UNIVERSITY OF WISCONSIN - MADISON

A Characterization of Five Native Wisconsin Roses and their Hips

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Joint Master of Science in Horticulture and Agroecology

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Index

Index of Figures

Index of Tables

Preface

The objective of this thesis is to describe the current status of research on the properties of rose hips, which are valuable for their medicinal properties and use as ornamental features on landscape plants. The research undertaken was to characterize five species of roses native to North America, which had not been studied before. In this way, the thesis serves to pave the way for future research on these species. The results described here strongly suggest that further research on these, and perhaps other North American species, would be valuable to undertake.

This thesis is divided into four Chapters. The first chapter describes the origins of roses, their current uses, and the medicinal properties of the rose fruits, called hips. It then describes in detail the five species of native Wisconsin roses that are the focus of the study. The second chapter is structured as a draft manuscript. It contains an abstract and introduction that briefly summarize the current research and uses of roses described in Chapter 1. Following this introduction, the methodology and results of the study are presented. The implications of the results are discussed at the end of Chapter 2. Chapter 3 is a reflection on the research process and an outline of subsequent research that should be undertaken given the results described in Chapter 2. The fourth chapter serves as an addendum, containing climatological data that may have impacted the results of this study.

List of Abbreviations

CP: Curtis Prairie

dd: Double distilled

EH: Eagle Heights Community Garden

LSP: University of Wisconsin Lakeshore Preserve

PBC: Pheasant Branch Creek Conservancy

UW: University of Wisconsin-Madison

Chapter 1: An Overview of Roses

1.1 History and Uses of Roses

Roses, the flowering woody shrubs of the genus *Rosa*, are easily one of the most iconic and recognizable horticultural crops, having been cultivated for ornamental and herbal uses for over 5000 years (Shepherd, 1954; Gudin, 2010). Every year, people in the U.S. spend hundreds of millions of dollars on rose flowers for holiday gifts and on shrubs for their landscapes (USDA, 2010; Bonarriva, 2003). What many may not realize, however, is that roses are also used in a number of different ways. The petals, fruits, and seeds provide cosmetic products such as rose water and rose oil, which are used in perfumes and lotions. Their fruits and petals have also been consumed as food in soups, jams, and teas throughout history around the world, due to their health-promoting properties. Today we know that the fruits and petals of roses contain high levels of Vitamin C and many other beneficial phytochemicals (Mikanagi et al., 2000; Smulders et al., 2011; Uggla, 2004; Uggla et al.). These chemical constituents of the rose fruit, as in many other species, are of growing interest to researchers due to their potential health benefits. The general public is growing more interested in wild and native plants as well, especially those that provide edible or otherwise useful products. The increasing number of farmers' markets, community gardens, and ecological restoration businesses, as well as a growing public call for non-synthetic health and beauty products clearly point to a growing interest in and market for native edible plants and plant-based products (USDA, 2014b; Censkowsky et al., 2007). While roses are native throughout the temperate regions of the Northern Hemisphere, North American rose species represent an under-studied group of plants that could be valuable for a wide variety of uses.

In the United States, the most economically important use for roses is from landscape and potted roses. According to the USDA's census of horticultural specialties (USDA, 2010), shrub roses sold wholesale for $209,212,000, landscape roses in plugs for $16,287,000, and small potted roses for

$30,983,000. This vastly outstrips the value of roses grown in the U.S. for cut flowers:$23,475,000. However, imports of cut roses totaled $205,695,000, mostly produced in Columbia and Ecuador (Bonarriva, 2003), so the total economic value of cut roses in the US is comparable to the value of roses as ornamental plants. Regulations governing the import and export of live plants lead to little import or export of horticultural shrubs such as roses in the US (Arita, Mitchell, & Beckman, 2015).

Internationally, Europe is a large consumer of cut roses, importing over 5.4 billion roses in 2014, most of which are produced in Kenya, Ecuador, and Ethiopia (Vanderelst & Zolichova, 2015; CBI, 2015). India is also growing to become a leading producer of cut roses for Europe and Asia (ITC, 2012). The Netherlands are the main producer and importer of floriculture products, including roses, in Europe and they serve as a hub to (re)export these products to the rest of the European Union (CBI, 2015; ITC, 2012). The EU also produces over 200,000 tonnes (220,462.262 tons) of nursery products and 180,000 tonnes (198, 416.036 tons) of potted plants (Vanderelst & Zolichova, 2015). At least some of that production is likely roses, given their popularity (in the US, over 40% of deciduous shrubs produced for sale are roses (USDA, 2010)), but the data are not specific. Rose oil, one of the most important additives to cosmetics worldwide, is produced from the species *R. damascena.* Between 1991 and 2001, rose oil, produced mainly in Turkey and Bulgaria, sold from $1,800-$4,000 per kg, making it a highly valuable commodity (Gunes, 2005). The value of rose hips as such has apparently been not been studied by economists. However, it is estimated that hips have been harvested wild from over 11.8 million hectares of land throughout the world, totaling over 7.7 tons of fruit; thus making rose hips one of the most popularly harvested medicinal plants in the world (Censkowsky et al., 2007).

The family Rosaceae is the 19th most diverse family of plants found around the world, and is particularly diversified within the northern hemisphere. It contains many extremely economically importance species, including apples, cherries, strawberries and roses. Roses are described as having at least 150 different species, which makes it one of the more moderately diverse genera within the

Rosaceae family (Hummer & Janick,2009).The majority of roses are native to Eurasia, and it is thought that the genus' origins lie on that landmass, but the research is unclear (Bruneau et al. 2007). However, roses are distributed throughout Asia, North America, Europe and North African temperate and subtropical climates (Hummer & Janick, 2009). Traditionally, rose species have been used for ornamental purposes, food, beverages, and medicinal products throughout their range of distribution. The latter use is due to their phytochemical properties (Smulders et al., 2011; Hummer & Janick, 2009). Today, given their ubiquitous nature as popular landscaping plants, many species of Asiatic roses have become naturalized or invasive in other environments throughout the world because they are the sources of many popular rose cultivars (Hummer & Janick, 2009).

Though roses are not commonly recognized in North America as a source of food, ethnobotanical research has shown that roses have been used by indigenous peoples throughout North American for their medicinal, spiritual, and edible properties (Moerman, 2009). A surge in recent studies of North American native species (Barry et al., 2008; Sanderson and Fillmore, 2010; Sanderson and Fillmore, 2012; Ghose et al., 2013) point to a growing interest in their production for phytochemical and edible purposes and public interest in healthy foods and locally produced food products has been growing. This latter fact is demonstrated by the increase in numbers of farmers markets from 1,755 in 1994 to 8,628 in 2014 (USDA, 2014a) and the increased production of organic food from approximately 400 million dollars worth of produce to over 3 billion dollars from 2002 to 2012 (USDA 2004, 2014).

There is also growing interest in ecological restoration work, which emphasizes the use of native plants. Educational institutions are creating or expanding programs that prepare students for careers in restoration, a reflection of the growth of restoration businesses (Nelson et al., 2008) that rely on native wild plants to reestablish ecosystems damaged by humans. Roses that produce hips are almost all wild roses, as this trait has been bred out of many cultivated varieties. All of the aforementioned trends

mean that native wild roses, as recognizable and well-loved flowers that have edible components, are positioned to meet needs for many different potential markets.

1.2 Rose Taxonomy

The taxonomy of the genus *Rosa* has been studied intensely due to their popularity as landscape plants (Smulders et al., 2011). Consequently, researchers and breeders of roses have primarily been interested in the color, flowering time(s), disease resistance, and scent of roses, thought there have been some studies concerning the medicinal properties of rose hips as well (Smulders et al., 2011). Of the approximately 150 different species in the genus *Rosa*, there are 10 species and 70 cultivars grown specifically for the ornamental value of their hips ("Roses with Hips", 2015.) and approximately 11 species potentially grown for hip production, with *R. canina* and *R. dumalis* being the primary species for commercial purposes (Uggla, 2004; Günes, 2010; Celik et al., 2009; Andersson, 2009). A definitive number is difficult to obtain given the complicated taxonomy of the genus. Though many agree on at least 150 species, some taxonomists argue that there are as few as 100 or as many as 300 different species. This complexity arises from roses' long history of cultivation, the tendency of related species to hybridize, and the fact that the species are often characterized by highly variable morphological characteristics while having similar genetic profiles (Uggla, 2004; Smulders et al., 2011; Hummer & Janick, 2009).Koopman et al. (2008) describe the situation in the following:

> "Most of the taxonomic confusion in the genus *Rosa* originates from the complicated evolutionary history of the wild species, combined with a long history of cultivation and interbreeding of selected genotypes. The complexity is caused by several factors, often in conjunction: (1) extensive hybridization, both ancient and recent; (2) absence of clear differences between many of the species, partly due to their recent radiation; (3) incomplete lineage sorting (a common feature in recently diverged species); and (4) polyploidy (with multiple/hybrid origins for the polyploids in at least some of the species). A second source of confusion is the use of morphology as the basis of *Rosa* classifications…morphological characters are often under severe selection pressure, for example when growth conditions (rapidly) change. The selection pressure may on the one hand result in character similarity for evolutionarily divergent species adapting to similar conditions, and on the other hand striking differences between related species adapting to different conditions. The genus *Rosa* contains examples of both."

