Chapter 19

Species for Medicinal and Social Use with an Emphasis on *Theobroma cacao* L. (Cacao), *Nicotiana tabacum* L. (Tobacco), *Actaea racemosa* L. (Black Cohosh), and *Humulus lupulus* L. (Hops)

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Abstract This chapter explores plants that are used for medicinal and social uses. It first gives a brief overview of taxa that are found throughout North America, how and where they are conserved and how they are distributed. It then looks at four economically important taxa, *Theobroma cacao* L. (cacao), *Nicotiana tabacum* L. (tobacco), *Actaea racemosa* L. (black cohosh), *Humulus lupulus* L. (Hops), as case

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studies of how medicinal and social plants have been used over the centuries and how their wild relatives have been conserved and how we can expect these plant to be used in the future.

Keywords Medicinal crop · Wild collected · Social-use crop · Crop wild relative

19.1 Overview of Species Used for Medicinal and Social Use

19.1.1 Historic and Modern Use Worldwide

Plants have been central to human culture since antiquity. This chapter will focus on species that have played a role in medicinal and social settings. Worldwide, more than 80% of the population in developing countries relies on herbal medicine, and its use in developed countries is increasing (Canter et al. 2005). Historically, a wide range of plant species have been used by a multitude of cultures across the world, reflecting a diversity of traditional pharmacopeia. An estimated 50,000–80,000 plant species are used currently for medicinal purposes around the world (Chen et al. 2016). Both the United States and Mexico fall among the top ten countries, in terms of number of medicinal plant species; the United States having slightly less than 3000 species, representing about 12% of its flora, and Mexico having about 2500 species, representing 9% of its flora (Chen et al. 2016). Even in boreal Canada, at least 546 taxa were used by First Nations (Uprety et al. 2012). Approximately two-thirds of medicinal species are collected from the wild, which is creating sustainability concerns (Canter et al. 2005). Recommendations have been made for the conservation and sustainable use of medicinal plants (Hamilton 2004) and include field cultivation, breeding, and molecular fingerprinting, as well as tissue culture and genetic transformation.

19.1.2 Challenges to Cultivation and Crop Improvement

Medicinal and social-use crops span the production gamut from large-scale commercial production, small acreage, and backyard production to direct harvest of wild species from managed or unmanaged natural landscapes. Medicinal and social-use crops may also be used for multiple purposes. Plant part, plant preparation, and agronomics can differ depending on the use, adding to the complexities of cultivation. Although the challenges of cultivation vary by species, all share the common element of dealing with biotic stress (disease, insects) and abiotic stress (drought, heat), particularly in the face of climate change and the resulting changes occurring in pest distributions and weather patterns. Although it is not possible in this chapter to adequately address the cultivation challenges faced by all North American species utilized for medicinal and social purposes, a common thread among all species is that challenges in cultivation and commercialization can be addressed by capitalizing on the inherent diversity found in naturally occurring populations. The genetic resources available to improve medicinal and social-use crops span a range of germplasm that includes cultivated and wild forms (i.e., wild utilized species) of the crop species, as well as species related to the crop (crop wild relatives, CWR). This broad range of germplasm has great potential as a source of variation for many different traits. A general challenge in using diverse germplasm is lack of availability of diverse and well-characterized genetic resources, especially for crops used for specific cultural or ceremonial purposes. A challenge to the increased use of related wild species is the lack of knowledge about the potential for hybridization between CWR and the specific crop. A final challenge is to ensure that these resources, especially those found in the wild, are effectively conserved both in situ and ex situ.

19.1.3 Conservation and Sustainable Use

Globally, approximately two-thirds of medicinal species are obtained directly from the wild (Canter et al. 2005). Many medicinal and social-use plants occur on endangered and threatened plant lists. Table 19.1 lists medicinal species listed in the US crop wild relative inventory of Khoury et al. (2013), ranked as vulnerable or imperiled by (NatureServe 2017).

As with many wild plant species, habitat loss is a major factor contributing to vulnerability (e.g., *Echinacea*; Kindscher 2006). Other factors that contribute to species rarity include habitat specificity, distribution range, population size, species diversity, growth rate, and reproductive system (Chen et al. 2016). Overharvesting is a cyclical problem depending on demand. Although not officially listed, many medicinal species are at risk from overharvesting, and close monitoring is needed to ensure sustainable harvest. A ranking tool has been developed to quantify the vulnerability of temperate North American species to overharvest (Castle et al. 2014). The ranking tool supports a species watch list published by the United Plant Savers (https://www.unitedplantsavers.org) (United Plant Savers 2017). RootReport (http://www.rootreport.frec.vt.edu) is a collaborative website housed at Virginia Technical University, Virginia, that tracks US native medicinal plant harvest, production, and markets and provides resources to support the sustainable harvest and production of medicinal plants. In addition to monitoring for the negative impacts of overharvest, in situ and ex situ conservation are important strategies.

19.1.3.1 Ex Situ and In Situ Conservation

Medicinal and social-use taxa are present in many genebanks; significant collections include the NPGS (USA), the Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (Germany), and the National Agriculture and Food Research Organization (NARO) genebank in Japan. The NPGS medicinal plant collection is housed at the USDA, Agricultural Research Service, North Central Regional

Table 19.1 Vulnerable or threatened medicinal plant species occurring in crop wild inventory of the United States (Khoury et al. 2013)

Taxon	Common name	Rank[a]
Abies fraseri (Pursh) Poir.	Fraser fir	G2
Artemisia australis Less.	Hinahina	G3
Artemisia palmeri A. Gray	San Diego sagewort	G3
Artemisia porteri Cronquist	Porter's wormwood	G2
Croton alabamensis E.A. Sm. ex Chapm. var. Alabamensis	Alabama croton	G3 T3
Croton alabamensis E.A. Sm. ex Chapm var. Texensis	Texabama croton	G3 T2
Echinacea angustifolia var. strigosa	Narrowleaf purple coneflower	G4HQ
Echinacea atrorubens Nutt.	Topeka purple coneflower	G3
Echinacea laevigata (C.L. Boynt. & Beadle) S.F. Blake	Smooth coneflower	G2G3
Echinacea paradoxa (Norton) Britton var. neglecta R.L. McGregor	Bush's purple coneflower	G2T1
Echinacea paradoxa (Norton) Britton var. Paradoxa	Ozark coneflower	G2 T2
Echinacea sanguínea Nutt.	Sanguine coneflower	G3G5
Echinacea tennesseensis (Beadle) Small	Tennessee coneflower	G2
Eurybia furcata (Burgess) G.L. Nesom	Forked aster	G3
Guaiacum sanctum L.	Holywood lignum vitae	G2
Hypericum adpressum W.P.C. Barton	Creeping St. John's wort	G3
Hypericum chapmanii P. Adams	Apalachicola St. John's wort	G3
Hypericum cumulicola (Small) W. P. Adams	Highlands scrub St. John's wort	G2
Hypericum graveolens Buckley	Mountain St. John's wort	G3
Hypericum harperi	Sharp-lobe St. John's-wort	G3G4
Hypericum lissophloeus P. Adams	Smooth-barked St. John's wort	G2
Hypericum mitchellianum Rydb.	Blue ridge St. John's wort	G3
Lindera melissifolia (Walter) Blume	Pondberry	G2G3
Panax quinquefolius L.	American ginseng	G3G4
Papaver alboroseum Hultén	Pale poppy	G3G4

[a] NatureServe rank (NatureServe 2017); *G2* imperiled, at high risk of extinction due to very restricted range, very few populations (often 20 or fewer), steep declines, or other factors; *G3* vulnerable, at moderate risk of extinction due to a restricted range, relatively few populations (often 80 or fewer), recent and widespread declines, or other factors; *G#G#* range rank, a numeric range rank (e.g., G2G3) is used to indicate the range of uncertainty in the status of a species or community

Plant Introduction Station (NCRPIS), located in Ames, Iowa, and conserves ~210 medicinal taxa. Many taxa are also conserved in botanical gardens and arboreta. In Mexico, the IB-UNAM Botanic Garden has been active in sharing knowledge about indigenous plants, and the Oaxaca Botanic Garden has been promoting their use in public landscapes (Hawkins 2008). Although Maunder et al. (2001) found that European botanical gardens housed a number of medicinal species, few systematic conservation plans were in place, and efforts tended to be skewed toward ornamental species. However, the Botanical Garden Consortium International has since developed a global priority species list and action plan for conserving medicinal plants (Hawkins 2008). In temperate North America, the United Plant Savers has established a network of over 100 botanical sanctuaries that preserve habitat that harbors a diversity of wild medicinal species (http://www.unitedplantsavers.org). Public lands also afford a level of protection for medicinal and social use species that are commercially harvested. In the United States, USFS and BLM regulate collection, but there is room for improvement (Robbins 2000). In Canada there is no formalized system to protect native stands of commercially harvested species (Westfall and Glickman 2004). In Mexico a collaborative effort between the National Autonomous University of Mexico (UNAM), the National Commission for the Knowledge and Use of Biodiversity (CONBIO), and the KEW Royal Botanical Gardens has resulted in the collection and security of ex situ storage of useful native Mexican species, including many medicinal and social-use species (Rodríguez-Arévalo et al. 2017).

19.2 Case Studies

While it is possible to talk about the general status of medicinal and social-use genetic resources, it is impossible in this chapter to cover all species. Instead, we explore individual case studies of some of the most economically important species within this broad category. To this end, this chapter presents snapshots of cacao, tobacco, hops, and black cohosh – all important medicinal and social-use crops with important genetic resources in North America.

19.2.1 Cocoa (*Theobroma cacao* L.)

19.2.1.1 Summary

Cocoa is one of the most recognized products across the world due its major commercial product of chocolate. Chocolate holds a special place in many cultures across the world. Although cocoa is a crop of historic, economic, social, and ecological importance, there is still limited information about the distribution of native wild-type cocoa and no legal protection of native varieties. There is an urgent need to invest in the preservation of cacao germplasm resources to ensure that this crop will continue to thrive.

19.2.1.2 Origin of the Crop and Brief History of Use Worldwide

The genus *Theobroma* comprises 21 species, and includes *Theobroma cacao* L. (Cuatrecasas 1964). Most species of this genus are distributed exclusively in South and Central America; however, *Theobroma cacao* L. and *Theobroma bicolor*

Humb. & Bonpl. are distributed in southeastern Mexico, with *T. bicolor* being locally known as “pataxte” (CacaoNet 2012). Cocoa was important to the Mayan culture; it was used as currency, and therefore drinking chocolate represented the height of luxury. Historic information from antiquity is represented in a series of painted or engraved vessels found in the tombs of the Mayan nobles (Ogata 2002). Cocoa seeds are fat-rich and are used as a source of cocoa solids and butter for chocolate making and for the cosmetic industry. The center of origin has been well studied (Cuatrecasas 1964; Cheesman 1944; Dias 2001; Miranda 1962), with recent work suggesting cocoa originated in South America and was brought north by humans (Bartley 2005; Motamayor et al. 2002).

19.2.1.3 Modern-Day Use

Cocoa *(Theobroma cacao* L.) has great economic importance, being cultivated by greater than 2 million producers in greater than 50 countries. Each year, in the humid tropics, more than three million metric tons of dried cocoa beans are produced to be consumed in developed countries. Cocoa was exclusively a New World crop until 1890, when cultivation began in Africa (Ogata et al. 2006), where today the highest volume of production occurs. More than 20 million people around the world depend directly on the cultivation of cocoa for their livelihood with ~90% of cocoa production coming from small farms of less than five hectares. In 2015 in Mexico, cocoa was cultivated on 61,397 hectares with a production of 28,006 tons worth $1,034,792,000 Mexican pesos (SIAP 2016). Cocoa is cultivated in the states of Chiapas, Tabasco, and Guerrero (Avendaño-Arrazate et al. 2011). In Tabasco, cocoa is grown in the Chontalpa region, which includes the municipalities of Paraiso and Cardenas, in the central region that includes Nacajuca and Jalpa, and in the mountainous regions of Teapa, Jalapa, and Tacotalpa. In the state of Chiapas, it is cultivated in the northern region in the municipalities of Pichucalco, Ostuacan, Reforma, and Juarez and in the southern regions of Tapachula, Huixtla, Tuxtla Chico, Tuzantan, Cacahoatan, and Huehuetan (Fig. 19.1; SIAP 2016; Gutiérrez-López et al. 2016; Avendaño-Arrazate et al. 2011).

