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# **Clover and Special Purpose Legumes Germplasm Status Report**

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## Abstract

The Clover and Special Purpose Legume (CSPL) Crop Germplasm Committee (CGC) is responsible for all temperate and tropical non edible legumes except alfalfa and the annual *Medicago* species. This includes some 230 species of *Trifolium* and an additional 100 or more genera of temperate and tropical forage legumes. These species are a major component of grassland agriculture and are of paramount importance for meat and milk production and for soil conservation. These legumes are adapted to a wide range of soil and climatic conditions. The need for forage legumes as a critical component of sustainable agriculture production systems is more important today than ever. There is very little private research being conducted with these legumes. Therefore, public support is essential to continue to supply the agricultural community with adapted clovers and special purpose legumes.

Since our 1996 report, the committee has made excellent progress with our stated goals. We have identified core subsets in all major species and have regenerated seed of the red and white clover and trefoil core subsets. We have evaluated a variety of biochemical, morphological and agronomic characters for the red and white clover core subsets and the trefoil core subset. This information is in GRIN. Evaluations of descriptors for over 40 traits on over 25 species have been completed during this period (Evaluation Summary, Appendix 1).

In this report we highlight: 1). Present germplasm activities by the states and USDA-ARS; 2). status of crop vulnerability in the clovers and special purpose legumes; 3). needs for collection, preservation, evaluation, and enhancement in the clovers and special purpose legumes; and 4). recommendations for priority actions in these legumes. In general, forage-oriented research activities with these legumes have declined since 1987, but interest in using these species to support sustainable farming systems, phytoremediation and value-added products such as pharmaceuticals is increasing. Most species are not vulnerable to germplasm erosion, but specific needs for collection, germplasm exchange and conservation are highlighted. Regeneration of present collections (both older accessions and newly collected materials) is deemed as high priority to improve the quality of working collections and provide backup in NCGRP. Germplasm evaluation and enhancement of selected traits is seen as a continuing high priority need.

Specific recommendations include:

1. Reinstate funding for germplasm evaluations.
2. Conduct seed increases of annual and perennial clovers and other temperate and tropical legume accessions, with emphasis on replacing open-pollinated seed lots with seed produced under controlled pollination using original seed; increasing accessions that require special handling due to low seed quality and quantity and increasing seed of wild species.
3. Systematic evaluation of all descriptors for core collections of *Trifolium* spp. and update and standardize existing GRIN evaluation data
4. Collect annual and perennial *Trifolium* spp. and other special use legumes from Europe, Africa, and from the Western and Eastern U.S with a special emphasis on obtaining species closely related to the cultivated species.
5. Integrate the tropical legume collection at Ona, FL (formerly at IRREC, Ft. Pierce, FL) into the USDA NPGS including assignment of PI numbers.
6. Develop and incorporate descriptors for all special purpose legumes into GRIN.
7. Establish core collections of other CSPL species.

8. Work with appropriate agencies to establish *in situ* preservation sites for native U.S. CSPL species.
9. Collect *Rhizobium* strains for western North American clovers.

The CSPL-CGC members have a high level of dedication and commitment to germplasm activities. The loss of germplasm evaluation funding to budget changes at the ARS Forage and Range research Lab at Logan, Utah in the mid 1990's has eliminated funding for evaluation efforts in our species since 1998. We feel our evaluation activities have been extremely productive and merit continued and increased support. We sometimes feel that the advice and counsel of the committee is not sought in the early stages of germplasm decisions, but we seek to continue to work with the NPGS leadership to improve the quality of the collections.

## I. Introduction

In the report which the Clover and Special Purpose Legume (CSPL) Crop Germplasm Committee (CGC) submitted in March 1987 and updated in 1996 we stated that the overall economic worth of the clovers and special purpose legumes to U.S. agriculture is difficult to assess since statistical reporting services have ceased to report the usage of these species or have combined the information with other species. This situation continues unchanged and documentation of economic worth of these commodities remains a subject of considerable speculation. Nevertheless, these legume species remain a major component of grassland agriculture and are of paramount importance for meat and milk production and for soil conservation. The clovers are adapted to a wide range of soil and climatic conditions. Unlike alfalfa, which is grown extensively on the better soils in the North Central area of the U.S., the clovers and other special purpose legumes have a much wider area of adaptation and ability to adjust to much wetter and more acid soils. The perennial clovers, primarily red clover (*Trifolium pratense* L.) and white clover (*T. repens* L.), are grown extensively in the North Central and Northeastern areas and in the South as winter annuals (Smith, et al., 1985; Van Keuren and Hoveland, 1985). According to Gibson and Hollowell (1966) white clover grows in every state of the U.S. and occurs widely in pastures throughout the humid and irrigated regions. Red clover, probably the most important forage legume other than alfalfa, has long been recognized as a hay and silage crop and has recently become important in Eastern U.S. for deferred fall and winter grazing. Annual clovers, such as arrowleaf (*T. vesiculosum* L.), berseem (*T. alexandrinum* L.), crimson (*T. incarnatum* L.), and sub (*T. subterraneum* L.), are extensively grown for winter grazing in the Southeastern states. Other clovers (rose - *T. hirtum* All., strawberry - *T. fragiferum* L., ball - *T. nigrescens* Viv., and persian - *T. resupinatum* L.) are of significant importance for hay and pasture in specialized areas throughout the Southern and Western regions of the U.S. (Van Keuren & Hoveland, 1985). Birdsfoot trefoil (*Lotus corniculatus* L.) has taken on increased importance in the area of pasture renovation, especially on marginal lands. Numerous species of the genera *Aeschynomene*, *Astragalus*, *Coronilla*, *Desmanthus*, *Desmodium*, *Lathyrus*, *Lespedeza*, *Lotus*, *Lupinus*, *Melilotus*, *Onobrychis*, and *Vicia*, to name a few, are also of significance to agriculture as forage legumes and in the area of soil conservation and reclamation. The CSPL are used in orchards and vineyards as winter ground cover and for habitat for predaceous arachnids and insects in biological control programs. In addition, CSPL contribute to the N available to subsequent crops and all species are considered to be extremely effective for improvement of soil tilth. . An increasingly important area of use of CSPL is for wildlife habitat enhancement. Some are used directly as food sources while others contribute to cover. A second area of increasing importance is the use of CSPL as phytonutrients. For example, the phytoestrogen compounds found in red clover, are now widely used in nutritional supplements manufactured to relieve peri- and menopausal complaints.

Various estimates have been reported based on seed disappearance figures which indicate that in excess of 10 million ha are seeded to clovers and special purpose legumes annually (Taylor, 1985 and Taylor and Quesenberry 1996). Approximately 4 million ha of red clover are grown annually. Assuming a yield of 5 metric ton per hectare and at \$80.00 per ton, the economic worth of this species alone is 1.6 billion dollars annually or \$400 per hectare. This does not consider the value of red clover as a pasture or as a seed crop, the latter returning between 23 and 27 million dollars annually (Smith, et al., 1985). In contrast, the estimated net worth of alfalfa (*Medicago sativa* L.) to our agricultural economy is 5.0 billion dollars annually. Therefore, red clover alone is worth one-third that of alfalfa and when considered collectively the forage legumes other than alfalfa have a significant impact on our food and fiber production. Estimates for white clover are exceedingly difficult because of its volunteering nature that varies from year to year. In some so-called "white

clover years” many, if not most, pastures in eastern United States contain large amounts of volunteered white clover. From the tropical legume standpoint, the acreage involved is small and almost entirely limited to Florida, the Gulf Coastal regions, and the Rio Grande Valley of the continental USA. However, research conducted in this area has broad implications to Caribbean countries where these legumes have great potential for improvement of animal production.

## II. Present Germplasm Activities

### A. Germplasm Collections:

1. The location and number of accessions of *Trifolium* species in the USDA-NPGS, are summarized in Table 1. Primary repository sites and curators at these sites are Griffin, GA - Dr. Gary Pederson; Lexington, KY - Dr. Norman Taylor; and Pullman, WA - Dr. Stephanie Greene. The two P.I. stations have the larger number of accessions, but the *Trifolium* curator collection at Lexington, KY has the largest number of different species. In addition, a large number of increases are located in the *Trifolium* curator collection at Lexington.
2. A listing of the other special purpose legume genera which fall under the scope of the CSPL CGC is contained in Table 2. Primary repository sites and curators at these sites are Ona, FL - UF/IFAS; Griffin, GA - Dr. Brad Morris; Ames, IA - Mr. David Brenner; and Pullman, WA - Dr. Clare Coyne, and Dr. Barbara Hellier.

### B. Primary Research Locations:

Research associated with clovers and special purpose legumes is being conducted at numerous locations throughout the U.S. Some of these programs conduct primarily forage management and evaluation research, but major integrated breeding and evaluation programs are located in Alabama, Florida, Kentucky, Minnesota, Texas, Utah and Wisconsin. Compared to a similar listing in a 1987 report, this list reflects a continued decline in both SAES and ARS positions conducting research on clovers and special purpose legumes. Since 1987, positions have been terminated at California, Colorado, Florida, Georgia, Idaho, Michigan, Mississippi, Missouri, Ohio, Pennsylvania, South Carolina and West Virginia. Only four private firms are involved in red and white clover cultivar development to a limited extent (less than 5% of their total effort). Since 1996 new or redirected programs by ARS have been initiated in Oklahoma and Kentucky.