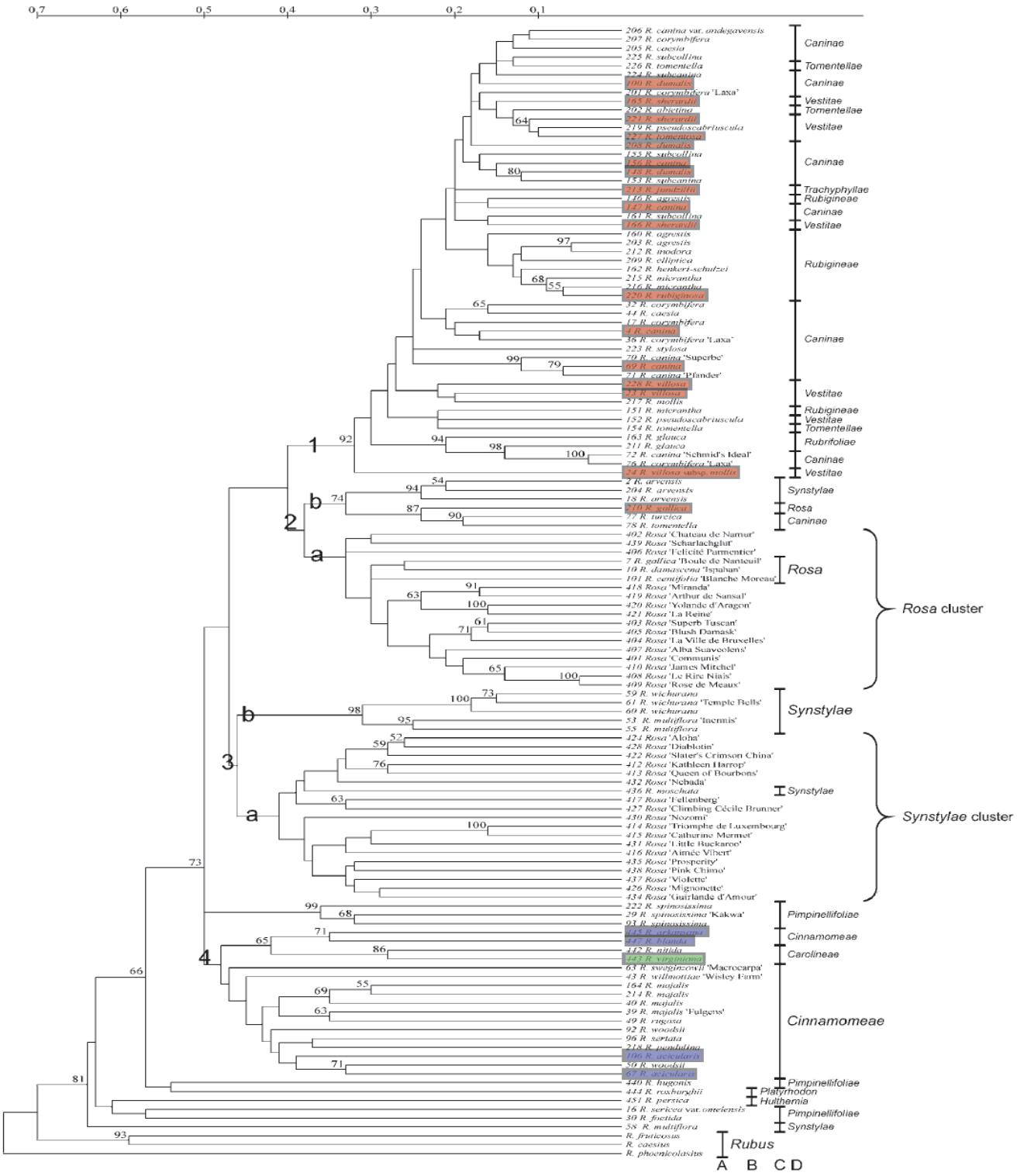

Figure 1.1 (adapted from Koopman et al., 2008): A phylogeny of many rose species and cultivars, delineated into sections. The phylogeny was created by analyzing AFLP markers arising from 7 primer combinations. The authors used UPGMA cluster analysis and Wagner parsimony to create the tree. Hybrid cultivars, other than one *Rosa x centrifolia* and *Rosa x damascena*, were not included. Species highlighted in red are Eurasian roses whose hips have been studied, the one highlighted in green is a North American species previously studied, and those highlighted in blue are species studied herein.

Regardless of the number of species recognized, the genus *Rosa* is generally divided into a number of sections, some of which are monophyletic, but many of which are polyphyletic (See Figure 1.1). The section *Caninae* (called dog-roses) contains the species that have been most well-characterized in terms of the physical and chemical characteristics of their hips (see Table 1.1). The aforementioned *R. canina* and *R. dumalis* are members of this section. Dog-roses are unusual in several ways. The first is that they undergo a novel, incomplete, meiosis resulting in unequal numbers of chromosomes in the gametes. Pollen grains have 7 chromosomes (the base number for genus *Rosa*), while ova contain 21, 28, or 35 chromosomes, depending on species' ploidy level. The ploidy level is most often 5*x*, but can be 4*x* or 6*x* (Uggla, 2004; Smulders et al., 2011). Additionally, the hips of species within section *Caninae* have a unique aroma and taste, which is part of the reason for their popularity as foodstuffs (Smulders et al., 2011; Uggla, 2004). However, the exact chemical source of this aroma has not been described in the research. The sections *Caninae* and *Cinnamommeae* are the primary sections with species grown for their hips (Koopman et al., 2008; Smulders et al., 2011). Additional studies are focused on condensing differentiated species into geographic variations of a single species, particularly in North American species (Bruneau et al., 2005; Joly & Bruneau, 2007; Mercure & Bruneau, 2008).

Section *Cinnamomeae*, which contains North American species as well as species of Eurasian origin, is the largest section of the genus (approximately 80 species). Recent genetic work suggests that it be combined with the entirely North American section, *Carolinae.* This would result in making this section by far the most diverse section of the genus (Joly & Bruneau, 2007; Koopman et al., 2008). Presumably, this diversity of these sections has resulted from allopolyploid speciation and intense diversification (Smulders et al., 2011). Species in the sections *Cinnamomeae* and *Carolinae,* such as those being analyzed within this study, have had very little genetic impact on cultivated roses; cultivated roses mainly originate from sections *Synstylae, Gallicanae,* and *Pimpinellifoliae.* The only two species

from section *Cinnamomeae* that have had discernable genetic impact on cultivated varieties of roses, *R. rugosa* and *R. cinnamomea*, are both native to Eurasia (Smulders et al.; 2011).

1.3 Chemical Composition of Eurasian Roses

Studies concerning the properties of rose hips have primarily focused on roses within the section *Caninae*, as they have been used for food, herbal supplements, and cosmetics in Eurasian cultures (particularly Scandinavia and Turkey) for centuries thanks to their vigorous growth habits, noticeable scent, and unique flavor (Uggla, 2004; Uggla et al., 2005).Existing studies have observed high concentrations of vitamin C, vitamin E precursors, citric acid, carotenoids, phenolic compounds, and flavonoids in rose hips. They have also reported high levels of Omega-3 fatty acids in the seeds, and that many of the compounds present in rose hips are superior antioxidants. (Ercisli, 2007; Uggla, 2004; Andersson, 2009; Barros et al., 2010; Günes, 2013; Smulders et al., 2011; Demir et al., 2014). Several other studies have been undertaken on these roses concerning agronomic traits such as fruit size, fruit mass, color, and yield (Kovacs et al., 2005; Celik et al., 2009; Uggla et al., 2003). Table 1.1 lists the various studies on rose hip chemical properties and summarizes their observations.