19.2.1.4 Challenges in Cultivation

While Mexico is a large cocoa producer, the average yield of the main producing states (Tabasco and Chiapas) of 470 kg/ha (SIAP 2016) is low compared to other exporting countries where yield is approximately 1 ton per hectare. Low yields of cocoa in Mexico are mainly due to the following:

(a) Advanced age of plantations with 40–80% older than 25 years (Avendaño-Arrazate et al. 2011)
(b) The poor performance of old cultivars having low yield potentials (Hernández-Gómez et al. 2015)
(c) Low planting densities

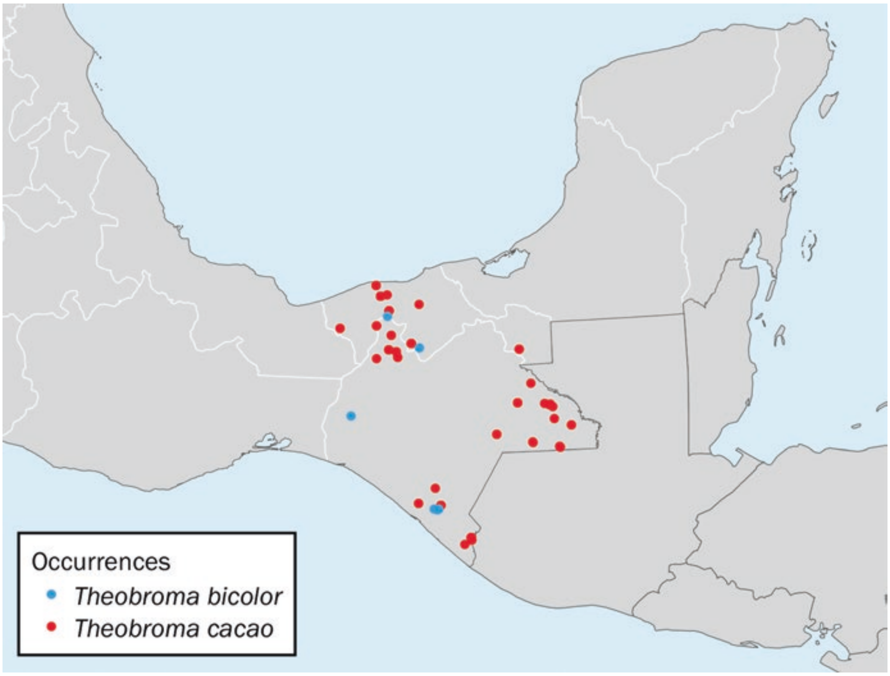

Fig. 19.1 Distribution of *Theobroma bicolor* L. and *Theobroma cacao* L. occurrence points located within areas of Mexico listed as native to the species by GRIN Taxonomy (USDA ARS National Plant Germplasm System 2017). Occurrence locations also exist for both species in many parts of central and southern Mexico, but their status as wild types is uncertain

(d) The cumulative damage of pests and diseases, with the major diseases being black pod disease caused by *Phytophthora palmivora* Butler, progressive tree death caused by *Ceratocystis fimbriata* Ellis & Halst, and recently moniliasis, caused by *Moniliophthora roreri* (Cif.) H.C. Evans, Stalpers, Samson & Benny, which has decreased yields in Soconusco, Chiapas, by up to 80% (Phillips-Mora et al. 2006; Phillips 2003)
(e) Little or incorrect management of plantations, which includes no shade management or pruning, inadequate fertilization, and inefficient control of pests and diseases
(f) Poor postharvest management (no controlled fermentation), leading to reduced quality and market price

19.2.1.5 Nutritional Use

Cocoa contains ~300 volatile compounds, including esters, hydrocarbolactones, monocarbonyls, and pyrroles, among others. The important flavor components are aliphatic esters, polyphenols, unsaturated aromatic carbonyls, diketopiperazines, pyrazines, and theobromine (Kalvatchev et al. 1998). Cocoa products and their uses are described in Table 19.2.

Table 19.2 Cocoa products and their uses

Products	Uses
Cocoa	Chocolate
Cocoa butter	Moisturizing creams, soaps
Cocoa pulp	Production of alcoholic and nonalcoholic beverages
Peel of the fruit	Animal feed, compost
Ashes of the shell	Soap, fertilizer
Cocoa juice	Preparation of jellies and jams
Cocoa powder	Ingredient in chocolate drinks and desserts, such as ice cream, mousses, sauces, cakes, and biscuits
Nutraceutical roles	(1) Treating patients to regain their weight; (2) stimulating nervous system of patients with hepatitis, exhaustion, or weakness; and (3) improving digestion, as cocoa/chocolate counteracts the effects of stunted or weak stomachs, stimulates the kidneys, and improves bowel function. In addition, chocolate/cocoa treatments have been performed for anemia, lack of appetite, mental fatigue, low breast milk production, tuberculosis, fever, gout, kidney stones, low sexual appetite, and low virility
Other	Antibacterial, antimycotic, and antiviral activity, the latter may be related to cocoa flavonoids

Source: Kalvatchev et al. (1998), Dillinger et al. (2000), Kalvatchev et al. (1998)

19.2.1.6 Crop Wild Relatives of the Crop

Theobroma cacao L. (2n = 2x = 20) belongs to the Malvaceae family and is classified into three main morphogeographic groups: outsider, criollo type, and trinitarian (Cheesman 1944). Cocoa populations from the Amazon basin belong to the outsider group, which can be further subdivided into an outlying group from the upper Amazon region and the outsider group from the lower Amazon region. The wild-type (criollo) group contains populations present from Central America to northern Venezuela and Colombia, while the trinitarian group is considered to be a group of hybrid materials between the outsider and the wild type (Bartley 2005).

The criollo type is characterized by elongated fruits with a pronounced asymmetry and acute point. The pod surface is usually rough, green, and often with splashes of red to purple, and seed embryos are large and white. This material produces the highest-quality chocolate (Cuatrecasas 1964; Ogata 2003). However, criollo type individuals are poor in performance and susceptible to disease. For these reasons, criollo type cocoa has been displaced from plantations by more productive varieties resistant to disease, but with lower quality (Avendaño-Arrazate et al. 2011). In Mexico, there is a wide diversity of criollo type cocoa genotypes that have not yet been exploited due to the lack of systematic studies (Avendaño-Arrazate et al. 2010). In Mexico, criollo cocoa has practically disappeared from commercial plantations. Only 5% of the interviewed producers reported having exclusively native cocoa, while 13% reported having native cocoa associated with other types (Avendaño-Arrazate et al. 2011).

19.2.1.7 Utilization

The evaluation and selection of clones began between 1945 and 1948 with the selection of 350 trees on farms from the municipalities of Tuxtla Chico and Cacahoatan, Chiapas. Clones were multiplied and established at Rosario Izapa Experimental Field of the National Institute of Forestry, Agriculture and Livestock Research (INIFAP), and the preliminary evaluation was initiated. Of these collections, 26 clones were selected in 1962, from which, 13 clones with superior production were selected as a basis for the genetic improvement program. The establishment of a germplasm bank occurred in 1980 with collections from Mexico and Central and South America. A collection of ~175 accessions was obtained, of which 125 clones have been characterized morphologically. Varietal descriptors of cocoa were created for the International Union for the Protection of Plant Varieties (UPOV), and these were adopted worldwide as a reference for the registration of improved cocoa varieties (Avendaño et al. 2014). Further evaluation and selection of interclonal crosses were carried out in field experiments in Rosario Izapa and other localities of Tabasco and northern Chiapas. Interclonal crosses involving Mexican clones and introductions from other countries were evaluated for production, adaptation, and resistance to *P. palmivora* and *Moniliophthora roreri* under natural conditions. In these studies, the progeny of clone "Pound 7" has been identified as most outstanding. Interclonal hybrids generally perform better than the "Amelonado" variety traditionally grown in the region of Tabasco and northern Chiapas.

Starting in 2009 in the region of Soconusco, Chiapas, INIFAP began the participative genetic improvement project that includes a very close collaboration between researchers and producers (Martin and Sherington 1997). Specifically, producers are involved in the breeding program, including setting objectives, generating variability, selecting and testing, as well as seed production and distribution (Rios et al. 2000). The methodology consists of (a) socialization of the project and definition of the criteria of selection of trees with the participating producers; (b) searching for, selecting, and labeling trees; (c) in situ morphological characterization and agronomic behavior of the selected trees; (d) evaluation of the response to pathogens; and (e) propagation of basal suckers and evaluation of the disease resistance in at least three environments of Soconusco, Chiapas. More than ten producers participated in the project. The criteria for selection were disease tolerance (moniliasis), high yield, quality, and aroma (wild-type characteristics). Five trees were selected and resistance testing was performed. Of these, two varieties were found to be tolerant to moniliasis, "Regalo de Dios" and "Arcoiris," and three with characteristics of wild type, "Rojo Samuel," "Rojo Gustavo," and "Verde Gustavo" (Avendaño-Arrazate et al. 2013).

19.2.1.8 Wild Utilized Species

Pataxte (*Theobroma bicolor*) is the most important relative of commercial cocoa. Pataxte is classified in Mexico as a semidomesticated species which has its own market (Ogata 2002). The major uses of pataxte are the same as for cocoa – drinks, candies, and marmalades (García et al. 2002). Dried pataxte seeds are a snack in South America (Avendaño-Arrazate et al. 2010). The pataxte pulp and beans are mainly used to manufacture confectioneries (marzipan, nougat, and marshmallow), chocolate (mixture of seeds of *T. cacao* and *T. bicolor*), fresh drinks (gruel, pozol, powder, and popo), and as fresh fruit (García et al. 2002; Bressani and Furlan 1997). A common preparation in the state of Oaxaca is a drink known as "popo" that in Nahuatl means "smoke," perhaps in reference to the foam produced when the drink is whipped with a wooden blender, traditionally made from a branch of *Quararibea funebris* (Key) Vischer, where flowers of pataxte are also used for other beverage known as "tejate." The drink "popo," also known by foreigners as "capuchino oaxaqueño," is made with slight modifications by the Nahuas, Mixe-Popolucas, Zoque-Popolucas, Mazatecos, and Chinantecos (Galvez-Marroquín et al. 2016). Recent work mentions its utility as a better source of antioxidants than *T. cacao* (Kalvatchev et al. 1998), and several studies recommend the use of *T. bicolor* as a substitute for cocoa butter alone or in combination, although in comparison with commercial cocoa it contains less fat. The fruit in general contains 127 volatile compounds, with high concentrations of ethylene acetate (36%).

Today, pataxte is associated with cocoa production systems, serving as a shade tree or as part of backyard orchards, with a wide variation of fruit shapes and sizes (Fig. 19.2). However, like cocoa, fruits of this species are susceptible to moniliasis *(Moniliophthora roreri* [Cif. & Par.] Evans), providing an alternate host for the pathogen. It is for this reason that producers are eliminating it, and in some regions it is disappearing (Mendoza-López et al. 2012). In the Soconusco and Chiapas northern regions, it is associated with cocoa; however, both regions lack management, and the few existing trees are being cut down (Gálvez-Marroquin et al. 2016). In the 1980s a considerable area was planted in the Chinantla region of Oaxaca, particularly Valle Nacional, San Felipe León, San Mateo Yetla, San Juan Lalana, Ojitlan, Usila, and San Jose Chiltepec. The state of Oaxaca is the only Mexican state that consumes large amounts of pataxte, and this demand represents a profitable opportunity for producers motivated by the price at the regional level (Mendoza-Lopez et al. 2012). Pataxte represents an alternative crop to improve the income of producers in southeastern Mexico; however, the lack of availability of improved varieties, including those with resistance to moniliasis, lessens its appeal. That is why it is necessary to generate technological packages that allow its cultivation in a sustainable way for the producer and in this way help to preserve it.

19.2.1.9 Conservation Status of CWR and WUS

Criollo-type cocoa is still found in the natural protected area of Montes Azules (Lacandon Jungle, Chiapas), a federal reserve where cocoa is preserved. There is a need for in situ conservation in cocoa and for promoting this strategy among producers of cocoa and for developing varieties that the market needs. By creating participatory breeding programs, where the transfer and use of varieties are more effective, producers can have additional income and avoid displacement from the

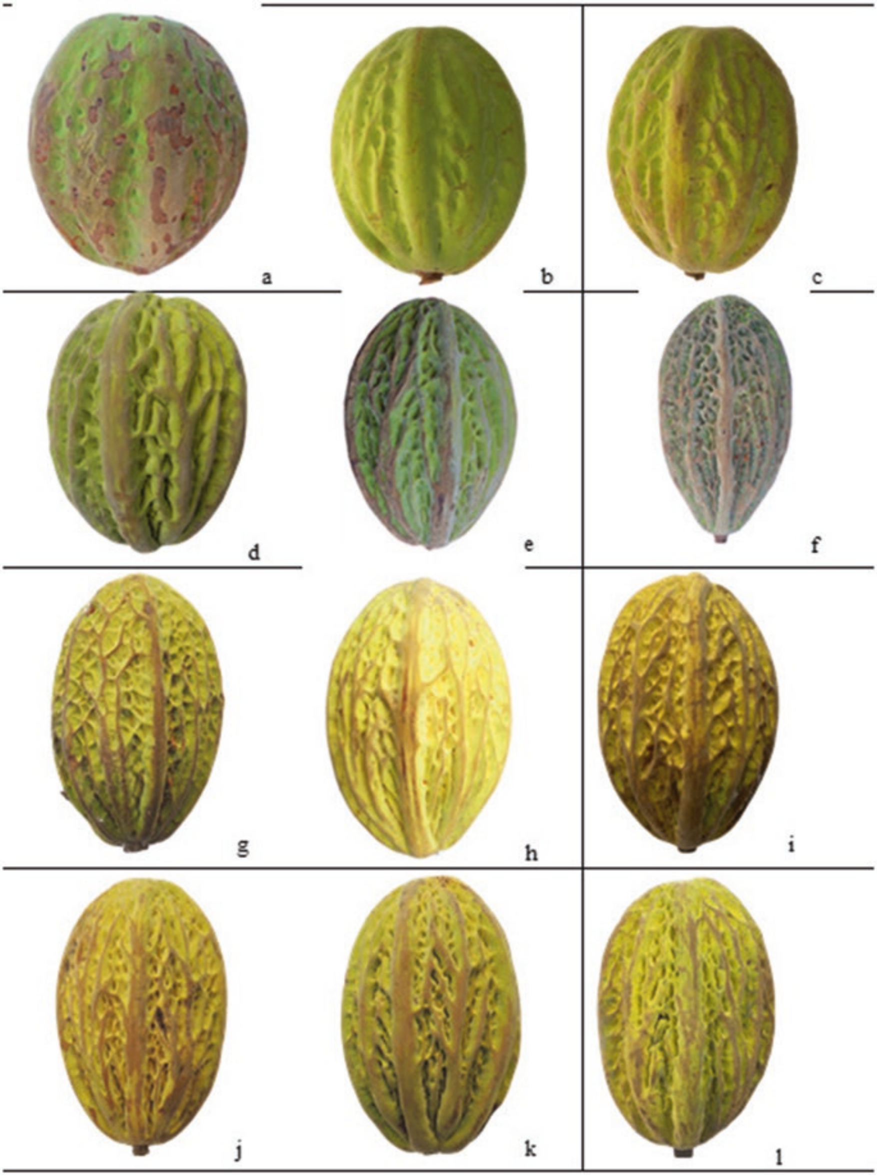

Fig. 19.2 Diversity of fruit forms of pataxte *(T. bicolor* L.*)* distributed in Mexico. (Source: Galvez-Marroquín et al. 2016)

countryside or, in this case, the replacement of the cultivation of cocoa by other crops. Finally, the protected natural areas of Mexico, mainly the Montes Azules in Chiapas where native cocoa is still found, should be invested in heavily to educate both technicians and producers for the conservation and sustainable use of this resource. An associated ethnic group, the Chol, live in the city of Palenque, Chiapas, and are supported by the National Commission of Natural Protected Areas, although this does not necessarily imply that in situ species conservation is being performed (Avendaño-Arrazate et al. 2011). As wild-type cocoa is highly valued by market niches in Europe, this can be an incentive to continue the conservation and sustainable use of wild-type cocoa. The challenge is to provide institutions that promote in situ conservation with financial resources for training and capacity building, so that the various players in the value chain of cocoa increase their awareness of preserving and maintaining Mexican wild-type cocoa. In the case of *T. bicolor*, its conservation is promoted in cocoa plots, because on average each plot has five to ten trees of *T. bicolor*.

