GENETIC AND CHEMICAL VARIATION IN NORTH AMERICAN POPULATIONS OF THE MEDICINAL PLANT WILD TARRAGON (*ARTEMISIA DRACUNCULUS* L.)

by

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ABSTRACT OF THE DISSERTATION

Genetic and Chemical Variation in North American Populations of the Medicinal Plant Wild Tarragon (*Artemisia dracunculus* L.)

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Artemisia dracunculus L. (wild tarragon; Asteraceae) is a polymorphic, herbaceous perennial with a distribution spanning western North America (NA), Eastern Europe and most of temperate Asia. Wild tarragon has been widely used as a folk remedy for numerous ailments and seven compounds (davidigenin, sakuranetin, 6-demethoxycapillarisin, 5-O-caffeoylquinic acid, 4,5-di-O-caffeoylquinic acid, 2',4-dihydroxy-4'-methoxydihydrochalcone and 2',4'-dihydroxy-4-methoxydihydrochalcone), previously isolated from the plant, have shown bioactivity in studies on type 2 diabetes. The species is known to have diploid to decaploid individuals and the production of some phytochemicals has been shown to vary between cytotypes. Focusing on populations in the U.S., four main areas were investigated, 1) the geographical distribution of cytotypes, 2) the influence of cytotype, environment, and genetics on qualitative and quantitative

variation of the seven compounds, 3) essential oil diversity, and 4) genetic diversity and structuring of the populations. The main findings of these investigations were that diploids and polyploids were found in Eurasia, while only diploids were found in NA. In qualitative chemical investigations, decaploid plants were found to contain all the target compounds, while only sakuranetin, trace amounts of 6-demethoxycapillarisin, and complex mixtures of various caffeoylquinic and di-O-caffeoylquinic acids were detected in diploids from the U.S. In the quantitative analyses, sakuranetin levels varied between wild individuals and their cultivated clones, but the same four sites had the highest average production in both wild and common garden conditions. Essential oils extracted from NA populations represent a number of new chemotypes for the region. Primary components included (Z)-β-ocimene, methyl eugenol, methyl chavicol and α-terpinolene. Many of the samples had significant concentrations of the phenylacetylenes capillene, 5-phenyl-1,3-pentadiyne and 1-(4-Methoxyphenyl)-2,4-pentadiyne. The isocoumarinic acetylene, capillarin, was also found in the majority of samples but in low amounts. Four different genetic diversity indices were calculated and all returned comparable values, with ~78% genetic variation within populations and ~22% of the variation between populations. In a cluster analysis, based on genetic distances, populations with high sakuranetin production were grouped together. In conclusion, ploidy level was correlated with the presence of medicinal compounds, while both genetics and environment were found to influence quantitative variation of sakuranetin.

For Hillary and Eliyah

लोकाः समस्ताः सुखिनो भवन्तु

Lokah samastah sukhino bhavantu

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לחיים

(L'Chaim!)

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Chapter 1

Overview of the taxonomy, ethnobotany and phytochemistry of *Artemisia dracunculus* L. (Asteraceae)

Abstract

A brief introduction to the genus *Artemisia* L. is followed by a review and detailed chronology of the taxonomic history of *Artemisia dracunculus* L. (tarragon, Asteraceae). This is followed by a review of the ethnobotanical uses, phytochemical content and the biological activity of *A. dracunculus.* The species has a distribution that covers western North America, Eastern Europe, as well as Eastern and Central Asia and currently, floristic treatments vary in their recognition of infraspecific taxa. The *Flora of the USSR* recognizes five varieties, the draft treatment of *Artemisia* for the *Flora of China* has five varieties (four of those being different than those listed in Flora of the USSR), and the *Flora of North America* does not recognize any infraspecific taxa. The herb has been widely used as a folk remedy for numerous ailments and has been shown to contain a diverse assortment of chemical compounds including terpenes, coumarins, isocoumarins, flavonoids, caffeoylquinic and dicaffeoylquinic acids, polyacetylene derivatives, sesquiterpenoids and alkaloids. A number of these compounds, as well as various phytochemical extracts derived from the plant, have documented antifungal, antibacterial, anti-diabetic, anti-mutagenic or anti-thrombolytic activities.

Introduction

The genus Artemisia L. The genus *Artemisia* L. (Asteraceae) contains upwards of 500 species, is the largest genus in the tribe Anthemideae, and one of the largest genera in the family (Bohm and Stuessy, 2001; Torrell et al., 1999; Torrell and Vallès, 2001; Watson et al., 2002). Species in this genus often dominate dry regions of the mid to high latitudes of the Northern Hemisphere. Much of the western United States is dominated by sagebrush ecosystems where *Artemisia* species serve as a source of food for various types of animal wildlife (McArthur and Sanderson, 1999; Watson et al., 2002). *Artemisia* species are most commonly shrubs, rarely perennial herbs and even more rarely annual or biennial herbs (Bremer, 1994; Vallès and McArthur, 2001). Some of the better known species can be recognized by common names such as sagebrush, mugwort, wormwood, absinthe, armoise, sweet Annie, etc. *Artemisia* species are predominantly wind-pollinated and can produce copious amounts of pollen. In areas where they grow in abundance, *Artemisia* spp. can therefore be a major cause of allergies in humans (Lewis et al., 1983; Giner et al., 1999; Wopfner et al., 2002).

The circumscription of the genus *Artemisia* has changed considerably over the centuries. Some taxonomists have treated the group as a large single genus with over 400 species while others have divided the genus up into as many as thirteen genera including *Elachanthemum*, *Artemisia*, *Crossostephium*, *Picrothamnus*, *Sphaeromeria*, *Seriphidium*, *Neopallasia*, *Turaniphytum*, *Mausolea*, *Stilpnolepis*, *Filifolium*, *Ajaniopsis* and *Kaschgaria* (Heywood and Humphries, 1977; Bremer, 1994; Vallès and McArthur, 2001; Watson et al., 2002; Riggins, 2008). One of the oldest classification concepts was developed by Tournefort (1700), who recognized three distinct groups: *Artemisia*,

Absinthium, and *Abrotanum* among these plants. Linnaeus (1753) combined these groups into the single genus *Artemisia*. In 1817, Cassini described the new genus *Oligosporus*, based on presence of functionally staminate disk florets, and segregated it out from Linnaeus' *Artemisia*. During an extensive analysis of the genus, Besser (1829, 1832, 1834, 1835a, 1835b, 1836, 1841a, 1841b, 1845) developed a generic concept with four sections, *Abronatum* (later called *Artemisia*), *Absinthium*, *Seriphidium*, and *Dracunculus*, based on the presence or absence of ray florets, the sex and fertility of the ray and disc florets, and the presence or absence of hairs on the receptacle. His treatment was adapted and published by De Candolle (1837) and Hooker (1829) and has been the most widely followed and has formed the foundation for subsequent taxonomic works on *Artemisia*.

The traditional circumscription was generally maintained until early 1900's when Rydberg (1916) segregated out a number of additional genera in North America as well as recognizing the old-world section *Seriphidium* as a distinct genus from a newly created section *Tridentatae*, which contained only the North American species that were formerly placed in *Seriphidium*. Later in the 20th century, a number of other genera were also segregated from *Artemisia* by Poljakov, who developed a complex classification for *Artemisia* with many new series and sections (Poljakov 1961b; Ling 1982, 1991a, 1991b, 1994, 1995; Bremer and Humphries, 1993). A detailed history of these, and other, changes in generic and sub-generic circumscriptions was presented by Riggins (2008).

Recent regional treatments of *Artemisia* vary in both their species and sectional circumscriptions. For the treatment used in the *Flora of North America*, Shultz (2006) recognized five subgenera, including: *Dracunculus*, *Absinthium*, *Artemisia*, *Tridentatae*, and *Seriphidium* (the latter is restricted to the Old World). In a draft treatment for the

Flora of China, Ling et al. (in prep) recognize *Seriphidium*, *Crossostephium*, *Ajaniopsis*, *Kaschgarica*, *Ajania*, *Filifolium*, and *Stilpnolepis* as distinct genera from *Artemisia* and divides *Artemisia* s. str. into seven sections: *Dracunculus*, *Absinthium*, *Artemisia*, *Abrotanum*, *Albibractea, Viscidipubes and Latilobus*. Recent molecular evidence and phylogenetic analyses based on ITS data, strongly suggests that *Seriphidium* falls within *Artemisia s.str.* and that *Artemisia s.l.* is only monophyletic with the inclusion of a number of segregate genera including *Artemisiastrum*, *Filifolium*, *Mausolea*, *Picrothamnus*, *Sphaeromeria* and *Turaniphytum* (Torrell et al., 1999; Watson et al., 2002; Vallès et al., 2003; Sanz et al., 2008).

Riggins (2008) conducted the most extensive molecular phylogenetic analysis of *Artemisia* to date. He found that a narrowly circumscribed *Artemisia* is paraphyletic, and that the traditionally used sections within *Artemisia* will need to be recircumscribed as well. The molecular systematics studies including *Artemisia* species have been complicated by incongruent ITS and cpDNA analyses, which some authors attribute to abundant interspecific hybridization, as well as rampant polyploidy throughout the genus (Kornkven et al., 1998; Riggins, 2008). A revision of *Artemisia*, and its related genera, is necessary and will change many of the traditionally accepted ideas regarding the circumscription of the genus and associated groups.

Human use of Artemisia species. *Artemisia* species are utilized by humans for numerous applications. Some species, especially those with either silver-colored foliage, highly dissected leaves, or a dense mat-like growth are used as ornamental garden plants. Most of these species are also usually rather drought tolerant and are often used in areas with

low water availability. Such ornamentals include *Artemisia stelleriana* (commonly called old woman, dusty miller [name also used for *Senecio cineraria*], beach sage, or beach wormwood), for which a number of cultivars are available, *A. absinthium, A. afra, A. caucasica, A. frigida, A. lactiflora, A. ludoviciana, A. vulgaris,* and the putative hybrid *Artemisia* 'powis castle' (*A. arborescens* x *A. absinthium*) and others (Bailey, 1949; Burnie et al., 2001). Many taxa of *Artemisia* have been introduced to areas beyond their natural range of distribution and some such as *A. annua*, *A. verlotiorum and A. vulgaris* have become naturalized or invasive weeds (Global Compendium of Weeds, 2007).

In the area of food, herbs, and beverages, *A. dracunculus* (tarragon) is the primary species utilized as a culinary herb, and it is used regionally (Russia, Estonia, Armenia, etc.) in the production of a carbonated, non-alcoholic beverage called "Tarkhun" (тархун). This species is also used in the production of condiments, pickled vegetables, cheeses and infused vinegars (Uphof 1968; Aglarova et al., 2008). *Artemisia absinthium*, *A. genipi*, *A. umbelliformis*, and *A. petrosa* are used in the production of the alcoholic liquors absinthe and genepi, and *A. pontica* is used in the production of vermouth (Vallès and McArthur, 2001; Mucciarelli and Maffei, 2002; Woerdenbag and Pras, 2002).

Artemisia species produce terpenes, biterpenes, triterpenes, polyacetylenes, sesquiterpene lactones, flavonoids, coumarins and glycosides (Greger, 1977; Tan, 1998; Mucciarelli and Maffei, 2002) and many of these chemical compounds have been found to have biological activity. The most widely known and successfully marked compound is artemisinin, obtained from *Artemisia annua*. In combination with other compounds artemisinin is currently one of the most effective treatments against malaria and as of 2009, eighty countries have officially adopted artemisinin-based combination therapies as

their first line of treatment against malaria (World Health Organization, 2009a). Artemisinin has also been shown to induce apoptosis in cancer cells, selectively kill cancer cells *in vitro,* and retard the growth of implanted fibrosarcoma tumors in rats (Singh & Lai, 2001, 2004).

Artemisia annua is not the first species of *Artemisia* that was used for commercial production of medicine. The seeds of *A. santonicum* were traditionally used as an emmenagogue, stimulant, stomachic and as a vermifuge (Mitchell, 1857). Isolated in 1830, the compound santonin was used to expel worms from the gastrointestinal tract as early the 1850's (Culbreth, 1917; Birladeanu, 2003), and a preparation of santonin, mixed with a candy flavoring to make it more palatable, was the first product of the then infant company Charles Pfizer & Company (Pfizer Inc., 2009). Eventually the use of this remedy was discontinued due to adverse side effects and the development of other, safer anti-parasitic treatments (Waller et al., 2001).

Many *Artemisia* species are used in traditional medicine practices around the world. *Artemisia absinthium*, *A. dracunculus*, *A. leucodes*, and *A. scoparia* have all been used in Central Asian traditional medicine (Khalmatov, 1964; Khalmatov et al., 1984; Khodzhimatov, 1989). Moerman (1998) lists over 20 different *Artemisia* species that Native American populations traditionally used for the treatment of a multitude of different ailments. Some of the most well known species used in traditional medicine by various cultures around the world are *Artemisia vulgaris*, *A. absinthium*, *A. capillaris* (particulary in Japan), *Artemisia herba-alba* (North Africa, Middle-East), and *Artemisia ludoviciana* subsp. *mexicana* (Mexico). As a result of this extensive human use of these and other species, the phytochemical and pharmacological literature dealing with

Artemisia is extensive and various biological activities of many species have been investigated (Khafagy et al., 1971; Yamahara et al., 1989; Al-Khazarji et al., 1993; Marrif et al., 1995; Lee et al., 1998; Tan, 1998; Wake et al., 2000).

Artemisia dracunculus. *Artemisia dracunculus* L. (wild tarragon) has a distribution that covers western North America, Eastern Europe, as well as Eastern and Central Asia (Figures 1.1 &1.2; Hall and Clements 1923; Poljakov, 1961b; Ling et al., in prep; Greuter, 2006-2009; USDA, ARS, NGRS, 2010; USDA, NRCS, 2010). In North America it is found in the western part from Alaska south to northern Mexico and from California in the west, through the Intermountain and Rocky Mountain regions, to the central and northern plains states, portions of the upper Mid-West, and south to Texas. On the eastern edge of its range the species becomes rarer extending to Wisconsin (with a local state listing of S2 and as a species of concern), Missouri (local state listing of SH [occurred historically in the state and may still be extant]), and Illinois (listed as endangered; Illinois Endangered Species Protection Board, 2009; Wisconsin Natural Heritage Inventory Program, 2009; Missouri Natural Heritage Program, 2010). The species is on record as being found in a few states in the Northeast but these are most likely rare escapees from cultivation and there is no historical documentation of the species growing wild in these areas (USDA, NRCS, 2010; Fernald, 1950; Erichsen-Brown, 1989; Gleason and Cronquist, 1991; Shultz, 2006). In the western United States, the plant can be found in a number of different ecosystems including forests types such as ponderosa pine, fir-spruce and pinyon-juniper, as well as in more open ecosystems such as sagebrush, desert shrub, mountain grasslands, plains grasslands and prairie. A detailed compilation of

habitats and plant communities in which *A. dracunculus* has be found in the U.S. has been assembled by Groen (2005).

In Canada it can be found in Yukon Province as well as all southern provinces from British Columbia to Ontario (USDA, NRCS, 2010). The wild distribution of the species in Europe is complicated by a long history of cultivation and the plant can now be found growing throughout most of Europe (Figure 1.2 and see discussion in Chapter 2). In Asia the species is documented as growing in Turkey (possibly only as a cultivated introduction), the Caucasus region, Central Asia (Kazakhstan, Kyrgyzstan, Tajikistan, Uzbekistan) Iran, Afghanistan, Pakistan, northern India, Mongolia, northern and western China, and the Russian Siberia and Far East (Poljakov, 1961b; Davis, 1975; Kaul and Bakshi, 1984; Ling et al., in prep).

In addition to its wide distribution, the species can be found growing at a wide range of altitudes. In the United States specimens have been collected from just above sea level in the coastal areas of southern and central California up to elevations over 3,050 m (10,000 ft) in the Rocky Mountains of Colorado and 3,650 m (12,000ft) in Sierra Nevada and White Mountains of California (*Consortium of California Herbaria, 2010*). Kapustina et al. (2001) reported a maximum altitude of 3,700 m (12,000 ft) for the species in Uzbekistan and Henderson and Hume (1873) reported it from Ladakh (in the state of Jammu and Kashmir, India) and Yarkand (now part of Xinjiang Uyghur Autonomous Region, China) at 3,962 m (13,000 ft). Pampanini (1927) refers to specimens that were collected by T. Thomson in Tibet at altitudes from 4,267-4,877 m (14,000 to 16,000 ft).

***Taxonomic history* of *A. dracunculus*.** Prior to the advent of the Linnaean classification, the plant now known as *Artemisia dracunculus* was well known, and listed or described in many early botanical works, materia medica and herbals. Although some authors, and many websites, note *A. dracunculus* as being documented by Dioscorides and Pliny, it is difficult to confirm this ancient documentation of *Artemisia dracunculus* because of the lack of standardized plant names during that time. For example, in their translation of Pliny's *Naturalis Historia*, Bostock and Riley (1855) note that the plant referred to as "dracontion" in book XVIII, chapter 12, refers to wheat. The issue is more confused by another plant called "dracunculus" first mentioned in Book XXIV, chapter 52. Bostock and Riley identified this plant as *Artemisia dracunculus*., but later "dracunculus" and "dracontium" are discussed in more depth in chapters 91-93 of Book XXIV, where these names are attributed to *Arum dracunculus*, *A. colocasia* and/or *A. maculata* of the Araceae. Another plant, also called "dracunculus" in referred to in Book XXV, chapter 6. Pliny notes that this plant is different than that in Book XXIV ch. 91, but the description he gives is not at all reminiscent of *Artemisia dracunculus*. In their translation of Strabo's *Geographica*, Hamilton and Falconer (1892), also refer to "dracontium" as an *Arum* species.

Simeon Seth, the famous 11th century physician, is said to have described the plant with the name 'tarchon', an Arabic name, which is still used for *A. dracunculus* in a number of regions (Dodoens, 1616). The renowned Islamic scholars Avicenna and Ibn al-Baitar and Al-Biruni described its use in their Materia Medicas as early as the 11th and 13th centuries, respectively (Greenhill, 1848; Aglarova et al., 2008). Modern translations and interpretations of those texts have also revealed substantial confusion over the names

of plant described there in. Habib (1980) discussed confusion over the identity of a plant Al-Biruni called tarkhun-al-Rumi which has been attributed to ne *Anethum pyrethrum* by some researchers and *Artemisia dracunculus* by others. Tarragon was definitely included and described in the works by Mattioli (1563), L'obel (1576), Dodoens (1616), Bauhin (1671), Tournefort (1700), Boerhaave (1710), Blackwell (1737), Linnaeus (1737), and many others. The names for the plant in these texts often change, due to different regional colloquial names, but usually included one or more of the following words: 'artemisia', 'absinthium', 'abrotanum', 'draco', 'dracunculus', 'taracon' or 'tarchon', often followed by descriptors. Linnaeus described the species in his first edition of *Species Plantarum* with the distribution noted as "*Habitat in* Sibiria, Tataria" (Linnaeus, 1753).

Due to the abundant morphological variation found in the species, *A. dracunculus* has a fairly complex taxonomic history. This history was further confounded by the fact that both European and North American botanists were concurrently describing varieties solely based on Asian or American plants respectively. Many on the Asian varieties had similar characteristics as the North American varieties but were given different names. Additionally, in some cases the same varietal name was coincidentally used by both a European and North American botanist. A chronologic list for the specific and infra-specific names associated with *A. dracunculus* is presented in Table 1.1. Using a name published by Pallas (1793), *Artemisia glauca* Pall. ex Willd. was described and published by Willdenow (1803). Willdenow (1809) also published *Artemisia inodora* with the primary difference from *A. dracunculus* being a lack of fragrance and taste. The designation as a species of *A. glauca* was based mainly on its gray pubescence.

In 1813, Frederic Pursh published *Flora Americae Septentrionalis*, in which he recorded the presence of *Artemisia dracunculus* in the native flora of the United States for the first time. According to Pursh (1813), the inclusion of the species was based on specimens from the Lewis and Clark expedition of 1804-1806. In this flora Pursh also described a new species, *Artemisia dracunculoides* Pursh, from specimens collected along the Missouri River in 1811 by John Bradbury. In his own account of his travels through America, Bradbury (1817) noted that *Artemisia dracunculus* was common along the Missouri River. Pursh distinguished *Artemisia dracunculoides* from *A. dracunculus* by having thinner leaves and smaller capitula (Pursh, 1813). *Artemisia dracunculoides* was thereafter used instead of *A. dracunculus* in many of the regional American floras for the following 100 years.

Nuttall (1818) described the new species *A. cernua* Nutt. and cited Pursh's descriptions of *A. dracunculus* and *A. dracunculoides* as synonyms, but states that "upper leaves like those of *A. dracunculus*, but neither aromatic nor agreeably scented". Even the world expert in *Artemisia*, Willibald Von Besser expressed confusion (Besser, 1835), in regards to Hooker's *A. dracunculus* var. *glauca* (Hooker, 1829). Besser was confused because in the text it was cryptically followed by the word "fastigiata". It is unclear if "fastigiata" was meant as an additional name or as part of the descriptive treatment, but Besser seemed to think that it was publishing a taxon (*A. fastigiata* Besser) he had used in an unpublished manuscript. Besser puts Hooker's *A. dracunculus* var. *glauca* under his listing of *A. glauca* var. *fastigiata* Besser. Additionally, he listed and described the varieties *A. glauca* var. *incana* Besser, *A. glauca* var. *subcanescens* Besser (with syn. *A.*

glauca var. *latifolia* Ledeb.), *A. glauca* var. *glabra* Turtschan [likely Turczaninow] as well.

In that same work, Besser only described two varieties of *A. dracunculus.* The cultivated culinary herb, currently known as French tarragon, was named *A. dracunculus* var. *sativa*, while wild tarragon was called *A. dracunculus* var. *inodora*. De Candolle (1837) also describes Besser's two varieties and lists the species names *A. redowskii* Ledeb. and *A. inodora* Willd. as synonyms under *A. dracunculus* var. *inodora* Besser.

In their *Flora of North America*, Torrey and Gray (1843) described three new varieties of the species, *A. dracunculoides* var. *brevifolia*, *A. dracunculoides* var. *incana*, and *A. dracunculoides* var. *tenuifolia*. The differentiation of these varieties was based on the degree of pubescence and variation in leaf size and shape.

Watson (1888) described the new species *A. dracunculina* from material collected by C.G. Pringle in Chihuahua Mexico. The main characteristics distinguishing *A. dracunculina* from *A. dracunculus* and *A. dracunculoides* were "a very loose panicle and heads (capitula) on filiform peduncles 2 to 4 lines long", but no further explanation is given as to what unit the lines mentioned refer to (Watson, 1888).

Only a decade later, Nelson (1900: 273) described *Artemisia aromatica*. He distinguished *A. aromatica* from *A. dracunculoides* by referencing Pursh's description of *A. dracunculoides*, "…which [*A. dracunculoides*] is much larger, freely branched, the branches drooping (ramis nutantibus) and the heads fewer flowered", and differs from the three varieties of Torrey and Gray, which he stated are only forms of *A. dracunculoides* (Nelson, 1900: 273). Nelson also noted that another major feature distinguishing *A.*

aromatica from *A*. *dracunculoides* was the overpowering fragrance of *A*. *aromatica* (Nelson, 1900).

Rydberg published the most extreme classification, splitting *A. dracunculus* into seven narrowly defined species: *A*. *aromatica*, *A*. *cernua*, *A*. *dracunculina*, *A*. *dracunculoides*, *A*. *dracunculus* and *A*. *gracillima,* as well as describing the variety *A. dracunculoides* var. *wolfii* (Rydberg, 1905; Rydberg, 1916). The seven species were separated using morphological characters of the synflorescence shape, degree and/or type of pubescence, involucre size, comparative length of inner and outer involucral bracts, number of florets per capitulum and orientation of inflorescence branches.

Hall and Clements (1923) published a study on North American species of *Artemisia* where they reviewed the species, varieties and synonyms associated with *A. dracunculus*, *A*. *dracunculoides*, and *A*. *glauca*. They also provided their own infraspecific classification in which they describe three new subspecies of *A. dracunculus*: subsp. *typica*, subsp. *glauca* and subsp. *dracunculina*. While Rydberg retained *A*. *aromatica* at the species level, citing differences in the relative lengths of the inner and outer involucral bracts and the number of flowers in each, Hall and Clements dismissed *A*. *aromatica* as being the same as the Old World *A*. *dracunculus*.

Jepson (1925) lowered the rank of *Artemisia glauca* to *Artemsia dranunculus* var. *glauca*, but this combination had previously been executed by Besser, and was therefore superfluous. Similiarly, *Artemisia dracunculina* was reduced to the rank of variety in *Artemisia dracunculoides* by Blake (1940).

Fernald (1945), gave his opinion on the taxonomic difficulties of the "*dracunculoides* complex". He aptly describes the difficulties found when dealing with these characteristics:

> "I get no satisfaction trying to separate *A. dracunculoides* Pursh (1814) from *A. glauca* Pall. (1804)...At best they seem to be confluent forms of one species, the degree of pubescence or glabrousness and of glaucescence or greenness being most difficult to distinguish" (Fernald, 1945: 247).

In his opinion, of all the variants included in *A. dracunculoides s.l.*, only one grouping, *A. dracunculina* S.Wats., stood out as possibly having stable and distinct characters that could support its use as a unique variety. According to Fernald, the defining characteristics for this taxon were a loose inflorescence and nodding or pendulous and long-peduncled heads, with peduncles 4 mm or longer (Fernald, 1945). He finally made the combination *Artemisia glauca* var. *dracunculina*, stating correctly that the species name *A. glauca* had priority over *A. dracunculoides*. Interestingly, there is no mention of the species *A. dracunculus* in Fernald's work, but he does proceed to heavily criticize the earlier work of Hall and Clements.

The taxonomic shuffling continued into the second half of the 20th century. Poljakov (1961a) resurrected Cassini's genus *Oligosporus* and transferred *Artemisia dracunculoides*, *A. dracunculus* and *A. glauca* into *Oligosporus*. Following this resurrection a number of new combinations were made to transfer varieties and subspecies of *Artemisia dracunculus* to *Oligosporus* as well. This included Á. Löve & D. Löve's combination of *A. dracunculus* subsp. *glauca* which was changed into *Oligosporus dracunculus* subsp. *glauca,* and Weber's transferring of *A. dracunculus*

subsp. *dracunculina* to *Oligosporus dracunculus* subsp. *dracunculinus* (Love and Love 1982; Weber 1989). During the 20th century a number of varieties and forms of *A. dracunculus* were described from Europe and Asia as well. Pampanini described a number of forms based on specimens collected in Asia, but these forms have generally not been recognized in major regional floras or were put in synonymy with other taxa (Pampanini 1927, 1929; Ling et al., in prep).

For the recent treatment in *Flora of North America*, Shultz (2006) recognized one species, *A. dracunculus* L., and no varieties or subspecies at all. For the treatment in *Flora of the USSR*, Poljakov (1961b) listed five varieties of *A. dracunculus*: *A. dracunculus* var. *pratorum* Krasch., *A. dracunculus* var. *turkestanica* Krasch., *A. dracunculus* var. *pilosa* Krasch., *A. dracunculus* var. *humilis* Kryl. and *A. dracunculus* var. *redowskyi* Ledeb and maintained *A. glauca* as a separate species. *Artemisia pamirica* C. Wink. and *A. dracunculiformis* Krasch. were listed as separate species by Poljakov (1961b), although *A. dracunculiformis* Krasch. was listed as a synonym of *A. dracunculus* by Czerapanov (1995). Poljakov (1961b) also recognized *A. glauca* Pall. ex Willd. as a separate species as well as the varieties *A. glauca* var. *incana* Bess. and *A. glauca* var. *humilis* Kryl.

For the *Flora of China*, Ling et al. (in prep.) listed *A. dracunculus* var. *dracunculus*, *A. dracunculus* var. *pamirica* (C. Wink.) Y. R. Ling & Humphries, *A. dracunculus* var. *turkestanica* Krasch., *A. dracunculus* var. *changaica* (Krasch.) Y. R. Ling, and *A. dracunculus* var. *qinghaiensis* Y. R. Ling.

The characteristics used to describe varieties unique to Asia are often the same as those that have been used to describe different varieties in North American populations.

For example, *A*. *dracunculus* var. *redowskyi* Turcz. (described from Asia) is noted as having drooping capitula on long peduncles in spreading, paniculate inflorescences. These are the same characteristics used to describe *A*. *dracunculus* subsp. *dracunculina* (S. Watson) Hall and Clements in the U.S. There are also varieties that are described as having larger capitula (3-4 cm rather than 2-3 cm) reported from both continents as well. The juvenile pubescence of *A*. *dracunculus* var. *pilosa* Krasch. (Asian variety) is very reminiscent of *A*. *dracunculoides* var. *incana* Torrey and Gray (North American variety), and *A*. *dracunculus* var. *humilis* Kryl. (Asian variety) is described as having a short habit with narrow-paniculate or nearly raceme-like inflorescences, which is often described as being the habit of *A. dracunculus* in the more northern areas of its distribution in North America (Fernald, 1950). Although the varieties do not match perfectly, it is interesting to note that many of the same variable characteristics occur in separate subtaxa described from North America and Asia.

Use of A. dracunculus in folk medicine. Over twenty different Native American tribes were documented as using wild tarragon (*Artemisia dracunculus*) for a very wide array of ailments (heart palpitations, wounds and bruises, swelling, rheumatism and arthritis, aches and pains, eye diseases, skin diseases, difficult labor, dysentery, excessive menstrual flow, etc.) as well as to repel insect pests (Moerman, 1998). The use of wild tarragon, primarily as a medicine, has been documented in many different cultures throughout the world. The plant has been noted for traditional use in the treatment of edema, scurvy, oral diseases, gastritis and dyspepsia, to improve appetite, and as a carminative and anti-helminthic in Uzbekistan (Khalmatov, 1964; Khalmatov et al.,

1984). According to Khodzhimatov (1989), in Tajikistan a decoction or tea of flowering branches or flower heads is used to treat gastritis as well as to clear the respiratory tract of phlegm, as a vermifuge, and as a mouthwash to treat bleeding gums. He also reports that a bath in water, in which the aboveground parts have been steeped, is used to treat skin diseases (scabies, eczema, fungal, etc). In Pakistan the plant is mainly used as an herb in cooking and as fodder for horses, and in Ladakh (India) it is used to treat throat infections (Kletter and Kriechbaum, 2001; Hayat et al., 2009). In Iranian folk medicine the plant was used to treat epilepsy, as an anticoagulant, for cleaning and diluting the blood, and as a treatment for dizziness and headache (Sayyah et al., 2004; Yazdanparast et al., 2000; Shahriyary and Yazdanparast, 2007). Steinmetz (1957) and Uphof (1968) report the following general uses and qualities for *A. dracunculus*: increasing appetite, stomachic, diuretic, aromatic, cooling, carminative, antiscorbutic, emmenagogue, vermifuge and to treat toothaches, but does not attribute the uses to any specific country or region.

Phytochemistry and bioactivity of A. dracunculus. *A. dracunculus* has been shown to contain a diverse array of chemicals consisting of terpenes, coumarins, isocoumarins, flavonoids, caffeoylquinic and dicaffeoylquinic acids, polyacetylene derivatives, sesquiterpenoids, alkaloids and others (Kletter and Kriechbaum, 2001; Deans and Simpson, 2002; Aglarova et al., 2008; Bhutia and Valant-Vetschera, 2008). Because of their biological activity, their use in chemotaxonomy, the flavonoids of *Artemisia dracunculus* have been of particular interest to many researchers and this class of compounds has been investigated in a number of phytochemical studies. A list of the

flavonoid and flavonoid glycosides that have been documented in these studies and, when available, the provenance of the investigated plant material, is provided in Table 1.2.

The bioactive phytosterols beta-sitosterol and stigmasterol have been isolated from *Artemisia dracunculus* (Tunmann and Mann, 1968; Mallabaev et al., 1969). Unlike these two common phytosterols, which are widely distributed throughout the plant kingdom, some very unique nitrogenous compounds have also been found in *A. dracunculus*. Kavvadias et al., (2000) found endogenous production of the two benzodiazepines, delorazepam and temazepam, in sterilely cultivated *A. dracunculus* cell cultures. Extracts and fractions prepared from these cultures exhibited significant binding activity to human benzodiazepine receptors (Kavvadias et al., 2000). Saadali et al. (2001) identified the insecticidal compounds alkamides, pellitorine, neopellitorine A and neopellitorine B from this species, but did not provide details regarding the provenance of the plant material that was used.

Coumarins and coumarin derivatives such as daphnetin methylene ether, daphnetin 7-methyl ether, 6-Methoxy-7,8-methylenedioxycoumarin, scoparone, scopoletin, coumarin, herniarin, aesculetin (esculetin) and aesculin (esculin) have all been isolated from wild tarragon (Herz et al., 1970; Hofer et al., 1986; Murray and Stefanovic, 1986; Wollenweber et al., 1989; Saadali et al., 2001, Aglarova et al., 2008). Bhutia and Valant-Vetschera (2008) found that scoparone accumulated solely in the inflorescences, while herniarin was found predominately in the leaves and with lesser amounts in the inflorescences. A number of the coumarins have shown biological activity. Saadali et al. (2001) found that herniarin was insecticidal and Silvan et al. (1996) found that herniarin, aesculin and scopoletin had strong anti-inflammatory activity. Scoparone exhibits

cytotoxic activity against murine leukemia P-388 cells (Yen et al., 2008) and shows vasorelaxant and immunosuppressive activity (Huang et al., 1991). Herniarin has also been shown to exhibit strong anti-allergenic properties (Watanabe, 2005).

A variety of different isocoumarins and isocoumarin derivatives including 3-(1Z-butenyl)-isocoumarin, 3-(1E-butenyl)-isocoumarin, 7-hydroxyartemidin, (E/Z)-artemidin, artemidiol, artemidinol, capillarin, 8-hydroxyartemidin, 8-hydroxycapillarin and others, have been isolated from wild tarragon (Mallabaev et al., 1970; Mallabaev et al., 1971; Mallabaev and Sidyakin, 1974; Mallabaev and Sidyakin, 1976; Greger, 1977; Greger and Bohlmann, 1979; Rutskikh et al., 2000; Yazdanparast et al., 2000; Lutz-Kutschera et al., 2003; Engelmeier et al., 2004). Isocoumarins have been shown to be produced from different biosynthetic pathways than coumarins, and their presence has been shown to vary between different *A. dracunculus* cytotypes (Greger, 1979). Engelmeier et al. (2004) showed that some of these compounds have antifungal activity against rice blast fungus (*Pyricularia grisea*) and that the presence of a 3-butyl side chain is a prerequisite for high activity. Information regarding the seasonal accumulation and region variation of coumarins and isocoumarins in *A. dracunculus* has been summarized by Aglarova et al. (2008).

***Anti-diabetic compounds found in A. dracunculus*.** The compounds davidigenin, 2',4-dihydroxy-4'-methoxydihydrochalcone, 2',4'-dihydroxy-4-methoxydihydrochalcone, sakuranetin, 6-demethoxycapillarisin, 5-O-caffeoylquinic acid, and 4,5-di-O-caffeoylquinic acid have been shown to be active in a number of different pathways

associated with the diabetic condition (see Introduction in Chapter 3 for discussion of specific bioactivities).

The nomenclature of chlorogenic acids can be confusing to those unfamiliar with this group of compounds. 5-O-caffeoylquinic acid is commonly called chlorogenic acid, but in actuality chlorogenic acids are also a family of esters formed between certain *trans*-cinnamic acids and quinic acid. They are generally involved in responses to biotic and abiotic stress by plant but 5-O-caffeoylquinic acid has been found to be an intermediate in the lignin biosynthesis pathway. These chlorogenic acids (including 5-O-caffeoylquinic acid) can be divided into groups based on the type, number and position of the acyl residues. Mono-, di-, tri- and tetra-esters of caffeic acid are referred to as caffeoylquinic, dicaffeoylquinic, tricaffeoylquinic and tetracaffeoylquinic acids, respectively. Tricaffeoylquinic and tetraaffeoylquinic acids seem to be primarily found in the Asteraceae (Clifford, 1999; Clifford, 2003; Mondolot et al., 2006).

5-O-caffeoylquinic acid has been shown to have a wide array of biological activities including immunomodulatory effects (Lin et al., 1999; Chiang et al., 2003), causing hormonal (corticotropin) reduction (Ina et al., 2004), antimutagenic activity (Yoshimoto et al., 2002), and antioxidant effects (Zang et al., 2003; Kono et al., 1997). 4,5-Di-O-caffeoylquinic acid has been isolated from a number of plant species in unrelated families, including *Ipomoea batatas* L. (Convolvulaceae), *Dipsacus asper* Wall. (Dipsacaceae), *Coffea canephora* Pierre ex Froehn. (Rubiaceae), *Securidaca longepedunculata* Frasen. (Polygalaceae) and others, and this compound has been documented as having a wide array of biological activities including antioxidant, antimutagenic and antiviral effects (Mahmood et al., 1993; Islam et al., 2002; Yoshimoto

et al., 2002; Clifford et al., 2003; Hung et al., 2006; Truong et al., 2007). In addition to *Artemisia dracunculus*, 4,5-Di-O-caffeoylquinic acid has been isolated from a diverse range of species within the Asteraceae family including *Artemisia herba-alba* Asso. (Kim et al., 2004), *Bidens pilosa* L. (Chiang et al., 2004; Yang et al., 2006; Matsumoto et al., 2009), *Cynara scolymus* L. (Schütz et al., 2004), *Dichrocephala bicolor* (Roth) Schltdl. (Lin et al., 1999), *Hieracium pilosella* L. and *Hypochaeris radicata* L. (Zidorn et al., 2005), *Gnaphalium stramineum* Kunth (Rastrelli et al., 1998), *Taraxacum mongolicum* Hand-Mazz. (Shi et al., 2008), *Taraxacum officinale* F.H. Wigg. (Schütz et al., 2004), and *Xanthium* spp. (Han et al., 2006).

Davidigenin (4,2',4'-trihydroxydihydrochalcone) has been previously isolated from *Euphorbia portlandica* L. (Madureira et al., 2004), *Viburnum davidii* Franch. and *V. lantanoides* Michx. (Jensen 1977). In pharmacodynamic studies of compounds isolated from a component (*Glycyrrhiza glabra* L. or *G. uralenesis* Fisch. ex DC.) of some traditional Kampo and Chinese medicines, davidigenin was isolated from urine and plasma of the experimental animals and was determined to be a hydrogenated metabolite of liquiritigenin (Taniguchi et al., 2000; Kamei et al., 2003). *In vitro*, davidigenin has been shown to induce apoptosis in human lung fibroblasts, inhibit the release of leukotrienes (an anti-allergenic activity) and has exhibited antitussive effects *in vivo* (Homa et al, 2000; Kamei and Morita, 2001; Liu et al., 2002).

Sakuranetin (5,4'-dihydroxy-7-methoxyflavanone) was first isolated from the bark of *Prunus yedoensis* Matsumura and was subsequently found to present in a number of *Prunus* species including *P. aequinoctialis*, *P. nipponica*, *P. maximowiczii*, *P. jamasakura*, *P. avium* (Narasimhachari and Seshadri, 1949); Hasegawa and Shirato,

1957 a; Hasegawa and Shirato, 1958; Miyazawa et al., 2003; Vinciguerra et al., 2003). Sakuranetin has been most thoroughly studied in rice (*Oryza sativa* L.). Originally isolated from ultraviolet-irradiated leaves, extensive analyses have shown that it acts as an antifungal phytoalexin (Dillon et al., 1997). In addition to *Prunus* and *Oryza*, sakuranetin has also been identified in an assortment of species, from a wide range of families, including *Potentilla viscosa* Donn ex Lehm. and *Rubus parviflorus* Nutt. (Rosaceae; Wollenweber and Doerr, 2008), *Betula ermani* Cham., *Betula pendula* Roth, and *Populus davidiana* Dode (Betulaceae and Salicaceae; Wollenweber and Egger, 1971; Chen and Liang, 2006; Shul'ts et al., 2005), *Ribes nigrum* L. (Grossulariaceae; Atkinson and Blakeman, 1982), *Piper crassinervium* Kunth and *Piper marginatum* Jacq. (Piperaceae; Danelutte et al., 2003; Lago et al., 2004; Reigada et al., 2007), *Arachis hypogaea* L. and *Mimosa hostilis* (Mart.) Benth. (Fabaceae; Ohsaki et al., 2006; Dean et al., 2008), *Phoradendron robinsonii* Urb. (Loranthaceae; Rivero-Cruz et al., 2005), *Terminalia fagifolia* Mart. (Combretaceae; Garcez et al., 2006), various species in the genera *Thymus* L., *Origanum* L. and *Cunila* D. Royen ex L. (Lamiaceae; Horwath et al., 2008; Skoula et al., 2008), *Lippia dulcis* Trev. and *Lippia graveolens* H.B.K. (Verbenaceae; Ono et al., 2006; Lin et al., 2007; Ogawa et al., 2007(Ogawa et al., 2007) *Xanthorrhoea hastilis* (Liliaceae; Ogawa, et al., 2007) the fern *Notholaena nivea* var. *flava* (Pteridaceae; Bravo et al., 2003) and the basidiomycete polypore fungus *Phellinus igniarius* (Mo et al., 2003). Sakuranetin has also repeatedly been isolated from bee propolis, as well as from the nests of bees. In a number of the studies the propolis and nesting material has been traced to plant exudates collected from species including

Populus tremuloides Michx. and *Eucalyptus citriodora* Hook. (Christov et al., 2006; Freitas et al., 2008).

Sakuranetin has also been found in multiple species of the Asteraceae family, including *Blumea glomerata* DC., *Chromolaena odorata* (L.) R.M.King & H.Rob., *Dittrichia graveolens* (L.) Greuter, *Heterothalamus alienus* (Spreng.) Kuntze, *Inula viscosa* (L.) Aiton, and *Ophryosporus heptanthus* (Sch. Bip. ex Wedd.) R.M. King & H. Rob. (Abid and Qaiser, 2003). Sakuranetin has been found to have antifungal activity, amtimutagenic activity, anti-allergenic properties *in vivo*, and anti-inflammatory activity *in vitro* (Bravo et al., 2003). Saito et al. (2008) observed that sakuranetin induces adipogenesis and stimulates glucose uptake into adipocytes.

In addition to being documented in *Artemisia dracunculus*, 6-demethoxycapillarisin has been found in extracts of *Rosa rugosa* (Rosaceae), *Mimosa tenuiflora* (Fabaceae), and in the Asteraceae, in the species *Achillea ageratum* and *Artemisia capillaris* (Hashidoko, 1996).

Bioactivity of A. dracunculus extracts. Many studies have documented the bioactivity of various crude extracts prepared from *A. dracunculus*. In an *in vitro* assay, Shahriyary and Yazdanparast (2007) and Yazdanparast et al. (2008) found that a crude methanol extract, and a chloroform fraction of wild tarragon from Iran reduced platelet adhesion and aggregation, and reduced protein secretion *in vitro*. Extracts of wild tarragon have exhibited a number of different anti-diabetic activities and a more detailed review of these activities can be found in Chapter 3.

A number of studies have been conducted using various assays for antioxidant and/or radical scavenging activity. Bahramikia et al. (2008) used an ethanolic extract of plant material collected near Arak, Iran, to assess radical scavenging and antioxidant ability using the 3-ethylbenzothiazoline-6-sulfonate (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assays and the ferric reducing ability potential (FRAP) assay. In these assays *A. dracunculus* exhibited low radical scavenging and antioxidant capabilities when compared with *Satureja hortenisis*. Using essential oil isolated from wild-growing plant material collected in the Central Alberta Prairies of Western Canada, Lopes-Lutz et al. (2008) showed that although *A. dracunculus* has antioxidant (b-carotene/linoleic acid assay) and radical scavenging (DPPH assay) activity, the activity of the oil is much less than that of positive controls (oregano oil and BHT). Similar results were found in a study using essential oil isolated from plants growing in Turkey (Kordali et al., 2005). Caillet et al. (2006) found that an aqueous extract and ethanolic extracts of *A. dracunculus* had mild anti-oxidant activity and pro-oxidative activities respectively. Together these studies show fairly consistent results that when compared with plants and compounds that have strong anti-oxidant activity, *A. dracunculus* has low antioxidant and radical scavenging ability regardless of the type of extract or provenance of plant material.

The antimicrobial effects of tarragon extracts and essential oil have been investigated by many different researchers. Benli et al. (2007) found that a methanol extract of the plant had better antimicrobial effects than chloroform and acetone extracts when tested against *Pseudomonas aeruginosa*, *Shigella sp.*, *Listeria monocytogenes* and two different strains of *Escherichia coli*. Essential oil of tarragon was found to have low

inhibition of methicillin-resistant *Staphylococcus aureus* (MRSA) in a disc diffusion assay for inhibitory activity (Chao, 2008). In a study to determine the antibacterial activity of the essential oil in the vapor phase against five food borne bacteria, Nedorostova et al. (2009) found that the essential oil did not have significant antibacterial effects. Curini et al. (2006) also found that oil isolated from the cultivar 'Piemontese', collected in central Italy, also had low antibacterial activity. In inhibition test with 10 different bacterial species, Deans and Svaboda (1988) found that individual components of the essential oil from French tarragon (anisaldehyde, p-cymene, eugenol, limonene, linalool, menthol, cis-ocimene, α-phellandrene, α-pinene and β-pinene) caused 2-3 times more inhibition than an equal amount of the total extract. They also noted that although French tarragon is noted for its high amount of methyl chavicol (65-81% of total oil), this compound had little to no inhibitory activity. Further discussion of essential oil bioactivity is presented in Chapter 4.

Artemisia dracunculus has a long history of use as a medicinal plant and many of the compounds found in the plant have been shown to have biological activity. It is important to reiterate that researchers have also documented distinct morphological and chemical variation in *A. dracunculus* and it is possible that this variation in chemical content could result in differences in the bioactivity of extracts prepared from different cytotypes and/or populations of *A. dracunculus*.

Table 1.1. A chronologic list of synonyms and available subspecies, variety, and form names of *A. dracunculus*. Taxonomic name with auctor is listed in the left column, and author and first publication in the right column.

Taxon	Publication
Artemisia dracunculus L.	Linnaeus, 1753
A. glauca Pall. ex Willd.	Willdenow, 1803
A. inodora Willd.	Willdenow, 1809
A. dracunculoides Pursh	Pursh, 1813
A. nutans Fras. ex Pursh	Pursh, 1813
A. redowskyi Ledeb.	Ledebour, 1815
A. cernua Nuttall	Nuttall, 1818
Oligosporus condimentarium Cass.	Cassini, 1825
A. dracunculus var. *glauca* Besser	Hooker, 1829
A. nuttalliana Besser ex Hook.	Hooker, 1829
A. dracunculus var. *sativa* Besser	Besser, 1835a
A. dracunculus var. *inodora* Besser	Besser, 1835a
A. glauca var. *glabra* Turcz.	Cited in Besser, 1835a
A. glauca var. *incana* Besser	Besser, 1835a
A. glauca var. *latifolia* Ledeb.	Cited in Besser, 1835a
A. glauca var. *subcanescens* Besser	Besser, 1835a
A. glauca var. *fastigiata* Besser	Besser, 1835a
A. dracunculus var. *redowskyi* Turcz.	Turczaninow, 1838
A. dracunculoides var. *incana* Torr. & Gray	Torrey and Gray, 1843
A. dracunculoides var. *tenuifolia* Torr. & Gray	Torrey and Gray, 1843
A. desertorum var. *macrocephala* Franchet	Franchet, 1883
A. dracunculina S. Wats.	Watson, 1888
A. pamirica C. Winkl.	Winkler, 1890
A. aromatica A. Nels.	Nelson, 1900
A. dracunculus f. *humilis* Kryl.	Krylov, 1904
A. glauca f. *humilis* Kryl.	Krylov, 1904
A. dracunculoides var. *wolfii* Rydberg	Rydberg, 1905
A. gracillima Rydberg	Rydberg, 1916
A. dracunculus subsp. *typica* Hall & Clements	Hall & Clements, 1923
A. dracunculus subsp. *glauca* (Pall. ex Willd.) Hall & Clements	Hall & Clements, 1923
A. dracunculus subsp. *dracunculina* (S. Wats.) Hall & Clements	Hall & Clements, 1923
A. dracunculus var. *glauca* (Pall. ex Willd.) Jeps.	Jepson, 1925
A. glauca var. *cernua* (Nutt.) Bush	Bush, 1928
A. glauca var. *dracunculoides* (Pursh) Bush	Bush, 1928
A. dracunculus f. *chinensis* Pamp.	Pampanini, 1929
A. dracunculus f. *falciloba* (Mattf.) Pamp.	Pampanini, 1929
A. dracunculus var. *inodora* Pamp	Pampanini, 1929
A. dracunculus f. *intermedia* Pamp.	Pampanini, 1929
A. dracunculus subf. *oblonga* Pamp.	Pampanini, 1929
A. dracunculus f. *pinnata* Pamp.	Pampanini, 1929

Taxon	Publication
A. dracunculus var. *subdigitata* (Mattf.) Pamp.	Pampanini, 1929
A. dracunculus f. *thomsonii* Pamp.	Pampanini, 1929
A. simplicifolia Pampanini	Pampanini, 1934
A. dracunculoides var. *glauca* (Pall. ex Willd.) Munz	Munz, 1935
A. dracunculus var. *pilosa* Krasch.	Krascheninnikov, 1936
A. changaica Krasch.	Krascheninnikov, 1937
A. dracunculoides var. *dracunculina* (S. Wats.) S.F. Blake	Blake, 1940
A. glauca var. *dracunculina* (S. Wats.) Fernald	Fernald, 1945
A. dracunculiformis Krasch.	Krascheninnikov, 1946a
A. dracunculus var. *turkestanica* Krasch.	Krascheninnikov, 1946b
A. dracunculus var. *pratorum* Krasch.	Krascheninnikov, 1948
A. glauca var. *megacephala* B.Boivin	Boivin, 1955
Oligosporus changaicus (Krasch.) Poljakov	Poljakov, 1961a
O. dracunculoides (Pursh) Poljakov	Poljakov, 1961a
O. dracunculus (L.) Poljakov	Poljakov, 1961a
O. glaucus (Pall. ex Willd.) Poljakov	Poljakov, 1961a
O. pamiricus (C. Winkler) Poljakov.	Poljakov, 1961a
O. dracunculus subsp. *glauca* Á. Löve & D. Löve	Love and Love, 1982
A. dracunculus var. *changaica* (Krasch.) Y.R. Ling	Ling, 1982
Draconia dracunculus (L.) Soják	Soják, 1983
A. dracunculus var. *pamirica* (C. Winkl.) Y.R. Ling & Humphries	Ling, 1988
A. dracunculus var. *qinghaiensis* Y.R. Ling	Ling, 1988
O. dracunculus subsp. *dracunculinus* (S.Wats.) Weber	Weber, 1989

Table 1.2. Studies investigating flavonoid type compounds in *A. dracunculus* L.

Author	Compound	Source material
Lukovnikova, 1965	quercetin-3-rutinoside	
Chumbalov et al., 1969	quercetin hyperoide	wild (southern USSR)
	isorhamnetin 7-a-D-galactopyranoside p-hydroxybenzoate	
Chumbalov and Mukhamed'yarova, 1970	kaempferol, luteolin, quercetin-3-O-rutinoside, quercetin-3-O-rhamnogalactoside	wild (Southern USSR)
Ryakhovskaya et al., 1970	quercetin-3-O-rutinoside	
Hoffmann and Herrmann, 1982	kaempferol-3-rhamnoglucoside, patuletin-3-β-d-glucoside, patuletin-3-O-rhamnoglucoside, quercetin-3-rhamnoglucoside	
Balza and Towers, 1984;	narigenin, 3,5,4'-trihydroxy-7-ethoxyflavanone	wild (Canada)

Author	Compound	Source material
	3,5,4'-trihydroxy-7,3'-dimethoxyflavanone	wild (Canada)
Balza et al., 1985	5,3'-dihydroxy-7,4'-dimethoxyflavanone	wild (Canada)
Vienne et al., 1989	quercetin-3-O-glucoside, quercetin-3-O-rhamnoglucoside, quercetin-3-O-rhamnogalactoside,	French, wild
	quercetin, patuletin-3-O-glucoside, patuletin -3-O-rhamnoglucoside, patuletin -3-O-rhamnogalactoside,	wild
Wollenweber et al., 1989	kampferol, kaempferol-3-methyl ether, quercetin, querceti-3-methyl ether, querceti-3'-methyl ether, querceti-3,7,3'-methyl ether, sakuranetin [naringenin methyl ether], naringenin-4'-methyl ether, eriodictyol-7-methyl ether, eriodictyol-3'-methyl ether, eriodictyol-7,3'-methyl ether	wild (Arizona)
Jakupovic et al., 1991	eriodictyol, hesperetin, naringenin	wild (Mongolia)
Kurkin et al., 1996	annagenin [4',5,6,7,8-pentahydroxy-3'-methoxyflavone], estranogoside, naringenin, pinocembrin [5,7-dihydroxyflavanone]	wild (Gribovskii variety)
Kurkin et al., 1997	estranogoside [4',5,6,7,8-pentahydroxy-3'-methoxyflavone 8-O-a-L-rhamnopyranoside], pinocembrin-7-O-B-D-glucopyranoside	wild (Gribovskii variety)
Onuchak et al., 2000	annagenin, estranogoside, naringenin, pinocembrin	wild (Gribovskii variety)
Justesen and Knuthsen, 2001	isorhamnetin, kaempferol, luteolin, quercetin	
Logendra et al., 2006	davidigenin, 2,4-dihydroxy-4-methoxydihydrochalcone	wild (purchased from Sheffield's Seed Co. Inc.)
Wang et al., 2006	sakuranetin [4',5-dihydroxy-7-methoxyflavanone; naringenin-7-methyl ether]	wild (purchased from Sheffield's Seed Co. Inc.)
Bhutia et al., 2008	eriodictyol-7,3-dimethyl ether, eriodictyol-7-methyl ether, sakuranetin [naringenin-7-methyl ether], eriodictyol-7-methyl ether	wild (Kyrgyzstan)

Figure 2.1. Distribution of *A. dracunculus* L. in the U.S. and Canada; *A. dracunculus* has been documented in states and provinces colored green. [Reproduced with permission. USDA, NRCS, 2010]

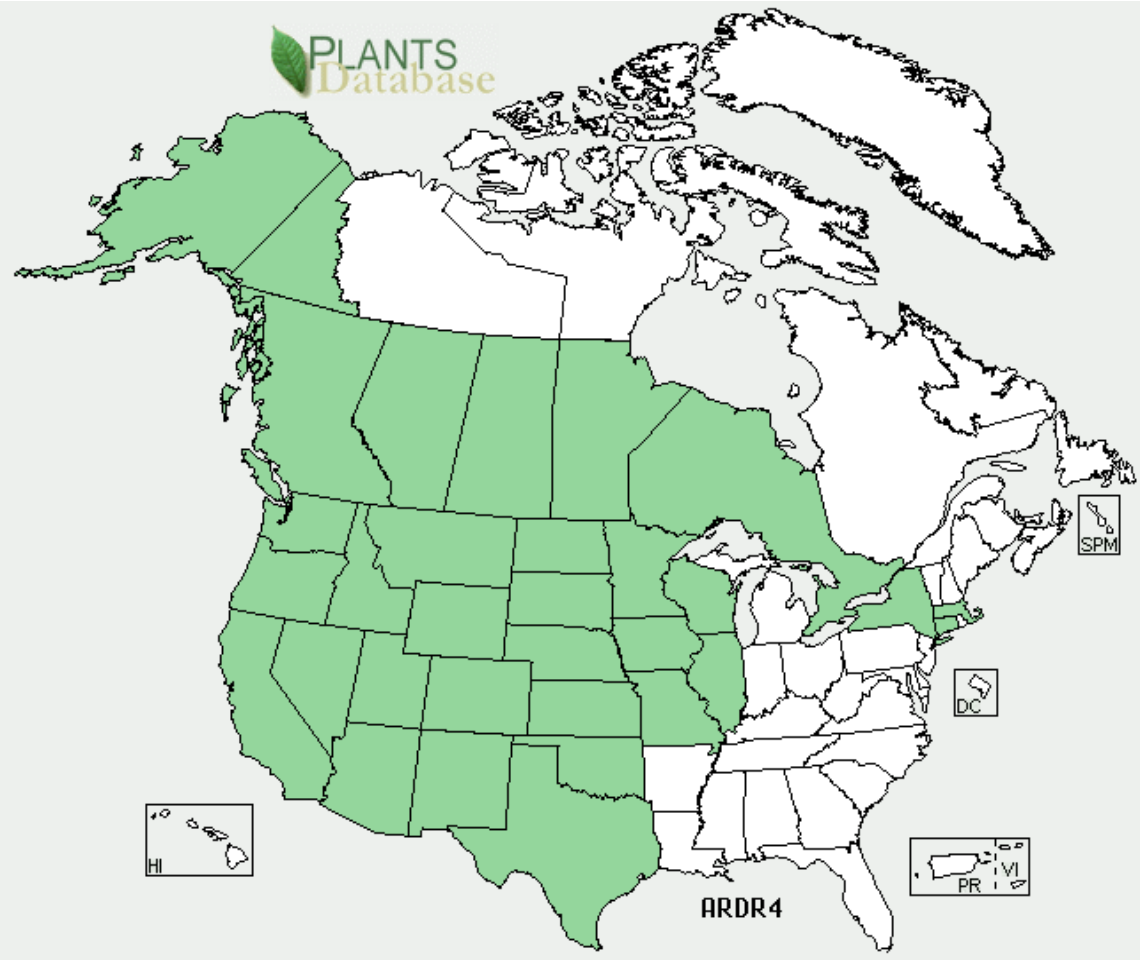
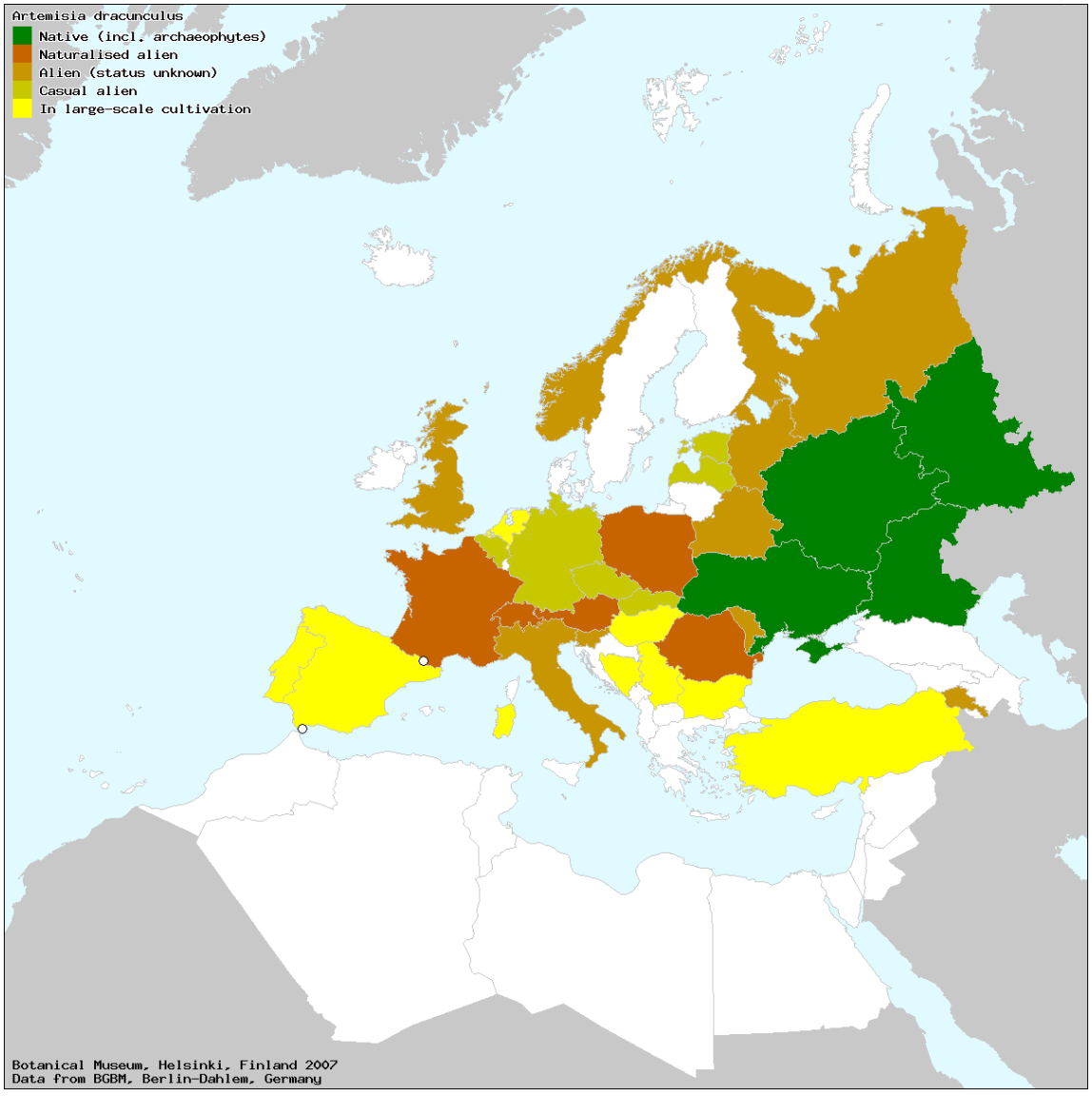


Figure 1.2. Partial distribution of *A. dracunculus* in Europe and the Mediterranean region; grey = areas are not covered by the Euro+Med PlantBase, yellow = areas in large scale cultivation, light brown = casual alien, brown = alien (status unknown), red-brown = naturalized alien, green = native and white = areas that require further data, [Reproduced with permission of the Botanic Garden and Botanical Musem, Berlin (Germany), and the Botanical Museum, Helsinki (Finland)]



Chapter 2

Assessing the geographic distribution of wild tarragon (*Artemisia dracunculus* L.) cytotypes using meiotic chromosome counts and flow cytometry

Abstract

Artemisia dracunculus L. (wild or Russian tarragon), is an polymorphic, herbaceous perennial with a widespread distribution that spans western North America, parts of temperate Asia, as well Central Asia and Eastern Europe. The species is documented as having from diploid to decaploid ploidy levels. A literature review of the geographical occurrences of cytotypes revealed a lack of records from North America. In order to fill in this gap in the cytogeographic distribution, meiotic chromosome counts and flow cytometry were used to determine the ploidy level of 27 individuals from 16 different populations throughout the western United States. The results show that both diploids and polyploids are found in Europe and Asia, while only diploid populations were found in North America with the exception of one hexaploid record from Arizona which was found in the literature.