A brief description of activities at the major locations follows:

#### Alabama:

The major thrust of research is on breeding sunnhemp (*Crotalaria juncea* L.), crimson clover (*Trifolium incarnatum*), sericea lespedeza (*Lespedeza cuneata*), vetches (*Vicia* spp.), red clover (*Trifolium pratense*), white clover (*Trifolium repens*), and caley peas (*Lathyrus hirsutus*). Research conducted includes genetics, ecophysiology, germplasm evaluation, breeding techniques for forage crops and utilization of these species. Improved cultivars of sericea lespedeza, common vetch (*V. sativa*), hairy vetch (*V. villosa*), crimson clover and caley peas have been released in recent years.

#### Arkansas:

Major focus of legume research at the USDA-ARS station near Booneville is on the agroecology, management, and establishment of clovers and special purpose legumes. This program is associated with the South-Central Family Farms Research Unit.

#### Florida:

Major effort in clover and special purpose legume research is associated with the University of Florida Institute of Food and Agricultural Sciences, and at the USDA Subtropical Agricultural Research Station (STARS) at Brooksville, FL. Breeding effort is centered at Gainesville with a focus on red, white, and crimson clover. Additional breeding research is being conducted on *Arachis* and *Desmodium*. Evaluation research is being conducted on these and several other miscellaneous tropical legumes. A significant germplasm maintenance program for tropical legumes is located at Ona with a focus on germplasm evaluation. Germplasm is also being evaluated at Ona, often under animal grazing. Research at the USDA STARS involves evaluation of germplasm and breeder lines of *Trifolium* spp., *Arachis glabrata*, and native legumes. Germplasm evaluation in northwest Florida at Quincy and Jay has focused primarily on *Trifolium* spp.

#### Georgia:

Research focused on management and germplasm evaluation of *Trifolium* spp., *Lotus corniculatus* and other special purpose legumes is being conducted at the University of Georgia, Athens.

Research focused on new uses of the special purpose legumes is being conducted at the USDA, ARS, Plant Genetic Resources Conservation Unit (PGRCU), Griffin, GA. Research efforts on the genetic diversity, molecular characterization, and phytochemical characterization for dietary fiber, lectin, and isoflavones among *Lablab purpureus* genotypes is being conducted at the USDA, ARS, PGRCU, Griffin, GA. in cooperation with Phyllogix, Inc., Waltham, MA.

Research continues on the genetic variability of *Mucuna pruriens* for root-knot nematode reduction when used as a soil amendment. Regeneration research continues with emphasis on those special purpose legume genera that are difficult to increase including *Leucaena* species. Working collections of annual *Trifolium* spp. and numerous genera of tropical special purpose legumes are maintained at the PGRCU.

#### Kentucky:

A major research effort on the genetics, pathology, taxonomy, and breeding in *Trifolium* is concentrated at the University of Kentucky, Lexington, KY. Emphasis is on basic research at the molecular and whole plant level in numerous species with developmental research directed primarily to red and kura clover. The *Trifolium* species curator collection maintained by N.L. Taylor is located at Lexington. ARS forage research programs have recently expanded at Lexington.

#### Minnesota:

A major research effort on improving seedling vigor and developing pasture ideotypes of *Trifolium ambiguum* is being conducted at the University of Minnesota. Other research activities are investigating establishment techniques, fertility requirements, dinitrogen fixation potential, grazing management strategies, and seed production potential. Breeding research continues on *Lotus corniculatus* emphasizing glyphosate tolerance, disease resistance, and enhanced winter hardiness.

In cicer milkvetch (*Astragalus cicer*), selection for improved palatability to grazing ruminants has led to the development of populations with improved grazing potential for the upper Midwest.

#### Texas:

Basic and applied research on annual clovers is conducted at Beeville, Stephenville and Overton, Texas with major emphasis on breeding, management, and utilization in diverse environments. At Overton, Texas the emphasis is on breeding, genetics, and management of *Trifolium* species, *Melilotus alba* and *Lablab purpureus*. Research at Beeville, Texas has focused on germplasm evaluation, selection and management of various tropical legumes and recently on *Desmanthus bicornutus* and other *D. spp.* from the southern USA and Mexico. Research at Stephenville is focused on both native and introduced forage legumes. These programs are funded by the Texas Agricultural Experiment Station. Management and physiology of various forage legumes are part of a broad forage research program at Lubbock, Texas funded by Texas Tech University.

#### Washington:

Working collections of the perennial *Trifolium* species and *Lotus* species are maintained and evaluated at the USDA National Temperate Forage Legume Genetic Resources Unit, in Prosser, WA. Research activities include population genetic studies using molecular markers to better understand how in situ reserves can be established, using *Trifolium thompsonii* as a model species.

#### Wisconsin:

The major emphasis of the Wisconsin clover genetics and improvement program is on host plant resistance; genetics, cytology, cytogenetics and tissue culture; germplasm development; and breeding strategies for red and kura clover and birdsfoot trefoil. The major goals are to elucidate genetic mechanisms and their interactions which contribute to the improvement of these species and to develop high quality, persistent germplasm. To this end both basic and applied research is jointly supported by ARS and the Wisconsin AES.

#### Utah:

At the USDA-ARS Forage and Range Research Lab. in Logan, Utah one SY works on the improvement of alfalfa (*Medicago sativa* L.) and special purpose legumes. The breeding efforts of special purpose legumes include birdsfoot trefoil (*Lotus corniculatus* L.), cicer milkvetch (*Astragalus cicer* L.), kura clover (*Trifolium ambiguum*), sainfoin (*Onobrychis viciifolia* Scop.), and Utah sweetvetch (*Hedysarum boreale*). The major effort is in improving seedling vigor, tolerance to extended periods of drought and persistence. Some research is conducted at Utah State University on management and livestock utilization of special purpose legumes.

### **III. Status of Crop Vulnerability**

#### **A. *Trifolium* species:**

Genetic vulnerability among the cultivated clovers, *T. pratense*, *T. repens*, *T. hybridum*, *T. incarnatum*, *T. vesiculosum*, etc. was reviewed by Taylor, et al. (1977) and Morris and Greene (2001). The conclusion reached in 1977, which still holds today, is that the trend in number of cultivars and genetic diversity within cultivars in the United States is downward because of emphasis

on other crops and narrowing of gene base by breeding. Although broad genetic diversity exists in the germplasm collections of most cultivated perennial clover species because they are inherently variable due to a cross-pollinated, heterozygous, and heterogeneous nature, there has been no systematic attempt to preserve obsolete U.S. clover cultivars, which have considerable usefulness in breeding efforts due to their high level of agronomic adaptation. As clover breeding and research programs are phased out, and seed companies discontinue commercial varieties, obsolete cultivars are likely to be lost- especially older cultivars. Research with molecular markers suggested limited genetic variability among U.S. cultivars and NPGS accessions of *Trifolium incarnatum* (Steiner, et al., 1995). Morris and Greene (2001) describe several conditions that are contributing to the erosion of genetic diversity within the genus on a global scale. For the cultivated species, traditional varieties are being abandoned in favor of highly productive forage crops such as alfalfa and modern-day clover cultivars. Native and naturalized ecotypes of cultivated species are also threatened by changing land-use patterns (Morris and Greene 2001). Taylor, et al. (1977) concluded that germplasm resources of *Trifolium* species related to cultivated clovers are believed to be in a more hazardous position than the cultivated species. The NPGS collection has very limited representation of species that are closely related to the cultivated clover species. Greene (1998) reported that only four percent of the NPGS *Trifolium* collection consisted of species in the secondary and tertiary gene pool of the cultivated species. Morris and Greene (2001) reported that 36 species of *Trifolium* are recognized by the IUCN as threatened species. Representation of wild species has broadened considerably since 1987 as a result of a number of collection trips that focused on *Trifolium* species from uncollected regions in Eastern Europe and the United States. Germplasm collection expeditions in Europe have been completed to Romania, the former Yugoslavia, twice to Bulgaria, and to the Republic of Georgia. These expeditions have added about 450 collections of over 50 different species to the USDA Plant Germplasm System, including new accessions of *T. vesiculosum* and related species, and two species not formerly in the U.S. collection. With completion of these trips, only about fifteen *Trifolium* species native to Europe and the Middle East are not represented in the U.S. collection by one or more accessions; however, many species still are limited in numbers of accessions. About eight species native to Africa are also not represented in the U.S. collection.

The second focus has been *Trifolium* species from the Western United States. Our review of the USDA collection during 1987 showed that about 30 *Trifolium* species native to the Western U.S. were not represented in the collection and that numerous others were represented by only limited numbers of collections. Three collection expeditions to this region [California (1994), Oregon and Washington (1994), and California (1995)] resulted in collection of over 140 accessions. Included in this group were over 25 species not previously contained in the USDA NPGS, including some seven species listed by the USDA as potentially threatened or endangered. These collections have greatly improved the diversity in the USDA NPGS collection. Presently, only about five species native to the Western U.S. are not represented in the USDA NPGS collection.