In 1942 Mexico initiated collection and census of clonal nurseries of the collected germplasm, selecting local germplasm, and introduced germplasm in the Experimental Field Rosario Izapa-INIFAP. Likewise, INIFAP has reported that most of the genetic material protected in its germplasm banks is wild in origin (López et al. 1990). From these clonal nurseries and in their selection programs, important genotypes have been derived including Amelonado, Calabacillo, RIM (Rosario Izapa, Mexico), Guayaquil, Ceylan, wild type, Colegio de Postgraduados Germoplasma (CP1, CP6, (INIFAP 68 and 67 derived from wild type)), and INIFAP (INIFAP 75 and 76 derived from Amelonado). The "Rosario Izapa" germplasm collection is one of the two national collections of INIFAP cocoa germplasm, with a total of 176 accessions of trinitarians, outsiders, and a collection of Mexican wild-type cocoa. The other collection is located in the Huimanguillo Experimental Field of INIFAP in Tabasco. The cocoa collection at the Rosario Izapa Experimental Field was established in the early 1980s and currently has accessions from eight countries in Latin America and Mexico: Costa Rica (UF, CC, CATIE, Santa Clara, Diamantes), Colombia (ICS), Venezuela (Ocumare, Chuao, Porcelana), Guatemala (SPA), Brazil (SIAL, RB, Catongo, EEG) (SGU) and Mexico (RIM, La esmida pentagona, PICH, TAB, P, CHI, OST, Santa Ana), *T. mammosum*, T. *bicolor* and *Herrania* spp., and 70 wild type accessions reproduced through buds (materials from Yucatan, Tabasco, Chiapas, Oaxaca, and Veracruz mainly). In addition, it has 30 accessions of materials reproduced by seeds from the Lacandon Jungle of Chiapas. The collection has been an important resource in the search for resistance to *Phytophthora palmivora* Butler, *P. capsici* Leonian, *Colletotrichum gloeosporioides* M. B. Dickman, *Ceratocystis fimbriata* Ellis & Halst., and *Moniliophthora roreri* (Cif.) H. C. Evans, Stalpers, Samson, & Benny. In addition to different characteristics of commercial interest, there has been extensive characterization of the germplasm (Avendaño et al. 2014; UPOV 2011). The germplasm bank has helped produce cocoa germplasm that is tolerant to *P. palmivora*, such as INIFAP-H12 and INIFAP H-13 and tolerant to moniliasis (CAERI-1, CAERI-2); varieties of high performance and quality, such as RIM-24, RIM-44, RIM-56, RIM-88, and RIM-105; and wild-type varieties such as CAERI and Lacandon.

There has been less effort to collect *T. bicolor*, although during 2012 and 2013, collections were made in southern Mexico, and currently a genebank has been established in the Experimental Field Rosario Izapa-INIFAP with 20 accessions derived from seed. There are fewer economic resources to keep the collection alive and to have a permanent breeding program. The best rationale for funding is that there is a need to characterize native cocoa germplasm for quality and aroma that can be used for gourmet chocolates.

One of the main challenges to conservation is keeping the collection alive, as collections must be maintained as clonally propagated trees (Cheesman 1944; Cuatrecasas 1964). In living collections, there is limited water availability, and there is the potential for natural disasters. Another challenge is the possible arrival of diseases, such as witches broom caused by *Moniliophthora perniciosa*. The future of cocoa worldwide depends on the use of germplasm for the generation of new varieties with resistance to pests and diseases, quality characteristics, and good adaptation to climatic changes (Zhang and Motilal 2016).

19.2.1.10 Suggestions on How to Improve Conservation

Although cocoa is a crop of ancestral, economic, social, and ecological importance in Mexico, there is still no systematized information about the distribution of native wild-type cocoa. The information found in the herbaria is old and in most cases, the places where collections were made are now pastures (as in the case of the jungle region of Chiapas). In addition, there is no legal protection of native varieties. Germplasm conservation is located in a single institution, INIFAP, and accessions have not yet been fully characterized. Most research is dedicated to genetic improvement, health, and postharvest. Phytosanitary problems such as moniliasis, little or no management of plantations, and the advanced age of producers and plantations are leading to the abandonment and demolition of plantations and, as a consequence, the loss of genetic diversity. Therefore, it is urgent and necessary to continue with the national collection and morphological, physiological, biochemical, and molecular characterization. Advances in rescuing wild-type cocoa from Mexico and the conservation of genotypes in germplasm banks have not been concluded, and there is a risk of losing the great genetic and cultural richness of the species. The safeguard and disposal of the high organoleptic quality of the Mexican wild-type cocoa represent a significant advance for the future of cocoa. An area that needs further exploration and research is cryopreservation of clonal plant parts or seed, as an alternative to the ex situ conservation of genetic diversity of wild-type cocoa of Mexico.

19.2.2 Tobacco (Nicotiana L.)

19.2.2.1 Summary

Tobacco (*Nicotiana* spp.) use dates back thousands of years ago in North America; it has been used medicinally, socially, and ceremonially for much of this timeframe. Tobacco has been a major economic species worldwide for over 500 years. There has been widespread use of CWR in tobacco as well the genus being a workhorse for basic plant science.

19.2.2.2 Origin of the Crop and Brief Use History

Although tobacco (*Nicotiana* spp.) is not a food crop, its use likely stretches back to the very origins of agriculture, with the earliest known evidence dating to 2500–1800 BC in northern Peru (Rafferty 2002). It subsequently spread throughout the American continents such that usage of various *Nicotiana* species by Native Americans was widespread at the time of European arrival to the New World. At the time of contact, *N. tabacum* L. was being grown in the northern regions of South America, the Caribbean, and Mexico (although it is unclear in this region if the "tobacco" noted was *N. tabacum* L., *N. rustica* L., or both). *Nicotiana rustica* L. was the preferred species of the Mississippian tribes of eastern North America, while Native peoples of western North America made use of locally available species such as *N. attenuata* Torr. ex S. Watson and *N. quadrivalvis* Pursh. Among Native Americans, plants were either actively or passively grown depending on the tribe, and the leaves (or calyces in the case of *N. quadrivalvis*) were dried and smoked or ground and mixed with lime to form a type of lozenge (Linton 1924).

Large-scale cultivation of *N. tabacum*, modern commercial tobacco, was initiated in the Caribbean by Spaniards in the late 1500s. Tobacco use and cultivation was rapidly spread by European sailors as they traveled along trade routes to Europe, Asia, and Africa during the early 1600s (Collins 2013). Over the centuries many different market classes of tobacco were developed with different smoke flavor profiles and leaf textures. The current classes are burley, flue-cured, dark fire- and air-cured, cigar wrapper and filler, and oriental. While tobacco has been consumed in many different forms since antiquity, today the primary uses of tobacco leaf are for the manufacture of cigarettes, cigars, and smokeless tobacco (i.e., chewing tobacco). The nicotine dispensed by electronic cigarettes primarily comes from cheap waste materials discarded from the manufacture of other tobacco products. According to the Food and Agriculture Organization of the United Nations, 7.4 million tons of cured tobacco leaf was harvested from 4.2 million hectares of land in 2013, generating a gross production value of over USD 19 billion (FAOSTAT 2017). The total value of manufactured tobacco products on the global market in 2013 was USD 605.1 billion, with cigarettes accounting for over 90% of all revenue (USDA, ERS).

19.2.2.3 Cultivation

19.2.2.3.1 Agronomic Practices

Nicotiana tabacum is a tropical perennial species that is grown as an annual crop. Agronomic practices involve the germination and growth of seedlings, the transplanting of young plants into the field, growth of plants to maturity, harvesting, and curing. Each market class of tobacco has its own unique set of practices regarding row and plant spacing, fertilization, when or if the inflorescence is removed (topping), whether the leaves are harvested individually (priming) or if the whole plant is harvested by cutting the stalk, and how the leaves are ultimately dried during the curing process (reviewed in Johnson and Reed 1994). Tobacco is primarily grown on silt or sandy loam soils, where the soil is actively cultivated into large ridge rows around the base of plants in order to prevent lodging and for drainage. For flue-cured tobacco, the predominant market class, the inflorescence is removed shortly after the initiation of flowering. This allows for increased development of the leaves, particularly those of the upper stalk positions, and also triggers desirable chemical changes in the leaves (including an increase in nicotine content). Approximately 2 weeks after topping, harvest will begin as the lower leaves start to yellow due to nitrogen starvation. Collected leaves are subjected to a very specific regimen of heat and humidity control during curing, which will result in the leaf turning a golden hue and having a sweet aroma.

19.2.2.3.2 Pests, Diseases, and Climatic Limitations

While many pathogens impact tobacco production (reviewed by Lucas 1975), the two most economically important pathogens in North America are *Phytophthora nicotianae* Breda de Haan and *Ralstonia solanacearum* (Archibald) Robbs, the casual agents of the black shank and bacterial wilt diseases of tobacco, respectively. These two pathogens are particularly devastating because they often lead to complete plant death before harvest. A number of different viral diseases also are important globally, including *Tobacco mosaic virus* (TMV), *Potato virus Y* (PVY), *Tobacco etch virus* (TEV), and *Tomato spotted wilt virus* (TSWV). Due to the natural insecticidal properties of nicotine, few herbivorous insects consume significant tobacco leaf tissue, but there are two species, the tobacco hornworm (*Manduca sexta* L.) and budworms (*Heliothis virescens* (Fabricius)), which can cause considerable damage. Aphids are also a problem as their exudates promote mold growth during curing that reduces quality and value.

Tobacco is amenable to a wide array of soil and climatic conditions, and it is actively grown on all arable continents, with China, Brazil, and Zimbabwe being the major producers along with the United States. It has historically been grown in North America in Mexico, throughout the Caribbean, in the United States from Florida up into Pennsylvania and Wisconsin, and as far north as Canada. A cultivar which grows well in Florida will often grow equally well in Pennsylvania, as latitude and altitude tend to have little impact on phenotype. This is at least partly due to the fact that almost all tobacco varieties are day-neutral, despite being the species in which photoperiodism was first described (Garner and Allard 1920). While tobacco is known to be drought tolerant, requiring only about 1 in of precipitation per week, fields are often irrigated during long dry spells to preserve yield. Conversely, tobacco does not grow as well in soils that retain a lot of moisture, and its growth is limited in extreme latitudes (and altitudes) where a sufficient window of time does not exist between the last and first frost dates.

19.2.2.4 Crop Wild Relatives (CWR)/Wild Utilized Species (WUS) of the Crop

19.2.2.4.1 General Description of Genus

Nicotiana L., a member of the Solanaceae family, is comprised ~75 naturally occurring species, the exact number being debatable as species once considered distinct have been consolidated by various authorities but not by others. In his treatise of *Nicotiana*, Goodspeed (1954) provided the following description of the genus:

> Tall, soft-woody subarborescent shrubs to diminutive annuals. Indument varied, often moist- or viscid-glandular, seldom lacking. Leaves alternate, petioled or sessile, the blade entire. Flowers scentless or fragrant at dusk, pedicelled, in terminal mixed panicles with evident central axis, false racemes, false racemes secondarily converted to flat pinnate panicles, or a variety of loosely expanded or remotely glomerate systems with some too much dichotomy, rarely flowers associated with leaves instead of bracts. Calyx regular or irregular, 5-toothed or cleft, commonly much shorter than corolla, always persistent, usually somewhat enlarged on fruit. Corolla regular or slightly irregular, tubular, infundibular or salverform, the tubular portion often differentiated into a distinct tube ("tube proper") and distinct throat, the limb 5-cleft, shallowly 5-lobed or nearly entire, in bud contorted-plicate, rarely imbricate, at anthesis erect, spreading or recurving, unaffected by light intensity or loosely folding during the day and expanding at dusk. Stamens 5, free filaments equally or unequally inserted on corolla at some point below limb, commonly at base of corolla throat if throat is present, equal or unequal in length, usually included or nearly so, sometimes obsolescent; anthers with or without connective, dehiscing along longitudinal suture. Ovary bilocular, oblique in relation to surrounding whorls, base adnate to thick, sometimes nectiferous, annular hypogynous disk, placental cushions on the central dissepiments, ovules numerous, anatropous; style terminal; stigma slightly grooved. Capsule membranous- or slightly woody-walled, lower portion indehiscent, upper dehiscent by rather long septicidal and very short loculicidal cleavages, he former commonly cutting the partition in patterns which leave part attached to the wall, part to the placentae. Seeds minute, reniform, globose, elliptic, oblong or angular, one seed coat, surface honeycombed- to fluted-reticulate, infrequently obscurely wrinkled-reticulate or -pitted. Embryo straight, arcuate, hemicyclic or bent. Chromosome number chiefly 12 or 24 pairs. Natives of South America, North America, Australia and the South Pacific. (p. 331)

Also characteristic of the genus is the abundant production of a certain class of bicyclic alkaloids that include nicotine, nornicotine, anatabine, and anabasine. The extensive morphological diversity and geographic distribution of species led to the widespread use of a sub-genus sectional classification that was originally outlined by Goodspeed (1954) and has been modified by Knapp et al. (2004) to account for relationships only recently elucidated by molecular analyses.