Introduction

Artemisia dracunculus L. (wild or Russian tarragon), is an herbaceous perennial with a widespread distribution that spans western North America, parts of temperate Asia, as well Central Asia and Eastern Europe (Hall and Clements, 1923; Poljakov, 1961; Hultén, 1968; USDA, NRCS, 2010).). Due to its widespread use by humans, the species

is thought to have been spread beyond its native region into Western Europe and England (Sutton et al., 1985; Greuter, 2006-2009). Because there is a great deal of morphological variation, the species has long been the source of taxonomic confusion. Variation in the type and amount of pubescence, leaf size and lobing, pedicel length, capitulum size, number of florets per capitulum, and synflorescence shape, led earlier authors to describe a number of separate species, subspecies and varieties in North America (Torrey and Gray, 1841; Watson, 1888; Nelson 1900; Rydberg, 1905, 1916; Hall and Clements, 1923; Fernald, 1945). These taxa are not currently recognized in the Flora of North America (Shultz, 2006).

The species is best known for the unique variety, *Artemisia dracunculus* var. *sativa* Besser, or French tarragon, which is used as a culinary herb. It is commonly used in the preparation of béarnaise sauce, for making flavored vinegars as well as a variety of other culinary applications. This variety is a sterile tetraploid and has to be propagated clonally (Rousi, 1969; Sutton et al., 1985; Deans and Simpson, 2002), and this aspect of the plants reproduction was documented as early as 16th century by Dodoens (1563). French tarragon is favored for its spicy licorice flavor, and has high concentrations (60-80%) of methyl chavicol (estragole) in its essential oil (Werker, 1994; Deans and Simpson, 2002; Arabhosseini, 2006). Wild tarragon is noted as having a different essential oil profile, with sabinene, elemicin, trans-isoelemicine or *trans*-anethole as the major oil constituent and contains little or no methyl chavicol (Albasini et al., 1983; Deans and Simpson, 2002). As a result, it is considered to have an inferior flavor to French tarragon. Although rarely used in cooking, wild tarragon has a long history of use in various traditional medicine systems and has been used by peoples in the Old and New

World to treat a wide variety of illnesses (Khalmatov, 1964; Khalmatov et al., 1984; Khadzhimatov, 1984; Moerman, 2003). Empirical research on the medicinal activity of wild tarragon has shown that the plant contains compounds that affect type 2 diabetes and diabetes related illnesses. Swanson-Flatt et al. (1989) found that tarragon significantly reduced the hyperphagia and polydipsia associated with streptozotocin diabetes. More recently, an extract of wild tarragon has been shown to reduce hyperglycemia in both chemically induced and genetically diabetic mice and reduce expression of an important enzyme which is normally over-expressed in insulin-resistant diabetic rats (Ribnicky et al., 2006; Govorko et al., 2007; Schmidt et al., 2007). The extract was also found to inhibit aldose reductase, an enzyme which is involved in the cause of a number of secondary conditions associated with the diabetic condition and bioassay-guided fractionation led to the isolation of a number of different active compounds (Logendra et al., 2006; Schmidt et al., 2007).

Ploidy level in A. dracunculus. Polyploidy is particularly common in *Artemisia* species and is well documented (Vallès and McArthur, 2001; Vallès et al., 2001a, 2001b, Torell and Vallès, 2001; Hoshi, 2003; Garcia et al., 2004, 2006; Pellicer, 2007a, 2007b). Studies have shown that *Artemisia dracunculus*, like the majority of *Artemisia* species, has a base chromosome number of x = 9 (Rousi, 1969; McArthur and Sanderson, 1999; Torell and Vallès, 2001) and that the species has both diploid plants ($2n = 2x = 18$), as well as a whole series of polyploid individuals ($2n = 3x = 27$), ($2n = 4x = 36$), ($2n = 6x = 54$), ($2n = 8x = 72$) and ($2n = 10x = 90$; Vallès and McArthur, 2001).

Polyploidy is an important factor to consider when investigating plants for medicinal activity. Many physiological processes including enzyme production, photorespiration and secondary metabolite production have been shown to vary between diploid and polyploid individuals of the same species (Levin, 1983). Chemical variation in various cytotypes of a single species has been documented for many species including *Artemisia dracunculus* (Vienne et al., 1989; McArthur and Sanderson, 1999; Engelmeier et al., 2004). For example, Greger (1979) showed that root polyacetylenes exhibited qualitative variation in different cytotypes of *A. dracunculus* (hexaploid, octoploid and decaploid cytotypes), as well as the closely related sprecies *A. glauca* (often considered synonymous with *A. dracunculus*), *A. pamirica* (currently considered a variety of *A. dracunculus*) and *A. dracunculiformis* (Ling, 1988; Shultz, 2006). Based on the presence of capillen, *A. glauca*, *A. dracunculiformis* and *A. pamirica*, and the decaploid *A. dracunculus* were grouped together, while the three other *A. dracunculus* samples (an octaploid, hexaploid and putative octoploid) formed a separate group which lacked capillen and contained artemidins. Additionally, the decaploid *A. dracunculus* cytotype was also found to contain butynyl-isocoumarins not found in any of the other cytotypes or taxa.

In conjunction with ongoing phytochemical studies of North American populations of *Artemisia dracunculus*, a literature review of all available *A. dracunculus* ploidy records was conducted and the collection locations of the plant material were plotted onto maps to determine the global distribution of cytotypes. Additionally, flow cytometry and traditional microscopy were used to assess the genome size and ploidy level of populations across the western United States. Flow cytometry was utilized

because it provides a fast and accurate way to assess the ploidy level of non-flowering material. Because of the potential medicinal value of compounds produced by *A. dracunculus*, and because it has been previously shown that phytochemical content can depend on ploidy level, knowing the geospatial distribution of the cytotypes and their chemical variation is important for future selection of plant material for investigations of biological activity.

Materials and Methods

Records of literature documenting chromosome numbers were obtained from the Missouri Botanical Garden's digital database Index to Plant Chromosome Numbers (http://mobot.mobot.org/W3T/Search/ipcn.html) as well as from numerous journal articles and books (Cave, 1964; Moore 1973; Goldblatt, 1981; references cited in Table 1). The geographic coordinates of the collection localities cited in each article were estimated using Google Earth 5 (http://earth.google.com/) and these records were plotted onto maps using ArcView GIS 9.0 (Environmental Systems Research Institute, 2009). In cases where the plant material was cultivated, and the locality of the source material was not stated, the locality of the where the analysis was conducted was used and marked as cultivated on the maps.

Artemisia dracunculus L. plants were grown from wild-collected root cuttings of individuals from populations throughout the western United States (see Appendix 1). Root cuttings of plants were collected from each site and cultivated in 11" polyethylene pots at the Rutgers University/NJAES greenhouses. In addition to wild material from North America, French tarragon plants were purchased from Pantry Garden Herbs

(Freeman, MO), a single wild tarragon sample from Kyrgyzstan was cultivated from seed and tarragon seed purchased Sheffield's Seed Co., Inc (Locke, NY) labeled with a collection locale of Amsterdam were also grown. Voucher specimens from the collection sites are deposited at the Rutgers University Chrysler herbarium (CHRB). As each plant began to develop reproductive tissue, immature capitula were collected (prior to florets opening) and immediately fixed in 3:2:1 chloroform:glacial acetic acid:ethanol. The capitula were left in the fixative for approximately one week and then transferred to propionic-carmine stain for one week. Individual flowers were removed from the capitulum under a dissecting scope and the anthers were excised in a few drops of 35 % glacial acetic acid. Pollen was manually removed from the anthers by "squashing" the anthers with forceps. A cover slip was applied, covered with a paper towel and pressed down with moderate pressure with the goal of spreading out the pollen grains and arranging them into a single layer. Meiotic chromosome counts of the pollen were conducted at 400 and 1000x on a Nikon compound microscope fitted with an Olympus BX41 digital camera. Images were processed with Magnafire 2.0 and in some cases Adobe Photoshop was used to alter the contrast and brightness of the image in order to enhance the visibility of the chromosomes. Because chromosomes do not always lie in the same focal plane, the image for sample 17-132 consists of a combination of two images of the same pollen grain taken at different focal lengths and merged into one photo in order to capture all the chromosomes.

Ploidy level and DNA 2C values were estimated using flow cytometry. *Zea mays* L. ssp. *mays* cv. B73 was used as an internal standard. For this cultivar, Rayburn et al. (1993) determined there to be 9.7 picograms of DNA per 4C nucleus which is

4645.5mbp/2C nucleus and 2322.75mbp/1C assuming that 1 pg DNA = 0.978 X 10^9 base pairs (Doležel et al., 2003). In order to suspend the nuclei and to remove secondary metabolites, approximately 25 mm^2 of fresh, young leaves from each sample were placed in a Petri dish with 1 mL 2-methyl-2,4-pentanediol-based extraction medium (MEB buffer) following the protocol of Peterson et al. (1997) with the following exception: the final pH of the medium was adjusted to 5.0 instead of 6.0. The plant tissue was chopped with razor blades and each suspension of nuclei was filtered through a clean 30µM Celltrics® nylon mesh screen (Partec GmbH, Münster, Germany) and centrifuged at 9000rpm for 2 min. The supernant was discarded and the pellet was resuspended in 200 µL Galbraith's buffer (Galbraith et al., 1983). 50 µL of propidium iodide solution (1mg/mL) and 12µL RNase (4mg/mL) was added to each sample and mixed by pipetting. Samples were kept on ice until measurement. For the preparation of the Maize B73 standard, the same protocol was followed except the tissue was initially chopped in Galbraith's buffer rather than MEB buffer. Flow cytometry was conducted at the University of Dentistry and Medicine of New Jersey – Rutgers University joint institute of Environmental and Occupational Health Sciences Institute using a Coulter Cytomics FC500 (Beckman Coulter, Miami, FL). Voltage for the analysis was set to 410 and each sample was automatically stopped after 1500 events. For the analysis, 200 µL of prepared sample was mixed with 30 µL of maize standard. The total nuclear DNA content was calculated by multiplying the known DNA content in Maize B73 (2322.75 megabases) by the Q x-mean of *Artemisia dracunculus* samples and dividing by the Maize B73 Q x-mean. The amount of DNA in picograms (pg) was calculated assuming that 1 pg DNA = 0.978 X 10^9 base pairs (Doležel et al., 2003). Ploidy level estimates were made by

comparing the estimated genome size of wild tarragon samples with known ploidy levels, as determined by chromosome counts, to the estimated genome size of samples with unknown ploidy levels.

Results

The literature review revealed that that the majority of recorded chromosome counts (~50) were conducted using cultivated or wild-growing material from populations in Europe and Asia (Table 2.1). Relatively few reports were found from North America with five records reporting diploid chromosome levels and a single record reporting a hexaploid sample from Arizona. The worldwide distribution of the cytotypes is presented in Figure 2.3 and the distribution in North America, Europe and Asia are presented in figures 2.4, 2.5 and 2.6 respectively. The diploid cytotype is widely distributed and can be found in Across Asia and Europe. Wild tetraploids also have a large distribution and can be found in both Europe and Asia, while hexaploids have a more restricted distribution and seem to be restricted to Eastern Europe and the central portion of Asia. Wild occurring octoploids are only known from Central Asia and decaploids are only known from cultivation with the exception of one sample from Poland.

Ploidy level was determined for 27 individual plants representing 16 different populations in the western United States. The ploidy levels of 10 individuals were identified using only chromosome counts of meiotic pollen grains, 13 individuals were analyzed using only flow cytometry, and 4 individuals were analyzed with both chromosome counts and flow cytometry (Table 2.2). Figure 2.1: A-N shows the meiotic chromosomes from the studied North American samples. All these samples were found to

be diploid (n=9; Fig. 2.1: A-N; Table 2.2). The plant cultivated from purchased seed, originating from Amsterdam, was found to be decaploid (n=45; Fig. 2.1: P). Figure 2.2 shows representative histograms of a sample with a known ploidy level of n=9, a sample with an unknown ploidy level, maize B73, and the histograms for the French tarragon and Kyrgyz samples. Table 2.2 also presents the flow cytometry results for each analyzed sample including the Q x-mean, estimated 2C value, chromosome number and estimated ploidy level. All North American plants were found to be diploid with 2C values ranging from 3.49 to 5.07 pg. Ploidy levels for the French tarragon (2n=36, 4x) and the Kyrgyz sample (2n=72, 8x), were estimated by dividing the 2C values by the average of of diploid, *A. dracunculus* 2C values. The *Artemisia dracunculus* cytotypes found in the literature, as well as the new records, are shown on the distribution maps in Figures 2.3, 2.4, 2.5 and 2.6).

Discussion

Artemisia dracunculus L. is well known as a widespread, polymorphic species with a remarkable series of polyploids (Tables 2.1 & 2.2; Figures 2.1 & 2.3). The samples included in this study represent populations from four different states in the western United States and include the first records of *A. dracunculus* ploidy levels from Colorado, Utah and Nevada. Although only six records of ploidy levels for *A. dracunculus* were found in the literature, the analysis of an additional 27 individuals from four different states only revealed diploid individuals.

Estimates for the genome size of *A. dracunculus* have previously been reported in the literature. Torrell and Vallès (2001) reported a 2C genome size of 23.22 pg for the

decaploid cytotype and Pellicer et al. (2007) reported 2C genome sizes of 5.94 pg for a diploid *A*. *dracunculus* plant originating from Russia, 11.82 pg for a tetraploid from Russia, 15.71 pg and 15.58 pg for hexaploids from Kazakhstan and Arizona, and 24.58 pg for the decaploid cytotype. The genome size estimates made for *A. dracunculus* plants used in this study were considerably lower than those presented in the previous studies. This discrepancy could be due to differences in the flow cytometry systems and/or standards used rather than actual differences in genome size (Doležel et al., 1998). Because of the differences in the estimated ploidy level, it is important to compare the relative genome sizes of only the samples used in this study, as the measurements are not directly comparable to those of other reports. For example, although the 2C value of the Kyrgyz sample used in this study was 15.91pg, which is approximately the same size as the hexaploids reported by Pellicer et al., it is four times the average North American diploid 2C value. Similarly, the 2C genome size of the French tarragon was also lower than those previously reported for tetraploids, but again is approximately two times the 2C value found in this study.

Wild tarragon is generally considered to be native to North America. The first recorded wild collection of the species on the continent was made in 1804 by Meriwether Lewis, during the Lewis and Clark Expedition. The specimen was collected near the mouth of the White River in South Dakota on September 15, 1804 and the record was first published in Frederick Pursh's *Flora Americae Septentrionalis* (Pursh, 1813; Moulton, 1987; Reveal et al., 1999). This region was far outside the area with permanent European settlements. In that same work, Pursh described a new species, *Artemisia dracunculoides* Pursh (now considered a synonym of *A. dracunculus*), based on a

specimen collected by John Bradbury in 1811. Bradbury's specimen has a collection locality of Upper Louisiana (Pursh, 1813; Nuttall, 1818), and in his account of his travels, Bradbury states that *A. dracunculus* is "common on the Missouri [River]" (Bradbury, 1817). By 1841, Torrey and Gray had described three varieties of *Artemisia dracunculoides* in their Flora of North America. Their first two varieties had a much expanded range, "Missouri! Common from near St. Louis to the Rocky Mountains! and north to Saskatchawan!" (Torrey and Gray, 1841). From these early descriptions of the distribution of *Artemisia dracunculus* it seems evident that the species was in North America prior to the arrival of Europeans.

Erichsen-Brown (1989), proposed that the species was brought to North America from Asia by Native Americans crossing the Bering land bridge. She presented only circumstantial evidence and admitted that real confirmation of this theory would only come with the material evidence of *A. dracunculus* pollen being found at a dated archaeological site. This would only be truly substantiated by a lack of wild tarragon pollen in strata laid down prior to the migration of people across Beringia. In a species with rampant polyploidism, lack of cytotypic diversity (due to there being only a few human mediated introductions to North America) could lend support to this theory. Although there is a lack of cytotypic variation in the North American populations, morphological and chemical (essential oil & flavonoid) variation does show that there are unique characteristics between different populations in North America and between North American and Asian populations (see Chapter 3 and 4). Based on paleopalynologic and molecular phylogenetic data, Riggins (2008) suggests that multiple *Artemisia* species have moved from Asia to North America or from North America to Asia by crossing the

Beringian land bridge, which served as a migration corridor. So it seems quite plausible that *A. dracunculus* could have migrated, unaided by human influence, during these periods of reduced ice coverage.

Diploid individuals of *Artemisia dracunculus* have been found across nearly the entire distribution of the species (Figure 2.3). As the diploid condition can be considered the ancestral state, and the distribution of this cytotype is so widespread, the species has not necessarily had polyploidy act as a method for overall range expansion, as has been documented in other species (Soltis et al., 2004; Hijmans et al., 2007). It is still possible that the polyploidy cytotypes have colonized more inhospitable habitats regionally, but this has yet to be determined. After the diploid cytotypes, the tetraploid cytotype has the largest geographical distribution. This cytotype covers a vast area spreading from Eastern Europe to the Russian Far East and has been found in remote areas of Kashmir, Inner Mongolia and Kazakhstan. The area in which this cytotype has been found is covered by formidable ecological and geologic barriers, and through which the natural movement of plant material would be more unlikely. Separated by great distances and geographic barriers, individuals of the tetraploid cytotype have either arisen independently, multiple times or the tetraploid condition is quite old and has had adequate time to spread across Asia.

Based on literature records, it seems that the triploid cytotype is much rarer, and has only been documented twice in the northern part of India. The hexaploid cytotype has been documented numerous times, but has a more limited distribution than the tetraploid cytotypes. The hexaploid has only been found in areas just west of the Ural Mountains, and further east, in a north-south corridor comprised of Central Asia and Central Siberia.

No hexaploids have been found in eastern Asia, but curiously, a single record of a hexaploid has been reported from material collected in Arizona. This anomaly either represents a new polyploidization event or is a mistake. A hexaploid would likely be formed from diploid and tetraploid progenitors but currently there are no other records of polyploidy in North America. The record would seem more probable if there was any other documentation of polyploidy on this continent. The octoploid cytotype is another rare strain which has only been documented in the wild from a few collections made in Central Asia (Kyrgyzstan, Uzbekistan, and Kazakhstan).

Seeds of the unique decaploid strain have been in circulation since at least the 1960's. Most of the cytological records for plants with this particular ploidy level originate from material held in botanical gardens of Europe and seeds purchased from companies listing the source of the seed as being in the Netherlands. French tarragon has been in cultivation for many centuries, is widely cultivated for culinary use, and is commonly sold. As a result of widespread use in cooking and traditional medicine, the distribution of these particular cytotypes, and possibly the other cytotypes, has been altered, making the true historic distribution and origin of cytotypic races difficult to determine. More importantly, there is differing bioactivity between, and within, the different cytotypes. Selection of plants for bioassays and medicinal applications is highly dependent on the source material and this complex distribution of cytotypes must be considered when collecting germplasm for such uses. Knowing the general distribution of the cytotypes will allow for more targeted collecting and provide a foundation for future studies of this interesting example of complex cytotypic distribution.

Table 2.1. Ploidy levels of *A. dracunculus* in the literature; * = *Artemisia glauca* Pall. ex Willd. (synonym used in original article); † = plants in cultivation and original source unknown; North American records are in bold.

AUTHORS	LOCATION	PLOIDY LEVEL
Weinedel-Liebau, 1928	Germany, Berlin-Dahlem Botanical Garden	n=9†
Kawatani & Ohno, 1964	**U.S.A, Arizona, Tucson**	**2n=18**
	Belgium, Leuven (Louvain)	2n=54,72†
	Poland, Krakow	2n=36
	Russia, Tomsk	2n=18†
	Uzbekistan, Tashkent	2n=54
Khoshoo & Sobti, 1958	India	2n=36*
Koul, 1964	India	2n=36
Love & Solbrig, 1964	Canada, Manitoba	2n=18
Rousi, 1969	France, Paris	2n=36†
	Sweden, Alnarp	2n=90†
	Finland, Helsinki	2n=90†
	Germany, Marburg Botanical Garden	2n=90†
	Denmark, Kastrup	2n=90†
	Sweden, Hammenhög	2n=90†
	Turku, Finland	2n=90†
Podlech and Bader, 1974	Afghanistan, Bamyan	2n=18
	Afghanistan, Wardak (Maidan)	2n=18
	Afghanistan, Kapisa	2n=18
Powell et al., 1974	**U.S.A., California, Riverside Co.**	**2n=18**
Zhukova et al., 1977a	Russia, Yakutia (Sakha Republic), Chersky	2n=18
	Russia, Yakutia (Sakha Republic), Chersky	2n=18
	Russia, Yakutia (Sakha Republic), Chersky	2n=18
	Russia, Yakutia (Sakha Republic), Omolon River	2n=18
	Russia, Yakutia (Sakha Republic), Shandrin River	2n=18
	Russia, Yakutia (Sakha Republic), Semiruch Point	2n=18
Zhukova and Petrovsky, 1977	Russia, Chukotka	2n=36
Greger, 1979	Uzbekistan, near Tashkent	2n=72
Kiel, 1979	**U.S.A., Arizona, Santa Cruz Co**	**2n=18**
Rostovtseva, 1979	Russia, Novosibirsk Oblast, Novosibirsk, around Akademgorodok	2n=54
Morton, 1981	**Canada, Alberta Province,Vermilion**	**2n=18**
	Canada, Alberta Province, Fort Saskatchewan	**2n=18**

AUTHORS	LOCATION	PLOIDY LEVEL
Krasnikova et al., 1983	Russia, Tuva Republic, Mongun-Taiginskii Region	2n=18*
Sundberg, 1983	**U.S.A., Arizona, Cochise Co.**	**2n=18**
Kaul & Bakshi, 1984	India, Kashmir, Lower Munda	2n=18*,27*,36*
Krogulevich, 1984	Russia, Irkutsk Oblast, Erbogachen	2n=18
	Russia, Irkutsk Oblast, Nakanno	2n=18
Bakshi, 1985	India, Kashmir, Lower Munda	2n=27*,36*
Krasnikov, 1985	Russia, Altai Republic, Kosh-Agachskii District	2n=18
Mendelak & Schweizer, 1986	Russia, North Yakutia (Sakha Republic)	2n=18
Malakhova, 1990	Russia, Tomsk Province, around Tomsk, Potopovs meadows	2n=54
	Russia, Tomsk Province, around Tomsk, Anikino	2n=54
	Tomsk Oblast, Zyryansky raion, around Bogoslovka	2n=54
	Tomsk Oblast, Zyryansky raion, around Холдеева	2n=54
	Russia, Tomsk Province, Kozhevnikovsky region, around Urtam	2n=18*
	Russia, Tomsk Province, Kozhevnikovsky region, around Desyatova	2n=36*
Krasnikov & Lomonosova, 1990	Russia, Novosibisrk Province, Iskitimsky District	2n=18
Lavrenko & Serditov, 1991	Russia, Komi Republic, Syktyvkar, Ezhva	2n=18*
	Russia, Komi Republic, Syktyvkar, Chov	2n=54
Murín, 1997	Seeds purchased (Royal Sluis)	2n=90†
Wang, L.-s., 2000	China, Inner Mongolia, Hailar District	2n=36
Vallès et al., 2001	Kazakhstan, Almatynskaya oblast	2n=36
	Kazakhstan, Zhambulskaya oblast	2n=36
	Kazakhstan, Chimkentskaya oblast	2n=54
Torrell and Vallès, 2001	Seeds purchased in market (Amsterdam)	2n=90†
Kreitschitz & Vallès, 2003	Poland, Lower Silesia, Wroclaw	2n=90
Pellicer et al., 2007	Russia, Chita Oblast, Kyra Raion, Near Village of Kyra	2n=18
	Russia, Volgograd Oblast, left shore of Khoper River	2n=36
	Russia, Tuva Republic, Near the City of Kyzyl, summit of hills	2n=36
	U.S.A., Arizona, Globe County, Pinal Mountains	**2n=54**
Sánchez-Jiménez et al., 2009	China, Inner Mongolia, Ulanqab province, Yin Shan, Da Qing Shan	2n=18

Table 2.2. New records of ploidy level for U.S. populations of *A. dracunculus*; chromosome count (CC); flow cytometry (FC).

SAMPLE	STATE	COUNTY	LOCATION	METHOD	Q x-MEAN	2C VALUE (picogram)	2C VALUE (megabase pairs)	2n, PLOIDY LEVEL
2-11	Colorado	Clear Creek	Georgetown	FC	168	4.58	4476.82	18, 2x
6-38	Utah	Wayne	Bicknell	CC	–	–	–	18, 2x
8-49	Nevada	Mineral	Walker Lake	CC	–	–	–	18, 2x
8-50	Nevada	Mineral	Walker Lake	CC	180	4.90	4796.60	18, 2x
8-53	Nevada	Mineral	Walker Lake	CC,FC	141	3.84	3757.33	18, 2x
9-56	California	Plumas	Feather River	FC	150	4.09	3997.16	18, 2x
12-75	Colorado	Sedgwick	Julesburg	FC	186	5.07	4956.48	18, 2x
14-100	California	San Bernardino	Yucaipa	FC	139	3.79	3704.04	18, 2x
14-103	California	San Bernardino	Yucaipa	FC	163	4.44	4343.58	18, 2x
15-113	California	San Diego	old hwy 395	CC	–	–	–	18, 2x
15-114	California	San Diego	old hwy 395	FC	144	3.92	3837.28	18, 2x
16-120	California	San Diego	Julian	FC	131	3.57	3490.86	18, 2x
16-124	California	San Diego	Julian	FC	184	5.01	4903.19	18, 2x
17-131	California	Inyo	Onion Valley	CC	–	–	–	18, 2x
17-132	California	Inyo	Onion Valley	CC,FC	128	3.49	3410.91	18, 2x
18-140	California	Plumas	Crescent Mills	FC	132	3.60	3517.50	18, 2x
18-141	California	Plumas	Crescent Mills	CC	–	–	–	18, 2x
18-145	California	Plumas	Crescent Mills	CC	–	–	–	18, 2x
19-154	California	Lassen	Susanville	FC	172	4.69	4583.41	18, 2x
20-160	California	Inyo	Rt 168	CC	–	–	–	18, 2x
20-163	California	Inyo	Rt 168	CC	–	–	–	18, 2x
22-180	California	Inyo	Nine Mile Cyn 1	CC,FC	159	4.33	4236.99	18, 2x
22-183	California	Inyo	Nine Mile Cyn 1	CC	–	–	–	18, 2x
23-191	California	Tulare	Nine Mile Cyn 2	CC,FC	151	4.11	4023.81	18, 2x
24-203	California	Los Angeles	Elizabeth Lake	CC	–	–	–	18, 2x
24-204	California	Los Angeles	Elizabeth Lake	FC	159	4.33	4236.99	18, 2x
26-220	California	San Bernardino	Running Springs	FC	136	3.71	3624.09	18, 2x
Seed	Amsterdam	–	–	CC	–	–	–	90, 10x
French.Tarr.	Purchased	–	–	FC	318	8.66	8473.99	36, 4x
Kyrgyz	Kyrgyzstan	Inner Tian-Shan	Naryn	FC	584	15.91	15562.29	72, 8x
maize B73	–	–	–	FC	178	4.85	4743.30	–
Average diploid *A. dracunculus*	–	–	–	–	–	4.20	4111.59	–

Figure 2.1. Meiotic chromosomes levels in pollen of *Artemisia dracunculus*; (A) 6-38, n=9; (B) 8-49, n=9; (C) 8-50, n=9; (D) 8-53, n=9; (E) 15-113, n=9; (F) 17-131, n=9; (G) 17-132, n=9; (H) 18-141, n=9; (I) 18-145, n=9; (J) 20-160, n=9; (K) 20-163, n=9; (L) 22-180, n=9; (M) 22-183, n=9; (N) 23-191, n=9; (O) 24-203, n=9; (P) decaploid from purchased seed, n=45.

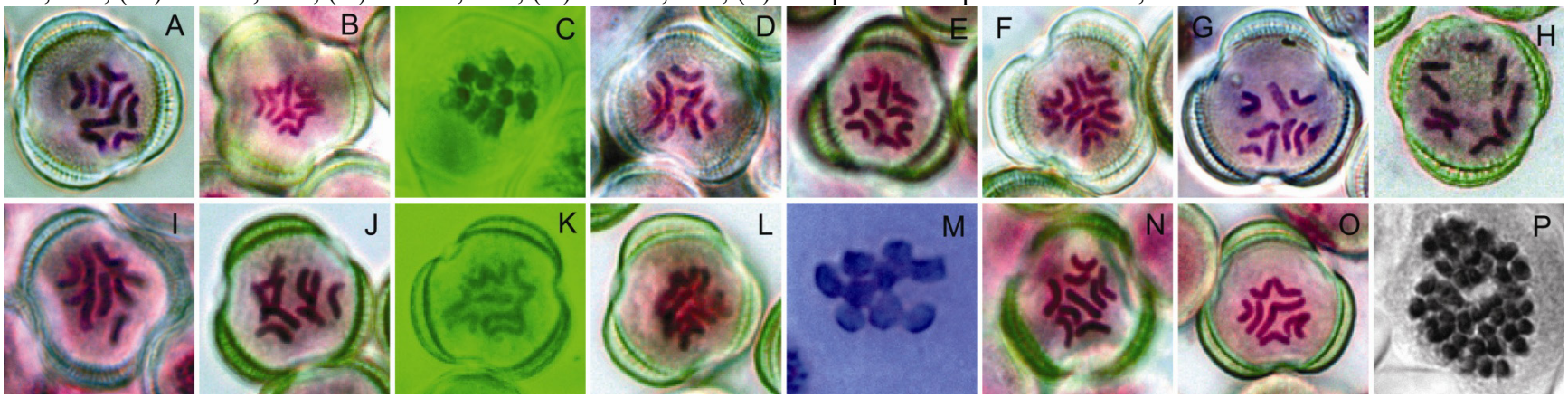


Figure 2.2. Histogram of fluorescence intensity for sample 8-53, ploidy known: n=9 (A); Histogram of fluorescence intensity for sample 14-103, ploidy unknown (B); Maize B73 (C); Maize B73 [peak Q] and French tarragon [peak C] (D); sample 14-103 [peak Q] and French tarragon [peak C] (E); Maize B73 [peak Q] and Kyrgyz plant [peak C] (F).

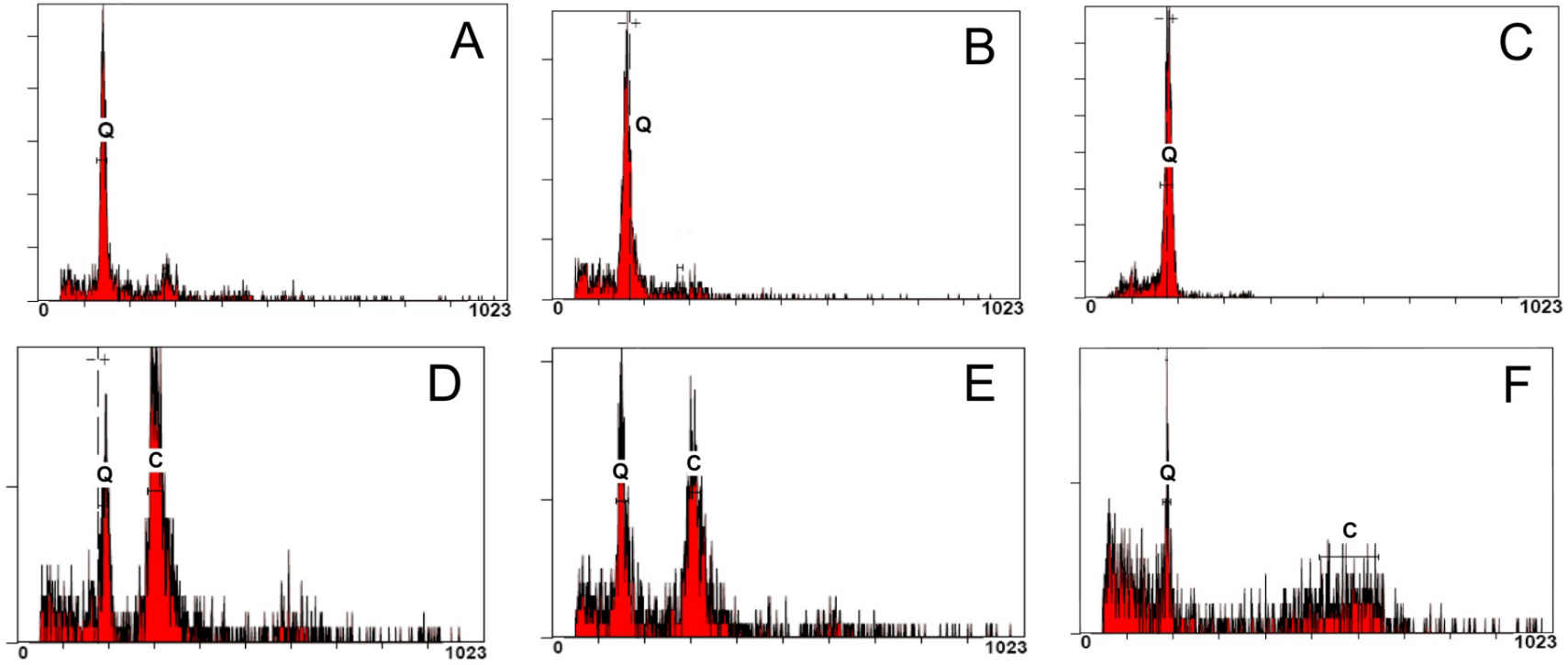


Figure 2.3. Global distribution of *A. dracunculus* cytotypes. Many records were available for Europe and Asia, while only six records from North America were found in the literature; The diploid and tetraploid cytotypes have widespread distributions, while the distributions of the hexaploid and octoploid cytotypes are more restricted. The decaploid cytotype is only known from cultivation, except for one wild collection in Poland, which may have been introduced to the site. cult. = ploidy level derived from a cultivated plant. Red stars indicate new records resulting from this study.

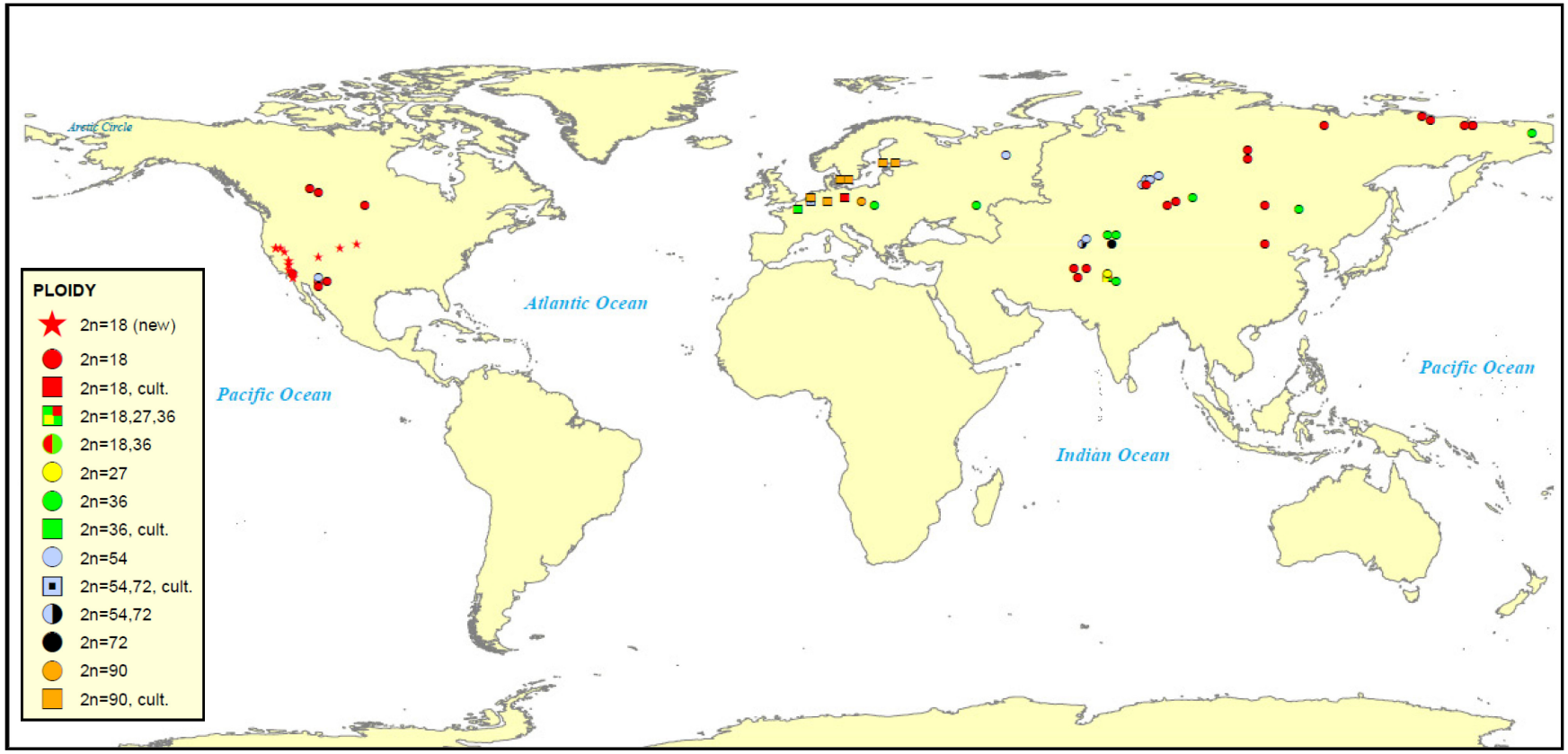


Figure 2.4. Distribution of *A. dracunculus* cytotypes in Asia. Asia has the highest diversity of cytotypes with all known cytotypes except 10x being documented.

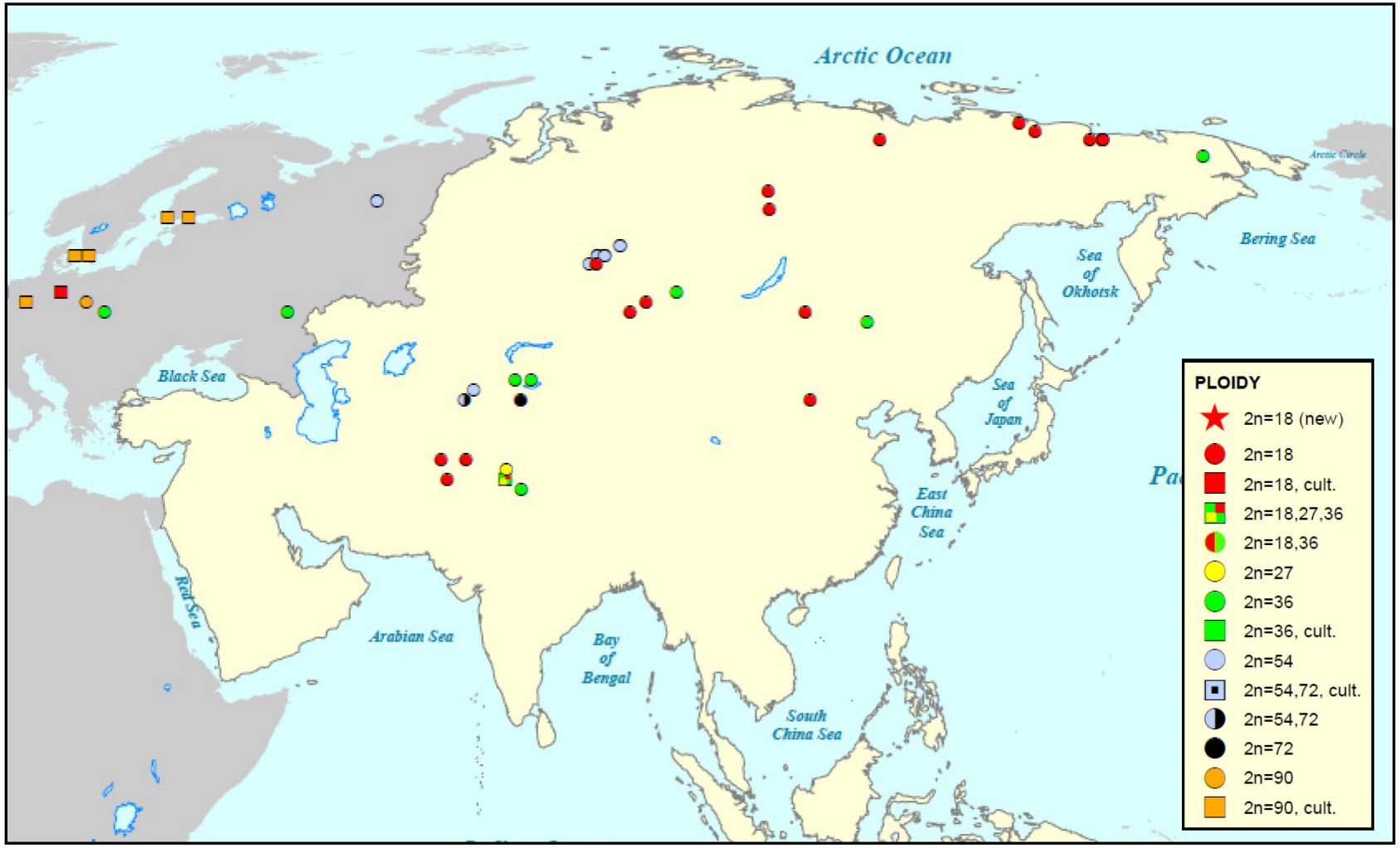


Figure 2.5. Distribution of *A. dracunculus* cytotypes in Europe. Although many different cytotypes records come from Europe the majority are derived from cultivated plant material (cult.).

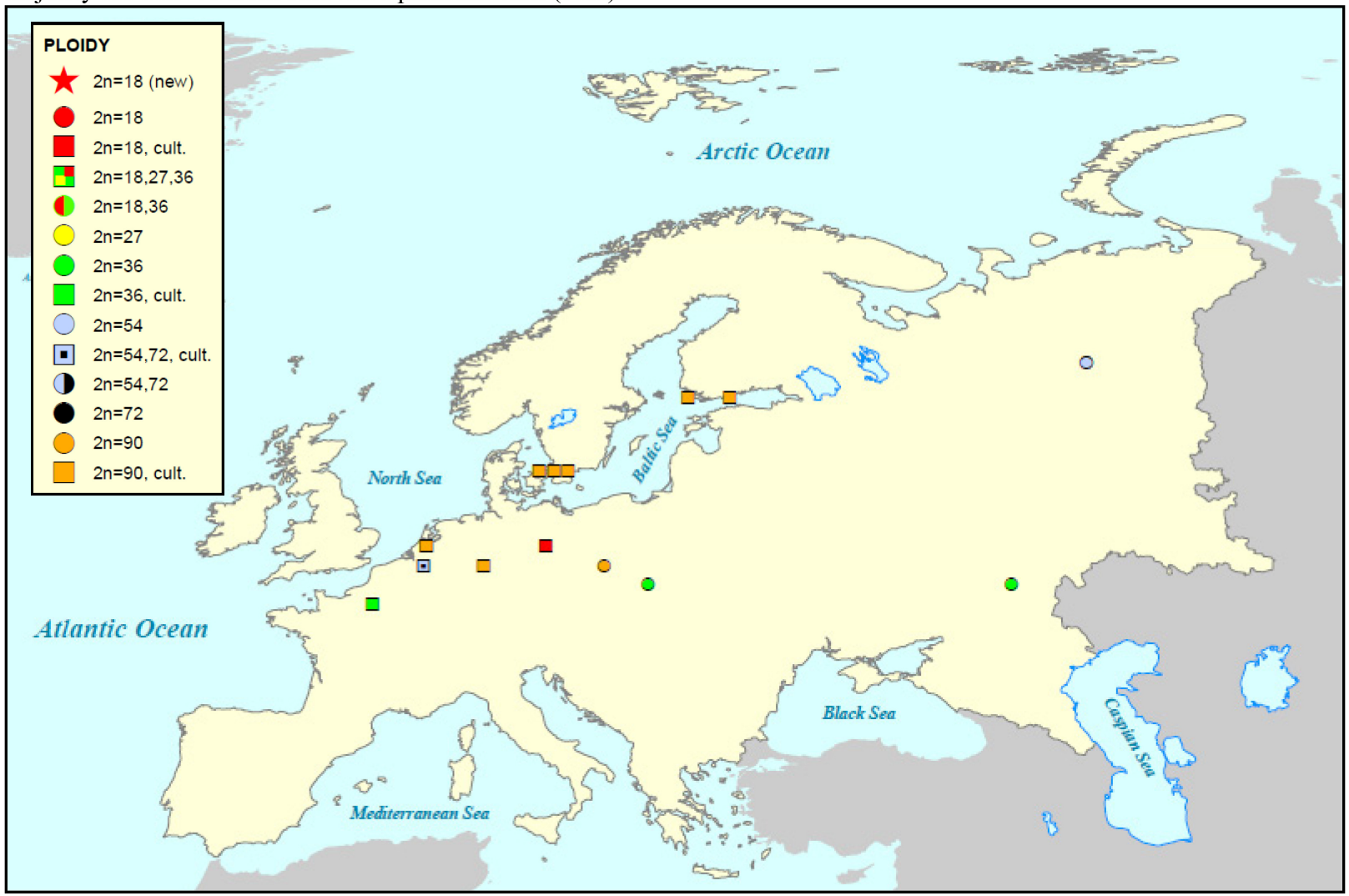
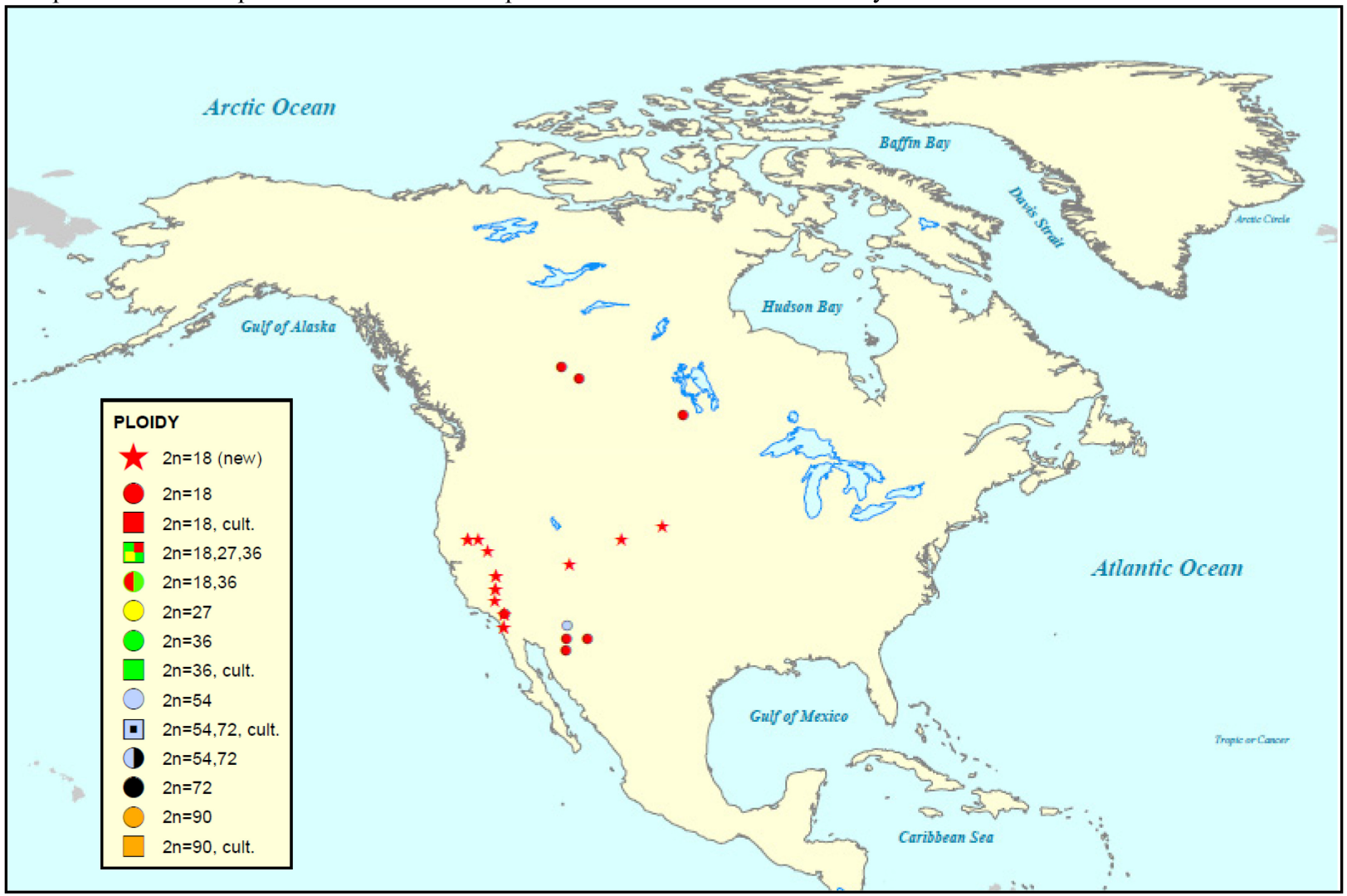


Figure 2.6. Distribution of *A. dracunculus* cytotypes in North America (NA). Only diploids have been found in NA, with the exception of one hexaploid record. Red stars represent new records from this study.



Chapter 3

Screening North American populations of wild tarragon (*Artemisia dracunculus* L.) for anti-diabetic compounds

Abstract

Ethanolic extracts of 104 individuals of *Artemisia dracunculus*, collected from 22 populations throughout the western United States, were screened for the presence of seven anti-diabetic compounds using liquid chromatography-mass spectrometry. The presence of sakuranetin, 6-demethoxycapillarisin were confirmed, mixtures of caffeoylquinic and di-O-caffeoylquinic acids were found as well, while the compounds davidigenin, 2',4-dihydroxy-4'-methoxydihydrochalcone and 2',4'-dihydroxy-4-methoxydihydrochalcone were not detected in North American plants. Root cuttings from those same individuals were collected and grown in a common garden in order to determine if environmental factors influenced the production of the compounds. A quantitative analysis was conducted to measure the amount of sakuranetin in both the field-prepared and cultivated extracts and when compared, some individuals had extreme changes in the amount of sakuranetin found in the wild versus common garden extracts. Sites 9, 14, 16, 17, 18 and 19 had the individuals with the highest sakuranetin content and the highest average content. A Kruskall-Wallis test showed that when averaged by population, differences between the sakuranetin content of field-prepared extracts were statistically significant. The sakuranetin content of 62 cultivated individuals from the wild North American populations ranged from 3.38 μg/g in sample 18-141 to 147.69 μg/g

in 14-100. Decaploid plants, grown from purchased seed, were found to contain all the target compounds and had sakuranetin content that ranged from 337.99 to 556.21 μg/g. In order to assess if the level of individual sakuranetin production in the wild was correlated with the level of sakuranetin production in the common garden, a Spearman's rank correlation analysis was conducted between extracts prepared from individuals growing in the field and their clones grown in the common garden. This analysis showed that the rank of each individual's wild and cultivated sakuranetin production was moderately correlated returned a value for $\rho = 0.414$ with a P-value = 0.0012. Spearman's rank correlation analysis was also conducted on the average sakuranetin production by site. The rank of the average site sakuranetin level in the wild and in the common garden were significantly correlated with $\rho = 0.696$ and strongly supported with a P-value = 0.0025. In both the wild, and the common garden conditions sites 9, 14, 16 and18 were in the highest ranked sites, while site 2 was ranked lowest. Site 17 and 19 were two of the highest ranked sites in the wild, but their sakuranetin production drastically dropped in cultivation. A correlation analysis between the amount of sakuranetin in the field-prepared extracts and the altitude of the collection site did not show any significant correlation.

Introduction

Artemisia dracunculus L. (tarragon) has a long history of human use as a culinary herb and medicine and throughout its native range (western North America, Asia and Eastern Europe) human populations have utilized the plant for the treatment of a wide variety of diseases (see Chapter 1 for distribution and ethnobotanical review). Like many

other species in the genus *Artemisia*, *A. dracunculus* produces a wide array of phytochemicals including monoterpenoids, sesquiterpenoids, flavonoids, coumarins, isocoumarins, polyacetylenes, and alkaloids (Bhutia and Valant-Vetschera, 2008; Engelmeier et al., 2004; Greger, 1977; Greger and Bohlmann, 1979; Greger, 1979; Lutz-Kutschera et al., 2003; Saadali et al., 2001; see Chapter 1 for a review of phytochemicals found in *A. dracunculus*). Both qualitative and quantitative variation of phytochemicals within different varieties and cytotypes of *Artemisia dracunculus* has been documented. This variation may be particularly important because differences in the chemical content of wild tarragon collections could have an impact on the bioactivity of medicinal preparations.

The first portion of this study was conducted to assess for the presence or absence of specific anti-diabetic compounds in *A. dracunculus* extracts prepared from populations found throughout the western United States. The compounds davidigenin (1), 2',4-dihydroxy-4'-methoxydihydrochalcone (2) 2',4'-dihydroxy-4-methoxydihydrochalcone (3), sakuranetin (4), 6-demethoxycapillarisin (5), 5-O-caffeoylquinic acid (6), and 4,5-di-O-caffeoylquinic acid (7) have been shown to be active in a number of different pathways associated with the diabetic condition and have been selected as the focus of this study (Figure 3.1; Kennedy and Ramachandran, 2000; Wang et al., 2004, 2006, 2008; Logendra et al., 2006; Ribnicky et al., 2006; Govorko et al., 2007).

Because the presence/absence screening results showed that sakuranetin was the compound most regularly found in measurable quantities in North American plants, and because it was not in a complex mixture (like the caffeoylquinic and di-O-caffeoylquinic acids), a quantitative analysis was conducted to determine the amount of sakuranetin in

each extract. A Kruskall-Wallis test (analysis of variance by ranks) was conducted to assess if differences in sakuranetin content between-collection localities of field-prepared samples were statistically significant. To determine which groups differ significantly, a nonparametric Tukey-like multiple comparisons test was conducted as well. Using the results of the quantitative chemical analysis of wild-prepared samples, correlation analyses of genetic variation and the production of sakuranetin are presented in Chapter 5.

Because the chemical content of plants can exhibit intra-specific variation between genotypes, and can be influenced by environmental conditions, clones were made from each wild plant, and were cultivated in a common garden at Rutgers University. To assess if the quantity of sakuranetin produced by individuals changed when clones of the wild plants were grown in a common garden, sakuranetin content of the wild-growing plants was compared to that of the cultivated plants using Spearman's rank correlation analysis. This analysis was used to answer the question: did the individuals that produced low levels of sakuranetin in the wild continue to produce low levels in the common garden experiment, and did the individuals that produced high levels of sakuranetin continue to do so as well. This test functioned as an indirect measure of the genetic control of the amount of sakuranetin produced by individuals.

Additionally, to find if there were particular populations that had a genetic predisposition for increased sakuranetin production, Spearman's rank correlation analysis was also conducted using the average sakuranetin level of each site. For this analysis the quantity of sakuranetin for all individuals from each site were averaged and the averages of all the sites were ranked sequentially from lowest to highest and the same was done for the average sakuranetin level for plants from each site grown in the common garden. This

was used to determine if the populations that produced high levels of sakuranetin in the wild retained high sakuranetin production in the common garden.

