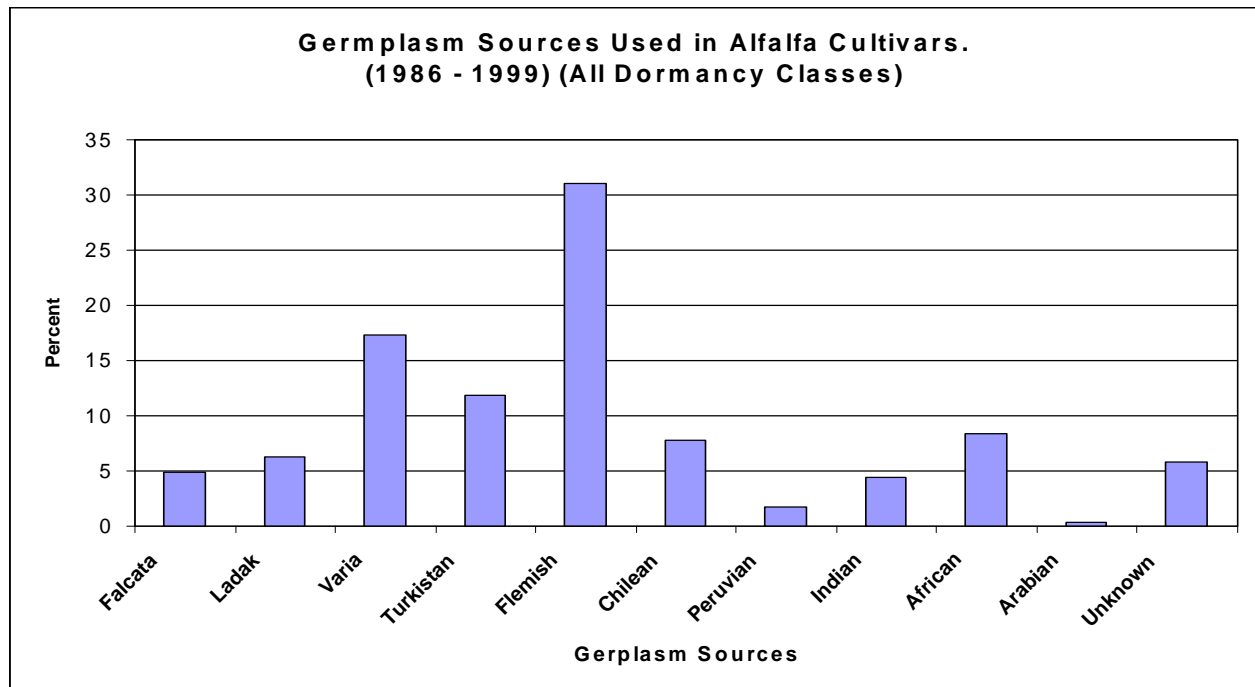


Figure 4.

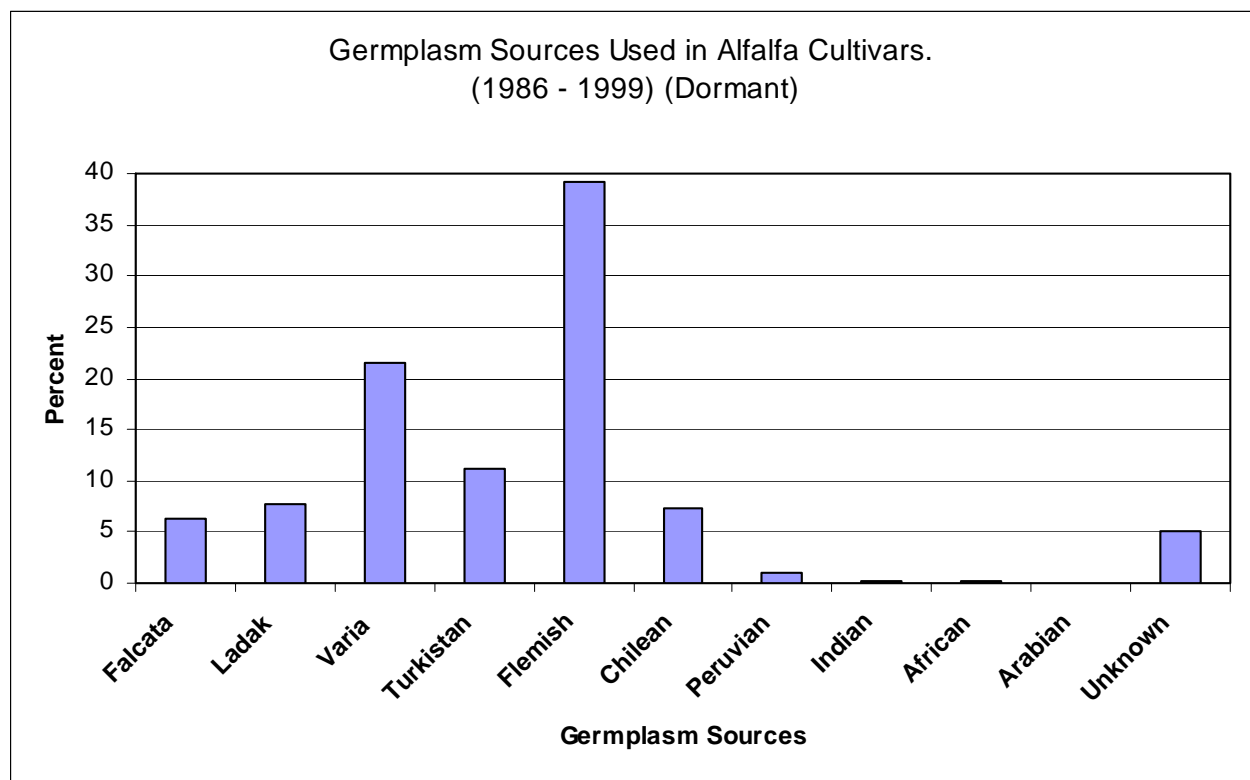
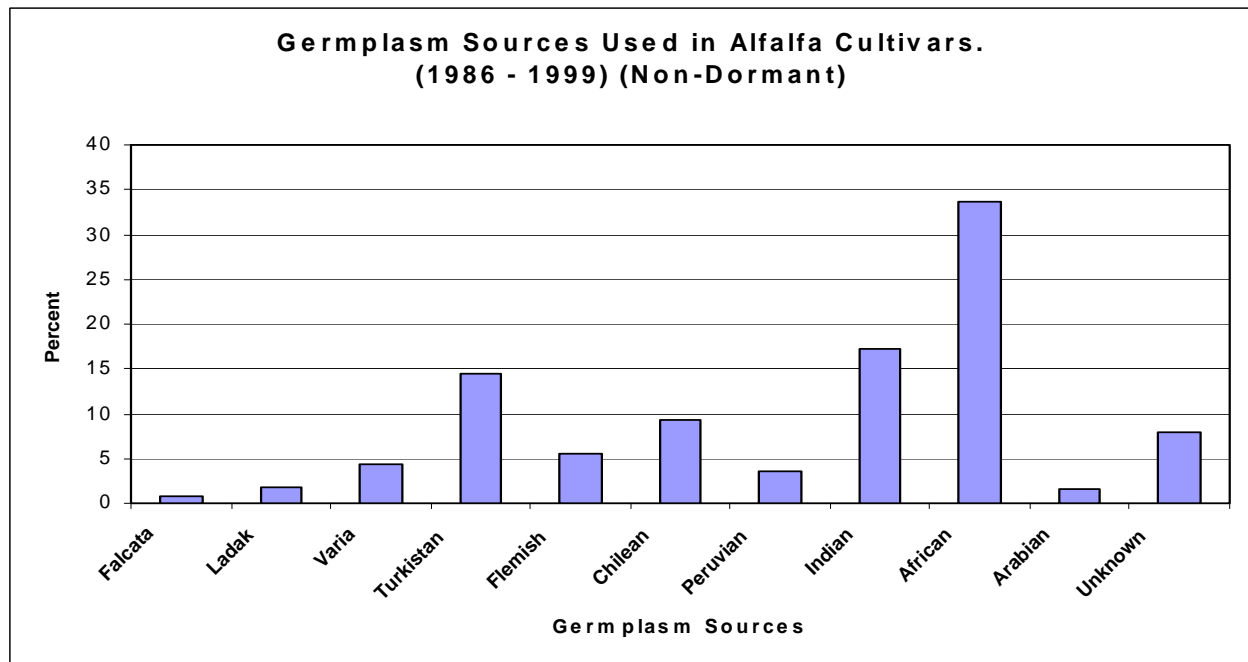


Figure 5.



1997 List of Threatened Alfalfa Relatives and Other Medicago Species

The following *Medicago* species have been listed by the World Conservation Monitoring Center as having a world status of vulnerable or rare:

*M. cancellata**
M. citrina
M. cyrenaea
*M. pironae**
*M. rhodopea**
M. shepardii
M. strasseri

* Note that this list includes three species considered to be wild relatives of cultivated alfalfa.

Chapter 3 Alfalfa Breeding

Introduction

The first breeding efforts in the U.S. date back to the 1850's for non-dormant alfalfas and the early 1900's for dormant types of alfalfa. Most alfalfas grown in the early years were selections of the best materials imported from Europe and Russia. In the Midwest in the early 1900's the emphasis was the selection for winter hardiness. From alfalfa's initial introduction until 1955, about 33 recognized alfalfa cultivars or regional strains were grown in the U.S. and Canada. About half of these were directly introduced from Europe, Asia or the Near East. Between 1958 and 2000, organized alfalfa breeding expanded tremendously and the number of recognized cultivars increased to about 400. From the 1900's until the 1950's almost all of the new alfalfa cultivars were developed by the USDA and public university programs. The goal of these programs was primarily to

increase winter hardiness and the development of bacterial wilt resistance. Starting in the 1950's the alfalfa seed industry began. Since their inception, private alfalfa breeding programs in the U.S. have been responsible for the rapid increase in numbers of recognized alfalfa cultivars. During the period between 1955 and 1960, approximately 20% of cultivars were released from private breeding programs. This proportion increased to more than 95% between 1985 and 1990 and nearly 100% between 1990 and 2000. Private breeding programs increased in number, size and sophistication from about 1965 to 1985. During the late 1980s, competition for market share caused several mergers within the private sector. At present, there are about 8 commercial alfalfa breeding programs with annual research expenditures ranging from nearly \$100,000 with 1.0 scientist-year (SY) to more than \$2 million with five or more SYs. The rate of release of new alfalfa cultivars in the U.S. has increased steadily over the last 90 years. About one cultivar was released every three years between 1901 and 1940; one each year between 1941 and 1960; about 17 per year between 1981 and 1985; about 30 each year between 1985 and 1990; about 60 per year between 1990 and 1995, and about 100 per year between 1996 and 2000.

Increased breeding by industry was accompanied by a reduction in the number of public breeding programs, and by a change in the direction of public research from breeding new cultivars to developing new breeding procedures and improved germplasm (Barnes et al., 1988). The transition from applied alfalfa breeding to more fundamental research within public agencies contributed to the development of several comprehensive research efforts. Examples of research areas include:

- study of the morphology and anatomy of alfalfa as it relates to productivity and persistence.
- improvement of nitrogen fixation.
- improvement in grazing tolerance.
- transfer of genes between ploidy levels and *Medicago* species.
- development of methods to maximize heterosis for yield.
- improvement of forage quality by improving nitrogen concentration in the forage
- elevating insect resistance using novel methods of insect inhibition.
- development of genetic maps using molecular genetic markers.
- description of phenotypic and genetic relationships between different alfalfa germplasm sources.
- the use of tissue culture in selection programs.
- transformation of alfalfa with foreign genes.

More generally, public researchers working with alfalfa also continue to provide valuable knowledge in plant genetics, physiology, and growth, including resistance to biotic and abiotic stresses. Public research in cooperation with industry partners has lead the way in developing potentially new uses of alfalfa for a bio-fuel for the production of electricity, bioremediation of soils with high levels of nitrogen, and as a factory for the production in bioreactors of industrial enzymes.

In discussing research directions for the 1990s and beyond, the Alfalfa Crop Germplasm Committee (ACGC) listed the following as new uses or value-added traits that could be developed in alfalfa (Minutes of the Alfalfa CGC, October 9-10, 1990, Kansas City, MO):

- | | |
|--|-------------------------------|
| ○ Grazing tolerance | ○ Restoration ecology |
| ○ Wildlife adaptation | ○ Root starch for cattle feed |
| ○ Low-input sustainable agriculture systems | ○ Kitty litter |
| ○ Paper pulp production | ○ Green manure |
| ○ Ethanol production | ○ Soil nitrate removal |
| ○ Experimental system for protease inhibitor | ○ Ornamental use |

The increased commitment to alfalfa improvement in the private sector has been associated with a concomitant decline in the number of public alfalfa researchers. The wide acceptance of the best proprietary cultivars by the 1980s confirms the efficacy of private breeding programs, and suggests

that an appropriate balance has been reached between fundamental public research and more applied private research. However, the balance between public and private programs may now be in danger because of reductions in support for public alfalfa research programs. The termination of a number of public alfalfa breeding and genetics programs also means that many breeder's germplasm collections may be subject to loss.

Alfalfa Crop Germplasm Committee (ACAC)

The Alfalfa Crop Germplasm Committee consists of 21 members representing federal, state and private interests. There are representatives of several scientific disciplines including: breeding, genetics, cytogenetics, entomology, pathology, physiology, microbiology, and molecular biology. In addition there is geographic representation of breeders from the major alfalfa growing areas of the U.S.