19.2.2.4.2 Distribution, Habitat, and Abundance

In North America, there are seven extant native species of *Nicotiana*. Goodspeed (1954) postulated that three distinct diploid ($n = 12$) lineages of *Nicotiana* migrated from their South American point of origination into Central and North America as early as the Upper Pliocene, where they subsequently combined to form several endemic amphiploid ($n = 24$) species. However, recent genomic analyses of *Nicotiana* by Chase et al. (2003) and Clarkson et al. (2004) suggest that the third lineage was likely already an amphiploid during its migration and that it gave rise to all members of *Nicotiana* section *Repandae* (*N. nudicaulis* Watson, *N. repanda* Willd. ex Lehm, *N. stocktonii* Brandegee). The modern descendants of the two proposed diploid migrants are believed to be *N. attenuate* Torr. ex S. Watson and *N. obtusifolia* Martens & Galeotti, which are thought to have hybridized at least twice upon their arrival to North America. *N. clevelandii* A. Gray is the resultant amphiploid from an older hybridization while *N. quadrivalvis* arose from a more recent hybridization event (Clarkson et al. 2004).

Additionally, there have been four introductions of *Nicotiana* species into North America where the species are now considered naturalized. *N. acuminate* (Graham) Hook, *N. glauca* Graham, and *N. plumbaginifolia* Viv were all introduced from their native ranges in South America by unknown means. However, all are so thoroughly established and have been for well over a century that there was a prolonged debate over whether they were native and the implications that had on attempts to decipher the natural history of the genus. *N. glauca* and *N. plumbaginifolia* are now known as problematic invasive species on a global scale (Florentine et al. 2006; Gairola et al. 2016). *Nicotiana rustica* was also introduced as an agricultural species to Mexico, the eastern United States, and Canada, presumably through Native American trade, and while it can be considered a naturalized introduction, it has only rarely been reported in the wild. Occasionally other species, including *N. tabacum*, *N. longiflora* Cav, and *N. alata* Link & Otto, are seen growing as weedy escapes from some form of cultivation. The horticultural species, *N. x sanderae* W. Watson, can also be found growing ornamentally in North American flower beds.

While all of the North American species of *Nicotiana* prefer sandy and gravely disturbed soils, they exhibit individual preferences over a range of habitats (Fig. 19.3; Table 19.3). North American species clustered regionally by latitude and longitude, which corresponds to lower amounts of precipitation than for their relatives in South America (Kawatoko 1998). Within the regional cluster, *N. attenuata* Torr. ex S. Watson and *N. quadrivalvis* were further separated out from the rest of the group by their intolerance of higher temperatures. Goodspeed (1954) subdivided the native species further into four regional groups: Mexican desert is singularly populated by *N. obtusifolia* Martens & Galeotti which has strong preferences for arid landscapes and high temperatures; Great Basin includes *N. attenuata* which prefers montane habitats and is known as a postfire annual; Californian includes both *N. quadrivalvis* and *N. clevelandii*, but *N. clevelandii* is restricted to lower elevations where temperatures are greater; and Mexican semiarid is comprised of *N. nudicaulis*, *N. repanda*, and *N. stocktonii*.

19.2.2.4.3 Utilization

Formal tobacco breeding began in earnest very early in the twentieth century and by the 1930s the need for novel germplasm was recognized, leading the United States Department of Agriculture to conduct collecting expeditions into Central and South America in order to obtain diverse germplasm (Chaplin et al. 1982). This new

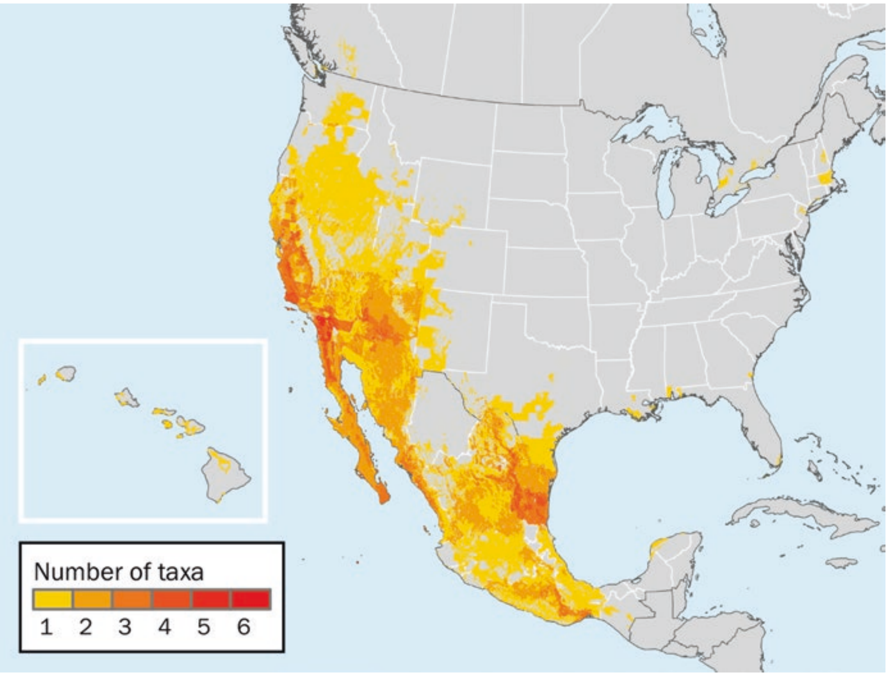

Fig. 19.3 Species richness map of modeled potential distributions of North American *Nicotiana* taxa, based on climatic and edaphic similarities with herbarium and genebank reference localities. Warmer colors indicate areas where greater numbers of taxa potentially occur in the same geographic localities. Full methods for generation of map and occurrence data providers are given in Appendix 1

primary pool of *N. tabacum* germplasm contained many plant architecture traits which would be used to increase yield capacity. Resistance to a number of pathogens was also found within these materials, but in many cases, the resistance was found to be inadequate in degree of protection or found to be unworkable due to complications in gene transfer (Burk and Heggestad 1966). Thus, in parallel with studies aimed at understanding speciation and polyploidy, tobacco breeders began utilizing CWRs as early as the 1940s for transferring disease resistance. The long, rich history of manipulating *Nicotiana* species includes utilizing techniques such as intra- and interspecific crossing (including bridges, chromosome doubling, somatic cell hybridization), intergeneric cellular hybridization and nuclear transfer, grafting, mutagenesis (chemical, ionizing radiation), and all known iterations of genetic transformation (reviewed in Lewis 2011).

While many of the North American species have disease resistance traits that would be beneficial additions to cultivated tobacco germplasm (Table 19.4), most interspecific crosses are very difficult, and efforts to transfer advantageous alien germplasm are often met with significant sterility barriers. Even successful transfers are often plagued with substantial yield losses or unacceptable phenotypes resulting

Table 19.3 North American tobacco CWRs

Group	Species	Range	Altitude (m)	Environment
Native	*N. attenuata* Torr. ex S. Watson	Baja, Mexico to S Canada; Great Basin	0–2600	Semi-desert; disturbed soils along roadsides
	N. clevelandii A. Gray	S Baja into S California and SE Arizona	0–500	Often seen growing under mesquite canopy; dry sandy soils along roads and coasts
	N. nudicaulis S. Watson	NE Mexico, primarily Nuevo Leon and Tamaulipas	300–2100	Dry, shaded rocky crevices
	N. obtusifolia M. Martins % Galeotti	Mexico, including Baja; SW United States	0–2300	Arid environments; gravel and rocky soils along roadside
	N. quadrivalvis Pursh	W United States, especially California; rare in Missouri	0–2000	Sandy soils, especially along creeks; full sun
	N. repanda Willd.	S Texas; Nuevo Leon and Tamaulipas, Mexico	0–600	Moist ground along streams
	N. Stocktonii Brandegee	Revillagigedo Archipelego (Socorro and Clarion Islands)	0–50	Valleys and rocky coasts; sandy gulches
Naturalized	*N. acuminata (Graham) Hook.*	Central California to Nevada; N Oregon and Washington	0–2000	Rocky soil, arid hillsides, disturbed soil along washes and roadsides
	N. glauca Graham	Mexico; SW and W United States; Hawaii	0–2300	Disturbed soils of roadsides and riverbanks
	N. plumbaginifolia Viv.	Cuba; Florida keys; Mexico	0–2100	Moist gravel or sand bars of streams, also scattered along roadsides; partial shade
	N. rustica L.	Rare in Mexico, New England, Appalachia, and Ontario	0–2100	

Data compiled from Goodspeed (1954), Kawatoka (1998), and herbarium records

from linkage drag. Due to these breeding hurdles, tobacco CWRs have not yet been evaluated for beneficial agronomic traits outside of disease resistance. However, in spite of these obstacles, successful introgressions have been made, such as the transfer of what is believed to be a single dominant black shank resistance gene, known as *Php*, from *N. plumbaginifolia* to *N. tabacum* that confers immunity to infection by race 0 of *Phytophthora nicotianae* Breda de Haan (Chaplin 1962). Genetic factors from *N. rustica*, which is also resistant to black shank, have likewise been transferred to tobacco, although the nature and utility of these factors are still being investigated. Unfortunately, we are likely past the golden era of tobacco breeding (1940s–1980s),

Table 19.4 Diseases of tobacco and sources of resistance in North American CWRs

Group	Species	An[a]	BRR[b]	BSh[c]	BM[d]	BSp[e]	CN[f]	FLS[g]	FW[h]	GW[i]	PM[j]	RKN[k]	RV[l]	TSWV[m]	TEV[n]	TMV[o]	TS[p]	WF[q]
Native	*N. attenuata* Torrey ex S. Watson										x							x
	N. clevelandii A. Gray												x					
	N. nudicaulis S. Watson	x									x	x						x
	N. obtusifolia M. Martens & Galeotti	x			X						x							
	N. repanda Willd										x							x
	N. repanda Willd.	x		x			x	x			x	x	x			x		x
	N. stocktonii Grandegee	x										x						
Introduced-Naturalized	*N. acuminata* (Graham) Hook.										x		x			x		x
	N. glauca Graham	x	x								x		x		x		x	
	H. plumbaginifolia Viv.			x												x		x
Introduced-Ag/Hort	*N. x sanderae*	x									x					x		x
	N. tobacum L.		x	x	X				x	x	x	x				x		

[a] Anthracnose; causal organism *Colletotrichum destructivum*

[b] Black root rot; causal organism *Thielaviopsis basicola*

[c] Black shank; causal organism *Phytophthora nicotianae*

[d] Blue mold/Downy mildew; causal organism *Peronospora tabacina*

[e] Brown spot; causal organism *Alternaria alternata*

[f] Cyst nematodes; causal organisms *Globodera tabacum* subsp. solanacearum and *G. tabacum* subsp. Tabacum

[g] Frogeye leaf spot; causal organism *Cercospora nicotianae*
[h] Fusarium wilt; causal organism *Fusarium oxysporum*
[i] Granville wilt; causal organism *Ralstonia solanacearum*
[j] Powdery mildew; causal organism *Erysiphe cichoracearum*
[k] Rootknot nematodes; causal organisms *Meloidogyne* spp.
[l] Rattle virus
[m] Tomato spotted wilt virus
[n] Tobacco etch virus
[o] Tobacco mosaic virus
[p] Tobacco streak virus
[q] Wildfire; causal organism *Pseudomonas syringae* pv tabaci
Adapted from Burk and Heggestad (1966)

and the few programs that remain are left with cost-benefit analyses that tend not to favor breeding approaches involving interspecific hybridization. Progress is still being made however, as an introgression conferring black root rot resistance was recently introduced from *N. glauca* that appears to have less linkage drag than the traditional source from *N. forsteri* Roem. & Schult (Trojak-Goluch and Berbeć 2011).

Efforts to utilize *Nicotiana* CWRs as sources of disease resistance genes in conventional breeding are further hampered by insufficient data on the reactions of CWRs to pathogens. In Table 19.4 all incidences of reported pathogen resistance or tolerance among North American tobacco CWRs are recorded, but only a few sources have been examined in any detail. Often in early screenings, only one accession of each CWR was used as representative of the entire species. Screening a larger germplasm pool for each CWR is likely to yield more positive results, such as the recent discovery of blue mold resistance in a specific accession of *N. obtusifolia* (Heist et al. 2004). These classical screening studies were also often conducted with crude inoculation techniques that likely overwhelmed plants and skewed results. Thus, studies should be repeated in a manner that attempts to best mimic natural processes and what we now know about pathogen modes of infection.

