

# Wild *Humulus* Genetic Resources at the U.S. National Clonal Germplasm Repository

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## Abstract

During the past 23 years the *Humulus* genetic resource collection at the U. S. Department of Agriculture (USDA), Agricultural Research Service (ARS), National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon, has greatly benefited from many international plant collecting trips. Significant contributions from North America include several private and USDA sponsored expeditions to Arizona, Colorado, Iowa, Kentucky, Manitoba, Minnesota, Missouri, Montana, Nebraska, New Mexico, New York, North Dakota, Saskatchewan, and Wisconsin. In addition, USDA-sponsored foreign expeditions to Albania, Armenia, China, Kazakhstan, and Russia, have also expanded the representation of global *Humulus* diversity. More than 199 wild hop accessions are preserved at the NCGR from these expeditions. Locality information from the plant collecting expeditions is loaded to the publicly accessible Germplasm Resource Information Network (GRIN) database. Seed from these accessions are inventoried, labeled, and stored at -20°C. Germination protocols are being reviewed and revised. Seedlings from these accessions are being evaluated for disease resistance, morphological traits and chemical and genetic profiles. Genotypes with specific quality traits are being preserved as plants growing in screened enclosures, protected from virus or other pathogen infection. Specific genotypes are cultured in vitro. These tissue cultures are preserved at 4°C for up to 2 years. Meristems from tissue cultures are cryopreserved and stored at a remote location for long-term, back-up preservation. Plant propagules including stem cuttings, rhizomes, tissue cultures, or seeds, are available for distribution to international researchers upon request.

## INTRODUCTION

The NCGR-Corvallis, was established in 1981, to collect, maintain, distribute, and evaluate diverse genetic resources of temperate fruit, nut and specialty crop genera, such as *Humulus* L., hops. The NCGR-Corvallis preserves specific genotypes or hop cultivars as potted plants under screen, with tissue culture and cryogenic back-up. Examples of wild species are represented by seed. A core collection for hops has been defined to represent a broad spectrum of diversity in about 10% of the total collection.

During the past 23 years, 15 USDA and privately-sponsored plant collecting expeditions have donated hop plant material to the Repository (Table 1, 2). These trips are described below.

## PLANT COLLECTION TRIPS

Each year the USDA designates funding specifically for plant exploration. Qualified plant explorers annually submit proposals to request sponsorship from this funding. While many grants request foreign travel, domestic trips are also possible through this funding source.

### Eastern and Midwestern United States

Since the 1980's, Dr. Richard Hampton, USDA, ARS Plant Pathologist retired, has been a lead plant collector and major force encouraging ex situ preservation of Native American *Humulus*. Through his efforts on more than 5 trips (Table 1) through Canada

and the United States, the locations of significant native *Humulus* resources have been identified. He described the habitat and variability of *H. lupulus* var. *lupuloides* in the upper Midwestern North America as a critical source of American hop germplasm (Hampton et al., 2001; 2002). Hampton et al. (2001) stated that 5 of 11 of his selected study sites had complete loss of hops or serious damage to the hop population at that vicinity. In these Midwestern localities he observed a reduction in hop populations over the course of 10 years where soil moisture decreased as a consequence of historic transition from riparian to prairie habitat (Hampton et al., 2001). He suggested that protection of sensitive riparian hop habitats may be warranted. He also collected *Humulus lupulus* var. *pubescens* from Missouri and *H. japonicus* from Eastern states (Table 1). Seeds from his collection trips along with extensive collection notes have been deposited at the NCGR-Corvallis.

### **Western United States**

In 2002 and 2003, with funds provided by USDA plant exploration grants and from the NCGR-Corvallis, Native American hops were collected from Colorado, New Mexico and Arizona (Hummer et al., 2002; 2003; Table 1). Jim Oliphant, Doug Cook, Jodi Jackson, and Scott Dorsch participated in these collecting trips. In preparation for the trip, locality data from herbarium specimens of major universities in the region were consulted.

During the 2002 trip to Colorado, much variation in morphology and aroma was observed in hop populations. Hop distribution in the Rocky Mountains was scattered and uncommon. The herbarium locality information was invaluable to locate populations, although no clear predictors of potential habitat based on associated species or topology were evident (Hummer et al., 2002). *Humulus* appeared to have an opportunistic, fragmented distribution pattern and might be undergoing range expansion or extinction. Hop distribution was more fragmented on the east slope of the Rockies than on the west. On the east slope, the drier climate and loss of habitat through human development may contribute to fewer localities supporting hop populations.