Finally, to assess the effects of environmental conditions on sakuranetin production, multiple clones were made from three individuals originating from three different collection sites. These clones were grown in three different environmental situations 1) shade/no fertilizer 2) sun/no fertilizer and 3) sun/with fertilizer.

Inter- and intra-specific variation of phytochemicals. Variation and quality control is currently one of the main concerns within the area of medicinal plant use (Raskin et al., 2002). Both, variation in genetic controls of gene expression by differing genotypes, as well as environmental influence on the expression of those genes, can affect the production of various secondary metabolites (Lila, 2006). For example, flavonoid synthesis in plants can be influenced by a variety of biotic and abiotic factors including UV light radiation, drought, ozone, nutrient availability, diseases, herbivory and developmental stage (McDougal and Parks, 1984; Tomas-Barberan et al., 1988; Coronado et al., 1995; Liu et al., 1995; Chaves et al., 2001; Cuadra et al., 1997; Cooper-Driver and Bhattacharya, 1998; Lalova, 1998; Markham et al., 1998; Simmonds 1998; Saleem et al., 2001; Karlova, 2006). Kodama et al. (1992) found that sakuranetin levels increased in rice leaves after they had been exposed to ultraviolet light, and after infection with the fungus *Pyricularia oryzae* (=*Magnaporthe grisea*). Zidorn and Stuppner (2001) noted that ecological factors influenced absolute flavonoid content in *Leontodon* (Asteraceae) inflorescences, whereas relative amounts of particular flavonoids, as percentages of the total flavonoid content, seem to be genetically controlled.

Correlation of flavonoid production with altitude has been documented in a number of different studies (Nikolova and Ivancheva, 2005; Zidorn et al., 2005). This is attributed to be a response to increased UV light radiation at higher altitudes, but this correlation may be compound specific, as some flavonoids do not fit this trend. Nikolova and Ivancheva (2005) found that apigenin content in *Veronica chamaedrys* L. was highest in alpine populations, but that in *Artemisia vulgaris* L., the production of quercetin 3,7,3'-trimethyl ether was not related to the altitude of the plants.

Temporal variation of phenolics (terpenoids, coumarins and flavonoids) has also been documented in the genus *Artemisia*. Wilt and Miller (1992) found that over half of the individual phenolics present in persistent leaves of *Artemisia tridentata* Nutall ssp. *wyomingensis* Beetle & Young varied significantly over two years and the concentration of the 11 most highly variable phenolics exhibited the most change between seasons and not within. Their results also revealed that within-individual variability was lower than variation between individuals.

Pavelkovskaya et al. (1967) found that *A. dracunculus* plants growing in conditions with higher moisture levels and at higher altitudes exhibited increased total flavonoid content. They also found that maximum flavonoid production occurred during flower-bud formation and in the beginning of blooming period. In a similar study, Manadilova and Alyukina (1981) reported slightly different results. They also reported two periods of maximum flavonoid accumulation, but those stages were flower-bud formation and at the end of flowering. They also found that while different growth conditions affected the formation of phenolic compounds, variation in flavonoid synthesis was not as significantly affected. Increased air humidity did not affect flavonoid

content, but increased temperature (40°C) did decrease total flavonoid formation. Seasonal variation of essential oil production by *Artemisia dracunculus* was reported by Deans and Svaboda (1988), who found that while limonene and α-pinene concentrations remained constant throughout the growing season, methyl chavicol levels were highest mid-season and trans- and cis-ocimene [E-β-ocimene and Z-β-ocimene] were highest in early- and late-season with the lowest levels found mid-season.

In addition to variation in chemical production as a result of environmental and temporal conditions, many studies have looked at the variation of chemical groups within taxonomic groups as well. For example, polyacetylenes and related compounds have been shown to segregate various infra-generic groups within *Artemisia* (Greger, 1982; Riggins and Clausen, 2003). In a chemotaxonomic study of flavonoids found in *Betula* species, Lahtinen et al. (2006) found that diploid species did not contain any of the flavanones that were present in the leaves of other polyploid species. Because a number of the biologically active compounds in wild tarragon are flavonoids, and due to the high amount of polyploidy in wild tarragon, a similar finding could make selection of the proper plant material critical.

Variation in chemical production by conspecific individuals with different ploidy levels has also been documented in wild tarragon. Both French and wild tarragon have been analyzed to determine if there are differences in their phytochemical compositions. Analyses of these tarragon varieties have shown marked qualitative variation and that flavonoids can exhibit distinctly segregated distribution within varieties of *A. dracunculus*. Vienne et al. (1989) investigated the presence of various flavonols in wild tarragon and French tarragon. They found that both types of tarragon contained quercetin

glycosides, but only the Russian tarragon contained patuletin glycosides. Greger (1979) conducted an analysis of polyacetylenes, using roots extracts prepared from *A. dracunculus* (four plants were sampled with varying ploidy levels: 6x, 8x, a putative 8x, and 10x), *A. glauca* (2x, often considered a synonym of *A. dracunculus*), *A. pamirica* (often considered a variety of *A. dracunculus*), and *A. dracunculiformis*. The results showed that these taxa, and even varying cytotypes of *A. dracunculus*, exhibited distinct variation. Based on the presence or absence of specific compounds, the taxa were nearly separated into two groups. One group, based on the presence of capillen, was composed of *Artemisia glauca*, *A. dracunculiformis* and *A. pamirica*, and the decaploid *A. dracunculus* cytotype. The second group was composed of the other *A. dracunculus* cytotypes (6x, 8x and putative 8x) which all lacked capillen, but did contain artemidins not found in the first group. The decaploid *A. dracunculus* was the only sample that contained butynyl-isocoumarins and, based on overall polyacetylene chemistry, showed a closer relationship to *A. glauca, A. pamirica* and *A. dracunculiformis* that to the 6x and 8x *A. dracunculus* cytotypes (Greger, 1979). These studies have clearly shown that there can be distinct variation in the production of phytochemicals within different varieties of *A. dracunculus*, and between different cytotypes as well.

Artemisia dracunculus and diabetes. Type 2 diabetes is a complex metabolic disorder causing elevated blood glucose levels. It is generally the result of a progressive decrease of insulin activity (insulin resistance), and decreased pancreatic insulin secretion, due to failure of insulin producing β-cells (Inzucchi, 2002; Ashiya and Smith, 2007). According to the World Health Organization more than 220 million people worldwide suffer from

diabetes and an estimated 1.1 million people died from it in 2005 (World Health Organization, 2009b). According to the National Center for Chronic Disease Prevention and Health Promotion, 23.6 million people or 7.8% of the U.S. population have diabetes, and in adults, type 2 diabetes accounts for about 90 to 95 percent of all diagnosed cases of diabetes (National Institutes of Health, 2008).

Swanson-Flatt (1991) listed tarragon as a traditionally used plant medicine for the treatment of diabetes in the United Kingdom and early bioactivity research showed that tarragon significantly reduced hyperphagia (increased appetitite) and polydipsia (excessive thirst) associated with streptozotocin-induced diabetes in mice (Swanson-Flatt et al., 1989). Tarragon also slowed body weight loss but did not significantly alter plasma glucose or insulin concentrations. More recent research has shown that an extract of commercially available wild tarragon reduced hyperglycemia in both streptozotocin-induced and genetically diabetic KK-Aγ mice and that the extract significantly reduced the expression of phosphoenolpyruvate carboxykinase (PEPCK) mRNA (Ribnicky et al., 2006). This enzyme catalyzes the first step in liver glucose production and is normally over-expressed in insulin-resistant diabetic rats Using *in vitro* skeletal muscle cell cultures (from both human and obese Zucker rats), an alcoholic extract of *A. dracunculus* was found to improve glucose disposal in these insulin sensitive tissues (Wang et al., 2004). A bioassay-guided fractionation experiment using quantitative polymerase chain reaction (qPCR) to measure decreased PEPCK expression led to the isolation of two compounds, 6-demethoxycapillarisin and 2',4'-dihydroxy-4-methoxydihydrochalcone (Govorko et al., 2007). The experiment showed that 6-demethoxycapillarisin exhibited stronger inhibitory activity on PEPCK expression and when combined with insulin it

decreased PEPCK expression twice as much as insulin alone. Like insulin, 6-demethoxycapillarisin was shown to function by activating the PI3K pathway, in contrast to the effect of 2',4'-dihydroxy-4-methoxydihydrochalcone, which was not associated with this pathway.

Additional bioassay guided fractionation experiments revealed that four compounds (4,5-di-O-caffeoylquinic acid, davidigenin [4,2',4'-trihydroxydihydrochalcone], 6-demethoxycapillarisin, and 2',4'-dihydroxy-4-methoxydihydrochalcone) contained in wild tarragon extract, inhibited aldose reductase (Logendra et al., 2006). In cells, excess glucose is converted to sorbitol by the aldose reductase enzyme and then accumulates in the cells causing of a number of secondary diseases associated with the diabetic condition. In an *in vitro* analysis assessing the bioactivity of fractions derived from an alcoholic extract of *A. dracunculus*, three compounds showed inhibitory effects on protein tyrosine phosphatase-1B activity. Protein tyrosine phosphatase-1B is a key enzyme in the insulin signaling pathway and acts as a negative regulator and studies have shown that mice lacking the protein tyrosine phosphatase-1B (PTP-1B) have enhanced insulin sensitivity. The compounds with inhibitory effects on PTP-1B activities were identified as sakuranetin (4,2',4'-trihydroxydihydrochalcone), 2',4'-dihydroxy-4-methoxydihydrochalcone and its positional isomer 2',4-dihydroxy-4'-methoxydihydrochalcone (Kennedy and Ramachandran, 2000; Wang et al., 2006; Wang et al., 2008).

Materials and methods

***Plant material and extract preparation*.** In total, 116 extracts and 119 root cuttings were collected from twenty-six sites throughout the western United States. Plant samples consisted of *Artemisia dracunculus* L. var. *dracunculus* (104 extracts and 107 root cuttings [samples 18-145, 18-148 and 19-155 had root cuttings collected, but no field-prepared extracts were made from these individuals]), *A. campestris* L. subsp. *caudata* (Michaux) H. M. Hall & Clements (a member of subgen. *Dracunculus*; 5 samples), *A. ludoviciana* Nutt. subsp. *incompta* (Nuttall) D. D. Keck (subgen. *Artemisia*; 2 samples), *A. ludoviciana* Nutt. subsp. *ludoviciana* (3 samples), and *A. ludoviciana* Nutt. subsp. *candicans* (Rydberg) D. D. Keck (1 sample) (Appendix 1, Table A.1). Each sample was named using the site number followed by the unique individual identifier (eg., 26-220 is site 26, individual 220). For comparison, French tarragon plants purchased from Pantry Garden Herbs (Cleveland, MO), a single plant from seed collected in Kyrgyzstan, and three plants grown from purchased seeds (Sheffields Seeds Co., Locke, NY) were also cultivated. In the results, these individuals are abbreviated FRENCH, KYRGYZ and RUGH, respectively. In order to collect all the plants at the same time of the year, collection expeditions were conducted during the last week of August and the first week of September, in 2006 and 2007. A phytochemical extract, a root cutting, and a herbarium voucher were collected from each plant sample. Herbarium vouchers were deposited in the Rutgers University's Chrysler herbarium (CHRB). Five plants were sampled at each locality with a few exceptions (Appendix 1, Table A.1). To prepare the phytochemical extracts, one gram of fresh leaves was collected from within the synflorescence (branched upper portion of flowering plant) of each plant sampled. The leaf sample was

ground with a mortar and pestle in 10 mL of 95% ethanol and poured into a scintillation vial. An additional 10 mL of ethanol was used to rinse the mortar and added to the vial. The ground material was stored in the dark for 24 hours and then filtered, through a folded Kimwipes EX-L (Kimberley-Clark), into a new scintillation vile. Upon returning to Rutgers University, the extracts were kept in the dark at 36° C until the isolation and analysis were conducted.

The root cuttings collected in 2006 were planted in 7" pots using CANADA mix and were cultivated in the Rutgers University research greenhouses in New Brunswick, NJ. Due to inadequate storage in moistened paper towels during the collection trip, a number of the root cuttings decayed and could not be cultivated. In the spring and fall of 2007 all cultivated plants were trimmed back to just above the level of the soil to produce a new flush of stems. Root cuttings collected in 2007 were planted in the same manner as the 2006 material. During the 2007 collection trip, root cuttings were stored in dry potting mix rather than moistened paper towels. As a result, those collected in 2007 had a much higher survival rate. In April of 2008, all the surviving cultivated plants [62 wild-collected *A*. *dracunculus* plants, 3 *A*. *dracunculus* grown from purchased seed, 5 wild-collected *A*. *ludoviciana* plants, 1 *A*. *dracunculus* from Kyrgyz seed, and French tarragon plants] were transplanted into 11" pots, trimmed back to just above the level of the soil and placed in an outside growing area. At that time, 9 g of Scotts Osmocote Classic 14-14-14 slow release fertilizer (Vero Beach, FL) was applied to the surface of the soil of each pot. Each plant was subsequently watered bi-weekly and fertilized monthly with 5.5g Scotts Peters Professional 20-20-20 fertilizer (Vero Beach, FL). To reduce environmental variability, all cultivated plants were watered thoroughly the day before

they were harvested, and all were harvested on the same day in October 2007. The phytochemical extraction process for the cultivated plants was conducted in the same manner as for the wild-collected plant samples.

***Phytochemical analysis*.** Five mL of each phytochemical extract was dried in a centrifugal evaporator and the dry solids were re-dissolved in a mixture of 2 mL Millipore H_20 and 2 mL ethyl acetate (EA) using a vortex. The samples were placed in a sonicator at 20° C for 10 minutes to aid in the dissolving process. The samples were vortexed again for 1 minute and then run in a centrifuge for 5 minutes at 3000 rpm. To ensure that the solids completely dissolved in the ethanol, vortexing and sonication were repeated for samples that did not re-dissolve after the first treatment. The upper EA fraction was collected, placed in a new tube, and 2 mL of new EA was added to the H_20 fraction in the original tube. The EA/ H_20 sample was then vortexed, sonicated, vortexed and centrifuged in the same manner as previously described. The EA fraction was again collected, added to the first EA fraction, and the process was repeated once more to obtain a total EA fraction of 6 mL. The EA fraction was dried in the centrifugal evaporator and re-dissolved in 500μL of 95% ethanol. The samples were then centrifuged for 5 minutes at 3000 rpm and 20°C and ~200 μL was pipetted into a vial for liquid chromatography/mass spectroscopy (LC-MS) analysis.

The 7 investigated compounds were identified using liquid chromatography/mass spectroscopy (LC/MS). Each sample (30 μL) was analyzed using a LC-MS system composed of a Waters (Milford, MA) LC-MS Integrity™ system composed of a solvent delivery system with a W616 pump and W600S controller, W717plus auto-sampler,

W996 photodiode array detector (PDA). UV data were collected & analyzed with the Waters Millennium® v. 3.2 software. After the 996 PDA detector the eluent flow was guided to a Varian 1200L (Varian Inc., Palo Alto, CA) triple quadrupole mass detector with electrospray ionization interface (ESI), operated in negative ionization mode. The electrospray voltage was -4.5 kV, heated capillary temperature was 240° C, sheath gas was air. The mass detector was set to scan from 65 to 1500 atomic mass units. Data from the Varian 1200L mass detector was collected, compiled and analyzed using Varian's MS Workstation, v. 6.41, SP2. Compounds were separated on a Phenomenex® Luna C-8 reverse phase column, size 250 x 4.6 mm, particle size 5 μm, pore size 100 Å, equipped with a Phenomenex® SecurityGuard™ pre-column. The mobile phase consisted of 2 components: Solvent A (0.5% ACS grade acetic acid in double distilled de-ionized water, pH 3-3.5), and Solvent B (100% Acetonitrile). The mobile phase flow was adjusted at 0.5 ml/min, and a gradient mode was used for all analyses. The initial conditions of the gradient were 85% A and 15% B; for 30 minutes the proportion reached 5% A and 95% B which was kept for the next 3 minutes; from minute 38 to minute 41 the gradient went back to initial conditions. A 10 minute equilibration interval was included between subsequent injections.

Identification of the compounds was based on deprotonated molecular ions in negative ionization mode and their retention times (Table 3.1). Due to the diversity of caffeoylquinic acid and chlorogenic acid derivatives in the extract, and because of the crude nature of the extract, distinct chromatogram peaks for 4,5-Di-O-caffeoylquinic acid and 5-O-caffeoylquinic acid could not be obtained. As a result, all plants were screened for a mixture of dicaffeoylquinates with a parent ion of 515 and a retention time of 20

minutes, and a mix of chlorogenates with a parent ion of 353 and a retention time of 14 minutes. The liquid chromatography protocol that was used did not sufficiently separate the isomers, 2',4-dihydroxy-4'-methoxydihydrochalcone and 2',4'-dihydroxy-4-methoxydihydrochalcone. Because of this, plants were screened only for the presence of the molecule and not the specific isomers.

Sakuranetin quantification. The quantity of sakuranetin was calculated for both wild-growing and cultivated samples. In order to prepare a standard curve, sakuranetin was purchased from Extrasynthese (Genay, France). A master mix of 1mg/1mL 95% ethanol was used to prepare samples with concentrations of 120, 240, 480, and 960ng/30µL respectively. The solutions were prepared and run three times: once at the beginning of the sample analyses, once in the middle of the analyses and once after all the samples had been run. The data collected for each amount from all three standard runs were averaged and plotted to yield a composite standard curve for sakuranetin. In order to determine percent recovery, extracts prepared from *Artemisia ludoviciana* were spiked with 960 and 1920 ng of sakuranetin, respectively. This species was used because it was found to lack sakuranetin (samples 4-31, 4-32, 4-33, 4-34, 4-35, 5-36 in Table 3.2).

Statistical analysis. Because sample size within groups was small (n=5, except group 6 and 11 where n=2), and the variances of the populations were heterogeneous, a non-parametric method, the Kruskall-Wallis test (analysis of variance by ranks) was utilized to test for between-population (collection localities) differences of sakuranetin content for the wild-growing samples. In this analysis each sample is ranked from lowest to highest

based on sakuranetin content and the sum of the ranks for each population is calculated. The Kruskall-Wallis test statistic, *H*, was calculated following using the formula described in Zar (1984):

$$H = \frac{12}{N(N+1)} \sum_{i=1}^{k} \frac{R_i^2}{n_i} - 3(N+1),$$

where *k* is the number of groups, n_i is the number of observations in group *i*, $N = \sum_{i=1}^{k} n_i$ (total number of observations in all groups), and R_i is the sum of the ranks of the n_i observations in group *i*. Because there were five samples (observations) that were found to lack sakuranetin, these samples represented ties for the lowest rank. The rank assigned to each of the tied ranks was the mean of the ranks that would have been assigned to these ranks had they not been tied and received sequential ranks. So, instead of ranking them 1, 2, 3, 4, 5 and 6, each received a rank of (1+2+3+4+5+6)/6=3.5. A correction factor, $C = 1 - \frac{\sum T}{N^3 - N}$, was used to account for the presence of tied ranks, where

$$\sum T = \sum_{i=1}^{m} (t_i^3 - t_i)$$

and where t_i is the number of ties in the *i*th group of ties and *m* is the number of tied groups. The corrected value of *H* was calculated as $H_c = \frac{H}{C}$. Because the number of groups (*k*) was larger than 5, critical values for H_c were assessed using the critical values of the χ^2 distribution with *k* -1degrees of freedom and α=0.05 (Zar, 1984). Because the Kruskall-Wallis test only tests the null hypothesis that there are no differences between groups, even when the null hypothesis is rejected it is not known which groups differ

from which other groups. All that is known is that at least one group is different from the others.

To determine which groups differ significantly from other groups, a nonparametric Tukey-like multiple comparisons test was conducted following the methods described by Zar (1984). For this test the rank means for each group were determined and organized from lowest to highest value. Pairwise differences were then calculated, starting with the difference between the largest and smallest rank means, followed by the highest and next to lowest, and continuing with difference between the highest and each increasing rank mean. The standard error was determined using the equation

$$\mathrm{SE} = \sqrt{\left(\frac{N(N=1)}{12} - \frac{\sum T}{12(N-1)}\right)\left(\frac{1}{n_A} + \frac{1}{n_B}\right)},$$

which accounts for tied ranks and unequal population size. The test statistic Q was calculated as $Q = \frac{\bar{R}_B - \bar{R}_A}{\mathrm{SE}}$, with $\bar{R}$ being the rank mean. Critical values for this test were taken from Zar (1984) and an α of 0.05 was used.

The wild-growing samples were compared to the cultivated samples using Spearman's rank correlation coefficient. Only the samples which had extracts from both wild-growing and cultivated plants were included in the analysis. For Spearman's rank correlation the quantity of sakuranetin for each sample from the field prepared extracts was ranked sequentially from lowest to highest (1-59) and the same was done for the extract prepared from the cultivated material of each individual. This analysis assesses how much hierarchical change there was between the individuals rank within the two different groups (wild-growing vs. cultivated). In essence, this analysis was used to

answer the question did the individuals that produced a low level of sakuranetin in the wild continue to produce low levels in the common garden experiment, and did the individuals that produced a high level of sakuranetin continue to do so as well. Spearman's rank correlation analysis was also conducted using the average sakuranetin level of each site. For this analysis the quantity of sakuranetin for all individuals for each site were averaged and the averages of all the sites were ranked sequentially from lowest to highest and the same was done for the average sakuranetin level for plants from each site grown in the common garden. This was used to determine if the populations that produced high levels of sakuranetin in the wild remained the high producer in the common garden. Both Spearman's rank correlation analyses were conducted using the statistical program R version 2.9.2 and the cor.test command in the "stats" package (R Development Core Team, 2009).

***Environmental comparison parameters*.** Nine clones of each individual were made from three different plants (9-56, 16-124 and 18-140). The clones were made by taking cuttings of the roots and potting them in 7" pots with CANADA mix. The root cuttings were allowed to become established in a greenhouse for one month and were then moved outside and subjected to three different growing regimes: full sun with fertilizer [bi-monthly application of 2.75g Scotts Peters Professional 20-20-20 fertilizer (Vero Beach, FL)], full sun without fertilizer, and shade [grown under 50% black shade cloth] without fertilizer. All plants were grown for five months and harvested at the same time to ensure consistency. Extracts were prepared using the protocol already described.

Results

Of all the samples that were analyzed, only the extracts of plants grown from purchased *Artemisia dracunculus* seeds contained all the target compounds and were the only extracts that contained davidigenin and 2,4-dihydroxy-4-methoxydihydrochalcone. Davidigenin, sakuranetin, 6-demethoxycapillarisin, and 2,4-dihydroxy-4-methoxydihydrochalcone were not detected in *Artemisia campestris*, *A. ludoviciana*, French tarragon and the wild tarragon plant from Kyrgyzstan. Table 3.2 presents the presence/absence data of the targeted compounds for all samples and representative mass spectra for the target compounds are shown in Figure 3.2.

All extracts, from both wild-growing and cultivated samples, were found to contain complexes of chlorogenates and dicaffeoylquinates. Due to the simple preparation and partitioning of the extracts, and the specific liquid chromatography parameters, 5-O-caffeoylquinic acid (5-CQA) and 4,5-Di-O-caffeoylquinic acid (4,5-DiCQA) were co-eluted with related compounds and did not produce isolated peak. 3-O-caffeoylquinic acid (3-CQA) and 4-O-caffeoylquinic acid (4-CQA), isomers of 5-O-caffeoylquinic acid, have the same parent ion (353), as well as similar retention times. In a similar fashion, 3,4-Di-O-caffeoylquinic acid (3,4-diCQA) and 3,5-Di-O-caffeoylquinic acid (3,5-diCQA) share the same parent ion (515), and have similar retention times as 4,5-DiCQA acid. A revised liquid chromatography protocol with adjusted solvent flow rates would be necessary to adequately separate and quantify the 5-O-caffeoylquinic acid and 4,5-Di-O-caffeoylquinic acid from related compounds. Preliminary results from a

revised LC protocol revealed that the wild prepared sample 14-104 contained primarily 4,5-diCQA, with lesser amounts of 3,5-diCQA and 3,4-diCQA, as well as an unknown diCQA in very small concentrations (J. Munafo, per. comm.). 1,3-diCQA and 1,5-diCQA were found in significant amounts in some Asteraceae such as artichoke (*Cynara*) and one of these could possibly be the unidentified compound (Schutz et al., 2004). In the chlorogenate mix, 5-CQA was the most abundant, followed by 4-CQA, and very little 3-CQA. Additionally, feruloylquinic acids (FQAs) were found in the extract, with 5-FQA being the most abundant with small amounts of 4-FQA and 3-FQA (J. Munafo, per. comm.).

Ninety-nine of the 104 wild-growing North American *Artemisia dracunculus* plants were found to contain sakuranetin and 71 of those extracts were found to contain 6-demethoxycapillarisin. Based on the very small peak height and area of the selective ion monitoring chromatogram, these samples most likely contain only trace quantities of the latter. Exceptions to this were found in plants from sites 2, 5, 7, 8, 9 and 17 (Table A.1). The individuals 2-12, 2-14, 2-15, 5-36, 7-43, 8-49, 9-55, 9-56, 9-57, 17-130, 17-132 and 17-133 were found to have relatively larger peak areas of 6-demethoxycapillarisin relative to other field prepared extracts, which had trace amounts. The presence of 6-demethoxycapillarisin was found to be variable within individuals, as some of the plants that were found to contain 6-demethoxycapillarisin in the field-prepared extracts did not have detectable levels in the cultivated plants. This is not necessarily surprising, as many of the collections seemed to have trace amounts of 6-demethoxycapillarisin in the field-prepared extracts. Plant 2-12 (from site 2) cultivated in the common garden maintained a substantial peak for 6-demethoxycapillarisin while other individuals either had a very

small relative area or did not contain detectable amounts of the compound. It is important to note that the plants cultivated from purchased seeds had peak areas for 6-demethoxycapillarisin approximately 10-50 times greater than was found in plant 2-12 (Figure 3.3).

All of the North American collections of *Artemisia dracunculus*, that were cultivated in the common garden, were found to contain sakuranetin. Because sakuranetin was the most prevalent compound in all the extracts, and the compound could be purchased for use as a standard, it was quantified for all the wild-growing and cultivated plants of *A. dracunculus*. To assess the amount of sakuranetin recovered from the samples during quantification, average sample recovery was calculated based on the peak areas of the mass spectra from *A. ludoviciana* samples spiked with sakuranetin. The average recovery was ~68%. The amounts of sakuranetin used in the recovery test were very low and represent the low end of the range of sakuranetin content in the *A. dracunculus* samples. It is likely that recovery tests conducted with larger amounts of sakuranetin would have even higher percent recoveries. Table 3.3 presents the uncorrected amount of sakuranetin (μg) found in each gram of plant material for all plants. The sakuranetin content of the field-prepared *Artemisia dracunculus*, *A. campestris and A. ludoviciana* is presented in Figures 3.4 and 3.5. Overall, the level of sakuranetin in the field-prepared extracts of *A. dracunculus* varied greatly. While five samples had no detectable sakuranetin, the other samples ranged from 1.78 μg/g in sample 12-77 to 155.72 μg/g in sample 16-121. Sites 9, 14, 16, 17, 18 and 19 had the individuals with the highest sakuranetin content.

The average sakuranetin content calculated from all individuals in each wild site is shown in Figure 3.6. The sites with highest averages were sites 19, 14, 17, 9, 18 and 16. To determine if the variation between sites was statistically significant, the Kruskall-Wallis analysis was used to test the null hypothesis, that the sakuranetin production was the same for all the wild sites. The rankings used for this analysis are presented in Table 3.4 and the values used to calculate H_c are found in Table 3.5. The value for the Kruskall-Wallis test statistic was calculated to be H_c= 90.31. This value is highly significant when compared to the critical value determined for this analysis, $\chi^2_{0.05,21}$=32.671. Furthermore, $\chi^2_{0.001,21}$=46.797, so for this analysis the P<0.001 for H_c. Based on the results of this analysis the null hypothesis is rejected, and an alternative hypothesis, that sakuranetin production was not the same for all the wild sites, is strongly supported. To determine which groups differ significantly from other groups, a nonparametric Tukey-like multiple comparisons test was conducted. In this pairwise test the null hypothesis for each comparison was that sakuranetin content is the same in both sites. The rank values used are shown in Table 3.6 and the results are presented in Table 3.7. The overall conclusion of this test is that the sites with the rank means ranked from 6-22 have significant difference from sites with rank means ranked 1-5. This finding reflects the fact that although some sites had the highest averages sakuranetin content, the wide variation of the measurements within many of the sites made them statistically similar. It should be noted that the power of the test is very low when compared to a parametric test such as Tukey's (Zar, 1984).

Sakuranetin levels, measured in 62 cultivated individuals from the wild North American populations of *A. dracunculus*, are shown in Figure 3.7. Like the plant samples

collected in the wild, the samples from individuals cultivated in the common garden also varied in sakuranetin content, ranging from 3.38 μg/g in sample 18-141 to 147.69 μg/g in 14-100. The sakuranetin content of the plants grown from purchased seeds ranged from 337.99 to 556.21μg/g. This finding is noteworthy, as this amount of sakuranetin is two to four times more than the highest sakuranetin content found in the North American material from the wild or the common garden.

Figure 3.8 compares sakuranetin levels of extracts prepared from individuals in the wild and those same individuals in the common garden. A number of individuals had extreme changes in the amount of sakuranetin found between the wild and common garden extracts. For example, while 18-141 was one of the plants with the highest sakuranetin content in the wild (117.02 μg/g), it had the lowest content in the common garden. Interestingly, all the individuals from sites 2, 6, 8 (except sample 8-49), 10, 14, 17, 20, 22, 23, 25 and 26 had higher sakuranetin levels in the common garden than in the wild, while site 9 (except 9-58), 16 (except 16-124), 18 (except 18-144), 19 and 21 had higher sakuranetin levels in the wild. Sites 15 and 24 were mixed with half of each group being higher in the wild and half higher in the common garden.

In order to assess if the level of individual sakuranetin production in the wild was correlated with the level of sakuranetin production in the common garden, a Spearman's rank correlation analysis was conducted on individuals that had both wild-collected and common garden extracts. The value of Spearman's rank correlation coefficient (ρ) may range from -1 to +1, with a value of 0 having no correlation. The null hypothesis for this analysis was that the rank of an individual in the wild is independent of the rank in the cultivated situation. This analysis returned a value for $\rho = 0.418$ with a P-value = 0.001.

This value supports the rejection of the null hypothesis and shows that, although the rank of each individual's wild and cultivated sakuranetin production was moderately correlated, the correlation was strongly supported (Table 3.8 and 3.9).

Spearman's rank correlation analysis was also conducted on the average sakuranetin production by site. Figure 3.9 displays the average sakuranetin content of each site in both the wild, and the common garden (arranged by lowest to highest cultivated average). The rank of the average site sakuranetin level in the wild and in the common garden were significantly correlated with $\rho = 0.696$ and strongly supported with a P-value = 0.0026 (Table 3.10 and 3.11). In general, the sites that had high average sakuranetin production in the wild were also the sites that had the highest average sakuranetin production in the common garden. In both the wild, and the common garden conditions sites 9, 14, 16 and18 were in the highest ranked sites, while site 2 was ranked lowest. Exceptions to this general finding were also found. Site 17 and 19 were two of the highest ranked sites in the wild, but their sakuranetin production drastically dropped in cultivation. It should be noted that the sakuranetin levels of the individuals in these sites were extremely wide ranging.

In some cases, exposure to ultraviolet radiation in areas of higher altitude has been shown to correlate with an increase in the production of certain flavonoids. Because the wild-collected extracts of *A. dracunculus* were prepared from samples growing at sites with altitudes ranging from to 341 to 9193 feet above sea level (Appendix 1, Figure A.1.1) a correlation analysis was conducted to assess if sakuranetin levels correlate with increase in altitude. Although sakuranetin has been shown to increase with exposure to ultraviolet radiation in rice (Kodama et al., 1992; Rakwal et al. 1996), the analysis

between the amount of sakuranetin and the altitude of the collection site did not show a significant correlation between these two factors or with latitude (Table 3.12).

The experiment conducted to assess the influence of environmental conditions on sakuranetin production had low survival rate of clones (17 of the original 27 clones survived). The sakuranetin content of each surviving clone and the averaged sakuranetin content of replicates for each sample (9-56, 16-124 and 18-140), in each treatment, is shown in Table 3.13. A graph of the average for each sample in each treatment is shown in Figure 3.10. With the sakuranetin of the clones averaged, samples 9-56 and 16-124 both produced the highest sakuranetin content under the conditions of full sun with the addition of fertilizer. This was not seen in sample 18-140, who had the highest sakuranetin in the full sun and with no fertilizer treatment. Because of the very small sample size of cloned individuals per treatment, statistical analyses could not be conducted, but this preliminary study does suggest that the production of sakuranetin is influenced by environmental conditions. A more extensive experiment would be necessary to accurately assess how environment conditions affect the production of sakuranetin.

Discussion

This study has shown that there can be significant variation in the chemical content of the different *A. dracunculus* individuals and that this variation is an important factor to consider when selecting material for bioassay investigations. Many of the target compounds were not detected in North American populations, and sakuranetin, the

compound which was found in nearly all of the *A. dracunculus* samples, exhibited extreme quantitative variation.

Although sakuranetin is an interesting compound to man because of its medicinal qualities, in plants it serves as a defensive agent against pathogens and ultra-violet light. Interestingly, the production of the compound has been found to be constitutive in some species, while it is inducible in others. For example, in rice, sakuranetin has been shown to be formed *de novo* in response to fungal infection, while in *Ribes nigrum* it is preformed, and accumulates constitutively in leaf glands (Atkinson and Blakeman, 1982; Dixon, 2001). The results from this study show that in *A. dracunculus*, sakuranetin is most likely formed constitutively as well. The results also show that on average, some populations continually produce higher levels of sakuranetin. This is likely due to the higher expression of an enzyme or enzymes in the biosynthetic pathway of the compound. In rice, the enzyme naringenin-7-O-methyltransferase has been shown to convert naringenin into sakuranetin and it is probable that this enzyme is responsible for the production of sakuranetin in *A. dracunculus* as well.

The results suggest that the North American populations lack the capacity to produce some of these medicinal compounds or that they are produced in undetectable quantities. A subset of the plants (27 wild-collected *A. dracunculus* plants, 1 Kyrgyz plant, 1 French tarragon plant, 1 *A. dracunculus* from purchased seed) used in this study were also analyzed to determine their ploidy level (Chapter 2, Table 2.2). The seeds that were purchased, and that contained all the target compounds were found to be decaploid. The plant from Kyrgyzstan and the French tarragon, in which davidigenin, sakuranetin, 6-demethoxycapillarisin, and 2,4-dihydroxy-4-methoxydihydrochalcone were not

detected, were determined to be octoploid and tetraploid, respectively. Nearly all the wild-growing North American populations contained sakuranetin, most had trace amounts of 6-demethoxycapillarisin and were devoid of davidigenin and 2,4-dihydroxy-4-methoxydihydrochalcone. A subset of these plants was tested for ploidy level (26 individuals) and all were determined to be diploid.

These findings are similar to those of Greger (1979), who also found that phytochemical content was associated with ploidy level, and that the decaploid cytotype had a unique assembly of compounds. It is also interesting to note that the tetra-, and octoploid plants of *A. dracunculus* lacked nearly all of the target compounds, while the diploid and decaploid both contained the sakuranetin and 6-demethoxycapillarisin. This also agrees with Greger's findings in which a hexaploid and octoploid strain differed from the decaploid strain, while the diploid (referred to as *A. glauca* by Greger) was more allied to the decaploid.

Polyploidization has been shown to cause dramatic change in physiological processes, and increases in the production of secondary metabolites has been documented in many different taxa (Levin, 1983; Dhawan and Lavania, 1996; Lavania 2005), but it seems that, for these particular compounds, this is not exhibited in any of the polyploids except the decaploid. It would be interesting to screen diploid samples collected in Europe and Asia and assess their sakuranetin and 6-demethoxycapillarisin content as well as for davidigenin and 2,4-dihydroxy-4-methoxydihydrochalcone. Based on the findings of Greger (1979) and the results of this study it seems quite possible that the production of these compounds is unique to the decaploid strain. Since the decaploid strain is

presumably derived from diploids originating in Europe or Asia, it is possible that the diploid progenitors of this decaploid could produce all the target compounds.

Further investigations into the history of the polyploidization of these different strains could be informative in the determination of why there is this unique variation found throughout the different cytotypes. An allopolyploid hybridization event in the formation of the decaploid *A. dracunculus* could have resulted in the introduction of novel genes and gene products, or the unique chemical profile may be the product of autopolyploidy and result from inter-specific variation. The latter may very well be the case, as it seems that this species has a propensity for genomic duplication as evidenced by widespread documentation of individuals within the polyploidy series (2x-10x). Because sakuranetin content was found to vary between populations, and even between the same individual growing in two different environments, it is likely that there is polygenic controls on the production of the compound.

With its diversity of ploidy levels, *Artemisia dracunculus* provides an interesting look into genomic evolution, and this genetic diversity is reflected in its variable chemistry. The findings of this study show that the proper selection of germplasm material for the development of botanical therapeutics is of the utmost importance, and that the provenance, and more specifically the cytotype and genotype, of the plant material are essential to developing an effective and uniform product. In the case of the sakuranetin content of *Artemisia dracunculus*, both nature and nurture, or genetics and environmental conditions, seem to be contributing factors to its intra-specific production and quantity. Standardization of botanical products can only be achieved when the plant

material is uniform in composition and that starts with the selection of proper germplasm material.

Table 3.1. Molecular ions (in negative ionization mode) of target compounds, and their retention times.

Compound	Mass $[M - H]^-$	Approximate retention time (minutes)
davidigenin	257	26-27
6-demethoxycapillarisin	285	25
sakuranetin	285	30
2,4-dihydroxy-4-methoxydihydrochalcone	271	30-32
chlorogenates	353	14
dicaffeoylquinates	515	20

Table 3.2 Presence/absence of target compounds in extracts of both wild and cultivated plants.

sample	davidigenin (257; RT 26-27)		chalcone (271; RT 30-32)		6-De-MeO-Cap (285; RT 25)		sakuranetin (285; RT 30)		chlorogenates (353; RT 13-15)		Caffeoylquinates (515; RT 20)	
1-1	–		–		–		–		+		+	
1-2	–		–		–		–		+		+	
1-3	–		–		–		–		+		+	
1-4	–		–		–		–		+		+	
1-5	–		–		–		–		+		+	
2-11	–	–	–	–	+	–	+	+	+	+	+	+
2-12	–	–	–	–	+	+	+	+	+	+	+	+
2-13	–	–	–	–	+	–	+	+	+	+	+	+
2-14	–		–		+		+		+		+	
2-15	–		–		+		–		+		+	
3-21	–		–		+		+		+		+	
3-22	–		–		–		–		+		+	
3-23	–		–		–		–		+		+	
3-24	–		–		–		–		+		+	
3-25	–		–		–		–		+		+	
4-31	–		–		–		–		+		+	
4-32	–		–		–		–		+		+	
4-33	–		–		–		–		+		+	
4-34	–		–		–		–		+		+	
5-36	–		–		+		–		+		+	
6-37	–		–		–		+		+		+	
6-38	–	–	–	–	+	–	+	+	+	+	+	+
7-39	–		–		–		+		+		+	
7-40	–		–		+		+		+		+	
7-41	–		–		+		+		+		+	
7-42	–		–		+		+		+		+	

sample	davidigenin (257; RT 26-27)		chalcone (271; RT 30-32)		6-De-MeO-Cap (285; RT 25)		sakuranetin (285; RT 30)		chlorogenates (353; RT 13-15)		Caffeoylquinates (515; RT 20)	
7-43	–		–		+		+		+		+	
8-49	–	–	–	–	+	+	+	+	+	+	+	+
8-50	–	–	–	–	+	–	+	+	+	+	+	+
8-51	–	–	–	–	+	–	+	+	+	+	+	+
8-52	–		–		+		+		+		+	
8-53	–	–	–	–	+	–	+	+	+	+	+	+
9-54	–	–	–	–	+	–	+	+	+	+	+	+
9-55	–	–	–	–	+	–	+	+	+	+	+	+
9-56	–	–	–	–	+	–	+	+	+	+	+	+
9-57	–	–	–	–	+	–	+	+	+	+	+	+
9-58	–	–	–	–	+	–	+	+	+	+	+	+
10-64	–	–	–	–	+	–	+	+	+	+	+	+
10-65	–	–	–	–	+	–	+	+	+	+	+	+
10-66	–		–		–		+		+		+	
10-67	–		–		–		+		+		+	
10-68	–		–		–		+		+		+	
11-69	–		–		–		+		+		+	
11-70	–		–		–		+		+		+	
12-74	–		–		+		+		+		+	
12-75	–		–		+		+		+		+	
12-76	–		–		+		+		+		+	
12-77	–		–		+		–		+		+	
12-78	–	–	–	–	+	–	+	+	+	+	+	+
14-100	–	–	–	–	+	–	+	+	+	+	+	+
14-101	–	–	–	–	+	–	+	+	+	+	+	+
14-102	–	–	–	–	+	–	+	+	+	+	+	+
14-103	–	–	–	–	+	–	+	+	+	+	+	+
14-104	–		–		+		+		+		+	

sample	davidigenin (257; RT 26-27)		chalcone (271; RT 30-32)		6-De-MeO-Cap (285; RT 25)		sakuranetin (285; RT 30)		chlorogenates (353; RT 13-15)		Caffeoylquinates (515; RT 20)
15-110	–		–		+		+		–		+
15-111	–		–		+		+		–		+
15-112	–		–		+		+		–		+
15-113	–		–		+		+		–		+
15-114	–		–		+		+		–		+
16-120	–		–		+		+		+		+
16-121	–		–		+		+		+		+
16-122	–		–		+		+		+		+
16-123	–		–		+		+		+		+
16-124	–		–		+		+		+		+
17-130	–		–		+		+		+		+
17-131	–		–		+		+	+	+	+	+
17-132	–		–		+		+	+	+	+	+
17-133	–		–		+		+	+	+	+	+
17-134	–		–		+		+	+	+	+	+
18-140	–	–	–	–	+	–	+	+	+	+	+
18-141	–	–	–	–	+	–	+	+	+	+	+
18-142	–	–	–	–	+	–	+	+	+	+	+
18-143	–		–		+		+	+	+	+	+
18-144	–		–		+		+	+	+	+	+
19-150	–	–	–	–	+	–	+	+	+		+
19-151	–	–	–	–	+	–	+	+	+		+
19-152	–	–	–	–	+	–	+	+	+		+
19-153	–	–	–	–	+	–	+	+	+		+
19-154	–	–	–	–	+	–	+	+	+		+
20-160	–	–	–	–	–	–	+	+	+		+
20-161	–		–		–		+		+		+
20-162	–		–		–		+		+		+

sample	davidigenin (257; RT 26-27)		chalcone (271; RT 30-32)		6-De-MeO-Cap (285; RT 25)		sakuranetin (285; RT 30)		chlorogenates (353; RT 13-15)	Caffeoylquinates (515; RT 20)
20-163	–	–	–		–	–	+	+	+	+
20-164	–	–	–		–	–	+	+	+	+
21-170	–		–		+		+		+	+
21-171	–		–		+	–	+	+	+	+
21-172	–		–		+	–	+	+	+	+
21-173	–		–		+		+		+	+
21-174	–		–		+	–	+	+	+	+
22-180	–	–	–	–	–	–	+	+	+	+
22-181	–	–	–	–	+	–	+	+	+	+
22-182	–	–	–	–	+	–	+	+	+	+
22-183	–	–	–	–	+	–	+	+	+	+
22-184	–	–	–	–	–	–	+	+	+	+
23-190	–		–		–		+		+	+
23-191	–		–		–		+		+	+
23-192	–		–		–		+		+	+
23-193	–		–		–		+		+	+
23-194	–		–		–		+		+	+
24-200	–		–		–		+		+	+
24-201	–		–		+		+		+	+
24-202	–		–		+		+		+	+
24-203	–		–		+		+		+	+
24-204	–		–		+		+		+	+
25-210	–		–		–		+		+	+
25-211	–		–		–		+		+	+
25-212	–	–	–	–	–	+	+	+	+	+
25-213	–	–	–	–	–	–	+	+	+	+
25-214	–	–	–	–	–	–	+	+	+	+
26-220	–	–	–	–	–	–	+	+	+	+

sample	davidigenin (257; RT 26-27)		chalcone (271; RT 30-32)		6-De-MeO-Cap (285; RT 25)		sakuranetin (285; RT 30)		chlorogenates (353; RT 13-15)	Caffeoylquinates (515; RT 20)
26-221	–	–	–	–	–	–	+	+	+	+
26-222	–	–	–	–	–	–	+	+	+	+
26-223	–		–		–		+		+	+
26-224	–	–	–	–	–	–	+	+	+	+
26-228						–		+		
RUGH-1		+		+		+		+	+	+
RUGH-2		+		+		+		+	+	+
RUGH-4		+		+		+		+	+	+
RUGH-4_1		+		+		+		+	+	+
French 1		–		–		–		–	+	+
French 2		–		–		–		–	+	+
French 3		–		–		–		–	+	+
French 4		–		–		–		–	+	+
French 5		–		–		–		–	+	+
French 6		–		–		–		–	+	+
French 7		–		–		–		–	+	+
French 8		–		–		–		–	+	+
French 9		–		–		–		–	+	+
Kyrgyz		–		–		–		–	+	+

Table 3.3. Quantity of sakuranetin in *Artemisia campestris*, *A. ludoviciana and A. dracunculus* (wild and cultivated).

sample	wild leaf tissue (μg/gram)	cult. leaf tissue (μg/gram)
1-1	0	N/A
1-2	0	N/A
1-3	0	N/A
1-4	0	N/A
1-5	0	N/A
2-11	0	N/A
2-12	1.95	7.71
2-13	2.07	4.41
2-14	2.05	8.65
2-15	0	N/A
3-21	3.15	N/A
3-22	0	N/A
3-23	0	N/A
3-24	0	N/A
3-25	0	N/A
4-30	0	N/A
4-31	0	N/A
4-32	0	N/A
4-33	0	N/A
4-34	0	N/A
4-35	0	N/A
5-36	0	N/A
6-37	9.87	N/A
6-38	2.37	10.94
7-39	2.20	N/A
7-40	6.48	N/A
7-41	21.63	N/A
7-42	18.58	N/A
7-43	57.35	N/A
8-49	56.87	41.58
8-51	16.40	53.04
8-52	15.70	10.85
8-53	8.41	N/A
9-54	80.02	76.68
9-55	112.42	46.95
9-56	102.89	61.31
9-57	67.95	19.83
9-58	29.09	32.96
10-64	6.33	8.48
10-65	2.37	5.94
10-66	1.94	N/A

sample	wild leaf tissue (μg/gram)	cult. leaf tissue (μg/gram)
10-67	1.97	N/A
10-68	1.88	N/A
11-69	1.84	N/A
11-70	2.25	N/A
12-74	3.45	N/A
12-75	1.95	N/A
12-76	6.73	N/A
12-77	1.78	N/A
12-78	1.81	N/A
14-100	82.37	147.69
14-101	82.88	88.71
14-102	75.06	93.76
14-103	33.57	92.67
14-104	22.57	N/A
15-110	34.95	N/A
15-111	9.68	N/A
15-112	35.27	N/A
15-113	18.81	11.43
15-114	34.96	45.92
16-120	110.85	N/A
16-121	155.72	N/A
16-122	142.04	N/A
16-123	117.27	107.62
16-124	110.30	137.26
17-130	94.59	N/A
17-131	126.44	27.60
17-132	78.24	3.39
17-133	5.41	5.18
17-134	25.75	7.94
18-140	95.80	54.48
18-141	117.02	3.78
18-142	122.90	90.49
18-143	71.47	62.59
18-144	35.67	80.39
19-150	86.68	7.05
19-151	129.71	27.14
19-152	50.72	33.57
19-153	11.58	10.53
19-154	8.89	8.93
20-160	6.48	40.93
20-161	3.11	N/A
20-162	5.48	N/A
20-163	4.51	13.24

sample	wild leaf tissue (μg/gram)	cult. leaf tissue (μg/gram)
20-164	3.51	13.56
21-170	70.35	N/A
21-171	35.10	21.93
21-172	45.90	33.25
21-173	23.55	N/A
21-174	9.44	6.165
22-180	54.93	N/A
22-181	15.95	N/A
22-182	11.61	30.03
22-183	9.12	35.45
22-184	5.30	61.06
23-190	4.15	N/A
23-191	4.55	7.41
23-192	2.50	N/A
23-193	4.34	N/A
23-194	3.76	N/A
24-200	92.25	N/A
24-201	33.53	18.23
24-202	12.14	26.26
24-203	32.93	9.20
24-204	8.96	39.54
25-210	5.05	N/A
25-211	5.11	N/A
25-212	4.54	46.11
25-213	4.28	23.49
25-214	3.12	9.54
26-220	3.38	43.38
26-221	2.78	38.14
26-222	2.88	66.55
26-223	2.40	22.69
26-224	2.53	33.11
RUGH1	N/A	458.30
RUGH2	N/A	556.21
RUGH4	N/A	337.99
RUGH4-1	N/A	420.62

Table 3.4. Sakuranetin content of wild plants with individual ranks used in the Kruskall-Wallis test (tied ranks averaged).

sample	μg/g leaf	rank	number of individuals/site(*n*)	rank sum (*R*)
2-11	0.00	3.5		
2-12	1.95	12.0		
2-13	2.07	16.0		
2-14	2.05	15.0		
2-15	0.00	3.5	5 (site 2)	50.0
3-21	3.15	28.0		
3-22	0.00	3.5		
3-23	0.00	3.5		
3-24	0.00	3.5		
3-25	0.00	3.5	5 (site 3)	42.0
6-37	2.20	54.0		
6-38	6.48	20.0	2(site 6)	74.0
7-39	21.63	17.0		
7-40	18.58	46.0		
7-41	57.34	64.0		
7-42	56.86	62.0		
7-43	14.64	81.0	5(site 7)	270.0
8-49	16.40	80.0		
8-50	15.70	58.0		
8-51	8.41	61.0		
8-52	80.02	59.0		
8-53	112.41	48.0	5(site 8)	306.0
9-54	102.89	87.0		
9-55	67.95	97.0		
9-56	29.09	94.0		
9-57	6.33	82.0		
9-58	2.37	68.0	5(site 9)	428.0
10-64	1.94	44.0		
10-65	1.97	19.0		
10-66	1.88	11.0		
10-67	3.45	14.0		
10-68	1.95	10.0	5(site 10)	98.0
11-69	6.73	9.0		
11-70	1.78	18.0	2 (site 11)	27.0
12-74	1.81	30.0		
12-75	82.37	13.0		
12-76	82.88	47.0		
12-77	75.06	7.0		

sample	µg/g leaf	rank	number of individuals/site(*n*)	rank sum (*R*)
12-78	33.57	8.0	5(site 12)	105.0
14-100	22.57	88.0		
14-101	34.95	89.0		
14-102	9.68	85.0		
14-103	35.27	71.0		
14-104	18.81	65.0	5(site 14)	398.0
15-110	34.95	72.5		
15-111	110.85	53.0		
15-112	155.72	75.0		
15-113	142.04	63.0		
15-114	117.27	72.5	5(site 15)	336.0
16-120	110.30	96.0		
16-121	94.59	104.0		
16-122	126.44	103.0		
16-123	78.24	99.0		
16-124	5.41	95.0	5(site 16)	497.0
17-130	25.75	92.0		
17-131	95.80	101.0		
17-132	117.02	86.0		
17-133	122.90	42.0		
17-134	71.47	67.0	5(site 17)	388.0
18-140	35.66	93.0		
18-141	86.68	98.0		
18-142	129.71	100.0		
18-143	50.72	84.0		
18-144	11.58	76.0	5(site 18)	451.0
19-150	8.89	90.0		
19-151	6.48	102.0		
19-152	3.11	78.0		
19-153	5.48	55.0		
19-154	4.51	49.0	5(site 19)	374.0
20-160	3.51	45.0		
20-161	70.35	26.0		
20-162	35.10	43.0		
20-163	45.90	36.0		
20-164	23.55	31.0	5(site 20)	181.0
21-170	9.44	83.0		
21-171	54.93	74.0		
21-172	15.95	77.0		
21-173	11.61	66.0		

sample	µg/g leaf	rank	number of individuals/site(*n*)	rank sum (*R*)
21-174	9.12	52.0	5(site 21)	352.0
22-180	5.30	79.0		
22-181	4.15	60.0		
22-182	4.55	56.0		
22-183	2.50	51.0		
22-184	4.34	41.0	5(site 22)	287.0
23-190	3.76	33.0		
23-191	92.25	38.0		
23-192	33.53	22.0		
23-193	12.14	35.0		
23-194	32.93	32.0	5(site 23)	160.0
24-200	8.96	91.0		
24-201	5.05	70.0		
24-202	5.11	57.0		
24-203	4.54	69.0		
24-204	4.28	50.0	5(site 24)	337.0
25-210	3.12	39.0		
25-211	3.38	40.0		
25-212	2.78	37.0		
25-213	2.88	34.0		
25-214	2.39	27.0	5(site 25)	177.0
26-220	2.53	29.0		
26-221	0.00	24.0		
26-222	1.95	25.0		
26-223	2.07	21.0		
26-224	2.05	23.0	5(site 26)	122.0

Table 3.5. Values used to calculate the Kruskall-Wallis test statistic

k	*ΣT*	*C*	*H*	H_c	*v*	$X^2_{0.05,21}$	$X^2_{0.001,21}$
22	210	0.9998	90.289	90.306	21	32.671	46.797

Table 3.6. Rank sums and rank means values of wild plants use to conduct nonparametric multiple comparisons.

site	rank sum (R)	sample size	rank mean ($\bar{R}$)	rank mean ranks
3	42	5	8.4	1
2	50	5	10	2
11	27	2	13.5	3
10	98	5	19.6	4
12	105	5	21	5
26	122	5	24.4	6
23	160	5	32	7
25	177	5	35.4	8
20	181	5	36.2	9
6	74	2	37	10
7	270	5	54	11
22	287	5	57.4	12
8	306	5	61.2	13
15	336	5	67.2	14
24	337	5	67.4	15
21	352	5	70.4	16
19	374	5	74.8	17
17	388	5	77.6	18
14	398	5	79.6	19
9	428	5	85.6	20
18	451	5	90.2	21
16	497	5	99.4	22

Table 3.7. Nonparametric multiple comparisons.