In addition to its utility as a resource for breeding commercial tobacco, the genus *Nicotiana* has become a very powerful resource for investigating basic aspects of plant science. Historically, the genus as a whole has been a model for understanding the process of intraspecific hybridization (Smith 1968) and the evolution of polyploid species (McCarthy et al. 2016). *N. tabacum* and *N. benthamiana* Domin (an Australian species), which are known for their ease of genetic modification, are used to study an array of topics so diverse that it could fill an entire book. Excluding the aforementioned, *N. attenuata* may be the most well-studied *Nicotiana* species because it is widely used as a model to investigate ecological systems, including responses to herbivory (Kim et al. 2011) and plant-pollinator interactions (Kessler et al. 2012). *N. glauca* has also been used in the study of plant-pollinator interactions (Nattero and Cocucci 2007), often with specific interest in its invasive nature, as well as for the bioremediation of heavy metals from soils (Shingu et al. 2005).

19.2.2.5 Conservation Status of CWR and WUS

19.2.2.5.1 In Situ

Conservation of genetic resources is critical in order to allow for continued genetic improvement of a cultivated species and to provide flexibility for dealing with new production or industry needs. Many *Nicotiana* species are cosmopolitan due to their weedy nature, but special concern should be taken with those species which have narrow ranges, namely, the three members of *Nicotiana* section *Repandae*. While not listed as threatened, there is ample cause for concern regarding the status of *N. stocktonii* since it is only found on two islands within the Revillagigedo Archipelago, Mexico. The islands, which remain uninhabited except for a very small naval base, have been protected since 1994 as a biosphere reserve by the Mexican government, and the region has recently been inscribed as a UNESCO World Heritage site. A large portion of the range of *N. nudicaulis* is protected by the Parque Nacional Cumbres de Monterrey, which also protects much of the range of *N. repanda* along with the neighboring Reserva de la Biósfera El Cielo. Additional reserves and protected natural areas safeguard a large swath of Baja and the northern end of the Gulf of California, which is important for the conservation of *N. clevelandii*.

In the United States, *N. attenuata* (which is designated as "sensitive" in the state of Washington), *N. glauca*, and *N. obtusifolia* have long been known as poisonous weeds for livestock grazing in the southwestern regions of the country, and early USDA livestock researchers recommended that they be eradicated from grazing areas (Marsh et al. 1927). However, in the grazing lands now managed by the US Bureau of Land Management (BLM), *Nicotiana* species have a layer of protection, and seeds are actively saved for conservation and ecosystem restoration as a part of the Seeds of Success program. In fact, between BLM lands and other US preserves, much of the western and southwestern United States where *Nicotiana* species are known to occur is at least protected from development. Little is known, however, about the locations in which *N. rustica* grows in the eastern United States. Care should be taken to locate these regions and protect them as the plants found there are likely the direct descendants of those previously grown by Native Americans and thus have significant cultural and historical value.

19.2.2.5.2 Ex Situ

A global survey of *Nicotiana* genetic resources that is currently underway has revealed that almost all CWRs are poorly represented in ex situ germplasm collections, generally, with some preliminary data presented in Table 19.5 as it regards to North American *Nicotiana* CWRs (JM Nifong unpublished). The data show that, while large numbers of accessions from the primary germplasm pool of tobacco are often represented within collections, few, if any, CWRs are maintained. *N. rustica* was spread globally in conjunction with *N. tabacum*, and it is still grown for local consumption in some parts of the world. Like *N. tabacum*, it displays a large array of phenotypic diversity, and thus large numbers of this species are also maintained by germplasm collections, representing cultivars, breeding lines, and global sampling. With the exception of *N. attenuata*, which is bolstered by significant sampling done by the Seeds of Success program throughout much of its range, *Nicotiana* CWRs are poorly represented.

While global data may suggest that there are two dozen accessions of a particular species, this is an overestimate of the actual genetic diversity being maintained. Accessions have tended to be exchanged among collections, and thus several accessions can often be traced back to a common source collection event. As an extreme example, a single *Nicotiana repanda* Willd. ex Lehm. accession was collected by the University of California Botanical Garden (UCBG) and shared with

Table 19.5 North American tobacco CWRs present in ex situ germplasm collections

Institute	*N. attenuata*	*N. clevelandii*	*N. nudicaulis*	*N. obtusifolia*	*N. quadrivalvis*	*N. repanda*	*N. stocktonii*	*N. acuminata*[a]	*N. glauca*[a]	*N. plumbaginifolia*[a]	*N. rustica*[a]	*N. tabacum*[a]
IPK Gatersleben	0	1	2	5	4	2	0	6	3	3	112	355
Botanical Garden, Univ. of Nijmegen	8	2	4	4	4	8	2	3	11	3	48	53
CRA-Unitá di Ricerca per le Colture alternative al Tabacco	2	1	2	3	4	3	2	3	2	2	147	1424
Millennium Seed Bank	1	1	0	5	0	1	0	1	10	1	5	11
All other EURISCO members	3	6	2	1	5	2	3	2	2	3	196	4499
Imperial Tobacco, Bergerac	2	1	2	3	4	1	2	2	0	1	35	867
US Nicotiana Germplasm Collection	4	3	1	8	10	2	3	4	6	3	87	1928
Seeds of Success	28	0	0	3	1	1	0	1	0	0	0	0
All other collections in study	3	3	4	4	4	7	4	7	6	2	130	5780
Total	51	18	17	36	36	27	16	29	40	18	784	15,168

[a] These data include accessions not originating in North America

the Imperial Tobacco collection in Bergerac, France, and then duplicated at IPK Gatersleben in Germany, and the IPK accession was subsequently obtained by the Botanical Garden at the University of Nijmegen in the Netherlands. In addition, the IPK collection has another accession of *N. repanda* that has a designated UCBG origin that is likely the same as the one it ultimately received from Bergerac. Thus, four accessions of *N. repanda* can all be traced back to a probable singular collection event. In many long established germplasm collections, adequate source information is not available for older accessions, and it is believed that these types of duplications are common.

19.2.2.5.3 Ways to Improve Conservation

The long-term status of in situ preservation for North American *Nicotiana* CWRs looks promising as significant portions of their ranges are currently covered by designated protected natural areas of the US or Mexican governments. However, adequate survey data is lacking, and little is known about the structure of wild populations, which makes any assessment of the actual utility of the protected ranges in preserving the genetic diversity of these species difficult. *Nicotiana stocktonii* should mandate priority over other species when conducting future studies as it is probably the most vulnerable to loss due to its precarious existence on only two volcanic islands. Entire populations of *N. stocktonii* could easily be lost due to an eruption or passing hurricane, as the species prefers cliffs and washes where it could be swept away in a downpour. For the species that continue to be utilized by Native Americans for ceremonial purposes, partnerships could be made in a manner similar to those outlined by Nabhan (1985) for both the in situ and ex situ preservation of these genetic resources.

The overall low number of accessions of North American *Nicotiana* species maintained in global germplasm collections should be cause for concern. While direct exchange of materials among germplasm collections may seem like the go-to option for increasing the genetic diversity held at any given site, it is not believed that further *Nicotiana* CWR exchange among collections will be beneficial at this point due to the likelihood that existing accessions share a common source history. Further collecting expeditions would be valuable to secure important CWRs for future study, utilization, and conservation.

19.2.3 Black Cohosh (Actaea racemosa L.*)*

19.2.3.1 Summary

Black cohosh (*Actaea racemosa* L.) is a native North American medicinal plant traditionally harvested for its rhizomes and roots. Black cohosh-based products have been consistently listed as a top-selling dietary supplements from 2002 to 2017. Due to increasing commercial demand, there is a need to develop sustainable propagation protocols suitable for large-scale production purposes to replace current methods of wild harvesting from native populations.

19.2.3.2 Introduction

Actaea racemosa, formerly *Cimicifuga racemosa* (L.) Nuttal, is a member of the Ranunculaceae family which comprise 2450 species distributed in 62 genera (Weakley 2015). Native only to the eastern forests of North America, *A. racemosa* populations range from Ottawa, Canada, to Georgia, USA. The genus *Actaea* is characterized by perfect, actinomorphic flowers with shedding sepals, small staminodes, and many stamen which form elongated racemes or panicles (Compton and Culham 1998a). *A. racemosa* is a long-lived herbaceous perennial, derived from multi-annulated rhizomes up to 30 cm long and 15 cm wide with few to many roots. A typical mature rhizome from a native population averages approximately 15 cm in length and 2–3 cm in width with roots 8–23 cm long and 1–5 mm in diameter (Ramsey 1965). Rhizomes typically have few to many curved stems bearing foliage and/or fruit along with few to many buds which remain dormant throughout the growing season. Originally classified in the *Actaea* genus (1753) by Linnaeus, *A. racemosa* was later transferred, with reservation, into the *Cimicifuga* genus by Nuttal in 1818 as *Cimicifuga racemosa* (L.) Nutt (Compton and Culham 1998a). Reclassified through morphological analysis and DNA sequence variation analysis, *C. racemosa* L. was reinstated into the expanded *Actaea* genus as *Actaea racemosa* L. (Hasegawa 1993; Kyung-Eui et al. 1997; Compton and Culham 1998b). Many countries still refer to it as *Cimicifuga racemosa* as opposed to *Actaea racemosa.*

19.2.3.2.1 Origin

Actaea racemosa L. is an endemic North American plant whose medicinal use by Native Americans predates European settlement and continues today. Eastern Native American tribes, including the Cherokee, Delaware, Iroquois, Micmac, and Penobscot, predominantly used the herb for pain management and to combat inflammation (Upton 2002). Historical literature also recorded indigenous peoples use of *A. racemosa* for menstrual pain with cramping, sore throats (as a gargle), and rheumatism (Pengelly and Bennett 2012). *A. racemosa* was also used in emergency medicine treating snake bites, “for which purpose it [was] bruised and applied to the wound; and at the same time a little of the juice was to be taken internally” (Pengelly and Bennett 2012). In 1830, the species was included in the Pharmacopoeia of the United States, after many settlers began incorporating the species in medical practices. At the time, it was referred to as “black snakeroot.” *A. racemosa* became even more popular in 1844 when a physician, John King, recommended it to treat rheumatism. Other physicians were known to use it to treat endometritis, sterility, menorrhagia, dysmenorrhea, and amenorrhea, as well as to increase milk production in breastfeeding women (Anon 2003).

The rhizomes of *A. racemosa* have been harvested and used as medicine, including the Lydia Pinkham famous patented "Vegetable Compound" medicine, of the early 1900s (Upton 2002), which reached $3 million in sales in 1925 (Lewis 2011). In Germany, *A. racemosa* has been prescribed by physicians since 1940 to treat premenstrual, dysmenorrheal, and menopausal neurovegetative symptoms. Today only menopausal neurovegetative symptoms (such as hot flashes and profuse sweating) are accepted as indications for use. According to the Commission E Monograph, only the dried rhizome is used for the relief of menopausal symptoms (Committee on Herbal Medicinal Products 2010). To date, approximately 56 human clinical trials have been conducted investigating the safety and/or efficacy of *A. racemosa* for premenstrual and menopausal symptoms (PUBMED search 2/24/2017). Unadulterated, it is recognized to have no toxicity, although it is not recommended for pregnant, breastfeeding mothers or individuals with estrogen-driven tumors.

Due to the growing concerns over the potential risks of breast cancer, heart disease, and stroke from the use of conventional hormone replacement therapy (HRT) treatments currently on the market, many health professionals are now looking to natural substances to treat menopausal symptoms. This positive view coupled with continued promising clinical trial results of *A. racemosa* as a HRT continues to drive demand for the plant. As a result, *A. racemosa*-based products have consistently been listed as one of the top 10 selling herbal dietary supplements for more than a decade (Smith 1968).

19.2.3.2.2 Current Harvest and Challenges to Cultivation

Most commercial *A. racemosa* material used for medicinal purposes is wild harvested exclusively from eastern North American hardwood forests where it grows as a native understory, shade-tolerant, hardy perennial. Ninety-five percent of this harvest is thought to be exported to Europe. Owing to increasing harvest pressures, *A. racemosa* is listed among the top species of concern on both The Nature Conservancy and The United Plant Saver's lists of medicinal species at risk due to wild-collection. Additionally, in 1999, *A. racemosa* was recommended for inclusion in Appendix II of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) (CITES 2000).

Potential challenges to cultivation include various pathogens which have been recorded. *A. racemosa* is susceptible to leaf spot, root rot, and damping off (*Rhizoctonia solani* J.G. Kühn 1858), especially under crowded conditions. Leaf spot fungi include *Ascochyta actaeae* Bres (CT, NC, NY), *Ascochyta* sp. Lib (Canada), *Ectostroma afflatum* (Schwein) (VA), and *Phyllosticta* sp. (ID, MT, NC). Nematode infestations include *Ditylenchus destructor* Thorne, *Meloidogyne* sp. Göldi (rootknot) (NJ); root and stem rot, *Leptosphaeria clavigera* (Cooke & Ellis) Sacc. (GA), *Ophiobolus nigro-clypeata* Riess (GA), *Pythium* (MO), *Rhizoctonia solani* J. G. Kühn (damping off of seedlings); rust, *Puccinia recondite* Dietel & Holw. (NC, TN, Canada); *Puccinia rubigo-vera* (D. C.) (OH, MD, PA, VA, West

Germany); *Puccinia rubigo-vera* var. *agropyrina* (Erikss.) (MD, NC, OH, VA); and smut, *Urocystis carcinodes* (Berk. & M.A. Curtis) (NC, NY, OH, PA, TN, VA, Germany). 22 accessions of *A. racemosa* have been propagared for 10 years and never experienced a serious pathogen issue (McCoy 2013).