Many of these types of chemicals are generally known to have positive health impacts on humans and thus research has been undertaken to determine if rose hips have any medical value. For example, it has been reported that rose hip treatments reduce inflammation and pain associated with rheumatoid arthritis to a small-to-moderate degree (Andersson et al., 2012; Christensen et al., 2008; Chrubasik et al., 2006; Chrubasik et al., 2008; Chrubasik-Hausmann et al., 2014; Wenzig et al., 2008; Willich et al., 2010). Rose hips have also been purported as potentially having impacts on weight gain, cholesterol levels, and blood pressure (Chrubasik et al., 2008; Andersson et al., 2011; Andersson et al., 2012). Other research notes a very high anti-oxidative capacity in rose hip preparations and potential antimicrobial properties, including suppression of MRSA (Methicillin-resistant *Staphylococcus aureus:* an antibiotic-resistant “superbug”) (Yilmaz & Ercisli, 2011; Yi et al., 2007; Wenzig et al., 2008). Non-

Author(s)	Study Year	Source of Samples	Species Studied	Observed Phytochemicals	Other Variables Studied
Andersson	2009	Field-grown in Balsgård, Sweden	*R. rubiginosa, R. dumalis, R. dumalis*(hybrid)*, R. spinosissima*	α- and γ-tocopherol, lutein, zeaxanthin, β-carotene. In *R. spinosissima,* neochrome, neoxanthin, violaxanthin, 1 unknown xanthophyll and 3 unknown carotenes. In the other species, rubixanthin, lycopene, prolycopene, γ- and ζ-carotene. Esterified carotenids were found in all species, but not identified	Looked at change over time in phytochemical composition
Barros et al.	2010	Trás-os-Montes, Portugal	*R. canina*	Total carbohydrates, total protein, total fatty acids, Sugar content (Fructose, glucose, and sucrose), α-, β-, and γ- tocopherol, ascorbic acid, β-carotene, lycopene, total phenolics, total flavanoids	Also measured DPPH scavenging ability and reducing power
Günes	2010	Ag Research Station in Tokat, Turkey	*R. dumalis, R. canina, R. jundzillii, R. villosa, R. hirtissima*	total soluble solids, ascorbic acid	Vegetative and flower bud burst, flowering time, and hip harvest time. Also, mass, fruit length/width, flesh ratio
Uggla et al.	2003	Field grown in Balsgård, Sweden (from seed gathering throughout Sweden, Denmark, and Norway)	*R. dumalis* ssp*. dumalis, R. dumalis* ssp. c*oriifolia, R. rubiginosa, R. villosa* ssp. *mollis*	ascorbic acid	Fruit mass, percent dry matter, and percent fruit flesh
Türkben et al.	2010	Wild specimens in Bursa, Turkey	*R. canina*	ascorbic acid, lycopene, phenolic compounds (particularly quertecin and (+)-Catechin) (using LC-MS analysis)	Dry matter, minerals
Ercisli	2007	Erzurum, Turkey	*R. canina, R. dumalis* ssp. *boissieri, R. dumalis* ssp. a*ntalyensis, R. villosa, R. pulverulenta, R. pisiformis*	total phenolics, total soluble solids, total fat, fatty acid composition, ascorbic acid	mineral content, pH, dry matter, color
Adamczak et al.	2012	Throughout Poland, mostly wild but some from botanical gardens	*R. agrestis, R. canina, R. dumalis, R. glauca, R. indora, R. jundzillii, R. rubiginosa, R. sherardii, R. tomentosa, R. villosa, R. zalana*	citric acid, ascorbic acid, total flavonoids,	
Celik et al.	2009	Van region of Turkey	*R. canina, R. dumalis* ssp. *boissieri, R. iberica, R. foetida R. pulverulenta, R. pisiformis, R. hemisphaerica*	ascorbic acid, total soluble solids	fruit mass, fruit length/width, flesh ratio, shoot length/diameter, flowering duration, time from flower to harvest
Demir et al.	2014	Gümüşhane, Turkey	*R. canina, R. dumalis, R. dumalis* ssp. *boissieri, R. gallica, R. hitissima*	Total phenolics, total flavonols, tartaric esters, organic acid content (ascorbic, citric, malic), sugars (glucose, fructose) many different phenolic compounds were identified (gallic acid, protocatechuric acid, 4-hydroxy benzoic acid, catechin, vanillic acid, procyanidin-B2, syringic acid, (-) epicatechin, 4-methyl catechol, epicatechin gallate, caftaric acid, 2,5-dihydroxy benzoic acid, chlorogenic acid, t-caffeic acid, p-coumaric acid, ferrulic acid, sinapic acid, t-resveratrol), as well as numerous volatiles (too many to list here)	total antioxidative capacity,
Ghose et al.	2013	Prince Edward Island, Canada, wild samples and cuttings from same planted at research station	*R. virginiana, R. carolina*	total flavonols, tannins, fatty acids, tiliroside, anthocyanin	also covers genetic diversity with respect to metabolite diversity as its prime focus
Yi et al.	2007	British Columbia, wild samples	*R. nutkana, R. pisocarpa, R. woodsii*	Total phenolics	Total antioxidative capacity, lecithin lipsome oxidation, antimicrobial activity

Table 1.1: Published Research on Rose Hip Phytochemical Properties

reported include use as an anti-enzymatic browning agent in fruit products (Zocca et al., 2011) and as a skin whitener (Fujii et al., 2014).

In general, these studies used commercial preparations derived from *R. canina* or directly harvested tissues of closely related specie. Some of the detailed studies on phytochemical composition have noted that unique phytochemical compounds are found in different rose species, and that amounts of these compounds varies widely between species and between genotypes within a species (Uggla, 2004; Adamczak et al., 2012; Ercisli, 2007; Celik et al., 2009; Demir et al., 2014). Variability also exists for physical traits, though the variability is most highly expressed between species as opposed to within a particular species (Günes, 2013).

1.4 Chemical Composition of North American Roses

In summary, very few studies have been conducted on the chemical composition of the hips of North American native species, their physical characteristics, and their potential uses for health-rated products (Ghose et al., 2013; Barry et al., 2008; Sanderson & Fillmore, 2010; Yi et al., 2007). However, there is some research on the chemical composition of the flower petals, which may share a similar phytochemical profile to the hips (Mikanagi et al., 1995; Mikanagi et al., 2000). These roses all belong to the sections *Cinnamomeae* and *Carolinae.* Aside from the work of Mikanagi and co-authors on flower petals (1995, 2000), the chemical composition of only two species, *R. carolina* and *R. virginiana,* has been studied in detail (Ghose et al., 2013). Yi and co-authors (2007) do perform an analysis of total phenolics and anti-oxidative capacity on 3 other North American species, however (see Table 1.1).

1.5 Morphology, Phenology, and Ethnobotanical History of Five North American Native Roses

We studied five species of roses native to central and eastern North America: *R. acicularis*, *R. arkansana, R. blanda, R. carolina,* and *R. palustris*. These species are all native to Wisconsin, yet are not well-studied due to their limited impact on cultivated species. Since relatively few studies have been conducted on the hips of North American rose species, there exists a gap in the literature that could

prove valuable to investigate considering the potential health benefits of rose hips. We selected these species as they belong to sections that have been reported to have the most diverse overall phytochemical profiles (Smulders et al., 2011).This diversity in phytochemicals is thought to arise from the genetic diversity in these sections, due to the tendency towards polyploidy and significant inter-specific hybridization. In this study, total phenolic levels were measured as way to estimate overall phytochemical composition and potential for development, given the role phenolic compounds play in the medicinal benefits of rose hip treatments. Soluble solids were also measured as a way to assess the potential for edibility, as typically representative of sucrose content and sweetness. A description of the morphological, biogeographical, and ethnobotanical traits of the studied species follows.

1.5.1 *Rosa acicularis*

Rosa acicularis, commonly called Prickly Wild Rose, native to multiple locations within North America and Eurasia. It has a circumpolar distribution with two major subspecies, ssp. *acicularis*, found mainly in Eurasia and Alaska, and ssp. *sayi* (also known as ssp. *bourgeauiana*), found primarily in northern and central North America (see Figure 1.2) (Gleason & Cronquist, 1991; Stephens, 1973). It is an open-formed, colonial shrub that generally grows to a height of 1-2 m. It is often found in upland woods as well as on hillsides, rocky outcrops, and stream banks. The canes of *R. acicularis* are often densely covered with prickles of highly variable size and shape, hence its common name. The flowers (Figure 1.3) are solitary, a pink or deep rose shade, and bloom in early June. Flowers are formed on lateral branches from the previous year's stems. The hips (Figure 1.4) are globose to ellipsoid and generally ripen in late August through September (Gleason & Cronquist, 1991; Stephens, 1973). The genus *Rosa* has a base chromosome number of n=7. *R. acicularis* can be either hexaploid or octoploid (2*n*= 42 or 2*n*=56), though *R. acicularis* ssp. *sayi* is usually hexaploid (Bruneau et al., 2007; Gleason & Cronquist, 1991).