The Alfalfa Crop Germplasm Committee address critical issues facing the National Plant Germplasm System (NPGS) especially related to the genus *Medicago*. These include:

1. Identify gaps in the *Medicago* collection and develop proposals to fill these gaps through germplasm exchange and collaborative collecting trips.
2. Assist in germplasm regeneration projects.
3. Prioritize traits for evaluation and develop proposals to implement the evaluations.
4. Develop germplasm enhancement proposals to implement the utilization of germplasm.
5. Assist the *Medicago* curator in identifying duplication in the collections. Assist the *Medicago* curator and GRIN personnel in correcting passport data and ensuring that standardized, accurate and useful information is entered into the GRIN database.
6. Identify closed-out programs and other germplasm collections in danger of being lost and developing plans to rescue the important material in these programs.
7. Working with quarantine officials to identify and ensure new techniques for pathogen identification which will assist in the expeditious release of plant germplasm.
8. Evaluating the potential benefits and problems associated with the development and use of core germplasm subsets.
9. Maintaining current reports on the status of alfalfa and related species for the Congress, ARS National Program Staff and Administrators, State administrators and other key individuals involved with the NPGS.
10. Communicating germplasm recommendations to user groups such as federal, state, and industry researchers, and the international community.

Formation

The Alfalfa Improvement Conference (now the North American Alfalfa Improvement Conference - NAAIC) has since its inception in 1934 been concerned with alfalfa germplasm. In the early days of the conference there was need for germplasm exchange among scientists, seed production of experimental strains in the western U.S., development of a standardized system of note taking, and testing alfalfa cultivars for disease and insect pests, yield, and persistence. Several committees grew out of the Alfalfa Improvement Conference which were related to germplasm, they included: variety testing, release of new cultivars, available breeding lines, lists of cultivar descriptors, development of standardized tests for characterizing pest of alfalfa, and the National Alfalfa Variety Review Board. The Alfalfa Crop Germplasm Committee was formerly organized in 1969 as the Germplasm Committee of the Alfalfa Improvement Conference.

Accomplishments of the Alfalfa Crop Germplasm Committee.

- 1970 Developed a program for carefully controlled seed increase of the *Medicago* collection using isolation cages. Previous to this the germplasm collection was increased using open pollination.
- 1977 Developed the statement on the genetic vulnerability of alfalfa.
- 1977 Developed a plan to produce seven (7) regional pools of alfalfa germplasm as a means of preserving germplasm and evaluating germplasm for suitability of growth in a region.
- 1978 Alfalfa is included as one of the first crops to be included in the Germplasm Resources Information Project (now the Germplasm Resources Information Network - GRIN).
- 1979 Alfalfa seed increase was initiated in Reno, Nevada of at least one pound per accession under isolation cages.
- 1980 Plant explorations were undertaken in Chile, Bolivia, Peru, Ecuador, Turkey and old stands of alfalfa in the U.S. and Canada.
- 1981 Evaluation of the germplasm collection was initiated using standardized tests for 9 diseases, 5 insects, and 3 nematodes. Data was entered into GRIN.
- 1982 Germplasm collection trips were successful in collecting 561 accessions in the USSR and Morocco.
- 1984 The Karl Lesins Collection from Canada composed of over 3000 annual and 600 perennial accessions were added to the U.S. germplasm collection.
- 1985 Germplasm enhancement program developed.
- 1986 Seed increase project is transferred to Prosser, Washington.
- 1987 Committee membership expanded beyond only plant breeders to include representation of various disciplines including, cytogenetics, entomology, pathology, microbiology, and molecular biology.
- 1987 Transfer of the *Medicago* collection from Ames, IA to Pullman, WA.
- 1988 Annual medic seed increase initiated in Riverside, CA.
- 1990 Evaluations of the germplasm collection using standardized tests for agronomic traits such as fall dormancy, winter and frost injury, forage quality, salt tolerance, and tolerance to acid soils. Data entered into GRIN.
- 1991 Core subsets were developed for perennial and annual *Medicago* species as well as *Rhizobium*. Data are entered into GRIN.
- 1993 *Medicago* Germplasm Newsletter published.
- 1994 Majority of the germplasm collection has been evaluated for 35 agronomic and pest resistance traits. Data are available in GRIN.
- 1995 Curator of the *Medicago* collection is employed.
- 1996 Plant explorations were undertaken in Mongolia and North Caucasus Mountains of Russia.
- 1997 Seed increase of alfalfa collection under caged conditions largely complete. Increase of annual medic collection largely completed.
- 1998 Passport and evaluation data in GRIN was updated and duplicate accessions identified.

Table 3. List of Institutions and locations where germplasm research is taking place.

Institution	Location	Agronomic	Physio- logical	Diseases	Insects	Nematodes	Bio-Tech	Pre- breeding	Cultivar Develop- ment
USDA-ARS	Manhattan, KS	X	X	X			X	X	
USDA-ARS	Beltsville, MD	X	X	X			X	X	
USDA-ARS	St. Paul, MN	X	X	X		X	X	X	
USDA-ARS	Stoneville, MS	X		X				X	
USDA-ARS	Logan, UT	X	X				X	X	
USDA-ARS	Prosser, WA	X		X		X	X	X	
University of Arizona	Tucson, AZ	X	X				X	X	
University of California	Davis, CA	X	X	X	X		X	X	X
University of Georgia	Athens, GA	X	X				X	X	X
Iowa State University	Ames, IA	X					X	X	X
Purdue University	West Lafayette, IN	X	X		X		X		
Kansas State University	Manhattan, KS	X	X					X	
University of Minnesota	St. Paul, MN	X							
New Mexico State University	Las Cruces, NM	X	X				X		
Cornell University	Ithaca, NY	X						X	X

Ohio State University	Columbus, OH			X					
Oklahoma State University	Stillwater, OK	X			X				
University of Wisconsin	Madison, WI	X		X			X	X	
University of Wyoming	Laramie, WY	X					X	X	
ABI Alfalfa	Ames, IA	X	X	X	X	X			X
Cal/West	Woodland, CA	X	X	X	X	X			X
Dairyland Seeds	Clinton, WI	X	X	X	X	X			X
Forage Genetics	West Salem, WI	X	X	X	X	X	X	X	X
Great Plains	Apex, NC	X	X	X	X	X			X
Pioneer Hi-Bred	Johnston, IA	X	X	X	X	X	X	X	X
WL Research	Evansville, WI	X	X	X	X	X	X		X

Chapter 4. National Plant Germplasm System *Medicago* Germplasm Collection

Introduction

The National Plant Germplasm System Collection of *Medicago* germplasm is currently located at the Western Regional PI Station in Pullman, Washington. Stephanie Greene is the curator of the collection who can be contacted at the USDA-ARS, Washington State University Irrigated Agricultural Research and Extension Center, Prosser, WA 99350 Phone: (509) 786-9265, FAX: (509) 786-9370, e-mail: sgreene@ars-grin.gov. The current collection contains a total of 7,554 accessions with 4,108 perennial accessions representing 30 species and 3,446 accessions representing 60 annual species (Appendix 1).

History

The beginning of the U.S. *Medicago* germplasm collection traces back to 1898. Accessions were collected directly in the field by plant explorers and scientists or obtained from agricultural institutes, seed companies and U.S. Embassy agricultural attaches. About 65 collection trips that focused on alfalfa germplasm have been made in the last 100 years (Table 4). The majority of accessions that are available today were received after the 1940s. During the 1950s, 60s and 70s the collection grew by an average of 1400 accessions per year. During the eighties, more than 4200 accessions were added to the collection. During the nineties, the collection grew by 1400 accessions. The collection currently contains over 7000 accessions representing 90 species from 94 countries.

Table 4. Alfalfa germplasm collection trips made by U.S. scientists in the last 100 years

YEAR	COLLECTOR	COUNTRY
1898	N.E. Hansen	Russia
1899	Lathrop, D. Fairchild	Peru, Argentina
1899	C. Sprenger	Italy
1906	N.E. Hansen	Europe, Siberia, E. Asia
1909	N.E. Hansen	Central Asia
1910	F. Meyer	Russia
1924	H.L. Westover	South America
1925	D. Fairchild	North Africa
1925	P.H. Dorsett	China
1926	D. Fairchild	Northern Africa
1929	H.L. Westover	Russia
1930	H.L. Westover	Turkestan, Europe
1930	P.H. Dorsett, W.J. Morse	China
1936	H.L. Westover; F.L. Wellman	Turkey; Iran
1937	W. Koelz	Afghanistan
1939	W. Koelz	Afghanistan
1940	W. Koelz	Iran
1948	J.R. Harlan	Turkey
1951	S.P. Kohli	India
1951	W.A. Archer	Ethiopia
1952	R.K. Godfrey	Turkey
1953	H.S. Gentry	Afghanistan
1954	E.E. Smith	Afghanistan
1954	H.S. Gentry and E.E. Smith	Pakistan
1955	H.S. Gentry	Iran

1955	J.L. Stephens	E.Africa
1956	H.S. Gentry and H.A. Schoth	Europe
1958	H.S. Gentry and H.A. Schoth	Dinaric Alps
1968	W.H. Skarda	USSR
1972	D.R. Dewey	Iran
1972	J.L. Schwendiman	Turkey, Iran
1973	I. Forbes Jr., J.S. Gladstone	Morocco, Spain
1975	D.R. Dewey	Iran
1975	W.R. Lanford and B.L. Burson	Brazil
1976	P. K. Knowles	Afghanistan
1977	D. R. Dewey	USSR
1977	R.R. Smith; W.R. Landford	Greece, Italy; USSR
1978	D.R. Dewey and A.P. Plummer	USSR
1978	G.B. Polignando	Algeria
1980	M.D. Rumbaugh	N W. America
1980	W.L. Graves	North Africa
1981	J.H. Elgin; W.F. Lehman, M.D. Rumbaugh	Turkey; Bolivia, Peru, Ecuador
1982	M.D. Rumbaugh	Morocco,USSR
1983	M.D. Rumbaugh	Morocco
1984	M. D. Rumbaugh and N.L.Taylor	Romania
1986	D.A. Johnson	Pakistan,Nepal
1987	W. Kaiser	Spain
1988	K. Asay; N.L. Taylor,	USSR; Yugoslavia
1989	F.J. Muehlbauer, W.J. Kaiser and C.R. Sperling	Syria and Turkey
1989	D. Dewey and K. B. Jensen	Novosibirsk and Altai Mountains
1989	P. Beuselink, W. Graves, and J. Kirkbride Jr.	Morocco
1990	K.F.M. Reed and B.S. Dear	USSR
1991	D. A. Johnson and M.D. Rumbaugh	Xinjiniang, China
1991	L. Teuber, D. Mowry , T.A. Campbell	Mongolia
1993	G.A. Pederson and K.H. Quesenberry	S.Bulgaria
1993	D.A. Johnson and K.B. Jensen	N. Xinjiang, China
1993	G.R.Smith and C.Hoveland	Georgia
1994	D. A. Johnson and D. P. Sheehy	Mongolia
1994	W.L.Graves and P.J.Cunningham	N. Africa
1995	A.A. Afonin, M.D. Rumbaugh, W. Graves	Caucasus Mountains, S. Russia
1996	T. Lumpkin	Kazakhstan,Kirgizia, and Uzbekistan
1996	W.J. Kaiser and R. Hannan	Bulgaria
1996	D.A.Johnson and D.P.Sheehy	China
1997	D. A. Johnson, L.K. Holzworth,T.A.Campbell, J.D.	China
1998	D. A. Johnson, D. P. Sheehy, M.E. Majerus, S.R.Winslow	Mongolia
1999	R.C. Johnson ,H. Bockelman	Ukraine

In the early part of the century, alfalfa germplasm was obtained and selectively distributed by the USDA Office of Foreign Plant Introduction, in Beltsville, Maryland. In the 1940's the Regional Plant Introduction Stations were established to increase, evaluate, and distribute plant introductions. As plant introductions came into the United States, they were distributed to the four Regional PI Stations for evaluation. By 1949 the North Central Regional Plant Introduction Station (NCRPIS) in Ames, Iowa became the main site responsible for the increase, evaluation and distribution of alfalfa plant introductions. In 1971, the USDA National Plant Germplasm System was established,

with the broader mission of preserving and encouraging the use of germplasm as a guard against genetic erosion and crop vulnerability. In 1977, NCRPIS set up a specific cooperative agreement with University of Nevada, Reno, to increase the alfalfa accessions under caged conditions. This project was terminated in 1986. In 1987 the alfalfa collection was transferred from Ames, IA to the Western Regional Plant Introduction Station (WPIS), in Pullman, WA. A Specific Cooperative Agreement was set up with Washington State University, Prosser to increase the alfalfa accessions under caged conditions.

Efforts in the last two decades have focused on systematically regenerating the entire collection using controlled pollination, since prior seed increases had been contaminated by open pollination. A second important emphasis in the last two decades has been a germplasm evaluation program directed by the ACGC and administered by WRPIS. The objective of the ACGC has been to obtain a set of standard descriptors on newly regenerated material, since previous evaluations had been conducted on accessions representing open pollinated populations. Each year evaluation proposals were reviewed and funded based on priorities determined by the ACGC.

By 1988, the USDA alfalfa CRIS project was funded at \$160,000. This supported regeneration efforts at Prosser, WA, in the form of supplies and field labor. It also provided support for the germplasm evaluation program managed by the ACGC. In 1993, an alfalfa curator was hired to manage the collection, which had previously been managed by the Research Agronomist, Dr. R.C. Johnson, at the Western Regional PI Station. Whereas the salaries of R.C. Johnson and R. Peaden had not been supported by the Alfalfa CRIS, the alfalfa curator's salary was. In 1995, the *Trifolium* (5000 accessions) and *Lotus* (700 accessions) collections were transferred from the Northeastern Regional Plant Introduction Station in Geneva, New York, to WRPIS, in Pullman. The Alfalfa CRIS was rewritten to include the clover and trefoil collections, but no additional funds accompanied the collection transfer. With a flat budget and expanded responsibilities, funds have focused exclusively on regeneration activities. The ACGC-directed germplasm evaluation program was ended in 1997. One of the final projects of the ACGC evaluation program was to develop core subsets of the annual and perennial species.

Core Subsets

Introduction

A core subset is a small subset of accessions from the entire collection that: 1) contains most of the genetic variability that exists in the overall collection, 2) identifies duplications in the collection, 3) recognizes where there is a lack of germplasm representing a species or geographic location, 4) simplifies evaluation especially for difficult and/or expensive traits, and 5) increases the utilization of the collection. There are currently two (2) core subsets related to *Medicago*, one each for the perennial and annual *Medicago* species. In addition, development of a *Rhizobium meliloti* core collection is currently under development.