The largest suppliers of wild-harvested rhizome materials are based in the southeastern portion of North America where the highest-quality plant material is reported to originate (Upton 2002). As the rhizome is collected, there is concern over the sustainability of current wild-harvesting practices as future demands increase. Overharvesting is thought to currently be the number one true threat to the survival of the species in the wild. Both rhizomes and roots are harvested for commercial medicinal purposes and standardized to various concentrations of three triterpene glycosides – actein, 27-deoxyactein, and cimiracemoside (Upton 2002). Because *A. racemosa* occurs in moist cove habitats, there is concern that erratic climate patterns could potentially adversely alter future populations. Current efforts are underway to identify populations with potential drought resistance.

19.2.3.2.3 Current Production

From 2000 to 2010, 2.7 million dry pounds of *A. racemosa* entered the world market. This equates to 40.4–54 million *A. racemosa* plants harvested for the medicinal herb trade over 10 years (Davis and Persons 2014). A 2010 American Herbal Products Association report compiled of surveys from raw materials suppliers in North America noted that the harvest (as aggregate tonnage) of dried plant, both root and rhizome, grew over 379% from 2600 cultivated in 1999–9862 in 2010. Wild-harvested tonnage experienced a similar growth of 216% with 145,367 pounds of root and rhizome being harvested in 1999 to 314,695 pounds in 2010 (Dentali and Zimmerman 2012). The American Botanical Council, 2015 herb market report describes consumer spending on herbal dietary supplements in the United States as having reached an all-time high in 2015. Consumers spent approximately $480 million more on herbal products in 2015 than in the previous year – an increase that marks the 12th consecutive year of growth for these products. *A. racemosa* products were sixth in total sales from multi-outlet channel stores and were reported to have reached $43 million in the United States alone (Source- SPINSscan Natural/IRI) (Smith 1968). As a result, wild harvesting has increased significantly and will threaten future populations throughout its limited range.

19.2.3.3 Crop Wild Relatives

There are 28 closely related species to *A. racemosa* with eight found in North America, two in Europe, and 18 in Asia (Compton and Culham 1998a; Weakley 2015). It should be noted that there are numerous reports of toxicity and misidentification between the various species and only *A. racemosa* is used for commercial medicinal products. The North American species, *A. racemosa*, provides the majority of plant material for commercial and medicinal use, though some Asian species are also used for their medicinal properties. It is believed that the closely related *Actaea podocarpa* DC (formerly *Cimicifuga americana*), *Actaea pachypoda* Elliott, *Actaea rubifolia* (Kearney) Kartesz, *Actaea rubra* (Ait.) Willd, *Actaea elata* (Nutt.) Prantl, and *Actaea cordifolia* DC are erroneously harvested as *A. racemosa*, though their populations are much smaller and are at higher risk of extirpation (Lyke 2001; McCoy 2004a, b). An additional variety, *Actaea racemosa* var. *dissecta* (Gray) J. Compton, which has not been verified in its natural range for the past 40 years, was recently discovered in North Carolina for the first time (McCoy 2004a, b). All of these *Actaea* species share similar foliage and habitat characteristics, and thus could be easily misidentified for *A. racemosa* when not in reproductive stage (Weakley 2015; McCoy2004a, b). In a typical population, a large proportion of individuals are in the vegetative stage of their life cycle which makes identification to species level difficult due to similar ternate foliage and branching morphology (McCoy 2004a, b; Kaye and Kirkland 1999; Cook 1993). The species are however easily distinguished by their floral morphology and staggered flowering dates. *A. pachypoda* typically blooms from April to May and August to October, and *A. racemosa* blooms from May to July and *A. podocarpa* from July to September (Radford et al. 1968; McCoy 2004a, b). There are no data available comparing chemical compositions among the newly revised *Actaea* genus. Also, with the recent reclassification of *Actaea* to include the former *Cimicifuga* and *Souliea* genera, it is necessary to revise and publish updated taxonomic keys which distinguish the species (Compton and Culham 1998a; Hasegawa 1993).

Widely distributed across deciduous forests of eastern North America, *A. racemosa* reaches peak abundance in mesic cove forests of the southern Appalachians (Fleming et al. 2010; (NatureServe 2017). In rich cove habitats where *A. racemosa* is commonly found, it shares its distinctively characteristic two to three alternately compound leaf form with many other cove genera including *Astilbe* (Saxifragaceae), *Aruncus* (Rosaceae), *Caulophyllum* (Leonticaceae), *Aralia* (Araliaceae), *Angelica*, and *Ligusticum* (Apiaceae) (Weakley 2015; McCoy 2004a, b). All of these species, in addition to the associated members of the *Actaea* genus, are potential adulterants in products containing *A. racemosa*, if wild harvesters are not familiar with the local flora:

> Adulteration of black cohosh, mainly with herbal ingredients from Chinese *Actaea* species, remains a problem in the dietary supplement industry. In the absence of easily recognizable morphological features, e.g., when cut or powdered roots and rhizomes, or root and rhizome extracts are purchased, authentication of black cohosh material is difficult

noted Stefan Gafner, PhD, chief science officer of the American Botanical Council and technical director of the Botanical Adulterants Program. Researchers from the NY Botanical Garden developed a DNA fingerprinting technique to identify *A. racemosa* in products using amplified fragment length polymorphisms (AFLP) (Zerega et al. 2002). In their study, 262 AFLP markers were generated with one proving to be unique to *A. racemosa* when compared to the three closely related species, *A. pachypoda*, *A. cordifolia*, *and A. podocarpa*. More recently 36 analytical methods have been identified, including high-performance liquid chromatography

(HPLC), nuclear magnetic resonance (NMR), flow injection mass spectrometry (MS), DNA-based tests, high-performance thin-layer chromatography (HPTLC), and macroscopic and microscopic analyses (Upton 2002; Harnly et al. 2016).

19.2.3.4 Habitat

Kaye and Kirkland (1999) state that some *Actaea* species are "light-flexible" herbs rather than "shade restricted" and thus are "shade-tolerant" as opposed to "shade-dependent." Light-flexible herbs are defined as "herbs which tolerate full sun and shade, but are restricted to neither" (Kaye and Kirkland 1999; Kaye 2000). Light-flexible herbs tolerate a wide range of conditions and respond favorably to increased light due to canopy gaps. Increased flowering, seed production, seedling recruitment, and survival result from these increases. Traditionally, *A. racemosa* requires 70% shade for propagation; but it has now been accepted that it can tolerate more light than previously thought. In a preliminary study by the Yellow Creek Botanical Institute (Graham County, NC), *A. racemosa* rhizome sections were planted in full sun in 2001 for observation. Plants in full sun were stunted during the first year of growth, but emerged vigorously and produced flowers and seed in year two, and were vigorous and disease free in year three (Suggs 2003). *A. racemosa* is reported to thrive under a wide variety of soil types, including loam, sand, shale, and clay (Cech 1999). It has been further speculated that the native range of *A. racemosa*, which is limited to the eastern portion of North America (Fig. 19.4), is due to seed distribution method via a dry dehiscent

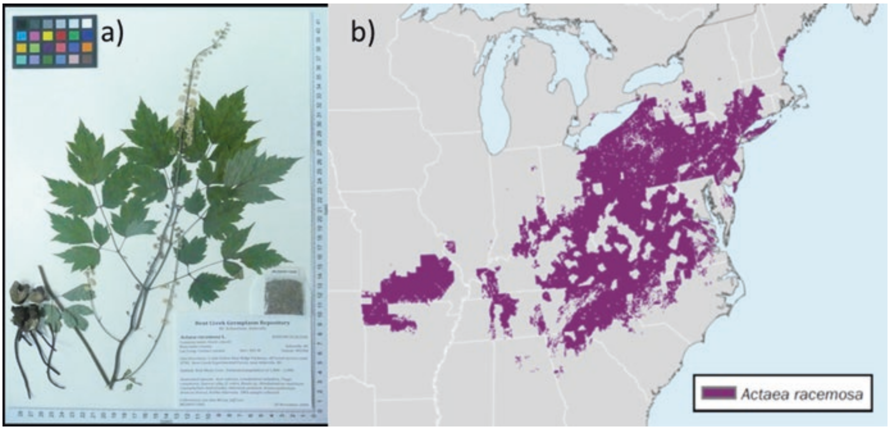

Fig. 19.4 (**a**) *Actaea racemosa L.* voucher specimen. USDA, NRCS. 2004. The PLANTS Database, Version 3.5 (http://plants.usda.gov). National Plant Data Center, Baton Rouge, LA 70874-4490 USA. (**b**) Modeled potential distribution of *Actaea racemosa L.* based on climatic and edaphic similarities with herbarium and genebank reference localities. Full methods for generation of maps and occurrence data providers are given in Appendix 1

follicle which limits the distribution of seeds to the immediate area surrounding the parent plant (Compton and Culham 1998a). Closely related *Actaea* species with fleshy fruits such as *A. pachypoda* and *A. rubra* have much larger native ranges due to seed dispersal by birds, although their populations are much smaller in size. These various facts imply that *A. racemosa* may possibly be propagated without shade given adequate moisture and weed control.

19.2.3.5 Conservation

Wild harvesting *A. racemosa* began long before Europeans settled the region in the early 1700s (Chamberlain et al. 2002; Sanders and McGraw 2005). With increased knowledge of the plant's therapeutic properties, market demand and harvest pressures have dramatically impacted this medicinal plant. It has been estimated that millions of kilograms of plant material have been extracted from Appalachian forests, with little effort to manage the plant species as a natural resource (Chamberlain et al. 2002). Growing concern for the conservation and sustainability of *A. racemosa* in the wild over the last 20 years has led to increased efforts to understand the ecological impacts of harvesting on natural populations (Small et al. 2011).

The American Herbal Products Association (AHPA-2007) has estimated that between 1997 and 2005, more than 1 million kg of *A. racemosa* roots and rhizomes were wild harvested from the Appalachian forests. Because plant reproduction and population expansion occur primarily through regrowth of buds from belowground rhizomes (Predny et al. 2006), wild harvesting presents a problem for long-term survival because the vast majority of black cohosh sold commercially is collected from native populations (Predny et al. 2006; McCoy 2004a, b; Davis and Greenfield 2003; Chamberlain et al. 2002). As *A. racemosa* sales maintain their top 10 status as a popular natural product, these conservation challenges will have to be addressed. To better understand wild-harvest impacts, researchers with the US Forest Service Southern Research Station investigated the likelihood of postharvest recovery by studying the effects of 2–4 years of experimental harvest on natural *A. racemosa* populations in the George Washington and Jefferson National Forest in southwest Virginia. It was found that after 2–3 years of intense harvest (66% plant removal), significant reductions in foliage area, stem production, and mean and maximum plant height occurred. After 3 successive years of experimental harvest, treatments were terminated to assess population regrowth. Populations experiencing intensive harvest showed no evidence of recovery after 1 year. Results suggest that *A. racemosa* is highly responsive to harvest intensity and that low to moderate harvest intensities and/or longer recovery periods will be necessary for prolonged and sustainable harvests of wild populations (Small et al. 2011).

Despite the current status of *A. racemosa* being "apparently secure" (N4) in the United States (NatureServe 2017), global projections of increased use suggest a 10–30% decline in *A. racemosa* populations over the next decade unless sources of cultivated plant material are established (NatureServe 2017). Of 15 major medicinal herb buyers contacted, 80% named black cohosh as one of the top three herbs most difficult to find at that time. Thus, demand for cultivated black cohosh will increase as wild-harvested populations become fewer in number and unable to keep up with demand. As growers in Germany and Canada have found, this could be a significant opportunity for forest farmers wanting to participate in the industry. As the supply of black cohosh continues to diminish, prices are expected to rise steadily (Davis and Persons 2014). Commercial production will grow as naturally occurring populations will not satisfy the expected increase in demand of 30–40% annually over the next 3–5 years. Lack of significant cultivation protocols creates an opportunity for private forest landowners or cultivators to fill the gap in supply as wild populations continue to decline (Davis and Persons 2014).

19.2.3.6 Suggested Methods to Improve Conservation

As a result of the increasing commercial demand for *A. racemosa*, there is a need to develop propagation protocols suitable for large-scale production purposes to replace current methods of harvesting from wild populations. Propagation studies have been completed, with the following objectives:

1. Determine optimal rhizome propagule division size for successful regeneration.
2. Analyze triterpene glycoside concentrations.
3. Quantify survival rates after 3 years of production.
4. Evaluate net yield results.

Experimental sites included a shade cloth structure in an agricultural research field, a shaded forest interior, and a shaded, disturbed forest edge. Plant emergence, growth, and survival were assessed at each site over a 3-year period. Optimal rhizome division size for propagation was a 10–30 g section originating from terminal rhizome portions. Rhizome survival averaged 97% among all treatments tested by year 3 at three sites. No differences in mean triterpene glycoside concentrations were detected between rhizome size classes or sites tested. Mean cimiracemoside concentrations ranged from 0.80 to 1.39 $mg.g^{-1}$ d/w tissue, 0.47 to 0.92 $mg.g^{-}1$ for deoxyactein, and 10.41 to 13.69 $mg.g^{-1}$ for actein (Fig. 19.5). No differences in triterpene levels were detected between flowering and nonflowering plants, nor were yields reduced. Net yields from a shade cloth production site were 9 and 17 times higher than for disturbed forest edge and forest interior site, respectively. The results of this study indicate that *A. racemosa* is a strong candidate for commercial propagation under adequate site selection (McCoy et al. 2006).