Recent phylogenetic analysis using molecular techniques and DNA sequencing has changed our concepts regarding *Trifolium* species relationships (Ellison et al. 2005). One significant change is the inclusion of white clover in the new Section *Trifoliastrum*. Eight of the fourteen species in this new Section appear to be genetically close enough to white clover to be possible ancestors and/or to be potential genetic resources for improvement programs (Ellison et al. 2005).

## B. Other CSPL genera

Special purpose legumes including species in the following genera *Aeschynomene*, *Astragalus*, *Canavalia*, *Centrosema*, *Chamaecrista*, *Crotalaria*, *Dalea*, *Desmanthus*,

*Desmodium*, *Glycyrrhiza*, *Indigofera*, *Lathyrus*, *Lespedeza*, *Leucaena*, *Lupinus*, *Onobrychis*, *Senna*, *Strophostyles*, *Stylosanthes*, *Trigonella*, and *Vicia* are wide spread throughout the United States. While it is possible to describe the distribution of these species assigning occupied acreage to them would not be possible. Most are found on rangelands and as individuals would not be considered of high economic importance. Exceptions might include sainfoin (*Onobrychis viciifolia*), cicer milkvetch (*Astragalus cicer*), partridge pea (*Chamaecrista fasciculata*), illinois bundleflower (*Desmanthus illinoensis*), *Desmodium* spp., and *Lespedeza* spp. which are used for improving rangelands with introduced species. Individual species from the list of genera would most frequently be used when reseeding many of the rangelands in the United States.

With few exceptions disease and insect pests of the species within these genera are not well described in the literature. This lack of knowledge can primarily be attributed to the relatively small economic importance of the individual species. The exceptions would be with those species that are more commonly available in the commercial markets. Among the species sainfoin, *Desmanthus*, and *Lespedeza* would be the most widely planted and are known to be susceptible to root and crown diseases. Even so, the vulnerability of these and other species should be considered low. Table 2 shows the large number of accessions available in each genus. This is a highly diverse collection and likely represents much of the diversity for these genera. The genetic diversity within an individual accession, or cultivars where available, would be substantial and would not be at risk from a biotic stress like highly homozygous crops.

In most of these CSPL genera, collections of the widespread and economically important species are sufficiently large so that extensive collecting is not warranted. Both the widespread and minor species should be collected in under-represented areas. Regional and global ecosystem and geographic information system databases are excellent sources of information about these environments (Steiner and Greene, 1996). However, there are some specific germplasm needs that can be met through further collection efforts.

There is increasing interest in incorporating native legumes into wild fire revegetation activities, particularly in the Western United States. The USDA collections contain a limited number of native rangeland legume species, particularly in the genera *Astragalus*, *Onobrychis*, and *Glycyrrhiza*. Collecting is needed to provide geographically adapted germplasm that can be used to develop leguminous species to support revegetation efforts. Many native legumes contain species that are vulnerable, especially in the genus, *Astragalus*. An exploration in 2004 focused on the collection of rare and common native legumes in the arid rangelands of Oregon and Idaho. Further collecting is warranted of native legume species indigenous to the United States, especially those species that have been identified as promising in supporting revegetation activities and species that are at risk for extinction.

A number of minor and endangered species are absent from the collections and should be acquired. For example, Greene (1999) reported that eight new world *Lotus* species were identified as threatened or endangered by the IUCN in 1994, and that none of these species were represented in ten major ex situ collections that were surveyed. Twenty-one old world species were similarly identified by the IUCN, and only nine were represented in the major ex situ collections surveyed (Greene 1999)

*Lotus* species are present throughout most of the USA and are used primarily as a pasture

and hay crop for ruminant livestock. Lotus species tend to tolerate poorer soil conditions than other forage legumes such as alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) There is a lack of vulnerability in Lotus, specifically in birdsfoot trefoil (*Lotus corniculatus* L.) due to the varieties used in agriculture being synthetics resulting from intermating numerous parent plants which results in a heterogeneous mixture. Since the varieties are produced through cross-pollination, each plant is heterozygous and a unique genotype. A core collection representing the majority of the diversity present in birdsfoot trefoil has been developed (Steiner et al., 2001)

Birdsfoot trefoil is susceptible to numerous pathogens and pests under certain environmental conditions. The highest impact crop diseases are the crown and root rot complexes which become increasing more prevalent and are most destructive in the more humid and warmer regions of the USA. *Lotus* is also susceptible to root-knot and root-lesion nematodes which can increase susceptibility to pathogens. Occasionally fungal pathogens such as *Rhizoctonia* and *Mycocleptodiscus* have limited stand productivity and/or longevity.

The tropical legume collection at Ona, FL (originally at Ft. Pierce) represents a major resource of these genera for the United States. This collection was developed primarily by the dedication of one faculty member, Dr. A. E. Kretschmer, Jr., and has been transferred to Ona, FL under the management of Dr. Rob Kalmbacher but could be in jeopardy upon his retirement. Other major world collections of clovers and special purpose legumes exist at CSIRO, Australia; CIAT, Columbia; ILCA, Ethiopia; EMBRAPA, Brazil; and VIR, Russia. The larger collections of tropical legume genera are located at CSIRO, CIAT and EMBRAPA. Many cultivars of tropical legumes are direct releases of plant introductions with minimal selection for improvement; however, more bred cultivars are being released.

#### **IV. Germplasm Needs**

Morris and Greene (2001) concluded that current gaps in the NPGS *Trifolium* collection include 1) cultivars and landraces of red and white clover originating from China, Japan, South America and South Africa; 2) obsolete cultivars developed by the United States, 3) minor-use species; and 4) wild relatives of cultivated species.

##### **A. Collection**

###### **1. *Trifolium* species**

Numbers of accessions of the two primary cultivated species (red and white clover) holdings are relatively large and core collection subsets have been designated. Core subsets have also been designated in rose, persian, berseem and subterranean clover, all of which are represented by more than 100 accessions. Crimson and arrowleaf clover are represented by limited numbers of accessions; however, recent collection expeditions and germplasm exchanges have added new accessions of these species. Addition collections of crimson and arrowleaf from areas of origin would add diversity to the limited existing germplasm accessions.

As indicated in III. above, collection trips since the 1987 report have been completed to Eastern Europe and the Western U.S. to collect clovers. All these expeditions have focused on less common species represented by only a few accessions, rather than the cultivated species. At present, about 30 *Trifolium* species considered as valid by Zohary and Heller in The Genus *Trifolium* are still not represented in the NPGS. About 12 of the species listed by Zohary and Heller are of doubtful authenticity, being based on only one herbarium specimen, or needing further study. Areas of

Europe and the Middle East where collections are still deficient and where uncollected species may be found include Albania, Italy, Greece, Sicily, France, Turkey, Armenia, Azerbaijan, Iran, and Iraq. Areas of Africa where uncollected species are known include Ethiopia, Uganda, Kenya, Tunisia, and Algeria. These areas are widely scattered, but an exploration trip to Albania would be beneficial since many wild trifolium species are located there. Also an exploration trip to Greece and Italy would be beneficial to collect the following wild relatives of red and white clover: *T. diffusum*, *T. pallidum*, *T. nigrescens*, and *T. uniflorum*

Additional collection efforts also are needed in the Eastern and Western U.S. Of the seven clover species native to the Eastern U.S., three are represented by less than three accessions and one (*T. polymorphum*) is only represented by one accession from South America. Additionally, at least five clover species from the Western U.S. are not collected and several are represented by three or fewer accessions. These areas need additional collection efforts.

### 2. *Lotus* species

*Lotus* species should be collected from the under-represented areas. A large number of accessions of the cultivated species *L. corniculatus* exist in the present U.S. collection, but the genus consist of about 150 species of which acquiring these species would be of value. Specifically, the Asian members of the *L. corniculatus* group should be acquired, especially from China and Central Asia and any additional accessions collected in near-equatorial highlands (Ethiopia, Yemen, etc.); Himilayan regions; and eastern and southeastern Black Sea regions (Georgia). The South American *L. corniculatus* and *L. tenuis* material, especially from humid regions of Argentina and Uruguay, are a potential source of resistance to fungal diseases. Species in North and East Africa should be further collected as a source of adaptation to arid conditions. The accessions of the natives in North America have not been systematically collected using the methods developed for Eurasia (these are taxonomically classified as "Lotus", but unrelated to the Eurasian taxa).

The remainder of the genus is of interest for systematic studies and for landscaping. Novel diversity in the Canary Islands, Azores and other Atlantic islands should be acquired, in some cases by exchange with other collections. There is one endangered taxa in California. Many new world species are absent from the collection.

### 3. Other temperate special purpose legumes

#### *Astragalus*

The NPGS should not attempt to acquire all the 2,000 species of this genus. *Astragalus canadensis* should be well collected for use as a forage. Additional accessions of the selenium accumulating species *bifulcatus* and *ramosus* should also be collected. Additional collections are needed of *A. membranaceus*. Nine *Astragalus* taxa are on the endangered species list. Most of these are native to the Western United States and should be collected and added to the NPGS, especially *A. purshii* and *A. filipes* which are of interest in rangeland and wildfire revegetation efforts.