Comparison (B vs A)	$(\bar{\boldsymbol{R}}_B - \bar{\boldsymbol{R}}_A)$	SE	Q	$Q_{0.05}$, 22	conclusion
22 vs 1	91	19.077	4.77	3.699	reject H_o
22 vs 2	89.4	19.077	4.69	3.699	reject H_o
22 vs 3	85.9	19.077	4.50	3.699	reject H_o
22 vs 4	79.8	19.077	4.18	3.699	reject H_o
22 vs 5	78.4	19.077	4.11	3.699	reject H_o
22 vs 6	75	25.237	2.97	3.699	accept H_o
22 vs 7	67.4	19.077	3.53	3.699	accept H_o
22 vs 8	64	19.077	3.35	3.699	accept H_o
22 vs 9	63.2	19.077	3.31	3.699	accept H_o
22 vs 10	62.4	19.077	3.27	3.699	accept H_o
22 vs 11	45.4	25.237	1.80	3.699	accept H_o
22 vs 12	42	19.077	2.20	3.699	accept H_o

Comparison (B vs A)	$(\overline{\boldsymbol{R}}_B - \overline{\boldsymbol{R}}_A)$	SE	Q	$Q_{0.05}$, 22	conclusion
22 vs 13	38.2	19.077	2.00	3.699	accept H_o
22 vs 14	32.2	19.077	1.69	3.699	accept H_o
22 vs 15	32	19.077	1.68	3.699	accept H_o
22 vs 16	29	19.077	1.52	3.699	accept H_o
22 vs 17	24.6	19.077	1.29	3.699	accept H_o
22 vs 18	21.8	19.077	1.14	3.699	accept H_o
22 vs 19	19.8	19.077	1.04	3.699	accept H_o
22 vs 20	13.8	19.077	0.72	3.699	accept H_o
22 vs 21	9.2	19.077	0.48	3.699	accept H_o
21 vs 1	81.8	19.077	4.29	3.699	reject H_o
21 vs 2	80.2	19.077	4.20	3.699	reject H_o
21 vs 3	76.7	19.077	4.02	3.699	reject H_o
21 vs 4	70.6	19.077	3.70	3.699	reject H_o
21 vs 5	69.2	19.077	3.63	3.699	accept H_o
21 vs 6	65.8	25.237	2.61	3.699	accept H_o
21 vs 7	58.2	19.077	3.05	3.699	accept H_o
21 vs 8	54.8	19.077	2.87	3.699	accept H_o
21 vs 9	54	19.077	2.83	3.699	accept H_o
21 vs 10	53.2	19.077	2.79	3.699	accept H_o
21 vs 11	36.2	25.237	1.43	3.699	accept H_o
21 vs 12	32.8	19.077	1.72	3.699	accept H_o
21 vs 13	29	19.077	1.52	3.699	accept H_o
21 vs 14	23	19.077	1.21	3.699	accept H_o
21 vs 15	22.8	19.077	1.20	3.699	accept H_o
21 vs 16	19.8	19.077	1.04	3.699	accept H_o
21 vs 17	15.4	19.077	0.81	3.699	accept H_o
21 vs 18	12.6	19.077	0.66	3.699	accept H_o
21 vs 19	10.6	19.077	0.56	3.699	accept H_o
21 vs 20	4.6	19.077	0.24	3.699	accept H_o
20 vs 1	77.2	19.077	4.05	3.699	reject H_o
20 vs 2	75.6	19.077	3.96	3.699	reject H_o
20 vs 3	72.1	19.077	3.78	3.699	reject H_o
20 vs 4	66	19.077	3.46	3.699	accept H_o
20 vs 5	64.6	19.077	3.39	3.699	accept H_o
20 vs 6	61.2	25.237	2.43	3.699	accept H_o
20 vs 7	53.6	19.077	2.81	3.699	accept H_o
20 vs 8	50.2	19.077	2.63	3.699	accept H_o
20 vs 9	49.4	19.077	2.59	3.699	accept H_o
20 vs 10	48.6	19.077	2.55	3.699	accept H_o
20 vs 11	31.6	25.237	1.25	3.699	accept H_o
20 vs 12	28.2	19.077	1.48	3.699	accept H_o
20 vs 13	24.4	19.077	1.28	3.699	accept H_o
20 vs 14	18.4	19.077	0.96	3.699	accept H_o
20 vs 15	18.2	19.077	0.95	3.699	accept H_o

Comparison (B vs A)	$(\overline{\boldsymbol{R}}_B - \overline{\boldsymbol{R}}_A)$	SE	Q	$Q_{0.05}$, 22	conclusion
20 vs 16	15.2	19.077	0.80	3.699	accept H_o
20 vs 17	10.8	19.077	0.57	3.699	accept H_o
20 vs 18	8	19.077	0.42	3.699	accept H_o
20 vs 19	6	19.077	0.31	3.699	accept H_o
19 vs 1	71.2	19.077	3.73	3.699	reject H_o
19 vs 2	69.6	19.077	3.65	3.699	accept H_o
19 vs 3	66.1	19.077	3.46	3.699	accept H_o
19 vs 4	60	19.077	3.15	3.699	accept H_o
19 vs 5	58.6	19.077	3.07	3.699	accept H_o
19 vs 6	55.2	25.237	2.19	3.699	accept H_o
19 vs 7	47.6	19.077	2.50	3.699	accept H_o
19 vs 8	44.2	19.077	2.32	3.699	accept H_o
19 vs 9	43.4	19.077	2.27	3.699	accept H_o
19 vs 10	42.6	19.077	2.23	3.699	accept H_o
19 vs 11	25.6	25.237	1.01	3.699	accept H_o
19 vs 12	22.2	19.077	1.16	3.699	accept H_o
19 vs 13	18.4	19.077	0.96	3.699	accept H_o
19 vs 14	12.4	19.077	0.65	3.699	accept H_o
19 vs 15	12.2	19.077	0.64	3.699	accept H_o
19 vs 16	9.2	19.077	0.48	3.699	accept H_o
19 vs 17	4.8	19.077	0.25	3.699	accept H_o
19 vs 18	2	19.077	0.10	3.699	accept H_o

Table 3.8. Sakuranetin content of individuals ranked 1-59 (lowest to highest). Only samples with both wild and cultivated extracts were included.

sample	sakuranetin in wild (μg/g leaf)	sakuranetin in cult. (μg/g leaf)	rank wild	rank cult.
2-11	2.37	10.94	1	9
2-12	56.86	41.58	2	3
2-13	14.64	62.78	3	12
6-38	16.40	53.04	5	18
8-49	8.41	10.85	42	39
8-50	80.02	76.68	29	49
8-51	112.41	46.95	30	44
8-53	102.89	61.31	21	17
9-54	67.95	19.83	47	51
9-55	29.09	32.96	54	43
9-56	6.33	8.47	52	47
9-57	2.37	5.94	43	23
9-58	0.00	7.71	33	31
10-64	1.95	4.41	19	11
10-65	2.07	8.65	4	5
14-100	82.37	147.69	48	59
14-101	82.88	88.71	49	53
14-102	75.06	93.76	45	56
14-103	33.57	92.67	36	55
15-113	18.81	11.43	31	19
15-114	34.95	45.92	37	41
16-123	117.27	107.62	56	57
16-124	110.30	137.26	53	58
17-131	126.44	27.60	58	29
17-132	78.24	3.39	46	1
17-133	5.41	5.17	18	4
17-134	25.75	7.94	32	10
18-140	95.80	54.48	51	45
18-141	117.02	3.78	55	2
18-142	122.90	90.49	57	54
18-143	71.47	62.59	44	48
18-144	35.66	80.39	39	52
19-150	86.68	7.05	50	7
19-151	129.71	27.14	59	28
19-152	50.72	33.57	41	34
19-153	11.58	10.53	26	16
19-154	8.89	8.93	22	13
20-160	6.48	40.92	20	38
20-163	4.51	13.24	14	20
20-164	3.51	13.56	12	21
21-171	35.10	21.93	38	24
21-172	45.90	33.25	40	33

sample	sakuranetin in wild (μg/g leaf)	sakuranetin in cult. (μg/g leaf)	rank wild	rank cult.
21-174	9.44	6.16	25	6
22-182	11.61	30.03	27	30
22-183	9.12	35.45	24	35
22-184	5.30	61.06	17	46
23-191	4.55	7.41	16	8
24-201	33.53	18.23	35	22
24-202	12.14	26.26	28	27
24-203	32.93	9.20	34	14
24-204	8.96	39.54	23	37
25-212	4.54	46.11	15	42
25-213	4.28	23.49	13	26
25-214	3.12	9.54	10	15
26-220	3.38	43.38	11	40
26-221	2.78	38.14	8	36
26-222	2.88	66.55	9	50
26-223	2.39	22.69	6	25
26-224	2.53	33.11	7	32

Table 3.9. Results of Spearman's ranked correlation analysis for wild and cultivated individuals.

Spearman's rho (ρ)	degrees of freedom	P-value
0.414	16	.0013
	15	0.0019

Table 3.10. Sites averaged and ranked for sakuranetin content 1-17 (lowest to highest) for sites with both wild and cultivated environments.

site	wild avg (μg/g)	cult avg (μg/g)	rank wild	rank cult.
site 2	1.34	6.92	1	1
site 10	4.35	7.21	5	2
site 23	4.55	7.41	6	3
site 6	2.37	10.94	2	4
site 17	58.96	11.03	13	5
site 21	30.14	20.45	12	6
site 20	4.83	22.57	7	7
site 24	21.89	23.31	9	8
site 25	3.98	26.38	4	9
site 15	26.88	28.67	11	10
site 26	2.79	40.77	3	11
site 8	24.08	42.06	10	12
site 22	8.68	42.18	8	13
site 9	78.47	47.55	15	14
site 18	88.57	58.35	16	15
site 14	68.47	105.71	14	16
site 16	113.78	122.44	17	17

Table 3.11. Spearman's ranked correlation analysis for sakuranetin averages of each site.

Spearman's rho (ρ)	degrees of freedom	P-value
0.696	15	0.0026

Table 3.12. Correlation analysis of sakuranetin content of individual samples with altitude of collection site.

	sakuranetin	altitude	latitude
sakuranetin	1	–	–
altitude	-0.234	1	–
latitude	-0.142	–	1

Table 3.13. Sakuranetin produced by clones of three different plants grown in three different environmental condition regimes.

sample	treatment	μg/g of leaf sample	average/sample/treatment
9-56a	Fertilizer/Sun	73.09	
9-56b	Fertilizer/Sun	101.68	87.39
9-56c	No Fertilizer/Shade	7.08	
9-56d	No Fertilizer/Shade	7.09	7.08
9-56e	No Fertilizer/Sun	2.91	
9-56f	No Fertilizer/Sun	17.50	
9-56g	No Fertilizer/Sun	4.64	8.35
16-124a	Fertilizer/Sun	58.68	
16-124b	Fertilizer/Sun	34.47	46.58
16-124c	No Fertilizer/Shade	31.06	
16-124d	No Fertilizer/Shade	26.64	28.85
16-124e	No Fertilizer/Sun	52.35	52.35
18-140a	Fertilizer/Sun	59.03	
18-140b	Fertilizer/Sun	58.41	58.72
18-140c	No Fertilizer/Shade	7.39	7.39
18-140e	No Fertilizer/Sun	87.71	
18-140f	No Fertilizer/Sun	74.07	80.89

Figure 3.1. Structures of seven bioactive compounds screened for in this study: davidigenin (A), 2',4-dihydroxy-4'-methoxydihydrochalcone (B), 2',4'-dihydroxy-4-methoxydihydrochalcone (C), sakuranetin (D), 6-demethoxycapillarisin (E), 5-O-caffeoylquinic acid (F), and 4,5-di-O-caffeoylquinic acid (G).

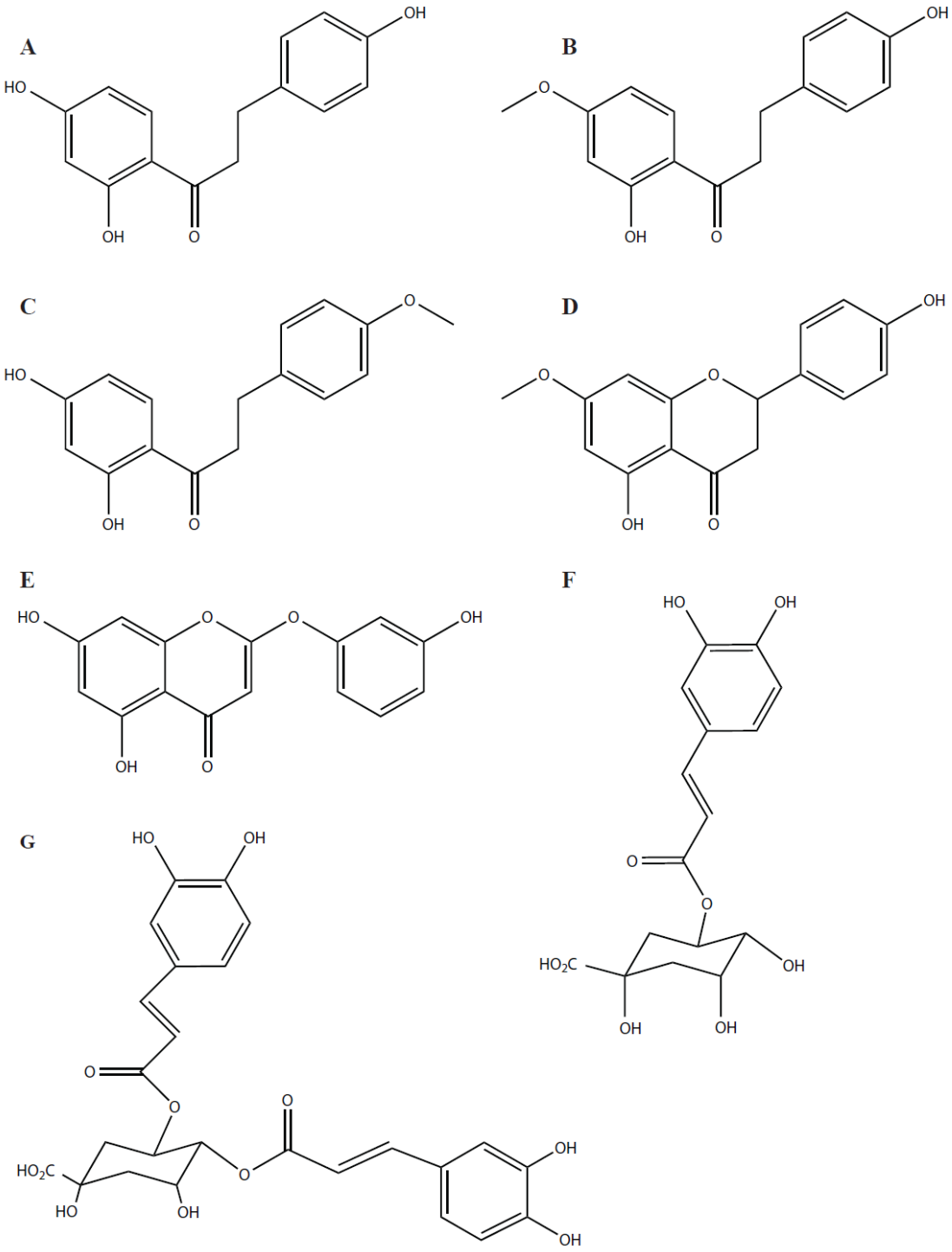


Figure 3.2. Representative chromatograms of target compounds.

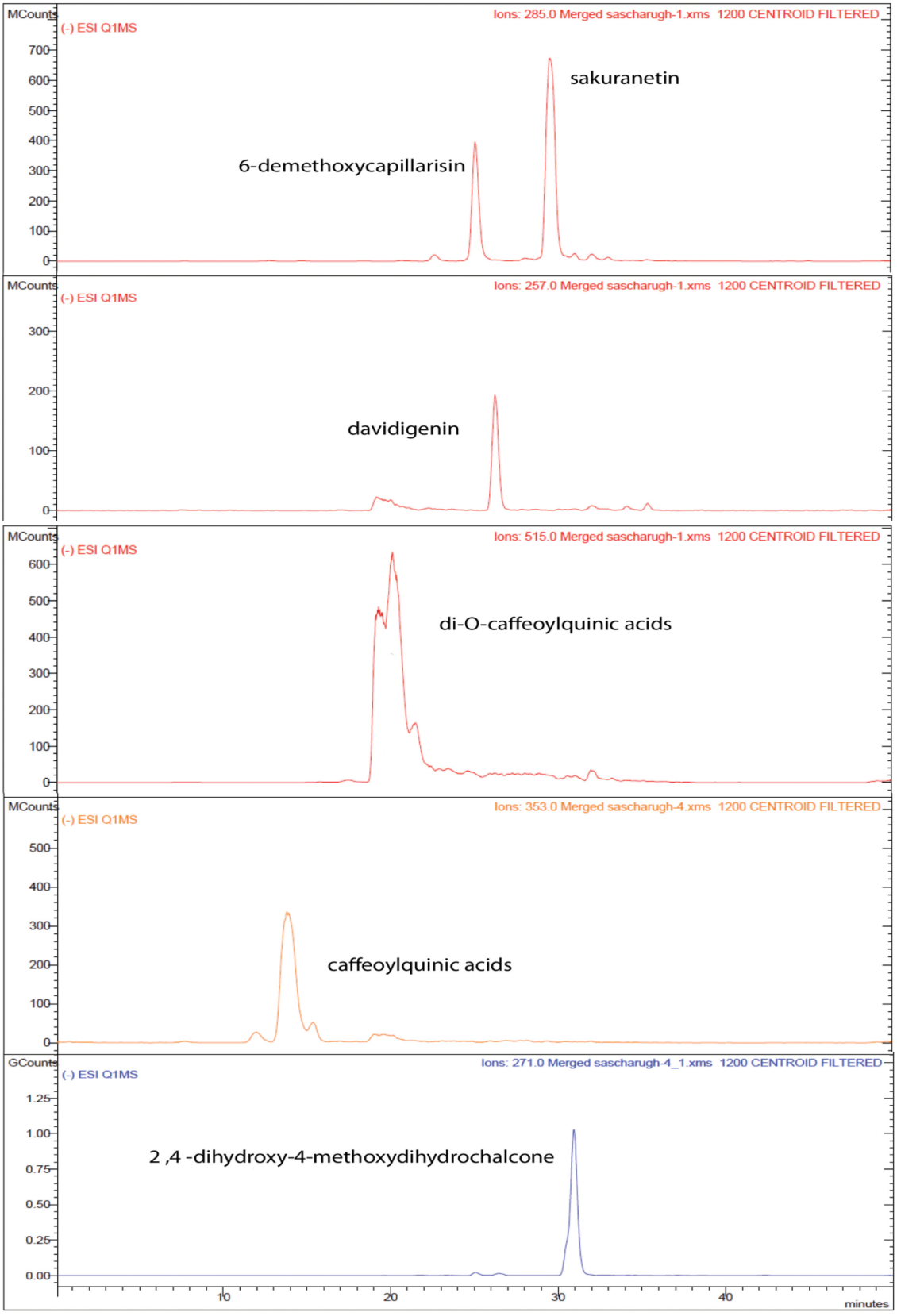


Figure 3.3. Comparison of peak areas for 6-demethoxycapillarisin (retention time 25-26); samples RUGH-2 (A), RUGH-4 (B), 2-12 (C).

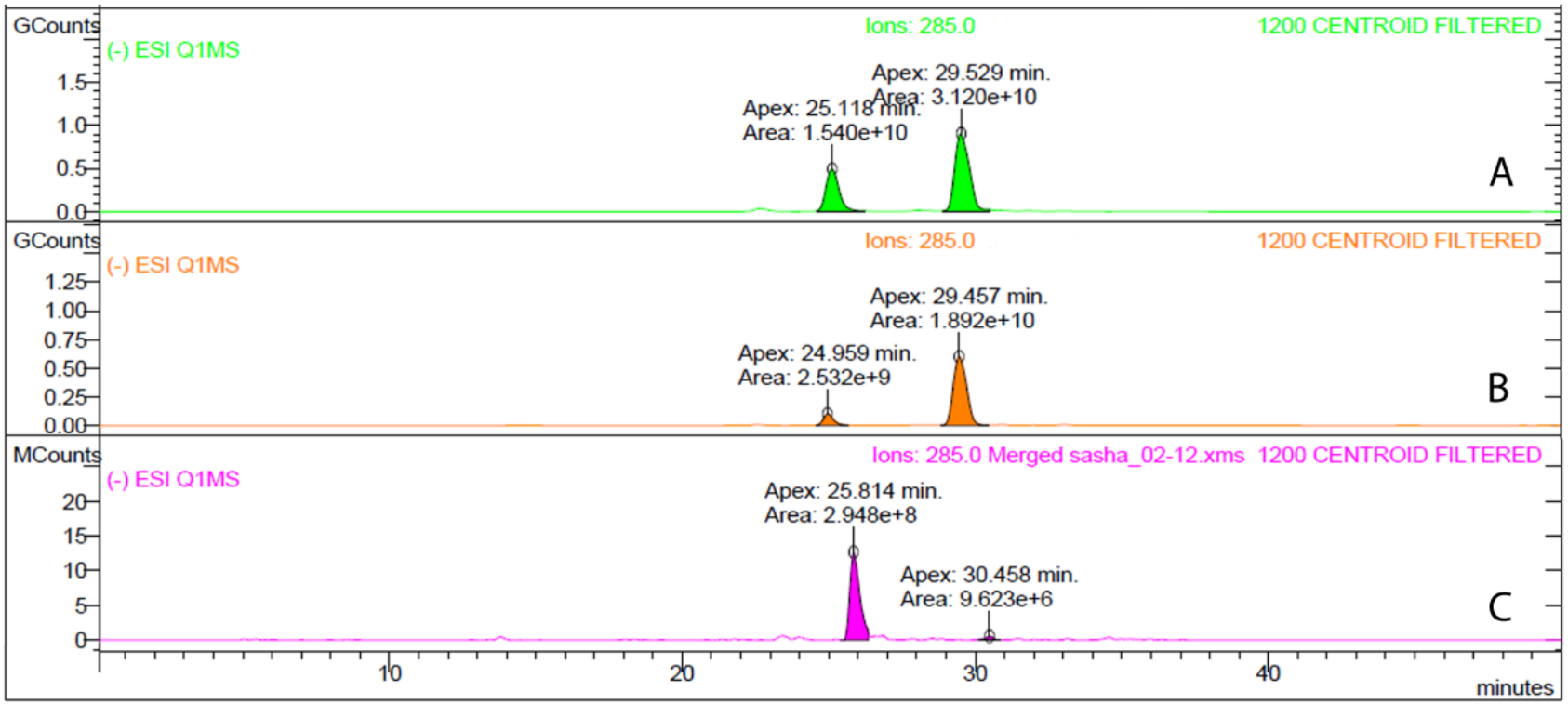


Figure 3.4. Sakuranetin content of extracts prepared from all wild growing plants (*Artemisia dracunculus*, *A. campestris* [samples 1-1:1-5] *and A. ludoviciana* [samples 4-30:4-35 and 5-36]) collected in 2006. The labels on the X-axis indicate the number of the collection site and the specific individual (detailed locality information for collection sites is provided in Appendix 1).

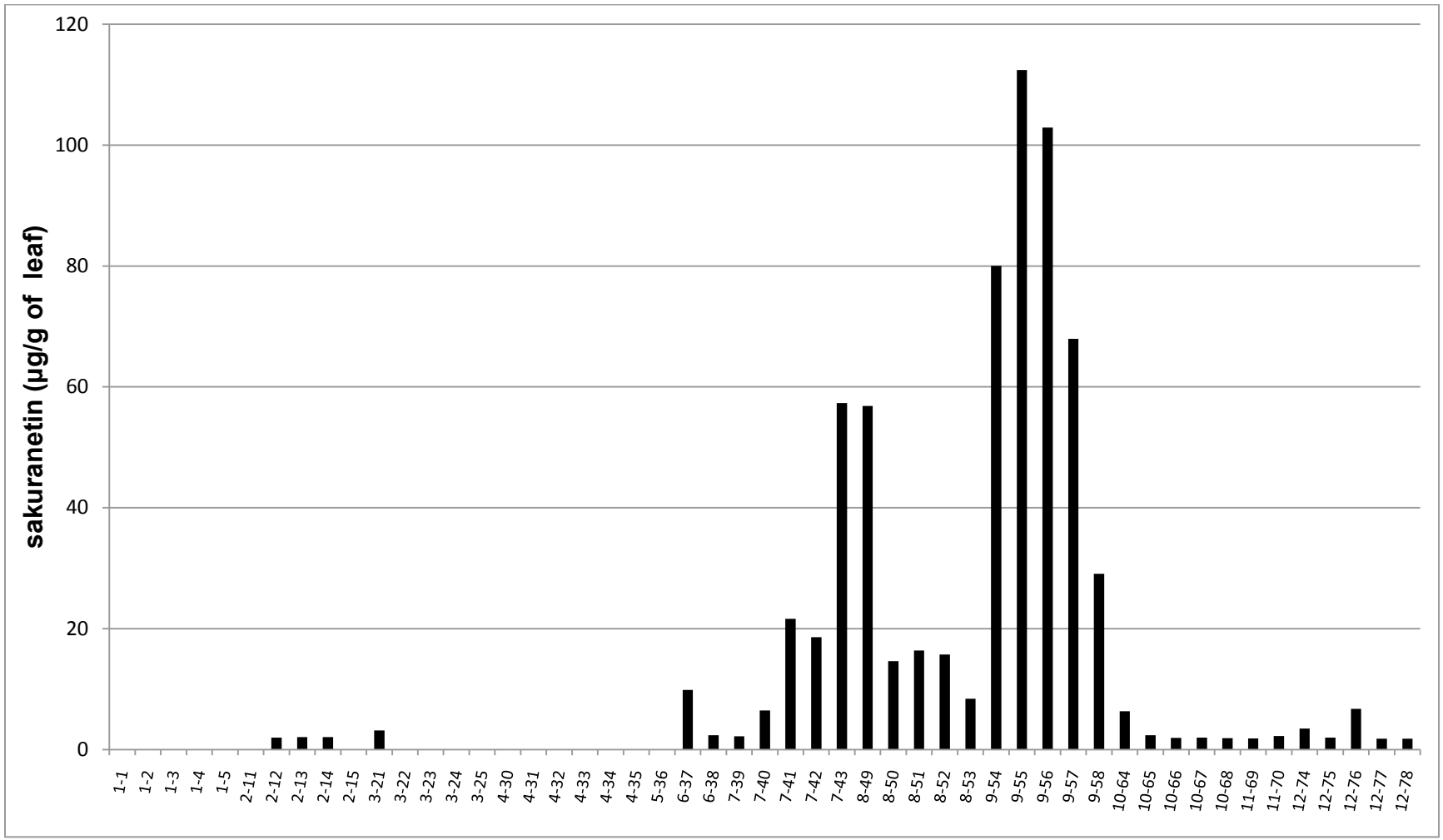


Figure 3.5. Sakuranetin content of extracts prepared from all wild growing plants (only *Artemisia dracunculus*) collected in 2007. The labels on the X-axis indicate the number of the collection site and the specific individual (detailed locality information for collection sites is provided in Appendix 1).

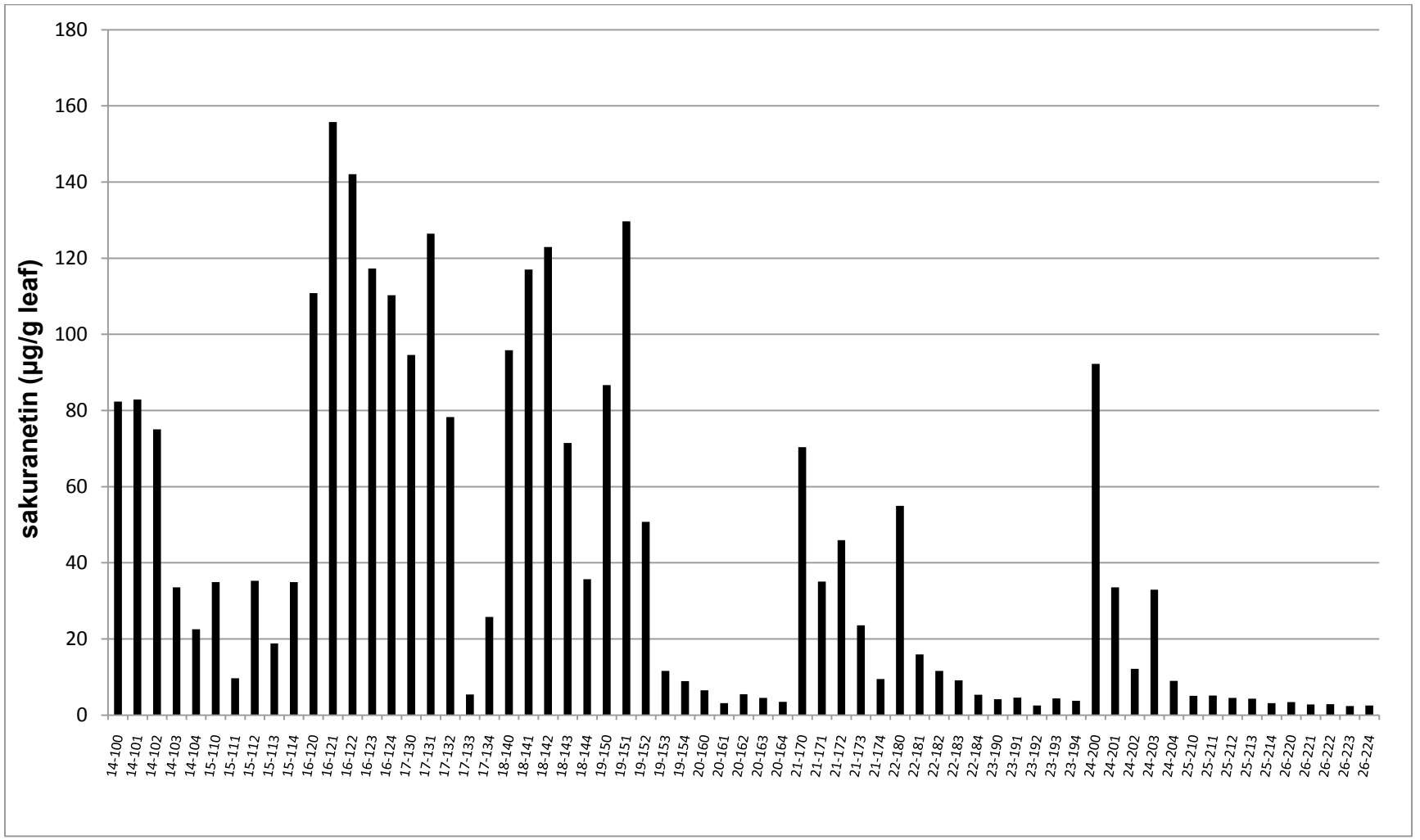


Figure 3.6. Average sakuranetin levels for wild sites of *A. dracunculus*. (Error bars represent the range of measurements at each site)

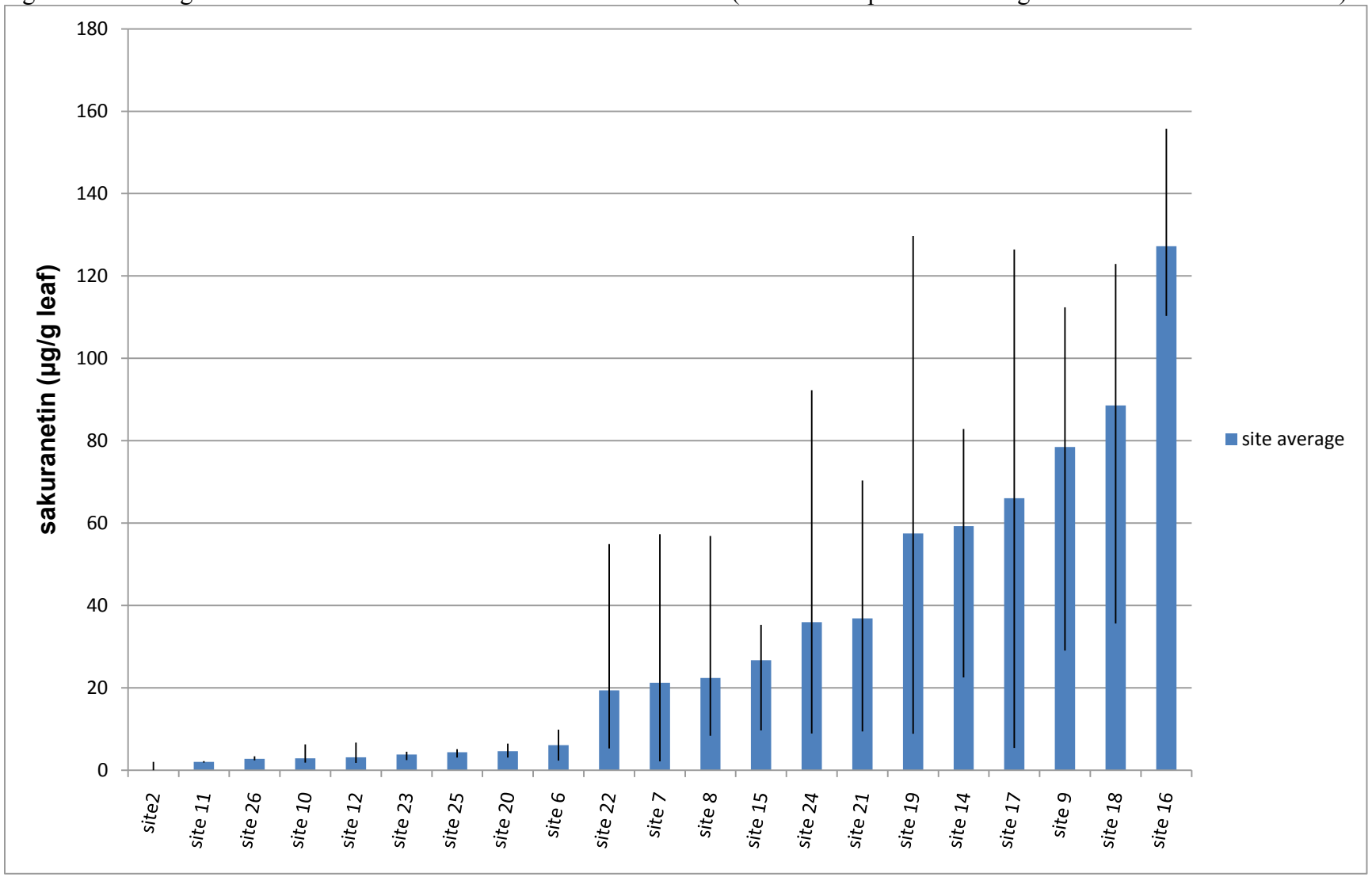


Figure 3.7. Sakuranetin content of *A. dracunculus* clones collected from different populations and cultivated in a common garden.

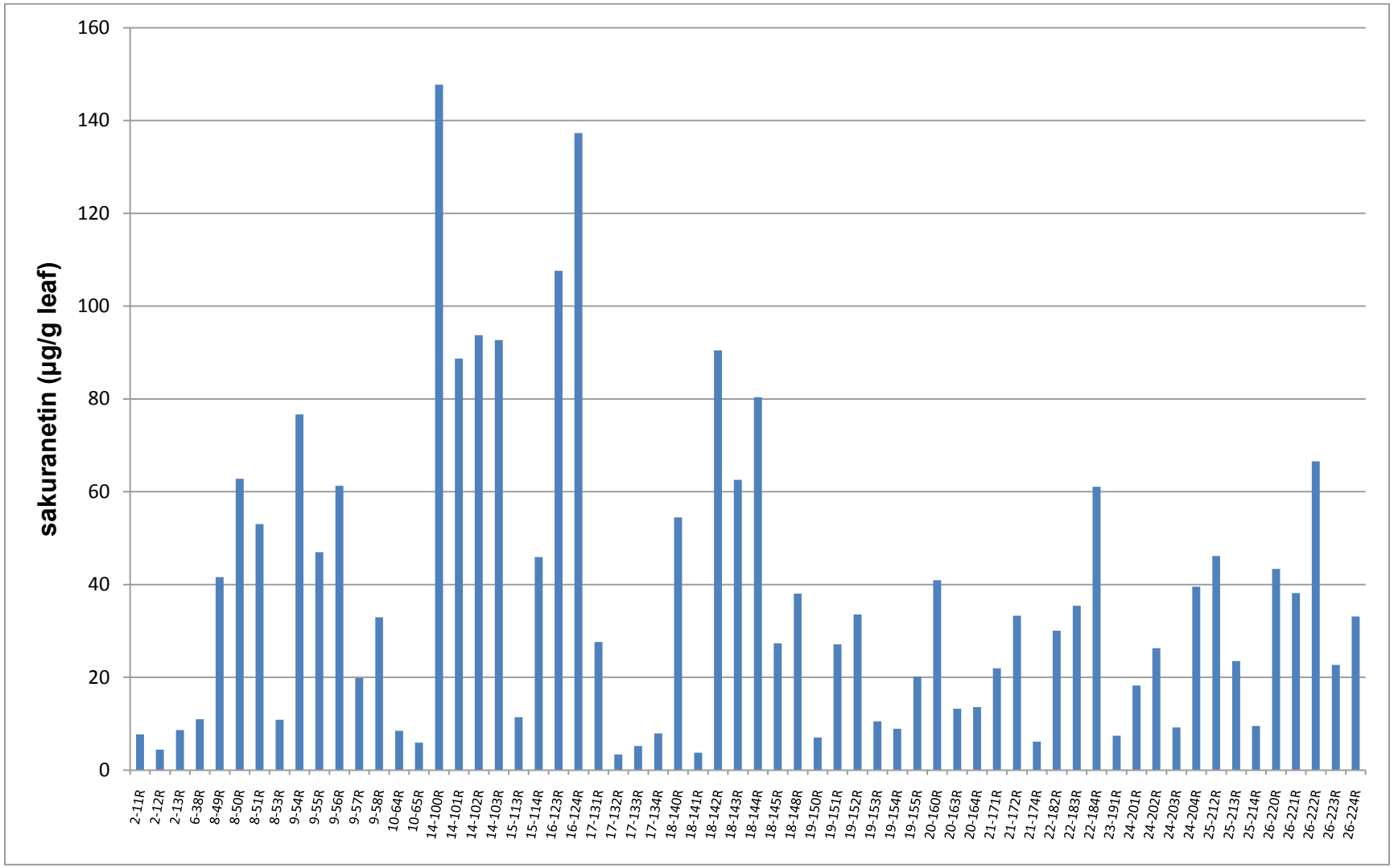


Figure 3.8. Comparison of the sakuranetin content of individuals in the wild and their cultivated clones.

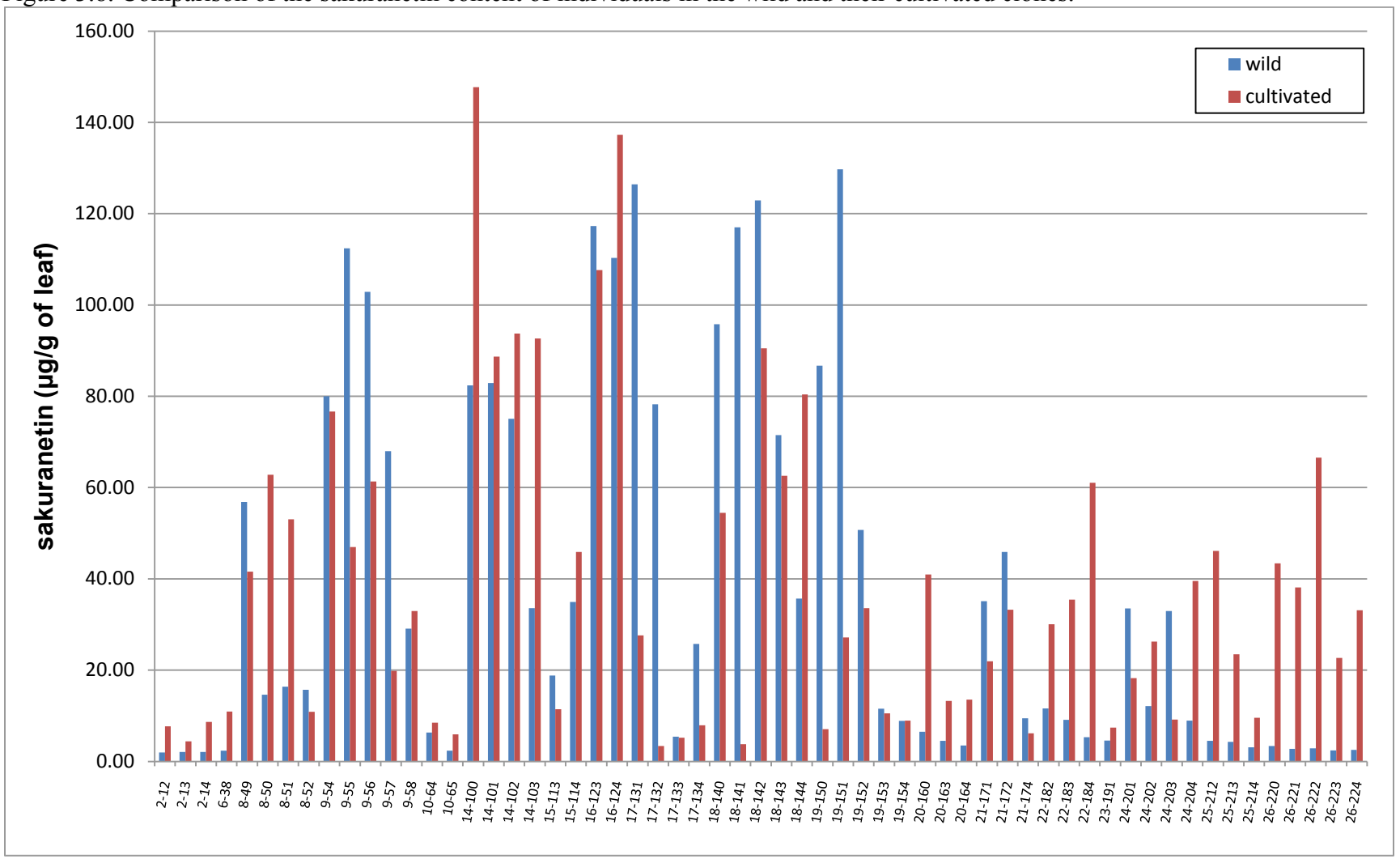


Figure 3.9. Average sakuranetin content of sites from wild and cultivated plants (arranged lowest to highest sakuranetin content in cultivated plants; error bars represent the range of measurements for each site; R = plants cultivated at Rutgers University).

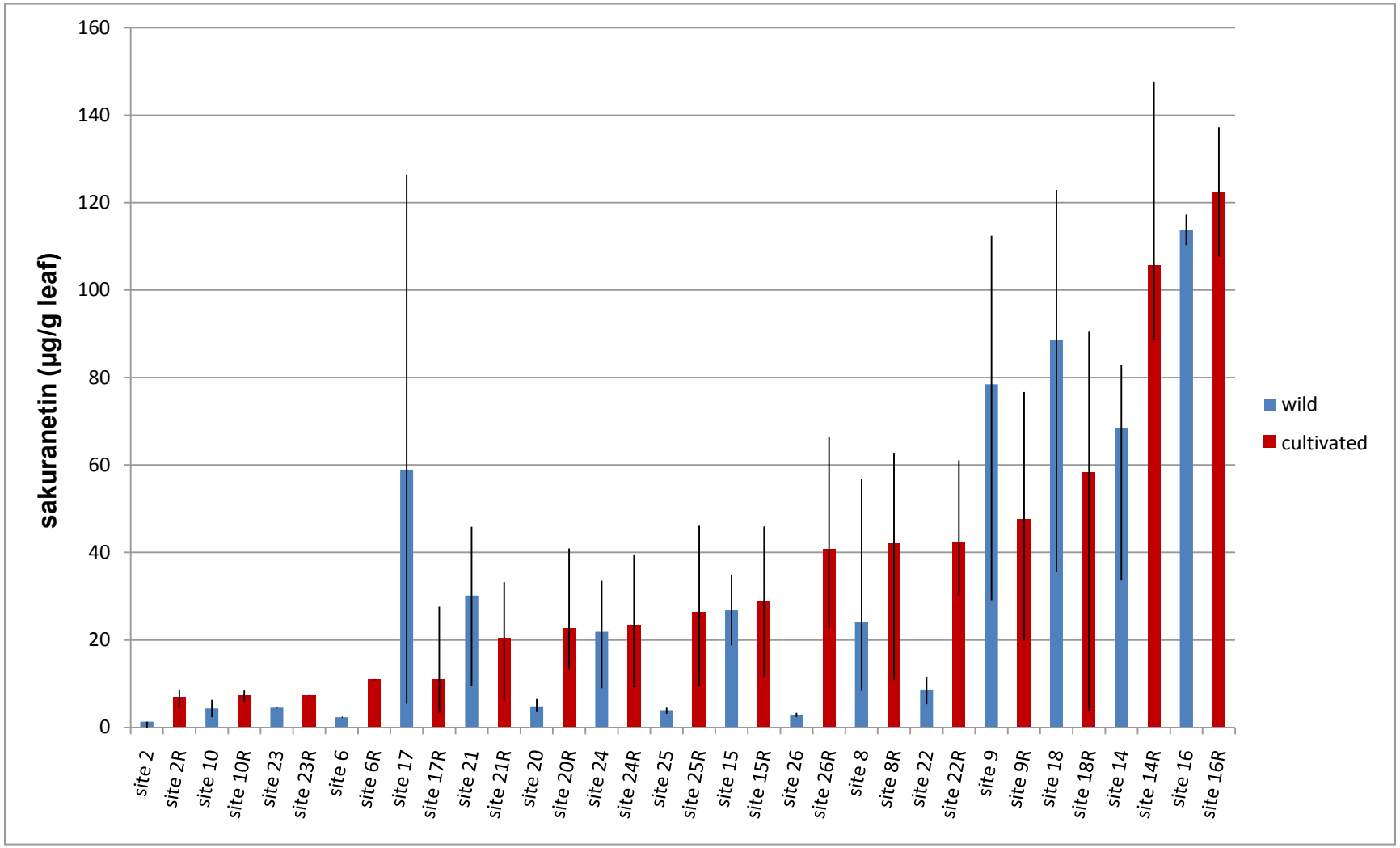
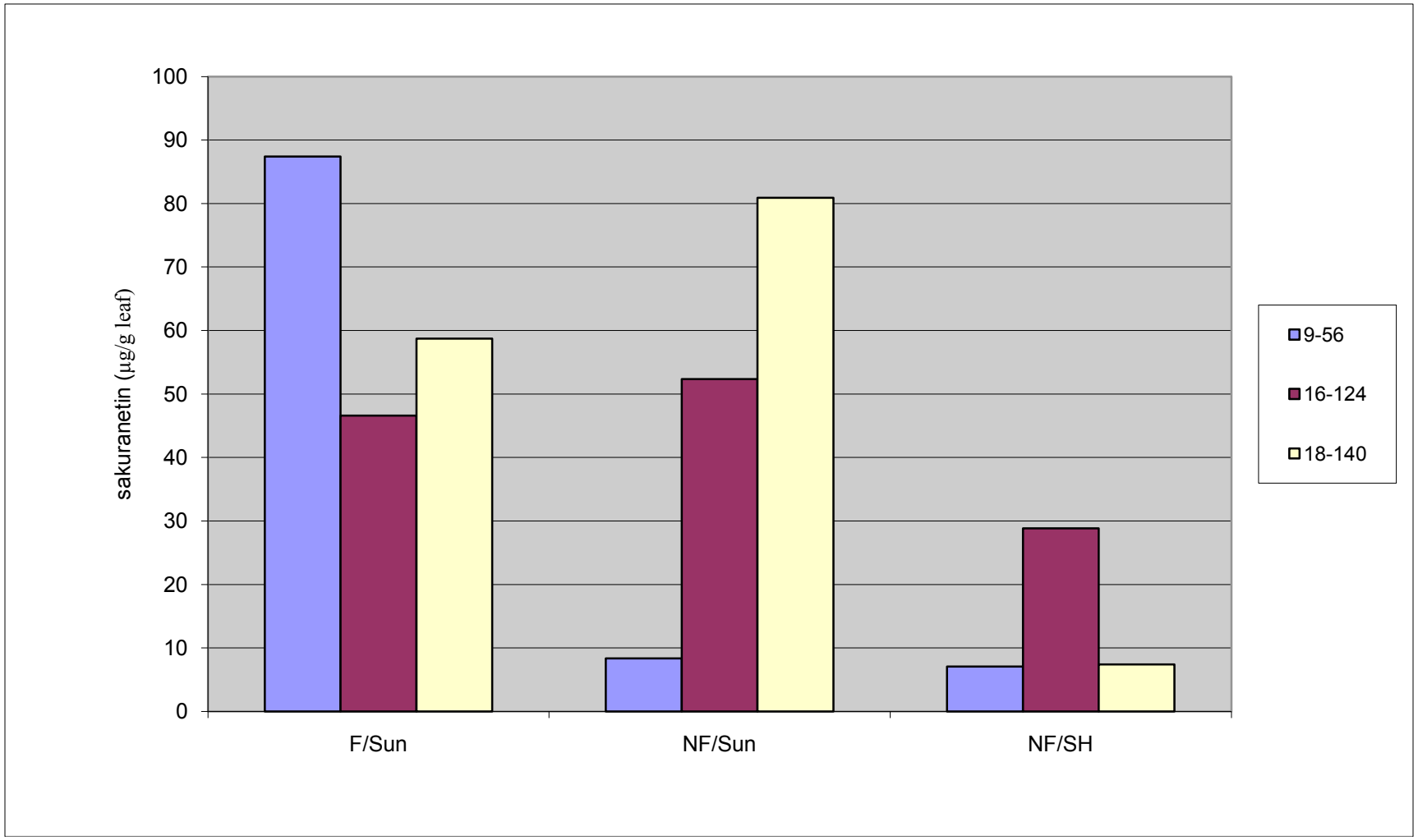


Figure 3.10. Average sakuranetin content of clones grown in different environmental conditions; F = grown with fertilizer treatments, NF = no fertilizer, SH = grown in shade, Sun = grown in full sun.



Chapter 4

Essential oil diversity in North American populations of wild tarragon (*Artemisia dracunculus* L.)

Abstract

Essential oil extracted from 63 cultivated individuals, originating from 18 different North American populations of *Artemisia dracunculus*, 1 cultivated individual from Kyrgyzstan and cultivated French tarragon plants were analyzed by gas chromatography-mass spectroscopy. The samples exhibited a variety of different volatile profiles with a number of different primary components including (Z)-β-ocimene (22 samples), methyl eugenol (15 samples) and, methyl chavicol (10 samples) and α-terpinolene (5 samples). Many of the samples had significant concentrations of the polylacetylenes capillene, 5-phenyl-1,3-pentadiyne and 1-(4-Methoxyphenyl)-2,4-pentadiyne. The isocoumarin acetylene, capillarin, was also found in the majority of (53) samples but was generally found in low amounts (less than 4%). Six major clusters were formed by UPGMA cluster analysis, with groupings based on high proportions of 1) methyl chavicol, 2) methyl eugenol, 3) α-terpinolene, 4) capillene, 5) 5-phenyl-1,3-pentadiyne or 6) (*E*)-β-ocimene/(*Z*)-β-ocimene. These clusters represent a number of new chemotypes for *Artemisia dracunculus*. Additionally, a sample from Kyrgyzstan was found to have a unique profile with its main constituents being myrcene (27.15%), (Z)-artemidin (25.85%) and limonene (11.62%). Bornyl acetate (3.759%), (*E*)-artemidin

(2.315), camphene (1.07%), dracumerin (0.499%), β-sesquiphellandrene (0.322%), 4-terpineol (0.264%) and neryl acetate (0.213%) were found in the Kyrgyz sample as well.

Introduction

Artemisia dracunculus L. (Asteraceae) is best known for the variety, *Artemisia dracunculus* var. *sativa* Besser, also called French tarragon, which is commonly used as a culinary herb. The unique characteristics of this type of tarragon were written about as early as 1563 by Mattioli. Two of the major characteristics distinguishing French tarragon from wild tarragon (*Artemisia dracunculus* var. *dracunculus* L.) are differences in their smell and flavor. French tarragon is favored for its spicy, licorice-like flavor, which has been attributed to high amounts (60-81%) of methyl chavicol [estragole, methyl chavicol, 1-allyl-4-methoxybenzene] in its essential oil (Thieme and Nguyen, 1972; Albasini et al., 1983; Balza et al., 1985; Deans and Svoboda, 1988; Lawrence, 1979; Werker et al., 1994; Tomitaka et al., 1997; Arabhosseini et al., 2006). Other unique characteristics of French tarragon are its inability to produce viable seeds and a less robust growth habit. Because of its sterility, it must be propagated asexually by cuttings or root division (Rousi, 1969; Sutton et al., 1985).

Recently, use of gas chromatography-olfactometry techniques, such as aroma extract dilution analysis (AEDA), have been used with the goal of determining the compounds most responsible for the odor of particular foods, herbs and spices (Abbott et al., 1993; Grosch, 1994; Schieberle, 1995). Human smell receptors exhibit selectivity and varying sensitivity to different volatile molecules. A molecule to which humans are highly sensitive may only be present in small amounts, yet provide the highest impact

towards flavor, while compounds found in greater abundance, but which are not as impactful, may not be contributing much to sensorial experience (Greger and Schieberle, 2007). Using AEDA to investigate the impact of flavor constituents in French tarragon, Zeller and Rychlik (2007) found that β-ionone and eugenol made the greatest contribution to the flavor of French tarragon, while methyl chavicol was of lesser importance than has been traditionally thought.

Although methyl chavicol content can be very high in French tarragon, it is important to note that time of harvest has been shown to influence chemical composition of essential oil profiles. Deans and Svaboda (1988) found that methyl chavicol was most abundant in plants harvested at mid-season (weeks 5-8 of a 13 week growing season) and dropped more than 50% by the end of the season, while trans- and cis-ocimene (*E*-β-ocimene and Z-β-ocimene) content more than doubled from the mid- to late-season (weeks 9-13)and reached peak concentration at week 12. In experiments that controlled for environmental conditions, Suchorska et al. (1992) found that elongated day length and constant temperature caused an increase in elemicin and a decrease in methyl chavicol, β-pinene, sabinene and ocimene in wild tarragon.

Many studies have investigated the essential oil composition of both French and wild tarragon, but the vast majority of studies on wild tarragon has used plant material originating from European or Asian populations. Wild tarragon has been documented as having sabinene, elemicin, *trans*-isoelemicine or *trans*-anethole as the major oil constituent, and has generally been shown to contain little or no methyl chavicol (Albasini et al., 1983). Recent studies of *Artemisia glauca* (often synonymized with *A. dracunculus*; Shultz, 2006) have reported the aromatic acetylene, capillene, as a major

component of essential oil extracted from plants in a number of different areas in Russia (Altai Krai, Troitsk District; Altai Republic, Kosh-Agachsk District; Altai Krai, Ust′-Pristansk District, and Novosibirsk Oblast, Novosibirsk District; Polyanskaya et al., 2007). Additionally, demethylcapillene (benzyldiacaetylene) was found to be the major component in plants from Khakasiya Republic, Bogradsk Region and Altai Krai, Burlinsk Region (Polyanskaya et al., 2007).

Acetylenes (hydrocarbon compounds with a carbon to carbon triple bond) are a distinct group of compounds that have been found in over twenty different plant families, and are especially abundant in the Asteraceae. Species in the genus *Artemisia* has been found to contain structurally diverse acetylenes including aliphatic, aromatic, spiroacetal enol ether and isocoumarinic acetylenes (Bohlmann et al., 1973; Greger, 1977; Crosier et al., 2006). Acetylenes have often been isolated from different *Artemisia* species and a number of different compounds including capillen, capillarin, 8-hydroxycapillarin, capillarin isovalerate dehydrofalcarinone, dehydrofalcarinol, dehydrofalcarindiol and dehydrofalcarinonol have all been isolated from the roots of *Artemisia dracunculus* (Greger, 1977; Greger, 1979; Jakupovic et al., 1991). In addition to capillene, other acetylenes such as capillin, (*E*)-hex-4-en-2-ynylbenzene, 1-(4-methoxyphenyl)-2,4-pentadiyne, and capillarin were isolated from *A. dracunculus* as well (called *A. glauca* in reference; Polyanskaya et al., 2007). Verma et al. (2008) also reported of *A. dracunculus* with high amounts of polyacetylenes. Although the source of the plant material was not stated, the analysis, conducted using plant material grown in Srinagar, India revealed capillene and 5-phenyl-1,3-pentadiyne as the major constituents of the oil. Srinagar is located in the Indian state of Jammu and Kashmir, where *A. dracunculus* (*A. glauca*) is

documented as growing wild, so the plant material used by Verma may have been from local populations.

Studies assessing variability of *A. dracunculus* essential oil from populations located in different areas of Russia, found significant variation of acetylene versus isocoumarin content, between regional populations (Rutskikh et al., 2000; Aglarova et al., 2008). Plants from the Altai Mountain Republic were found to contain a number of different aromatic polyacetylenes and lacked isocoumarins, while plants from the other regions lacked the aromatic polyacetylenes and contained isocoumarins This type of distinction, between the accumulation of aromatic acetylenes and isocoumarinic acetylenes, was also seen in root extracts of *A. dracunculus* cytotypes (Greger, 1979).

Although *A. dracunculus* has a wide distribution across western North America, few phytochemical studies have been conducted using wild material from this region. Lopes-Lutz et al. (2008) found that essential oil extracted from plant material from Central Alberta Prairies – Western Canada, consisted mainly of monoterpene hydrocarbons and phenylpropanoids (90.9% of the total oil) with methyl eugenol (35.8%), terpinolene (19.1%), and methyl chavicol (16.2%), as the predominant constituents. Using wild tarragon collected in Oregon, Pappas and Sturtz (2001) found that terpinolene (25.4%), *Z*-(β)-ocimene (22.2%) and β-phellandrene were the most abundant compounds. That study reported that the essential oil contained 4.8% capillene and 11.7 % 5-phenyl-1,3-pentadiyne (another aromatic polyacetylene). Meepagala et al. (2002) reported the relative abundance of 5-phenyl-1,3-pentadiyne (11%), methyl eugenol (9%), and capillarin (4%) in steam-distilled oil of *A. dracunculus.* As the Meepagala et al. study

was primarily focused on the identification of anti-fungal compounds, the entire chemical profile of the essential oil was not presented.

The essential oil from both French and wild tarragon has been investigated for a variety of biological activities including antithrombotic, anti-oxidant, antibacterial, and antifungal and tissue regenerating capabilities. The antibacterial effects of tarragon essential oil have been investigated by a number of researchers. To assess the results of the studies it is necessary to consider the nature of the assay conducted. The hydrophobic properties of essential oil can prevent it from diffusing through agar media, and therefore, in studies that use agar diffusion methods, results may exhibit reduced activity due to poor contact with the bacteria. In some studies this has been dealt with by combining the oil with dimethyl sulfoxide (DMSO) to allow for better diffusion. Although the provenance of the plant material is not given in all the studies, the results are consistent. Essential oil of tarragon was found to have low inhibition of methicillin-resistant *Staphylococcus aureus* (MRSA) in a disc diffusion assay for inhibitory activity (Chao, 2008). In a study to determine the antibacterial activity of the essential oil in the vapor phase against five food borne bacteria, Nedorostova et al. (2009) found that the essential oil did not have significant antibacterial effects. Curini et al. (2006) found that oil isolated from the cultivar 'Piemontese', collected in central Italy, also had low antibacterial activity, but did exhibit significant antifungal activity. In inhibition tests with 10 different bacterial species, Deans and Svaboda (1988) found that individual components of the essential oil from French tarragon (anisaldehyde, paracymene, eugenol, limonene, linalool, menthol, cis-ocimene, alpha-phellandrene, alpha-pinene and beta-pinene) caused 2-3 times more inhibition than an equal amount of the total extract. They also noted that

although French tarragon is noted for high amounts of methyl chavicol (65-81% of total oil) this compound as well as camphene, camphor, delta-3-carene and trans-ocimene had little to no inhibitory activity.

Using bioassay guided fractionation of wild tarragon essential oil from North American plants, Meepagala et al. (2002) determined that 5-phenyl-1,3-pentadiyne, methyleugenol, and capillarin exhibited antifungal effects on plant pathogenic *Colletotrichum* spp. Essential oil extracted from *A. dracunculus* growing in India was also found to have antifungal activity when tested on the growth of *Candida albicans*, *Sporotrichum schenkii*, and *Aspergillus fumigatus* (Mehrotra et al., 1993). Using the same cultivar, Zani et al. (1991) showed that essential oil from tarragon had DNA damaging activity in a *Bacillus subtilis rec*-assay, and that methyl chavicol was the causal compound. In that study, the oil did not exhibit activity in a *Salmonella* mutagenicity assay.

Although originally classified as GRAS (Generally Recognised As Safe), a number of studies have shown that at high doses, methyl chavicol is carcinogenic and genotoxic (mostly due to the metabolization into 1′-hydroxyestragole). After reviewing of the toxicological literature, the European Commission Scientific Committee on Food could not establish a safe exposure limit and recommended reductions in exposure and restrictions in use (European Commision Scientific Committee on Food, 2001). Methyl chavicol was selected for toxicity testing by the National Toxicology Program (an interagency program between the National Institute of Environmental Health Sciences of the National Institutes of Health, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention, and the National Center for

Toxicological Research of the Food and Drug Administration). The findings of this three month investigation have been preliminarily released as a draft for review of Toxicity Report Series no. 82, but the draft is currently labeled as not for distribution or attribution. According to Smith et al. (2002), studies have clearly shown that the conversion methyl chavicol to 1′-hydroxyestragole is dose dependent and that the toxicological risk diminishes markedly at low levels of exposure. They also cite rodent studies that show that the metabolism, metabolic activation, and covalent binding implicated in toxicity and carcinogenicity of methyl chavicol are minimal in the dose range of 1−10 mg/kg body weight, which is approximately 100–1000 times the anticipated human exposure to these substances. As such, in their opinion, exposure to methyl eugenol and estragole resulting from consumption of food, mainly spices and added as such, does not pose a significant cancer risk.

Based on the historic use of tarragon as an antiepileptic remedy in Iranian folk medicine, Sayyah et al. (2004) found that the essential oil produced from Iranian plant material exhibited anticonvulsant activity in studies on mice with two different types of induced seizures. In an *in vitro* study that screened the essential oils of many different plant species for possible antithrombotic activity, Tognolini et al. (2006) found that tarragon essential oil (with high p-allyanisole content, ~70%) exhibited high antiplatelet activity against three different platelet aggregation agonists. The oil also exhibited a good ability to reduce clot retraction. In an experiment on organ regeneration *in vivo*, rat liver regeneration was increased significantly by treatments comprised of injections of tarragon essential oil (Gershbein, 1977).