Joe-Ann McCoy, PhD, Director of the NC Arboretum Germplasm Repository located in Asheville, NC, currently maintains the USDA Agricultural Research Service National Plant Germplasm System (NPGS) collection of *A. racemosa* consisting of 22 populations representing its native range and maintained in controlled-pollination regeneration field cages. All populations have been propagated for over 10 years by the curator with control-pollinated seed stored in three seedbanks for long-term conservation. The NPGS collection is a valuable

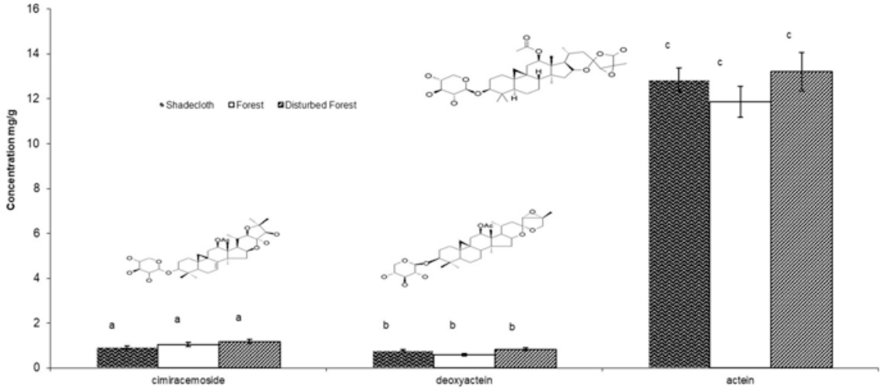

Fig. 19.5 Mean individual concentrations (mg/g dry weight) of cimiracemoside, deoxyactein, and actein from black cohosh rhizomes at three sites. Bars represent standard error. Bars with the same letter are not significantly different (α = 0.05)

resource for researchers and GMP compliant companies looking for taxonomically verified botanical reference materials for research. As majority of plant material is wild harvested, there is concern over the sustainability of current wild harvesting practices. These concerns, along with increasing demand, support the need to develop high-quality *A. racemosa* cultivars in order to create a sustainable supply of material to meet consumer demand and preserve native populations. The collection is currently being utilized for various research projects including phytochemical analysis of various compounds between populations, endophyte isolation, phylogenetic mapping, bioassays, in vitro studies, seed studies, propagation, cultivar, and demographic studies (Eisenstein et al. 2013; Pate et al. 2012; Clement et al. 2012).

19.2.4 Hops (Humulus L.*)*

19.2.4.1 Summary

The versatile hop plant, *Humulus* L., is a climbing vine with a perennial root. The genus includes three species, *H. japonicus* Siebold & Zucc., *H. lupulus* L., and *H. yunnanensis* Hu. The European hop (*H. lupulus*) is the species of primary economic importance from which all hop cultivars have been selected. This species has five botanical varieties distributed in Europe, Asia, and North America. Hop cones yield lupulin glands containing α and β acids and other compounds, which provide the bitterings, flavoring, and bacteriostatic properties needed for brewing beer. Hops has also been used for medicinal and pharmaceutical products, salad greens, ornamental decorations, fibers, and fodder. In 2014, 132,631 MT of hops, worth about $565 million US, were produced in 33 countries. The largest producers are Germany, Ethiopia, the United States, China, and Czechia. Major production challenges include fungal and viral pathogens, insect pests, and climate. Breeders and researchers seek disease resistance, dwarfing, low chilling, and improved and varied acid and flavor components from crop wild relatives. Conservation of hop plants in public and private genebanks includes growing containerized living plants under protected cultivation structures, tissue culture as backup plants, and seed and pollen stored in freezers. Broader collections of crop wild relatives are being developed through plant collection and ex situ preservation to increase diversity of global *Humulus* species available for research.

19.2.4.2 Introduction

The hop plant, *Humulus* L., is an herbaceous, dioecious or monoecious, climbing, dextrose-twining bine with a perennial root that is part of the hemp family (Cannabaceae). This plant grows wild to a length of >6 m in the temperate Northern Hemisphere (Small 1978) and tends to grow in riparian environments that are well-drained terraces along streams and rivers, in open areas, along hedge rows, and in deciduous woodlands of Eurasia (including Japan), northeastern and mid-western North America, and in moist locations in the island montane regions of southeastern United States. The genus likely originated in China, where all three species of the genus occur. Distinct populations of plants dispersed to the rest of Asia, Europe, and North America (Murakami et al. 2006), and likely speciated through geographic isolation.

Over the centuries, hop plants have been used for a variety of purposes including medicinal and pharmaceutical products, salad greens, ornamental decorations, pillow stuffing, textile fibers, and fodder (Hampton et al. 2001). The mature female flowers (also called cones, infructescenses, strobili, or commonly known as “hops”) are the primary economic product of the plant and are used in the production of beer. Hops contain resins found in lupulin glands containing α and β acids and essential oils, which are widely used as flavoring and aromatic agents in fermented liquors (Small and Catling 1995). The five common α acids are humulone, cohumulone, adhumulone, posthumulone, and prehumulone. The three main β acids are lupulone, colupulone, and adlupulone. Hop resins have bacteriostatic properties that are valuable for beverage preservation.

19.2.4.3 Origin and Brief History of Use

In the seventh century, monks in Carolingian monasteries began adding hops to preserve and flavor their beer. Hop cultivation probably began in Eastern Europe before the eighth century. From there hop cultivation spread to the rest of Europe (Neve 1991). However, cultivation for beer production began largely in the thirteenth century (Barth et al. 1994). Cultivation began in England in the early 1500s when production practices were adopted from Flemish growers (Burgess 1964).

As Europeans began colonizing the New World, they brought the knowledge and tradition of hop cultivation and beer brewing with them. English settlers introduced hops into the Southern Hemisphere in South Africa, New Zealand, and Australia in the 1800s. Early European settlers to North America picked native hops growing wild. Dutch settlers chose to import preferred dried hops from the old country. English settlers imported cuttings from England, and in 1629, the Massachusetts Company that began growing hops commercially in North America and New England became the first American hop-growing area (Barth et al. 1994). Production eventually migrated to New York, which had better soil and was closer to population centers.

By 1880, New York was producing 21 million pounds of hops annually. During the beginning of the twentieth century, downy mildew disease and hop aphids along with the advent of prohibition disrupted the New York hop growing and brewing industries. The main hop producing region shifted to the Pacific Northwest (Oregon, Washington, California, and Idaho), where hops were grown for export. The Pacific Northwest states continue to lead hop production in the United States.

19.2.5 Modern-Day Use and Agricultural Importance

In 2014, 132,631 MT of hops, worth about $565 million US, were produced in 33 countries (FAOSTAT 2017). Germany has the largest production of any country; Ethiopia, the United States, China (mainland), and Czechia were the next largest country producers. Brewing is by far the largest economically important use of hops; however, the nature of this use has been undergoing change over the past several decades. In the 1980s and 1990s, large national brewing companies dictated the research strategies for hop development. The breeding objectives were conservative and included maintaining stable α/β acid ratios in hops and increased disease and pest resistance. Recently microbrewing by small privately owned companies has increased greatly. There has been a resurgence of the need for diverse flavors and essential oils in hops for brewing, largely due to the microbrewing industry. Each microbrewery seeks individuality through new flavors and varied aroma profiles of their products. In 2014, the craft brewing industry contributed $55.7 billion to the US economy, providing more than 424,000 jobs (Brewers Association 2017). The demand for the availability of diverse hops from wild material and germplasm collections has had a parallel increase.

19.2.5.1 Challenges in Cultivation: Diseases, Pests, and Edaphic and Climatic Limitations

19.2.5.1.1 Fungal Diseases

Several fungal disease causing organisms have been distributed throughout the Northern Hemisphere. These diseases have not yet been reported in South Africa or New Zealand. Powdery mildew, caused by *Podosphaera macularis*, is a major pathogen. This organism may persist either as bud infections or as chasmothecia (sexually produced overwintering structures). Bud infections are the only confirmed overwintering inoculum source in the Pacific Northwest (Ocamb and Gent 2017). Once a field is infected, the disease usually recurs the following season. Spore movement within the field is the greatest threat for disease spread.

Powdery mildew grows between 54 °F and 85 °F and can tolerate more extreme temperatures especially during high humidity. In addition to leaves, flowers and cones may be infected. If a variety is susceptible, cones can be infected throughout most of their development. Growth stops in the infected area. Infected cones are stunted, malformed, and mature rapidly, leading to shattering and uneven crop maturity. Cultural control is recommended to reduce overwintering and buildup of early-season disease inoculum. Spores can move between fields, so management timing is important.

Another important disease is downy mildew, caused by *Pseudoperonospora humuli (Miyabe & Takah.) G.W. Wilson*, a fungus-like microorganism specific to hop. The disease is promoted by wet or foggy weather. The fungus persists from year to year in infected hop crowns or plant debris in the soil (Gent and Ocamb 2017). In early spring, spikelike infected bines rise among normal shoots. Tips of the normal branches may become infected and transformed into spikes. Leaves of all ages are attacked, resulting in brown angular spots. Flower clusters become infected, shrivel, turn brown, dry up, and may fall. Cones also are affected, becoming brown. Severe infection in some susceptible cultivars may produce a rot of the perennial crowns. Cultural control is recommended. Planting disease resistant or tolerant hop cultivars and diligently removing old bines are recommended.

A third significant fungal disease is black root rot, which is caused by *Phytophthora citricola Sawada*, a fungus-like microorganism that survives in soil by long-lived oospores. It also may survive in infected plant parts but not in dead tissue. The disease requires abnormally wet soils and is most often observed in areas of fields with soil or irrigation conditions that cause water to pool. The disease, normally restricted to certain areas of a field due to past irrigation and soil conditions, may become more distributed within a given field with increased use of drip irrigation. The symptoms include bine decline and wilt. The plant tissues become blackened and soft-rotted. Cultural control to avoid pooling water is recommended.

19.2.5.1.2 Virus Diseases

Hops can have many viruses (Eastwell and Ocamb 2017), including *Hop latent virus* (HpLV), *American hop latent virus* (AHLV), *Hop mosaic virus* (HpMV), and *Apple mosaic virus* (ApMV), all of which have been found in Pacific Northwest. The first two viruses (HpLV and AHLV) produce no symptoms and are not known to cause crop loss. HpMV, a *Carlavirus*, has not been a problem traditionally even when detected in a planting. ApMV, an *Ilarvirus* previously known in hop as *Prunus necrotic ringspot virus*, can cause up to 30% loss in cone production and also decrease α acid levels, but ApMV effects are cultivar dependent. HpLV, AHLV, and HpMV are transmitted by plant-to-plant contact and by the Damson-hop aphid (*Phorodon humuli*); HpMV is also transmitted by the green peach aphid, *Myzus persicae*. ApMV moves by plant-to-plant contact. The cultural control method of selecting certified pathogen negative planting material is recommended. Exclusion is an important means of virus control.

19.2.5.1.3 Insect Pests

The hop aphid (*Phorodon humuli* (Schrank)) is a frequent pest in Pacific Northwest. The aphid survives the winter as an egg on woody hosts of the genus *Prunus* (cherry, peach, or plum). Winged aphids move to the top of plant late in the spring. Aphid populations may build very rapidly and if left uncontrolled may result in defoliation. Sometimes mother aphids carry embryos that are carrying their own embryos. This reproduction strategy results in quick population growth. Scouting and natural predators can minimize aphid populations. The economic threshold for the Pacific Northwest of the United States is eight to ten aphids per leaf.

Other insect pests include the two-spotted spider mite (*Tetranychus urticae* C. L. Koch). Adults are small, eight-legged, spiderlike animals. They are pale green to yellowish to reddish, often with a dark spot on each side of the body. They suck plant juices from leaves and hop cones. Control options vary depending on the intensity of the infestation. The least invasive measures that may control mites include removing and destroying infested leaves or spraying the leaves with water to knock off the mites. Pepper or garlic sprays can be applied to plants as a deterrent to the mites. Flower borders such as African marigold and nasturtiums and garlic plants which promote beneficial insect predators may deter the mite where numbers are low in the early stages. Organic insecticides such as pyrethrum, insecticidal soap, nicotine, or diatomaceous earth can be used for control. When mite infestations have gone unchecked and are at high numbers, using commercial insecticides such as diazinon or malathion sprays may be the only option.

19.2.5.1.4 Edaphic and Climatic Limitations

Hops tend to grow best between the 35° and 55° latitude both in the Northern and Southern Hemispheres (Barth et al. 1994). The day length during the growing phase is significant to flowering and yield. The furthest north that hops are cultivated is the Chuvash region of Russia; Tasmania and Australia are the furthest south. To grow hops below 35° latitude in either hemisphere requires day length extension using artificial illumination. Despite these requirements, a number of countries at lower latitudes, such as Mexico, Colombia, Chile, Argentina, Brazil, Egypt, Kenya, Myanmar, and India, are experimenting in hop growing for production. Soil also is important to produce quality hops. A well-drained loam or sandy soil is favored for the best hop growth. Soils that are compacted or tend to become waterlogged are not suitable for hop cultivation due to disease issues.

19.2.5.2 Nutritional and Functional Use

Hops provide a complex flavor for beer, contributing as many as 800 chemical components. The major classes of compounds include the α acids, the β acids, essential oils, and esters (Stevens 1967). Essential oils contribute the major flavor and aroma to the beer. Although there are three main compounds (caryophyllene, humulene, and myrcene), 22 are significant in many brewed products. The esters, such as ethyl hexanoate, which produces a red apple anise aroma, are formed from the reaction of alcohol and the organic acids. They provide fruity flavors to beer. These are controlled by yeast, brewing time, and temperature. The five common α acids are humulone, cohumulone, adhumulone, posthumulone, and prehumulone. The α acids degrade during boiling to form iso-α acids which provide soft bittering qualities in beer. The three main β acids are lupulone, colupulone, and adlupulone. During fermentation, β acids add a harsh bittering quality. They also have bacteriostatic properties. The ratio of α acids/β acids, which is cultivar dependent, is important to brewers for their final product. Hop resins also have bacteriostatic properties that are valuable for beverage preservation. The associated tannins aid in clarification of the brews after boiling and give the flavor to beer (Snyder 1997; USDA 2017).