Perennial Medicago Core Subset

The perennial *Medicago* core subset was developed by Drs. Daniel Basigalup, Don Barnes (USDA/ARS) and Robert Stucker at the University of Minnesota in 1989 through 1991. The core subset was selected from 1105 accessions representing 47 different countries and 4 species (*M. sativa* including the 6 subspecies (ssp. *sativa*, ssp. *varia*, ssp. *falcata*, ssp. *coerulea*, ssp. *falcata*, and ssp. *ambigua*), *M. cancellata*, *M. platycarpa*, and *M. ruthenica*). The 1105 accessions were assembled into 18 different groups based on the original collection site and their geographic proximity, i.e. one group was accessions collected in Hungary, Greece, Italy and Romania. These accessions were evaluated at Rosemount, Minnesota in 1989 for 21 morphological traits and 8 quality traits. Additional evaluation data on these accessions were obtained from the GRIN

system including information on the resistance to 10 diseases and 5 insects, and salt and acid tolerance. Selection of the core was made by using cluster analysis to designate the most diverse accessions within each geographic proximity group. The core subset has 200 accessions (~18% of the evaluated accessions) with representatives from each species and geographic area (Basigalup, et. al., 1995).

Recommendations:

1. *The perennial Medicago core subset should receive high priority for increase, maintenance and evaluation of new traits, e.g. disease and insect resistance, quality traits, bypass proteins, water use efficiency, etc.*
2. *Specialty collections should be evaluated such as the highly non-dormant alfalfas (Steve Smith), the Chinese M. ruthenica collection (Austin Campbell and Larry Teuber), and the VIR collection in Russia. A core should be developed from this material by evaluating these collections in comparison to existing perennial Medicago core entries from the same geographic proximity group or species. Based on these evaluations the perennial Medicago core subset should be increased to represent the same frequency as the original core, i.e. about 15 - 20% of the specialty collection.*
3. *Additional germplasm collections are needed for secondary species ("wild" non-sativa species) from southeast China (Himalayan Mountains), southwest China, and southern Russia (Caucases Mountains).*
4. *The core subset should be expanded as the number of accessions in the collection markedly increases. The new entries should be grown out and evaluated in comparison to existing perennial Medicago core entries from the same geographic proximity group or species.*

Annual Medic Core Subset

The annual *Medicago* core subset was developed by Drs. Noa Diwan, Gary Bauchan (USDA-ARS, Beltsville, MD) and Marla McIntosh at the University of Maryland, College Park, Maryland from 1990 through 1992. The medic core subset was selected from 1220 accessions representing 34 annual *Medicago* species. These accessions were evaluated in Beltsville, Maryland in 1990 through 1992 for 15 morphological and agronomic traits (days to flower, days to full pod, biomass production within a species, biomass production among species, plant height, plant width (spread), growth habit, middle leaflet length, middle leaflet width, third internode length, pod production, pod spyness, number of flowers/raceme, number of pods/raceme, and seed size). All of these data are available in the GRIN system. The selection for the core was made using cluster analysis with the final selection of core accessions within each cluster based on geographic location. The annual medic core subset has 211 accessions (~17% of the evaluated accessions) and 34 annual *Medicago* species are represented (Diwan, et al., 1994). The core collection was evaluated in six (6) locations across the U. S. (Athens, GA, Beltsville, MD, Ithaca, NY, Logan, UT, St. Paul, MN, and Tucson, AZ) for 11 traits (days to flower, days to full pod stage, full pod stage, growth habit, biomass within species, biomass among species, pod production, pod spyness, plant height, plant width, and winter hardiness). All of these data are available in the GRIN system.

Recommendations:

1. *The annual Medicago core subset should receive high priority over the remaining collection for increase, maintenance and evaluation of additional traits especially freezing tolerance, disease and insect resistance.*

2. The annual Medicago core subset should be evaluated for freezing tolerance, either to extend the autumn growth in the Mid-West or for over-wintering in milder climates such as California and south Texas.
3. The annual Medicago core subset should be evaluated for disease resistance such as Anthracnose, Phytophthora, Fusarium, Rhizoctonia, Pythium, Leptosphaerulina, Phoma, and Rust. These diseases have been seen on some of the annual medics which have been grown in the U.S. If the annual medics are to be grown in the U. S., we need to know in advance if there are any disease problems.
4. The annual Medicago core subset should be evaluated for insect resistance to aphids and leafhoppers. These insects have been seen on some of the annual medics which have been grown in the U. S. If the annual medics are to be grown in the U. S., we need to know in advance if there are any insect problems.
5. The annual Medicago core subset should be evaluated for isozyme and molecular marker traits such as RFLPs, RAPDs, and microsatellites to further evaluate the collection for redundancies. The annual Medicago species are all self-pollinating and are thus relatively uniform within an accession.
6. No new collection trips are needed for the annual medics. There is an extremely large germplasm collection of 23,000 accessions located in Adelaide, South Australia. If additional accessions are needed of a particular species they can be easily obtained from this collection.

Rhizobium Collection

A comprehensive collection of *R. meliloti* isolates has been developed at St. Paul, Minnesota. It consists of a domestic collection (U. S. and Canadian) that was initiated by W. R. Ellis in 1979. That collection was formed by requesting alfalfa scientists in nearly all states and provinces to send nodules collected from up to four of the best adapted cultivars. We requested that whenever possible nodules from Saranac and Saranac AR be included in each locations' collection. An international collection was initiated by S. E. Viteri in 1987 using the same procedures of contacting alfalfa scientists. In addition, collections from Western Australia (Howieson), Nepal and Pakistan (Rumbaugh), Turkey (Elgin), and the USDA (Beltsville, VanBerkum) were also included in the Minnesota collection. Each isolate was isolated from an individual nodule, increased in culture, inoculated onto plants from a broadbased *Medicago sativa* gene pool (BIC-7), and then reisolated from an effective nodule. All isolates were further tested to insure that they were *R. meliloti*.

The entire collection was evaluated for reaction to 13 antibiotic and 14 bacteriophages. By considering these evaluations it was possible to eliminate similar isolates from each original collection location. For example, seventeen isolates from two Georgia fields were tested for bacteriophage reaction. Only two isolates had a similar profile of reactions. Similarly the two isolates were reconfirmed with the antibiotic evaluation. After all duplicate isolates were discarded the collection consisted of 641 isolates. All of these isolates were deposited in 1990 in the World *Rhizobium* Culture Collection in the Soybean and Alfalfa Research Lab., Beltsville, Maryland, Peter VanBerkum, curator. All of the isolates have been assigned a USDA accession number and have been placed in cold storage for long-term preservation.

Recommendations:

1. A core subset needs to be developed using the data available in the GRIN system utilizing cluster analysis to identify subgroups. The final core subset should consist of between 65 to 75 isolates.
2. Once the core subset is established, the core should be evaluated for tolerance to low pH conditions. The isolates should be evaluated for both free living tolerance and symbiotic tolerance to low soil pH conditions.
3. Once the core subset is established, a determination of the symbiotic effectiveness of the core collection on the *Medicago* species core subsets should be conducted.
4. Any additional *Rhizobium* isolates should be sent to Dr. VanBerkum. Once a significant number of isolates has been added to the collection they should be evaluated in comparison to the core subset.
5. Evaluate the collection for *Rhizobium* strains by plant genotype interaction. There is a reasonable amount of information from other *Rhizobia* that there can be a substantial strain by cultivar origin. Since we recently identified strains of *Rhizobia* that will infect both alfalfa and soybean in China it seems likely that we could expect some significant interactions between genotypes from diverse locations. In a study that Don Barnes and I reported on we noticed some marked difference in the *Rhizobia* obtained from the same genotypes in different locations.

Assessing the Collection for Current Gaps

The technical advances of molecular genetics, and interest in developing more sustainable agricultural systems, coupled with the continued rise in loss of biodiversity, have prompt us to closely examine the current contents of the collection to determine if it meets the needs of a broad array of users and conservation objectives. The Convention on Biological Diversity necessitates that this examination occur within the context that nations have sovereign rights over the genetic resources that originate within their boundaries.

General Recommendations:

- Acquisition must be undertaken following guidelines arising from the Convention on Biological Diversity
- Gaps in the U.S. collection can frequently be filled by other collections around the world.
- The ACGC needs to establish guidelines that define when efforts warrant the acquisition of germplasm for inclusion in the U.S. collection, versus making use of germplasm that is available from other collections.

Species Related to Alfalfa

Primary Gene Pool

- Cultivated, naturalized and wild forms of *Medicago sativa* sbsp. *sativa*
 - Modern and obsolete cultivars
 - Landraces
 - Ecotypes

- *M. sativa* ssp. *falcata*
- *M. sativa* nssp. *varia*
- *M. sativa* ssp. *glutinosa*
- *M. prostrata*

Secondary Gene Pool

- *M. sativa* nssp. *tunetana*
- *M. sativa* ssp. *caerulea*
- *M. sativa* ssp. *glomerata*
- *M. papillosa*
- *M. saxatilis*

Tertiary Gene Pool

-
- *M. cancellata*
- *M. daghestanica*
- *M. dzhawakhetica*

Primary Gene Pool (*Medicago sativa* subsp. *sativa*)

Obsolete and current cultivars

The following table indicates the number of alfalfa cultivars maintained in the NPGS collection. These accessions have been verified as registered cultivars that were directly donated by the country that released them.

Table 5. Number of cultivars and country of origin represented in the NPGS collection.

Country	Number	Country	Number
Afghanistan	1	Jordan	1
Algeria	2	Kazakhstan	8
Argentina	13	Kenya	2
Australia	12	Kyrgyzstan	3
Azerbaijan	2	Lebanon	1
Belarus	3	Mexico	8
Belgium	1	Mongolia	12
Brazil	2	Morocco	1
Canada	9	New Zealand	2
Chile	2	Peru	6
China	62	Poland	10
Czech Republic	1	Romania	9
Czechoslovakia	7	Russian Federation	51
Denmark	4	South Africa	4
Ecuador	1	Spain	15
Estonia	2	Sweden	7
Finland	1	Tajikistan	2
France	50	Turkey	3
Germany	12	Ukraine	11
Hungary	6	United Kingdom	6
India	4	United States	47

Iran	1	Uruguay	1
Israel	1	Uzbekistan	11
Italy	13	Yemen	2
Japan	7	Yugoslavia	6

United States

Should the NPGS collection preserve and distribute alfalfa cultivars that were developed in the United States but no longer commercially available? Currently 47 U.S. cultivars are preserved in the active collection. The National Seed Storage Laboratory (NSSL) preserves seed samples of cultivars (and breeding lines and germplasm) that have undergone *Crop Science* registration (CSR). There are currently over 350 alfalfa cultivars, breeding lines and germplasms that are stored at NSSL. Most of this material has not been assigned PI numbers nor is considered to be part of the active collection that is maintained and distributed from Washington State. Over 800 cultivars have been registered since the inception of the National Alfalfa Variety Review Board, with 100 being registered each year between 1996 and 2000. Obviously, not all these cultivars are CSR and have seed stored at the NSSL. Policy needs to be established regarding the preservation and distribution of obsolete US alfalfa cultivars in the NPGS.

Recommendations:

Given that funding can keep pace with a growing collection, the NPGS active collection should support the preservation of US cultivars that are no longer available through commercial sources. Although alfalfa is the fourth most widely grown crop in the US, NGO interest in conserving heirloom alfalfa varieties is poor to non-existent. Efforts need to be made to secure representatives of older varieties before seed stocks deteriorate. Materials registered with Crop Science should enter the active collection when the developer no longer distributes seed or it is not available from commercial sources. As non-CSR material is commercially retired, developers are encouraged to enter the obsolete cultivar into the NPGS collection. When PVP expires, cultivars should become part of the active collection, as PVP suggests the material is of exceptional value. At current levels of funding, a policy that preserves US cultivars as they are retired from commercial production cannot be implemented.

Cultivars of other nations

Recommendations

- *US users can make use of collections held in other institutes. An excellent source of information regarding the contents and availability of germplasm collections is the IPGRI Directory of Germplasm Collections <http://www.cgiar.org/IPGRI/doc/dbintro.htm>)*
- *Areas where collection and preservation of cultivars may be beneficial to both the United States (based on limited representation in US collection) and corresponding countries (based on limited support for ex situ conservation, and lack of availability) include the Middle East, South Africa, and Mexico.*
- *Nondormant alfalfa continues to be underrepresented in the US collection. Efforts should be made to acquire the alfalfa germplasm collected by ICARDA during the 1970s, 1980s and 1990s.*

Alfalfa Landraces, Primitive Cultivars

As of 1998, the NPGS collection contained about 600 alfalfa accessions classified as landrace

material, and 300 accessions that were classified as cultivated (i.e. data insufficient to make a determination if landrace or cultivar). Figures 6 and 7 indicate the geographic distribution of accessions that had sufficient locality information to obtain map coordinates of collection sites.

Figure 6. Distribution of landrace germplasm held in the NPGS collection.