These studies have shown that the essential oil of tarragon has varied biological activity, but there has been nearly no comparative analysis of tarragon originating from different geographic localities and few studies have compared bioactivity of French versus wild tarragon. The wide distribution and documented chemical variation of different populations, and even seasonal variation within populations, could provide individuals and populations with significantly differing bioactivity. Because of the large area of distribution for the species, and high amount of morphological variability, it is quite possible that there is variability in the major volatile components of plants from different populations as well. The goal of this research was to assess intra-specific variation by characterizing the essential oil profiles of individuals from different populations, in this case growing primarily in the western United States.

Materials and Methods

Plant Material. *A. dracunculus* plants were grown from wild-collected rootstocks of individuals from 20 different populations throughout the western United States (Table 4.1; see Appendix 1, Table A.1 and Figure A.1.2 for more detailed locality data). Each sample was named using the site number followed by the unique individual identifier (eg., 26-220 is site 26, individual 220). For comparison, French tarragon plants were purchased from Pantry Garden Herbs (Cleveland, MO) and an octoploid plant from seed collected in Kyrgyzstan were also cultivated. All plants were grown in environmentally-controlled conditions at Rutgers University's research greenhouses, New Brunswick, New Jersey. Plants were fertilized bimonthly with Scotts Peters Professional 20-20-20 balanced fertilizer (Vero Beach, FL) at a rate of 500 ppm, and the greenhouse was maintained at a

daytime temperature of 21-24°C, a night time temperature of 18-23°C, and with a day length of 14 hours. Because the plants exhibited phenological variability in flowering time, aboveground material was harvested from individual plants when they reached the flowering stage. Even after an extended growing period some plants never produced flowers. These individuals, from which only vegetative material was harvested, are marked with † in Table 4.1. Herbarium vouchers were deposited at the Chrysler Herbarium (CHRB) at Rutgers University.

Essential oil isolation and analysis. 100 g of leaves and inflorescences were removed from the main stems and were immediately hydrodistilled in 1000 mL of deionized water using a modified Clevenger apparatus. For distillation, the material was placed in a 2 L round bottom flask and heated with a 115 watt heater, initially set to power level 100 for 30 minutes and then reduced to power level 60 for 1.5 hours. The oil was allowed to cool and measured in a calibrated tube, and then stored in glass vials in -20°C. For the gas chromatograph analysis, 15 µL of each sample was pipetted into Eppendorf tubes. The tubes were filled to 1.5 mL with methyl-tertiary butyl ether. The samples were vortexed for 20 seconds and crystalline anhydrous Na_2SO_4 was added to the 0.1 mL line. The tubes were vortexed again for 20 seconds and then centrifuged at 13,000 rpm for 5 minutes. All the liquid was removed and put into glass vials for analysis. The volatile oil of one sample from each population (Table 4.1) was analyzed with a gas chromatograph (Agilent GC System, 6890 Series, Santa Clara, CA) coupled to a mass spectrometer (Agilent 5973 network Mass Selective Detector) and all samples were analyzed with a gas chromatograph coupled to a flame ionization detector (FID). 1 µL of sample was

injected with an autosampler (Agilent 7683 Series), at an inlet temperature of 220 °C, into a HP5-MS Column (30 m x 0.25 mm inner diameter, 0.25 μm film thickness). The temperature program was set at 60°C for 1 minute, rising 4°C/min to 200°C, which was held for 4 minutes for a total run time of 35 minutes/sample. Helium was set at a constant flow of 1 ml/min. Individual identifications of chemical compounds were initially made by matching spectra with those from mass spectral libraries (Wiley 275.L; NIST Chemistry WebBook, 2008) and verified by comparing the Kovat's index, retention time and mass spectra with those reported in literature (Ulubelen et al., 1984; Harada and Iwasaki, 1982; Engelmeier et al., 2004; Polyanskaya et al., 2007). The percent concentration is defined as the relative percentage of each chemical constituent compared to total extract as determined by uncorrected FID analysis.

Statistical Analyses. All analyses were performed using the software program R version 2.9.2 (R Development Core Team, 2009). A hierarchical agglomerative cluster analysis was conducted to group the extracts based on a distance analysis derived from a matrix composed of the relative percentage of 30 different compounds in each extract. Twenty-one of the compounds were chosen because they represent the total diversity of the six most abundant compounds in each extract excluding limonene and β-phellandrene (Tables 4.2 and 4.3). Although both limonene and β-phellandrene were found to be present in a number of the samples, some samples with both compounds had peaks that were not adequately separated and therefore could not be accurately quantified. Because of this, these compounds were excluded from the analysis. In addition to the six major components from each sample, eight other commonly found, or unique, compounds were included as well ((*E*)-Hex-4-en-2-ynylbenzene, allo-ocimene, dracumerin, (*E*)-artemidin,

(*E*)-nerolidol, γ-terpinene, L-linalool and spatulenol; Table 4.3). A Euclidean distance matrix was produced using the "dist" function and hierarchical agglomerative cluster analysis were conducted using the "hclust" function with method = "average" to select the UPGMA method. Principal component analyses (PCA), principal coordinate analyses (PCoA), graphical biplots and point and axis predictivity graphs were produced using the package BiplotGUI version 0.0-5. Axis and point predictivities were used to indicate how well individual points or axes were represented in various dimensions of the biplot. Because the methyl chavicol content of some samples accounted for the majority of variation displayed in the PCA and PCoA, additional PCAs and PCoAs were conducted using a scale transformed data set and a reduced data set, which excluded the Kyrgyz sample, all samples from site 18, and samples 26-220 and 26-222.

Separate correlation analyses of the percent concentration of 26 of the essential oil compounds (allo-ocimene, bicyclogermacrene, capillarin, capillene, (E)-β-ocimene, (E)-Hex-4-en-2-ynylbenzene, E-nerolidol, germacrene-D, L-linalool, methyl chavicol, methyl eugenol, methyl salicylate, myrcene, spatulenol, xanthoxylin, (Z)-β-ocimene, α-fenchene, α-phellandrene, α-pinene, α-terpinolene, β-pinene, γ-curcumene, γ-decalactone, γ-terpinene, 1-(4-Methoxyphenyl)-2,4-pentadiyne and 5-phenyl-1,3-pentadiyne) with altitude, and with latitude, were conducted using Microsoft Excel.

Results

Populations of *Artemisia dracunculus* in North America (NA) exhibit a variety of different volatile profiles. The relative percentages of six primary essential oil compounds (21 compounds in total) for individuals from eighteen North American (NA)

populations, one individual from Kyrgyzstan, and six French tarragon plants are presented in Tables 4.2 and 4.3 and the relative percentages of the additional eight essential oil compounds included in the PCAs and PCoAs are shown in Table 4.4. (Z)-β-ocimene was found to be the most common primary component and was the most abundant in 22 of the 63 North American samples (Table 4.2). The second most common primary component was methyl eugenol, with 15 NA individuals having it as the most abundant compound. Of particular interest from a fragrance perspective, were ten individuals (8-50, 26-220, 26-222 and all samples from site 18) that were found to contain high amounts of p-allyanisole (47-70%). The samples from site 18 have a notable pleasant odor that is similar to that of French tarragon, and which was found to have methyl chavicol contents greater than 80%.

The individual sample from Kyrgyzstan was quite unique when compared to all other samples analyzed in this study. Unlike the other samples, its major constituents were myrcene, (Z)-artemidin and limonene (Table 4.2 and 4.3). Additionally, a number of compounds were found in the Kyrgyz sample that were not present in any other samples including (Z)-artemidin (25.854%), bornyl acetate (3.759%), (*E*)-artemidin (2.315) [trace amount tentatively identified in 19-153], camphene (1.07%), dracumerin (0.499%), β-sesquiphellandrene (0.322%), 4-terpineol (0.264%), neryl acetate (0.213%), etc. This sample also seems to represent a unique essential oil chemotype when compared to others described in the essential oil literature.

Another unique group of samples is composed of eight individuals from sites 19, 20, 21, 24 and 26, who were found to have capillene as the major component of the essential oil fraction (Table 4.2). Although capillene has been found in essential oil

extracted from NA plants, this is the first example of where it is the primary component (Pappas and Sturtz, 2001). Other *Artemisia* species in the subgenus *Dracunculus*, such as *A. capillaris* and *A. campestris* have been shown to have high amounts of capillene as well (Miyazawa and Kameoka, 1976a; Miyazawa and Kameoka, 1976b; Juteau et al., 2002). Although only one sample, 9-56, was found to have 5-phenyl-1,3-pentadiyne as its primary component (35.953%), this aromatic polyacetylene was found in high quantities (as much as 30%) as the second major component in many of the other samples. Additionally, 1-(4-Methoxyphenyl)-2,4-pentadiyne, was identified in a twenty-one samples as well. The isocoumarin acetylene, capillarin, was also found in the majority of (53) samples but was generally found in low amounts, with the highest percent concentration being between 3% and 4% of the extract and many of the samples were found to contain both an isocoumarin and polyacetylenes.

Six major clusters were formed UPGMA cluster analysis (Figure 4.1) showing groups rich in 1) methyl chavicol, 2) methyl eugenol, 3) α-terpinolene, 4) capillene, 5) 5-phenyl-1,3-pentadiyne and 6) (*E*)-β-ocimene/(*Z*)-β-ocimene. These rough clusters represent a number of new chemotypes for *Artemisia dracunculus* and could possibly be segregated into sub-groups based on the abundance of other compounds (Table 4.2). It is important to note that individuals from different populations may be of the same chemotype and that individuals within a population may be different chemotypes. The sample from Kyrgyzstan is quite unique and is sister to a cluster of all other samples.

The principal coordinate analysis (Figure 4.2) produced discrete groupings for the samples with high methyl chavicol and for high methyl eugenol content. The methyl chavicol group is divided into sub-clusters for French tarragon and wild tarragon samples

as was seen in the cluster analysis. This wild tarragon group is composed of all the individuals from site 18 in northern California, two out four individuals from site 26 in southern California, and a single individual from site 8 in southern Nevada. Two additional groups composed of nearly all other samples are also discernible, as well as three outliers - samples 2-11, 12-75 and the Kyrgyz sample. Interestingly, even with the unique profile of the Kyrgyz sample its point is located relatively close to the cloud composed of North American samples with a diversity of primary compounds. 71.1% of the variation in the samples is accounted for by the first two principal components (Table 4.5). When the loading value for compound 6 is considered (Tables 4.6 and 4.7), its influence on the first principal component is extremely high (-.936) and in effect, the variation in methyl chavicol drowns out the other variables, as seen in the PCA biplot in Figure 4.4 and Figure 4.5 (biplot with the axis for methyl chavicol [compound 6] highlighted). Therefore, the linear combination of the variables that has the largest possible variation (the 1st principal component) does not adequately represent the amount of difference that is found between all the remaining samples including the distinctly unique Kyrgyz sample. To avoid this suppression, a PCA, utilizing a scale transformation of the data (transforms each variable to unit variance) is presented in Figure 4.6. Now the Kyrgyz sample is completely isolated, while the remaining samples are clustered into one large group. Although this biplot, with the scale transformation, only represents 37% of the variance, it clearly removed the effect of methyl chavicol.

In order to determine how well the biplot in figure 4.4 approximates the distribution of variance, axis predictivities for the biplot are presented in Figure 4.7 and point predictivities are shown in Figures 4.7 and 4.8. The predictivities plots show how

well a point or axis is represented in the biplot. The further to the right it appears, the better represented it is in the first (or horizontal) biplot dimension. The closer it is to the top of the diagram, the better the point or axis is represented overall by both the first and the second biplot dimension. The contribution of the second biplot dimension is indicated by the vertical distance between the diagonal line and the point or axis. (La Grange et al. 2009).

As can be seen in figure 4.7 compound 6 is the variable well represented by component 1, while compounds 8, 16 and 25 are well represented by component 2. A further look at the point predictivities (rather than axis predictivities), shows that all the samples with high methyl chavicol content is well represented by component 1 while the rest of the samples vary in the degree that they are represented by component 2, with the Kyrgyz sample nearly unrepresented (Figures 4.7 and 4.8). An additional principal component analysis, using a data matrix with the Kyrgyz and high methyl chavicol containing samples removed, is presented in Figure 4.9. In this plot, 67.9% of the variation in the samples is represented by the first two principal components. By removing the samples that dominated the variation in the first principal component, more dispersal is seen between the remaining samples. As a result, the point predictivities show that the overall variation between samples is represented in the first two principal components (Figure 4.10). This analysis shows that PCoA and PCA should be utilized with the understanding that the results must be interpreted and it cannot just be assumed that these multivariate statistical methods will provide accurate and definitive explanations of variation that exist within a data set. Like the cluster analysis, the PCA biplots in figures 4.11 and 4.12 clearly show that distinct clusters do occur, but that the

various chemotypes (clusters) are not population specific. Examples of the Kovat indices and mass spectrum retention times for samples from sites 2, 6 and Kyrgyzstan are presented in Appendix 2. Correlation analyses to assess for relationships between the altitude at which the samples were collected and their percent concentration of 26 compounds did not reveal any strong correlations.

Discussion

This study shows that there is a number of different chemotypes in North American populations of *Artemisia dracunculus* and those individual populations often exhibit a heterogeneous composition with multiple chemotypes being present at one site.

Many of the samples contained particularly high percentages of the polyacetylenes capillene and 5-phenyl-1,3-pentadiyne and 1-(4-Methoxyphenyl)-2,4-pentadiyne. This differs from many of the studies focused on European and Asian populations which found sabinene, elemicin, trans-isoelemicine or *trans*-anethole as the most abundant compounds. These phenylacetylenic compounds are likely to function as defense compounds and have been shown to be feeding deterrents in experiments with *Pieris rapae crucivora* (white cabbage butterfly. Both have antimicrobial activity as well (Yashina and Vereshchagin, 1978; Yano, 1983). Wu et al. (2001) found that capillene, isolated from *A. capillaris*, inhibited induced blood platelet aggregation *in vitro*. In a similar fashion, *A. dracunculus* individuals rich in capillene could prove to have interesting biological activities. Other interesting compounds found in the essential oil of a number of individuals were xanthoxylin (brevifolin or 1-(2-hydroxy-4,6-dimethoxyphenyl)ethanone) and capillarin. Both were found in relatively low amounts

(highest accumulation for both compounds was less than 4.0%) but they have each been shown to have a number of significant biological activities including antispasmodic, antibacterial and antifungal activities (Filho et al., 1996; Meepagala et al., 2002). Xanthoxylin has also been shown to inhibit human cytochrome p450 3A4, an extremely important enzyme which is involved in the metabolism of many pharmaceuticals and foreign chemicals in the liver and intestine (Scott and Halpert, 2005; NCGC, 2010).

It is also interesting to note that a number of samples with pleasant odor and flavor characteristics also had higher percentages of methyl chavicol (samples 8-50, 26-220, 26-222 and all samples from site 18). Further analysis of these particular selections, using AEDA, would be useful in determining the most impactful flavor compounds from these plants. This methyl chavicol-rich germplasm may be useful in the development of a new line of tarragon with both the desirable flavor characteristics of French tarragon, as well as desirable traits of wild tarragon, such as increased biomass production and ability to produce seeds. It would be interesting to measure the methyl chavicol production by these individuals over the entire growing season in order to determine if there are similar fluctuations in methyl chavicol production to those observed in French tarragon (Deans and Svaboda, 1988).

The chemical composition from the essential oil of the Kyrgyz sample was completely unlike that of North American populations. Another study, using plant material obtained from Kyrgyzstan, recently reported a similar chemical profile (Bhutia and Valant-Vetschera, 2008). They reported that solvent extracts prepared from various parts of *A. dracunculus* plants contained quite significant amounts of artemidin and somewhat lesser amounts of dracumerin, which is similar to the results from this study.

Similarly, Engelmeier at al. (2004) also reported a butylisocoumarin-rich strain of *A. dracunculus* obtained from the botanical garden in Tashkent, Uzbekistan. Like the octoploid sample from Uzbekistan, which was used by Engelmeier et al. (2004), the Kyrgyz sample in the present study was determined to be an octoploid as well. These plants seem to have very different gene expression and metabolic products than the populations in North America and represent a very divergent Central Asian lineage of *A. dracunculus*.

Samples in NA do have essential oil profiles similar to those from some Siberian populations described by Polyankaya et al., (2007) and to a lesser extent by Rutskikh et al. (2000). There are no reports of ploidy levels for the populations sampled in these articles, but the essential oil profiles from Siberia populations were also quite different from the Central Asian octoploids. Rutskikh et al. also reported on populations from the Altai Republic which had high methyl chavicol content (42%), an accumulation of polyacetylenes and no isocoumarins. Similarly, the North American individuals with an abundance of methyl chavicol also exhibited accumulation of polyacetylenes and only trace amounts of the isocoumarin capillarin, although no samples from North America had capillarin contents above 4%. In many of the other North American populations polyacetylenes and isocoumarins were found in the same individuals. This finding is different from the Altai Republic populations, which did not find them in the same individuals.

As a species *Artemisia dracunculus* exhibits a large degree of morphologic, cytotypic and chemical variation. It is important that this variation is considered when wild tarragon essential oil is to be used in bioassays. It would behoove future researchers

interested in biological activity of *A. dracunculus* essential oil, as well as other types of phytochemical extracts, to conduct their research in concordance with a detailed analysis of the chemical composition of the extract, or at least document the precise source of the plant material. The novel chemotypes found in North American populations have interesting chemical compositions with an abundance of phenylacetylenes. Since many of the compounds found in these essential oil extracts have documented biological activities, it would be interesting to conduct a comparative analysis of the bioactivity of the different extracts.

Table 4.1. Plant collections used to produce essential oil samples for analysis by GC-FID and GC-MS*; Samples that were collected and extracted with only vegetative material are marked with †.

Sample	State	County	Locality	Latitude	Longitude
2-11*†	Colorado	Clear Creek	Georgetown	39.7354	-105.69
2-12	Colorado	Clear Creek	Georgetown	39.7354	-105.69
2-13	Colorado	Clear Creek	Georgetown	39.7354	-105.69
6-38*	Utah	Wayne	Bicknell	38.3044	-111.48
8-49*	Nevada	Mineral	Walker Lake	38.6211	-118.74
8-50	Nevada	Mineral	Walker Lake	38.6211	-118.74
8-51	Nevada	Mineral	Walker Lake	38.6211	-118.74
8-53	Nevada	Mineral	Walker Lake	38.6211	-118.74
9-55*	California	Plumas	Feather River	39.8196	-120.43
9-56†	California	Plumas	Feather River	39.8196	-120.43
9-57†	California	Plumas	Feather River	39.8196	-120.43
10-64*	Wyoming	Laramie	Happy Jack 1	41.1972	-105.27
12-75*	Colorado	Sedgwick	Julesburg	40.9744	-102.25
14-100*†	California	San Bernardino	Yucaipa	34.043	-117.06
14-101	California	San Bernardino	Yucaipa	34.043	-117.06
14-102	California	San Bernardino	Yucaipa	34.043	-117.06
14-103	California	San Bernardino	Yucaipa	34.043	-117.06
15-110*	California	San Diego	old hwy 395	33.2746	-117.15
15-113	California	San Diego	old hwy 395	33.2746	-117.15
15-114	California	San Diego	old hwy 395	33.2746	-117.15
16-120*†	California	San Diego	Julian	33.0883	-116.59
16-123	California	San Diego	Julian	33.0883	-116.59
16-124†	California	San Diego	Julian	33.0883	-116.59
17-131*	California	Inyo	Onion Valley	36.7717	-118.34
17-132	California	Inyo	Onion Valley	36.7717	-118.34
17-133†	California	Inyo	Onion Valley	36.7717	-118.34
17-134	California	Inyo	Onion Valley	36.7717	-118.34
18-140*	California	Plumas	Crescent Mills	40.0837	-120.92

Sample	State	County	Locality	Latitude	Longitude
18-141	California	Plumas	Crescent Mills	40.0837	-120.92
18-142	California	Plumas	Crescent Mills	40.0837	-120.92
18-143	California	Plumas	Crescent Mills	40.0837	-120.92
18-144†	California	Plumas	Crescent Mills	40.0837	-120.92
18-145	California	Plumas	Crescent Mills	40.0837	-120.92
18-148	California	Plumas	Crescent Mills	40.0837	-120.92
19-150*†	California	Lassen	Susanville	40.3353	-120.57
19-151	California	Lassen	Susanville	40.3353	-120.57
19-152	California	Lassen	Susanville	40.3353	-120.57
19-153†	California	Lassen	Susanville	40.3353	-120.57
19-155	California	Lassen	Susanville	40.3353	-120.57
20-160*	California	Inyo	Rt 168	37.2532	-118.16
20-163	California	Inyo	Rt 168	37.2532	-118.16
20-164	California	Inyo	Rt 168	37.2532	-118.16
21-171†	California	Inyo	Death Valley Rd	37.17	-118.21
21-172	California	Inyo	Death Valley Rd	37.17	-118.21
21-174*	California	Inyo	Death Valley Rd	37.17	-118.21
22-180*	California	Inyo	Nine Mile Cyn 1	35.8507	-117.94
22-181	California	Inyo	Nine Mile Cyn 1	35.8507	-117.94
22-182	California	Inyo	Nine Mile Cyn 1	35.8507	-117.94
22-183	California	Inyo	Nine Mile Cyn 1	35.8507	-117.94
22-184	California	Inyo	Nine Mile Cyn 1	35.8507	-117.94
24-200	California	Los Angeles	Elizabeth Lake	34.6612	-118.38
24-201	California	Los Angeles	Elizabeth Lake	34.6612	-118.38
24-202*	California	Los Angeles	Elizabeth Lake	34.6612	-118.38
24-203	California	Los Angeles	Elizabeth Lake	34.6612	-118.38
24-204	California	Los Angeles	Elizabeth Lake	34.6612	-118.38
25-210	California	San Bernadino	Hesperia	34.3656	-117.24
25-212	California	San Bernadino	Hesperia	34.3656	-117.24
25-213*	California	San Bernadino	Hesperia	34.3656	-117.24

Sample	State	County	Locality	Latitude	Longitude
25-214	California	San Bernadino	Hesperia	34.3656	-117.24
26-220	California	San Bernadino	Running Springs	34.2026	-117.09
26-221*†	California	San Bernadino	Running Springs	34.2026	-117.09
26-222	California	San Bernadino	Running Springs	34.2026	-117.09
26-224	California	San Bernadino	Running Springs	34.2026	-117.09
French 1 (F1) †	purchased				
French 2 (F2) †	purchased				
French 4 (F4) †	purchased				
French 8 (F8) †	purchased				
French 10 (F10)* †	purchased				
Kyrgyz*†	Kyrgyzstan	Naryn Province			

Table 4.2. 1st, 2nd and 3rd main components and their percentage of the total extract for each sample; 5-Ph-1,3-PD = 5-phenyl-1,3-pentadiyne; 1-(4-MP)-2,4-PD =1-(4-Methoxyphenyl)-2,4-pentadiyne; unknowns are labeled with their mass spectral retention times.

sample	main component	% total	2nd component	% total	3rd component	% total
2-11	methyl eugenol	30.413	(Z)-β-ocimene	27.556	methyl chavicol	14.188
2-12	methyl eugenol	66.094	(Z)-β-ocimene	18.115	methyl chavicol	6.827
2-13	methyl eugenol	51.231	(Z)-β-ocimene	30.618	(*E*)-β-ocimene	4.659
6-38	(Z)-β-ocimene	47.199	5-Ph-1,3-PD	26.361	β-phellandrene	6.395
8-49	(Z)-β-ocimene	43.831	(*E*)-β-ocimene	19.927	1-(4-MP)-2,4-PD	13.374
8-50	methyl chavicol	47.443	α-terpinolene	17.499	(*E*)-β-ocimene	9.029
8-51	(Z)-β-ocimene	41.538	capillene	27.666	(*E*)-β-ocimene	12.168
8-53	(Z)-β-ocimene	48.753	5-Ph-1,3-PD	15.962	(*E*)-β-ocimene	11.373
9-55	(Z)-β-ocimene	37.25	5-Ph-1,3-PD	30.03	(*E*)-β-ocimene	9.528
9-56	5-Ph-1,3-PD	35.953	(Z)-β-ocimene	32.666	(*E*)-β-ocimene	7.011
9-57	(Z)-β-ocimene	34.315	5-Ph-1,3-PD	26.098	(*E*)-β-ocimene	10.527
10-64	methyl eugenol	58.518	(Z)-β-ocimene	29.461	α-terpinolene	2.876
12-75	(Z)-β-ocimene	51.557	(*E*)-β-ocimene	19.745	methyl chavicol	14.831
14-100	α-terpinolene	40.79	5-Ph-1,3-PD	27.834	(Z)-β-ocimene	9.18
14-101	methyl eugenol	42.706	α-terpinolene	13.241	capillene	10.595
14-102	capillene	21.56	α-terpinolene	18.603	(*E*)-β-ocimene	13.107
14-103	methyl eugenol	64.471	(*E*)-β-ocimene	10.76	capillene	5.417
15-110	methyl eugenol	42.303	(*E*)-β-ocimene	24.789	(Z)-β-ocimene	15.848
15-113	methyl eugenol	46.205	α-phellandrene	12.822	(*E*)-β-ocimene	10.233
15-114	methyl eugenol	40.589	capillene	16.508	(*E*)-β-ocimene	14.009
16-120	methyl eugenol	40.214	(*E*)-β-ocimene	12.229	5-Ph-1,3-PD	11.584
16-123	α-terpinolene	17.724	capillene	17.202	α-phellandrene	16.46
16-124	α-phellandrene	27.381	α-terpinolene	19.603	(*E*)-β-ocimene	15.794
17-131	(Z)-β-ocimene	33.313	(*E*)-β-ocimene	24.249	methyl eugenol	11.32
17-132	(Z)-β-ocimene	43.527	1-(4-MP)-2,4-PD	21.677	capillene	12.7

sample	main component	% total	2nd component	% total	3rd component	% total
17-133	(Z)-β-ocimene	44.085	(*E*)-β-ocimene	18.746	methyl eugenol	14.654
17-134	methyl eugenol	44.862	α-terpinolene	15.578	(*E*)-β-ocimene	14.905
18-140	methyl chavicol	51.603	(Z)-β-ocimene	23.318	(*E*)-β-ocimene	11.887
18-141	methyl chavicol	50.577	(Z)-β-ocimene	39.072	(*E*)-β-ocimene	3.723
18-142	methyl chavicol	65.352	(Z)-β-ocimene	16.628	(*E*)-β-ocimene	6.429
18-143	methyl chavicol	68.754	(Z)-β-ocimene	25.598	(*E*)-β-ocimene	2.134
18-144	methyl chavicol	69.995	(Z)-β-ocimene	18.834	(*E*)-β-ocimene	6.523
18-145	methyl chavicol	60.841	(Z)-β-ocimene	29.935	(*E*)-β-ocimene	3.413
18-148	methyl chavicol	54.681	(Z)-β-ocimene	36.437	(*E*)-β-ocimene	2.759
19-150	(Z)-β-ocimene	47.789	5-Ph-1,3-PD	27.945	capillene	9.165
19-151	(Z)-β-ocimene	54.553	5-Ph-1,3-PD	28.857	capillene	8.228
19-152	(Z)-β-ocimene	55.232	5-Ph-1,3-PD	28.376	capillene	7.942
19-153	capillene	47.53	(Z)-β-ocimene	33.042	(*E*)-β-ocimene	12.03
19-155	(Z)-β-ocimene	46.347	5-Ph-1,3-PD	31.312	capillene	8.134
20-160	capillene	45.533	(Z)-β-ocimene	39.014	(*E*)-β-ocimene	4.351
20-163	(Z)-β-ocimene	30.682	capillene	26.846	(*E*)-β-ocimene	17.345
20-164	capillene	40.817	(Z)-β-ocimene	40.281	(*E*)-β-ocimene	11.222
21-171	(*E*)-β-ocimene	47.73	(Z)-β-ocimene	19.091	5-Ph-1,3-PD	9.321
21-172	capillene	35.662	(*E*)-β-ocimene	19.354	(Z)-β-ocimene	18.209
21-174	capillene	32.975	(Z)-β-ocimene	17.598	(*E*)-β-ocimene	17.476
22-180	(Z)-β-ocimene	28.017	α-terpinolene	23.296	α-phellandrene	18.648
22-181	α-terpinolene	27.921	(Z)-β-ocimene	20.552	capillene	14.947
22-182	(Z)-β-ocimene	39.231	(*E*)-β-ocimene	20.979	α-phellandrene	13.246
22-183	α-terpinolene	33.487	capillene	20.094	(Z)-β-ocimene	13.728
22-184	α-terpinolene	41.69	(Z)-β-ocimene	24.871	α-phellandrene	10.511
24-200	methyl eugenol	39.374	capillene	18.609	(Z)-β-ocimene	9.989
24-201	(Z)-β-ocimene	26.476	(*E*)-β-ocimene	18.884	α-phellandrene	14.302

sample	main component	% total	2nd component	% total	3rd component	% total
24-202	(Z)-β-ocimene	36.439	capillene	29.675	α-terpinolene	9.63
24-203	(Z)-β-ocimene	34.327	capillene	27.856	(*E*)-β-ocimene	21.688
24-204	capillene	30.745	(Z)-β-ocimene	29.444	α-terpinolene	20.484
25-210	α-terpinolene	24.869	(*E*)-β-ocimene	22.72	capillene	22.373
25-212	methyl eugenol	40.411	(Z)-β-ocimene	26.324	(*E*)-β-ocimene	9.914
25-213	methyl eugenol	42.03	α-terpinolene	14.976	(Z)-β-ocimene	12.836
25-214	methyl eugenol	55.216	α-terpinolene	15.105	(*E*)-β-ocimene	8.549
26-220	methyl chavicol	56.545	(*E*)-β-ocimene	11.084	(Z)-β-ocimene	7.625
26-221	(Z)-β-ocimene	45.709	5-Ph-1,3-PD	16.903	(*E*)-β-ocimene	11.619
26-222	methyl chavicol	56.484	5-Ph-1,3-PD	9.945	α-terpinolene	7.35
26-224	capillene	21.093	α-terpinolene	18.063	5-Ph-1,3-PD	16.484
French 1	methyl chavicol	82.871	(Z)-β-ocimene	6.198	(*E*)-β-ocimene	5.536
French 10	methyl chavicol	84.126	(Z)-β-ocimene	5.55	(*E*)-β-ocimene	5.232
French 2	methyl chavicol	80.753	(Z)-β-ocimene	6.298	(*E*)-β-ocimene	5.478
French 4	methyl chavicol	82.923	(Z)-β-ocimene	6.375	(*E*)-β-ocimene	5.501
French 8	methyl chavicol	83.886	(Z)-β-ocimene	5.578	(*E*)-β-ocimene	5.349
Kyrgyz	myrcene	27.152	(*Z*)-artemidin	25.854	limonene	11.617

Table 4.3. 4th, 5th and 6th main components and their percentage of the total extract for each sample; 5-Ph-1,3-PD = 5-phenyl-1,3-pentadiyne; 1-(4-MP)-2,4-PD =1-(4-Methoxyphenyl)-2,4-pentadiyne; unknowns are labeled with their mass spectral retention times

sample	4th component	% total	5th component	% total	6th component	% total
2-11	α-terpinolene	13.538	(*E*)-β-ocimene	4.385	xanthoxylin	2.479
2-12	(*E*)-β-ocimene	3.518	α-terpinolene	1.36	β-phellandrene	0.965
2-13	xanthoxylin	3.495	α-terpinolene	3.079	β-phellandrene	2.419
6-38	(*E*)-β-ocimene	5.415	capillene	3.538	α-terpinolene	2.432
8-49	α-terpinolene	6.592	α-phellandrene	2.55	capillarin	2.049
8-50	(Z)-β-ocimene	5.929	methyl eugenol	5.58	5-Ph-1,3-PD	5.544
8-51	methyl eugenol	9.805	β-phellandrene	2.661	α-pinene	0.815
8-53	β-phellandrene	9.084	capillarin	2.712	α-fenchene	2.447
9-55	capillene	6.273	α-phellandrene	4.093	α-terpinolene	2.934
9-56	capillene	6.096	α-terpinolene	5.666	β-phellandrene	4.455
9-57	capillene	9.974	α-terpinolene	3.601	β-phellandrene	3.197
10-64	(*E*)-β-ocimene	2.78	xanthoxylin	1.853	germacrene-D	0.661
12-75	α-terpinolene	2.433	methyl eugenol	1.919	germacrene-D	1.884
14-100	(*E*)-β-ocimene	8.116	capillene	2.864	germacrene-D	2.302
14-101	(*E*)-β-ocimene	9.321	5-Ph-1,3-PD	8.4	(Z)-β-ocimene	6.475
14-102	5-Ph-1,3-PD	12.58	(Z)-β-ocimene	9.335	α-phellandrene	5.156
14-103	α-terpinolene	4.636	5-Ph-1,3-PD	3.688	methyl chavicol	3.034
15-110	α-phellandrene	3.779	α-terpinolene	2.444	capillarin	2.109
15-113	(Z)-β-ocimene	7.761	α-terpinolene	6.383	β-phellandrene	2.901
15-114	(Z)-β-ocimene	9.158	α-terpinolene	6.468	xanthoxylin	2.574
16-120	α-phellandrene	10.711	α-terpinolene	7.697	(Z)-β-ocimene	4.552
16-123	5-Ph-1,3-PD	16.171	(*E*)-β-ocimene	6.402	methyl eugenol	4.581
16-124	(Z)-β-ocimene	14.707	α-fenchene	3.129	myrcene	2.93
17-131	5-Ph-1,3-PD	6.221	sabinene	5.622	terpinolene	2.362
17-132	(*E*)-β-ocimene	11.328	5-Ph-1,3-PD	3.224	α-fenchene	1.181

sample	4th component	% total	5th component	% total	6th component	% total
17-133	1-(4-MP)-2,4-PD	10.383	α-fenchene	1.599	bicyclogermacrene	0.995
17-134	5-Ph-1,3-PD	8.666	germacrene-D	1.473	capillene	1.422
18-140	α-phellandrene	0.7	α-terpinolene	1.907	β-phellandrene	2.606
18-141	β-phellandrene	2.439	germacrene-D	0.391	γ-curcumene	0.297
18-142	β-phellandrene	3.225	α-terpinolene	2.609	α-phellandrene	1.544
18-143	β-phellandrene	0.532	γ-curcumene	0.385	germacrene-D	0.363
18-144	β-phellandrene	0.733	α-terpinolene	0.699	α-fenchene	0.417
18-145	(*E*)-β-ocimene	1.159	germacrene-D	0.576	unknown 4.177	0.57
18-148	β-phellandrene	1.838	unknown 4.177	0.411	germacrene-D	0.364
19-150	β-phellandrene	3.471	(*E*)-β-ocimene	3.093	α-terpinolene	2.998
19-151	(*E*)-β-ocimene	3.424	unknown 4.177	0.488	γ-decalactone	0.406
19-152	(*E*)-β-ocimene	3.466	unknown 4.177	0.483	γ-decalactone	0.38
19-153	methyl salicylate	0.963	5-Ph-1,3-PD	0.887	unknown 14.836	0.709
19-155	β-phellandrene	5.084	(*E*)-β-ocimene	3.668	α-phellandrene	0.595
20-160	capillarin	3.396	β-pinene	1.835	1-(4-MP)-2,4-PD	0.892
20-163	methyl eugenol	16.105	capillarin	2.067	α-phellandrene	1.338
20-164	capillarin	2.897	5-Ph-1,3-PD	0.792	germacrene-D	0.575
21-171	methyl eugenol	9.243	capillene	5.006	capillarin	2.859
21-172	methyl eugenol	7.097	α-terpinolene	4.63	1-(4-MP)-2,4-PD	3.537
21-174	5-Ph-1,3-PD	7.399	methyl eugenol	6.166	α-terpinolene	5.436
22-180	(*E*)-β-ocimene	10.298	β-phellandrene	3.34	myrcene	2.152
22-181	(*E*)-β-ocimene	11.879	α-phellandrene	7.594	5-Ph-1,3-PD	5.202
22-182	α-terpinolene	7.766	capillarin	3.112	capillene	2.822
22-183	α-phellandrene	9.347	(*E*)-β-ocimene	7.813	capillarin	2.719
22-184	α-fenchene	6.947	myrcene	2.073	β-phellandrene	1.711
24-200	5-Ph-1,3-PD	9.258	α-terpinolene	8.939	(*E*)-β-ocimene	6.058
24-201	α-terpinolene	13.675	α-fenchene	3.645	methyl eugenol	2.849

sample	4th component	% total	5th component	% total	6th component	% total
24-202	(*E*)-β-ocimene	9.512	α-phellandrene	2.5	capillarin	1.998
24-203	α-terpinolene	3.555	capillarin	2.916	α-phellandrene	1.327
24-204	(*E*)-β-ocimene	7.304	capillarin	2.249	α-phellandrene	1.541
25-210	(Z)-β-ocimene	10.058	5-Ph-1,3-PD	6.817	capillarin	2.08
25-212	α-terpinolene	5.022	α-phellandrene	4.338	capillene	4.252
25-213	(*E*)-β-ocimene	5.123	α-phellandrene	4.073	capillene	4.019
25-214	5-Ph-1,3-PD	5.97	(Z)-β-ocimene	3.901	1-(4-MP)-2,4-PD	2.084
26-220	α-terpinolene	7.557	5-Ph-1,3-PD	4.9	methyl eugenol	3.453
26-221	1-(4-MP)-2,4-PD	6.611	capillene	4.915	α-fenchene	3.745
26-222	(Z)-β-ocimene	6.786	capillene	4.22	(*E*)-β-ocimene	4.163
26-224	α-phellandrene	15.93	(Z)-β-ocimene	10.003	(*E*)-β-ocimene	5.584
French 1	limonene	2.232	methyl eugenol	0.751	α-pinene	0.564
French 10	limonene	1.858	methyl eugenol	0.836	α-pinene	0.49
French 2	limonene	2.484	α-pinene	0.655	methyl eugenol	0.64
French 4	limonene	2.281	methyl eugenol	0.685	α-pinene	0.559
French 8	limonene	2.496	α-pinene	0.504	methyl eugenol	0.499
Kyrgyz	terpinolene	5.218	α-pinene	4.826	β-pinene	3.812

Table 4.4. Additional compounds included in cluster, PCoA and PCA analyses. Values represent the relative percentage of each extract.

sample	(*E*)-Hex-4-en -2-ynylbenzene	(*Z*)- artemidin	allo- ocimene	dracumerin	(*E*)- artemidin	(*E*)- nerolidol	γ - terpinene	L- linalool	spatulenol
2-11	0	0	0.196	0	0	0.059	0.105	0.121	0
2-12	0	0	0.101	0	0	0	0.055	0.048	0
2-13	0	0	0.216	0	0	0.14	0.075	0.078	0
6-38	0.034	0	0.153	0	0	0.087	0.088	0.116	0.282
8-49	0	0	0.292	0	0	0.078	0.14	0.139	0
8-50	0	0	0.046	0	0	0.046	0.191	0.155	0.055
8-51	0.253	0	0.26	0	0	0.068	0.093	0.242	0
8-53	0	0	0.182	0	0	0.092	0.102	0.04	0.14
9-55	0.082	0	0.207	0	0	0.092	0.116	0	0.359
9-56	0.081	0	0.124	0	0	0.182	0.038	0.081	0.166
9-57	0.135	0	0.205	0	0	0.432	0.045	0.093	0.289
10-64	0	0	0.198	0	0	0.075	0.118	0.058	0
12-75	0	0	0.279	0	0	0.065	0.087	0.044	0.06
14-100	0	0	0.058	0	0	0	0.368	0	0.184
14-101	0.111	0	0.047	0	0	0.051	0.094	0	0
14-102	0.264	0	0.054	0	0	0.284	0.154	0	0.038
14-103	0.056	0	0	0	0	0.101	0.042	0	0
15-110	0	0	0.113	0	0	0.108	0.072	0	0
15-113	0	0	0.062	0	0	0.115	0.212	0	0
15-114	0.146	0	0.066	0	0	0.063	0.068	0	0
16-120	0.058	0	0.034	0	0	0.033	0.271	0	0
16-123	0.205	0	0	0	0	0.051	0.362	0	0
16-124	0	0	0.105	0	0	0.127	0.481	0	0.563
17-131	0	0	0.125	0	0	0.14	0.033	0.1	0.936

sample	(*E*)-Hex-4-en -2-ynylbenzene	(*Z*)- artemidin	allo- ocimene	dracumerin	(*E*)- artemidin	(*E*)- nerolidol	γ - terpinene	L- linalool	spatulenol
17-132	0.117	0	0.178	0	0	0.044	0	0.275	0.422
17-133	0	0	0.277	0	0	0.279	0.228	0.304	0.09
17-134	0	0	0	0	0	0.24	0.108	0.106	0.135
18-140	0	0	0.126	0	0	0.241	0.035	0.046	0.058
18-141	0	0	0.292	0	0	0.138	0.057	0.063	0
18-142	0	0	0.112	0	0	0.05	0.051	0	0
18-143	0	0	0.158	0	0	0.048	0	0.016	0.013
18-144	0	0	0.13	0	0	0.042	0	0	0
18-145	0	0	0.123	0	0	0.126	0.04	0.084	0
18-148	0	0	0.264	0	0	0.114	0.038	0.069	0
19-150	0.133	0	0.197	0	0	0.145	0	0	0.13
19-151	0.105	0	0.295	0	0	0	0.095	0	0.13
19-152	0.107	0	0.288	0	0	0	0.097	0	0.129
19-153	0.397	?	0.237	0	0	0.256	0	0.041	0.072
19-155	0	0	0.284	0	0	0.084	0.042	0.059	0
20-160	0.377	0	0.222	0	0	0.052	0.087	0	0.087
20-163	0.264	0	0.215	0	0	0.086	0.113	0.03	0
20-164	0.281	0	0.252	0	0	0.052	0.09	0	0
21-171	0	0	0.125	0	0	0	0	0	0
21-172	0.353	0	0.143	0	0	0	0.115	0	0
21-174	0.295	0	0.137	0	0	0.106	0.086	0	0.072
22-180	0	0	0.193	0	0	0.203	0.423	0	0.176
22-181	0.198	0	0.152	0	0	0.017	0.267	0	0
22-182	0	0	0.259	0	0	0.213	0.366	0	0
22-183	0.292	0	0.107	0	0	0.087	0.281	0	0
22-184	0	0	0.168	0	0	0.154	0.377	0.031	0

sample	(*E*)-Hex-4-en -2-ynylbenzene	(*Z*)- artemidin	allo- ocimene	dracumerin	(*E*)- artemidin	(*E*)- nerolidol	γ - terpinene	L- linalool	spatulenol
24-200	0.088	0	0.06	0	0	0	0.091	0	0
24-201	0	0	0.201	0	0	0.13	0.357	0	0
24-202	0.301	0	0.243	0	0	0.109	0.156	0	0
24-203	0.27	0	0.172	0	0	0.142	0	0	0
24-204	0.312	0	0.209	0	0	0.218	0.213	0	0
25-210	0.249	0	0	0	0	0.348	0.234	0	0.334
25-212	0	0	0.194	0	0	0.2	0.005	0	0
25-213	0.043	0	0.112	0	0	0.228	0.13	0	0
25-214	0	0	0.032	0	0	0.08	0.216	0.061	0
26-220	0	0	0.077	0	0	0.059	0.085	0	0
26-221	0.055	0	0.271	0	0	0.203	0	0.062	0.114
26-222	0	0	0	0	0	0	0.111	0	0
26-224	0.22	0	0.067	0	0	0.121	0.282	0	0.05
French 1	0	0	0.061	0	0	0	0	0	0
French 2	0	0	0.053	0	0	0	0	0	0
French 4	0	0	0.051	0	0	0	0	0.015	0
French 8	0	0	0.049	0	0	0	0	0.019	0
French10	0	0	0.047	0	0	0	0	0	0
Kyrgyz	0	25.854	0	0.499	2.315	0.071	0.143	0.197	0

Table 4.5. The standard deviation, proportion of variance and cumulative proportion of variance for the PCA derived from all samples and shown in figure 4.3.

	standard deviation	proportion of variance (POV)	cumulative POV
Comp.1	29.57778	0.4716003	0.4716003
Comp.2	21.254328	0.2435217	0.715122
Comp.3	14.182787	0.1084341	0.8235562
Comp.4	11.503228	0.07133167	0.89488785
Comp.5	8.7849477	0.04160268	0.93649053
Comp.6	7.4751502	0.03012193	0.96661246
Comp.7	5.5152152	0.01639713	0.98300959
Comp.8	4.3072307	0.0100009	0.99301049
Comp.9	3.2267728	0.005612802	0.998623293
Comp.10	1.0401264	0.000583197	0.99920649
Comp.11	0.6977609	0.000262456	0.999468946
Comp.12	0.6129644	0.000202541	0.999671487
Comp.13	0.514414	0.000142649	0.999814136
Comp.14	3.72E-01	7.47E-05	1.00E+00
Comp.15	2.32E-01	2.90E-05	1.00E+00
Comp.16	2.19E-01	2.59E-05	1.00E+00
Comp.17	1.86E-01	1.86E-05	1.00E+00
Comp.18	1.59E-01	1.37E-05	1.00E+00
Comp.19	1.27E-01	8.70E-06	1.00E+00
Comp.20	1.05E-01	5.96E-06	1.00E+00
Comp.21	8.53E-02	3.92E-06	1.00E+00
Comp.22	5.74E-02	1.77E-06	1.00E+00
Comp.23	4.81E-02	1.25E-06	1.00E+00
Comp.24	3.81E-02	7.80E-07	1.00E+00
Comp.25	3.50E-02	6.59E-07	1.00E+00
Comp.26	2.84E-02	4.35E-07	1.00E+00
Comp.27	2.44E-02	3.20E-07	1.00E+00
Comp.28	1.87E-02	1.88E-07	1.00E+00
Comp.29	6.01E-09	1.94E-20	1.00E+00
Comp.30	0.00E+00	0.00E+00	1.00E+00

Table 4.6. The loadings (correlation of original variables with each principal component [1-16]) for the PCA derived from all samples and shown in figure 4.3.

	comp. 1	comp. 2	comp. 3	comp. 4	comp. 5	comp. 6	comp. 7	comp. 8	comp. 9	comp. 10	comp. 11	comp. 12	comp. 13	comp. 14	comp. 15
compd1															
compd2							-0.515	-0.194	-0.154	-0.203	-0.142	-0.114	-0.234	-0.125	
compd3											0.221	0.797	-0.542	-0.101	
compd4								-0.193	0.938	-0.175			-0.151		
compd5			-0.176	-0.149	-0.21	-0.103		0.87		-0.262	-0.128		-0.156		
compd6	-0.936						0.169			-0.125			-0.151		
compd7		-0.161		-0.45	0.743	0.317	0.173			-0.128			-0.175		
compd8															
compd9										0.788	-0.481	-0.141	-0.265	-0.177	
compd10															0.649
compd11			-0.5	-0.337	-0.132	-0.477	0.408	-0.352	-0.107	-0.17	-0.103		-0.157		
compd12															0.664
compd13												0.133	0.247		0.252
compd14										0.214	0.718	-0.478	-0.358		
compd15	0.166	-0.183	-0.307	0.785	0.37	-0.115	0.116			-0.125	-0.125		-0.13		
compd16	0.164	-0.508	0.671		-0.175	-0.323	0.188		-0.12	-0.155	-0.102		-0.157		
compd17															
compd18															
compd19															
compd20													0.103		-0.143
compd21															
compd22											0.176		0.233	-0.946	
compd23															
compd24	0.207	0.822	0.388	0.126	0.117	-0.113	0.152			-0.123	-0.101		-0.142		
compd25															
compd26							-0.526	-0.144	-0.158	-0.219	-0.164	-0.147	-0.256		
compd27													0.13		
compd28													0.1		
compd29				0.119	-0.429	0.722	0.376	-0.109	-0.171	-0.146	-0.159		-0.171		
compd30															

Table 4.7. The loadings (correlation of original variables with each principal component [17-30]) for the PCA derived from all samples and shown in figure 4.3.

	comp. 16	comp. 17	comp. 18	comp. 19	comp. 20	comp. 21	comp. 22	comp. 23	comp. 24	comp. 25	comp. 26	comp. 27	comp. 28	comp. 29	comp. 30
compd1							0.111			-0.201	0.531	0.668	0.447		
compd2					-0.129		-0.13		-0.63	-0.14	0.2				
compd3															
compd4															
compd5															
compd6															
compd7															
compd8									-0.207		-0.292	-0.299	0.867		
compd9	0.108														
compd10	-0.402	-0.24	0.381	0.372	-0.135	0.102	-0.111								
compd11															
compd12	0.142	0.564	-0.308	-0.266	0.19										
compd13	0.68	-0.48	-0.126	0.136		-0.302		0.123		-0.106					
compd14	0.126			0.136	-0.124										
compd15															
compd16															
compd17														0.997	
compd18															-0.994
compd19			-0.309		-0.1	-0.204		-0.85			-0.271	0.141			
compd20	0.316	0.501	0.171	0.737						0.146					
compd21				-0.135		-0.254	-0.134	0.202	-0.219	0.805		0.388			
compd22															
compd23						-0.125	-0.305	-0.273	0.243	0.325	0.634	-0.482	0.102		
compd24															
compd25	-0.3		-0.764	0.346	-0.178	0.117	-0.123	0.308			0.12				
compd26		-0.119							0.626	0.141	-0.206				
compd27	0.306	-0.115		-0.16	-0.158	0.826	-0.246	-0.144		0.174			0.109		
compd28		0.273		-0.124	-0.903	-0.167			0.143						
compd29															
compd30		-0.101				0.199	0.868		-0.128	0.287	0.203	-0.186			

Figure 4.1. UPGMA cluster analysis of *A. dracunculus* essential oil samples based on the percent concentration of 30 oil constituents.

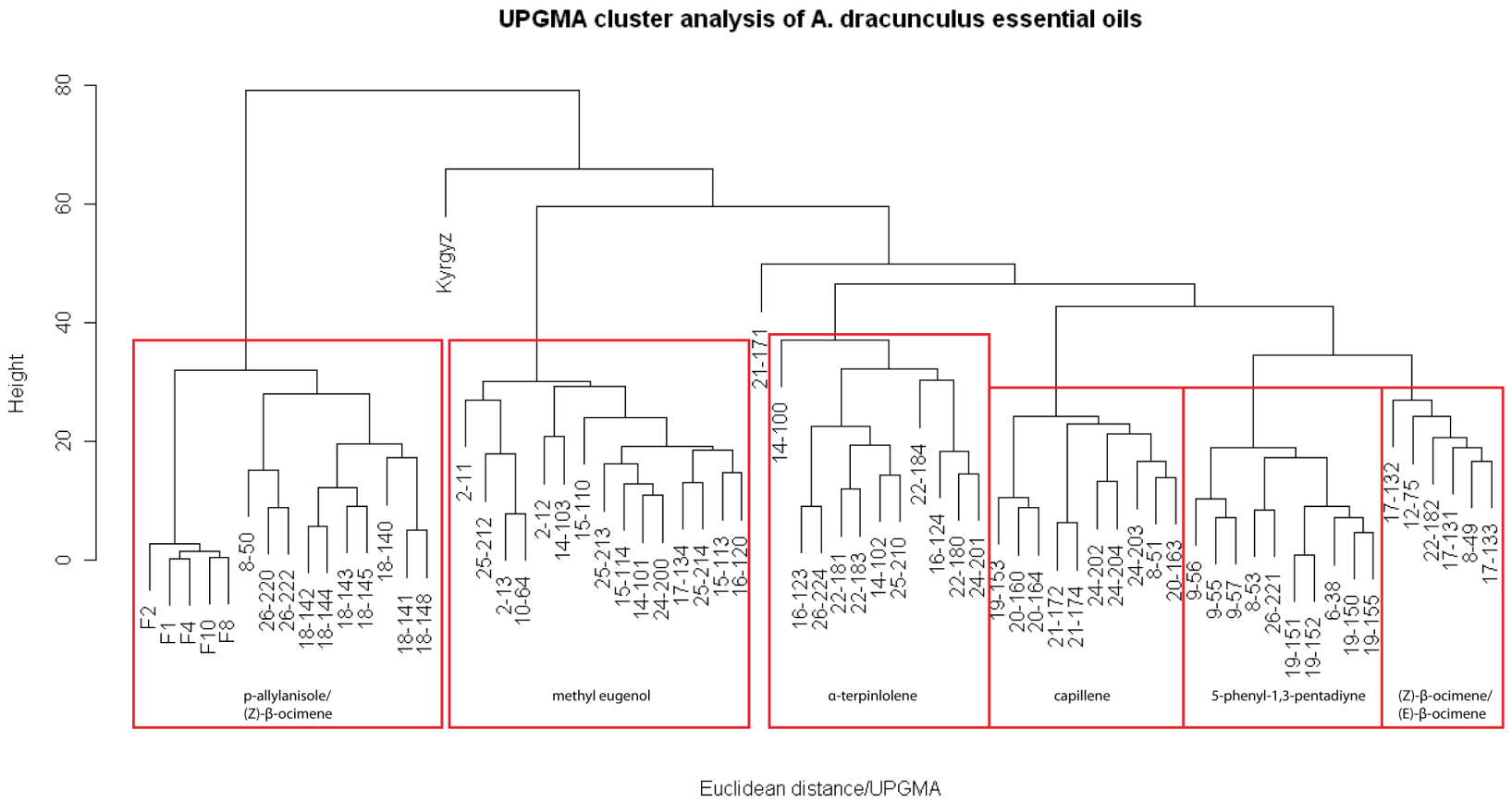


Figure 4.2. Plot of principal coordinate analysis of *A. dracunculus* samples based on essential oil constituents. Each point represents an individual extract and is labeled with the site number and individual number. The points are also color and shape-coded by collection site (French tarragon = 27 and Kyrgyz = 28) and clusters are defined by the red outlines.

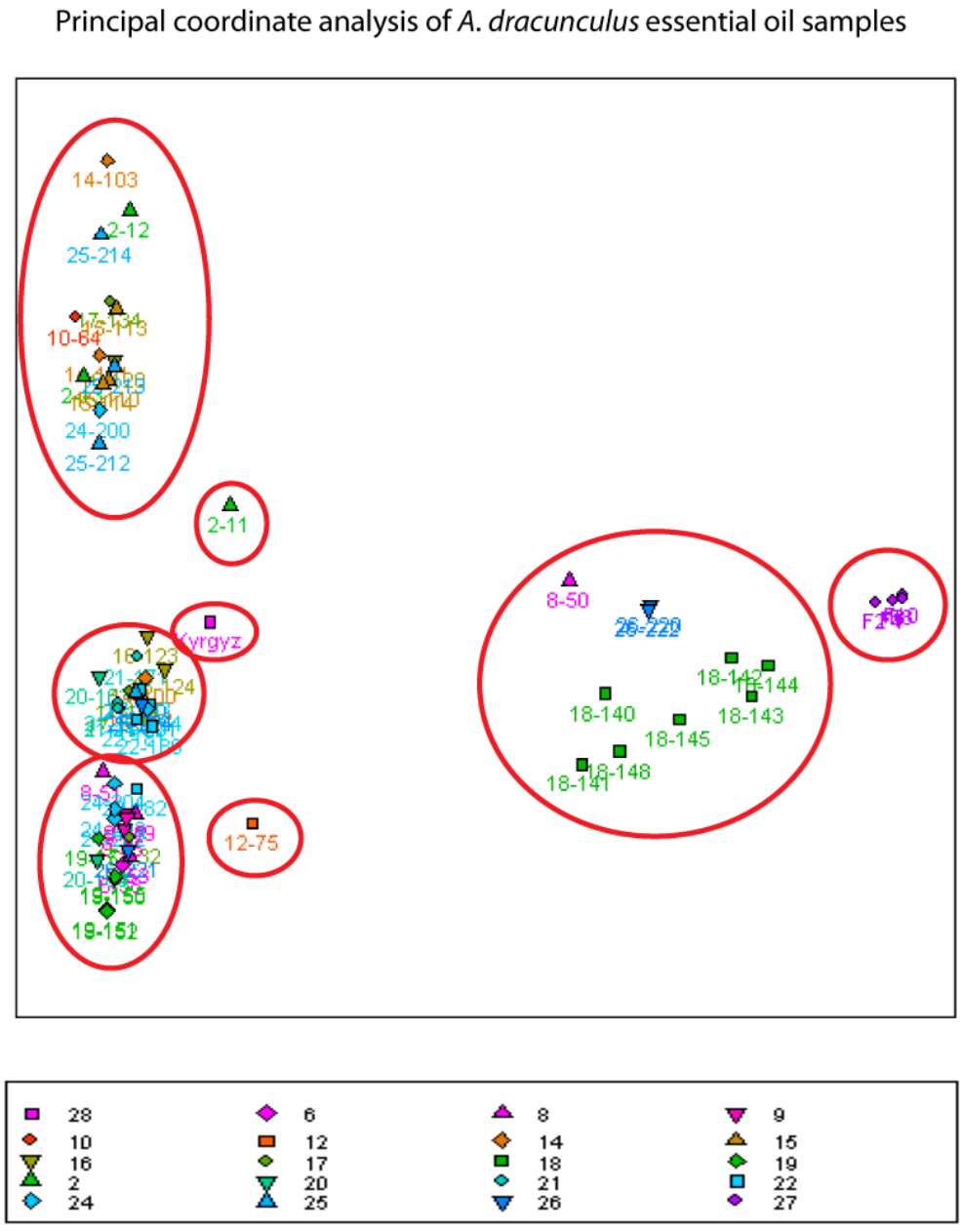


Figure 4.3. Plot of principal component analysis of *A. dracunculus* samples based on essential oil constituents. Each point represents an individual extract and is labeled with the site number and individual number. Each axis represents one of the compounds found in the extracts. The points are also color and shape-coded by collection site. The numbers in the legend are the site numbers (French tarragon = 27 and Kyrgyz = 28)

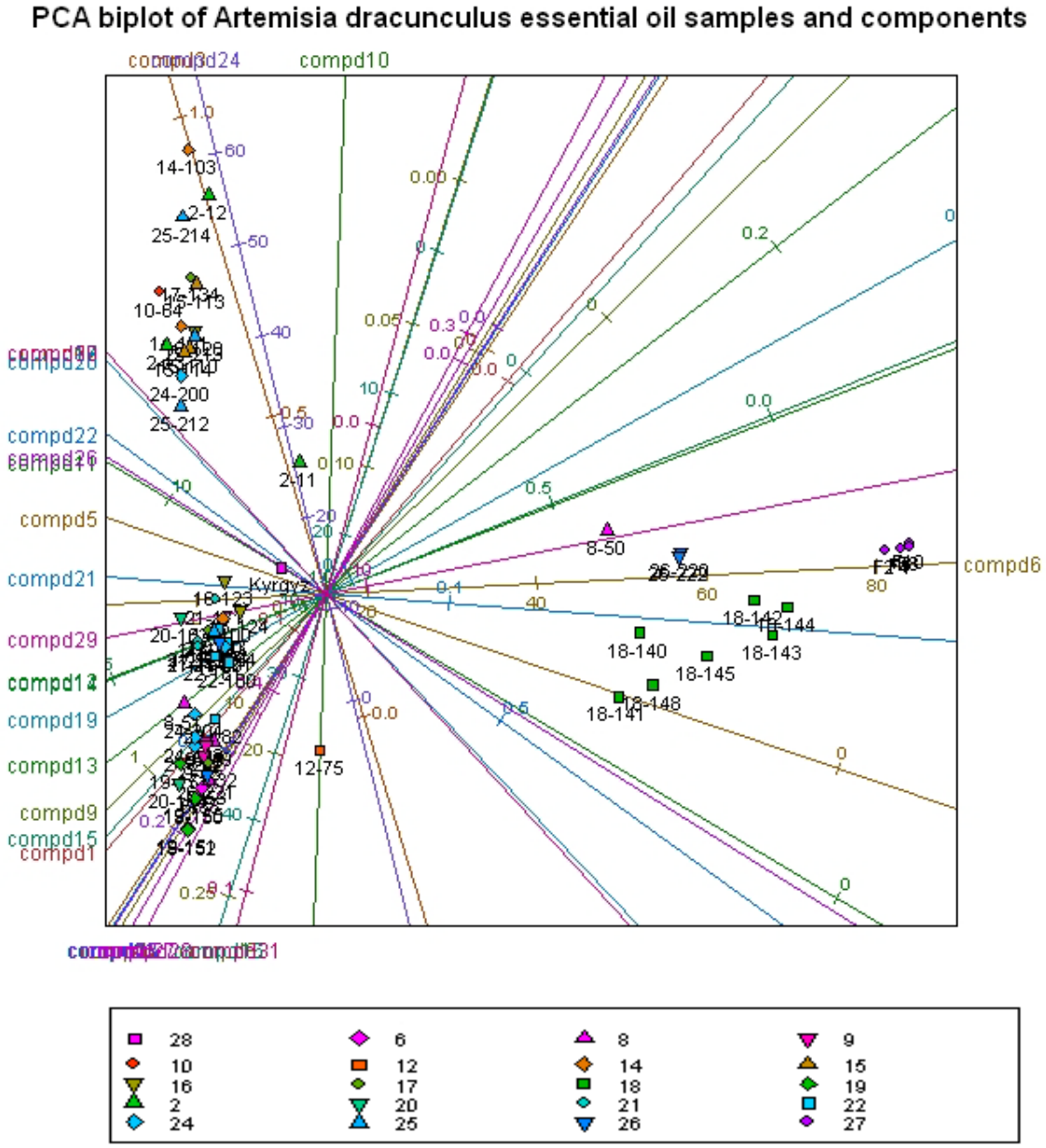


Figure 4.4. Biplot of principal component analysis with axis for compound 6 (methyl chavicol) highlighted. The cluster to the right is composed of the samples with high percentages of methyl chavicol. Each point represents an individual extract and is labeled with the site number and individual number. The points are also color and shape-coded by collection site. The numbers in the legend are the site numbers (French tarragon = 27 and Kyrgyz = 28).