#### *Coronilla*

*Coronilla varia* (now *Securigera varia*) should be collected in Africa, especially from south of the Sahara. The African material would have potential for adaptation in the southern United States. Europe should not be further collected. Because of (presumed) self-incompatibility, regeneration may be impossible if the parent population size is too small. Regeneration is difficult even if the original seed lot is adequate. The other *Coronilla* species should only be collected if the species are absent from the collection and the seed sample is excellent. These minor species are a low priority.

#### *Dalea* and *Marina*

Because of a new interest in using the annual *Dalea leporina* in crop rotations, the other 15 annuals (Barnaby, 1977) should also be acquired. Most of the annual species are native in the Southwest U.S. or Mexico. The perennial species are difficult to regenerate and there is little demand so we should acquire them only if there is a good reason (species endangerment) and a large, healthy original seed lot.

#### *Galega*

Acquisition is a very low priority. If we did get more we would want very large collections of seeds because they are difficult to regenerate. *Galega officinalis* is a noxious weed in the U.S. and, therefore, is not wanted for the collection. *Galega orientalis* is susceptible to leaf hopper damage in the U.S. An unusually pubescent form might resist the leaf hoppers better than our glabrous material and, therefore, would be desirable. One or two accessions of the other four *Galega* species could be acquired; most are African.

#### *Glycyrrhiza*

*Glycyrrhiza lepidota* is under investigation as a ground cover for the Western United States. New collections should be from diverse areas and environments. It is widespread in U.S. and Canada.

#### *Lathyrus*

Approximately 41 wild *Lathyrus* species are absent from the collection and should be acquired. Of the absent species 17 are European, nine North American, seven Mid Eastern, four South American, two Asian, one African, and one unknown.

#### *Melilotus*

All but one of the species as recognized by Stevenson (1969) are in the collection. Cultivars (if they exist) from Asia could be useful. We already have many cultivars from Russia. Materials of the following wild species are desirable for the collection, in this order:

(1) *Melilotus macrocarpus* is absent from the collection. It is a minor spice seed in Algeria and Morocco. Since it is the only *Melilotus* species gap it is the highest priority.

(2) *Melilotus suaveolens* is native to southeast Russia and adjacent northern China and Mongolia. Ten to 20 well documented accessions would be of potential economic use. Germplasm from highly humid Pacific coastal areas near Vladivostok would be most desirable because it might have resistance to diseases that prevent growing this species in humid areas of the United States. The form with large (7 to 8 mm long) flowers (Suvorov, 1950) is not present in our collection. We already have ten accessions for the species, including two obsolete United States cultivars.

(3) *Melilotus albus* and *Melilotus officinalis* with the fine-stem, multiple-stem growth form. Collections of these sweetclovers from extremely dry environments would also be useful

(4) *Melilotus infestus* is native to the Western Mediterranean region. Five to 10 well documented collections would be desirable because this species is known to be a source of resistance to sweetclover weevil. We have six accessions, but they are poorly documented.

(5) *Melilotus polonicus* is native north of the Caspian Sea. We have three accessions. Five to 10 well documented collections would be desirable.

(6) *Melilotus speciosus* is native to North-East Africa. Five to ten well documented collections would be desirable. We have four poorly documented accessions.

#### *Onobrychis*

Additional collections of western U.S. species would be useful to support rangeland revegetation efforts.

#### *Trigonella*

Nine wild species are absent from the collection and should be acquired. Of the absent species, seven are Mid Eastern, and two are European.

## *Vicia*

One of the species that has more economic potential in the USA is *V. villosa*; however, the number of accessions available is small. It should be collected in Southern Europe and the Eastern Mediterranean region. In addition, there are many *Vicia* species that are represented by only one or two accessions or are not represented at all. Most of those species are weedy and perhaps could be collected at the same time that a collection trip for other species is carried out in the region.

### 4. Other tropical legume genera

The CIAT Tropical Pastures' program and the Brazilian germplasm programs have had major collecting efforts focused on species with potential for growth on acid soils in the tropics. These programs have not focused on species at higher elevations, which might have cold (winter) tolerance for sub-tropical areas of coastal South Eastern U.S. Likewise the Australian CSIRO tropical legume collection has not generally focused on species, which are well adapted to the U.S. Additional efforts should be made to coordinate U.S. collections with the CIAT, Brazilian, and Australian collections.

Some forage type *Arachis* species have been collected as parts of U.S. expeditions focused primarily on the cultivated peanut species, *A. hypogea*. Thirty-five mostly nut producing *Arachis* accessions are presently being evaluated for seed production at the Indian River Research and Education Center (IRREC), Ft. Pierce, FL. (Ken, is this correct??)

Adequate genetic representation is available world wide of many of the tropical legume genera, although present U.S. holdings are limited in several genera. Major collections of *Aeschynomene*, *Macroptilium* and *Desmodium* spp. are presently stored at Ona, FL. The genus most lacking in representative species is *Aeschynomene* from Africa. Only about 45 of the 160 species are represented in the world germplasm banks yet four species, *A. americana* and *A. evenia* (USA), *A. falcata* and *A. brasilianum* (Australia), have been successfully commercialized. Many of the African species are found at 1000-3000 m altitude and should have better cold tolerance than the tropical American species.

The *Desmanthus* collection in the NPGS is limited although CSIRO has an extensive collection. Recent release of 4 cultivars of *Desmanthus bicornutus* in south Texas suggest that an attempt should be made to acquire accessions from CSIRO. *Desmanthus* is native to the Americas with documented collections from as far north as central Texas. Additional collections are needed as *Desmanthus* is well adapted to semiarid regions.

## B. Ex Situ Preservation

### Seed Storage

The CSPL CGC has recommended that seed of all collections be stored at sub-freezing temperatures to prevent loss of viability. This recommendation has been completed at the curator collections at the University of Kentucky and the AREC, Ona, FL and at SRPIS. At WRPIS, all original and regeneration seed lots are stored at sub-freezing temperatures, while distribution samples are maintained at 4 C. All locations should move to store all seed at sub-freezing temperatures ASAP.

### Regeneration

A major need for collections stored at the regional Plant Introduction Stations (Griffin, GA; Ames, IA; and Pullman, WA), is for isolated increase of accessions having low seed quality and quantity. These accessions require special handling such as sterile germination and transplant production, and in some cases, in vitro seed rescue efforts. For example, a recent report from SRPIS

at Griffin, GA indicated that about 30% of the annual *Trifolium* species and other special purpose legume accessions were not available for distribution due to low seed numbers. Through contact with Dr. Walter Graves in California through the late 1980s and early 1990s, seed of many of the self-pollinated annual clovers held at SRPIS was increased. Nevertheless, a large number of *Trifolium* spp. accessions from Africa and of cross-pollinated annual clovers are still in need of increase. The CSPL CGC has provided input regarding regeneration priorities over the past decade. The regeneration of the core collections of red and white clover is completed; however, many more accessions of these species remain in need of regeneration. We have also recently completed increases of *T. medium* and *T. ambiguum* collections through cooperation with various USDA and SAES cooperators. Efforts are underway at Prosser, WA to systematically regenerate the perennial *Trifolium* and *Lotus* collection using controlled pollination conditions and original seed, since previous increases were done under open pollination. Many of the perennial wild *Trifolium* spp. require specific growth conditions including cold temperature vernalization for adequate seed regeneration. All these species must be increased in isolation, usually in bee cages, to preserve genetic diversity. A primary need at present is regeneration of the perennial wild species accessions collected in the Western U.S. in 1994 and 1995. Funding was increased at Prosser, WA in 2000, which will allow for us to continue our systematic regeneration of the collections, and carrying out regeneration of accessions that require special handling. The genera at Ames, IA were assigned low regeneration priority by the CGC because of few requests for that germplasm. Regeneration plantings will, therefore, be at intervals of two or three years.

#### Passport Documentation

Documentation of the *Trifolium* collection was completed in 1998 (Greene 2001). Efforts are now focused on upgrading the passport documentation of the *Lotus* collection. The status of passport documentation on other genera, especially obscure taxa collected prior to 1979 should be examined and efforts made to improve GRIN documentation as much as possible. Complete documentation allows us to better manage collections through the identification of duplicate cultivars. This was done in the white clover collection (Greene and Pederson 1996)

#### Special Collections

##### University of Kentucky Collection

The University of Kentucky curator collection now consists of approximately 207 of the possible 230 valid species of the genus but is represented by only a few seeds of some species and only a few accessions of a number of species. This material is available only in small amounts and the requester must increase the seed supply for certain accessions. Seed distributions from the Kentucky collection are not reported in the GRIN system, so it is difficult to monitor the use of this collection. Additionally, there is an unknown level of overlap between accessions held at Kentucky and accessions held in the active collections at Pullman and Griffin. All accessions in the curator collection have been forwarded to NSSL including 10 new species added since 1996. All species have been documented by herbarium specimens and photographs for identification purposes and should be added to GRIN. Photographs of plants and seeds have been published (World of Clovers, Gillett and Taylor, 2001). Curator activities at Kentucky continue with seeds being increased as needed based on germination. Accessions now number 1016 and increases 737, for a total of 1737

samples of 207 species (Table 1). We recommend that curator activities at Kentucky in cooperation with the Regional Stations at Experiment, GA and Pullman WA be continued.