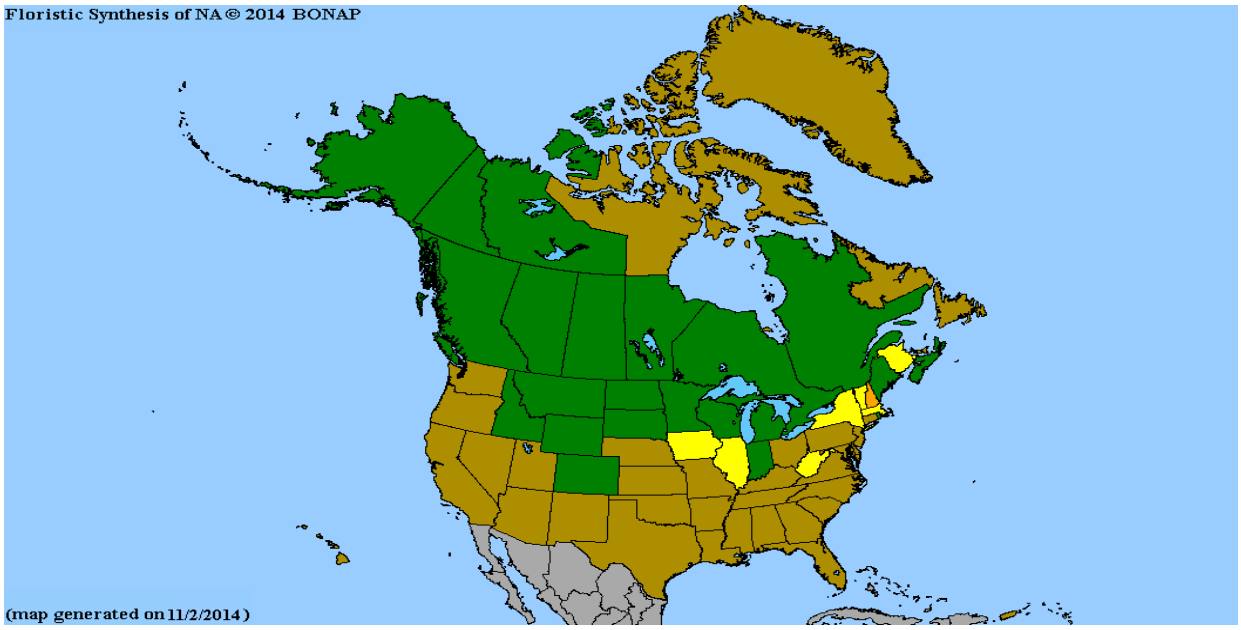

Figure 1.2: North American range of *R. acicularis* courtesy of the Biota of North America Program (BONAP) (Kartesz, 2015). Key: Dark Green is species present and native, Yellow is species present but rare, Orange is species extirpated from region.

Figure 1.3: *R. acicularis* flower © Kitty Kohout

Figure 1.4: *R. acicularis* hip © Kathleen E. Ervin

Given its wide range, this species has been used by many indigenous peoples throughout North America, and it has the most widely documented uses. Tribes in Alaska and the northern part of Canada, including the Upper Tanana and Eskimo, historically used *R. acicularis* hips as a raw food, in sauces, jellies, and jams and as a source of juice (Moerman, 2009; Jones, 2010; Guedon, 1974; Heller; 1953).The leaves were used to make tea, and the stems for preparations to treat colds and stomach troubles. A decoction of the bark has also been reported to induce vomiting (Heller, 1953; Moerman, 2009).

Other tribes throughout Canada and across the Northern US (Washington to New York, south into Illinois) used the hips as an aid to women in labor to hasten delivery of babies (Turner et al., 1990; Moerman, 2009; Nelson, 1983; Leighton, 1985; Herrick, 1997). The leaves were used in infusions to treat

blindness by the Cree (Leighton, 1985) and as components of body washes, treatment for bee stings, and food wrappings by the Okanagan (Moerman, 2009). The roots were consumed by women of the Thompson River Tribes after childbirth (Turner et al., 1990) and were used as a treatment for diarrhea in children by the Blackfoot (Moerman, 2009). The stems and branches have also been reported to have been boiled into decoctions to treat vomiting, diarrhea, or menstrual cramps (Turner et al., 1990). In addition to use as food or medicine, the hips were used as components of necklaces and toys (Leighton, 1985), and in various rituals to provide good luck or protection from bad spirits (Turner et al., 1990; Herrick, 1997; Moerman, 2009).

1.5.2 *Rosa arkansana*

Rosa arkansana (syn. *R. suffulta*), common name Dwarf Prairie Rose, is a short species: less than 1 m according to Gleason & Cronquist (1991 and less than 50 cm according to Stephens (1973). It is also distinguished by its densely prickly stems and colonial growth habit. It is native throughout central North America (Figure1. 5), found in dry prairie, plains, woodland margins, and open riverbanks (Stephens, 1973; Gleason & Cronquist, 1991). Flowers are whitish-pink to deep rose in color and open in June. Flowers are most commonly produced on corymbs that terminate the current year's growth, but also on the ends of lateral branches on older growth (Figure 1.6). The hips of *R. arkansana* are usually glabrous and sub-globose, forming striking rounded fruits which ripen in late August (Stephens, 1973; Gleason & Cronquist, 1991) (Figure 1.7). *R. arkansana* is tetraploid (2*n*=28) and it is hypothesized that it arose from populations of either *Rosa blanda* and/or *Rosa woodsii*. These latter species are purported to be genetically synonymous despite some morphological differences (Joly & Bruneau, 2007).

The use of *R. arkansana* by indigenous peoples is less well documented than that of *R. acicularis*, but there is evidence that multiple parts were used by a variety of groups. For example, the hips were used as dried food and in soups by the Lakota (Moerman, 2009). In addition, the Omaha made an infusion of the hips to treat eye troubles and also used hips as a food source in times of scarcity

(Gilmore, 1919). The Ojibwa (Chippewa) used the roots to make treatments for convulsions and bleeding wounds as well as to treat eye-related troubles (Gilmore, 1919; Densmore, 1913). The Ojibwa also used the petals for perfume and in jams and jellies (Moerman, 2009; Gilmore, 1919). Additionally, the Pawnee used charred stems as a treatment for burns and smoked the inner bark with tobacco (Gilmore, 1919).

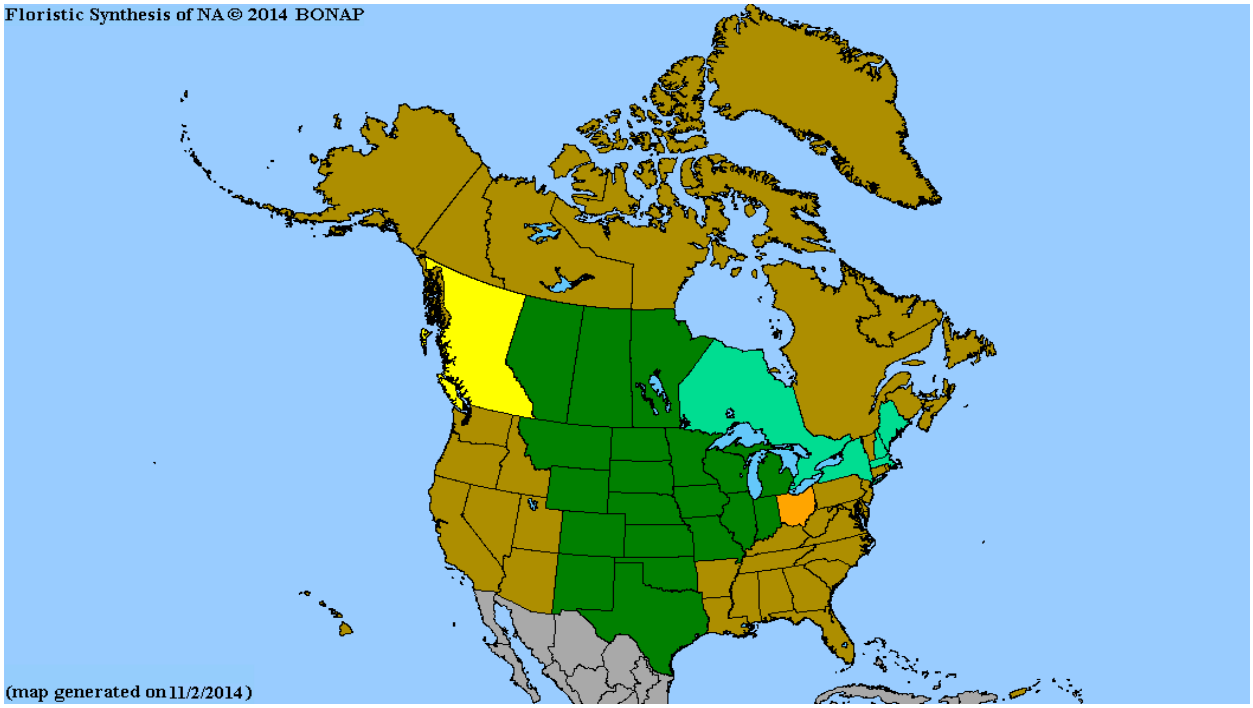

Figure 1.5: Range of *R. arkansana* presented by the Biota of North America Program (BONAP) (Kartesz, 2015). Key: Dark Green is species present and native, Light Green is species present and not rare, Yellow is species present but rare, Orange is species extirpated from region.

Figure 1.6: *R. arkansana* flowerfrom Pheasant Branch Creek Conservancy 6/12/2015

Figure 1.7: *R. arkansana* hip from Pheasant Branch Creek Conservancy 10/5/15

1.5.3 *Rosa blanda*

Rosa blanda, commonly called Smooth Wild Rose, is an erect, colonial shrub growing from 1 to 2 meters. Its common name comes from the fact that the flowering branches of the shrub usually have minimal or no prickles. It is found throughout central and eastern North America, north into Quebec and Ontario and south as far as Missouri (Figure 1.8.) It grows in dense thickets on dry hillsides, prairies, and dunes (Gleason & Cronquist, 1991; Stephens, 1973). Its flowers are produced in early June from lateral buds on the previous year's growth and grow both singly and in corymbs (Figure 1.9). The hips are smooth and globose, beginning to ripen in late August (Stephens, 1973) (Figure 1.10). *R. blanda* is diploid (2*n*=14) and is known to readily hybridize with other diploid species, including *R. rugosa* (Mercure & Bruneau, 2008; Joly & Buneau, 2007). As mentioned previously, genetic analysis and comparison of some morphological traits suggest that *R. blanda* is synonymous with *Rosa woodsii*. However, distribution of *R. woodsii* is generally more western (Joly & Bruneau, 2007). Since one of *R. woodsii*'s key differences is the presence of prickles, the primary morphological trait used for species identification of *R. blanda* may not always be accurate (Gleason & Cronquist, 1991).