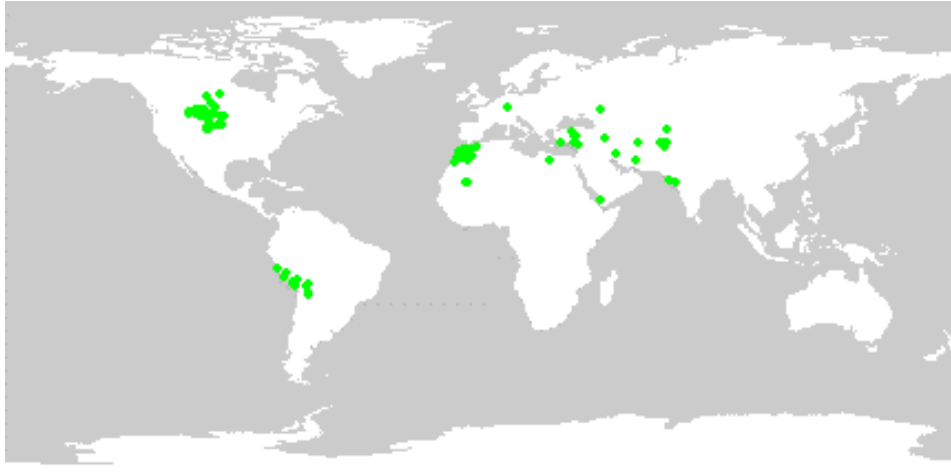
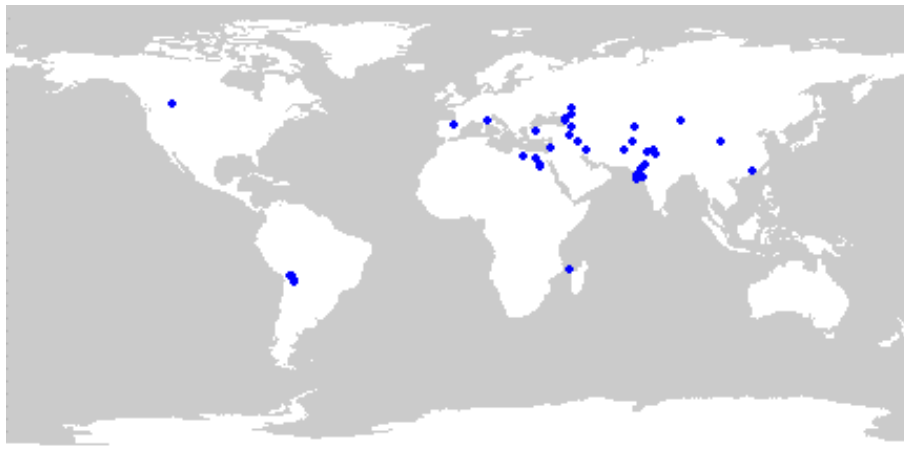


Figure 7. Distribution of cultivated germplasm held in the US collection.



Recommendations

- U.S users can make use of collections held in other institutes. Many of the European nations contain extensive collections of local landraces and primitive cultivars. For example, the European perennial *Medicago* database contains passport data on 2888 accessions of 32 taxa, including 505 land race accessions stored in 23 European genebanks <http://www.cgiar.org/ecpgr/platform/Crops/Medicago.htm> .
- Priority geographic areas recognized by the ACGC in 1988, and not yet collected include the Himalayan region of India, south and northeast regions of China, Sudan, Egypt and the Arabian Peninsula. Other geographic areas with limited representation of landrace materials include South Africa, Northern Kazakhstan, and adjacent Central Asian countries (i.e. Kyrgistan, Uzbekistan, Afghanistan), Iran and Iraq.

- Ⓒ *Past CGC reports have placed emphasis on cultivated alfalfa that is adapted to acid soils and saline soils. There is increasing interest in developing alfalfa with increased water use efficiency, so drought tolerant germplasm would also be valuable to collect*

Wild *Medicago sativa* subsp. *sativa*

As of 1998, the NPGS collection contained 369 accessions of alfalfa (*Medicago sativa* subsp. *sativa*) classified as wild germplasm. Figure 8 indicates the geographic distribution of accessions that had sufficient locality information to obtain map coordinates of collection sites.

Figure 8. Distribution of wild *Medicago sativa* subsp. *sativa* germplasm held in the NPGS collection.



Recommendations:

- Ⓒ *Considering that the center of origin for *Medicago sativa* subsp. *sativa* centers around Iran, Iraq, Azerbaijan and Turkmenistan, explorations to collect wild material there, and in adjacent areas such as Southern Kazakhstan, Uzbekistan, Afghanistan, and Tajikistan would be valuable, since wild-type *M. sativa* subsp. *sativa* from this region are under-represented in the US collection.*
- *Efforts should be made to acquire germplasm collected during the 1980's and 1990's through IPGRI-funded trips into Central Asia.*
 - *Priority geographic areas recognized by the ACGC in 1988, and not yet collected include Georgia, Crimea and Southern Caucasus mountains*

***Medicago sativa* complex**

Reflecting historic objectives of plant introduction, crop improvement and preservation of cultivated germplasm, 64 % of the collection consists of alfalfa accessions (*Medicago sativa* subsp. *sativa*). Of the approximately 3000 alfalfa accessions, 73 % represent cultivars, landrace or breeding material.

Wild relatives of cultivated alfalfa remain the most significant gap in the NPGS collection. The recent success of transferring the glandular hair trait from close relatives into alfalfa, providing resistance to potato leaf hopper has been recognized as a major accomplishment in alfalfa

breeding. Additionally, there are substantial breeding efforts engaged in introgressing *M. sativa* subsp. *falcata* germplasm, into alfalfa. In a survey of 63 *ex situ* germplasm collections, related species were significantly under-represented in other collections as well. Acquisition resources need to be focused on this group of germplasm to provide valuable germplasm for breeders, and ensure the conservation of these valuable resources.

Table 6. Number of accessions representing wild relatives (primary gene pool) of alfalfa in the NPGS collection.

Category	ssp. <i>glomerata</i> / <i>glutinosa</i>	<i>M.</i> <i>prostrata</i>	ssp. <i>falcata</i>	nothosp. <i>varia</i>
Breeding	0	0	11	0
Cultivar	0	0	21	106
Cultivated	2	0	50	14
Landrace	0	0	20	63
Uncertain	0	0	18	17
Wild	5	13	276	76
Totals	7	13	396	276

Recommendations:

Fill the following geographic gaps in US collection through collection and donations. Species are listed in priority order:

1. *Medicago sativa* subsp. *glomerata* (2x=16), subsp. *glutinosa* (2x=32) are grouped together since chromosome counts have not been obtained to distinguish among the two subspecies: Southern Europe, Caucasus region, Algeria, Tunisia
2. *Medicago prostrata*: Albania, eastern Austria, Greece, Czechoslovakia, Hungary, Romania, Yugoslavia
3. *M. sativa* subsp. *falcata*: Northern Libya, Eastern Morocco, Lebanon, Syria, Turkey, Central Asia (Turkmenistan, Afghanistan, Uzbekistan, Tajikistan, northern Kazakhstan), Northeastern China, Northwest India, Northern Pakistan, Southern Caucasus region, Ukraine, Northeastern Europe (Belarus, Lithuania, Latvia, Estonia)
4. *M. sativa* nothosp *varia*: Northwest Iran, Syria, Northern, Western, and Central Europe

Secondary Gene Pool

Only 1.5% of the total U.S. *Medicago* collection are species within the secondary gene pool. Representation of the secondary gene pool is also limited in other *ex situ* collections around the world.

Recommendations:

Fill the following gaps in US collection through collection and donations. Species are listed in priority order:

1. *M. saxatilis*: All areas of species distribution (see Appendix 1)
2. *M. papillosa*: Northern Armenia, Southern Georgia, Turkey
3. *M. sativa* nssp. *tunetana*: All areas of species distribution (see Appendix 1)
4. *M. sativa* ssp. *caerulea*: Crimea, Central Asia

Tertiary Gene Pool

With the exception of *M. marina*, species in the secondary gene pool are limited in the NPGS collection, and other *ex situ* collections around the world.

Recommendations:

Fill the following gaps in US collection through collection and donations. Species are listed in priority order:

1. *M. dzhawakhetica*: Caucasus Mountains, Russia
2. *M. daghestanica*: Caucasus Mountains, Dagestan
3. *M. rupestris*: Lower Crimea Mountains, Ukraine
4. *M. hybrida*: Southern France
5. *M. rhodopea*: Southern Bulgaria
6. *M. pironae*: Alps of northeastern Italy
7. *M. cancellata*: Russia (North Caucasus, Caspian region)

Acquisition priorities

Cultivated Medics

1. Most cultivated varieties of annual medic have been developed in Australia. Although they can be used in the southern latitudes of United States (notably California) they lack cold tolerance to more northern latitudes. Acquisition of accessions that are adapted to the northern boundaries of the species distribution may help us develop annual medic cultivars that can be used as cover crops or in ley farming in Washington, Montana and Wyoming.
2. The following cultivated species has been classified by Prosperi et al. as being vulnerable due to lack of representation in *ex situ* collections: *M. arborea*, *M. italica*, *M. littoralis*, *M. rugosa*, *M. scutellata*. *Priority should focus on obtaining complete ecogeographic coverage of these taxa.*

Non-cultivated Medicago species

Perennial species

Seventy five percent of the perennial *Medicago* species received a conservation status of

“A” by Prosperi et al. (A=endangered species, endemic or rare, very few accessions available in gene banks). With the exception of *M. ruthenica*, *M. arborea* and *M. marina*, the NPGS has few representatives of the perennial wild species.

Recommendations:

Efforts should be made to collaborate with other PGR institutes to conserve these resources in gene bank collections and in natural reserves.

Annual species

Representation of annual *Medicago* species is relatively strong in the NPGS collection since the inclusion of the Lesin’s collection from Canada, in 1984. The collection contains about 4000 accessions of 35 species. Although most of these accessions have been increased, many still have not been assigned PI numbers. Documentation of the Lesin’s accessions is sparse, and for many accessions, very few seeds were obtained.

Recommendations:

- *Efforts should focus on determining if all accessions of the Lesin’s collection should receive PI numbers. We need to collaborate with other PGR institutes to conserve those annual species that have potential use, or are vulnerable to genetic erosion.*
- *Efforts should be made to collaborate with other PGR institutes to conserve these resources in gene bank collections and in natural reserves.*

Collection Evaluations

Perennials

Since 1981 the perennial *Medicago* collection has been evaluated for 14 diseases, six (6) insects, chromosome number, seven (7) feed quality traits, 25 agronomic traits, and five (5) stress tolerant traits. All of the evaluations were conducted using standardized tests when they were evaluated. Scientists in the federal government, universities and private industry conducted the tests and provided the data which was imported into GRIN. Appendix 2 is a listing of the evaluations which have been conducted. They indicate the percentage of accessions for each species that have been evaluated for a specific descriptor. In some cases accessions have been evaluated multiple times for the same descriptor. The percentages reflect only a single evaluation event per accession.

Recommendations

- C *Efforts to verify ploidy have focused on M. sativa subsp. falcata, and M. sativa subsp. caerulea. Work needs to be done to confirm ploidy status of the Medicago sativa subsp. sativa collection*
- C *Approximately a third of the Medicago sativa subsp. sativa collection has been evaluated for disease, insect, growth and morphological descriptors. The remaining two-thirds of the collections should be evaluated. To fully utilize the collection, efforts need to focus on evaluating the remaining two thirds of the alfalfa accessions. This will not be possible without increasing the baseline budget of the NPGS Forage Legume CRIS.*

Annual Medics

The majority of annual medic accessions have data that characterize production, phenology and growth (Appendix 3). These data were acquired as accessions and were regenerated in Riverside, CA from 1988 to 1999 and as the annual medic core collection was developed in Beltsville, MD and evaluated in six (6) locations (Tucson, AZ; Athens, GA; Beltsville, MD; St. Paul, MN; Ithaca, NY and Logan, UT) across the U.S.

Recommendations

- *Data on only one disease descriptor has been gathered, and for only the core collection, thus, additional diseases need to be screened.*
- *Some morphological descriptors have been taken, however, the majority of the accessions in the collection do not have even basic morphological descriptors. Thus a complete set of morphological descriptors needs to be obtained.*

Over - all Evaluation Recommendations:

- Ⓒ *Efforts should focus on acquiring a complete set of descriptors on all accessions, especially all alfalfa accessions. A complete data set would allow users to understand the diversity within the collection, and enable users to fully use the collection.*
- Ⓒ *The currently established core subsets should be re-evaluated and adjusted once descriptor data has been obtained on the entire collection.*
- Ⓒ *Accession classification based on molecular markers needs to be compared to classifications based on morphological and ecogeographic descriptors. Can we identify a combination of descriptors (that are easily and efficiently obtained) that group accessions within the collection into meaningful clusters? Can we establish a method to routinely classify newly acquired accessions to ensure the collection continues to reflect a wide range of diversity but with a manageable number of accessions?*
- Ⓒ *Evaluations directed at traits that can broaden the use of alfalfa need to be carried out. For example, there is current interest in the use of alfalfa as a medicinal herb, for phytoremediation, use as biofuels, cover crop, etc. The production of organic hay is increasing. The genetic variation within the germplasm collection for these uses has been minimally explored .*
- Ⓒ *Accession evaluation and observation data needs to be acquired and placed into the GRIN database in a format that allows users to draw valid comparisons across all accessions evaluated for that trait.*

Germplasm Enhancement

Because the current PI collection of perennial *Medicago* spp. is large and mostly in "raw" form (i.e., without many of the traits necessary in current varieties), it is not practical for commercial breeders to use for developing alfalfa varieties with improved quantitative traits (forage and seed yield, quality, etc.). Current market competition demands rapid development of "new and improved" varieties; therefore, most breeders have insufficient time and resources to improve non-elite germplasm into forms useable for varietal development. Therefore, a national effort to genetically enhance these PI's is necessary for their utilization in programs to develop varieties.

Simply inherited traits from unadapted PI's may be incorporated into adapted backgrounds by backcrossing or by biotechnological methods available in the future. For quantitative traits, however, early use of PI's probably will be in the form of adapted or nearly adapted germplasm pools (GPP). These pools should have at least moderate levels of the economic traits available in current varieties, while containing large variability for traits that may be useful to improve alfalfa in the future. Alfalfa breeders need GPP's as sources to tap both for "new" genes conditioning traits currently recognized as important and for traits that may be needed in the future. These pools also would provide material for research in alternate uses of alfalfa, such as sustainable agriculture.