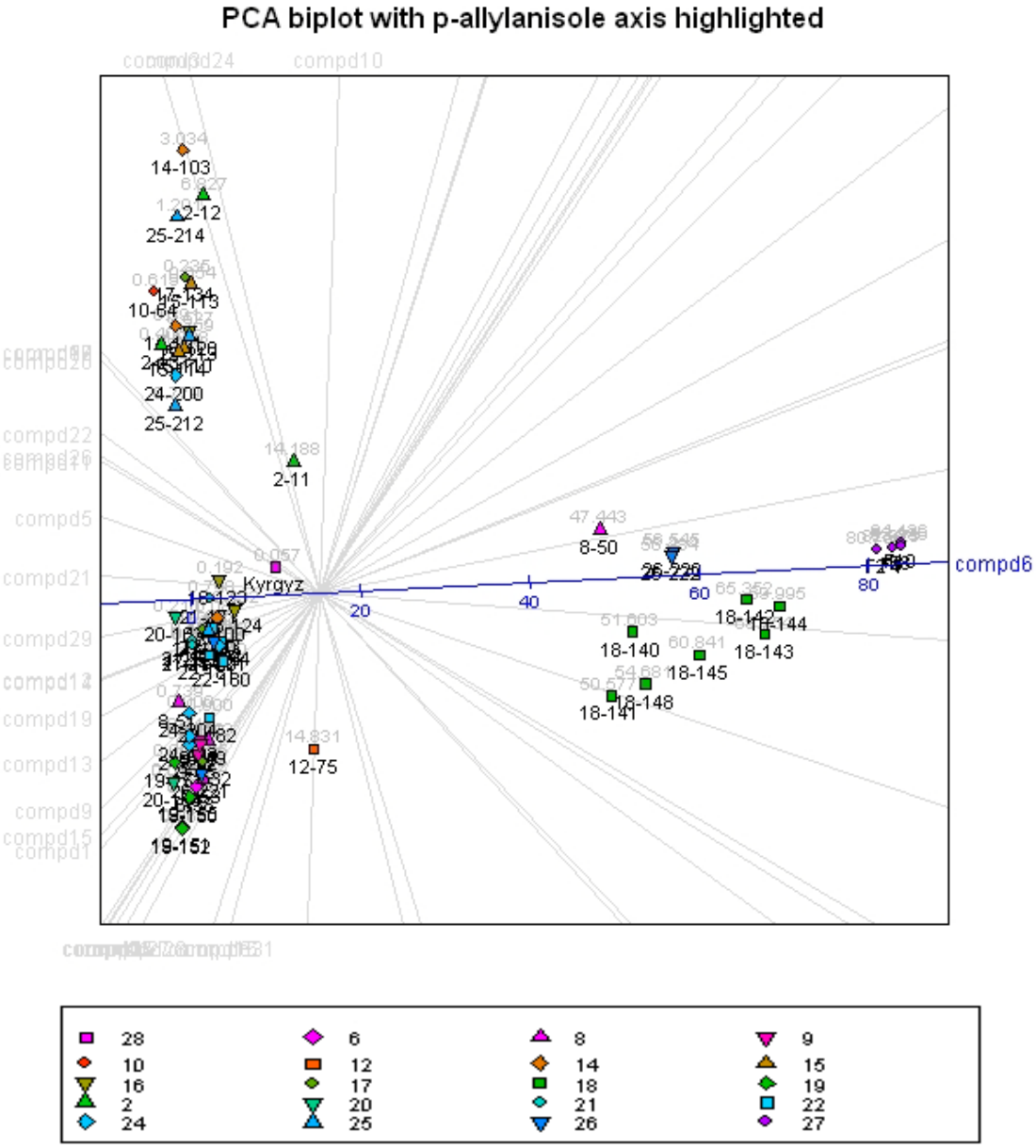


Figure 4.5. Biplot of principal component analysis after a scale transformation. The transformation clearly shows that the sample from Kyrgyzstan is extremely different from extracts prepared from North American and French tarragon plants. Each axis represents one of the compounds found in the extracts. Each point represents an individual extract and is labeled with the site number and individual number. The points are also color and shape-coded by collection site. The numbers in the legend are the site numbers (French tarragon = 27 and Kyrgyz = 28).

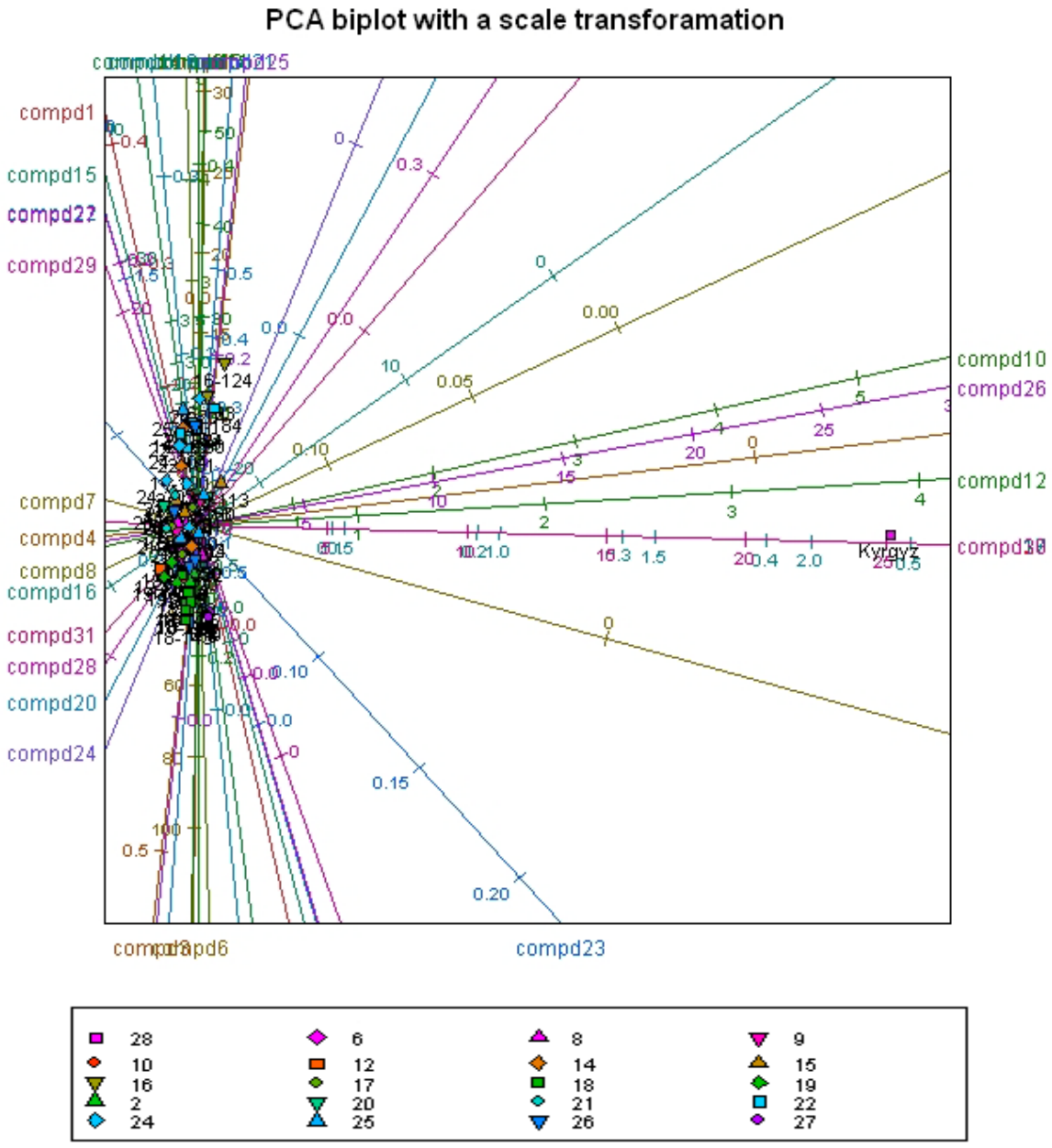


Figure 4.6. Axis predictivities for PCA of entire data set of essential oils in Fig. 4.3. Each point represents a compound (the axes of the PCA biplot) and the placement of the point on the graph represent how well that particular compound is represented by the first two principal components. This shows that compound 6 (methyl chavicol) is extremely well represented by the 1st principal component, while compound 24, 16 and 8 are progressively less represented in the PCA and the rest of the compounds have little to no representation.

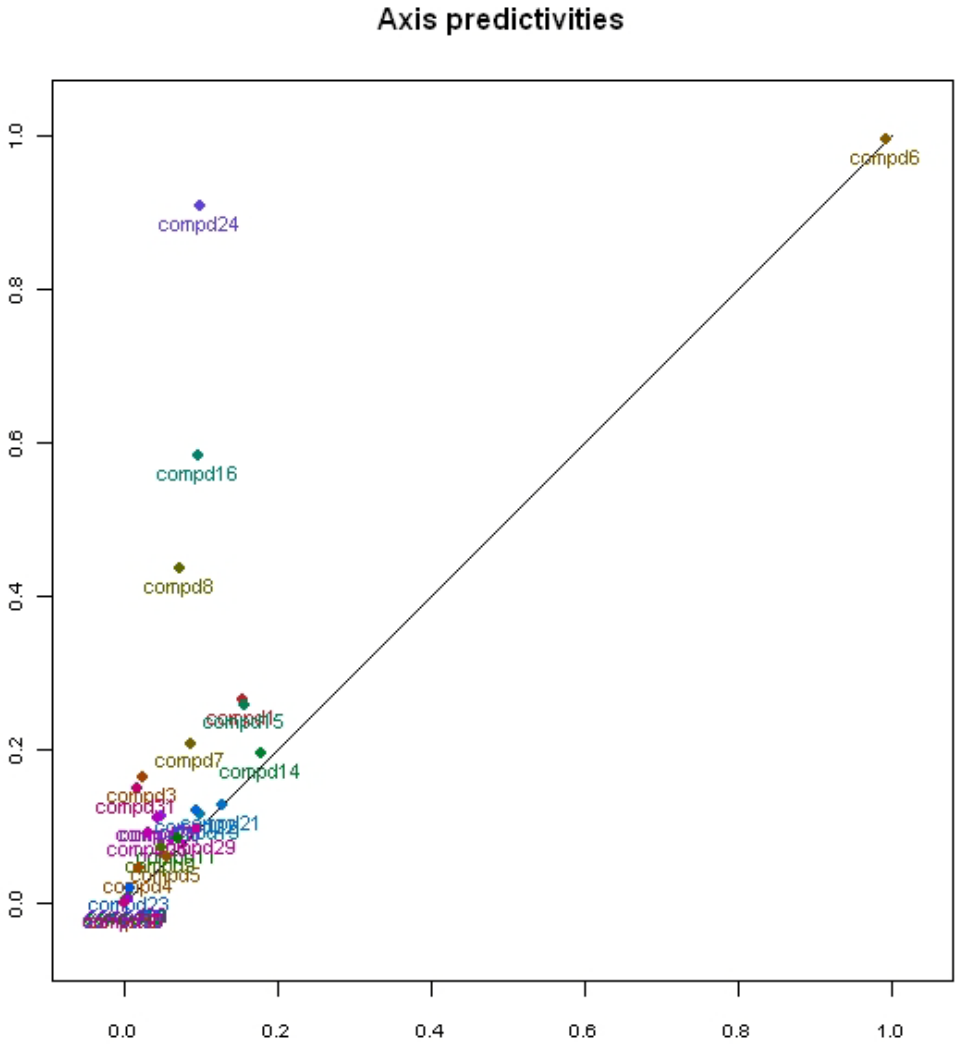


Figure 4.7. Point predictivities for PCA of entire data set of essential oil extracts as shown in Figure 4.3. Each point represents an extract (the points on the PCA biplot and label in the same manner). The placement of the point on the graph represents how well that particular extract is represented by the various dimensions. This shows that extracts high in compound 6 (methyl chavicol), which are in the upper right section of the plot, are extremely well represented by the 1st principal component. The extracts in the upper left are represented by the 2nd principal component and the extracts in the lower left are not well represented.

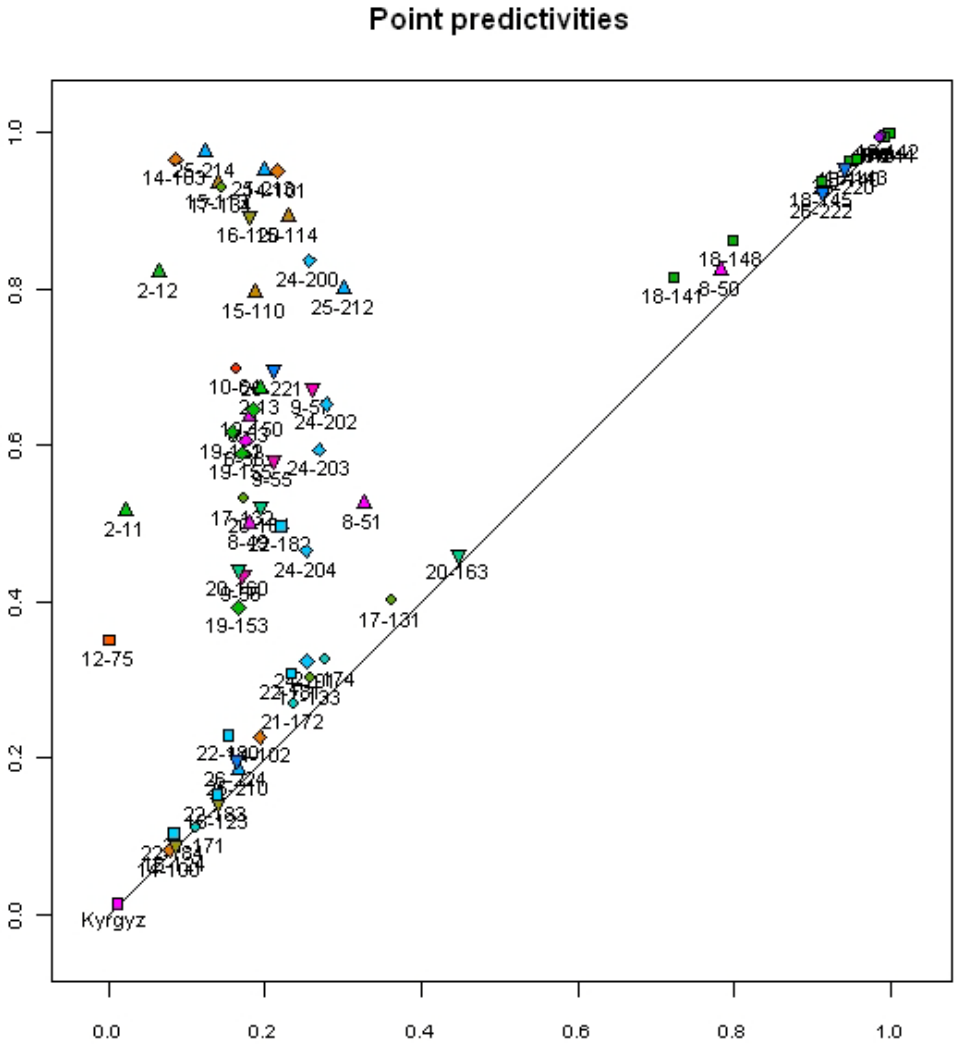


Figure 4.8. Point predictivities for PCA of entire data set of essential oils after a scale transformation, as shown in Figure 4.5. Each point represents an extract (the points of the PCA biplot and label in the same manner). The placement of the point on the graph represents how well that particular compound is represented by the various dimensions. This shows that the Kyrgyz extract, which is in the upper right section of the plot, is extremely well represented by the 1st principal component. The extracts in the middle-left are moderately represented by the 2nd principal component and the extracts in the lower left are not well represented.

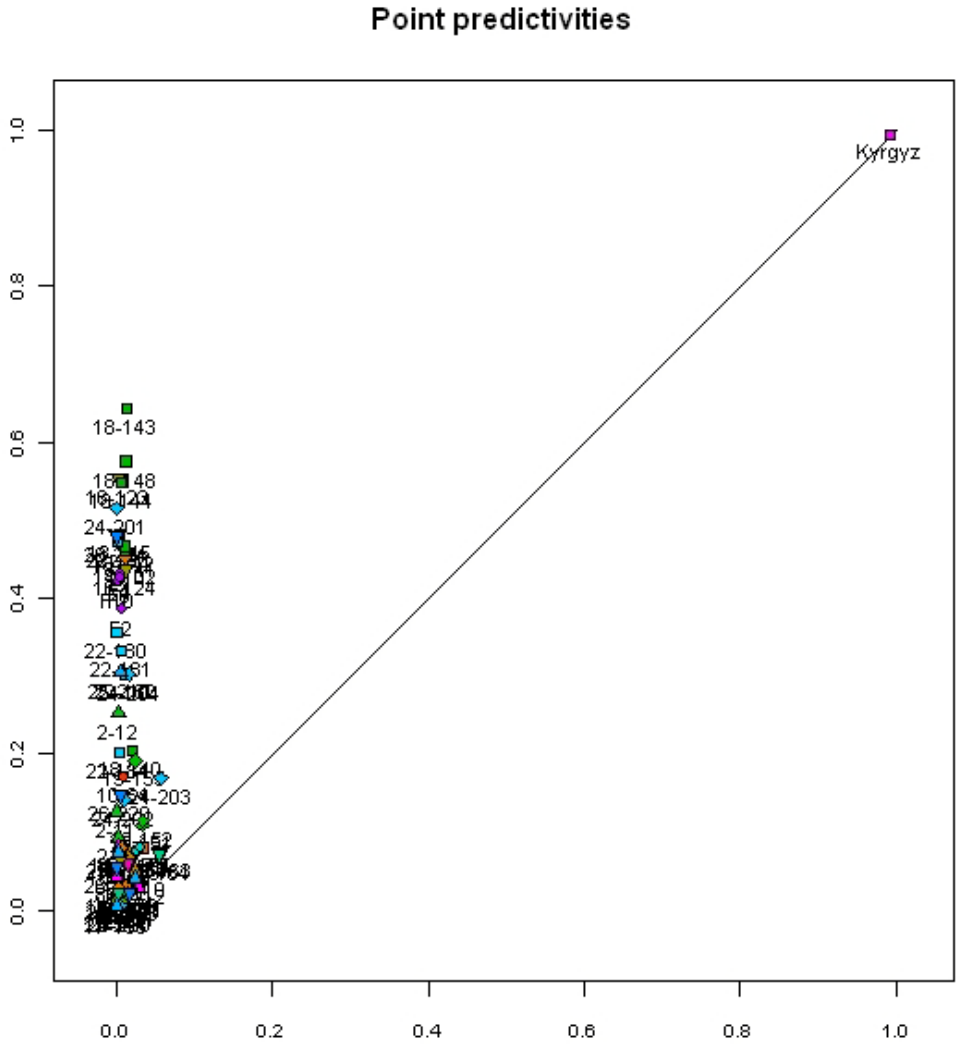


Figure 4.9. PCoA biplot of the reduced sample set which excludes the Kyrgyz and high methyl chavicol samples. Each point represents an individual extract and is labeled with the site number and individual number. The points are also color and shape-coded by collection site. The numbers in the legend are the site numbers.

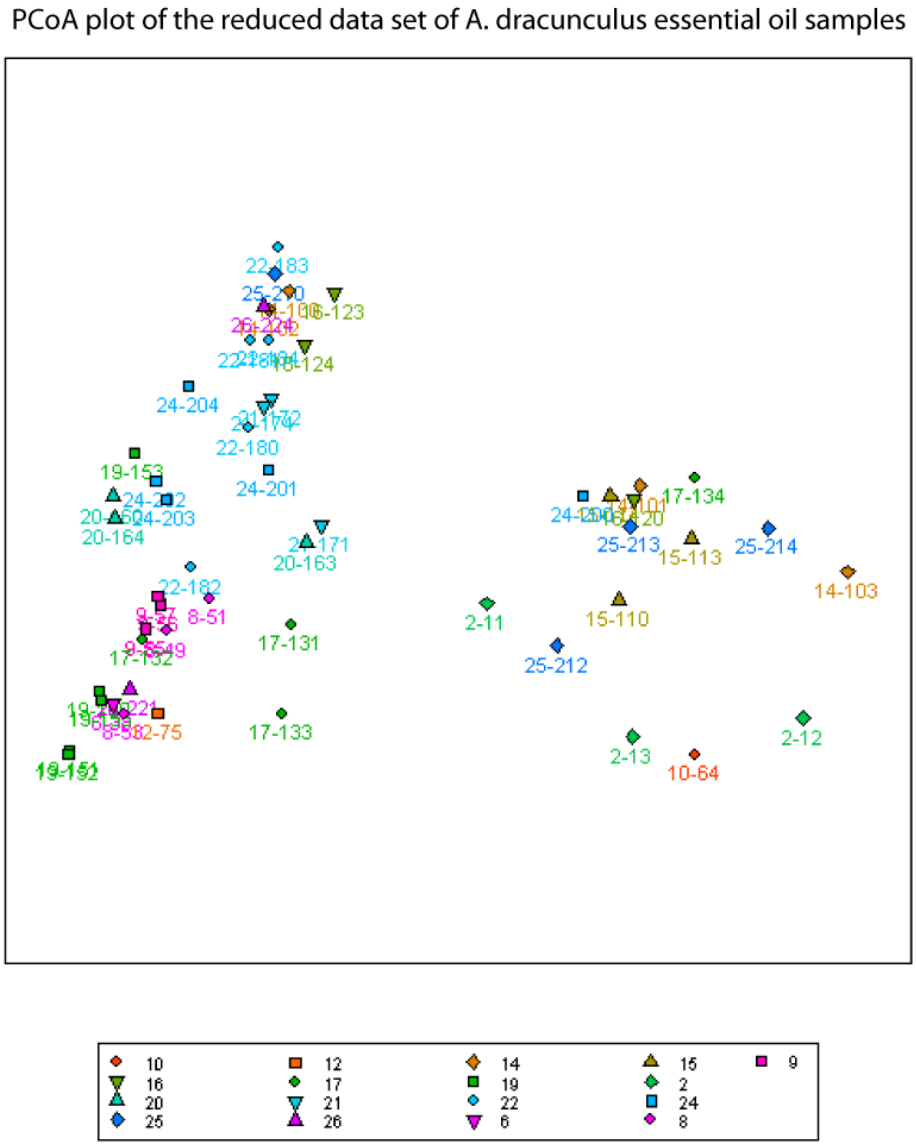


Figure 4.10. Point predictivities for the PCoA shown in Figure 4.9 which used a reduced sample set. Each point represents an extract (the points of the PCoA biplot and labeled in the same manner). The placement of the point on the graph represents how well that particular extract is represented by the various dimensions. This shows that many of the extracts are better represented in the first two principal cooordinates after the removal of the Kyrgyz extract and the extracts with high methyl chavicol content.

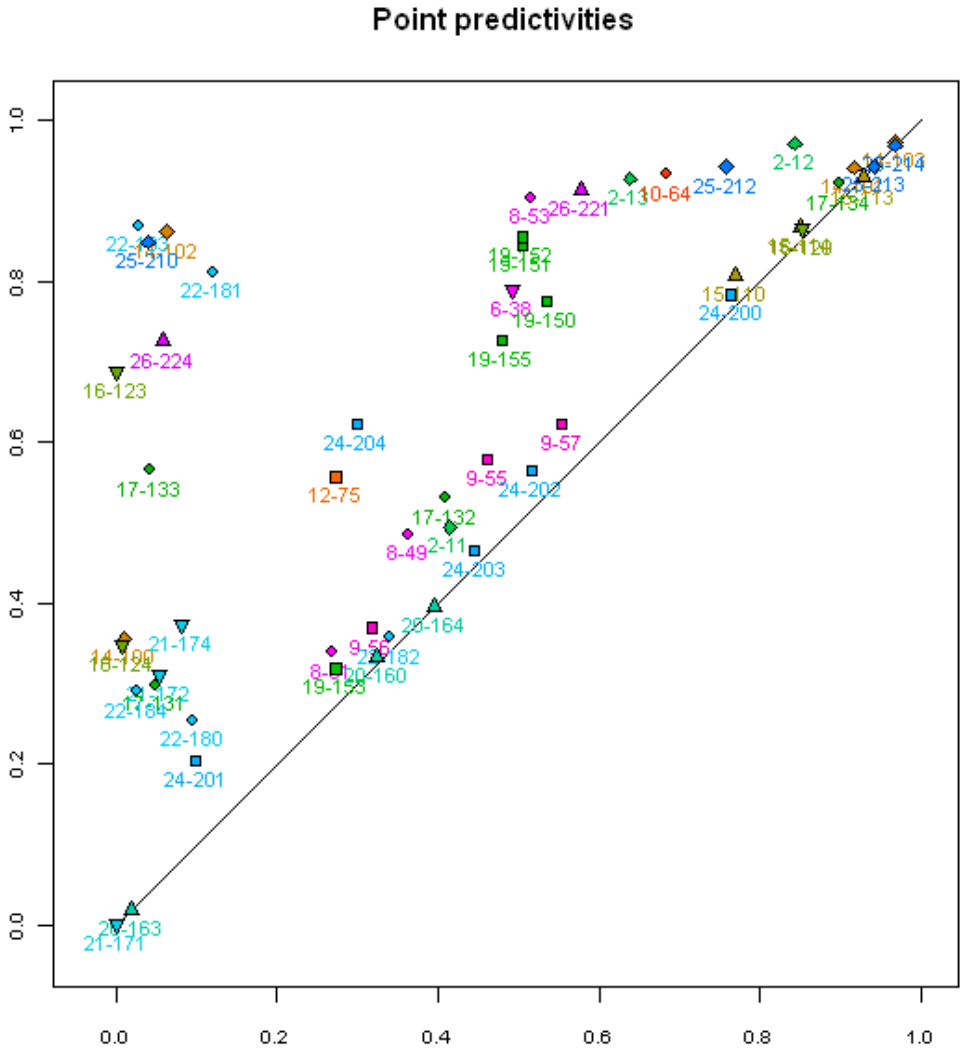


Figure 4.11. PCA biplot of the complete set of essential oil samples with convex hulls. Each point represents an individual extract and is labeled with the site number and individual number. Each axis represents one of the compounds found in the extracts. The points are also color and shape-coded by collection site. The numbers in the legend are the site numbers (French tarragon = 27 and Kyrgyz = 28). Each outline connects the points of a particular collection site. This plot shows that individuals with similar chemical composition can be found in different sites, and that sites have a diversity of chemotypes (with the exception of site 18 and French tarragon samples).

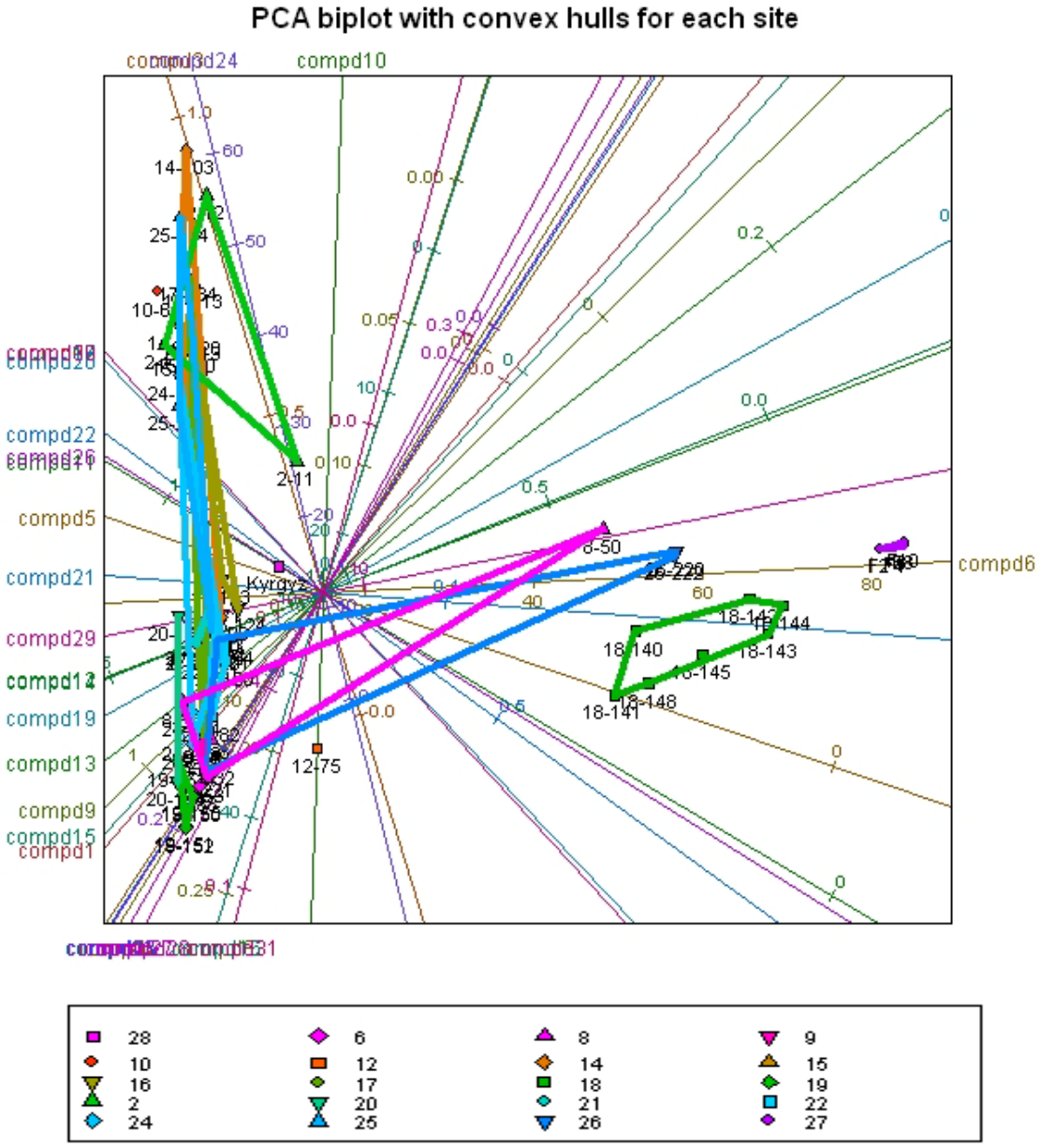
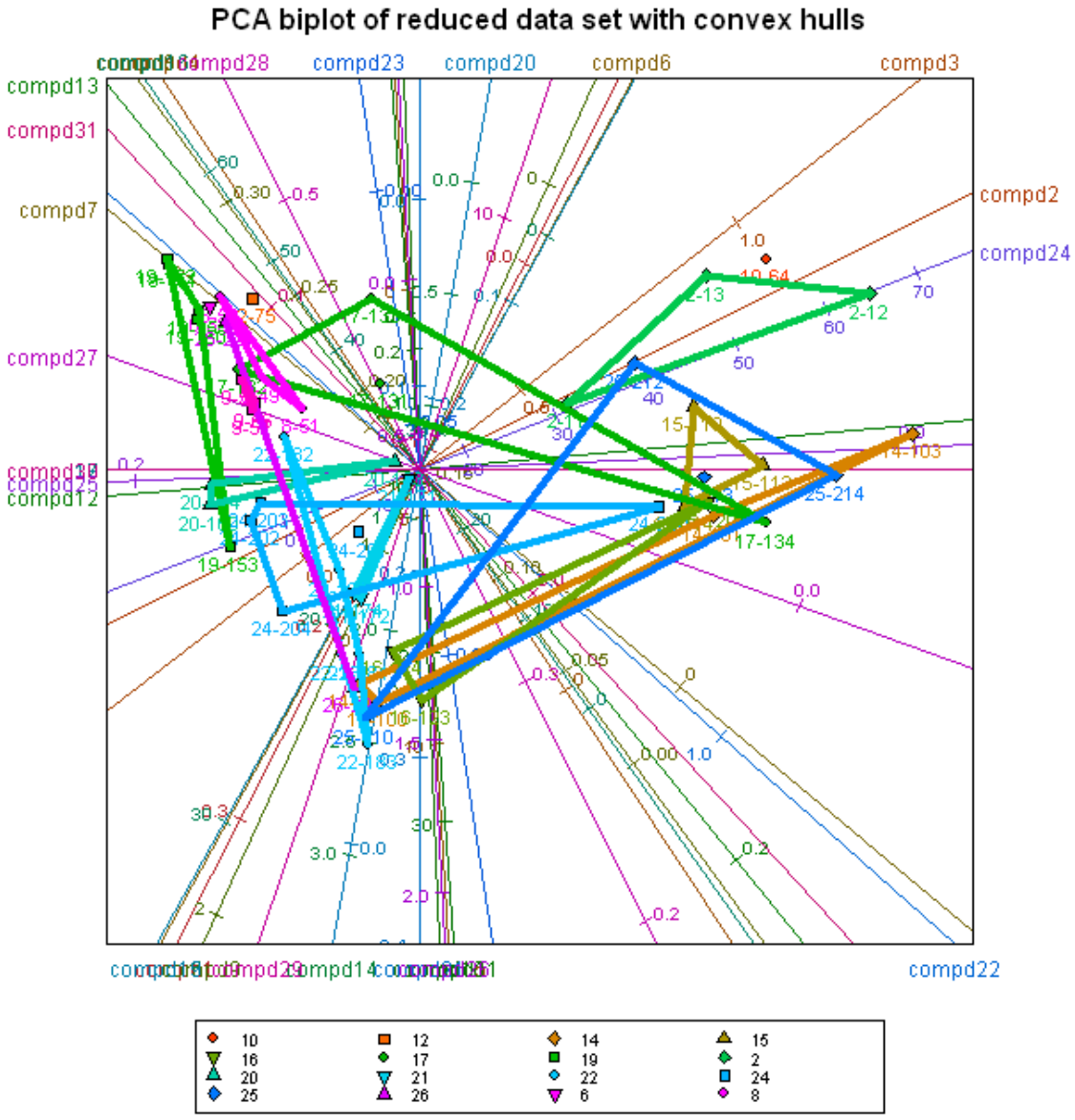


Figure 4.12. PCA biplot of the reduced sample set with convex hulls. Each point represents an individual extract and is labeled with the site number and individual number. Each axis represents one of the compounds found in the extracts. The points are also color and shape-coded by collection site. The numbers in the legend are the site numbers. Each outline connects the points of a particular collection site. This plot shows that individuals from the same collection sites are chemically diverse.



Chapter 5

Genetic diversity and relationships in North American populations of *Artemisia dracunculus* L. and correlation with sakuranetin production

Abstract

Amplified Fragment Length Polymorphism analysis (AFLP) was conducted to assess the genetic diversity found within and between North American populations of the medicinal plant *Artemisia dracunculus* (wild tarragon, Asteraceae). Four different diversity indices G_{ST}, G'_{ST}, D_{EST} and Φ_{PT} were calculated and all returned comparable values ranging from 0.221 to 0.259, showing a moderate amount of genetic diversity. The majority of genetic variation (78%) was within populations, and only 22% of the variation was between populations. A Mantel test showed significant correlation between the geographic distance and the genetic distance between populations. Spearman's ranked correlation analyses of sakuranetin content and within-population diversity indices did not show any correlations. A second Mantel test, to determine if genetic relatedness of wild tarragon populations is correlated with variation in sakuranetin production, did not show any correlation as well.

Introduction

Artemisia dracunculus L. (wild tarragon) is an herbaceous perennial that can be found growing wild in western North America, most temperate Asia, and Europe. In the western United States the species has a widespread distribution, is generally found in scattered populations, and inhabits a variety of environments, including roadsides,

riparian borders, prairies, grasslands and various types of forested areas (see Chapter 1 for additional distribution information). The species is primarily wind-pollinated although insect visitation of flowers has been documented (Müller, 1883). Self-pollination in wild tarragon has not been extensively studied, but using a decaploid strain of wild tarragon, Rousi (1969) conducted a pollination experiment and found that the strain was nearly self-incompatible, but did display normal seed set when cross-pollinated by another individual.

The primary goal for this study was to determine if genetic relatedness of wild tarragon populations is correlated with variation in phytochemical production. After conducting extensive cytological and chemical analyses, it was found that cytotypic variation was a significant factor influencing chemical presence, and that diploid populations in the U.S. lack a number of bioactive compounds that are present in a commonly cultivated decaploid strain of an unknown European or Asian origin (Chapters 2 and 3) One of the targeted compounds, sakuranetin, was present in U.S. populations of *A. dracunculus*, being detected in 99 out of 104 wild-collected chemical extracts (Chapter 3). This compound was therefore selected for further analysis in order to determine if there was a correlation between quantitative variation among populations of *A. dracunculus* and their genetic structure. Genetic diversity within and between the populations was conducted using amplified fragment length polymorphism (AFLP) markers. AFLP analysis is a PCR-based fingerprinting technique and was selected for this study because, unlike microsatellite markers, it requires no *a priori* sequence knowledge and has been shown to be more reproducible than RAPD markers (Vos et al., 1995; Jones et al., 1997; Mariette et al., 2002; Meudt and Clarke, 2007).

Differences in the production of secondary metabolites have been documented from the scale of taxonomic orders down to individuals. For example mustard-oil glucosides (glucosinolates) are found in primarily one order of vascular plants, the Brassicales, and betalains mainly in the Caryophyllales. Certain types of polyacetylenes and caffeoylquinic acids are only found in the Asteraceae, while other groups of compounds such as alkaloids and cardiac glycosides are distributed throughout many unrelated families and orders (Bohlmann et al, 1973; Rodman et al., 1998; Clifford, 1999; Cuénoud et al., 2002; Wink, 2003). The presence of the same compounds in distant groups could be a result of convergent development of the genetic machinery necessary to produce such compounds. For example, limonene synthase genes in gymnosperms and angiosperms have been shown to have similar functions but different origins (Theis and Lerdau, 2003; Wink, 2003). Alternatively, it could be that the basic ability to produce the compound is an ancestral feature, which all groups have, but that regulatory factors or mutations are leading to differential gene expression (Wink, 2003).

In some cases, the connection between genetic and chemical variation within a species has been determined. For example, the maize pathogen *Fusarium verticillioides* usually produces polyketide-derived mycotoxins called fumonisins. Analyses of naturally occurring variants of the fungus that are deficient in fumonisin, showed that this phenotype can be the result of single point mutations (Proctor et al., 2006). A single nucleotide insertion, that causes a frame shift in the coding region of the gene, or a nucleotide substitution, which introduces a premature stop codon in the coding region are two different mutations identified as the cause for loss of fumonisin production. In this example simple mutations provide an explanation for the presence or absence of

compounds, but in other cases, when the production of a compound varies quantitatively, the trait may be affected by multiple genetic loci as well as environmental effects (Remington and Purugganan, 2003).

In *Artemisia*, chemical variation between species, between populations within a single species, between individuals within a population, and between tissues within an individual have been documented, but in most cases the underlying cause of this variation is not well documented (Greger, 1977; Greger, 1979; Hoffmann and Herrmann, 1982; Vienne et al., 1989; Charles et al., 1990; Nikolova and Ivancheva, 2005; Bhutia and Valant-Vetschera, 2008). The most thoroughly examined *Artemisia* species for within-species variation of a phytochemical is *A*. *annua*. Detailed hybridization experiments and genetic linkage analyses have shown that the content of the important anti-malarial compound artemisinin varies in plants from different origins and is heritable, but under polygenic influence (Delabays et al., 2001; Graham et al., 2010).

Sakuranetin has been identified in unrelated taxa throughout many plant families. In some plants, such as rice, sakuranetin acts as a phytoalexin (synthesized de novo in response to external stimuli), while in *Ribes* it acts as a phytoanticipin (pre-formed inhibitor of infection; Dixon, 2001). The enzyme naringenin 7-O-methyltransferase has been shown to catalyze a reaction where the flavanone naringenin is methylated to yield sakuranetin, with S-adenosyl-L-methionine as the methyl donor (Rakwal et al., 1996). Associated regulatory genes for this enzyme have not been described, but differential regulation of enzyme production could be a possible explanation for varying amounts of sakuranetin produced by different populations.

Building on the findings of cytological and chemical analyses, the primary objectives of this genetic analysis study were to (i) provide a preliminary estimate of within- and among-population genetic variation for the twenty populations (sites) sampled in the western United States, (ii) determine whether there are geographical patterns to genetic diversity among these populations, and (iii) determine if within-population genetic diversity and between-population genetic distance is correlated with sakuranetin production.

Materials and methods

Plant material. A total of 190 samples were collected from 20 different populations across the western United States, including Colorado, Utah, Nevada, Wyoming and California. Sample size was based primarily on the number of plants found in a population. When available, material from ten individuals per population was collected for DNA extraction and AFLP analysis. (Table 5.1; see Appendix 1, Table A.1 and Figure A.1.2 for detailed locality information and Table 5.6 for linear geographic distances between populations). Fresh leaves were collected in the field and immediately put in silica gel desiccant. Upon returning to the laboratory the dried leaf material was permanently stored at -20°C.

DNA extraction and AFLP protocols. For DNA extraction, 20-30 mg of the silica-dried leaf tissue was ground to a powder using a FastPrep Bio101 Homogenizer (Thermo Scientific, Waltham, MA) and lysing matrix A tubes (Qbiogene, Solon, OH). Total genomic DNA was extracted using a DNeasy Plant Mini Kit system (Qiagen, Valencia,

CA), following the protocol of the manufacturer with the following modification: addition of 30 uL of β-mercaptoethanol to the 400 uL of extraction buffer AP1 used for each sample. To confirm the extraction process was successful, the initial extracts were run on a 1.2% agarose gel and DNA concentrations for all samples were determined using a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA).

AFLP reactions were conducted using an Applied Biosystems Plant Mapping Kit for Regular Genomes (Applied Biosystems, Foster City, CA) following the protocol of the manufacturer. Pre-selective amplification was done using the primer pair *Mse*I-C/*Eco*RI-A. Based on literature surveys for selective-amplification primer-pairs successfully used for species in the Asteraceae family, 36 different primer pairs were chosen for testing on samples from seven different populations. The majority of primer pairs did not amplify well, or did not exhibit polymorphisms, but from these tests, 3 primer-pairs, *Mse*I + CAC/*Eco*RI + ACA, *Mse*I +CTC/*Eco*RI + ACG and *Mse*I +CAG/ *Eco*RI +ACC were chosen for selective amplification. The resulting PCR products were analyzed using automated capillary electrophoresis using a ABI 3130xl genetic analyzer (Applied Biosystems, Foster City, CA). Raw data was analyzed with GeneMapper™ analysis software Version 3.7 (Applied Biosystems, Foster City, CA) and all loci were checked manually. A binary matrix (allele present = 1, allele absent = 0) was prepared in GeneMapper™ and exported for analysis.

Data analysis. Binary AFLP markers are generally assumed to be dominant and because dominant homozygotes and heterozygotes are indistinguishable, allele frequencies cannot be directly calculated. Assuming Hardy-Weinberg equilibrium it is possible to indirectly

estimate allele frequencies and expected heterozygosity. In order to obtain information on the within-population variation of the different populations, GenAlEx version 6.3 (Peakall and Smouse, 2006) was used to calculate the percent of polymorphic bands (*P*), expected heterozygosity (H_e), and the relative allelic diversity per population as measured by the Shannon index of diversity (*I*).

In order to compare the most commonly used measures of between-population genetic diversity a number of different diversity measurements were calculated. Popgene version 1.32 (Yeh et al., 2000) was used to calculate the Nei's coefficient of gene differentiation (G_{ST}). Although G_{ST} is a widely used measure of diversity, this calculation has recently been the focus of debate, and has been found not to be an appropriate method to measure diversity in many cases, but is more accurately a measure of migration rate (Hedrick and Goodnight, 2005; Jost, 2008). This measure is based on additive partitioning of heterozygosity and therefore it fails to provide accurate estimates of diversity. In particular, when there is near complete differentiation between populations it is possible to have lower G_{ST} values than when populations are poorly differentiated (Jost, 2008). Because G_{ST} has been a standard measure and is so commonly used, it is presented and compared with newer measurements of estimating diversity G'_{ST} and D_{EST} (Hedrick and Goodnight, 2005; Jost, 2008). G'_{ST} is a standardized measure of genetic differentiation in which the observed value of G_{ST} is standardized by the maximum level that it can obtain for the observed amount of genetic variation and was calculated according to Hedrick and Goodnight (2005). D_{EST} is unique in that it involves a partitioning of the genetic diversity into pure and independent within- and between-group components and therefore is not subject to the inaccuracies of G_{ST} (Jost, 2008;

Heller and Siegismund, 2009). D_{EST} was calculated using SPADE (Species Prediction And Diversity Estimation, version 2009/Feb) with 200 bootstrap replications; Chao and Shen, 2009). For another assessment of genetic diversity between populations, GenAlEx 6.3 was used to conduct an analysis of molecular variance (AMOVA) with 999 permutations, and to calculate Φ_{PT}. Φ_{PT} is calculated as the proportion of the variance among populations, relative to the total variance and represents the correlation between individuals within a population, relative to the total.

In order to evaluate "isolation by distance", a Mantel test was conducted with 999 permutations in GenAlEx 6.3 as well. This test assesses for correlation between two matrices, in this case a between-population, linear genetic distance matrix and a matrix of geographic distance between the populations. Using genetic distance matrices produced in GenAlEx 6.3, separate UPGMA dendrograms for samples, and populations, were produced using *Mega* version 4.0.2 and were port-processed with TreeDyn (198.3) and Adobe Illustrator CS4 (Sneath and Sokal, 1973; Chevenet et al., 2006; Tamura et al., 2007). The population-based tree has the sites with the highest average sakuranetin content labeled as such, and the sample-based tree has the primary compound of the essential oil extracted from a subset of the samples demarked (see Chapters 3 and 4 for specific details regarding chemical analyses). For all the genetic analyses, the samples from sites 6 and 7 were considered to be from a single population because of the close proximity of the original collection sites. For the same reason, the samples from sites 10 and 11 were pooled into a single population as well.

To assess for correlation between the within-population genetic diversity and the average sakuranetin content of wild populations, two different Spearman's rank

correlation analyses were conducted. The first was to assess correlation between the averages of H_e for each population and the average sakuranetin content of each population, and the second was to determine if the average *P* for each population and the average sakuranetin content of each population were correlated. A mantel test with 999 permutations was conducted to assess correlation between a canonical distance matrix of population/population sakuranetin content and a population/population genetic distance matrix. The sakuranetin distance matrix was produced using the software program R version 2.9.2 (R Development Core Team, 2009) with the package 'ade4' (Dray and Dufour, 2007) and the Mantel test was executed using GenAlEx 6.3.

Results

***AFLP patterns and polymorphism*.** Because of poor amplification, the primer pairs *Mse*I + CAC/*Eco*RI + ACA and *Mse*I +CTC/*Eco*RI + ACG produced unsatisfactory chromatographs for many of the samples and results from these primers were therefore excluded from the analysis. The primer pair *Mse*I +CAG/ *Eco*RI +ACC produced 103 polymorphic loci. No monomorphic loci were included in the analysis. Five samples failed to amplify and were also excluded; the analyses were conducted with a total of 185 individuals in 20 populations (Table 5.1).

Within-population genetic diversity. The percentage of polymorphic loci (%*P*) within the populations ranged from 16.5-69.9%, with a mean of 47.82%. The genetic diversity within each population expressed as expected heterozygosity (H_e) ranged from 0.054-0.188, with a mean H_e = 0.123 and Shannon indices (*I*) ranged from 0.083-0.298, with

mean of 0.196 (Table 5.1). The ranges expressed in these results show that there are distinct differences in the degree of genetic diversity in the individual populations sampled. When the least and most diverse populations are compared (sites 12 and 26, respectively) there is a more than three-fold increase of %*P* and H_e. To assess correlation between these three measures, Spearman's rank correlation analyses were conducted and returned the following coefficients: $r_s = 0.98$ between H_e and the *I*, $r_s = 0.868$ between H_e and *P* and $r_s = 0.929$ between the *I* and *%P* ($P < 0.0001$ for each correlation).

Among-population differentiation. Overall, the various methods of estimating among-population genetic variation returned very similar values. Φ_{PT} was calculated to be 0.221 and was found to be highly significant with P<0.001. The values calculated for G_{ST} and G'_{ST} were calculated to be 0.224 and 0.257, respectively. D_{EST} was 0.226 with a 95% confidence interval of 0.192-0.259. Values of the four different measures of genetic variation among populations Φ_{PT}, G_{ST} and D_{EST} are compared in Table 5.2. The AMOVA analysis revealed that 78% of the genetic diversity is found within the populations and 22% of diversity is among populations (Table 5.3).

Genetic vs. geographical structure and sakuranetin content. The Mantel test showed significant correlation between a genetic and geographic distance matrices with $r = 0.428$ and a *P*-value of 0.02 (Table 5.5; Figure 5.1). The UPGMA dendrogram generated from the population/population distance matrix is shown in Figure 5.2. Like the Mantel test, the UPGMA dendrogram also shows some association between the genetic relationships of populations that are from the same geographic area. In particular, sites 18 (Crescent

Mills) and 19 (Susanville) are both in northern California, are approximately 50 miles apart (by road) and are clustered together. Site 8 (Walker Lake, Nevada) and site 20 (Rt 168, Inyo Co., California) are ~150 miles apart and cluster together, site 3 (Villa Grove in south-western Colorado) and site 6/7 (Bicknell and Torrey in south-central Utah) are more than 400 hundred miles apart and sites 17, 18 and 19 are all in southern California. Other sites were sampled in southern California, but did not cluster together. The sites with high-averaging sakuranetin contents are found clustered in two different groups (Figure 5.2). The sample/sample dendrogram does not exhibit strong phylogeographic structuring. Some individuals from the same populations are clustered together but there are many other individuals mixed in from other populations. The primary essential oil components that have been mapped onto this dendrogram are quite variable in their distribution and do not form well-defined clusters (Figure 5.3).

Correlation of sakuranetin production with genetic diversity. Spearman's rank correlation analyses of average H_e for each population versus the average sakuranetin content of each population, and the average *%P* for each population versus the average sakuranetin content of each population, both showed negative correlation for within-population diversity and sakuranetin content, but both tests had non-significant *P*-values (Table 5.4). The Mantel test for the between-population genetic distance matrix and the canonical distance matrix of between-population average sakuranetin content (Table 5.6) showed these matrices to be uncorrelated.

Discussion

The results from this study show that there are distinct differences in the degree of genetic diversity within the sampled individual populations. The three-fold increase of the %*P* and H_e observed between sites 12 and 26 could be due to the small sample size for site 12 (*N*=5), but site 8, which also has a sample size of 5, has diversity measurements that are within the ranges of other sites with 10 samples. The mean of all populations, $H_e = 0.123$, is very similar to other estimates of within-population genetic diversity in the Asteraceae (Hamrick and Godt, 1997; Hamrick and Godt, 1996).

The amount of genetic diversity within a species has been attributed to a number of critical traits that affect gene flow, including life form (woody vs. herbaceous) breeding system (selfing vs. out-crossing) and seed dispersal mechanisms (Hamrick and Godt, 1996; (Nybom and Bartish, 2000). Particularly, long-lived, out-crossing taxa have been found to retain most of their genetic variability within populations. Species that are wind-pollinated are also more likely to have a higher degree of out-crossing and therefore more genetic homogenization between populations. Alternatively, due to increased genetic isolation of populations, annual, self-pollinating taxa are found to retain more of their genetic variability between populations.

The AMOVA analysis showed that the majority of genetic variation (~78%) was found within the populations, which is in keeping with what would be expected for an out-crossing perennial species. Similarly, Φ_{PT}, G_{ST} and D_{EST} all produced estimates of between-population genetic diversity of approximately ~22-25%. Although the majority of the diversity is found within the populations, 22-25% is a significant portion of the total genetic variation and shows that there is a substantial amount of differentiation between populations. It is interesting to note that the relative measures produced by the

different diversity indices compared in this study match those found by Heller and Siegismund (2009) in a meta-analysis comparing diversity indices (G_{ST}, G'_{ST} and D_{EST}). They found that G_{ST} was always numerically lower than D_{EST} and G'_{ST}, and that G'_{ST} was uniformly higher than D_{EST} and this is what was seen in the *A. dracunculus* analysis as well.

Artemisia dracunculus is a wind-pollinated, out-crossing species, and some genetic homogenization of the populations is expected. This is evident in the UPGMA dendrogram derived from the sample/sample genetic distance matrix. This tree shows that although many samples cluster with their fellow population members there are many misplaced individuals. Yet overall, populations do have significant divergence from each other. Wind-pollinated species are more likely to have a higher degree of out-crossing and therefore more genetic homogenization between populations, but if there is significant distance between populations genetic differentiation may still occur as a result of 'isolation by distance' (Wright, 1943; Hamrick and Godt, 1996). Nybom and Bartish (2000) found that in out-crossing taxa, RAPD-based estimates of between-population diversity were closely correlated with maximum geographic distance between sampled populations. Most of the sites sampled for the current study had significant distance between them and were often separated by substantial topographical and environmental features in the landscape. Because the populations sampled were distributed across a large portion of the western United States, geographic distance could be a cause of the between-population divergence that was observed. This idea is supported by the Mantel test which showed significant correlation of genetic distance and geographic distance.

The geographic range of this study represents a small fraction of the actual distribution of *A. dracunculus*. The species is circumboreal and it is possible a different degree of genetic partitioning would be found if the range of the study was increased. The genetic diversity of North American *A. dracunculus* may in fact be lower than that of *A. dracunculus* in Asia. If the species colonized via the Bering Strait, it is possible that the American populations are the result of founding populations that represent a fragment of the total diversity contained in the populations distributed across the vast region of Eurasia. If the species has a Eurasian origin, the populations there would have likely had a much greater expanse of time to allow for divergence and diversification. This is particularly evident in the occurrence of cytotype diversity. Although the species is known to have a long series of polyploidy cytotypes (2x-10x), only diploid individuals have been found in North America (see Chapter 2). According to the *Flora of the USSR* the species can be an aggressive agricultural weed (Poljakov, 1961b). This description is quite unlike the type of growth seen in the U.S. and may be due to the presence of polyploid populations with a more vigorous growth habit. Polyploidy in Asian populations may affect gene flow and genetic diversity. Differences in the abundance and density of populations, the ability to colonize more readily, and particularly reduced gene flow and reproductive isolation of cytotypes could create a very different population dynamic than that found in North America.

The overall genetic diversity found in *A. dracunculus* is reflective of the variation in morphological and content of volatile compounds presented in Chapters 4 and 6. The morphology of *A. dracunculus* has confounded botanists because of morphological gradations associated with leaf size, capitulum width, peduncle length and synflorescence

shape. This AFLP analysis shows that although there is some distinction between populations, there is likely significant gene-flow as well and this gene flow may be a factor contributing to the lack of distinct morphological groups. The chemical variation found in the essential oil profiles of these populations also shows this pattern of unresolved differentiation. Although distinct chemotypes were observed, different chemotypes could be found mixed in a single population and some distant populations were found to have individuals with very similar essential oil profiles.

A lack of correlation between genetic similarity and sakuranetin production was also observed in the populations of *A. dracunculus*. In rice, sakuranetin is involved in defense against fungal pathogens, and in experiments has been shown to be inducible by the application of UV light or fungal inocula (Kodama, 1992). In a study of rice genotypes, with varying susceptibility to the rice blast fungus *Pyricularia oryzae*, distinct qualitative and quantitative differences in phytoalexin production were found between genotypes, and there was a strong correlation between genotypic resistance and the accumulation of sakuranetin and other defensive phytoalexins (Dillon et al., 1997). The genetic relatedness of the genotypes was not discussed, but the disease resistant cultivars that produced high levels of sakuranetin were from Vietnam and Taiwan, respectively, and therefore probably not closely related.