The ethnobotanical data on *R. blanda* is somewhat sparse; however there are reports of use by the Potowatomi, the Ojibwa, and the Meskwaki as all three groups used the hips for medicinal purposes (Smith, 1933; 1923; 1928).Specifically, hips were used by the Ojibwa to treat stomach troubles and indigestion (Smith, 1923). Similarly, the Meskwaki used the hips to treat stomach ailments and as part of a decoction for the treatment of severe itches and piles (Smith, 1928). Infusions of the roots of *R. blanda* were used to treat inflamed eyes and headache by the Ojibwa (Smith, 1933; Hoffman, 1891) and the dried petals were used crushed and used as a treatment for heartburn (Smith, 1928; 1923). However, if we were to include *R. woodsii* into the assessment of uses, assuming that Joly & Bruneau's (2007) assessment of its synonymy with *R. blanda* is correct, there is more information on the historical use of this species.

R. woodsii hips were used to make infusions to treat sore throat and coughs, as well chewed to aid childbirth by the Thompson (Turner et al, 1990). The Thompson also used the stems to treat diarrhea and vomiting and the roots to treat colds and syphilis, as well as to aid women recovering from giving birth (Turner et al., 1990). The Arapaho used the achenes to treat muscular pain (Moerman, 2009) and Okanagan-Collville used the leaves to treat bee stings and as a component in body washes for sweat bathers (Moerman, 2009). The Pauite and Shoshoni both used assorted plant parts in the treatment of burns and the roots to treat diarrhea and colds, and the Shoshoni also used the roots as a diuretic and blood tonic (Train et al., 1941).

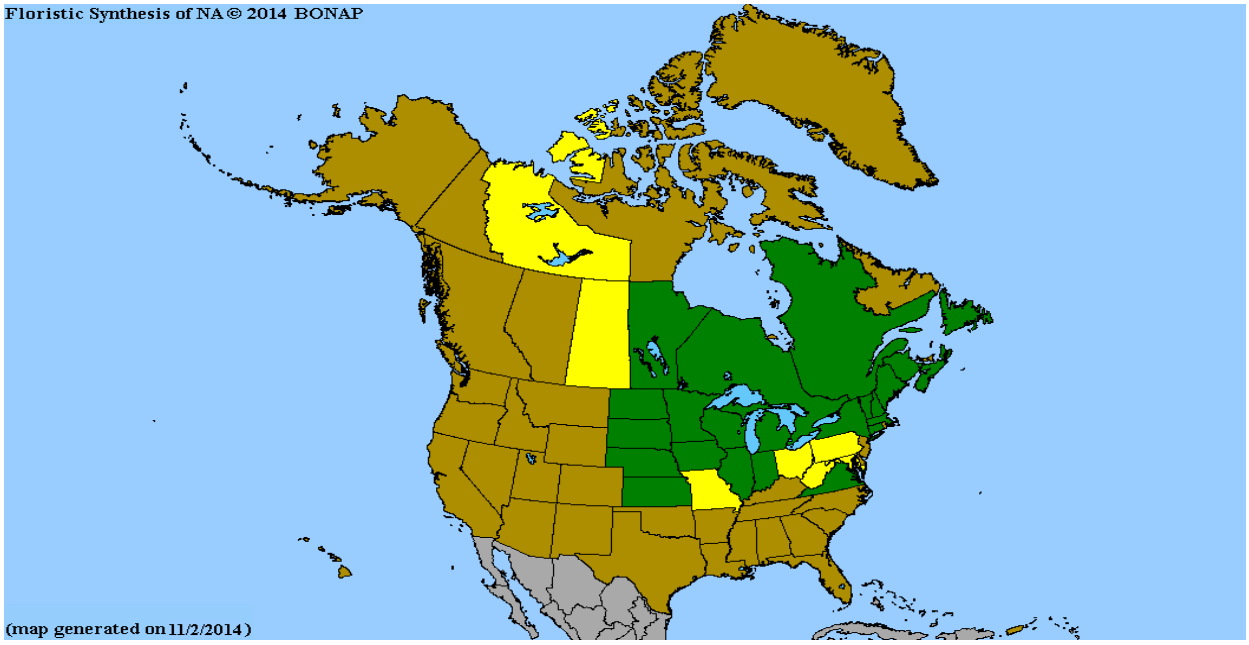

Figure 1.8: Range of *R. blanda* presented by the Biota of North America Program (BONAP) (Kartesz, 2015). Key: Dark Green is species present and native, Yellow is species present but rare

Figure 1.9: *R. blanda* flower from Pheasant Branch Creek Conservancy 5/28/15

Figure 1.10: *R. blanda* hips from Peasant Branch Creek Conservancy 8/31/15

1.5.4 *Rosa carolina*

Rosa carolina, also known as Pasture Rose is an upright, densely prickly shrub growing to the height of one meter. Gleason & Cronquist (1991) report that the shrub is colonial, though Stephens (1973) does not describe its growth habit, in a departure from his other descriptions of rose species. Given the extremely variable morphology of the genus, perhaps there are a variety of ecotypes. *R. carolina* is found along the eastern coast of North America, from Florida to northern Quebec and west as far as Texas and the Great Plains (Figure 1.11). Generally, it is found in dry prairies and upland woods, and usually in rocky soils (Gleason & Cronquist, 1991, Stephens, 1973).

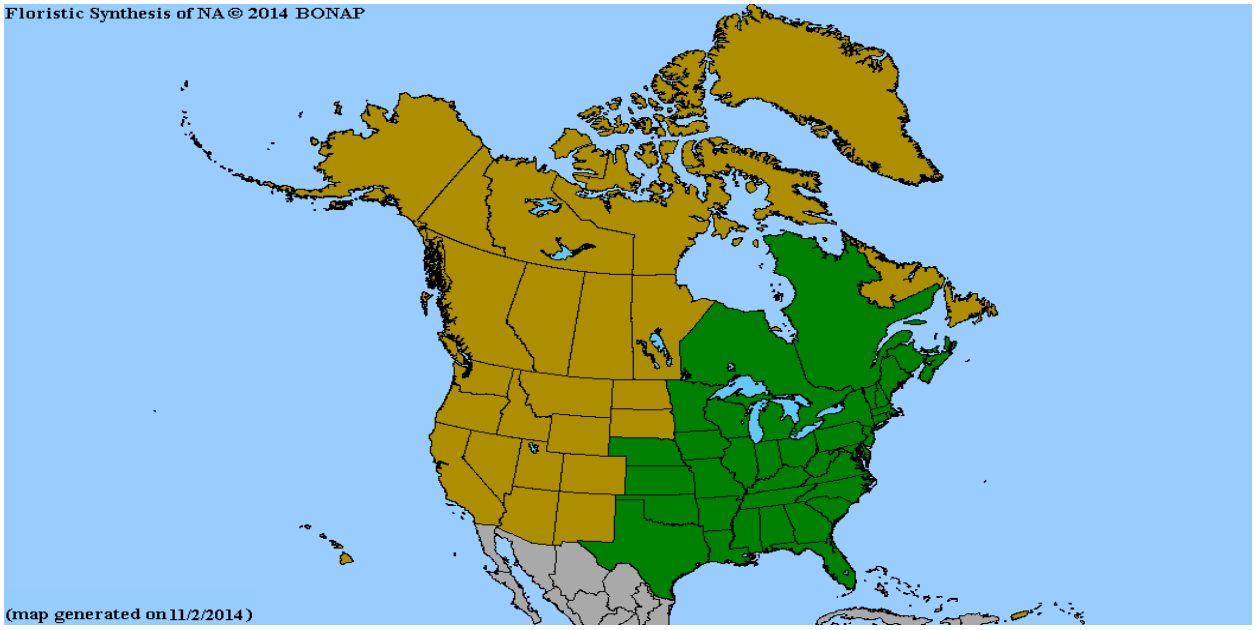

Figure 1.11: Range of *R. carolina* presented by the Biota of North America Program (BONAP) (Kartesz, 2015). Key: Dark Green is species present and native, Yellow is species present but rare

Figure 1.12: *R. carolina* flower from the University of Wisconsin Lakeshore Preserve 6/8/15

Figure 1.13: *R. carolina* hips from the University of Wisconsin Lakeshore Preserve 8/12/15

The flowers are pink and bloom both individually and in corymbs in early June on the stems of the current year (Stephens, 1973) (Figure 1.12). The hips are globose and stipitate-glandular, e.g. they are covered in small hairs which possess a gland at the tip. These glands are a key identifying feature of this species (Figure 13). *R. carolina* is tetraploid (2*n*=28) and its origins suggest that it arose as a hybrid between *R. blanda* and *R. palustris* (Joly & Bruneau, 2007). It easily hybridizes with *R. arkansana* and *R. virginiana*, and less frequently with *R. acicularis* and *R. palustris* (Gleason & Cronquist, 1991). *R. carolina* is not well studied in the ethnobotanical literature; however there is limited information stating that the Menominee used the hips as a treatment for stomach troubles (Smith, 1923).

