

TRIFOLIUM SPECIES GERMPLASM FROM WESTERN NORTH AMERICA

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ABSTRACT

The genus *Trifolium*, which consists of about 230 species of annual and perennial legumes, has been divided into eight taxonomic sections. Approximately 50 species are native to western North American inter-mountain regions and the states of California, Oregon, and Washington. Prior to this research, about 30 of the *Trifolium* species from western North America were not represented in the U.S. National Plant Germplasm System (NPGS). Three germplasm collection expeditions in 1994 and 1995 collected about 140 accessions encompassing at least 44 species, including at least 25 species not previously available in the NPGS. Of these, nine were listed as potentially threatened or endangered. Plants of some perennial species exhibited slow and limited seedling growth in the greenhouse before entering into a period of senescence. All annual species except *T. fucatum* (cross-pollinated) were self fertilized, whereas all perennials which have flowered appear to be cross pollinated. *In situ* conservation appears to be feasible for a number of these perennial species.

KEYWORDS

Red clover, *Trifolium*, germplasm conservation, *in situ* conservation

INTRODUCTION

The genus *Trifolium* consists of over 230 species (Zohary and Heller, 1984), but only about 12 species are used to any extent in planted pastures. Although some 65 clover species are native to the Americas, all commercialized clover species originate from Europe, Asia, or Africa. Because of this, most *Trifolium* germplasm research has focused on germplasm collection in central and southern Europe. Recent expeditions for collection of *Trifolium* species germplasm in Europe have been sponsored by the U.S. NPGS include: 1) Romania in 1984 that yielded 39 accessions of 13 *Trifolium* species (Taylor and Rumbaugh, 1986); 2) Yugoslavia in 1988 that produced 95 accessions representing 16 species (Taylor and Smith, 1990); 3 & 4) two to Bulgaria (1990 and 1993) (Quesenberry and Smith, 1992, Pederson and Quesenberry, 1994) which generated about 250 accessions representing about 55 *Trifolium* species; and 5) Republic of Georgia in 1993 which obtained over 60 accessions of *Trifolium* species representing about 10 perennial species (Smith and Hoveland, 1994). As a result of the above collections and previous collections, most of the *Trifolium* species described from Europe and Asia have been collected, although several African species remain uncollected.

In 1993 the Clover and Special Purpose Legume Crop Germplasm Committee of the U.S. NPGS determined that about 30 *Trifolium* species native to western North America were not represented in any of the world's germplasm collections. After review of taxonomic literature, the states of California, Oregon, and Washington were targeted for collection.

METHODS

In the summers of 1994 and 1995, N. L. Taylor and K. H. Quesenberry, sponsored by the U.S. NPGS, participated in three expeditions to collect germplasm of *Trifolium* species native to western North America. Taylor and Quesenberry collected in central and north central California in early June, focusing primarily on annual *Trifolium* species. During early August 1994, Quesenberry, Taylor, and W. M. Williams (Margot Forde Forage Genetic Resource

Center, Ag Research Grasslands, Palmerston North, New Zealand) collected in Oregon and southern Washington, focused primarily on perennial species. In August 1995, Quesenberry and Taylor collected in the eastern and southern Sierra Nevada Mountains of California with major focus on perennial species. All collection efforts relied on expertise of local botanists to identify sites in widely separated and often isolated locales.

RESULTS AND DISCUSSION

A total of 61 accessions encompassing 24 species were collected during phase 1 of this effort (Table 1). Included among the species collected in June 1994 were *T. amoenum* Greene, *T. buckwestorium* Isley, *T. obtusiflorum* Hook, *T. oliganthum* Steud., *T. polyodon* Greene, and *T. trichocalyx* Heller, which are listed as candidates for threatened or endangered status by the U. S. Fish and Wildlife Service. Only one accession of each species was collected, except for *T. polyodon* where two accessions were collected.

Most species were identified in very specific microhabitats. The relatively abundant rhizomatous perennial species *T. wormskioldii* Lehm. was found in moist areas along the Pacific coast as well as along inland streams. *T. obtusiflorum* was found at only one site along the banks of a ravine in Napa County. *T. polyodon* and *T. trichocalyx* were only collected on the Monterey Peninsular. Seeds of *T. amoenum*, thought to be extinct before a 1993 rediscovery, were obtained from plants grown from the original rediscovered plant.