#### IRREC Collection (Ona)

The tropical legume collection at Ona, FL contains more than 7000 accessions including 110 genera representing 550 species. Pertinent passport data include genus and species name, all available site collection (ie., latitude, longitude and altitude for most), other countries' corresponding accession numbers, and pertinent comments. A computerized list of the accessions that have been evaluated is near completion. This work will include all available passport data and other countries' ID numbers. These data will be compiled numerically and alphabetically as soon as funds for temporary help are available. Largest collections of forage legumes include 771 *Aeschynomene* (49 species), 446 *Centrosema* (17), 786 *Desmodium* (47), 374 *Leucaena* (12), 612 *Macroptilium* (12), 538 *Stylosanthes* (19), 374 *Vigna* (42), and 104 *Zornia* (8). Many are duplicates of those held by the SRPIS, CSIRO (Australia) and CIAT (Colombia). Seed supplies of many of these accessions are limited or in poor quality and there is a need to increase seed for storage distribution and exchange.

Since the 1970s, there have been various cooperative formal and informal agreements between the IRREC and the SRPIS. These agreements were terminated in 1995. Primarily tropical legumes species (mostly forage) were grown at the IRREC for seed increase and more than 1600 packets of seed have been returned for SRPIS storage. Also, many of the IRREC collections, particularly in *Macroptilium atropurpureum*, *Aeschynomene americana*, *A. villosa*, and *Alysicarpus vaginalis*, have been donated to the SRPIS and assigned numbers. This has increased the SRPIS holdings from a very few to an estimated 75% or more of the available world collection. There are still many non-PI accessions held in the Ona (formerly IRREC) germplasm bank that are not duplicated in the SRPIS bank. A concerted effort should be made to increase seed of these non-PI accessions so that samples can be assigned PI numbers and placed in the NPGS.

#### *In situ* conservation

*In situ* preservation should be considered as an alternative for many of the CSPL species, especially those native to the U.S. Recent correspondence between the *Trifolium* curator and Ned Garvey has begun the process of identifying potential sites for perennial Western U.S. *Trifolium* species. One preserve already exists for *T. thompsonii* and numerous other collections in the Western U.S. were made on public owned lands.

#### C. Evaluation

Descriptors for 12 species have been developed and approved by the CSPL CGC since the 1987 report. Beginning in 1988 and continuing until 1998, the CSPL CGC has allocated \$45,000 annually for germplasm evaluation. This funding has generated the majority of currently available descriptor data to the GRIN system. A brief summary of the species and respective descriptions evaluated is presented in Table 4. The majority of projects funded by the CGC have focused on descriptors identified as priorities by the CGC. These data have been generated by funding cooperators from state AES and USDA-ARS. Despite some earlier problems with input of this evaluation data into the NPGS, most of it is now accessible through GRIN. In 1998 and 1999 the core collections of white and red clover were evaluated. With the complex array of species for which the CSPL CGC has responsibility, many species still have few or no descriptors evaluated. In 1999,

germplasm evaluation monies were no longer available to the CSPL CGC, and as a consequence, no further evaluations have been carried out. Virtually no evaluation data is available through GRIN for the tropical legume species maintained by the USDA NPGS. Of high priority is to have reinstated, a source of funding that would continue to support an evaluation program.

#### D. Enhancement

Enhancement in cross pollinated *Trifolium* spp. is usually required to bring pest resistance up to a useable level. Often the level of available pest resistance is as low as one-10% of a given introduction. Simple identification is not adequate for the trait to be of benefit. In other cases intermediate tolerance can be improved to full resistance through enhancement efforts. Two to four cycles of recurrent phenotypic selection are often adequate. For these *Trifolium* species, enhancement efforts should receive additional funding. Broad genetic base populations of *Lotus tenuis* and *L. uliginosus* are being developed from NPGS collection accessions to be used to develop adapted North American cultivars. At present all enhancements have been carried out by SAES and USDA cooperators and then released as germplasms which are preserved in the NPGS. In most cases enhancement should be adequate to bring the level of a trait up to a point that a line could be released as a germplasm source, but not necessarily as a released cultivar. A procedure for review and naming of genetic marker stocks in the clovers was jointly coordinated by the CSPL CGC and the Trifolium Conference and has been published since our 1987 report (Quesenberry, et al., 1991). Development of molecular markers assisted selection procedures could improve progress in selection for desirable traits.

### V. **Recommendations**

#### A. Priority actions

1. Reinstatement of funding for germplasm evaluations.
2. Conduct seed increases of annual and perennial clovers and other temperate and tropical legume accessions, with emphasis on replacing open-pollinated seed lots with seed produced under controlled pollination using original seed; increasing accessions that require special handling due to low seed quality and quantity and increasing seed of wild species.
3. Systematic evaluation of all descriptors for core collections of *Trifolium* spp. and update and standardize existing GRIN evaluation data
4. Collect annual and perennial *Trifolium* spp. and other special use legumes from Europe, Africa, and from the Western and Eastern U.S with a special emphasis on obtaining species closely related to the cultivated species.
5. Integrate the tropical legume collection at Ona, FL (formerly at IRREC, Ft. Pierce, FL) into the USDA NPGS including assignment of PI numbers.
6. Develop and incorporate descriptors for all special purpose legumes into GRIN.
7. Establish core collections of other CSPL species.
8. Establish *in situ* preservation sites for native U.S. CSPL species.
9. Collect *Rhizobium* strains for western North American clovers.

#### B. Budget Recommendations General

1. Acquisition (collection)
 

Western U.S. <i>Lotus</i>	\$9,000
(Eastern U.S. <i>Trifolium</i>	\$7,000
Western U.S.)	\$9,000
Africa	\$15,000
Greece, Italy	\$15,000
2. Preservation (maintenance)
 

Germplasm regeneration (support needed for all CSPL collections)	\$300,000 (annual)
Compilation and integration of information on accessions in the system.	\$25,000
3. Evaluation \$100,000 (annual)
4. Enhancement \$100,000 (annual)

Details

1. A trip to California, Oregon and Washington focused on native *Lotus* species that may be rare or endangered. Most are not currently represented in the NPGS collection. Projected participants are P. Beuselinck, J. Kirkbride, J. Steiner, and W. Williams from New Zealand.  
 A trip or trips to be developed for collection of species native to the Eastern U.S, with focus on *T. polymorphum*, *T. calcaricum*, *T. virginicum*, *T. reflexum*, *T. carolinianum*, *T. bajariense*, and *T. stoloniferum*.  
 A trip to the Western U.S. focused on *T. brandegi*, *T. monathum*, *T. mucronatum*, and *T. siskiyouense*.  
 A trip to Greece and Italy to collect wild relatives of red and white clover and annual clovers such as crimson and arrowleaf.
2. Restoration of availability of accessions in working collections. Five year projection:
 

Annuals	300/yr @ 30/acc. = \$ 9,000/yr
Perennials	300/yr @ 300/acc.= \$15,000/yr
Tropicals	100/yr @ 50/acc. = \$ <u>5,000/yr</u>
Total	\$29,000/yr

Five year total - - - - - \$145,000

Compilation and integration of information on accessions in the system. This could be a onetime allocation or divided over several years to provide support for one or two persons to compile and integrate (including assigning PI numbers) current accessions in various collections. The funding to be used for travel, per diem and, if necessary, salaries for assistance. Estimated cost \$25,000.

3. Evaluation:

Increase funding to \$100,000 per year for at least eight more years to allow evaluation of descriptors on other special purpose legumes. Germplasm will be evaluated according to priorities established by CSPL CGC.

#### 4. Enhancement:

Development of genetic marker (molecular, biochemical, physiological and morphological), stocks and genome mapping of the major species; development of techniques for evaluation of germplasm for quality, disease and insect resistance; combination of several types of resistance in a single population are enhancement priorities that can be carried out once monetary support is provided. We visualize that for selected traits several cycles of recurrent selection may be needed to improve the level of a desired trait such that it can be readily incorporated into elite cultivars.

#### **IV. Reflections:**

The Clover and Special Purpose Legume Crop Germplasm Committee is responsible for all the *Trifolium* spp. (230) and over 100 other temperate and tropical special purpose legume genera. The composition of the committee was designed to have a wide range of scientists and experts which would adequately represent the numerous genera and species involved. We have strived to achieve a balance of effort among species while giving priority to the most widely cultivated species.

The major concentration of the CGC has been the cultivated clovers with the highest economic value and birdsfoot trefoil. Having now completed regeneration of core collections in this group of species, we are prepared to evaluate all descriptors on these species. This effort should make the germplasm collection more useful for both public and private users.