1.5.5 *Rosa palustris*

Rosa palustris, or Swamp Rose, is a highly branched and dense shrub up to two meters high. Its range overlaps significantly with that of *R. carolina*: it is found throughout the eastern seaboard of North American and stretching west into Texas and the Great Plains states (Figure 1.14). Unlike *R. carolina*, *R. palustris*, as its Latin and common name would suggest, is found almost exclusively in marshes, swamps, and riparian areas (Gleason & Cronquist, 1991; Weakley, 2015). The flowers are pink, strong-smelling,

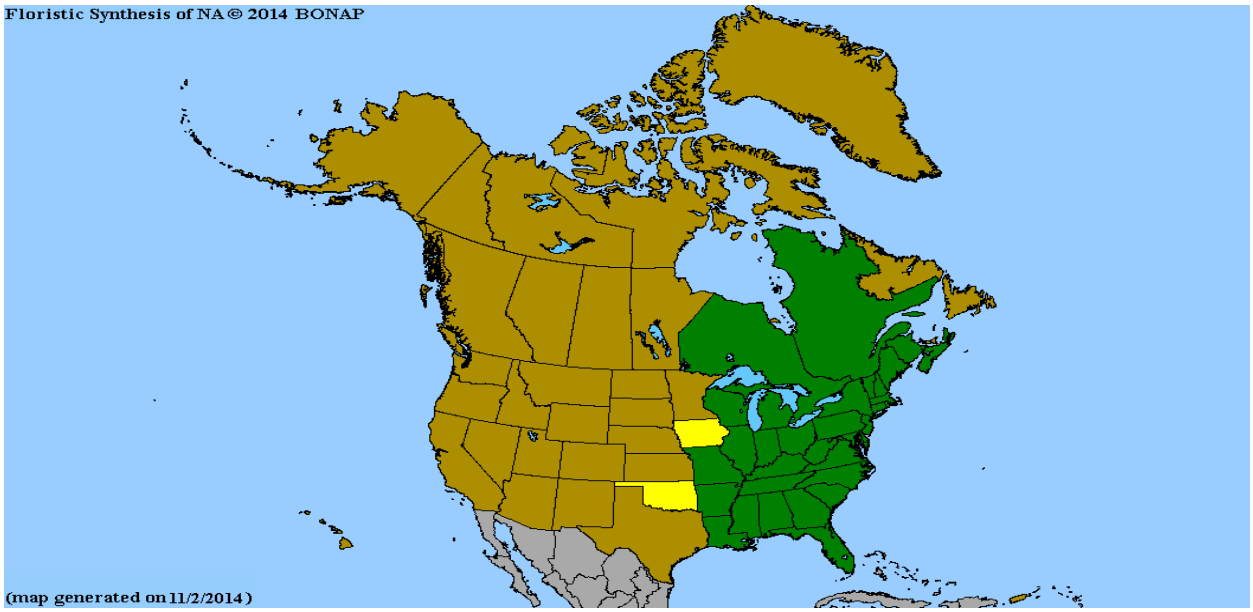

Figure 1.14: Range of *R. palustris* presented by the Biota of North America Program (BONAP) (Kartesz, 2015). Key: Dark Green is species present and native, Yellow is species present but rare.

Figure 1.15: *R. palustris* flower, Pheasant Branch Creek Conservancy, 6/12/15

Figure 1.16: *R. palustris* hips, Pheasant Branch Creek Conservancy, 8/25/15

and appear individually or in small corymbs in May on small branches off of the main stems of the plant (Gleason & Cronquist, 1991; Weakley, 2015) (Figure 1.15). The hips are red, globoid and stipitate-glandular, ripening September-October (Weakley, 2015) (Figure 1.16). As with *R. carolina*, the stipitate-glandular hips are a key identifying feature of the species. *R. palustris* can be distinguished from *R. carolina* by the absence or reduced amount of infrastipular prickles and more finely-toothed leaves (Gleason & Cronquist, 1991). *R. palustris*is diploid (2*n*=14) and is known to be the part of the hybrid origin (with *R. blanda*) of *R. carolina,* as well as being an evolutionary ancestor to *R. virginiana* (Joly & Bruneau, 2007). As with *R. carolina*, there is limited ethnobotanical data available. The sole source that was identified, Hamel and Chiltoskey (1975), notes that *R. palustris* roots were used by the Cherokee to treat intestinal worms and dysentery.

1.6 Chapter 1 References

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Chapter 2: A Characterization of Five Native Wisconsin Roses

2.1 Abstract

Roses are the single most popular genus of flowering plants for gardening in the United States and have been grown throughout the world for their aesthetic properties. Wild species in both Eurasia and North America have been harvested for their fruit for food and for medicinal purposes. The historical use of these fruit, called hips, has inspired a growing contingent of studies on their potential medicinal benefits. These properties stem from a diverse array of phytochemicals present within the hips, particularly phenolic compounds. Most of the existing research has targeted roses of the section *Caninae* (dog roses), native to Europe and Central Asia, but there has been a growing interest in species native to the Americas. Many North American species remain unstudied in terms of their phytochemical diversity. This study describes the phenology, morphology, and basic phytochemical composition of five species of roses native to Wisconsin. We determined that these five species have significantly higher levels of phenolic compounds than dog-roses and warrant further study. These data, with further study, could be valuable to industries that create supplements or phytochemical-based medicines as well as individuals who wish to grow native plants and medicinal herbs.

2.2 Introduction

Plants of the genus *Rosa* are easily one of the most iconic and recognizable horticultural crops in the world. Roses have been cultivated for over 5000 years, but many may not realize that they have many potential uses (Shepherd, 1954; Gudin, 2010). While roses are primarily used in landscapes and as cut flowers, comprising industries worth hundreds of millions of dollars in the U.S. and around the world, roses have also been used as food, perfumes, and medicine by many cultures (USDA, 2010; Vanderelst & Zolichova, 2015; Smulders et al., 2011; Hummer & Janick, 2009; Moerman, 2009). The popularity and multiple potential uses of roses place them at the crux of a number of emerging markets

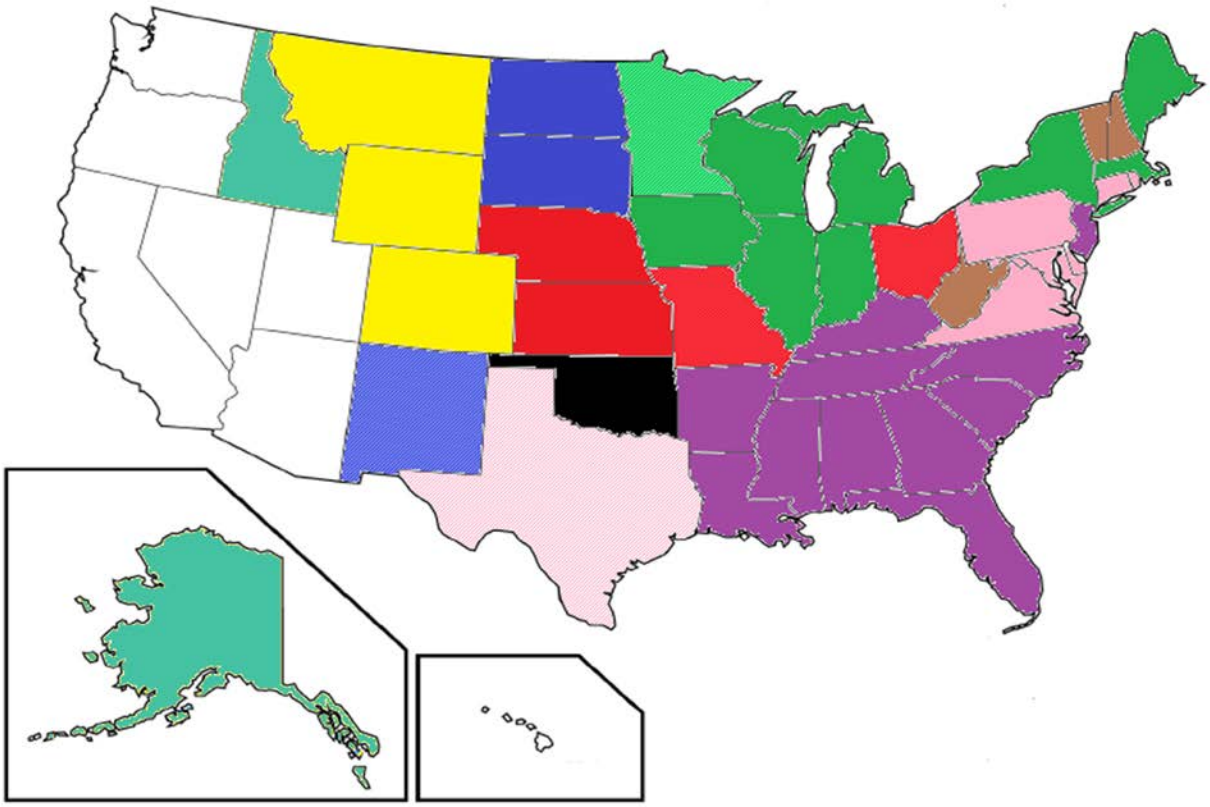
with the growth of plant-derived bioactive compounds in the medical and supplement industries and consumer-driven demand for locally produced food and native plant landscaping (Nelson et al., 2008; Uggla, 2004; USDA, 2014; Smulders et al., 2011).