An enhancement proposal was submitted to the ACGC in 1990 which outlined methods to develop alfalfa GPP's that should be useful to breeders in the USA. The proposal was developed by a subcommittee within the ACAC and it serves as an initial focal point from which the ACGC can plan for developing national and regional GPP's. The main objectives of the proposal are:

1. Develop a *national GPP* to serve as a source of new recombinants among the total diversity of germplasm available. This pool potentially would provide recombinants not obtained in the more genetically restricted regional pools described below.
2. Develop *regional GPP's* that are adapted to each of four regions in the USA. Under the typical management practices for alfalfa in a particular region, the regional pools should be agronomically more acceptable than the national GPP. They also should have at least moderate levels of the traits available in varieties currently used in that region, plus genes that may be used to improve cultivated alfalfa.

Recommendations:

- *The proposed enhancement program for the development of national and regional germplasm pools should be enacted. Appendix 4 provides details of the enhancement program.*
- *An ARS alfalfa research geneticist position was established in 1999 at Prosser, WA. with an emphasis to carry out additional germplasm enhancement work. Enhancement work by this position should continue to be encouraged and supported.*

NPGS Germplasm Collection Use 1993-1997

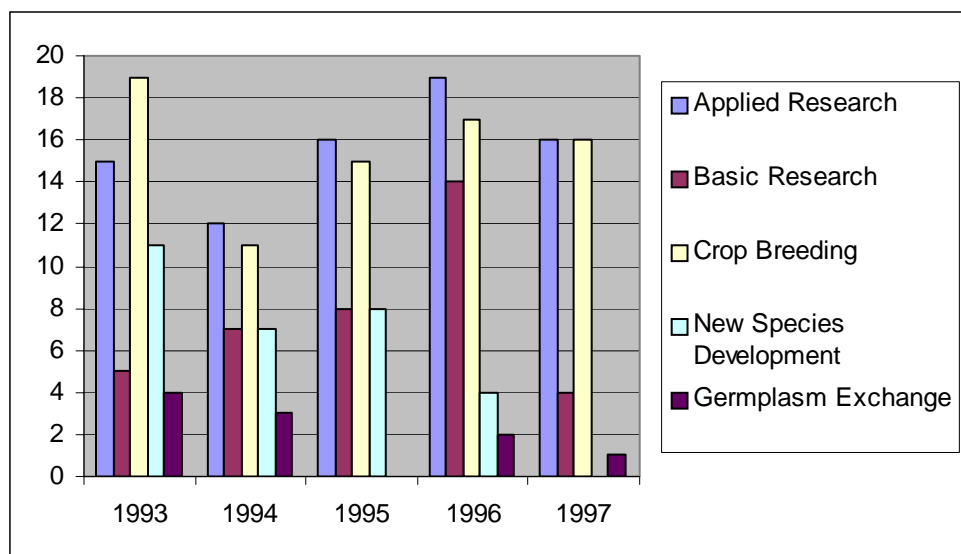
A total of 12,800 seed packets were distributed for 229 requests from 1993 through 1997. Scientists from U.S. universities were responsible for almost half of the seed requests. The remaining requests for perennial species were split about equal between USDA-ARS scientists, private breeding companies and foreign scientists. For annual species, private company requests accounted for only 4%. The majority of requested germplasm was intended for applied and basic research projects. A substantial amount of germplasm was requested for screening by crop breeding programs.

When collection use was examined by taxa, and size of collection factored out, use of germplasm representing the secondary and tertiary alfalfa gene pools has been higher on average, than use of the alfalfa collection in the last 5 years. However, 5400 seed packets were distributed from the *M sativa* ssp *sativa* collection. Requests for non-cultivated annual species has been greater than for the perennial species and is probably due to requests for the annual medic core collection .

Five (5) % of the orders for perennial species were for the alfalfa core collection, 13 % of orders for annual species were for the core subset.

Accession availability

Figure 9. Number of requests grouped by intended seed use as reported by requestor from 1993 to 1997.



Collection Preservation

The *Medicago* collection is housed and distributed from the USDA, ARS Western Regional Plant Introduction Station located in Pullman, WA. Original seed packets and regeneration seed lots are kept at 38-40 C° with 28 % relative humidity. Seed lots that are distributed are kept at the same conditions. Seed counts have been obtained on all packets except for original seed. Germination testing has been carried out for seed lots increased in 1996, 1997 and 1998. Original seed has not had germination tests, nor have baseline germinations been obtained on increase seed lots. Approximately 84 % of the accessions in the collection have a safety backup sample stored at NSSL. Accessions not backed up are those with very few seeds, or are currently queued for regeneration.

From 1979 to 1997, the majority of perennial accessions were regenerated using original or the oldest existing seed lot, under isolation in cages using up to 200 plants per population (this number varies with amount of seed available; number of plants harvested is placed in GRIN as a historic record). From 1987 to 1998, most of the annual medic species were increased at Riverside, California. Accessions in the *Medicago* collection that remain to be increased include those species that are difficult to regenerate, and accessions with very low seed counts or low seed viability. These accessions require special handling to ensure they are not lost during the regeneration process. Approximately 500 accessions fall into this category.

Recommendations:

- *Original seed needs to be inventoried with seed counts. Germination data on original seed lots needs to be obtained on all accessions with sufficient seed. Original and regeneration seed lots need to be stored at -15 to -20 C.*
- *Germination data needs to be obtained on increased material to establish a baseline to monitor seed viability and to provide a check to verify that regeneration procedures are high quality.*

- © *Research needs to be carried out to verify if our standard plant population size during regeneration is adequate for limiting genetic drift, and if genetic change in accessions is minimized during the standard regeneration process.*

Appendix 1. The genus *Medicago* according to Small and Jomphe, (1989), with geographic distribution, number of accessions per species¹ in the NPGS collection, and conservation status of each species as defined by Properi et al. (1996) .

Section	Subsection	Species	Subspecies	Geographic Distribution	NPGS Accessions	Status ¹
Dendrotelis		<i>M. arborea</i> L.		S Europe, Turkey	38	B-Cult
		<i>M. strasseri</i> Greuter, Matthas & Risse		Crete		A
<i>Medicago</i>		<i>M. sativa</i>		E. Turkey, Iran, Former USSR	77	B
		ssp. <i>caerulea</i> Schmalh.				
		ssp. <i>sativa</i> (L.) L & L.		Possible native range-Middle East, C Asia, now widely distributed	3042	C-CULT
		ssp. <i>glomerata</i> Balbis.		S Europe, N Africa, Caucasus	7	A
		ssp. <i>falcata</i> (L.) Arcangeli		S Europe, Former USSR		B-CULT
		var <i>falcata</i> Archang.		N Eurasia	419	-
		var <i>viscosa</i> Posp.		S Europe, N Africa, Caucasus	14	-
		ssp. <i>x varia</i> Martin		Europe, Iran, Syria, Turkey, Caucasus	287	-
		ssp. <i>x tunetana</i> Murbeck		S Europe, N Africa, Caucasus	6	-
		<i>M. papillosa</i> Boiss.		Turkey, S Russia	8	A
		ssp. <i>macrocarpa</i> (Boiss) Urban		Turkey	0	-
		ssp. <i>papillosa</i> Bioss.		Turkey, S Russia	1	-
		<i>M. prostrata</i> Jacq.		Albania, E Europe, Italy	13	A
		<i>M. rupestris</i> M. Bieb.		Crimea, W Caucasus	0	A
		<i>M. cancellata</i> M. Bieb.		European Russia	7	A
		<i>M. rhodopea</i> Velen.		Bulgaria	2	A
		<i>M. saxatilis</i> M. Bieb.		Crimea	1	A
		<i>M. daghestanica</i> Rupr. Ex Boiss		Dagestan	0	A
		<i>M. pironae</i> Vis.		Italy	4	A
		<i>M. hybrida</i> (Pourret) Trautv.		France, Spain	1	A
		<i>M. suffruticosa</i> Raymond ex DC.		France, Spain, Morocco	17	B
		<i>M. marina</i> L.		S Europe, N Africa, Middle East, Crimea	20	B
Carstiensae		<i>M. carstiensis</i> Jacq.		E Europe	1	A
Spirocarpos	Pachyspirae	<i>M. soleirolii</i> Duby		France, Italy, Algeria, Tunisia	11	A
		<i>M. italica</i> (Miller) Fiori		Mediterranean Basin	87	B-CULT

	<i>M. littoralis</i> Rohde ex Lois	Mediterranean Basin, E Europe, Caucasus	173	B-CULT
	<i>M. truncatula</i> Gaertner	Mediterranean Basin, E Europe, Caucasus	320	C-CULT
	<i>M. doliata</i> Carmign.	Italy, Spain, Algeria, Morocco	104	C
	<i>M. turbinata</i> (L.) All.	Mediterranean Basin	86	B
	<i>M. rigidula</i> (L.) All.	Mediterranean Basin, E Europe, Caucasus, Central Asia	148	C-CULT
	<i>M. rigiduloides</i> Small.	E Mediterranean, Middle East	192	
	<i>M. constricta</i> Durieu	E Mediterranean Basin, Iran, Iraq	52	B
	<i>M. lesinsii</i> E. Small	Mediterranean Basin	9	B-CULT
	<i>M. murex</i> Willd.	Mediterranean Basin	69	B-CULT
	<i>M. rugosa</i> Desr.	Mediterranean Basin	43	B
	<i>M. scutellata</i> (L.) Miller	Mediterranean Basin, Southern Ukraine, Crimea	61	B-CULT
Rotatae	<i>M. blancheana</i> Boiss.	E Mediterranean	18	B
	<i>M. rotata</i> Boiss.	Cyprus, Iraq, Israel, Jordan, Lebanon, Syria, Turkey	21	B
	<i>M. noeana</i> Boiss.	Iraq, Turkey	19	A
Intertextae	<i>M. shepardii</i> Post. Ex Boiss.	Turkey	4	A
	<i>M. intertexta</i> (L.) Miller	W Mediterranean Basin	17	B
	<i>M. ciliaris</i> (L.) Krockner	Mediterranean Basin, Iraq	64	C
	<i>M. muricoleptis</i> Tineo	France, Italy	8	A
	<i>M. granadensis</i> Willd.	Egypt, Israel, Syria, Turkey	13	B
Leptospirae	<i>M. sauvagei</i> Negre	Morocco	5	A
	<i>M. laciniata</i> (L.) Miller	N Africa, Arabian peninsula, India, Pakistan, Afghanistan	136	B
	<i>M. minima</i> (L.) Bartal.	Europe, N Africa, India., Russia	299	C
	<i>M. praecox</i> DC.	S and E Europe, Cyprus, Turkey, Crimea	23	B
	<i>M. coronata</i> (L.) Bartal.	E Mediterranean Basin, Iran, Iraq	24	B
	<i>M. polymorpha</i> L.	Europe, N Africa, Middle East, Crimea, Caucasus, Central Asia	685	C-CULT
	<i>M. laxispira</i> Heyn.	Iraq	0	A

		<i>M. arabica</i> (L.) Hudson	Europe, N Africa, Middle East, Crimea, Caucasus	79	C
		<i>M. tenoreana</i> Ser.	France, Italy, Yugoslavia	6	A
		<i>M. disciformis</i> DC.	Mediterranean Basin	50	B
		<i>M. lanigera</i> Winkler & B. Fedtsch.	Afghanistan, Turkmenistan, Tajikistan	1	A
Geocarpa E. Small		<i>M. hypogaea</i> E. Small	SE Mediterranean	0	A
Lupularia		<i>M. lupulina</i> L.	Europe, N Africa, Middle East, Asia	209	C-Cult
		<i>M. secundiflora</i> Durieu	S Europe, N Africa	2	B
Heyniana		<i>M. heyniana</i> Greuter	Greece	2	A
Orbiculares		<i>M. orbicularis</i> (L.) Bartal.	Mediterranean basin, Middle East, Central Asia	322	C
Hymenocarpos		<i>M. radiata</i> L.	Middle East, Russia, Central Asia	11	C
Platycarpae		<i>M. plicata</i> (Boiss)	Turkey	0	UNK
		<i>M. platycarpa</i> L. Trautv.	China, Mongolia, Central Asia	15	A
		<i>M. ruthenica</i> L. Trautv.	China, Korea, Mongolia, Russia	122	A
		<i>M. popovii</i> Sirj.	Central Asia	1	A
		<i>M. archiducis-nicolai</i> Sirjaev	Central China, NE Tibet	0	UNK
		<i>M. edeworthii</i> Sirjaev	Himalayas	3	A
		<i>M. cretacea</i> M. Bieb.	Russia, Former USSR	2	A
		<i>M. ovalis</i> (Boiss.) Sirjaev	S Spain, Morocco	0	UNK
Lunatae		<i>M. rostrata</i> (Boiss. & Bal.)	Turkey	0	?
		<i>M. biflora</i> (Griseb.) E Small	Turkey, Iran, S Trans-Caucasus	1	?
		<i>M. brachycarpa</i>	Turkey, Lebanon, Iraq, Transcaucasia	5	?
		<i>M. huberi</i> E. Small	SW Anatolia	0	?
Buceras					
	Erectae	<i>M. astroites</i> (Fisch. & Mey.)	E Mediterranean	0	?
		<i>M. halophila</i> (Boiss) E Small	S Anatolia	0	?
		<i>M. arenicola</i> (Huber-Mor)	Turkey	0	?
		<i>M. heldreichii</i> (Boiss.) E. Small	Turkey	0	?
		<i>M. phrygia</i> (Boiss.& Bal.)	Turkey, Syria, Iraq, Iran	0	?
		<i>M. fischeriana</i> (Ser.) Trautv.	Turkey, Iraq, Iran, European USSR	5	?
		<i>M persica</i> (Boiss.) E. Small	Iran, Iraq	0	?