In many of the North American *A. dracunculus* populations, a rust fungus, tentatively identified as *Puccinia tanaceti*, was found growing on the plants. It could be possible that the increased production of sakuranetin, in genetically unrelated populations, was a response to disease pressure. But, when individuals from these populations were cultivated, and no presence of rust was observed, they were still found

to have the highest sakuranetin production on average (see chapter 3). It seems that in this case, high levels of sakuranetin production are not a direct response to environmental conditions. Sakuranetin has been identified in unrelated taxa throughout many plant families. In some plants, such as rice, sakuranetin acts as a phytoalexin (synthesized de novo in response to external stimuli), while in *Ribes* it acts as a phytoanticipin (pre-formed inhibitor of infection; Dixon, 2001). The enzyme naringenin 7-O-methyltransferase has been shown to catalyze a reaction where the flavanone naringenin is methylated to yield sakuranetin, with S-adenosyl-L-methionine as the methyl donor (Rakwal et al., 1996). Associated regulatory genes for this enzyme have not been described, but differential regulation of enzyme production could be a possible explanation for varying amounts of sakuranetin produced by different populations. It may be that although all populations have the ability to produce sakuranetin, high amounts of sakuranetin production in unrelated populations is due to fixed increases in primary or regulatory gene expression and that this has arisen independently in different populations.

Table 5.1. Site information, sample size (*N*) and genetic diversity indices of the 20 *Artemisia dracunculus* populations; percent polymorphic loci (*%P*), expected heterozygosity (H_e), Shannon index (*I*), and standard error (SE).

site #	State	County	Locality	*N*	*%P*	H_e (SE)	*I* (SE)
site 2	CO	Clear Creek	Georgetown	8	53.40%	0.160 (0.017)	0.249 (0.025)
site 3	CO	Saguache	Villa Grove	10	46.60%	0.105 (0.015)	0.172 (0.022)
Site 6&7	UT	Wayne	Bicknell/Torrey	11	43.69%	0.113 (0.016)	0.180 (0.023)
site 8	NV	Mineral	Walker Lake	5	42.72%	0.121 (0.015)	0.193 (0.023)
site 9	CA	Plumas	Feather River	9	50.49%	0.139 (0.017)	0.218 (0.025)
site 10&11	WY	Laramie	Happy Jack 1&2	10	61.17%	0.139 (0.014)	0.230 (0.022)
site 12	CO	Sedgwick	Julesburg	5	16.50%	0.054 (0.013)	0.083 (0.019)
site 14	CA	San Bernardino	Yucaipa	8	54.37%	0.119 (0.014)	0.199 (0.021)
site 15	CA	San Diego	old hwy 395	10	31.07%	0.091 (0.016)	0.141 (0.023)
site 16	CA	San Diego	Julian	10	41.75%	0.082 (0.012)	0.140 (0.019)
site 17	CA	Inyo	Onion Valley	10	43.69%	0.115 (0.016)	0.183 (0.023)
site 18	CA	Plumas	Crescent Mills	10	33.01%	0.086 (0.015)	0.136 (0.022)
site 19	CA	Lassen	Susanville	9	31.07%	0.072 (0.012)	0.119 (0.019)
site 20	CA	Inyo	Rt 168	10	52.43%	0.120 (0.015)	0.197 (0.022)
site 21	CA	Inyo	Death Valley Rd	10	64.08%	0.154 (0.016)	0.250 (0.023)
site 22	CA	Inyo	Nine Mile Cyn 1	10	45.63%	0.140 (0.018)	0.215 (0.026)
site 23	CA	Tulare	Nine Mile Cyn 2	10	50.49%	0.138 (0.018)	0.216 (0.025)
site 24	CA	Los Angeles	Elizabeth Lake	10	58.25%	0.149 (0.018)	0.234 (0.025)
site 25	CA	San Bernardino	Hesperia	10	66.02%	0.172 (0.017)	0.273 (0.024)
site 26	CA	San Bernardino	Running Springs	10	69.90%	0.188 (0.017)	0.298 (0.024)
mean	–	–	–	9.25	47.82%	0.123 (0.004)	0.196 (0.005)

Table 5.2. Comparison of between-population genetic variation index values calculated from AFLP markers in 20 populations of *A*. *dracunculus*.

	Φ_{PT}	G_{ST}	G'_{ST}	D_{EST}
Value	0.221	0.224	0.259	0.226

Table 5.3. Analysis of Molecular Variance (AMOVA) for 185 individuals of *Artemisia dracunculus,* using 103 AFLP markers. The total data set contains individuals from 20 sites. Statistics include sums of squared deviations (SS), mean squared deviations (MS), variance component estimates and the percentages of the total variance (% of variation) contributed by each component.

Source	df	SS	MS	Est. Var.	% of variation
Among Pops	19	636.477	33.499	2.625	22%
Within Pops	165	1527.502	9.258	9.258	78%
Total	184	2163.978		11.882	100%

Table 5.4. Results from Spearman's rank correlation analyses of sakuranetin production and three within-population diversity indices.

	sakuranetin/ H_e	sakuranetin/ *I*	sakuranetin *%P*
Spearman's *r*	-0.367	-0.361	-0.252
degrees of freedom	18	18	18
P-value	0.1115	0.118	0.2832

Table 5.5. Pairwise canonical distance matrix of average sakuranetin content for 20 collection sites (S) used in a Mantel test to assess correlation between genetic distance and sakuranetin production.

S2	S3	S6/7	S8	S9	S10/11	S12	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	
0																				S2
289	0																			S3
938	741	0																		S6/7
5044	4938	4302	0																	S8
16438	16473	16110	13326	0																S9
120	178	865	5012	16465	0															S10/11
287	3	743	4940	16473	176	0														S 12
12973	12961	12503	9263	5029	12981	12962	0													S14
6021	5927	5307	1162	12479	5994	5929	8316	0												S15
25494	25623	25471	23714	12867	25559	25622	17223	23103	0											S16
14226	14231	13806	10725	3324	14241	14232	1824	9810	15748	0										S17
18185	18244	17931	15388	2709	18222	18245	7533	14595	10375	5882	0									S19
12611	12596	12129	8851	5441	12617	12596	476	7896	17570	2261	7929	0								S20
614	378	404	4667	16347	524	381	12785	5666	25605	14073	18144	12415	0							S21
8249	8182	7611	3775	10344	8234	8183	5973	2696	21485	7532	12581	5541	7947	0						S22
4315	4202	3558	810	13856	4280	4203	9870	1924	24069	11306	15877	9464	3925	4482	0					S23
446	189	582	4818	16426	346	192	12890	5812	25630	14168	18211	12522	200	8081	4079	0				site 17
8063	7993	7418	3558	10548	8046	7995	6192	2476	21645	7747	12775	5760	7756	243	4269	7891	0			site 18
572	331	452	4710	16372	480	334	12817	5708	25616	14102	18166	12447	53	7986	3969	151	7795	0		site 19
202	97	809	4981	16474	86	94	12977	5967	25595	14242	18238	12612	457	8214	4247	273	8026	411	0	site 20

Table 5.6. Linear pairwise geographic distance (km) matrix of 20 collection sites (S) used in a Mantel test to assess correlation of geographic and genetic distance between populations of *A*. *dracunculus*.

S2	S3	S6/7	S8	S9	S10/11	S12	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	
0																				S2
179	0																			S3
525	481	0																		S6/7
1130	1113	632	0																	S8
1258	1262	790	198	0																S9
166	345	621	1182	1289	0															S10/11
322	448	844	1430	1542	255	0														S12
1192	1094	689	531	709	1306	1514	0													S14
1250	1144	757	611	784	1370	1571	86	0												S15
1223	1111	741	645	823	1347	1543	115	56	0											S16
1152	1101	628	209	385	1230	1467	325	404	439	0										S17
1298	1305	836	248	51	1324	1578	754	828	868	431	0									S18
1267	1279	813	247	59	1290	1544	765	842	880	441	41	0								S19
1119	1076	598	160	347	1192	1431	371	452	485	56	395	401	0							S20
1126	1081	604	168	352	1200	1438	363	444	477	46	401	407	10	0						S21
1159	1092	634	316	492	1250	1478	216	295	331	108	538	549	157	149	0					S22
1164	1097	639	313	488	1255	1483	220	298	335	105	534	545	155	146	6	0				S23
1256	1175	737	441	602	1358	1578	139	191	240	235	643	660	289	279	138	138	0			S24
1185	1093	676	492	669	1295	1507	40	122	154	285	714	726	332	324	177	181	109	0		S25
1184	1089	679	513	691	1297	1506	18	103	132	307	736	748	353	345	199	203	129	23	0	S26

Figure 5.1. Relationship between linear geographic distances (km) between sites as calculated from GPS coordinates (GGD) and linear genetic distance between sites (GD).

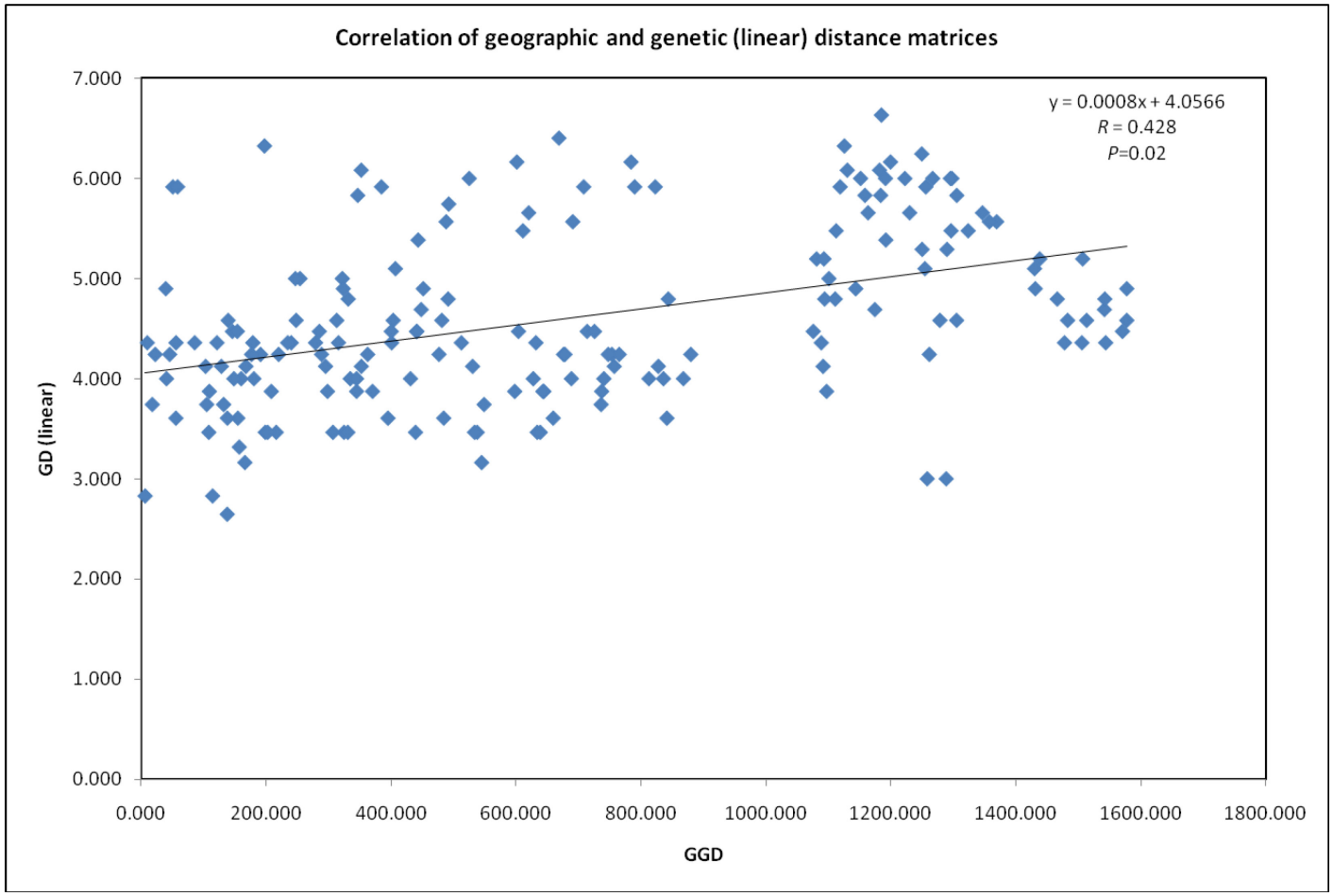
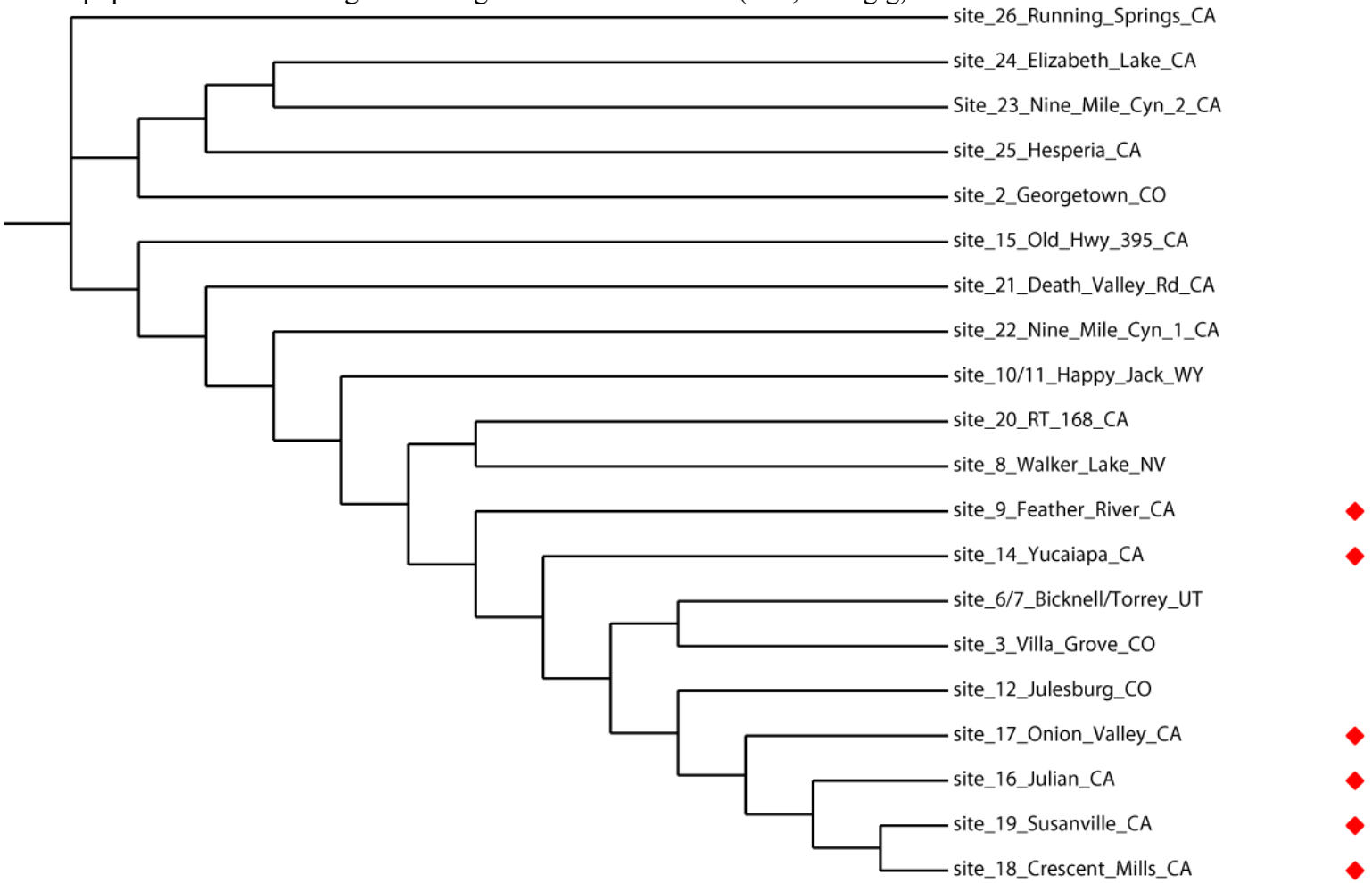


Figure 5.2. Unscaled UPGMA dendrogram derived from a population/population genetic distance matrix; the six populations with the highest average sakuranetin content (>50,000 ng/g) are marked with ◆.



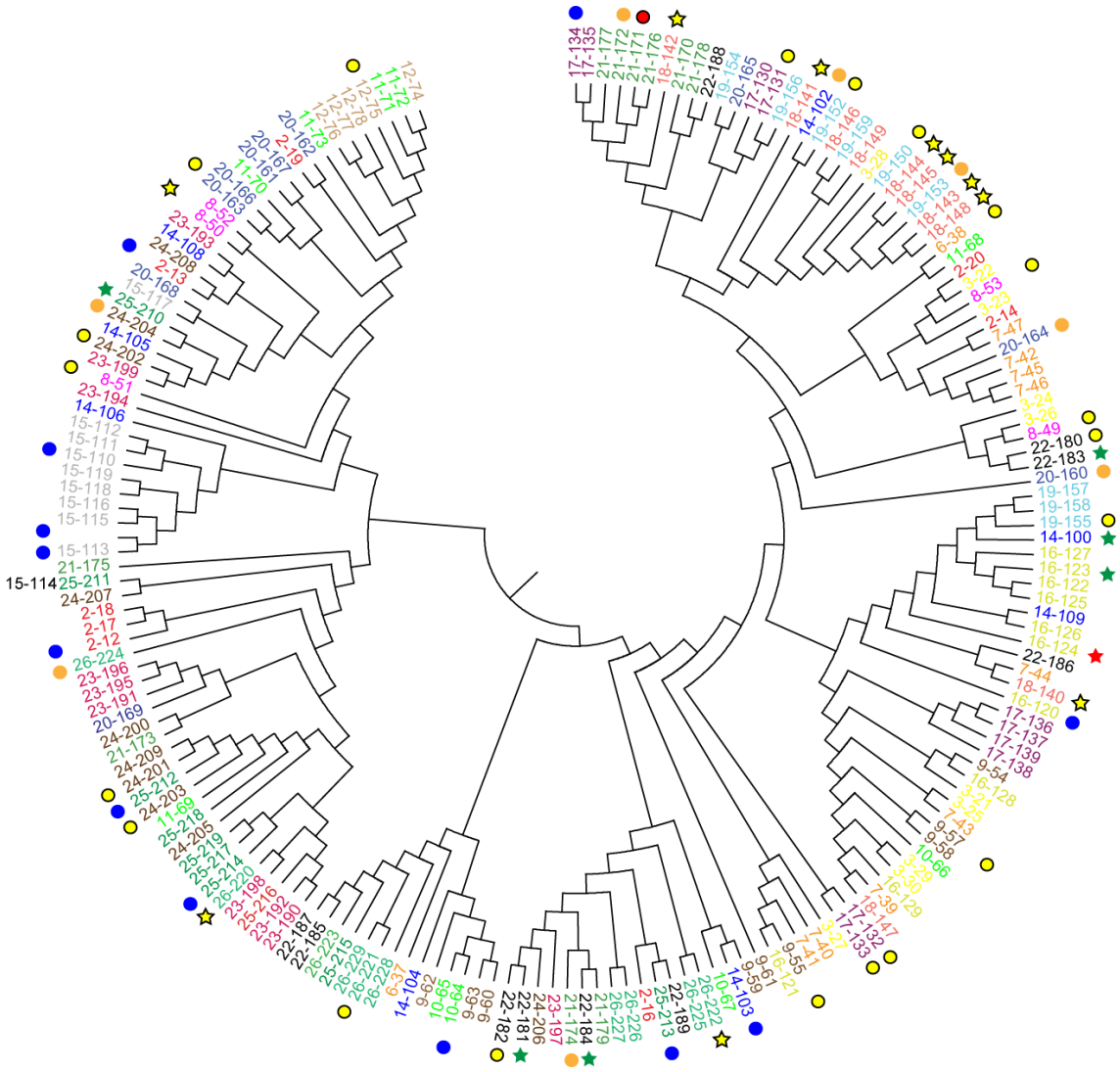


Figure 5.3. UPGMA dendrogram derived from a genetic distance matrix of 185 *A. dracunculus* samples. Samples are color coded by site. The symbols around the periphery represent the primary compound contained in essential oil extract of that individual (see chapter 4). E-β-ocimene = ●, Z-β-ocimene = ○, capillene = ●, methyl eugenol = ●, methyl chavicol = ☆, α-phellandrene = ★, α-terpinolene = ★. Only the individuals with symbols were extracted for essential oil analyses.

Chapter 6

Morphological analysis of North American specimens of *Artemisia dracunculus* L. with a particular focus on the subsp. *dracunculina* (S. Watson) Hall and Clements

Abstract

Artemisia dracunculus L. (wild tarragon) is a polymorphic species with a nearly circumglobal distribution. Over the past 150 years, populations in North America have been split into numerous sub-taxa by some, while others have only recognized one highly variable species. Two separate morphological analyses were conducted to examine some of the characteristics that have been used to define segregate and sub-specific taxa. The first analysis focused on elongated peduncles and wide, panicle-like synflorescences, traditionally used to delineate *Artemisia dracunculus* subsp. *dracunculina* (S. Watson) Hall and Clements. The second analysis investigated leaf morphology in live plants grown from root cuttings collected from western United States. The results of both morphological analyses gave similar conclusions. A great amount of morphological variation was found within the species, but there was no evidence for morphologically distinct sub-groupings that require subspecies or varietal taxonomic status. Additionally, both glandular, and non-glandular hairs were observed on North American *A. dracunculus* plants.

Introduction

Artemisia dracunculus L. (wild tarragon) is an herbaceous perennial well known for its wide morphological variation. Based on this variation, a number of authors have argued for the recognition of several infraspecific taxa (subspecies and varieties) within the species. A detailed account of the taxonomic history of *Artemisia dracunculus* including previously described species that are now synonyms, subspecies and varieties, is presented in Chapter 1. Wild tarragon is classified in section *Dracunculus* of the genus *Artemisia* L., which is distinguished from other sections by having sterile, functionally staminate disk florets and fertile peripheral florets, while the other sections have both fertile disk and peripheral florets (Figure 6.1). In addition to being functionally different, the two different floret types differ morphologically in *A. dracunculus* (Figure 6.2).

The plant is generally 0.5-1.5 m tall, with a woody caudex, rhizomes and fibrous roots (Hall and Clements, 1923; Poljakov, 1961b, Shultz, 2006). It has numerous erect stems, which are green, yellowish or reddish-brown, partially woody, particularly at the base, and usually glabrous and occasionally glaucous. The leaves are alternate, 4.5-12.0 cm long, linear-lanceolate, slightly coriaceous, often entire, but commonly irregularly lobed or variously trilobate, particularly towards the base of the plant, and usually glabrous. Inflorescences are capitula that are 2.0-6.5 cm wide, sub-globose to ovate, with short to long peduncles, and arranged in terminal or lateral racemiform to paniculiform synflorescenses. The phyllaries are green to light brown, herbaceous, but becoming scarious and hyaline at maturity, sometimes with a green or reddish-orange midrib, and the receptacle is naked. Florets are 1.0-2.0 mm long and light-yellow to yellowish-green or occasionally maroon-tinged at the apex. The peripheral florets are pistillate and fertile, and have a reduced corolla and a deeply cleft and revolute style, whose surface is covered

with papillae (Figure 6.2A, B and C). The disk florets are functionally staminate and have five connate petals with triangular lobes. Each disk floret has five connate stamens with introrse anthers and free filaments. The styles of the disk florets are shallowly cleft with short, terminal, penicillate appendages (Figure 6.2D and E). The fruits are oblong cypselae, 0.5-1.0 mm long, and brown and glabrous.

Because of the small size of the individual florets in *Artemisia* it is difficult to accurately study and measure the floral organs. The results of past studies of quantitative characteristics such as floret number per capitulum and leaf length have been disagreed upon and have not provided characteristics necessary to clearly define divisions between some intra-specific groups (Hall and Clements, 1923; Fernald, 1945). While multiple varieties are recognized in Asian Floras, the most recent regional treatment of *A. dracunculus* in the *Flora of North America* does not recognize any segregate or sub-specific taxa (Poljakov, 1961b; Shultz, 2006; Ling et al., in prep.). The primary morphological characteristics that have been used to segregate infra-specific taxa are leaf width and length, capitulum size, synflorescence shape (raceme-like or panicle-like), and peduncle length. An additional characteristic, which has been used to segregate the species *A. glauca* Pallas ex Willd. from *A. dracunculus*, is the presence of gray or silvery pubescence on the plant (Willdenow, 1803; Poljakov, 1961b).

To examine some of the characteristics that have been used to define segregate and sub-specific taxa in the *Artemisia dracunculus* complex, two separate morphological analyses were conducted. The first utilized herbarium specimens to assess five characters used historically to separate species, subspecies and varieties. Particular focus was on the two characters ‘elongated peduncles’ and ‘wide, panicle-like synflorescence’,

traditionally used to identify *Artemisia dracunculus* subsp. *dracunculina* (S. Wats.) Hall & Clements. The goal of this preliminary study was to determine if the variation of these morphological characters form a gradient with no clear boundaries or if there are discernibly unique morphological groups. The second analysis investigated leaf morphology and was conducted on live plants grown from root cuttings that were collected from different sites in the western United States. The goal of this analysis was to determine if variation in leaf morphology exhibited a gradient from small to large, or if distinct morphogroups could be observed. Additionally, anatomical observations were made in order to assess if the types of hairs found on North American *A. dracunculus* plants are similar to descriptions of hairs on Asian or European material which have been described in the literature.

Materials and Methods

For the first analysis, 33 specimens from Harvard University' herbarium (GH) were analyzed and measured for five morphologic characters: maximum leaf width (mm), panicle width (cm), length of the longest panicle branch (cm), and maximum peduncle length (mm) and maximum capitulum width (mm). The specific herbarium sheets included in the analysis were selected based on annotations made by M.L. Fernald and a portion of which represented sheets defined as subsp. *dracunculina*. Principal component analyses (PCA) and correlation analyses were conducted using SAS version 9.1 (reference). The software program R version 2.9.2 (R Development Core Team, 2009) with the package BiplotGUI version 0.0-5 was used to conduct a second PCA on a reduced data set of measurements (peduncle length, longest panicle branch and panicle

width) which are the specific characters used to define subsp. *dracunculina*. R version 2.9.2 and the package BiplotGUI were also used to produce graphical biplots and point predictivity and axis predictivity graphs (R for statistical, 2009; la Grange et al., 2009).

A second morphological analysis, which focused strictly on leaf size, was conducted using 49 live plants grown from root cuttings that were collected from 17 different populations in California, Nevada, Utah and Wyoming (Table 6.4; see Appendix 1, Table A.1 and Figure A.1.2 for collection site information). Plants were grown in a greenhouse at Rutgers University and at the time of measurement, all plants were in a non-flowering, vegetative state. Five separate measurements were made for each individual for leaf width (mm) and length (cm) and were averaged to acquire an average leaf length and width for each individual. Mature, fully expanded cauline leaves were measured. Leaf width was taken at the widest section of the leaf, below the start of the leaf lobes.

Microscopic observations of hairs were conducted using two different techniques. To observe hairs growing on fresh leaves, whole leaves were placed in a Petri dish and photographed with a Nikon Coolpix 8700 camera mounted to a Zeiss Stemi 2000C stereo microscope. Glandular hairs on the exterior of individual florets were examined by fixing immature, fresh capitula in a solution of 3:2:1 chloroform:glacial acetic acid: 95% ethanol. The capitula were left in the fixative for approximately one week and then transferred to propionic-carmine stain for one week. Individual flowers were removed from the capitulum under a dissecting scope and placed on a microscope slide in a drop of 35% glacial acetic acid. A cover slip was applied and images were taken at 400 and 1000x on a Nikon compound microscope fitted with an Olympus BX41 digital camera.

Images were processed with Magnafire 2.0 and in some cases Adobe Photoshop Elements 7.0 was used to alter the contrast and brightness of the images.

Results

***Analysis of herbarium specimens*.** Measurements taken from the herbarium sheets are presented in Table 6.1. Graphs showing the range for capitulum width, leaf width, panicle width and peduncle length are shown in Figures 6.3 and 6.4, with the samples in the graph organized from smallest to largest along the X axis (peduncle length $x10^1$ to make comparison more visible). Maximum peduncle length for the specimens ranged from 1.5 to 12.8 mm. Although both short- and long-peduncled specimens were found, the entire set of specimens represented a gradient with no clear delineation of groups based on peduncle length (Figure 6.5). This graph clearly shows that there is a strong correlation between the longest panicle branch and panicle width, and that these features are not associated with peduncle length, as has been described previously for *A. dracunculus* subsp. *dracunculina*. The correlation analysis of the five morphological characters shows that the length of the longest panicle branch and panicle width are strongly correlated (r = 0.85), whereas leaf width and capitulum width show weak correlation (r = 0. 42; Table 6.2).

PCA ordinations are presented for the first and second, first and third, and second and third principal axes in Figures 6.6, 6.7, and 6.8, respectively, with the specimens historically annotated as subsp. *dracunculina* demarcated. The first three axes account for 41%, 29% and 22% of the variation, respectively, and 91.14 % cumulatively (Table 6.3).

The second analysis, conducted with three of the characters (panicle width, longest panicle branch and peduncle length), traditionally used to define subsp. *dracunculina* also fails to show distinct groups (Figure 6.9A). Although 96.17% of the variation in the samples is accounted for by the first two components, the axis predictivities chart shows that the two characters, panicle branch length and panicle width, are almost completely represented by the 1st component while peduncle length is virtually unrepresented by the 1st component and poorly represented in the 2nd component (Figure 6.10A). After a scale transformation of the data, the axis predictivities chart shows that the peduncle length character is now almost completely represented by the two combined components and the plot of the samples show that the subsp. *dracunculina* specimens do group together but that grouping is not well isolated from the remaining *A. dracunculus* samples (Figures 6.9B and 6.10B). The point predictivities plots shown in figures 6.11A and 6.11B confirm that more of the samples are represented in the biplot after the scale transformation.

***Fresh specimens*.** The leaf measurements made from live plant material are shown in Table 6.4. Although the specimens exhibit a diversity of leaf morphologies (Figure 6.12A), the majority of the specimens form an indistinct cluster when graphed in a scatter plot (Figure 6.13). There a few unique outliers including the specimens with the smallest leaves collected in Wyoming, and the largest leaved-specimens collected in southern California (Figure 6.13). Although the collections from Wyoming represent the northernmost site for this study, correlation analyses of leaf width and leaf length

measurements with latititude do not support the idea that leaf size exhibits a gradient associated with latitude.

Observations of the hairs found on the florets revealed two types, non-glandular (Figure 6.14) and glandular (Figure 6.15). The glandular hairs were found to be multi-cellular, consisting of 8 or 10 cells, and varied in shape (both apically and basally thickened hairs were observed). Many of the specimens exhibited non-glandular hairs on young emergent shoots (Figure 6.14), while the mature leaves of all specimens examined were nearly glabrous, except for sample 20-163 (Figures 6.12B, C and D). This unique specimen was collected from a site in Inyo County, California and none of the other specimens collected from that site exhibited such a high amount of pubescence.

Discussion

The measurements from the herbarium specimens confirmed that there is a large amount of morphological variation in the species *A. dracunculus.* The analysis was conducted with a small set of samples and most likely represents a small portion of the morphological variation that actually exists in wild populations. When studied individually, each character used in this study displayed a well distributed gradient and did not provide any distinct groupings that could be used to segregate morphological variants.

Based on observations made while studying herbarium sheets of *Artemisia dracunculus*, and observations of the species growing in the wild, a number of problematic issues became apparent regarding the study of the morphology of this species. Study of the variation in morphology found in this species is complicated by the fact that

slight differences in the stage of development of the plant at the time of specimen collection (for the herbarium) can dramatically affect the size of the peduncles, capitula, phyllaries and florets. At maturity these parts are quite small (a capitulum can range from 2-3 mm wide) and the use of specimens determined to be at nearly the same stage of development was deemed to be critical.

Another issue with the study of herbarium collections arises from the fact that in some regions and environments the species can grow to be upwards of six feet tall with wide, spreading panicle-like synflorescences. Because of its height, the entire plant is rarely collected and it is often impossible to determine if the material presented on a herbarium specimen is just a fragment of this large panicle-like structure, or if what is presented is the entire upper portion of the plant. This can confound the study of the gross morphology of the species because the size and branching of the synflorescence has often been considered an important feature. Additional features that are often lacking from herbarium specimens of this species are cauline and basal leaves. In the United States, the species predominately grows in arid regions. By the time the plant has reached the stage of floral development (the most common stage to collect herbarium specimens), the lower leaves have often senesced. The lower portion of the plant is rarely collected with the exception being if the entire plant is small enough to fit on an herbarium sheet.

When plants from different regions were grown in a common garden experiment at Rutgers University, with abundant inputs of water and nutrients, the morphology of basal and cauline leaves were found to be extremely variable between populations. The scatter plot of cultivated samples did not provide distinct clusters based on these leaf measurements. There were two samples, from site 10, with particularly short, thin leaves.

These individuals do look unique, but increased sampling is needed to determine if there is a distinct morphological group based on small leaf size.

The hairs found on North American specimens are consistent with those of French and wild tarragon described by Worker et al. (1994) and Yaichibe et al. (1997). Both studies used seeds of cultivated wild tarragon from of unknown provenance, purchased from a commercial source in the Netherlands. The pubescence on sample 20-163 was unique. The entire plant was covered with dense pubescence even at maturity. Although this characteristic fits the description used for *A. glauca* very well, this unique specimen was collected from a site in Inyo County, California and none of the other specimens collected from that site exhibited a high amount of pubescence. Although this specimen is unique with regards to pubescence, it is very similar to the other specimens in genetic analyses, ploidy level and essential oil components and seems to be simple a mutant rather than a separate species (see chapters 2, 4 and 5).

Although not included in these analyses (specimens were observed during brief visits to various herbaria in the western U.S.), it was observed that *A. dracunculus* samples collected at high-altitudes (~3,350-3,660 m) may have unique morphological characters. Many of these samples share similar morphological features consisting of short plant height, raceme-like synflorescences with large capitula, and short, relatively wide leaves with rounded apices. Because samples with these similar characteristics have been collected on mountains with significant distance between them, this morphology may be due to convergent evolution and adaptation to high elevation, rather than because of a direct relationship between the distant populations. Similar adaptations such as large

capitula and reduced plant height are a trend in *Artemisia* species found growing in the Arctic (Tkach et al., 2008).

In general, the analyses conducted confirm the variation previously described for the *A*. *dracunculus* including differences in capitula width, pedicel length and panicle width. Although the measurements show this variation, the specimens represented a morphological gradient and neither analysis provides evidence of distinct groupings useful in delineating morphogroups. Therefore, this study supports the recognition of a single polymorphic species. Because such a limited number of specimens were used in these analyses, this can only be considered a preliminary investigation and is not conclusive.

Table 6.1. Morphological measurements of herbarium samples from GH; coll. no. = collection number; maxcapit = maximum capitulum width (mm); maxlewid = maximum leaf width (mm); lengtlongpbr = length of the longest panicle branch (cm); widpan = width of the panicle (cm); maxped = maximum peduncle length (mm).

collector	coll. no.	maxcapit	maxlewid	lengtlongpbr	widpan	maxped
Walker	43	3.1	2.2	8.0	6.2	2.9
Clokey	2946	3.3	2.0	9.0	10.6	2.1
Eastwood	80	3.0	3.0	7.5	6.9	3.1
Ewan	997	2.6	2.0	9.5	5.1	2.8
Baker	651	3.0	2.0	8.7	13.8	3.6
Spongberg	64_256	5.3	4.2	8.6	5.9	2.7
Engelmann	s.n.	3.0	2.5	2.5	2.2	2.1
Breitung	1696	3.0	2.0	7.0	3.7	2.1
Tolstead	799	3.0	3.0	5.8	2.8	1.9
Palmer	1876	4.0	4.0	8.1	3.7	1.5
Merrill	1008	3.0	4.0	2.7	1.6	1.7
Nelson	4132	4.0	4.0	6.8	3.9	3.1
Nelson	9	3.2	3.4	3.7	2.2	1.8
Scoggan	1057	3.2	4.3	17.2	11.8	1.5
Allen	s.n.	2.9	1.9	31.3	23.8	1.9
Sears	s.n.	3.1	6.9	25.9	19.1	2.2
Blumer	1372	3.9	7.8	42.6	28.0	3.1
June	s.n.	3.9	2.2	4.6	8.3	6.6
Pammel	1084	2.8	1.5	10.4	13.6	8.9
Eastwood	8683	3.2	1.4	12.2	12.9	7.9
Lemmon	s.n.	4.2	1.7	16.4	16.1	8.9
Gillespie	8647	4.1	2.8	14.4	26.2	6.9
Hinckley	2127	3.2	1.7	28.5	23.4	12.8
Hartman	778	3.8	4.7	8.8	8.9	9.2
Shreve	5368	4.2	2.2	25.8	18.7	8.1
Cutler	3038	4.5	2.6	9.7	7.7	8.2
Leiberg	868	4.9	2.7	23.4	23.7	5.3
Barrell	502-62	5.0	3.0	13.0	6.4	6.9
Thornber	s.n.	2.8	5.2	16.4	14.7	4.4
Norton	298	3.5	1.6	13.7	15.7	4.5
Sharsmith	3836	6.4	8.9	6.4	3.0	5.8
Hozinger	1538	4.4	2.5	22.2	17.4	5.5
Boivin	s.n.	4.5	6.9	18.1	5.8	4.4

Table 6.2. Correlation matrix of the morphological characters measured on herbarium specimens; see table 6.1 for key to abbreviations.

	maxcapit	maxlewid	lengtlongpbr	widpan	maxped
maxcapit	1	0.421	0.061	-0.013	0.269
maxlewid	0.421	1	0.209	-0.063	-0.227
lengtlongpbr	0.061	0.209	1	0.850	0.210
widpan	-0.013	-0.063	0.850	1	0.369
maxped	0.269	-0.227	0.210	0.369	1

Table 6.3. Eigenvalues, percentage variance and cumulative variance for the principal component analysis.

Axis	Eigenvalue	% variance	% Cumulative
1	2.037	40.75	40.75
2	1.432	28.65	69.40
3	1.087	21.74	91.14
4	0.339	06.79	97.93
5	0.104	02.07	1

Table 6.4. Measurements (cm) of five mature leaves from living *A. dracunculus* plants grown in a common garden; LL = leaf length; LW= leaf width; avgLL = average leaf length; avgLW = average leaf width.

sample	LW1	LW2	LW3	LW4	LW5	avg LW	LL1	LL2	LL3	LL4	LL5	avg LL
10-64	3.24	3.22	2.67	3.52	2.76	3.08	4.50	4.40	4.90	4.90	4.30	4.60
10-65	2.81	2.97	2.47	3.35	2.83	2.89	6.60	6.00	4.60	6.20	5.40	5.76
14-102	4.78	4.98	5.89	6.49	6.34	5.70	8.90	9.20	6.50	7.70	9.00	8.26
15-110	10.11	10.44	10.27	10.76	10.76	10.47	10.00	10.20	9.40	9.70	8.90	9.64
15-113	7.94	8.01	8.29	7.48	7.25	7.79	6.70	6.00	6.50	7.10	6.40	6.54
15-114	6.37	7.73	6.53	7.38	6.86	6.97	9.20	9.90	10.70	7.20	9.40	9.28
16-120	3.46	3.81	4.26	6.56	4.80	4.58	9.90	9.80	9.70	11.00	9.60	10.00
16-123	6.51	6.33	6.86	7.82	6.53	6.81	12.20	11.60	10.40	8.10	8.70	10.20
17-131	6.86	8.99	8.57	7.24	8.65	8.06	13.20	9.10	8.50	9.80	12.80	10.68
17-131	12.57	10.41	9.18	8.29	8.30	9.75	9.80	9.40	9.30	8.80	8.70	9.20
17-132	9.16	8.20	6.72	7.25	6.65	7.60	11.60	10.30	11.50	10.20	9.50	10.62
17-133	9.30	8.12	7.71	8.91	7.61	8.33	9.80	8.30	9.70	9.80	8.20	9.16
17-134	12.72	9.81	8.19	9.15	9.48	9.87	11.20	8.00	11.10	10.60	8.80	9.94
18-140	10.98	11.83	10.33	12.02	11.82	11.40	9.50	11.20	9.50	10.50	9.00	9.94
18-140	10.08	9.42	10.08	10.30	8.58	9.69	8.60	8.00	8.80	9.00	9.30	8.74
18-144	6.27	7.27	7.16	6.24	6.38	6.66	7.50	8.20	5.70	6.80	6.00	6.84
18-155	6.64	8.42	7.53	5.89	6.76	7.05	8.80	8.60	9.70	7.70	9.20	8.80
19-154	10.83	10.50	10.62	9.84	10.60	10.48	6.50	6.40	6.60	6.80	5.76	6.41
20-160	4.47	4.74	5.32	5.52	5.07	5.02	9.40	10.80	13.50	14.20	9.00	11.38
20-163	6.37	6.22	6.60	6.11	5.48	6.16	9.20	10.10	9.90	11.90	10.50	10.32
20-164	5.36	6.00	5.46	4.88	7.20	5.78	11.90	10.60	10.70	11.30	8.70	10.64
21-172	4.62	3.88	4.62	4.19	4.38	4.34	17.30	15.70	15.40	14.30	14.20	15.38
21-174	7.48	6.43	6.38	6.21	6.94	6.69	14.00	14.30	17.20	16.50	15.20	15.44
22-180	7.88	8.25	8.59	8.38	7.90	8.20	9.00	9.10	10.40	9.70	10.40	9.72

sample	LW1	LW2	LW3	LW4	LW5	avg LW	LL1	LL2	LL3	LL4	LL5	avg LL
22-181	7.32	7.15	7.43	6.53	6.93	7.07	9.20	9.40	9.60	10.70	9.80	9.74
22-182	7.62	8.13	7.19	6.07	6.07	7.02	11.00	12.50	13.60	11.60	12.00	12.14
22-183	8.22	9.22	8.18	8.02	7.82	8.29	9.80	9.50	9.60	9.90	10.20	9.80
22-184	7.16	6.51	6.91	7.22	6.88	6.94	10.70	9.90	10.10	10.30	10.50	10.30
23-191	9.77	7.46	6.26	7.13	7.12	7.55	8.80	9.20	8.20	8.40	7.90	8.50
24-200	6.75	7.55	6.42	7.45	6.45	6.92	7.30	8.20	8.30	6.80	6.90	7.50
24-201	6.47	4.52	5.81	5.18	5.96	5.59	9.50	8.80	8.90	7.50	9.20	8.78
24-202	9.30	7.35	6.95	8.54	6.91	7.81	9.10	8.20	8.40	9.20	9.80	8.94
24-203	8.30	7.53	9.14	6.60	9.70	8.25	11.00	10.00	11.20	9.60	11.10	10.58
24-204	6.69	5.65	6.01	7.48	6.19	6.40	8.70	9.40	9.20	9.70	7.20	8.84
25-210	4.69	3.94	4.53	4.23	4.33	4.34	8.40	7.50	7.70	7.20	7.40	7.64
25-212		7.06	6.39	6.56	6.77	6.70	6.00	6.10	6.60	5.80	6.40	6.18
25-213	5.51	6.15	4.98	5.53	5.25	5.48	8.20	7.20	8.00	7.70	8.60	7.94
26-221	6.96	5.53	5.00	6.24	4.71	5.69	10.10	11.00	10.80	11.50	9.50	10.58
26-222	10.58	13.28	8.27	11.49	7.50	10.22	10.50	12.70	10.70	11.20	14.40	11.90
6-38	2.93	2.38	4.29	3.82	2.77	3.24	12.00	8.30	7.20	10.80	8.70	9.40
8-49	21.68	18.48	14.49	14.92	16.53	17.22	13.50	13.60	14.00	11.00	10.30	12.48
8-50	8.50	8.45	7.29	10.26	7.46	8.39	11.00	11.70	11.50	10.25	11.70	11.23
8-50	8.82	11.50	10.44	10.86	11.30	10.58	14.00	15.20	15.90	12.00	12.20	13.86
8-51	9.44	7.43	3.30	8.11	7.80	7.22	10.50	9.80	11.20	9.50	9.00	10.00
8-53	5.46	5.81	6.21	4.81	5.99	5.66	11.20	13.10	12.90	11.20	12.50	12.18
9-56	6.95	7.51	6.64	6.31	7.01	6.88	8.00	6.90	7.60	6.70	6.40	7.12
9-57	8.76	7.70	8.25	10.05	6.73	8.30	7.00	8.60	9.50	10.30	9.20	8.92
9-58	6.56	7.22	5.97	5.87	5.80	6.28	8.90	8.50	7.40	7.40	7.00	7.84
Kyrgyz	10.20	11.01	12.21	11.87	11.94	11.45	10.70	10.20	12.20	11.30	10.96	11.07

Figure 6.1. Capitula of *A. dracunculus* A) four capitula exhibiting stigmas of the peripheral fertile florets and dehiscing pollen from the functionally staminate disc florets, B) capitulum with dehiscing anthers at anthesis spreading pollen, C) capitulum with receptive stigmas.

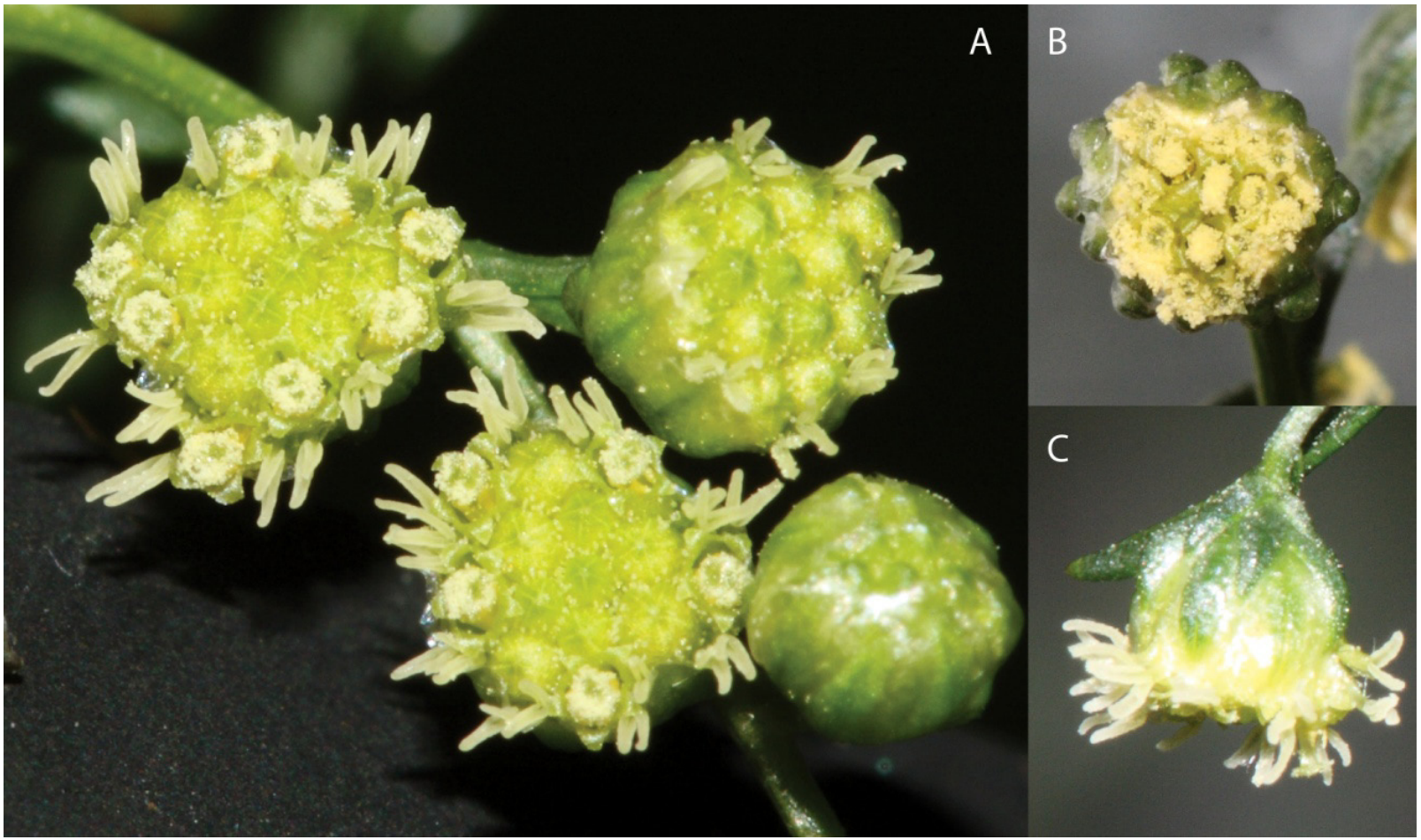


Figure 6.2. Floret dimorphism in *A. dracunculus*; A) pistil from a fertile peripheral floret, B) stigma of peripheral floret, C) maturing ovary of fertilized peripheral floret, D) style and stigma from a functionally staminate disk floret, E) penicillate stigma of a disk floret, F) anther with pollen grains from a disk floret.

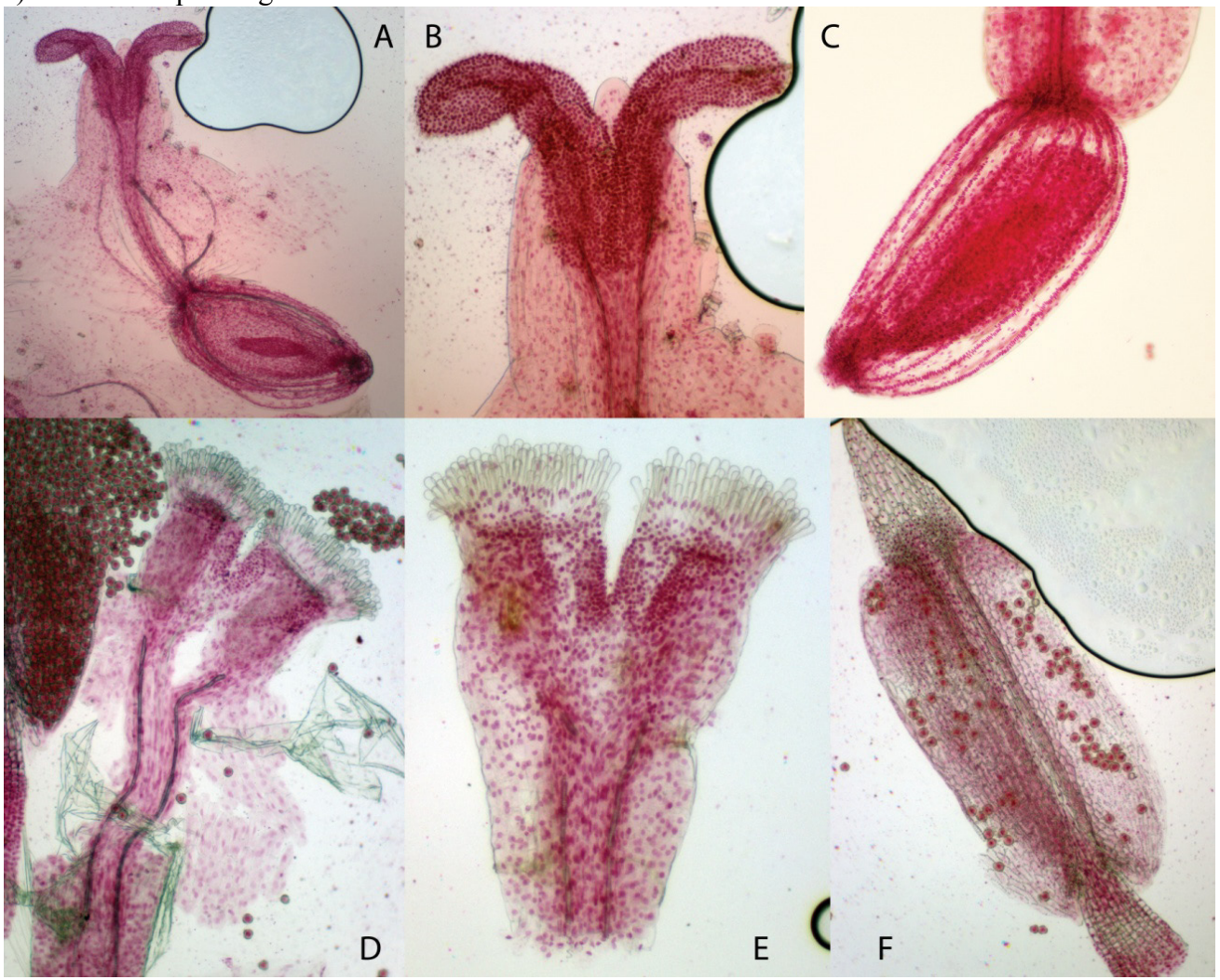


Figure 6.3. Measurements of morphological characters collected from herbarium specimens arranged from the smallest to largest; A) capitulum width, B) leaf width.

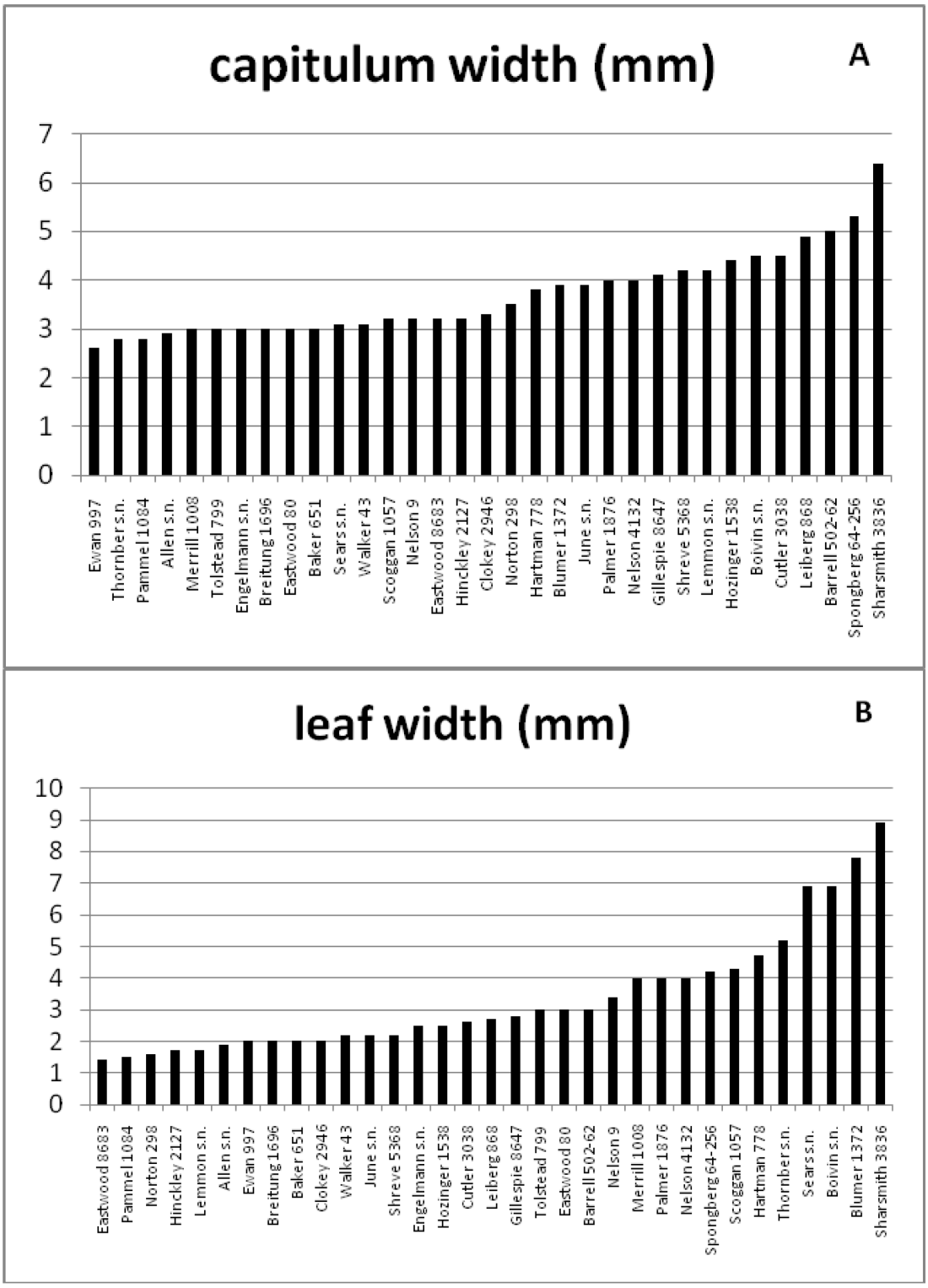


Figure 6.4. Measurements of morphological characters collected from herbarium specimens arranged from the smallest to largest; A) panicle width, B) peduncle length.

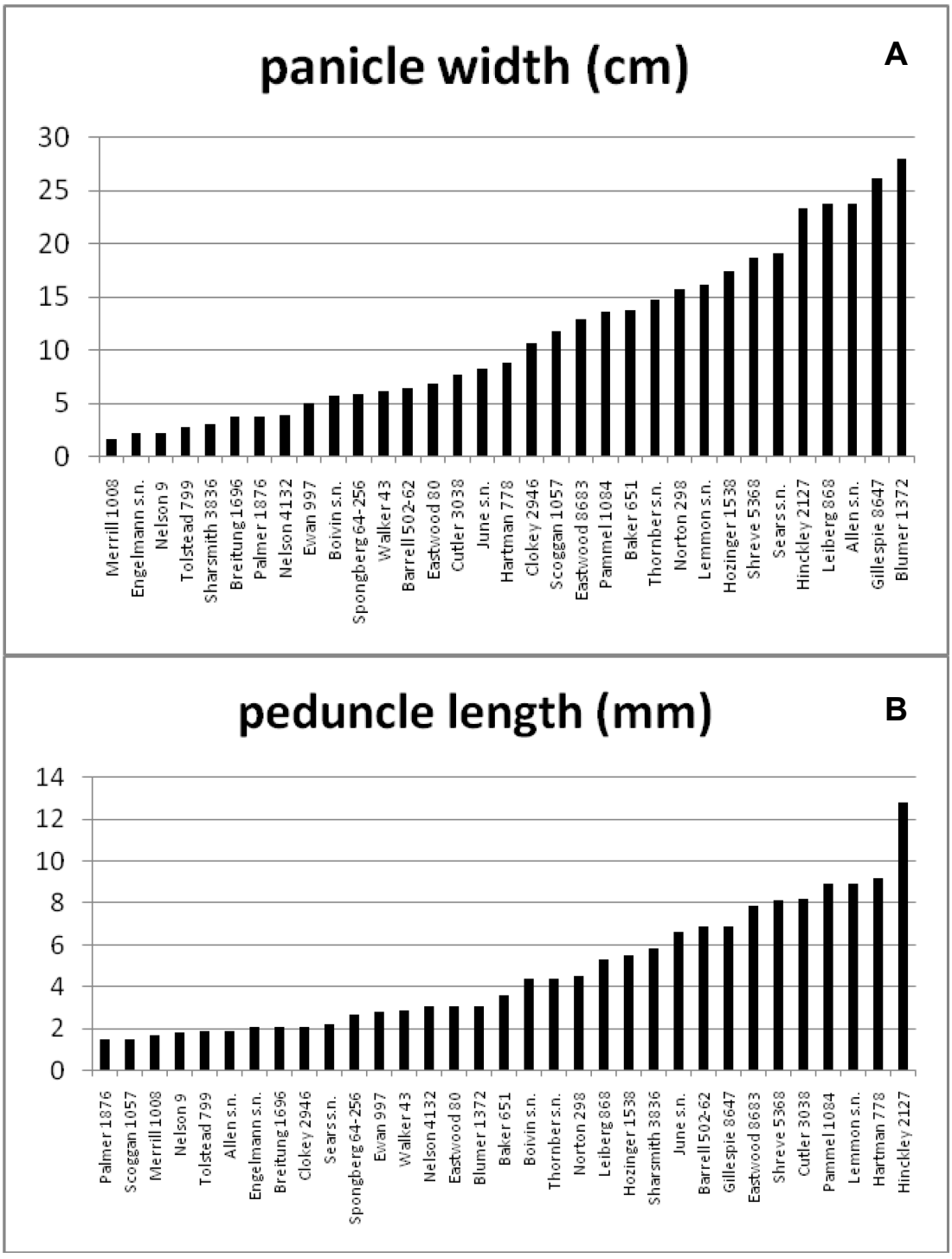


Figure 6.5. Measurements of panicle width, longest panicle branch, and peduncle length (measurements increased by 10^1), characters traditionally used distinguish *A*. *dracunculus* subsp. *dracunculina*.