19.2.5.3 Crop Wild Relatives and Wild Utilized Species

Humulus encompasses three species: *H. japonicus* Siebold and Zucc., from Asia originally, but now introduced throughout the world; *H. lupulus* L.*,* with the type species from Europe from which cultivated hops are derived; and *H. yunnanensis* Hu from Yunnan, China. The first species is an annual, while the other two are perennial (GRIN-Global 2017).

Humulus japonicus is a dioecious species native to Asia where it spreads as an aggressive weed. It has been introduced to the east coast of the United States where it has an exotic invasive weed status in several eastern states (Natureserve 2017). *H. japonicus* cytotypes have complex chromosome numbers. The base chromosome number for this species is $x = 8$. Female plants have $2n = 2x = 14 + 2$ (XX) = 16, and male plants have $2n = 2x = 14 + 3(XY_1Y_2) = 17$ chromosomes (Alexandrov et al. 2012). This species has not been used for breeding hop cultivars because crosses between *H. lupulus* and *H. japonicus*, or with *Cannabis* (a relative in the same family), produce inviable embryos (Tournois 1914; Winge 1914).

The *H. lupulus* cytotypes have a base chromosome number of $x = 10$ and a diploid formula of $2n = 2x = 20$. Tetraploids, $2n = 4x = 40$, are occasionally found in the wild or can be produced artificially by doubling the diploid set in the laboratory using colchicine. Tetraploids can be crossed with diploids to produce triploids, $2n = 3x = 30$. Triploid female cultivars, which are more vigorous plants, produce seedless hops which improves yield. Triploid males stimulate cone growth of normal diploid female cultivars while producing limited seed set.

Small (1978, 1980, 1981) divided *H. lupulus* into five botanical varieties: one Japanese, one European, and three North American. These varieties can be hybridized to produce fertile offspring. The Japanese, *H. lupulus* var. *cordifolius* (Miguel) Maximowicz, can be found on the islands of Hokkaido and Honshu. This taxon has been bred into a few Japanese hop cultivars. The European, *H. lupulus* var. *lupulus* L., grows throughout Northern Europe and provides the main germplasm from which most hop cultivars have been regionally selected or bred. The American varieties are *H. lupulus* var. *nemomexicanus* Nelson and Cockerell, which is endemic to the North American Cordillera; *H. lupulus* var. *pubescens* E. Small from the midwestern United States and Canadian provinces; and *H. lupulus* var. *lupuloides* E. Small of central and eastern North America. The European *H. lupulus* var. *lupulus* was introduced to North American from Europe for brewing by the colonists.

Humulus yunnanensis is not well described in western literature, and chromosome counts are not recorded. It is dioecious, and its leaves are less lobed and its cones longer than those of *H. japonicus*, though narrower than *H. lupulus*.

19.2.5.3.1 Distribution, Habitat, and Abundance

The North American distribution of the *Humulus lupulus* botanical varieties (Small 1978) are presented in Fig. 19.6. The American varieties within *H. lupulus* introgress where they are sympatric in the wild and can be hybridized in the laboratory for breeding purposes. The European hop, *H. lupulus* var. *lupulus*, was naturalized in North America after colonial introductions in the east coast from Ontario and Quebec, Canada, to New Hampshire, Vermont, New York, Pennsylvania, New Jersey, and Maryland. This introduced distribution is sympatric with that of *H. lupulus* L. var. *lupuloides* E. Small (Fig. 19.7), though it ranges further westward to the Dakotas, Nebraska, Montana, Manitoba, Saskatchewan, and Alberta.

H. lupulus var. *pubescens* E. Small ranges in the middle of the United States from Ohio through Indiana, Illinois, Arkansas, Missouri, Nebraska, and Kansas; *H. lupulus* var. *neomexicanus* A. Nelson & Cockerell (Fig. 19.8) is allopatric in the Cordillera of the southwestern states. Pleistocene pollen deposits confirmed that this species is autochthonous to the New World (Cushing 1963). These *Humulus* taxa are frequently found and are not listed as endangered or threatened in either the IUCN Red List or the US National Resources Conservation List.

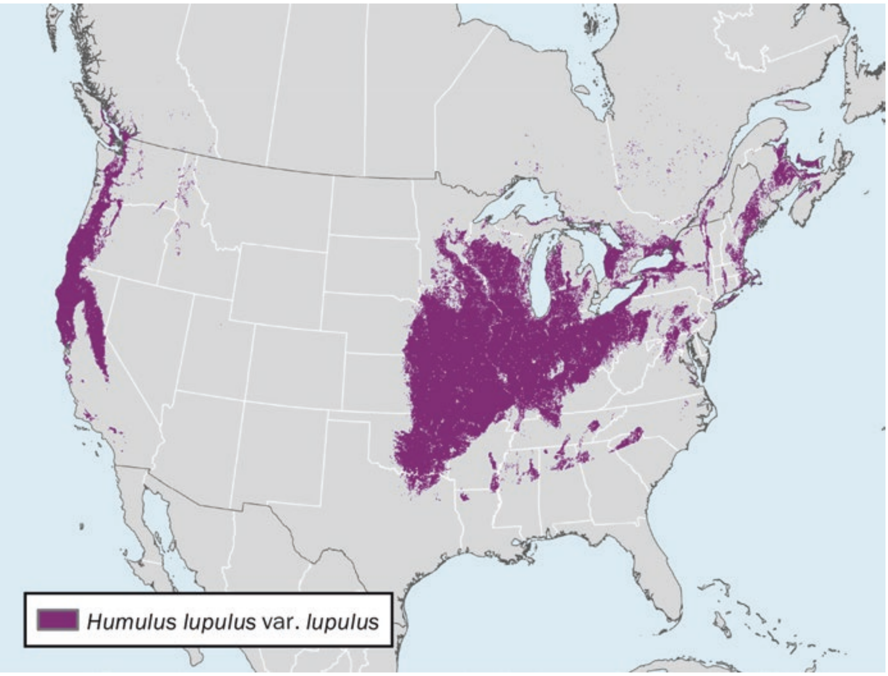

Fig. 19.6 Modeled potential distribution of *H. lupulus* L. var. *lupulus* (European hops) introduced into North America based on climatic and edaphic similarities with herbarium and genebank reference localities. Full methods for generation of maps are given in Appendix 1

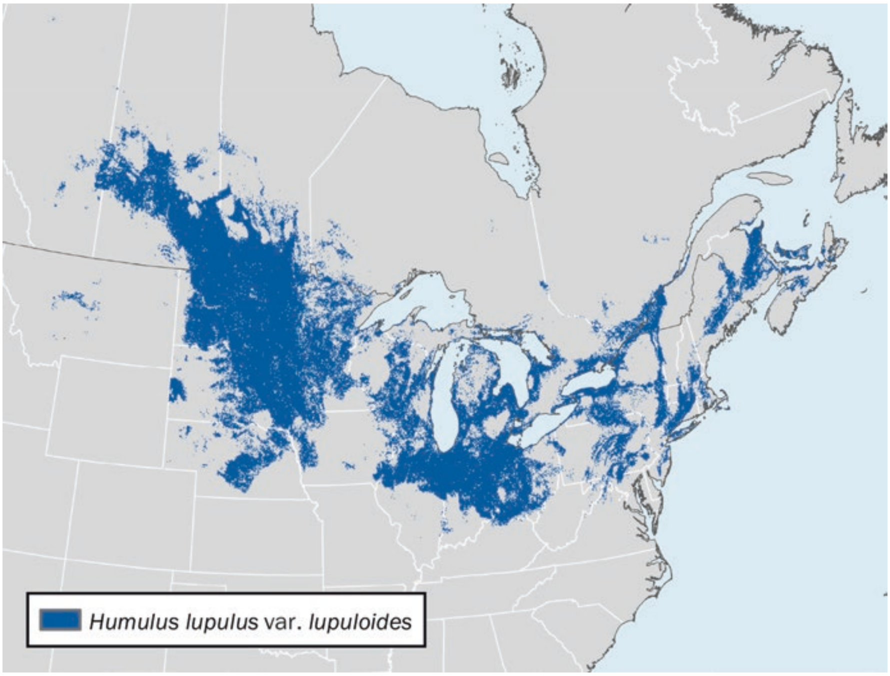

Fig. 19.7 Modeled potential distribution of *H. lupulus* L. var. *lupuloides* E. Small, native to the United States, based on climatic and edaphic similarities with herbarium and genebank reference localities. Full methods for generation of maps are given in Appendix 1

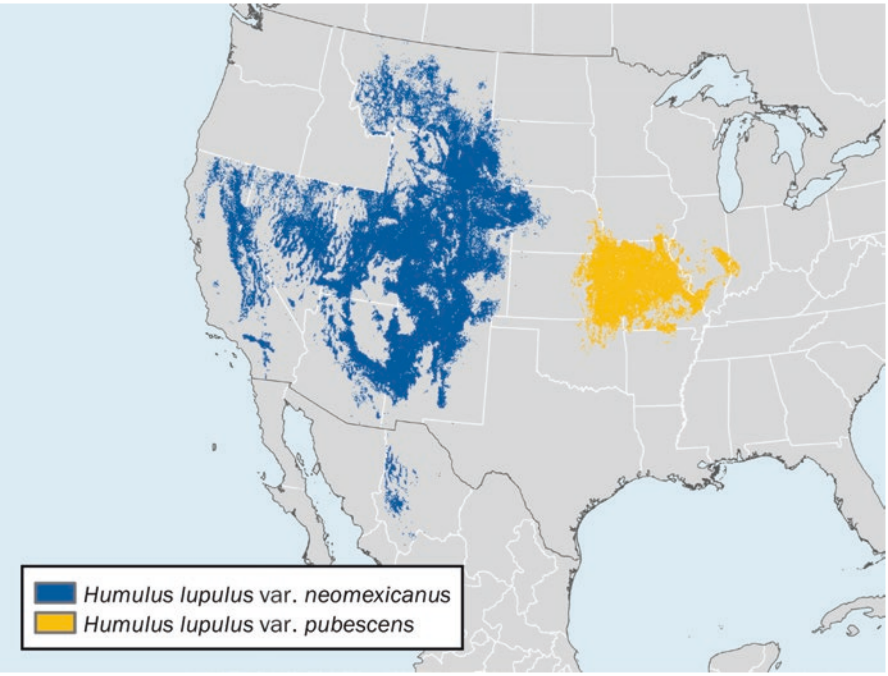

Fig. 19.8 Modeled potential distribution of *H. lupulus* var. *neomexicanus* A. Nelson & Cockerell native to the United States and *H. lupulus* var. *pubescens* E. Small native to the United States based on climatic and edaphic similarities with herbarium and genebank reference localities. Full methods for generation of maps are given in Appendix 1

19.2.5.3.2 Utilization: North American Breeding Contributions

Commercial hops are selected landraces from, or hybrid crosses of, the species *Humulus lupulus*. Most named cultivars were originally derived from European hops, *H. lupulus* var. *lupulus*. The desirable traits of this taxon include a relatively low content of soft resins, an α/β acid ratio that approaches one, low cohumulone, moderately low essential oil content, and relatively low myrcene in the essential oil fraction. Domesticated land races such as the German "Hallertauer," "Hersbrucker," "Saazer," and "Tettnanger" are regional selections that have become standards of the industry.

The first report of using North American native species as a parent for breeding hops was made by Professor E.S. Salmon (1934), who hybridized a wild female hop collected in 1916 from Morden, Manitoba, Canada, with a traditional English hop cultivar to increase the soft resin content in seedlings. From this cross, Salmon released "Brewers Gold," "Northern Brewer," and "Bullion." "Brewers Gold" was subsequently included in the pedigrees of many new cultivar releases from breeding programs throughout the world. Zimmerman et al. (1975) released "Comet," a cross including *H. lupulus* var. *neomexicanus* from Utah. For the past several decades, American wild hops were not preferred as breeding parents because of undesirable traits such as a low quantity of soft resins, less than 10% of cone weight (Haunold et al. 1993). Plants with α acids above 5% were rare, and the α/β acid ratio tended to be less than 50%. Native American hops tended to have high cohumulone and colupulone complexed with a pungent, unpleasant aroma (Haunold et al. 1993). However, American hops could also provide positive traits: dwarfing tendency, production precocity, early season flowering, high yield potential, disease and pest resistance, frost resistance, drought resistance, and novel chemistry. In 1986, the value of the contribution of native American hops to commercial brewing was estimated at $20 million annually (Prescott-Allen and Prescott-Allen 1986). Microbrewing, privatization, and individualization of hop breeding programs have caused many breeders and brewers to experiment with higher β-acid hops and a broad spectrum of essential oils previously thought unacceptable.

19.2.5.3.3 Conservation Status of CWR and WUS

No in situ conservation agreements have been made in the United States for the indigenous botanical varieties of *Humulus lupulus* to date. The US National *Humulus* Germplasm Collection is housed at the USDA ARS National Clonal Germplasm Repository in Corvallis, Oregon. This living collection is comprised of 330 accessions. In addition, 194 seedlots represent wild collected taxa. As a backup, ~110 in vitro cultured plantlets are stored at NCGR and at a remote backup site of the USDA ARS at Ft. Collins, Colorado. The tissue cultures are stored at 4 °C using semipermeable plastic bags. Plants can remain in the bags for up to 3 years without re-culturing. The primary collection is composed of European cultivars and American cultivars (particularly those released from Pacific Northwestern breeding programs), along with species representatives of crop wild relatives from around the world. Gaps in the collection include the Asian species, *H. yunnanensis*, and better representation of the wild botanical varieties of the Japanese, American, and European *H. lupulus*. Broader representation of CWR are being sought through plant collection and ex situ preservation to increase diversity of global *Humulus* species available for research.

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