	<i>M. medicaginoides</i> (Retz.) E. Small	SE Europe, SW USSR, Turkey, Iran, S and C Asia	1	?
	<i>M. rigida</i> (Boiss. & Bal.)	S Anatolia	0	?
	<i>M. crassipes</i> (Boiss) E. Small	Anatolia. Lebanon, N Iraq, W Iran	1	?
	<i>M. pamphylica</i> (Huber-Mor. & Sirjaev) E. Small	S Anatolia	0	?
	<i>M. carica</i> (Huber-Mor. & Sirjaev) E. Small	S W Anatolia	0	?
	<i>M. monantha</i> (C.V. Meyer) Trautv.	Middle East, S and C Asia	38	?
	<i>M. orthoceras</i> (Kar. & Kir.) Trautv.	Middle East, S and C Asia	0	?
	<i>M. polyceratia</i> (L.) Trautv.	W Mediterranean	10	?
Deflexae	<i>M. retrorsa</i> (Boiss.) E. Small	Afghanistan	0	?
Reflexae	<i>M. monspeliaca</i> (L.) Trautz.	W Europe, N Africa, E Mediterranean, Jordan, Iraq, Iran, USSR, C Asia	12	?
Isthmocarpae	<i>M. rhytidiocarpa</i> (Boiss. & Bal.) E. Small	S Anatolia	0	?
	<i>M. isthmocarpa</i> (Boiss. & Bal.) E. Small	C Anatolia	0	?

¹Conservation Status : A = Endangered species, endemic or rare, very few available accessions in gene banks; B = Species with threatened diversity due to a rapid modification of its ecosystem or due to its localization in regions subject to an important climate change, few available accessions; C = Species widespread, large number of available accessions, Cult = Genetically improved for agriculture; ? = unknown or undetermined (Prosperi et al. 1996)

Appendix 2. Evaluation status of the perennial *Medicago* species¹

Species	Cytology	Disease													
	CHR	ANTH	APHA	BACT	DOW	FUS	LSPT	LEPT	PHY	RHIZ	SCL	SPRB	STEML	STEM	VE
arborea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
cancellata	0	43	43	57	86	57	29	43	14	43	100	43	43	43	86
carstiensis	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
hybrid	0	33	33	11	11	11	11	17	17	22	39	17	22	17	28
papillosa	0	0	0	0	0	0	0	0	0	0	25	0	0	0	0
subsp. papillosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
pironae	0	0	0	0	0	0	0	0	0	0	50	0	0	0	0
platycarpus	0	7	7	13	7	13	0	0	7	7	40	7	7	7	13
polymorpha	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
prostrata	15	0	0	0	0	0	0	0	0	0	38	0	0	0	0
ruthenica	0	1	1	1	1	1	0	1	1	1	16	1	0	1	1
sativa nssp. tunetana	0	0	0	0	0	0	0	0	0	0	33	0	0	0	0
sativa nssp. varia	0	24	54	40	37	40	14	22	29	31	41	22	45	22	67
sativa subsp. caerulea	38	36	47	52	51	52	31	32	22	9	23	32	36	32	57
sativa subsp. falcata	63	15	18	19	13	19	9	11	8	11	10	11	14	11	23
sativa subsp. glomerata	0	0	0	0	0	0	0	0	0	0	43	0	0	0	14
sativa subsp. sativa	1	35	35	32	33	32	28	29	26	21	26	29	32	29	37
sativa var. viscosa	15	8	0	8	8	8	8	8	0	0	46	8	0	8	23
saxatilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
scutellata	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0
suffruticosa	0	0	0	0	0	0	0	0	0	0	15	0	0	0	0

CHR= chromosome number; **ANTH**= Anthracnose; **APHA**= Aphanomyces; **BACT**= Bacterial wilt ; **DOW**= Downey mildew; **FUSW**= Fusarium wilt; **LSPT** = Common leaf spot; **LEPT**= Lepto spot; **PHYR**= Phytophthora root rot; **RHIZ**= Rhizoctonia; **SCLE**= Sclerotinia; **SPRB**= Spring black stem; **STEMLF**= Stemphylium leaf spot; **STEM**= Summer black stem; **VERT**= Verticillium wilt

¹ Tables indicate the percentage of accessions for each species that have been evaluated for a specific descriptor. In some cases accessions have been evaluated multiple times for the same descriptor. The percentages reflect only a single evaluation event per accession.

Species	Insect						Chemical		
	BLUE	LYGUS	PEAA	POTLF	ROOT	SPOT	CLEAF	CSTEM	PBYPASS
arborea	0	0	0	0	0	0	0	0	0
cancellata	43	0	29	100	86	57	43	43	29
carstiensis	0	0	0	0	0	0	0	0	0
hybrid	11	6	11	22	22	22	22	22	17
papillosa	0	0	0	13	25	0	0	0	0
subsp. papillosa	0	0	0	0	0	0	0	0	0
pironae	0	0	0	50	50	0	0	0	0
platycarpus	0	7	0	7	13	7	7	7	0
polymorpha	0	0	0	0	0	0	0	0	0
prostrata	0	0	0	0	0	0	0	0	0
ruthenica	0	1	0	2	2	1	0	0	1
sativa nssp. tunetana	0	0	0	0	0	0	0	0	0
sativa nssp. varia	33	7	33	45	43	38	45	45	8
sativa subsp. caerulea	52	0	52	52	53	52	36	36	6
sativa subsp. falcata	12	0	12	24	24	14	14	14	3
sativa subsp. glomerata	0	0	0	14	14	0	0	0	14
sativa subsp. sativa	28	5	23	37	36	32	33	32	6
sativa var. viscosa	8	0	8	31	31	8	0	0	23
saxatilis	0	0	0	0	0	0	0	0	0
scutellata	0	0	0	0	0	0	0	0	0
suffruticosa	0	0	0	0	0	0	0	0	8

BLUE=Blue alfalfa aphid; **LYGUS**=Lygus bug ; **PEAA**= Pea alfalfa aphid; **POTLF**=Potato leaf hopper; **ROOT**= Clover root curculio; **SPOT**= Spotted alfalfa aphid; **CLEAF**= Crude protein in leaf ; **CSTEM**= Crude protein in stem; **PBYPASS**= By pass protein

Species	Growth							
	CSTD	CWID	FALL	GHAB	RECO	RTD2	SDLGV	TAPDIA
arborea	0	0	0	0	0	0	0	0
cancellata	43	43	43	29	29	43	43	43
carstiensis	0	0	0	0	0	0	0	0
hybrid	22	22	22	11	11	22	22	22
papillosa	0	0	0	0	0	0	0	0
subsp. papillosa	0	0	0	0	0	0	0	0
pironae	0	0	0	0	0	0	0	0
platycarpus	7	7	7	7	7	7	7	7
polymorpha	0	0	0	0	0	0	0	0
prostrata	0	0	0	0	0	0	0	0
ruthenica	1	1	1	0	1	1	1	1
sativa nssp. tunetana	0	0	0	0	0	0	0	0
sativa nssp. varia	45	45	45	24	22	45	45	45
sativa subsp. caerulea	36	36	36	30	18	36	36	36
sativa subsp. falcata	14	14	14	8	8	14	14	14
sativa subsp. glomerata	0	0	0	0	0	0	0	0
sativa subsp. sativa	33	33	33	20	19	33	33	33
sativa var. viscosa	0	0	0	0	0	0	0	0
saxatilis	0	0	0	0	0	0	0	0
scutellata	0	0	0	0	0	0	0	0
suffruticosa	0	0	0	0	0	0	0	0

CSTD=Crown stem diameter; **CWID** Crown width; **FALL**= Fall growth; **GHAB**= Growth habit; **RECO**= Recovery rate; **RTD2**= Secondary root diameter; **SDLGV**= Seedling vigor; **TAPDIA**= Tap root diameter

Species	Morphology												
	CBRAN	CORIG	CBUD	DCRO	DTAP%	DTAPP	FRTM	FLWC	LFSZ	MAT	RNUM2	RPOS2	UILEN
arborea	0	0	0	0	0	0	0	0	0	0	0	0	0
cancellata	43	43	43	43	43	43	43	0	43	43	43	43	14
carstiensis	0	0	0	0	0	0	0	0	0	0	0	0	0
hybrid	22	22	22	22	22	22	22	0	22	22	22	22	6
papillosa	0	0	0	0	0	0	0	0	0	0	0	0	0
subsp. papillosa	0	0	0	0	0	0	0	0	0	0	0	0	0
pironae	0	0	0	0	0	0	0	0	0	0	0	0	0
platycarpus	7	7	7	7	7	7	7	0	7	7	7	7	7
polymorpha	0	0	0	0	0	0	0	0	0	0	0	0	0
prostrata	0	0	0	0	0	0	0	15	0	0	0	0	0
ruthenica	1	1	1	0	1	1	1	0	1	0	1	1	1
sativa nssp. tunetana	0	0	0	0	0	0	0	0	0	0	0	0	0
sativa nssp. varia	45	45	45	45	45	45	45	0	45	45	45	45	8
sativa subsp. caerulea	36	36	36	36	36	36	36	0	36	36	36	36	6
sativa subsp. falcata	14	14	14	14	14	14	14	41	14	14	14	14	3
sativa subsp. glomerata	0	0	0	0	0	0	0	0	0	0	0	0	0
sativa subsp. sativa	33	33	33	32	33	33	33	0	33	32	33	33	6
sativa var. viscosa	0	0	0	0	0	0	0	0	0	0	0	0	0
saxatilis	0	0	0	0	0	0	0	0	0	0	0	0	0
scutellata	0	0	0	0	0	0	0	0	0	0	0	0	0
suffruticosa	0	0	0	0	0	0	0	0	0	0	0	0	0

CBRAN=Crown branching; **CORIG**= Crown origin; **CBUD**= Crown budding; **DCRO**= Depth of crown; **DTAP%**= Determinate tap root percentage; **DTAPP**= Determinate tap root position; **FRTM**= Fibrous root mass; **FLWC**= Flower color; **LFSZ**= Leaf size; **MAT**= Maturity; **RNUM2**=Secondary root number; **RPOS2**= Secondary root position; **UILEN**= Unifoliolate internode length

Species	Production								Stress-Abiotic				
	ADFL	ADFS	NDFL	NDFS	SDYLD	SDPROD	SDWGT	SLRAT	ACID	FRDAM	SGERM	SSTRESS	WININJ
arborea	0	0	0	0	0	0	9	0	0		0	0	0
cancellata	43	43	43	43	43	100	71	43	43	43	57	29	43
carstiensis	0	0	0	0	0	100	0	0	0	0	0	0	0
hybrid	22	22	22	22	22	50	61	22	11	22	33	28	22
papillosa	0	0	0	0	0	100	25	0	13	0	0	0	0
subsp. papillosa	0	0	0	0	0	100	0	0	0	0	0	0	0
pironae	0	0	0	0	0	100	50	0	50	0	0	0	0
platycarpus	7	7	7	7	7	47	27	7	27	7	7	7	7
polymorpha	0	0	0	0	0	0	0	0	0	0	0	0	0
prostrata	0	0	0	0	0	92	0	0	23	0	0	0	0
ruthenica	0	0	0	0	0	90	82	0	6	0	1	1	0
sativa nssp. tunetana	0	0	0	0	0	50	17	0	33	0	0	0	0
sativa nssp. varia	45	45	45	45	45	71	58	45	21	45	60	48	45
sativa subsp. caerulea	36	36	36	36	36	58	44	36	39	36	48	39	36
sativa subsp. falcata	14	14	14	14	14	45	24	14	10	14	18	16	14
sativa subsp. glomerata	0	0	0	0	0	86	0	0	29	0	14	0	0
sativa subsp. sativa	33	32	33	32	32	56	69	32	19	33	39	22	33
sativa var. viscosa	0	0	0	0	0	77	0	0	31	0	15	0	0
saxatilis	0	0	0	0	0	100	0	0	0	0	0	0	0
scutellata	0	0	0	0	0	0	0	0	0	0	0	0	0
suffruticosa	0	0	0	0	0	100	8	0	15	0	0	0	0

ADFL= Acid detergent fiber leaf; **ADFS**= Acid detergent fiber stem; **NDFL**= Neutral detergent fiber leaf; **NDFS**=Neutral detergent fiber stem; **SDYLD**= Seed yield; **SDPROD**= Seedling year productivity; **SDWGT**= 100 seed weight; **SLRAT**= Stem leaf ratio; **ACID**= Acid soil tolerance; **FRDAM**= Frost damage; **SGERM**= Salt germination; **SSTRESS**- Tolerance to salt stress; **WININJ**= Winter injury

Appendix 3. Evaluation status of annual medics.