We continue to recognize a need for regeneration of the existing collections of the clovers and other special purpose legumes. Progress has been made since our previous report, but the germplasm continues to be in need of seed regeneration. The CGC, in cooperation with the directors of the collections and the curator, has completed upgrades of storage conditions of CSPL at the various PI stations. Sub-freezing storage is still needed for the active collections at Ames and Pullman. Continued funding for seed increase is needed or many current accessions will be in such poor condition that it will be impossible to resurrect them.

The CGC prioritized several areas for collection of certain clovers and special purpose legumes in our 1987 report. Competent scientists were recruited to organize collection expeditions and assist in this acquisition. Most of these collection expeditions have been completed. Additional acquisition needs have been prioritized in this report and efforts will be made to recruit scientists to organize these efforts. Funding is needed to evaluate and increase recent and proposed collections.

Our committee has recognized that the large number of species in the collections which we oversee create management difficulties. The scope of committee membership has enabled us to remain current on contemporary germplasm management issues. We have developed core collections in species with large numbers of accessions to assist curators with maintenance and evaluation decisions. We have allocated evaluation monies to allow review and updating of passport records in the GRIN system for clovers and *Lotus* species. This information is now being entered into the GRIN system. We have reviewed the data for *T. repens*, and will need to conduct a similar type comprehensive review on a species by species basis to eliminate duplications in the collections.

Historically, private industry has devoted minimal to no fiscal resources to the maintenance and/or development of the clover and special purpose legume germplasm. Even with the vast area seeded to these species it is not foreseen that private industry will contribute significantly to future support for maintenance and/or the development of the CSPL germplasm. The species of concern have major, significant impacts on the economy of U.S. agriculture through direct production and use

and indirectly through soil conservation effects. Therefore, if the species in question are to be maintained and improved, support will have to come from public agencies.

In times of decreasing fiscal resources at all levels, the need for sound planning and well thought out decisions is paramount. The CSPL-CGC desires to be involved in giving advice when hard decisions have to be made, realizing that some decisions may not follow our views. Nevertheless, involvement of the committee early in the decision process will allow the group to feel a sense of ownership and responsibility. Early involvement should ensure a continued strong and viable committee.

## REFERENCES

- Ball, O.W. and Chrtkova-Zertova. 1968. *Lotus L.* p. 173-176 In T.G. Tutin (ed.) *Flora Europaea*. 2. Cambridge Univ. Press, Cambridge, England.
- Barnaby, R.C. 1977. *Daleae imagines*. Mem NY Bot Gard. 27:1-891.
- Ellison, N.W., A. Liston, J.J. Steiner, W.M. Williams and N.L. Taylor. 2006. Molecular phylogenetics of the clover genus (*Trifolium* –Leguminosae). Molecular Phylogenetics and Evolution (In Press)
- Gibson, P.B. and E.A. Hollowell. 1966. White clover. U.S. Dept. Agric. Handb. 314.
- Gillett, J.M. and N. L. Taylor. 2001. The World of Clovers. 455 pages. Ed. M. Collins. Iowa State University Press, Ames, Iowa.
- Greene, S.L. and G.A. Pederson. 1996. Eliminating duplicates in germplasm collections: a white clover example. Crop Science 36:1398-1400.
- Greene, S.L. 1998. U.S. clover germplasm collection: a century of collection, introduction and conservation. In Proceedings of the 15<sup>th</sup> *Trifolium* Conference, pg. 42, June 10-12, Madison, WI.
- Greene, S.L. 1999. Lotus genetic resources: maintaining diversity through conservation. In (ed. P. Beuselinck). Trefoil: the science and technology of Lotus. CSSA Special Publication 28, Madison, WI.
- Greene, S.L. 2001. Improving the quality of passport data to enhance germplasm use and management. Plant Genetic resources Newsletter 125:1-8.
- Morris, B. and S.L. Greene. 2001. Defining a multiple-use collection for the genus *Trifolium*. Crop Science 41:893-901
- Smith, R.R., N.L. Taylor and S.R. Bowley. 1985. Red clover. In N.L. Taylor (ed.). Clover-Science and Technology. Agronomy 25:457-470.
- Steiner, J.J. and S.L. Greene. 1996. Proposed ecological descriptors and their utility for plant germplasm collections. Crop Sci. 36:439-451.
- Steiner, J.J., E. Piccioni and M. Falcinelli. 1996. Germplasm diversity among cultivars and the NPGS crimson clover collection. Crop Sci. 36:in review.
- Steiner, J.J., P.R. Beuselinck, S.L. Greene, J.A. Kamm, J.H. Kirkbride, and C.A. Roberts. 2001. A description and interpretation of the NPGS birdsfoot trefoil core subset. Crop Sci. 41:1968-1980.

- Stevenson, G.A. 1969. An agronomic and taxonomic review of the genus *Melilotus* Mill. Can. J. Plant Sci. 49:1-20
- Suvorov, V.V. 1950. Sweetclover-*Melilotus*(Tourn,)Adans. Em. In Flora of the cultivated plants of the USSR. E.N. Sinskaya, (ed.) Vol. 13(1) pp. 426-627 Trans. from Russian as OTS 60-51198. U.S. Dep. Commerce, Washington D.C.
- Taylor, N.L. 1985. Clovers around the world. In N.L. Taylor (ed.). Clover-Science and Technology. Agronomy 25:1-6.
- Taylor, N.L., P.B. Gibson and W.E. Knight. 1977. Genetic vulnerability and germplasm resources of the true clovers. Crop Sci. 17:632-634.
- Taylor, N. L. and K. H. Quesenberry. 1996. Red Clover Science. 226 pages. Kluwer Academic Publishers. The Netherlands.
- Van Keuren, R.W. and C.S. Hoveland. 1985. Clover management and utilization. In N.L. Taylor (ed.). Clover-Science and Technology. Agronomy 25:325-254.

**Table 1. List of accessions by storage site (NPGS and Taylor Collections) of 211 *Trifolium* species†**

TAXON	Pullman (NPGS-W6)	Griffin (NPGS-S9)	Kentucky (Taylor)
<i>Trifolium acaule</i>	0	0	1
<i>Trifolium affine</i>	0	4	3
<i>Trifolium africanum</i>	8	0	6
<i>Trifolium aintabense</i>	0	0	3
<i>Trifolium albopurpureum</i>	0	3	6
<i>Trifolium alexandrinum</i>	0	123	12
<i>Trifolium alpestre</i>	53	0	16
<i>Trifolium alpinum</i>	6	0	1
<i>Trifolium amabile</i>	6	0	6
<i>Trifolium ambiguum</i>	150	0	90
<i>Trifolium amoenum</i>	0	1	2
<i>Trifolium andersonii</i>	4	0	2
<i>Trifolium andinum</i>	0	0	0
<i>Trifolium angulatum</i>	1	0	1
<i>Trifolium angustifolium</i>	1	37	9
<i>Trifolium apertum</i>	3	2	3
<i>Trifolium argutum</i>	0	5	8
<i>Trifolium arvense</i>	0	41	18
<i>Trifolium aureum</i>	3	16	4
<i>Trifolium baccarinii</i>	0	32	5
<i>Trifolium badium</i>	11	0	3
<i>Trifolium barbigerum</i>	0	7	13
<i>Trifolium batmanicum</i>	0	8	5
<i>Trifolium beckwithii</i>	4	0	2
<i>Trifolium bejariense</i>	0	1	4
<i>Trifolium berytheum</i>	0	1	2
<i>Trifolium bifidum</i>	0	2	9
<i>Trifolium bilineatum</i>	0	30	1
<i>Trifolium billardierei</i>	0	0	1
<i>Trifolium blancheanum</i>	0	0	1
<i>Trifolium bocconeii</i>	0	10	11
<i>Trifolium boissieri</i>	0	1	1
<i>Trifolium bolanderi</i>	6	0	2
<i>Trifolium brandegei</i>	1	0	1
<i>Trifolium breweri</i>	0	0	1

Trifolium brutium	0	0	2
Trifolium buckwestiorum	0	1	1
Trifolium bullatum	0	0	3
Trifolium burchellianum	40	0	5
Trifolium calcaricum	2	0	4
Trifolium calocephalum	0	2	1
Trifolium campestre	3	92	30
Trifolium canescens	21	0	3
Trifolium carolinianum	0	5	9
Trifolium caucasicum	8	0	1
Trifolium caudatum	1	0	1
Trifolium cernuum	0	5	4
Trifolium cheranganiense	3	0	2
Trifolium cherleri	0	54	9
Trifolium chilense	0	2	1
Trifolium ciliolatum	0	6	4
Trifolium clusii	0	1	4
Trifolium clypeatum	0	5	0
Trifolium constantinopolitanum	0	2	3
Trifolium cryptopodium	30	0	2
Trifolium cyathiferum	0	10	4
Trifolium dalmaticum	0	0	1
Trifolium dasiphyllum	2	0	2
Trifolium dasyurum	0	9	7
Trifolium decorum	0	31	1
Trifolium depauperatum	0	4	8
Trifolium dichotomum	0	0	3
Trifolium dichroanthum	0	2	3
Trifolium diffusum	1	30	6
Trifolium douglasii	3	0	1
Trifolium dubium	0	14	9
Trifolium echinatum	0	22	8
Trifolium elgonense	0	0	1
Trifolium eriocephalum	6	0	1
Trifolium eriosphaerum	0	1	2
Trifolium erubescens	0	1	1
Trifolium fragiferum	247	0	27
Trifolium fucatum	0	4	7