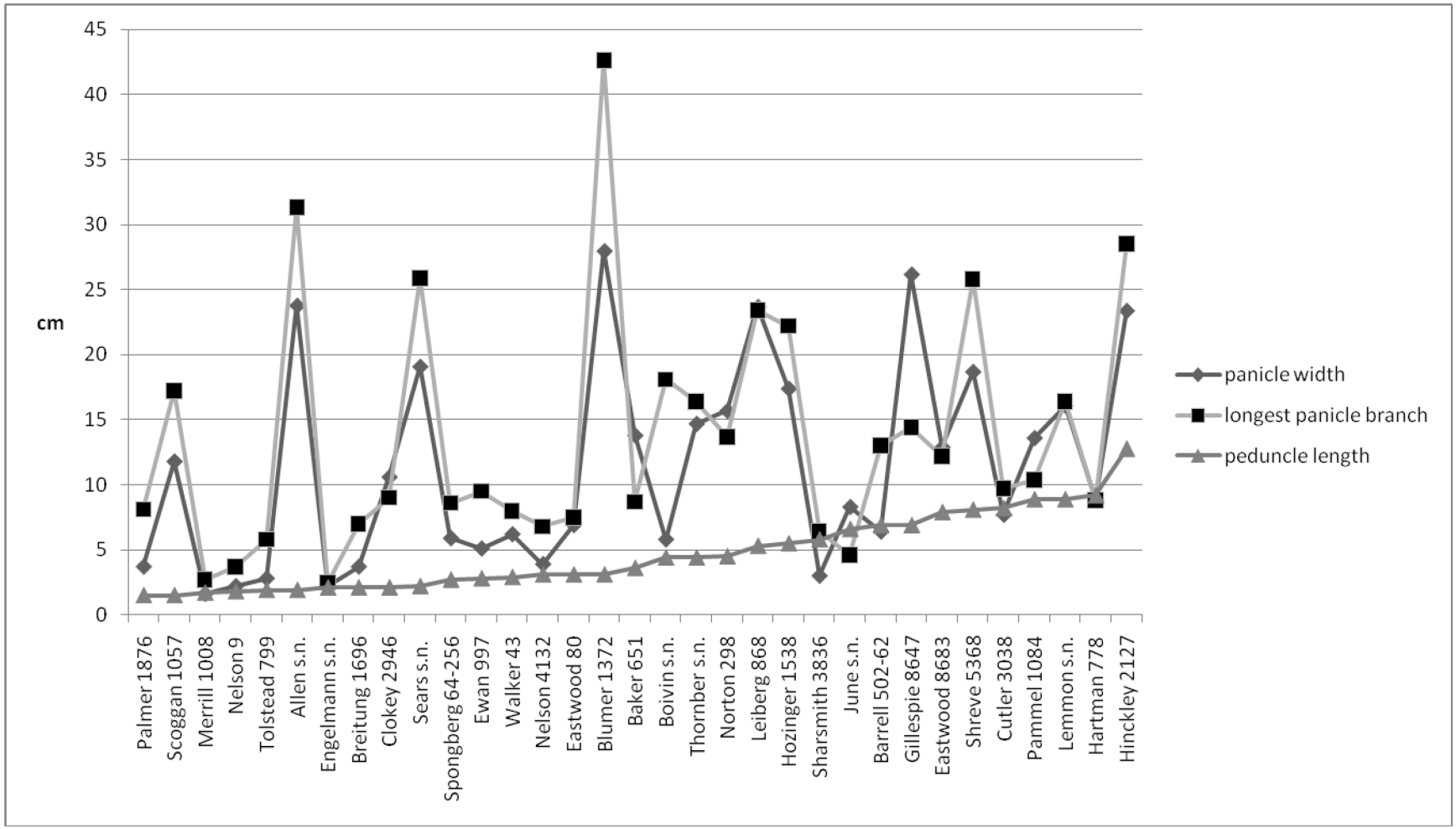


Figure 6.6. PCA of the 1st and 2nd components derived from the complete set of morphological measurements made on herbarium specimens. Those marked as *dracunculina* were annotated as such by M.L. Fernald.

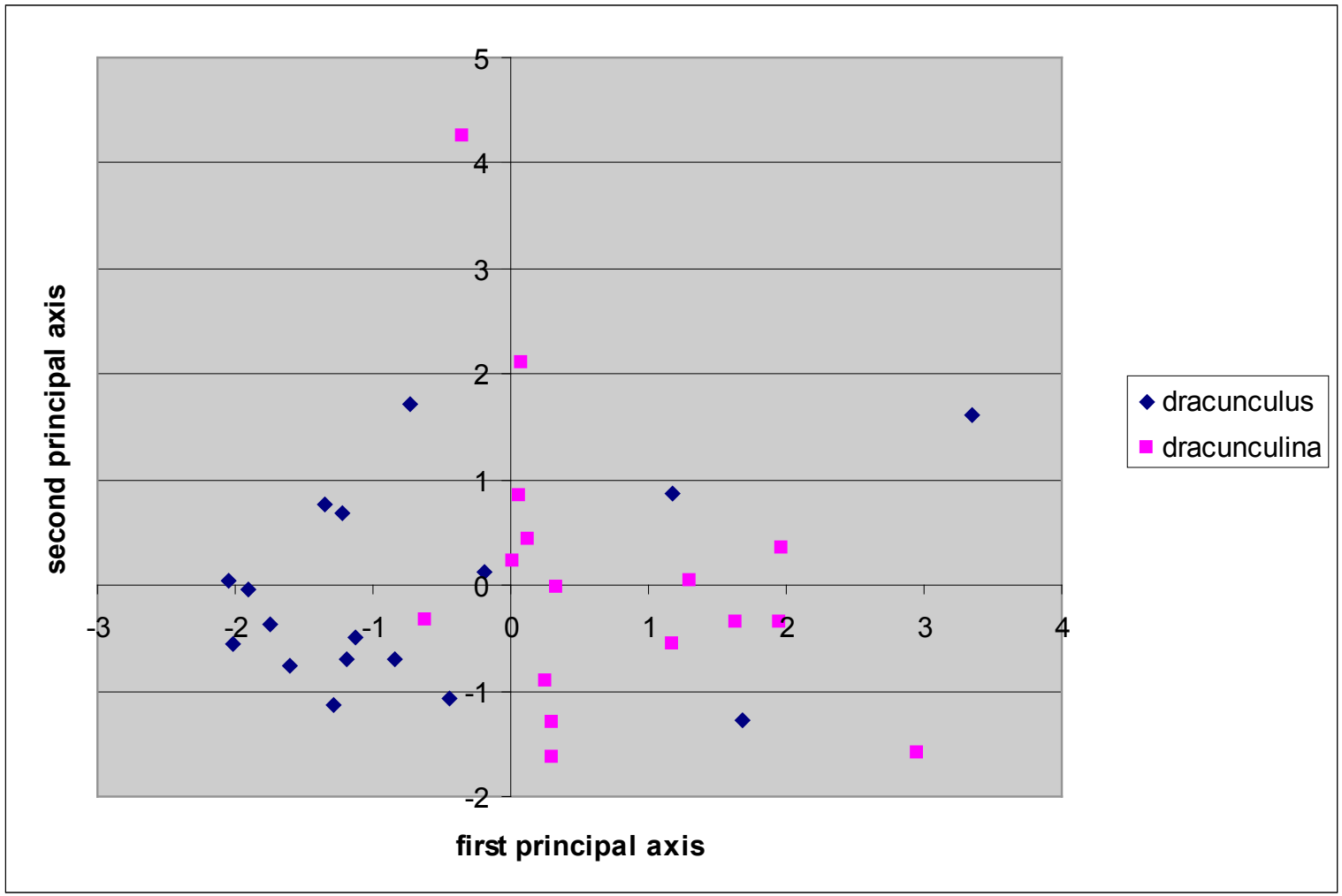


Figure 6.7. PCA of the 1st and 3rd components derived from the complete set of morphological measurements made on herbarium specimens.

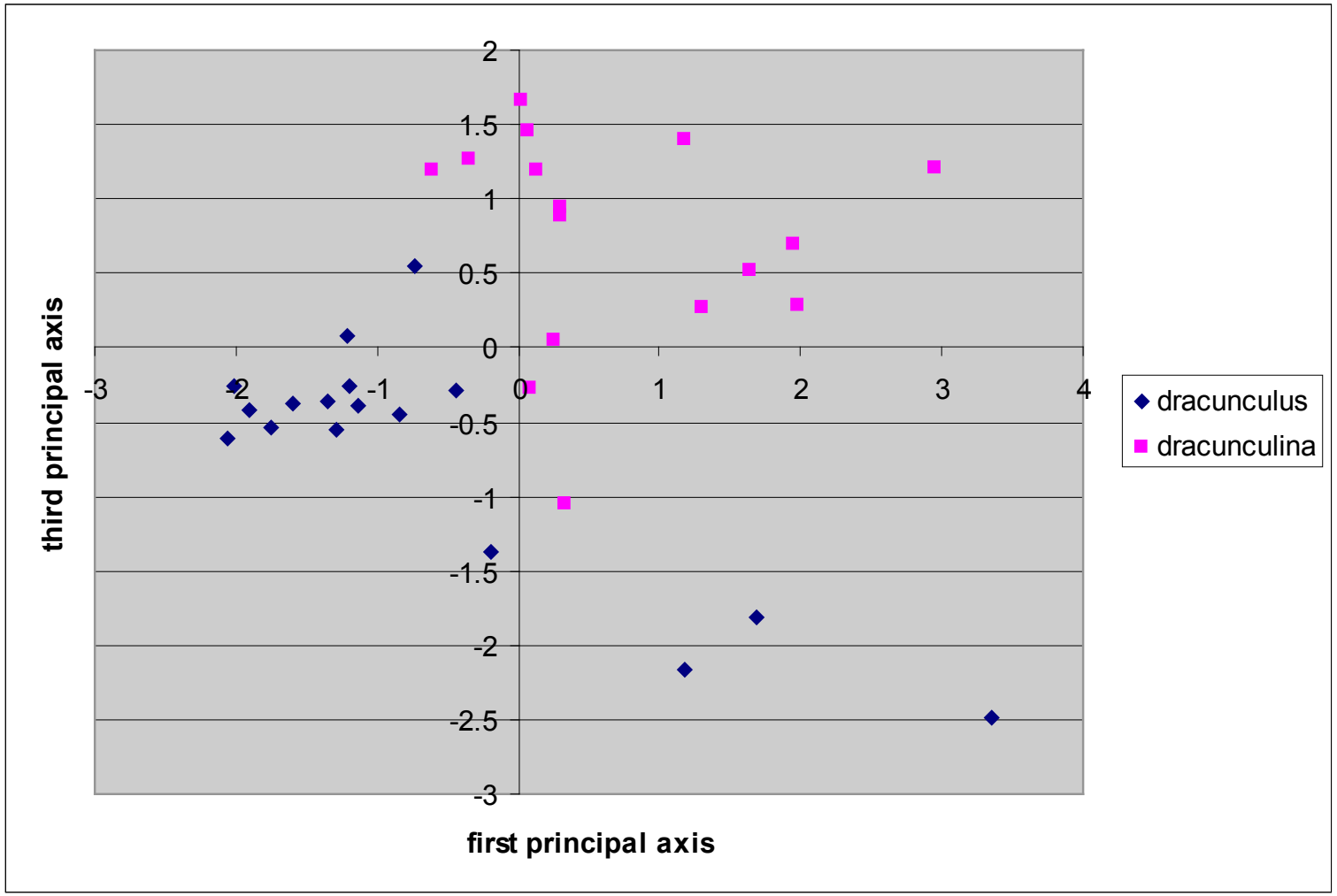


Figure 6.8. PCA of the 2nd and 3rd components derived from the complete set of morphological measurements made on herbarium specimens.

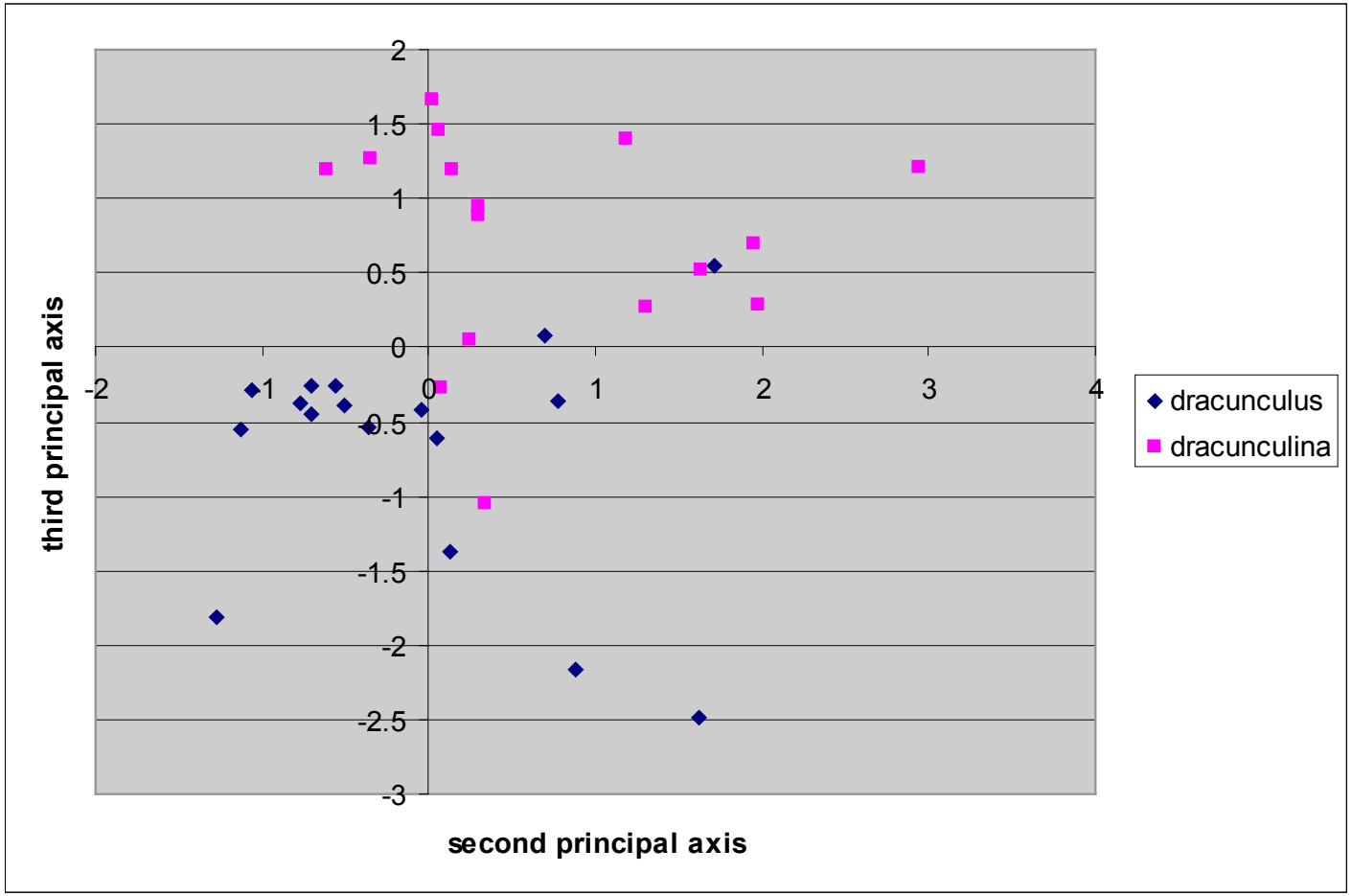


Figure 6.9. PCA biplot of the characters peduncle length, panicle width and panicle branch length; Group 1 = subsp. *dracunculus* specimens, group 2 = subsp. *dracunculina* specimens A) untransformed data set B) scale transformed data set.

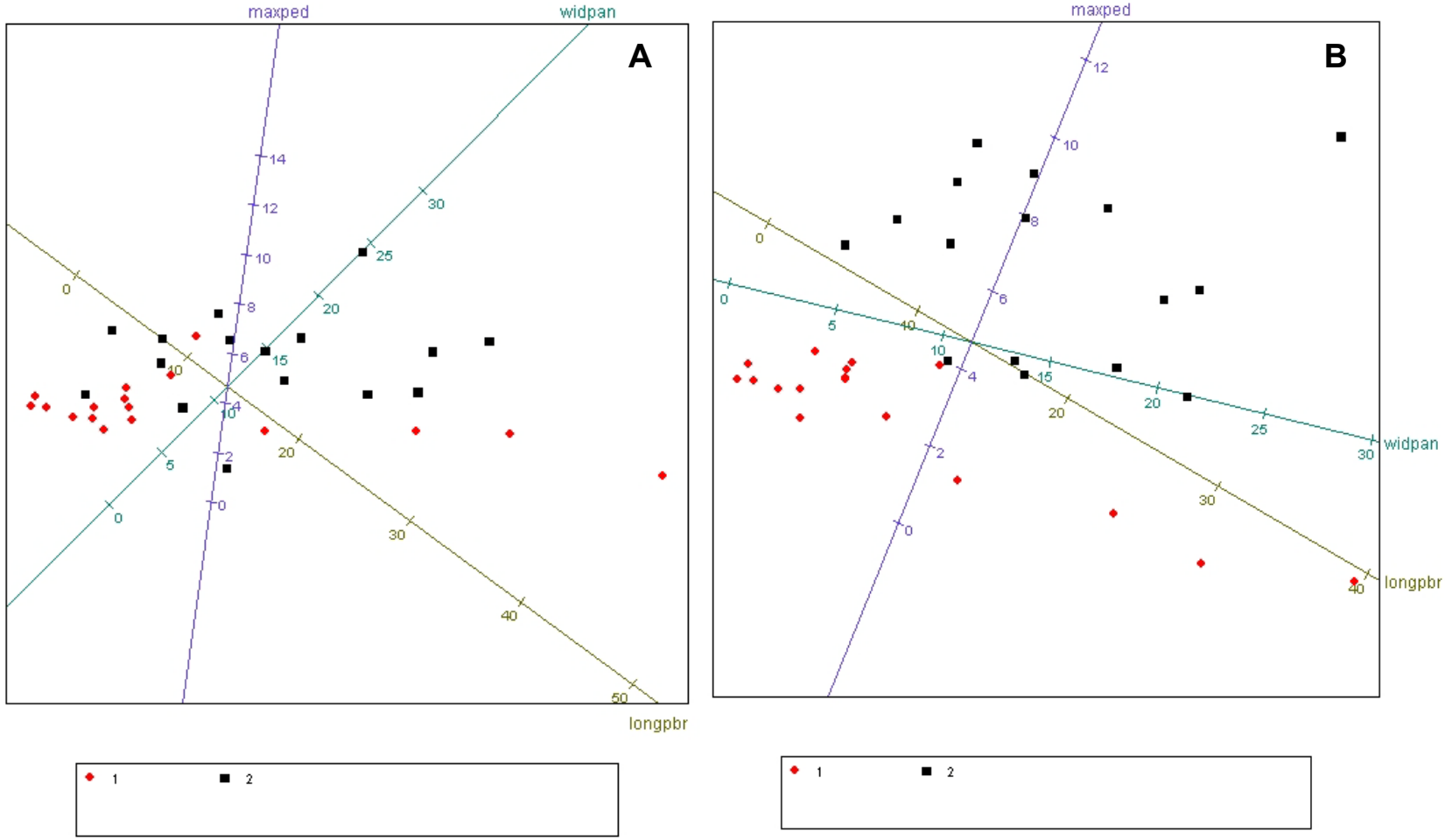


Figure 6.10. Axis predictivities for biplots in Figures 6.9A and B displaying how well the characters are represented by the dimensions of the biplot; A) untransformed data set, width of the panicle and longest panicle branch are highly represented by the 1st principal component and maxped is poorly represented B) after a scale transformation of the data set, maxped is better represent by the 2nd principal component.

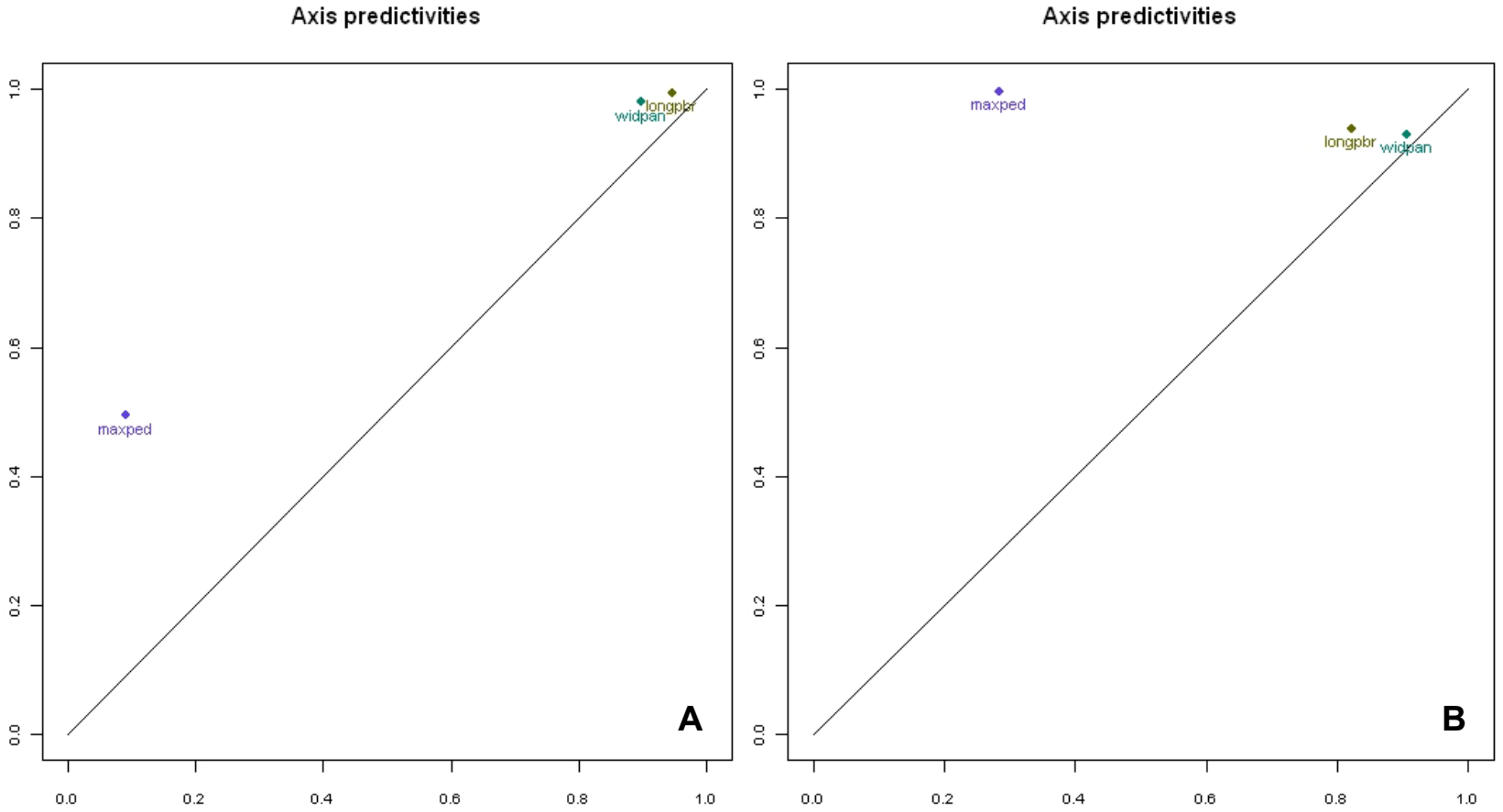


Figure 6.11. Point predictivities for biplots in Figures 6.9A and B displaying how well the samples are represented by the dimensions of the biplot; A) untransformed data set B) scale transformed data set has more of the sample represented in the first two principal components**.**

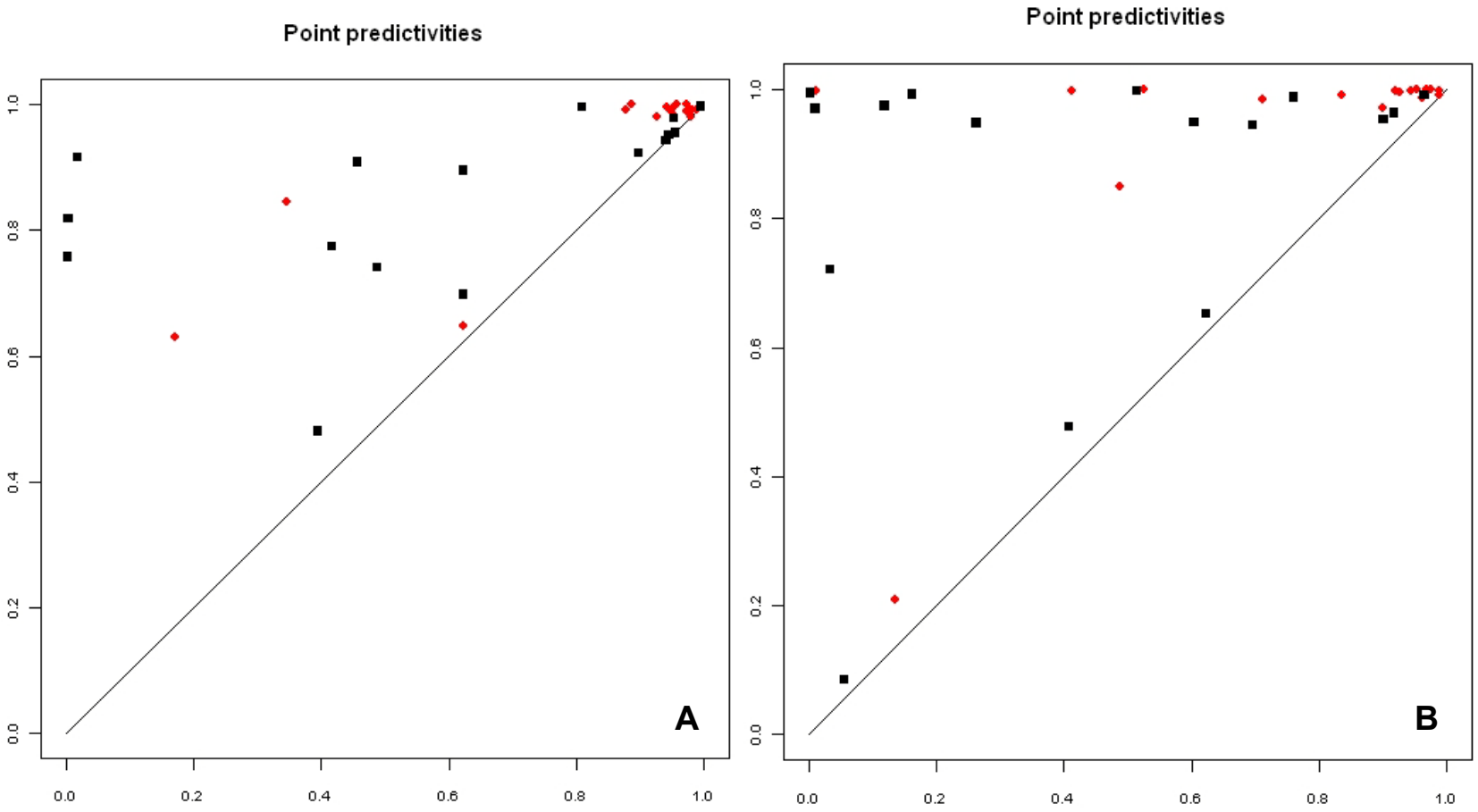


Figure 6.12. A) examples of morphological variation in leaves of *A. dracunculus* plants from various sites in the United States, B) typical leaves at maturity (glabrous), C&D) unique pubescent sample (20-163).

Figure 6.13. Measurements of leaf length and width taken from live *A. dracunculus* specimens.

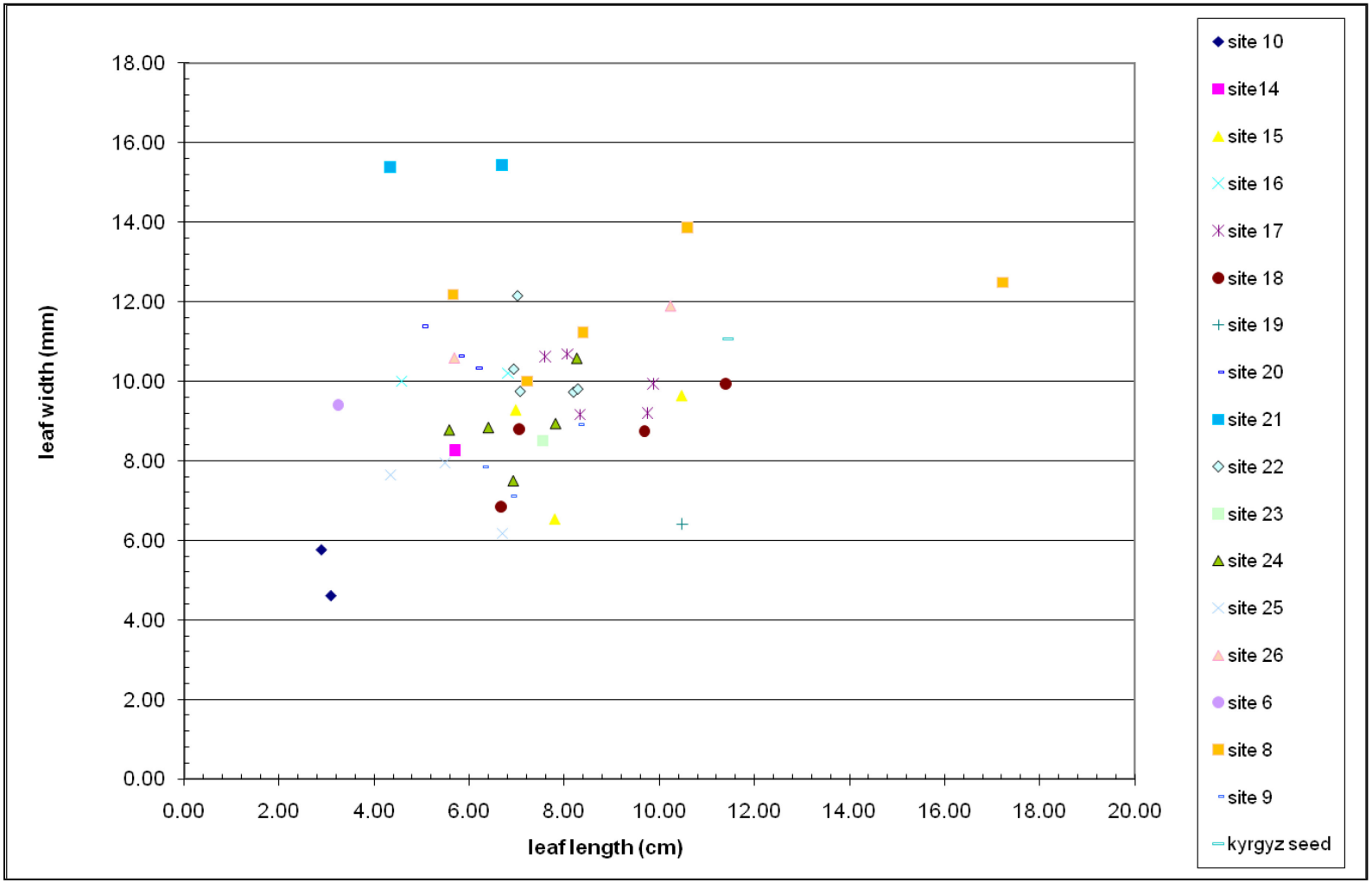


Figure 6.14. Non-gladular hairs on young vegetative shoots of *A. dracunculus*.

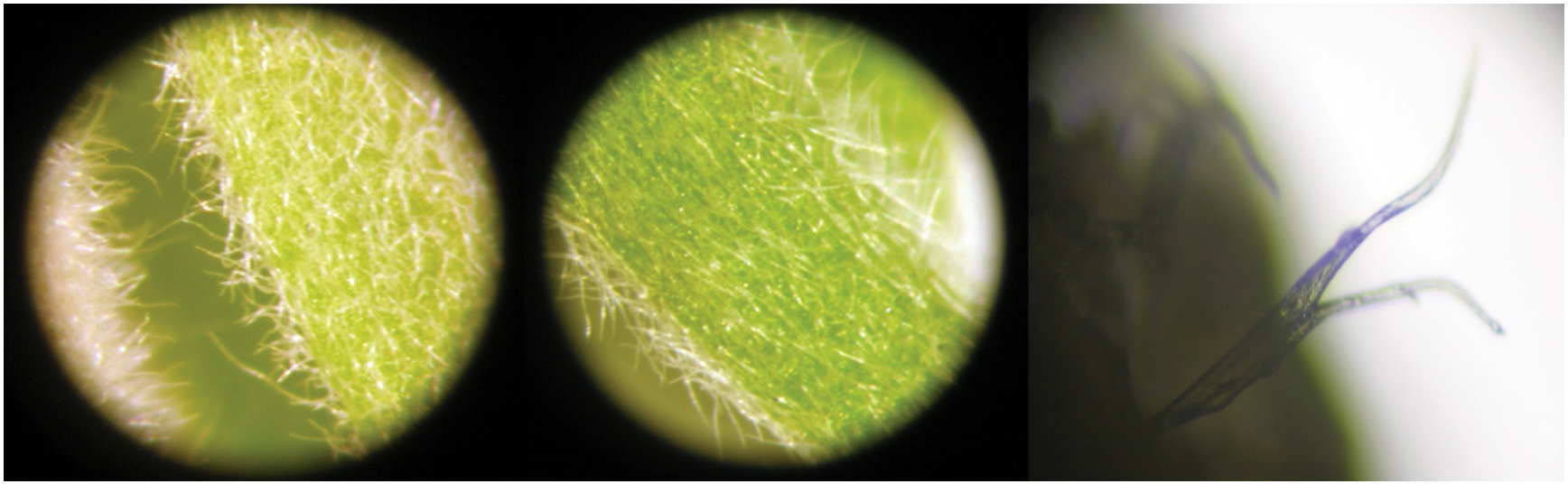
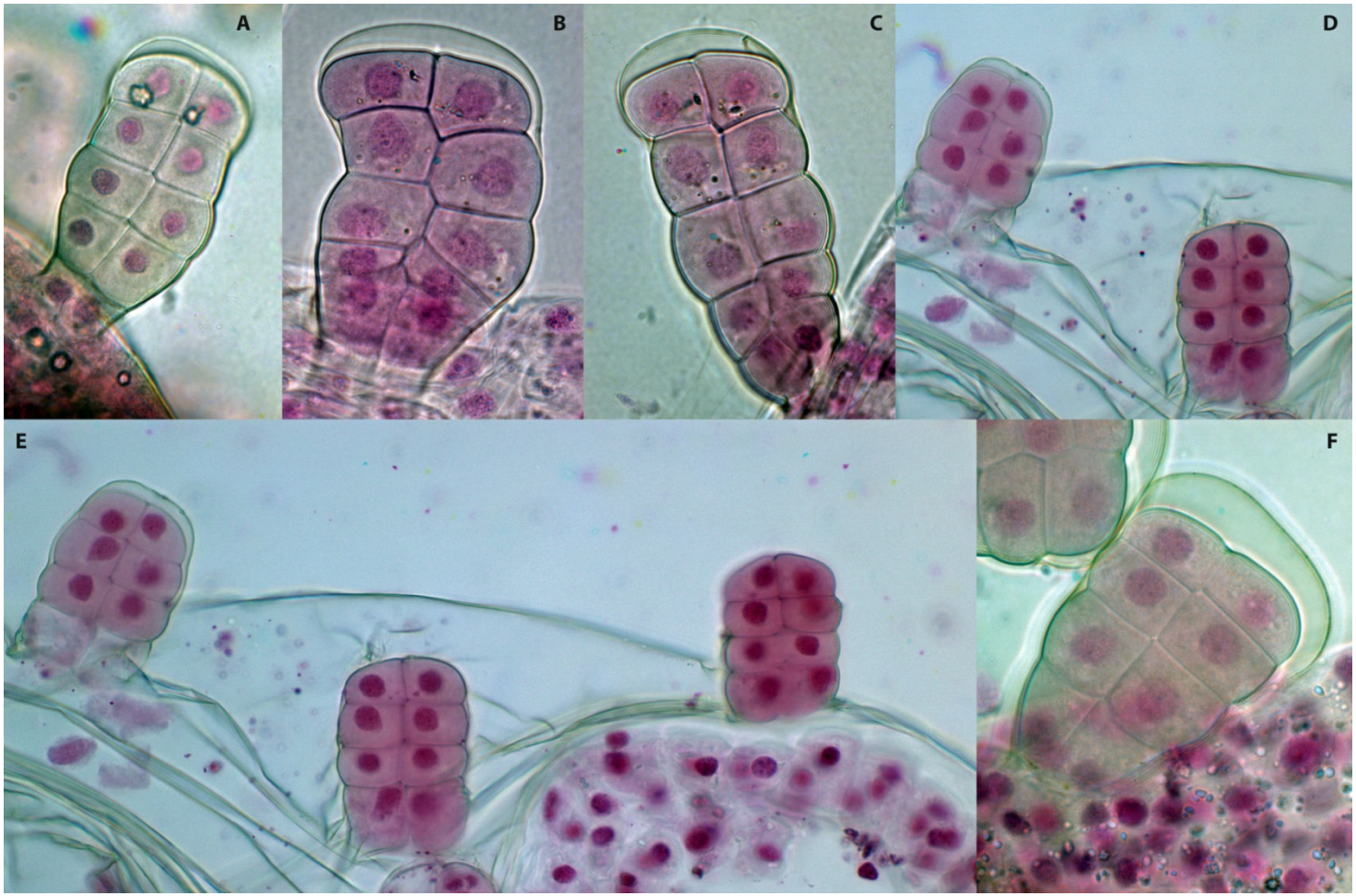


Figure 6.15. Glandular hairs on the outer surface of *A. dracunculus* corollas. A) sample 2-13, B&C) 10-64, D) 26-221, E) 19-154.



Appendix

Plant material and collection sites

Collections of wild growing *Artemisia dracunculus* plants used in research conducted for this dissertation were made in 2006 and 2007. In order to collect all the plants at the same time of the year, collection expeditions were conducted during the last week of August and the first week of September of both years. Sites were found by using locality records obtained via the Global Biodiversity Information Facility (GBIF) portal and the California Consortium of Herbaria database, as well as by direct examination of specimens from, BYU, CS, GH, KANU, NY, PH, RENO, RM, RSA, US and UT.

In total, 116 samples were collected from twenty-five sites throughout the western United States (Table A.1; Figures and A.1.1 and A.1.2). An effort was made to sample from populations across an altitudinal gradient (Figure A.1). Plant samples consisted of *Artemisia dracunculus* L. var. *dracunculus* (104 samples), *A. campestris* L. subsp. *caudata* (Michaux) H. M. Hall & Clements (a member of subgen. *Dracunculus*; 5 samples), *A. ludoviciana* Nutt. subsp. *incompta* (Nuttall) D. D. Keck (subgen. *Artemisia*; 2 samples) *A. ludoviciana* Nutt. subsp. *ludoviciana* (3 samples), and *A. ludoviciana* Nutt. subsp. *candicans* (Rydberg) D. D. Keck (1 sample; Table A.1). Each sample is named using the site number followed by the unique individual identifier (ex. 26-220 is site 26, individual 220). In 2006, root cuttings were collected from plants in the wild, wrapped in moistened paper towels, and stored in plastic bags until returning to Rutgers University. This method of storage was found to be too moist an environment and a number of the roots decayed and could not be cultivated (see Table 3.2). During the collection trip of

2007, the root cuttings were stored in plastic bags with dry potting mix and the survival rates for those roots were much higher. For comparison, French tarragon plants were purchased from Pantry Garden Herbs (Missouri, USA) and a single plant was cultivated from seed collected in the Central Asian country, Kyrgyzstan. Four plants were also grown from purchased seeds (Sheffield's Seed Company, NY).

Table A.1. Collection localities, coordinates (WGS84) and elevations. *Artemisia dracunculus* was collected at all sites except site 1*(*A. campestris* subsp. *caudata*), site 4†(*A. ludoviciana* subsp. *incompta* and *A. ludoviciana* subsp. *ludoviciana*), and site 5‡ (*A. ludoviciana* subsp. *candicans*).

collection site	country	state	county	locality	latitude	long	Altitude (ft)
site 01*	USA	Colorado	Kit Carson	Sand Creek	39.276	-102.993	4714
site 02	USA	Colorado	Clear Creek	Georgetown	39.735	-105.687	8420
site 03	USA	Colorado	Saguache	Villa Grove	38.141	-105.975	7820
site 04	USA	Utah	Emery	Bear Creek	39.391	-111.092	6506
site 05†	USA	Utah	San Pete	Sky line Dr	39.582	-111.314	10020
site 06‡	USA	Utah	Wayne	Bicknell	38.304	-111.484	6932
site 07	USA	Utah	Wayne	Torrey	38.299	-111.436	6854
site 08	USA	Nevada	Mineral	Walker Lake	38.621	-118.735	4031
site 09	USA	California	Plumas	Feather River	39.819	-120.429	4858
site 10	USA	Wyoming	Laramie	Happy Jack 1	41.197	-105.273	7666
site 11	USA	Wyoming	Laramie	Happy Jack 2	41.235	-105.274	7428
site 12	USA	Colorado	Sedgwick	Julesburg	40.974	-102.250	3452
site 14	USA	California	San Bernardino	Yucaipa	34.043	-117.058	2453
site 15	USA	California	San Diego	old hwy 395	33.274	-117.152	341
site 16	USA	California	San Diego	Julian	33.088	-116.593	4080
site 17	USA	California	Inyo	Onion Valley	36.771	-118.339	9193
site 18	USA	California	Plumas	Crescent Mills	40.083	-120.917	3491
site 19	USA	California	Lassen	Susanville	40.335	-120.568	4323
site 20	USA	California	Inyo	Rt 168	37.253	-118.161	6740
site 21	USA	California	Inyo	Death Valley Rd	37.169	-118.206	4480
site 22	USA	California	Inyo	Nine Mile Cyn 1	35.850	-117.939	4010
site 23	USA	California	Tulare	Nine Mile Cyn 2	35.864	-118.009	6262
site 24	USA	California	Los Angeles	Elizabeth Lake	34.661	-118.380	3340
site 25	USA	California	San Bernardino	Hesperia	34.365	-117.242	2947
site 26	USA	California	San Bernardino	Running Springs	34.202	-117.092	6060

Figure A.1. Elevation of collection sites; collections sites ranged from 341 ft to 10040 ft.

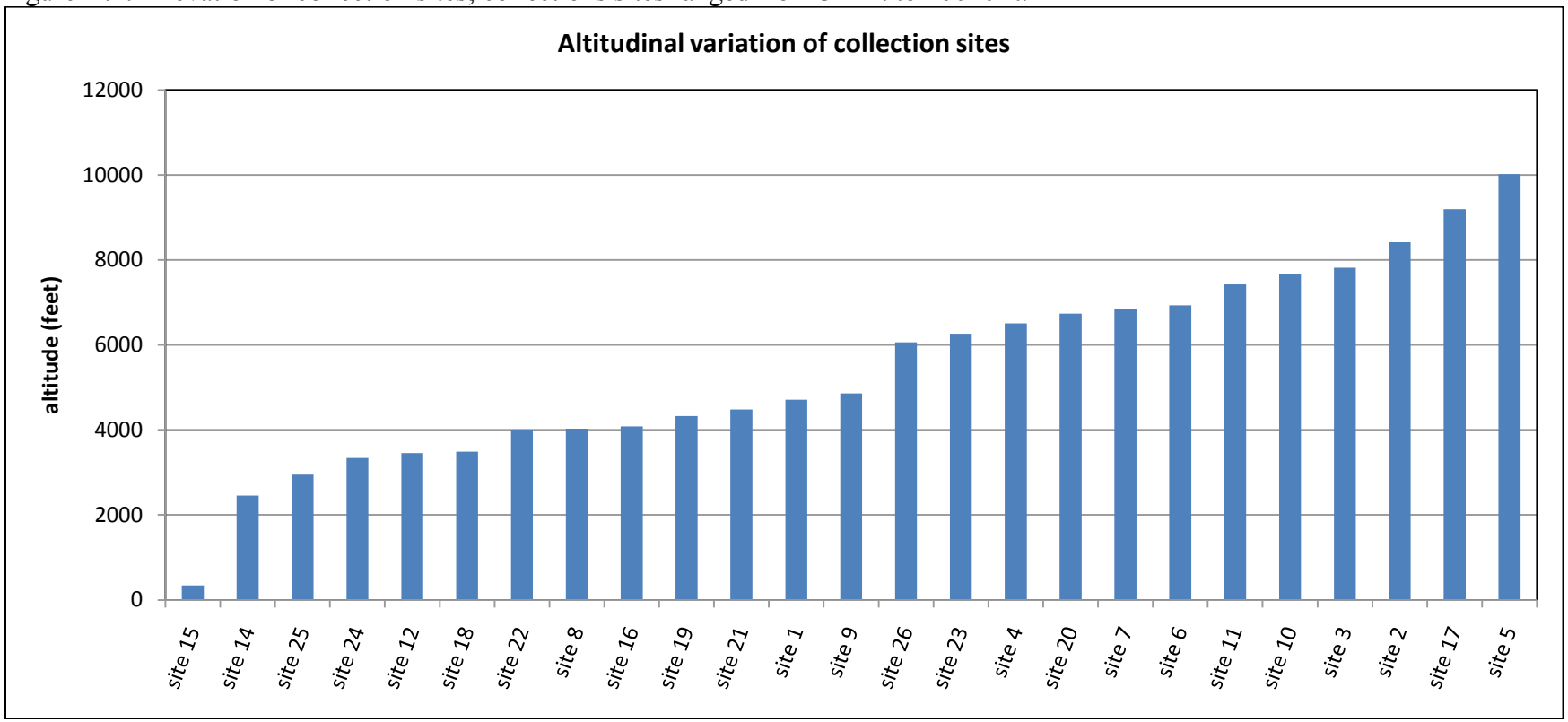


Figure A.1.2. Collection sites based on GPS coordinates (WGS84).

Appendix 2

Table A.2.1 Retention time (From HP5-MS Column), Kovat's index, and %Total (From FID) for essential oil samples from site 2.

R.T. MS	KI	Compound	% Total 2-11	% Total 2-12	% Total 2-13
3.554	825	unknown	0.054	0.048	0.153
4.177	863	unknown	0.36	0.48	0.325
5.732	938	alpha-pinene	0.068	0.03	0.098
6.057	952	alpha-fenchene	0.042	0.023	0.786
6.823	980	beta-pinene	0.148	0.091	0.305
7.149	992	myrcene	0.5	0.082	0.158
7.555	1006	α-phellandrene	1.169	0.133	0.211
7.606	1008	unknown	0.056	–	–
7.726	1013	unknown	–	–	–
7.903	1019	alpha-terpinene	0.272	0.058	0.06
8.126	1027	unknown	0.026	0.026	–
8.269	1032	limonene/β-phellandrene	0.651	0.965	2.419
8.532	1041	(Z)-β-ocimene	27.556	18.115	30.618
8.824	1051	(E)-β-ocimene	4.385	3.518	4.659
9.166	1062	gamma-terpinene	0.105	0.055	0.075
10.115	1090	alpha-terpinolene	13.538	1.36	3.079
10.47	1100	L-linalool	0.121	0.048	0.078
10.601	1105	unknown	0.043	–	–
11.384	1131	allo-ocimene	0.196	0.101	0.216
13.276	1187	unknown	0.045	–	–
13.55	1195	methyl salicylate	0.121	–	–
13.716	1199	methyl chavicol	14.188	6.827	0.4
14.705	1231	citronellol	0.077	0.054	–
14.956	1239	unknown	–	–	–
16.493	1284	5-phenyl-1,3-pentadiyne	0.097	–	–
18.768	1356	unknown	0.044	–	–
19.277	1371	Phenol, 2-methoxy-4-(1-propenyl)-, (e)-	0.052	–	0.062
19.54	1379	unknown	0.046	–	–
20.466	1407	methyl eugenol	30.413	66.094	51.231
20.923	1427	unknown	–	–	–
22.369	1470	γ-decalactone	0.048	–	–
22.729	1482	gamma-curcumene	0.329	0.352	0.504
22.809	1484	germacrene-D	0.421	0.552	0.448
23.198	1496	capillene	1.514	–	–
23.278	1499	bicyclogermacrene	0.221	0.426	0.258
23.569	1509	E,E alpha farnesene	0.112	–	–
25.198	1566	E-nerolidol	0.059	–	0.14
25.832	1587	unknown	0.055	–	–
27.816	1658	unknown		–	–
28.238	1673	xanthoxylin	2.479	–	3.495
33.788	1885	capillarin	0.183	–	–
36.377	1992	dursban	0.067	0.249	0.133

Table A.2.2. Retention time (From HP5-MS Column), Kovat's index, and %Total (From FID) for essential oil samples from site 6.

R.T. MS	KI	Compound	% Totoal 6-38
4.177	863	unknown	0.524
5.057	908	unknown	0.027
5.726	938	alpha-pinene	0.159
6.052	951	alpha-fenchene	1.756
6.372	964	unknown	–
6.817	980	beta-pinene	0.064
7.143	992	myrcene	0.109
7.549	1006	α-phellandrene	0.22
7.618	1009	cis-3 hexenyl formate	0.051
7.898	1019	alpha-terpinene	–
8.12	1027	m-cymene	0.027
8.281	1033	beta-phellandrene	6.395
8.549	1042	(Z)-β-ocimene	47.199
8.823	1051	(E)-β-ocimene	5.415
9.161	1062	gamma-terpinene	0.088
9.755	1080	unknown	0.047
10.092	1090	alpha-terpinolene	2.432
10.401	1098	unknown	0.039
10.47	1100	L-linalool	0.116
10.795	1112	unknown	–
10.972	1118	unknown	0.036
11.253	1127	unknown	0.034
11.378	1131	allo-ocimene	0.153
11.538	1136	unknown	–
11.864	1146	unknown	–
12.03	1151	unknown	–
12.201	1156	unknown	–
13.299	1188	unknown	–
13.396	1190	unknown	–
13.481	1193	unknown	–
13.527	1194	methyl salicylate	0.016
13.67	1198	methyl chavicol	0.032
13.79	1177	unknown	0.071
14.047	1187	unknown	0.092
14.699	1214	citronellol	0.705
14.95	1223	unknown	–
15.007	1226	unknown	–
15.167	1232	unknown	0.046
16.151	1268	unknown	0.065
16.265	1272	unknown	0.563
16.562	1283	5-phenyl-1,3-pentadiyne	26.361
16.916	1295	unknown	–
18.762	1356	citronellyl propionate	0.712
19.122	1367	unknown	0.111
19.42	1376	unknown	0.063
19.5	1378	unknown	0.075
19.837	1388	(E)-Hex-4-en-2-ynylbenzene	0.034

R.T. MS	KI	Compound	% Totoal 6-38
20.031	1394	unknown	0.095
20.917	1423	unknown	0.072
21.797	1452	unknown	0.051
22.723	1482	gamma-curcumene	0.366
22.803	1484	germacrene-D	0.843
23.203	1496	capillene	3.538
23.278	1499	bicyclogermacrene	0.377
23.563	1509	E,E alpha farnesene	0.052
24.049	1526	unknown	–
24.575	1544	unknown	–
25.204	1566	E-nerolidol	0.087
25.655	1581	spatulenol	0.282
26.198	1599	unknown	–
26.215	1599	unknown	–
27.347	1641	unknown	0.025
27.438	1644	unknown	–
27.833	1659	unknown	0.063
28.261	1674	unknown	0.041
36.377	1991	dursban	0.179

Table A.2.3. Retention time (From HP5-MS Column), Kovat's index, and %Total (From FID) for the essential oil sample extracted from the plant from Kyrgyzstan.

R.T. MS	KI	Compound	% Total Kyrgyz
4.188	863	unknown	0.331
5.474	927	unknown	0.038
5.566	931	thujene	0.053
5.743	939	alpha-pinene	4.826
6.114	954	camphene	1.07
6.732	977	sabinene	1.988
6.835	981	beta-pinene	3.812
7.189	993	myrcene	27.152
7.566	1007	1 phellandrene	0.329
7.743	1013	delta-3-carene	0.023
7.92	1020	alpha-terpinene	0.165
8.143	1028	p-cymene	0.032
8.286	1033	limonene	11.617
8.515	1041	(Z)-β-ocimene	3.457
8.835	1051	(E)-β-ocimene	3.547
9.178	1062	gamma-terpinene	0.143
9.538	1073	trans sabinene hydrate	0.029
10.115	1090	terpinolene	5.218
10.475	1101	L-linalool	0.197
10.612	1105	unknown	0.056
10.99	1118	unknown	0.113
13.047	1181	4-terpineol	0.264
13.293	1188	unknown	0.077
13.556	1195	methyl salicylate	0.185
13.687	1198	methyl chavicol	0.057
14.956	1239	cis-3 hexenyl isovalerate	0.468
15.15	1245	unknown	0.032
16.631	1288	bornyl acetate	3.759
18.774	1356	citronellyl acetate	0.196
19.128	1367	neryl acetate	0.213
19.74	1385	geranyl acetate	0.376
22.809	1484	germacrene-D	0.109
23.175	1495	trans alpha bergamotene	0.14
23.283	1499	bicyclogermacrene	0.242
24.06	1526	beta sesquiphellandrene	0.322
24.221	1532	unknown	0.043
25.209	1566	E-nerolidol	0.071
26.672	1616	unknown	0.048
27.147	1634	unknown	0.03
28.73	1690	unknown	0.131
31.993	1814	unknown	0.072
32.296	1826	*Z*-artemidin	25.854
33.696	1882	E-artemidin	2.315
35.468	1954	dracumerin	0.499
35.468	1954	unknown	0.076
36.291	1987	unknown	0.06

R.T. MS	KI	Compound	% Total Kyrgyz
36.377	1991	dursban	0.058

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Curriculum Vitae

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EDUCATION

Ph.D., Plant Biology Graduate Program, Rutgers, The State University of New Jersey New Brunswick, New Jersey (May 2010)
University of Pennsylvania, Departments of Landscape Architecture and Biology, non-matriculated (2001-2002)
B.Sc., Horticulture, Delaware Valley College, Doylestown, PA (2001)

TEACHING EXPERIENCE

2010 - Guest lecturer, The Byrne Seminars: Safe or Sorry? Herbal Medicine, Folklore and its Scientific Evidence
2009 - Teaching Assistant and guest lecturer, Advanced Plant Systematics/Plant Diversity and Evolution
2009 - Teaching Assistant, Plant Gene Transfer
2008 - Teaching Assistant, Plant Genetics
2008 - Lecturer, GIBEX week-long Training Course "Preserving and Evaluating Biodiversity through Screens-to-Nature technology", Maquipucuna Reserve, Ecuador
2008-2007 - Mentor, Biotechnology Research Project, Rutgers (1 student), "Genetic diversity of an *ex situ* collection of dawn redwood (*Metasequoia glyptostroboides*)"
2007 - Mentor, George H. Cook Scholars Program, Rutgers (1 student), "Persistence of non-native plant species introduced via 19th century ballast dumps" (this work received a national award in 2008)
2007 - Teaching Assistant, Advanced Plant Systematics/Plant Diversity and Evolution
2007 - Lecturer, GIBEX Screens-to-Nature and Bioexploration/Biodiversity Documentation Training "Enabling Pharmaceutical Bioexploration in Tanzania", Dar-es-Salaam, Tanzania
2006 - Teaching assistant, Advanced Plant Systematics/Taxonomy of Vascular Plants
2006 - Mentor, George H. Cook Scholars Program (1 student)
2006 - Mentor, The Douglass Project: Introduction to Scientific Research (2 students), "Persistence of non-native plant species introduced via 19th century ballast dumps"
2005 - Lecturer, Purdue/UAB Botanicals Research Center for Age-Related Diseases-2005 Workshop, "Botanical Standardization of Medicinal Plants"
2005 - Mentor, The Douglass Project for Rutgers Women in Math, Science and Engineering, Project SUPER summer enrichment program for undergraduate women, "Persistence of non-native plant species introduced via 19th century ballast dumps"
2005 - Lecturer, The International Cooperative Biodiversity Groups (ICBG) Central Asia Program. week-long "Training Course in Biodiversity Documentation", Tashkent, Uzbekistan and Bishkek, Kyrgyzstan.

RESEARCH/PROFESSIONAL EXPERIENCE

2010-New Jersey Department of Environmental Protection, Natural Heritage Program. Field botanist.

2009 - Floristic inventory, Van Kirk Island and the Hackensack Waterworks in conjunction with Bergen County Parks Department and Rutgers Department of Landscape Architecture

2008-2009 - Herbarium digitization project of rare and endangered plants and invasive species in the Rutgers University Chrysler Herbarium. Project coordinator and manager

2008 - Floristic inventory of proposed park land in Cutchogue, NY in conjunction with Rutgers Department of Ecology, Evolution and Natural Resources

2004-2009 - Collections Manager, Chrysler Herbarium, Rutgers University

2004-2009 - Supervisor, federal work study herbarium curatorial assistants

2004-2009 - Webmaster, Chrysler Herbarium, Rutgers University

2004-2009 - Webmaster, International Cooperative Biodiversity Group, Central Asia Program <http://icbg.rutgers.edu>

2002-2004 - Herbarium Curatorial Assistant, The Academy of Natural Sciences of Philadelphia

2001-2002 - Plant Protection Intern, The Morris Arboretum of the University of Pennsylvania

REFEREED SCIENTIFIC ARTICLES

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