Species	Cytology	Disease	Growth		Morphology					
	CHR	ANT	GHAB	WVIG	COIL	DAYSP	HTH	SPRD	SPIN	SCUR
arabica	0	3	67	86	0	3	3	3	3	0
blancheana	0	39	83	72	0	50	50	50	50	0
brachycarpa	0	0	0	0	0	0	0	0	0	0
ciliaris	0	9	77	73	0	8	9	9	8	0
constricta	0	6	62	60	0	6	6	6	6	0
coronata	0	8	100	100	0	8	8	8	8	0
disciformis	0	8	86	100	0	8	8	8	8	0
doliata	0	2	89	88	0	3	3	3	3	0
var. doliata	0	0	33	33	0	0	0	0	0	0
var. muricata	0	5	35	33	0	0	0	0	0	0
fischeriana	0	0	40	40	0	0	0	0	0	0
granadensis	0	31	100	100	0	23	23	23	23	0
heyniana	0	50	50	0	0	50	50	50	50	0
hybrid	0	0	22	22	0	0	0	0	0	0
intertexta	0	35	94	88	0	24	24	24	24	0
italica	0	10	71	66	0	7	10	8	7	0
laciniata	0	7	81	85	0	7	8	8	7	0
lanigera	0	100	100	100	0	100	100	100	100	0
lesinsii	0	22	89	89	0	11	11	11	11	0
littoralis	0	4	45	42	0	2	3	2	2	0
lupulina	0	7	48	49	0	8	8	8	8	0
medicaginoides	0	0	0	0	0	0	0	0	0	0
minima	0	1	71	86	0	1	2	1	1	0
monantha	0	0	32	32	0	0	0	0	0	0
monspeliaca	0	0	17	17	0	0	0	0	0	0
murex	1	7	72	86	0	4	7	6	4	0
muricoleptis	0	13	88	88	0	0	0	0	0	0
noeana	0	11	100	100	0	11	11	11	11	0
orbicularis	0	2	65	65	0	2	3	3	2	0
polyceratia	0	0	0	0	0	0	0	0	0	0
polymorpha	0	3	79	78	0	1	1	1	1	0
var. brevispina	0	17	80	89	0	20	20	20	20	0

var. polymorpha	0	11	68	78	0	14	14	14	14	0
var. vulgaris	0	0	69	69	0	8	8	8	8	0
popovii	0	0	0	0	0	0	0	0	0	0
praecox	0	9	70	83	0	9	9	9	9	0
radiata	0	36	55	18	0	36	36	36	36	0
rhodopea	0	0	50	50	0	0	0	0	0	0
rigidula	0	2	53	82	76	3	3	3	3	76
rigiduloides	0	2	59	76	100	1	2	1	1	100
rotata	0	29	86	86	0	29	29	29	29	0
rugosa	0	26	88	86	0	19	26	26	19	0
sauvagei	0	40	100	100	0	40	40	40	40	0
scutellata	0	28	80	80	0	30	31	31	30	0
secundiflora	0	0	100	100	0	0	0	0	0	0
shepardii	0	0	100	100	0	0	0	0	0	0
soleirolii	0	18	27	18	0	9	18	18	9	0
tenorea	0	17	83	100	0	17	17	17	17	0
truncatula	0	3	71	73	0	1	2	2	1	0
turbinata	0	6	74	79	0	3	9	7	3	0

CHR=chromosome number; **ANT**= anthracnose; **GHAB**=growth habit; **WVIG**= winter vigor; **COIL**= Coils on pods; **DAYS**=Days to full pod production; **HTH**=Plant height; **SPRD**= Plant maximal spread; **SPIN**= Pod spines; **SCUR**=Spine curvature

	Phenology	Production				
	DAYSF	SDWT	BMASS	BMSP	GMPR	PODP
arabica	89	89	89	3	80	89
blancheana	83	100	83	50	72	83
brachycarpa	0	60	0	0	0	0
ciliaris	77	92	77	9	64	77
constricta	69	96	69	6	50	69
coronata	100	92	100	8	96	100
disciformis	100	100	100	8	96	100
doliata	89	91	89	3	70	88
var. doliata	33	0	33	0	33	33
var. muricata	35	95	35	0	23	35
fischeriana	40	80	40	0	20	40
granadensis	100	100	100	23	100	100
heyniana	50	100	50	50	0	50
hybrid	22	6	22	0	22	0
intertexta	94	88	94	29	71	94
italica	71	63	71	10	64	70
laciniata	85	98	85	9	81	85
lanigera	100	100	100	100	100	100
lesinsii	89	100	89	11	89	89
littoralis	45	95	45	3	40	45
lupulina	53	61	53	8	44	53
medicaginoides	0	100	0	0	0	0
minima	88	89	88	2	84	88
monantha	32	61	32	0	32	32
monspeliaca	17	58	17	0	17	17
murex	87	94	87	7	83	87
muricoleptis	88	100	88	0	63	88
noeana	100	89	100	11	95	100
orbicularis	65	75	66	3	55	65
polyceratia	0	100	0	0	0	0
polymorpha	79	47	79	2	39	79
var. brevispina	91	100	91	20	86	91
var. polymorpha	81	98	81	14	64	81
var. vulgaris	69	69	69	8	69	69

popovii	0	100	0	0	0	0
praecox	83	91	83	9	83	83
radiata	55	100	55	36	18	55
rhodopea	50	0	50	0	50	50
rigidula	83	85	83	3	78	83
rigiduloides	79	99	79	2	66	79
rotata	86	76	86	29	67	86
rugosa	88	88	88	26	79	88
sauvagei	100	100	100	40	100	100
scutellata	89	97	89	31	77	89
secundiflora	100	100	100	0	50	100
shepardii	100	100	100	0	100	100
soleirolii	18	100	27	18	18	27
tenorea	100	100	100	17	100	100
truncatula	74	55	74	3	69	74
turbinata	81	84	81	9	76	81

DAYSF=Days to flower; **SDWT**=100 seed weight; **BMASS**=Biomass among species; **BMSP**= Biomass within species;
GMPR=Grams of seed produced ;**PODP**=Visual pod production

Appendix 4.

Germplasm Enhancement of Perennial Medicago Plant Introductions Development of National and Regional Germplasm Pools.

Objectives

- Develop a *national GPP* to serve as a source of new recombinants among the total diversity of germplasm available. This pool potentially would provide recombinants not obtained in the more genetically restricted regional pools described below.
- Develop *regional GPP's* that are adapted to each of four regions in the USA. Under the typical management practices for alfalfa in a particular region, the regional pools should be agronomically more acceptable than the national GPP. They also should have at least moderate levels of the traits available in varieties currently used in that region, plus genes that may be used to improve cultivated alfalfa.

Procedures

National Germplasm Pool

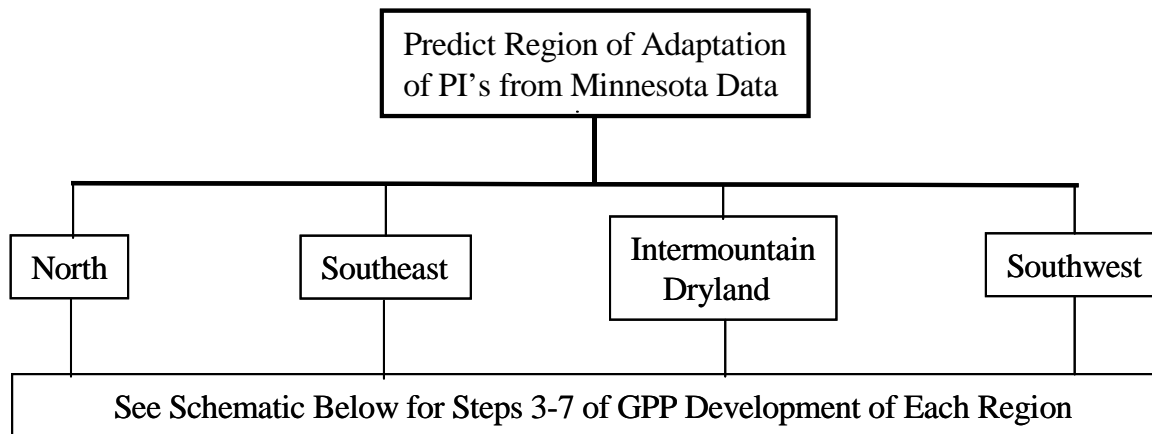
STEP 1: Bulk 100 seeds from each of the perennial PI's in the collection.

STEP 2: Plant 0.1 acre in field isolation to produce Syn. 1 seed. Three adjacent blocks should be planted and cut at different times to enhance overlap of pollination among the PI's in the regrowth.

STEP 3: To promote genetic recombination, produce Syn. 2 seed in field isolation. Fifty pounds of seed should be adequate to supply alfalfa researchers with a sample of the GPP. Seed should be produced in the Southwest to prevent natural selection for winter survival if a second year of production is necessary.

Regional Germplasm Pools

Four regions are suggested for developing GPP's: Northern, Intermountain Dryland, Southeast, and Southwest. Within each region, developing several pools organized by genetic and/or geographic origin would enhance opportunities for heterotic recombination in controlled cross-pollinations. Resource constraints, however, probably will allow for only a single GPP per region. The overall plan is to evaluate the PI's within the region where they are predicted to be most adapted according to the Minnesota research (Basigalup, 1989). Plants will be selected based on field performance, then crossed onto a bulk of current varieties used in that region to allow recombination of genes from the PI's with those from more adapted, elite backgrounds. Germplasm pools created in this manner should be simple to develop, while containing most of the genes in current varieties plus genes from the PI's that may enhance the rate of varietal improvements. Further improvements in the GPP's could be done by individual breeders according to their own strategies. Detailed steps of this plan are illustrated and outlined on the following pages.



STEP 1: *Predict adaptation of each of the PI's to specific regions* of the USA by using data from the perennial core subset collection (Basigalup, et al., 1992)

STEP2: *Establish regional committees* consisting of breeders, plant pathologists, entomologists, plant physiologists, and other interested alfalfa scientists to determine specific objectives and procedures for developing and improving GPP's in each region.

STEP 3: *Screen and select the most adapted plants.* In each region, establish spaced-plant nurseries (18" plant spacing) to evaluate the PI's that were predicted to be the most adapted to that region. In most regions, the PI's can be assigned among two or more breeders to reduce individual effort and to allow for selection in various environments. Locations and management conditions should be typical for alfalfa production in that region. Number of years of evaluation should be decided by the regional committee. In the North, for example, evaluation should span one or two winters to assess winter survival.

Select the most vigorous and agronomically acceptable plants. Discarded plants will be considered too unadapted for more immediate use in varietal development. Remnant seed of these PI's might be useful for long-term backcross or biotechnical programs to incorporate useful genes into adapted backgrounds.

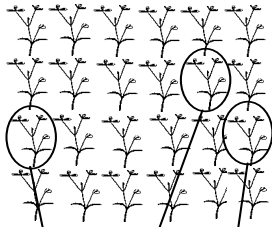
Two alternatives regarding selection method should be considered by the ACGC. The first is to phenotypically select plants from within each PI so that the GPP is equally represented by all PI's within that region. The second alternative is to select both among and within the PI's, similar to the "ear-to-row-to-ear" method described for corn (Lonnquist, 1964). Although this second type of GPP may not be as genetically variable at first, it probably will be more adapted. But consider this question: Does enough genetic variability exist within the PI's to justify the first type of selection?

STEP 4: *Hybridize selected PI's with improved varieties.* In an isolated field, cross-pollinate the selected plants to a bulk population of the most current varieties used in that region. This varietal bulk should consist of a number of varieties of diverse genetic background, and have a high level of expression (if available) for the economically important traits, such as pest resistance, in that region.

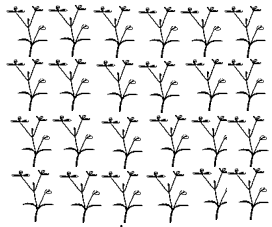
Backcrossing to the varietal bulk might be considered, but it might dilute the frequency of genes from the PI's to a level difficult for finding genes needed in the future.

STEP 5: *Identify hybrids.* Among progenies having the PI's as maternal parents, select plants with a pest resistance derived from the varietal bulk to identify progenies likely to be true hybrids between the PI's and the varietal bulk. Selecting for a simply inherited resistance, such as anthracnose (Race 1), would be easiest; especially if plants can be evaluated as seedlings.

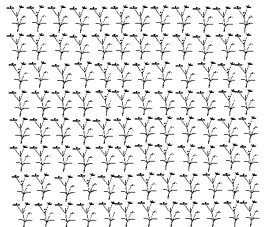
Regional Alfalfa Germplasm Pool Development



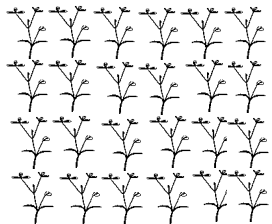
Step 3: Evaluate PI's for field performance. Select the most vigorous plants with good agronomic characteristics. 1 to 3 years.



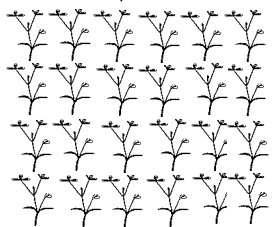
Step 4: Hybridize the selected PI's with a bulk of the most improved varieties used in the region. 1 year.



Step 5: Identify hybrids by selecting plants having PI's as maternal parents and having a simply inherited trait derived from the varieties. 1 year.



Step 6: Transplant hybrids to the field and intermate to promote genetic recombination (Syn. 1 generation). 1 year.



Step 7: Plant Syn. 1 seed to the field and intermate to further promote genetic recombination (Syn. 2 generation). 1 year.

STEP 6 & 7: *Promote genetic recombination.* Transplant hybrids to an isolated field and allow intermating to produce Syn. 1 seed. Then plant Syn. 1 seed to an isolated field (about 0.5 acre) to produce about 50 lbs. of Syn. 2 seed. This Syn. 2 seed would be available for distributing to researchers.

Result: GPP(s) semi-adapted to each region and having multiple pest resistance and other traits at levels where breeders can sample the pool and select plants with desirable traits for developing varieties. Adaptation of this type of GPP should be good enough to be useful to breeders for developing varieties. It would also serve as a reservoir of genes in a semi-adapted background for initial screening to identify genes that may be necessary for future breeding.

Anticipated time frame for developing useful regional GPP's is 5 to 7 years, depending on the traits desired for each region.

One additional suggestion is to develop a PI GPP for each region by intermating only the selected PI's; i. e., without crossing with the improved varieties. These GPP's may mask or dilute desirable traits less than would the PI x Variety GPP's. When searching for new traits, a logical sequence is to screen current varieties, regional PI x Variety GPP's, PI GPP's, the national GPP, then individual PI's.

Addition of New PI's and Improvement of the Regional GPP's.

Every 5 years, PI's collected since developing the regional GPP's should be evaluated and selected as was done when developing the original pools. Selected plants should be crossed with the most improved varieties used in that region, followed by identifying hybrids and subsequently producing Syn. 1 and 2 seed as described in Steps 3-7 for the original GPP's. Syn. 2 seed will be regarded as the Addition GPP's according to Hanson et al. (1972) terminology.

At this point more ideal procedures have been proposed by researchers to add new germplasm and improve the GPP. We anticipate, however, that both funds and researcher motivation needed for large-scale efforts may be available only for developing the original GPP's. Therefore, a simpler proposal is to make the Addition GPP available to researchers for incorporating into their own improved versions of the original GPP. For breeders who have not acquired seed of the original GPP by the time that the Addition GPP is available, seed of the Addition GPP should be mechanically mixed with the original GPP proportionate to those PI's already in the pool. Because adaptation of this Addition GPP should be nearly equal to that of the original GPP, mixing higher proportions into the pool as proposed by Barnes et al. (1977) is not necessary to offset the disadvantage in adaptation. And by crossing the new PI's to the most improved varieties, genes for newer traits in varieties will be added to the GPP, although at low to moderate frequencies.