In fact, the fruit of roses, called hips, have been the subject of an increasing number of studies regarding their medicinal properties. Research suggests that treatments made from powdered rose hips can reduce pain and inflammation caused by rheumatoid arthritis, positively impact weight and blood pressure management, provide potent antioxidative effects, and possess antimicrobial properties (Andersson et al., 2012; Andersson et al., 2011; Christensen et al., 2008; Chrubasik et al., 2006; Chrubasik et al., 2008; Chrubasik-Hausmann et al., 2014; Wenzig et al., 2008; Willich et al., 2010; Yilmaz and Ercisli, 2011; Yi et al., 2007). These findings lend credence to the many ethnobotanical reports of roses used as medicinal plants for symptoms as wide-ranging as eye inflammation, birthing pains, digestive illnesses, diarrhea, itching piles, and colds (Moerman, 2009; Heller, 1953; Turner et al., 1990; Gilmore, 1919; Densmore, 1913; Smith, 1933; Smith, 1928; Hoffman, 1891).

However, most research has focused on Eurasian species in the section *Caninae*, the dog-roses. These roses have had a long history of use and cultivation in Europe and Central Asia as food and as medicine. This historical use most likely accounts for numerous studies on the medicinal efficacy of rose hips (Smulders et al., 2011; Uggla, 2004; Uggla et al., 2005; Ercisli, 2007; Andersson, 2009). Despite this, there still remains a large gap in understanding of all rose species. Roses are found throughout temperate and sub-tropical climates in the Northern Hemisphere, but many of the species from North America have scarcely been studied beyond phylogenetic analyses (Smulders et al., 2011; Joly & Bruneau, 2007; Koopman et al., 2008). This could be due to the fact that these North American species have not been used in the breeding of cultivated varieties (Smulders et al., 2011). However, a few recent studies have begun to look at the potential uses of North American species (Ghose et al., 2013; Barry et al., 2008; Sanderson & Fillmore, 2010; Yi et al., 2007). Despite new interest, there remains much that

has not been explored. The sections *Cinnamomeae* and *Carolinae* contain the North American species and represent roughly one third to over one half of the estimated known rose species and may possess novel phytochemicals not present in Eurasian roses (Smulders et al., 2011; Koopman et al., 2008).

In an effort to explore more of this diverse part of the genus *Rosa*, this study analyzes five species of roses native to Wisconsin: *R. acicularis, R. arkansana, R. blanda, R. carolina,* and *R. palustris* (see Figure 2.1 for a map of the ranges of these species in the U.S.). These species' flowering and physical traits were observed to discern potential value as landscape plants and potential for cultivation. To assess the potential value for medicinal purposes, total phenolics were analyzed, in addition to a few other traits that may be of interest to industry or individuals. Phenolics are bioactive antioxidants which research suggest to be the source of the medicinal properties of rose hips, so this analysis will let us know whether these species warrant further, more detailed investigation (Ercisli, 2007; Uggla, 2004; Andersson, 2009; Barros et al., 2010; Günes, 2013; Smulders et al., 2011; Demir et al., 2014).



Legend:

R. acicularis	**X**	**X**	**X**					**X**	**X**				**X**
R. arkansana	**X**		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**			
R. blanda	**X**						**X**	**X**	**X**	**X**		**X**	**X**
R. carolina	**X**					**X**	**X**		**X**	**X**	**X**	**X**	**X**
R. palustris	**X**				**X**	**X**				**X**	**X**	**X**	**X**

Figure 2.1: A map showing the U.S. distribution of *R. acicularis, R. arkansana, R. blanda, R. carolina,* and *R. palustris.* Each color represents a unique combination of the species present in that state. Location data obtained from the Biota of North America Program (Kartesz, 2015).

2.3 Materials and Methods

2.3.1 Site Selection and Species Identification

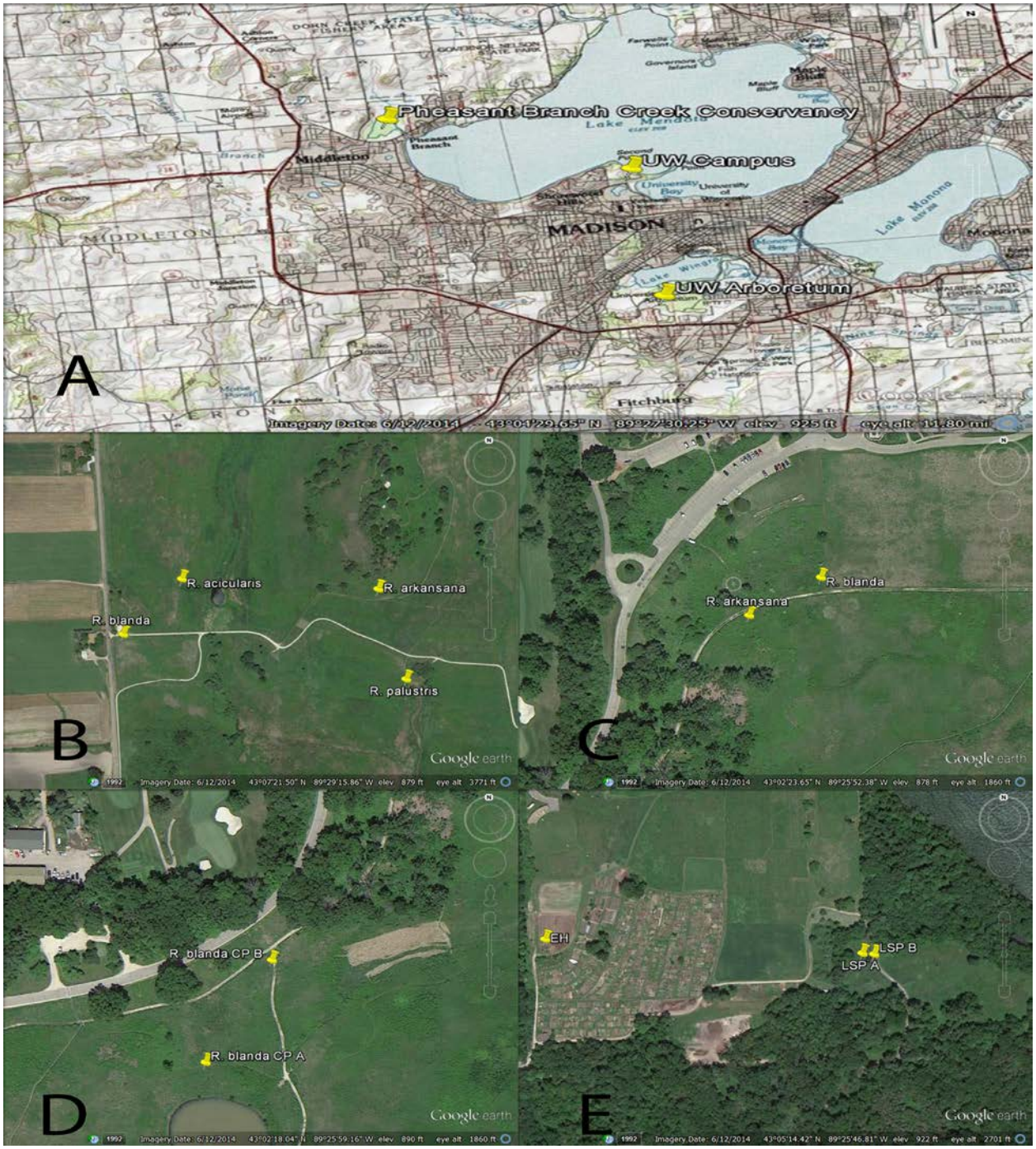


Figure 2.2: Maps of the Madison, WI area, with locations of samples marked. Image obtained via Google Earth software (Accessed 2016). **A:** Location of the primary sites in the Madison, WI area: Pheasant Branch Creek Conservancy (PBC), the University of Wisconsin Campus including the Lakeshore Preserve (LSP) and Eagle Heights Community Garden (EH), and the University of Wisconsin Arboretum including Curtis Prairie (CP) and the prairie near the Visitor's Center (VC) **B:** Locations of species at Pheasant Branch Creek Conservancy **C:** Locations of species present at the Visitor's Center Prairie **D:** Location of species in the Curtis Prairie **E:** Location of species in the Lakeshore Preserve and Eagle Heights

Due to the fact that native North American species of roses are neither traditionally cultivated nor readily available for purchase at floral retailers, this study required locating and identifying wild species. Multiple locations where native roses grew were identified. These included the UW-Madison's Lakeshore Nature Preserve, Pheasant Branch Creek Conservancy, and the UW Arboretum. Permission was obtained for taking observations and performing weekly harvests (see figure 2.2 and Table 2.1 for a map and site descriptions). Additionally, nurseries whose production was focused on native and/or edible plants were contacted, and a few species were grown in the greenhouse before being planted at a research site near the Eagle Heights Community Garden. Of the three species planted at Eagle Heights, *R. blanda, R. arkansana*, and the non-native *R. canina,* only the native species flowered during the period of study, and no species fruited prolifically enough to be included in any analysis.