During the 2003 trip to Arizona and New Mexico, the focus was on the southern, isolated *Humulus* localities. At many of the sites visited, no *Humulus* was found. This may be due to local extinction caused by degraded habitat or vegetation change (Hummer et al., 2003). The most stable habitat for *Humulus* was by perennial streams with well developed willow (*Salix* L.) cover. Another less common situation was to find hops at the base of rocks in a riparian corridor where the stream was at a distance or seasonal. Hops were less abundant in Arizona and New Mexico than in previous years, judging from herbarium locality information. Wild hops are undergoing local “extinctions” there, because many of the herbarium specimens from 50 to 100 years ago have no extant populations. Of 23 Southwestern sites visited, 10 had no hop populations. These losses represent significant erosion of the genetic diversity of the southern range of *H. lupulus* var. *neomexicanus*.

### **Foreign**

Thus far five foreign USDA sponsored plant collecting trips that were focused on other plant genera have also obtained wild *Humulus*. These trips have included remote locations in Albania, Armenia, China, Kazakhstan, and the Siberian Far East of Russia. While this does not provide significant insight into population dynamics in these countries, some genotypes from these seedlots may provide useful traits. For example, three selections from Sakand, Kazakhstan, have been observed to have an unusual form of hypersensitive response to hop powdery mildew caused by *Podosphaera macularis* Braun & Takamatus (Jackson per. comm.).

### **GERMPLASM PRESERVATION**

At the NCGR, hop plants are maintained as pathogen-tested potted plants in greenhouses and under screen. Hop clones are tested for American Hop Latent, Apple

Mosaic, Hop Latent, and Hop Mosaic viruses. Infected plants are subjected to pathogen elimination procedures involving thermotherapy and replaced with pathogen-negative genotypes. Testing records are maintained and provided for phytosanitary certification. Back-up plants are preserved as refrigerated tissue cultured plants in plastic bags for medium-term storage, from 1 to 5 years, and cryopreserved meristems in vials stored in liquid nitrogen for long-term storage of many decades.

### **Information Management**

The NCGR-Corvallis is a facility within the National Plant Germplasm System of the United States. The plant genetic resource information for the collection is maintained locally as well as on a publicly accessible database, called the Germplasm Resources Information Network (GRIN) at [www.ars-grin.gov/npgs](http://www.ars-grin.gov/npgs). Data on this site includes background information concerning locality and collector notes, inventory records, as well as observational evaluation records and voucher images. Queries of the database can be performed by entering a particular cultivar or genotype name, selection or collection numbers. Taxonomy of approved taxa and synonyms is available. Links to other botanical and horticultural references for *Humulus* are also available on this website.

### **Seed Preservation**

Seeds are usually donated to the NCGR-Corvallis as cone samples. Seeds are extracted from cones, dried and stored in labeled manila envelopes within larger computer-generated aluminum envelopes. Envelopes are placed in bins stored at -20°C in frost-free chest-freezers. Viability is monitored by germination and Triphenyl-tetrazolium chloride (TZ) tests. Back-up seedlots are separated and sent to the USDA ARS National Center for Genetic Resource Preservation in Fort Collins, Colorado, for remote storage.

Procedures for germination of most *H. lupulus* seeds recommend a minimum of 4 weeks in moist, pre-chilling conditions (about 5 °C) prior to germination conditions alternating between 15 and 25 °C. Germination then occurs within 2 to 4 weeks. Operculum removal can improve germination but is too labor-intensive for large seedlots (Paine, 1950).

### **Seedling Evaluation**

The NCGR-Corvallis staff is presently involved in many evaluation projects concerning the *Humulus* collection. Seed morphology and germination protocols are under investigation. Variation in prenylflavonoid content of wild hop species has been observed (Hummer et al., 2004a). Seedlings resistance to powdery mildew is being evaluated. Molecular markers are being used to assess genetic diversity in hops and address taxonomic questions (Bassil et al., 2004).