Time frame for developing Addition GPP's probably will be the same as for the original pools, but presumably with less effort because of fewer PI's.

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Compilation of Recommendations

Genetic Vulnerability (page 8)

There is a need to broaden the germplasm base used in alfalfa cultivars released by the alfalfa seed industry. The introduction of wild perennial germplasm and very non-dormant types from the Saudi Arabian Peninsula are examples of the beneficial attributes available.

Enhanced support for germplasm evaluation and enhancement projects would encourage the use of a broader germplasm pool by plant breeders which would lead to more diverse alfalfa cultivars

Core Subsets

Perennials (page 19)

1. The perennial *Medicago* core subset should receive high priority for increase, maintenance and evaluation of new traits, e.g. disease and insect resistance, quality traits, bypass proteins, water use efficiency, etc.
2. Specialty collections should be evaluated such as the highly non-dormant alfalfas (Steve Smith), the Chinese *M. ruthenica* collection (Austin Campbell and Larry Teuber), and the VIR collection in Russia. A core should be developed from this material by evaluating these collections in comparison to existing perennial *Medicago* core entries from the same geographic proximity group or species. Based on these evaluations the perennial *Medicago* core subset should be increased to represent the same frequency as the original core, i.e. about 15 - 20% of the specialty collection.
3. Additional germplasm collections are needed for secondary species ("wild" non-sativa species) from southeast China (Himalayan Mountains), southwest China, and southern Russia (Caucases Mountains).
4. The core subset should be expanded as the number of accessions in the collection markedly increases. The new entries should be grown out and evaluated in comparison to existing perennial *Medicago* core entries from the same geographic proximity group or species.

Annuals (Page 19 - 20)

1. The annual *Medicago* core subset should receive high priority over the remaining collection for increase, maintenance and evaluation of additional traits especially freezing tolerance, disease and insect resistance.
2. The annual *Medicago* core subset should be evaluated for freezing tolerance, either to extend the autumn growth in the Mid-West or for over-wintering in milder climates such as California and south Texas.
3. The annual *Medicago* core subset should be evaluated for disease resistance such as Anthracnose, Phytophthora, Fusarium, Rhizoctonia, Pythium, Leptosphaeria, Phoma, and Rust. These diseases have been seen on some of the annual medics which have been grown in the U.S. If the annual medics are to be grown in the U. S., we need to know in advance if there are any disease problems.
4. The annual *Medicago* core subset should be evaluated for insect resistance to aphids and leafhoppers. These insects have been seen on some of the annual medics which have been grown in the U. S. If the annual medics are to be grown in the U. S., we need to know in advance if there are any insect problems.

5. The annual *Medicago* core subset should be evaluated for isozyme and molecular marker traits such as RFLPs, RAPDs, and microsatellites to further evaluate the collection for redundancies. The annual *Medicago* species are all self-pollinating and are thus relatively uniform within an accession.
6. No new collection trips are needed for the annual medics. There is an extremely large germplasm collection of 23,000 accessions located in Adelaide, South Australia. If additional accessions are needed of a particular species they can be easily obtained from this collection.

Rhizobium (Page 20 - 21)

1. A core subset needs to be developed using the data available in the GRIN system utilizing cluster analysis to identify subgroups. The final core subset should consist of between 65 to 75 isolates.
2. Once the core subset is established, the core should be evaluated for tolerance to low pH conditions. The isolates should be evaluated for both free living tolerance and symbiotic tolerance to low soil pH conditions.
3. Once the core subset is established, a determination of the symbiotic effectiveness of the core collection on the *Medicago* species core subsets should be conducted.
4. Any additional *Rhizobium* isolates should be sent to Dr. VanBerkum. Once a significant number of isolates has been added to the collection they should be evaluated in comparison to the core subset.
5. Evaluate the collection for *Rhizobium* strains by plant genotype interaction. There is a reasonable amount of information from other Rhizobia that there can be a substantial strain by cultivar origin. Since we recently identified strains of Rhizobia that will infect both alfalfa and soybean in China it seems likely that we could expect some significant interactions between genotypes from diverse locations. In a study that Don Barnes and I reported on we noticed some marked difference in the Rhizobia obtained from the same genotypes in different locations.

Assessing the collection for current gaps (Page 21)

- Acquisition must be undertaken following guidelines arising from the Convention on Biological Diversity
- Gaps in the U.S. collection can frequently be filled by other collections around the world.
- The ACGC needs to establish guidelines that define when efforts warrant the acquisition of germplasm for inclusion in the U.S. collection, versus making use of germplasm that is available from other collections.

Primary Gene Pool

United States (Page 23)

Given that funding can keep pace with a growing collection, the NPGS active collection should support the preservation of US cultivars that are no longer available through commercial sources. Although alfalfa is the fourth most widely grown crop in the US, NGO interest in conserving heirloom alfalfa varieties is poor to non-existent. Efforts need to be made to secure representatives of older varieties before seed stocks deteriorate. Materials registered with Crop Science should enter the active collection when the developer no longer distributes seed or it is not available from commercial sources. As non-CSR material is commercially retired, developers are encouraged to enter the obsolete cultivar into the

NPGS collection. When PVP expires, cultivars should become part of the active collection, as PVP suggests the material is of exceptional value. At current levels of funding, a policy that preserves US cultivars as they are retired from commercial production cannot be implemented.

Cultivars of Other Nations (Page 23)

- US users can make use of collections held in other institutes. An excellent source of information regarding the contents and availability of germplasm collections is the IPGRI Directory of Germplasm Collections <http://www.cgiar.org/IPGRI/doc/dbintro.htm>)
- Areas where collection and preservation of cultivars may be beneficial to both the United States (based on limited representation in US collection) and corresponding countries (based on limited support for ex situ conservation, and lack of availability) include the Middle East, South Africa, and Mexico.
- Nondormant alfalfa continues to be underrepresented in the US collection. Efforts should be made to acquire the alfalfa germplasm collected by ICARDA during the 1970s, 1980s and 1990s.

Landraces and primitive cultivars (Page 24)

- U.S users can make use of collections held in other institutes. Many of the European nations contain extensive collections of local landraces and primitive cultivars. For example, the European perennial *Medicago* database contains passport data on 2888 accessions of 32 taxa, including 505 land race accessions stored in 23 European genebanks <http://www.cgiar.org/ecpgr/platform/Crops/Medicago.htm>) .
- Priority geographic areas recognized by the ACGC in 1988, and not yet collected include the Himalayan region of India, south and northeast regions of China, Sudan, Egypt and the Arabian Peninsula. Other geographic areas with limited representation of landrace materials include South Africa, Northern Kazakhstan, and adjacent Central Asian countries (i.e. Kyrgistan, Uzbekistan, Afghanistan), Iran and Iraq.
- Past CGC reports have placed emphasis on cultivated alfalfa that is adapted to acid soils and saline soils. There is increasing interest in developing alfalfa with increased water use efficiency, so drought tolerant germplasm would also be valuable to collect

Wild *Medicago sativa* ssp. *sativa* (Page 25)

- Considering that the center of origin for *Medicago sativa* subsp. *sativa* centers around Iran, Iraq, Azerbaijan and Turkmenistan, explorations to collect wild material here, and in adjacent areas such as Southern Kazakhstan, Uzbekistan, Afghanistan, and Tajikistan would be valuable, since wild-type *M. sativa* subsp. *sativa* from this region are under-represented in the US collection.
- Efforts should be made to acquire germplasm collected during the 1980's and 1990's through IPGRI-funded trips into Central Asia.
- Priority geographic areas recognized by the ACGC in 1988, and not yet collected include Georgia, Crimea and Southern Caucasus mountains

Medicago sativa complex (Page 26)

Fill the following geographic gaps in US collection through collection and donations. Species are listed in priority order:

1. *Medicago sativa* subsp. *glomerata* (2x=16), subsp. *glutinosa* (2x=32) are grouped together since chromosome counts have not been obtained to distinguish among the two subspecies: Southern Europe, Caucasus region, Algeria, Tunisia
2. *Medicago prostrata*: Albania, eastern Austria, Greece, Czechoslovakia, Hungary, Romania, Yugoslavia
3. *M. sativa* subsp. *falcata*: Northern Libya, Eastern Morocco, Lebanon, Syria, Turkey, Central Asia (Turkmenistan, Afghanistan, Uzbekistan, Tajikistan, northern Kazakhstan), Northeastern China, Northwest India, Northern Pakistan, Southern Caucasus region, Ukraine, Northeastern Europe (Belarus, Lithuania, Latvia, Estonia)
4. *M. sativa* nothosp. *varia*: Northwest Iran, Syria, Northern, Western, and Central Europe

Secondary Gene Pool (Page 26)

Fill the following gaps in US collection through collection and donations. Species are listed in priority order:

1. *M. saxatilis*: All areas of species distribution (see Appendix 1)
2. *M. papillosa*: Northern Armenia, Southern Georgia, Turkey
3. *M. sativa* nssp. *tunetana*: All areas of species distribution (see Appendix 1)
4. *M. sativa* ssp. *caerulea*: Crimea, Central Asia

Tertiary Gene Pool (Page 27)

Fill the following gaps in US collection through collection and donations. Species are listed in priority order:

1. *M. dzhawakhetica*: Caucasus Mountains, Russia
2. *M. daghestanica*: Caucasus Mountains, Dagestan
3. *M. rupestris*: Lower Crimea Mountains, Ukraine
4. *M. hybrida*: Southern France
5. *M. rhodopea*: Southern Bulgaria
6. *M. pironae*: Alps of northeastern Italy
7. *M. cancellata*: Russia (North Caucasus, Caspian region)

Cultivated Medics (Page 27)

1. Most cultivated varieties of annual medic have been developed in Australia. Although they can be used in the southern latitudes of United States (notably California) they lack cold tolerance to more northern latitudes. Acquisition of accessions that are adapted to the northern boundaries of the species distribution may help us develop annual medic cultivars that can be used as cover crops or in ley farming in Washington, Montana and Wyoming.

2. The following cultivated species has been classified by Prosperi et al. as being vulnerable due to lack of representation in *ex situ* collections: *M. arborea*, *M. italica*, *M. littoralis*, *M. rugosa*, *M. scutellata*. Priority should focus on obtaining complete ecogeographic coverage of these taxa.

Non-cultivated Medicago species (Page 27)

Perennial species (Page 27)

Efforts should be made to collaborate with other PGR institutes to conserve these resources in gene bank collections and in natural reserves.

Annual species (Page 28)

- Efforts should focus on determining if all accessions of the Lesin's collection should receive PI numbers. We need to collaborate with other PGR institutes to conserve those annual species that have potential use, or are vulnerable to genetic erosion.
- Efforts should be made to collaborate with other PGR institutes to conserve these resources in gene bank collections and in natural reserves.

Evaluations

Perennials (Page 28)

- C Efforts to verify ploidy have focused on *M. sativa* subsp. *falcata*, and *M. sativa* subsp. *caerulea*. Work needs to be done to confirm ploidy status of the Medicago sativa subsp. sativa collection
- C Approximately a third of the *Medicago sativa* subsp. *sativa* collection has been evaluated for disease, insect, growth and morphological descriptors. To fully utilize the collection, efforts need to focus on evaluating the remaining two thirds of the alfalfa accessions. This will not be possible without increasing the baseline budget of the NPGS Forage Legume CRIS.

Annuals (Page 28 - 29)

- Data on only one disease descriptor has been gathered, and for only the core collection, thus, additional diseases need to be screened.
- Some morphological descriptors have been taken, however, the majority of the accessions in the collection do not have even basic morphological descriptors. Thus a complete set of morphological descriptors needs to be obtained.

Over - all (Page 29)

- C Efforts should focus on acquiring a complete set of descriptors on **all** accessions, especially all alfalfa accessions. A complete data set would allow users to understand the diversity within the collection, and enable users to fully use the collection.
- C The currently established core subsets should be re-evaluated and adjusted once descriptor data has been obtained on the entire collection.
- C Accession classification based on molecular markers needs to be compared to classifications based on morphological and ecogeographic descriptors. Can we identify a combination of descriptors (that

are easily and efficiently obtained) that group accessions within the collection into meaningful clusters? Can we establish a method to routinely classify newly acquired accessions to ensure the collection continues to reflect a wide range of diversity but with a manageable number of accessions?

- C Evaluations directed at traits that can broaden the use of alfalfa need to be carried out. For example, there is current interest in the use of alfalfa as a medicinal herb, for phytoremediation, use as biofuels, cover crop, etc. The production of organic hay is increasing. The genetic variation within the germplasm collection for these uses has been minimally explored .
- C Accession evaluation and observation data needs to be acquired and placed into the GRIN database in a format that allows users to draw valid comparisons across all accessions evaluated for that trait.

Enhancement (Page 30 and Appendix 4 Page 45)

1. Develop a *national GPP* to serve as a source of new recombinants among the total diversity of germplasm available. This pool potentially would provide recombinants not obtained in the more genetically restricted regional pools described below.
2. Develop *regional GPP's* that are adapted to each of four regions in the USA. Under the typical management practices for alfalfa in a particular region, the regional pools should be agronomically more acceptable than the national GPP. They also should have at least moderate levels of the traits available in varieties currently used in that region, plus genes that may be used to improve cultivated alfalfa.
3. An ARS alfalfa research geneticist position was established in 1999 at Prosser, WA. with an emphasis to carry out additional germplasm enhancement work. Enhancement work by this position should continue to be encouraged and supported.

Collection Preservation (Page 31)

- C Original seed needs to be inventoried with seed counts. Germination data on original seed lots needs to be obtained on all accessions with sufficient seed. Original and regeneration seed lots need to be stored at -15 to -20 C.
- C Germination data needs to be obtained on increased material to establish a baseline to monitor seed viability and to provide a check to verify that regeneration procedures are high quality.
- Research needs to be carried out to verify if our standard plant population size during regeneration is adequate for limiting genetic drift, and if genetic change in accessions is minimized during the standard regeneration process.