A total of 43 accessions including about 17 species were collected in Oregon and Washington during phase two. Species collected in Oregon and Washington which were not previously in the NPGS include *T. douglasii* House, *T. eriocephalum* Nutt., *T. howellii* S. Wats, *T. kingii* spp. *productum* (Greene) Heller, *T. latifolium* (Hook) Greene, *T. leibergii* Weis & Macbride, *T. macrocephalum* (Pursh) Poir, *T. owyheense* Gilkey, *T. plumosum* Dougl. ex Hook and *T. rusbyii* spp. *oreganum* (Howell) Heller & Zoh. Of these *T. leibergii* and *T. owyheense* are also listed as candidates for threatened or endangered status.

Most of the species collected in phase two were perennials, but when collected in early August, several were completely dormant with only dry seed heads. The heads and leaves of *T. macrocephalum* had deteriorated, but individual florets with seeds were collected. *T. howellii* was found only at one site in Douglas County in a mountain meadow at about 4000 feet elevation, but was abundant along the wet stream banks. *T. latifolium* was collected on the top of Mt. Howard in north-eastern Oregon at 8150 feet.

A total of 21 collections of 10 mostly perennial species were collected in California during phase three. Included among these species were five new to the NPGS, *T. andersonii* A. Gray, *T. beckwithii* Brewer ex S. Wats., *T. lemmonii* S. Wats., *T. monanthum* A. Gray, and *T. macilentum* ssp. *dedeckeree* (Greene) Isley. Cooperators in California, Oregon, Washington, and Wyoming provided 13 additional collections encompassing four additional species, all new to the NPGS, *T. andinum* Nutt., *T. bolanderi* A. Gray, *T. gymnocarpon* Nutt., and *T. thompsonii* Morton, for a total of nine new species obtained in 1995. Some sites visited had abundant stands of species such as *T. longipes*, which at one location covered an area of several

hectares. Other populations were relatively small and isolated. Most collection sites were located on publicly owned lands, and consideration is currently underway to have some sites designated as *in situ* conservation sites for some species.

The three expeditions have resulted in about 140 collections of at least 25 species new to the NPGS, nine of which were listed as potentially threatened or endangered (Quesenberry and Taylor, 1996). Field collected seed quantities varied from a few seed to over 60 g of some accessions. Sample plants of all accessions have been grown in the greenhouse at the University of Kentucky and herbarium voucher specimens prepared from those that have flowered.

Several perennial species exhibited slow and limited seedling growth before entering into a period of senescence, and attempts to manipulate environmental conditions to improve growth and induce flowering have not been successful. All annual species except *T. fucatum* (cross-pollinated) set seed by self fertilization, whereas all perennials which have flowered appear to be cross pollinated. About nine additional *Trifolium* species from the Americas (four from Chile and five from the western US) remain as uncollected. All collections will be available through the U. S. NPGS when seed is increased. Original seeds of the 1994 California and Oregon collections were divided for storage in New Zealand. This expedition is a good example of international cooperation for unrestricted exchange of germplasm between countries.

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Table 1

Tentative listing of *Trifolium* species collected in western United States in 1994 and 1995 by N.L. Taylor, K.H. Quesenberry, and W.M. Williams.

Trifolium species	Number of collections	State(s)Where Collected
albopurpureum	3	CA
amoenum	1	CA
andersonii	3	CA
andinum	1	WY
appendiculatum	1	CA
barbigerum	5	CA
beckwithii	3	CA
bifidum	2	CA
bolanderi	6	CA
buckwestorium	1	CA
ciliolatum	5	CA
cyathiferum	8	OR
depauperatum	3	CA
dichotomum	1	CA
douglasii	3	OR, WA
dubium	1	CA
eriocephalum	4	OR
fucatum	4	CA
gracilentum	4	CA
gymnocarpon	2	WY
howellii	1	OR
hirtum	1	CA
kingii ssp. productum	1	OR
latifolium	2	OR
leibergii	2	OR
lemmonii	2	CA
longipes ssp. elmeri	1	OR
longipes	7	CA, OR
macilentum spp. dedeckeree	1	CA
macraei	2	CA
macrocephalum	3	OR, WA
microcephalum	9	CA, OR
microdon	4	CA, OR
monanthum	1	CA
obtusiflorum	1	CA
oliganthum	1	CA
owyheense	1	OR
plumosum	6	OR
polyodon	2	CA
rusbyii ssp. Oregonum	1	OR
thompsonii	1	WA
tridentatum	7	CA
trichocalyx	1	CA
variegatum	14	CA, OR
wormskioldii	10	CA, OR