Trifolium gemellum	0	4	0
Trifolium glanduliferum	0	4	1
Trifolium globosum	0	9	1
Trifolium glomeratum	0	28	9
Trifolium gracilentum	0	5	7
Trifolium grandiflorum	0	7	3
Trifolium gymnocarpon	0	0	1
Trifolium haussknechtii	1	2	1
Trifolium haydenii	0	0	2
Trifolium heldreichianum	10	0	4
Trifolium hirtum	0	63	11
Trifolium howellii	2	0	2
Trifolium hybrid	5	0	0
Trifolium hybridum	208	0	26
Trifolium incarnatum	0	36	41
Trifolium israeliticum	0	3	1
Trifolium isthmocarpum	0	24	2
Trifolium jokersti	0	0	1
Trifolium kingii var. productum	3	0	3
Trifolium lanceolatum	0	1	1
Trifolium lappaceum	3	28	19
Trifolium latifolium	2	0	1
Trifolium latinum	0	1	1
Trifolium leibergii	1	0	2
Trifolium lemmonii	2	0	1
Trifolium leucanthum	0	4	9
Trifolium ligusticum	1	5	3
Trifolium longidentatum	5	0	3
Trifolium longipes	11	0	2
Trifolium lucanicum	1	0	1
Trifolium lugardii	0	2	4
Trifolium lupinaster	32	0	1
Trifolium macilentum	1	0	0
Trifolium macraei	0	2	2
Trifolium macrocephalum	3	0	5
Trifolium masaiense	0	1	1
Trifolium mattirolianum	0	19	2
Trifolium medium	68	0	19

Trifolium medium var. medium	17	0	0
Trifolium meduseum	0	2	2
Trifolium michelianum	0	12	10
Trifolium micranthum	0	2	1
Trifolium microcephalum	0	10	13
Trifolium microdon	0	5	4
Trifolium miegeanum	0	1	2
Trifolium montanum	53	0	23
Trifolium monanthum	0	0	1
Trifolium multinerve	0	2	3
Trifolium mucronatum	0	0	1
Trifolium mutabile	0	1	3
Trifolium nanum	2	0	1
Trifolium nigrescens	0	41	9
Trifolium noricum	2	0	0
Trifolium obscurum	0	4	3
Trifolium obtusiflorum	0	2	0
Trifolium occidentale	0	0	2
Trifolium ochroleucum	34	0	15
Trifolium oliganthum	0	1	1
Trifolium ornithopodioides	2	0	1
Trifolium owyheense	1	0	1
Trifolium palaestinum	0	7	4
Trifolium pallescens	2	0	1
Trifolium pallidum	0	36	26
Trifolium pannonicum	25	0	12
Trifolium parryi	4	0	3
Trifolium patens	2	5	3
Trifolium patulum	5	0	3
Trifolium pauciflorum	0	22	2
Trifolium philistaeum	1	1	1
Trifolium phleoides	0	9	3
Trifolium physodes	23	0	7
Trifolium pichisermollii	0	2	3
Trifolium pignanii	2	0	1
Trifolium pilulare	0	8	3
Trifolium pinetorum	1	0	0
Trifolium plebeium	0	0	1

Trifolium plumosum	7	0	1
Trifolium polymorphum	1	0	14
Trifolium polyodon	0	2	1
Trifolium polyphyllum	3	0	3
Trifolium polystachyum	11	0	3
Trifolium pratense	1308	0	37
Trifolium pseudostriatum	0	0	4
Trifolium purpureum	3	18	5
Trifolium purseglovei	3	0	1
Trifolium quartinianum	0	20	2
Trifolium reflexum	0	4	10
Trifolium repens	813	0	35
Trifolium resupinatum	0	209	19
Trifolium retusum	0	11	4
Trifolium riograndense	1	0	2
Trifolium rubens	27	0	12
Trifolium rueppellianum	0	67	4
Trifolium salmoneum	0	1	1
Trifolium scabrum	0	28	8
Trifolium schimperi	1	20	3
Trifolium scutatum	0	4	3
Trifolium semipilosum	80	0	8
Trifolium setiferum	0	2	1
Trifolium simense	25	0	3
Trifolium somalense	2	0	1
Trifolium sp.	24	22	0
Trifolium spadiceum	0	9	2
Trifolium spananthum	0	0	2
Trifolium spumosum	2	16	5
Trifolium squamosum	0	10	6
Trifolium squarrosum	0	11	2
Trifolium stellatum	0	13	9
Trifolium steudneri	0	63	0
Trifolium stoloniferum	10	0	21
Trifolium stolzii	1	0	2
Trifolium striatum	2	17	11
Trifolium strictum	1	8	3
Trifolium subterraneum	0	310	3

<i>Trifolium suffocatum</i>	0	6	5
<i>Trifolium sylvaticum</i>	0	8	6
<i>Trifolium tembense</i>	0	135	11
<i>Trifolium thalii</i>	3	0	3
<i>Trifolium thompsonii</i>	2	0	1
<i>Trifolium tomentosum</i>	0	15	9
<i>Trifolium triaristatum</i>	2	1	1
<i>Trifolium trichocalyx</i>	0	1	0
<i>Trifolium trichocephalum</i>	6	0	1
<i>Trifolium trichopterum</i>	0	4	6
<i>Trifolium tridentatum</i>	0	6	7
<i>Trifolium tumens</i>	3	0	3
<i>Trifolium uniflorum</i>	2	0	4
<i>Trifolium usambarense</i>	0	1	3
<i>Trifolium variegatum</i>	0	12	10
<i>Trifolium vavilovii</i>	0	1	2
<i>Trifolium velebiticum</i>	7	0	4
<i>Trifolium velenovskyi</i>	0	0	1
<i>Trifolium vernum</i>	0	1	1
<i>Trifolium vesiculosum</i>	0	17	7
<i>Trifolium virginicum</i>	5	0	10
<i>Trifolium wentzelianum</i>	0	0	1
<i>Trifolium wigginsii</i>	0	0	7
<i>Trifolium willdenovii</i>	0	8	10
<i>Trifolium wormskioldii</i>	20	0	5
	3534	2129	1165

† **Overlap between NPGS collections and Taylor Collection is not currently known**

Table 2. Clover and Special Purpose Legume CGC Taxa other than *Trifolium*.

Genus	No.Ac.	Site	Curator	Other expert
Acacia	14		at six sites	
Adenocarpus	1	W6	Hellier	
Adesmia	3	S9	Morris	Quesenberry
Aeschynomene	231	S9	Morris	Quesenberry, Kalmbacher
Alhagi	1	W6	Hellier	
Alysicarpus	62	S9	Morris	Quesenberry
Amphicarpaea	1	S9	Morris	
Amorpha	29	W6	Hellier	
Anthyllis	72	W6	Hellier	
Arachis glabrata	99	S9	Pittman	Simpson
Argyrolobium	9	S9	Morris	
Astracantha	16	W6	Hellier	
Astragalus	634	W6	Hellier	Boe
Baphia	1	S9	Morris	
Biserrula	12	S9	Morris	
Bituminaria	13	S9	Morris	
Calicotome	1	W6	Hellier	
Calliandra	4 1	MA Y S9	Morris	
Calopogonium	12	S9	Morris	
Canavalia	30	S9	Morris	Lukefahr
Centrosema	73	S9	Morris	Quesenberry
Chamaecrista	62	S9	Morris	
Chamaecytisus	1 1	W6 S9	Hellier Morris	

Clitoria	27	S9	Morris	
Codariocalyx	2	S9	Morris	
Colutea	3	W6	Hellier	
Coronilla	34	NC7	Brenner	
Crotalaria	226	S9	Morris	Cook, Quesenberry, Mosjidis
Cyamopsis	1303	S9	Morris	
Cytisus	5	S9	Morris	
Dalea	44	NC7	Brenner	Boe
Dendrolobium	2	S9	Morris	
Desmanthus	93	S9	Morris	Ocuppaugh
Desmodium	253	S9	Morris	Boe
Dorycnium	12	S9	Morris	
Ebenus	6	W6	Hellier	
Eriosema	7	S9	Morris	
Galactia	12	S9	Morris	Quesenberry
Galega	40	NC7	Brenner	
Genista	11	W6	Hellier	
Glycyrrhiza	16	W6	Hellier	Boe
Gueldenstaedtia	1	W6	Hellier	
Halimodendron	1	W6	Hellier	
Hedysarum	109	W6	Hellier	
Hippocrepis	10	W6	Hellier	
Hymenocarpos	2	W6	Hellier	
Indigofera	118	S9	Morris	Quesenberry
Kummerowia	55	S9	Morris	
Lablab	108	S9	Morris	G. Smith
Lathyrus	625	W6	Coyne	