### **Plant Distribution**

The NCGR-Corvallis distributes about 3,000 accessions of temperate fruit, nut and specialty crops annually. During 2003, international requestors were shipped more than 290 hop accessions from the Repository collections. About one fourth of the shipped plants were sent to foreign requestors. *Humulus* is distributed as seeds, rhizomes, small rooted plants, and tissue cultures. Plant quarantine requirements are followed. Plants are pathogen-tested by the Repository staff, and inspected by Animal and Plant Health Inspection Service representatives. Phytosanitary certification is issued in compliance with the importation permit from the foreign country. Plants in the Repository collection are in the public domain and are freely distributed to researchers, according to the policy of the U.S. National Plant Germplasm System. The catalog of available *Humulus* accessions can be obtained through <http://www.ars-grin.gov/cor/humulus/huminfo.html>. Requests for germplasm can be made to the Curator of the NCGR-Corvallis.

### **CONCLUSIONS**

While the Repository has representatives of a number of wild species from the

mid-western and southwestern United States further exploration is warranted. Additional samples from the Northern mid-western states would be very appropriate because powdery mildew resistance genes seem more frequent in these than the southern populations. The Repository is lacking representatives of populations from many of the North eastern and western States.

Much of the genetic diversity in *Humulus* from southwestern populations is threatened and may continue to erode through local extinction. Additional ex situ preservation and potential development of in situ preservation areas in collaboration with National Parks, Nature Conservancy, and other governmental and non-governmental agencies may be appropriate in these delicate areas.

The Repository staff will strive to insure that a diverse collection of world and particularly Native American *Humulus* is available to breeders, developers and growers, to serve the needs of the hop industry and community.

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## Tables

Table 1. Plant collecting expeditions obtaining wild *Humulus* for the USDA ARS NCGR between 1981 and 2004.

<b>Participants or Donors</b>	<b>Date</b>	<b>Location, Country</b>	<b>Number of accessions</b>
Mel Westwood, Shimizuku	July 1981	Korea and Japan	2
Richard O. Hampton	July in 1981 to 1983	7 Midwestern US States	106
Richard O. Hampton	July to August 1987	Kentucky, West Virginia, Virginia, North Carolina	12
Shenan He, Kim Hummer	December 1991	Nanjing China	1
P. Forsline, G. Mink, E. Dickson	October 1993	Kazakhstan	2
Ned Garvey, Fred Muehlbauer	October 1996	Albania	1
Richard Hampton	October 1999	North Dakota, Saskatchewan, Manitoba	22
Richard Hannan	October 14, 2000	Kazakhstan	2
Kim Hummer, Andrey Sabitov, Pavel Cherbukin, Nick Vorsa	August 15, 2001	Khabarovsk, Russia	1
Richard Hampton, Doug Cook, Scott Dorsch, John Waddell	August and October 2001	Manitoba, Saskatchewan, North Dakota	179
Jim Oliphant, Jodi Jackson, Doug Cook, Scott Dorsch	September 2002	Colorado, New Mexico	70
Joseph Postman, Paul Meyer	September 2002	Armenia	2
Richard O. Hampton	October 2002	North Dakota	6
Doug Cook	July 2003	Oregon	2
Jim Oliphant, Jodi Jackson	September 2004	Arizona, Colorado	18

Table 2. Native *Humulus* species in the USDA ARS National Clonal Germplasm Repository, Corvallis, Oregon, August 2004.

Species	Origin state or country	Amount of accessions		
		Seedlots	Plants	
<i>Humulus japonicus</i>	China	1	0	
	Missouri		15	
<i>Humulus lupulus</i> var. <i>lupulus</i>	Albania	2	0	
	Armenia	3	0	
	Kazakhstan	4	0	
<i>Humulus lupulus</i> var. <i>lupuloides</i>	Iowa	0	18	
	Kentucky	0	12	
	Manitoba	47	4	
	Montana	0	41	
	Nebraska	1	11	
	North Dakota	15	0	
	Saskatchewan	137	0	
	Wisconsin	0	1	
	<i>Humulus lupulus</i> var. <i>pubescens</i>	Missouri	2	0
	<i>Humulus lupulus</i> var. <i>neomexicanus</i>	Colorado	48	17
New Mexico		7	9	
Arizona		5	7	
Total		272	135	