Lessertia	3	S9	Morris	
Leucaena	539	S9	Morris	Brewbaker
Lespedeza	150	S9	Morris	Mosjidis
Lotononis	22	S9	Morris	Quesenberry
Lotus	777	W6	Greene	Ehlke
Lysiloma	2	S9	Morris	
Macroptilium	206	S9	Morris	Kalmbacher
Macrotyloma	47	S9	Morris	Kalmbacher
Marina	3	NC7	Brenner	
Melilotus	952	NC7	Brenner	Gorz, G. Smith
Mimosa	5	S9	Morris	
Mucuna	44	S9	Morris	
Neonotonia	169	S9	Morris	Kalmbacher
Neptuna	1	S9	Morris	
Onobrychis	575	W6	Hellier	
Ononis	14	W6	BradleyHel lier	
Ophrestia	1	S9	Morris	
Ornithopus	54	S9	Morris	
Oxytropis	25	W6	Hellier	
Pachyrhizus	13	S9	Morris	
Pedimelum	1	S9	Morris	
Prosopis	2	W6	Hellier	
Pseudovigna	1	S9	Morris	
Psophocarpus	183	S9	Morris	
Psoralidium	6	W6	Hellier	
Psoralea	8	S9	Morris	
Pueraria	20	S9	Morris	

Retama	1	W6	Hellier	
Rhynchosia	31	S9	Morris	Quesenberry
Schleinitzia	1	S9	Morris	
Securigera Senna	104 48	NC7 S9	Brenner Morris	
Sesbania	43	S9	Morris	
Scorpiurus	80	W6	Hellier	
Sophora	7	W6	Hellier	
Spartidium	1	W6	Hellier	
Sphaerophysa	3	W6	Hellier	
Sphenostylis	14	S9	Morris	
Strophostyles	4 4	W6 S9	Morris	
Stylosanthes	112	S9	Morris	Kalmbacher
Sutherlandia	1	S9	Morris	
Tephrosia	89	S9	Morris	Quesenberry
Teramnus	33	S9	Morris	
Tetragonolobus	41	W6	Bradley	
Trigonella	245	W6	Coyne	
Vicia	1902	W6	Coyne	Mosjidis
Zornia	24	S9	Morris	Kalmbacher

**Table 3. Approximate Numbers of the Largest Collection of Tropical Forage Legume Species Maintained at Germplasm Bank at the IRREC, Ft. Pierce, FL.**

Genus	Species	Total
Aeschynomene	49	771
americana		312
villosa		67
Alysicarpus	7	82
Arachis	11	149
Calopogonium	3	67
Centrosema	17	446
Desmanthus	8	167
Desmodium	47	786
heterocarpon		111
Leucaena	12	374
leucocephala		265
Lotononis	22	86
Macroptilium	12	612
atropurpureum		413
Macrotyloma	7	82
Neonotonia	1	114
Phaseolus	11	74
Rhynchosia	12	79
Stylosanthes	19	538
guianensis		236
hamata		36
humulis		87
Teramnus	7	142
uncinatus		89
Vigna	42	374
adenantha		32
luteola		36
vexillata		60
Zornia	8	104
latifolia		27

**Table 4. Descriptors evaluated on Clover and Special Purpose Legume (CSPL) germplasm since 1988 as authorized by the CSPL Crop Germplasm Committee. (Nov 2005).**

<b>Genus/species</b>	<b>Descriptor</b>	<b>No. acc. evaluated</b>
<i>Astragalus cicer</i>	Chemical (Isoflavonoids)	42
<i>Lespedeza</i> spp.	Chemical (tannin)	79
<u><i>Lotus</i></u>		
<i>L. ann.</i> spp.	Cytology/fertility	51
<i>L. spp.</i>	Identification Morocco collection	72
" "	Molecular (RFLP's and RAPDS)	50
<i>L. corniculatus</i>	Chemical (tannin)	400
" "	Disease ( <i>Rhizoctonia</i> )	65
" "	Cytology	400
" "	Molecular (RFLP's and RAPDS)	128
" "	Chemical (chitinase)	100
" "	Molecular (ITS)	95
" "	Photoperiod and flowering	70
<i>L. tenuis</i>	Molecular (RFLP's and RAPDS)	30
<i>L. uliginosis</i>	Molecular (RFLP's and RAPDS)	80
<i>Macroptilium</i> spp.	Disease ( <i>Uromyces appendiculatus</i> )	100
<u><i>Trifolium</i></u>		
<i>T. alexandrinum</i>	Bean Yellow Mosaic Virus	100
" "	Nematode ( <i>Meloidogyne arenaria</i> race 1)	50
" "	" ( <i>Meloidogyne hapla</i> )	50
" "	" ( <i>Meloidogyne incognita</i> race 3)	50
" "	" ( <i>Meloidogyne javonica</i> )	50
<i>T. ambiguum</i>	Cytology	98
" "	Agronomic (five characters)	80
<i>T. fragiferum</i>	Agronomic (salinity)	96
<i>T. hirtum</i>	Chemical (Isoflavin - Genistein)	34
" "	Chemical (Isoflavin - Formononetin)	34
" "	Chemical (Isoflavin - Biocanin A)	34
" "	Agronomic (maturity and seed set)	50
" "	Agronomic (pH tolerance)	50
" "	Agronomic (waterlogged soils)	60
" "	Nematode ( <i>Meloidogyne arenaria</i> race 1)	35
" "	" ( <i>Meloidogyne hapla</i> )	35
" "	" ( <i>Meloidogyne incognita</i> race 3)	35
" "	" ( <i>Meloidogyne javonica</i> )	35
<i>T. incarnatum</i>	Agronomic (Fe chlorosis)	30
" "	Nematode ( <i>Meloidogyne arenaria</i> race 1)	25
" "	" ( <i>Meloidogyne hapla</i> )	25

**Table 4. Descriptors evaluated on Clover and Special Purpose Legume (CSPL) germplasm since 1988 as authorized by the CSPL Crop Germplasm Committee. (Nov. 2005) con't.**

<b>Genus/species</b>	<b>Descriptor</b>	<b>No. acc. evaluated</b>
<i>T. incarnatum</i> cont.	" ( <i>Meloidogyne incognita</i> race 3)	25
" "	" ( <i>Meloidogyne javonica</i> )	25
" "	Molecular (RFLP's and RAPDS)	35
<i>T. medium</i>	Cytology	88
" "	Agronomic (five characters)	88
<i>T. repens</i>	Nematode ( <i>Meloidogyne arenaria</i> race 1)	230
" "	" ( <i>Meloidogyne hapla</i> )	230
" "	" ( <i>Meloidogyne incognita</i> race 3)	230
" "	" ( <i>Meloidogyne javonica</i> )	230
" "	Agronomic (nine characters)	130
" "	Disease ( <i>Clyndrocladium crotolariae</i> )	200
" "	Chemical (cyanogenesis )	500
" "	Nematode ( <i>M. graminicola</i> )	550
" "	Agronomic eval of core collection	90
<i>T. pratense</i>	Disease( <i>Aphanomyces euteiches</i> )	400
" "	Disease ( <i>Stemphlium sarciniforme</i> )	400
" "	Nematode ( <i>Meloidogyne arenaria</i> race 1)	400
" "	" ( <i>Meloidogyne hapla</i> )	400
" "	" ( <i>Meloidogyne incognita</i> race 3)	400
" "	" ( <i>Meloidogyne javonica</i> )	400
" "	Chemical (Protein degradation )	100
" "	Disease ( <i>Clyndrocladium crotolariae</i> )	200
" "	Chemical (isoflavenoids)	40
" "	RAPD's and morphology from Caucasus coll.	40
" "	Agronomic eval. of core collection	100
" "	Isozyme variability of core collection	100
" "	Isozyme variability of Caucasus coll	15
<i>T. resupinatum</i>	Nematode ( <i>Meloidogyne arenaria</i> race 1)	40
" "	" ( <i>Meloidogyne hapla</i> )	40
" "	" ( <i>Meloidogyne incognita</i> race 3)	40
" "	" ( <i>Meloidogyne javonica</i> )	40
<i>T. subterraneum</i>	Chemical (Isoflavin - Genistein)	225
" "	Chemical (Isoflavin - Formononetin)	225
" "	Chemical (Isoflavin - Biocanin A)	225
" "	Fe Chlorosis	100
" "	Agronomic (hardseediness)	50
" "	Disease (Clover Yellow Vein Virus)	250
" "	Winter hardiness	50
" "	AFLP and morphological characterization	90
<i>T. vesiculosum</i>	Disease (fungal seedling diseases)	20
<i>T. spp.</i>	Ident. of Yugoslavian col.	90
" "	Regeneration	15

" "	Ident. of Bulgarian col.	226
" "	Ident. of Georgian col.	38
" "	Biochem. character. of West Trif.	138
" "	Identification of Western US Trif.	138
" "	Seed prod. on African species	30
" "	Molecular Taxonomy (ITS)	40
<i>T. spp.</i>	Regeneration of African species	32
<u><i>Vicia</i></u>		
<i>V. vilosa</i>	Agronomic (winter hardiness)	58
<i>V. sativa</i>	Chemical (cyanogenesis)	215
" "	Agronomic (root length)	200

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