Location	**Species (Location Label)**	**Site Description**	**GPS Coordinates**	**Year(s) Harvested**
Eagle Heights Garden Plot	*R. canina, R. blanda, R. arkansana* (EH)	Perennial garden planting with clayey loam soils. Sunny area which has been cultivated as a garden for over 10 years	43° 5'15.63"N 89°25'59.52"W	N/A
UW-Madison: Lakeshore Preserve	*R. carolina* (2 distinct morphologies, henceforth LSP A and LSP B)	Restored prairie, heavy clay soils. LSP A colonies are found throughout a sunny, open field. LSP B is found in the shade of a large pine tree.	43° 5'14.58"N 89°25'42.24"W	2014-2015
Pheasant Branch Creek Conservancy, Middleton, WI	*R. blanda*(PBC)	Restored prairie, loamy soil near gravel parking lot. Site is in full sun, but many canes are shaded by taller grasses	43° 7'19.60"N 89°29'28.20"W	2014-2015
Pheasant Branch Creek Conservancy, Middleton, WI	*R. acicularis*(PBC)	Restored prairie, burnt in spring of 2015, clay loam soil. Site is in full sun, and the plants are in sun near a walking path.	43° 7'23.34"N 89°29'22.94"W	2014
Pheasant Branch Creek Conservancy, Middleton, WI	*R. arkansana*(PBC)	Restored prairie, loamy soil, burnt in spring of 2015. On steep, sunny hillside, south-facing slope.	43° 7'22.19"N 89°29'5.92"W	2014-2015
Pheasant Branch Creek Conservancy, Middleton, WI	*R. palustris*(*PBC)*	Restored prairie in riparian zone, sandy loam soil near creekbed. Site is in full sun	43° 7'15.81"N 89°29'3.56"W	2014-2015
UW Arboretum Curtis Prairie, West	*R. blanda*(CP A)	Restored prairie, on a slight rise with loamy soil. Site is sunny and canes are surrounded by vegetation.	43° 2'15.69"N 89°26'0.96"W	2014-2015
UW Arboretum, Curtis Prairie: West	*R. blanda*(CP B)	Restored prairie, loamy soil in slight depression. Site is in full sun. Most canes largely exposed, but some are shaded by dogwoods.	43° 2'18.03"N 89°25'58.92"W	2014-2015
UW Arboretum, Curtis Prairie East, by Visitor's Center	*R. blanda*(VC)	Restored prairie with moist, clayey soils. Shrub is located at the intersection of two paths in full, unobstructed sunlight.	43° 2'24.22"N 89°25'51.09"W	2014-2015
UW Arboretum, Curtis Prairie East, by Visitor's Center	*R. arkansana*(VC)	Restored prairie with moist, clayey soils. Most plants shaded by taller grasses and forbs	43° 2'23.37"N 89°25'53.26"W	2014

Table 2.1: Location description and GPS coordinates of sample sites.

All species were identified using the dichotomous key in Gleason & Cronquist (1991) as well as assistance from the staff at Pheasant Branch Creek Conservancy and the UW Arboretum. While staff at the Lakeshore Preserve were unfamiliar with rose biology, they had previously noted two divergent morphologies: one distinctly shrub-like with small fruit, the other more colonial, with larger fruit and an earlier bloom time. Interestingly, both were keyed to *R. carolina*. Given that *R. carolina* may have arisen as a cross between the colonial *R. blanda* and the shrubby *R. palustris*, this divergence may simply be due to chance inherited characteristics and/or the typical high phenotypic variability characteristic of those roses. These two subsets were treated as separate samples in this study to identify any differences.

Additionally, the specimen labeled in Figure 2.2 C as *R. blanda* did key out to *R. blanda* using Gleason & Cronquist (1991). However, it also exhibited several differences from the other samples of *R. blanda*. These differences included later flowering than the other observed *R. blanda* specimens, a shrub-like growth habit instead of colonial, and hips which were unusually large and thick-walled compared to the other *R. blanda* samples. Since it was located close to the ornamental horticulture garden of the UW Arboretum, it is possible that this specimen is a hybrid of *R. blanda* and the popular garden rose *R. rugosa* (see Mercure & Bruneau, 2008).

2.3.2 Sample Collection and Morphological/Phenological Analysis

Rose hips were collected in the growing seasons of 2014 and 2015.Morphological traits measured included were wet fruit mass, dry fruit mass, dry matter percentage, and fruit perimeter. We were able to compare the fruit size and actual mass of fruit tissues (as opposed to mass from water and seeds) between species. This is valuable for determining which species may have the highest overall amounts of bioactive compounds and for landscape use, as larger hips may be of more winter interest due to being more apparent. Phenological traits observed included bloom period, cane height, number of flowers per cane, and overall flower density (calculated by dividing the mean number of flowers per

cane by the mean height). These traits are valuable when considering landscape use and are of interest for possible commercialization of hip production: higher flower density means more hips per area planted, which makes achieving economies of scale easier. There were significant differences in the harvest techniques and sample collection between years as the study was refined, as noted below.

2014

In 2014, initial site locations were identified and permission obtained for harvesting. Delays resulted in samples being harvested in mid-late September, after hips were fully red, and before species identification had fully occurred. Only healthy hips (less than 25%incidence of disease or insect damage) were collected by randomly picking from assorted canes within the shrubs/colonies present at each location. Aside from avoiding harvesting severely diseased or damaged hips, no deliberate attempts were made to select hips of a particular quality or developmental stage. After harvest and identification, the hips were weighed in bulk (each sample being a particular date, species, and location) and an average was taken (total weight by number of hips), frozen at 4° C, and cut in half. These half-hips were laid round-side down on a flatbed scanner, covered with a dark black box, and scanned. Hips that were seriously deformed by the process of cutting them in half were not scanned. Subsequent to scanning, the hips were lyophilized in a Labconco FreeZone Freeze Dry System (Kansas City, Missouri) and weighed again. The seeds were then removed and the hips weighed a final time. The remaining hip tissue was pulverized for phenolic analysis.

Images were analyzed using the Tomato Analyzer program (Rodríguez et al., 2010). This program measures the physical size of the fruit as well as color. Tomato Analyzer has two potential color measuring systems, one using the RGB color space, the other using CIELab (Rodríguez et al., 2010). This analysis uses the RGB color space option, which is an“(additive) system, as it measures the strength of each R (red), G (green), B (blue) color in each pixel to reproduce other colors. The additive RGB color space is a cube with each axis representing variance in one of the primary colors and a white reference

point. This color space is nonlinear and does not mimic the nature of color perception" and the values it provides range from 0 (no instance of the color) to 255 (maximum Redness/Greenness/Blueness) (Strecker et al., 2010).Accurate color analysis was assured by calibrating the scanner and the program with the X-rite ColorChecker.

2015

In 2015, all sites were visited every 3-4 days starting in early May in order to track bloom emergence and length of bloom time. Buds began emerging around 5/20/2015, and subsequently the progress of flowering was tracked until all species had stopped flowering on 7/21/2015. Starting 8/31/15, each sample site was visited every 7 days and 10-15 fruit were collected for analysis. Each day of harvest and the preceding 6-8 days are labeled as "Harvest Week *n*" in the analysis to facilitate comparisons between years. Table 2.2 denotes the specific date ranges for each Harvest Week. As in 2014, only fruit that had less than 25% of its surface marred by disease or insect damage were collected. After all flowers had bloomed, but before hips were harvested, the total number of hips on 5-10 canes of each specimen (or colony of specimens) were counted in order to assess the average number of flowers per cane. Hipless pedicels that were clearly part of a corymb but whose hips were no longer present were included in this count as well.

Unfortunately, multiple unexpected challenges occurred. During the period after flowering and counting flowers per cane, but before harvesting the hips, several plants were destroyed at Pheasant Branch Creek Conservancy and the Lakeshore Preserve. At the Lakeshore Preserve, herbicide was used to remove invasive species, and a number of smaller colonies of *R. carolina* LSP A were destroyed. At Pheasant Branch Creek Conservancy, the largest *R. palustris* shrub at that location was mistakenly identified as the invasive *R. multiflora* by a work group and cut down. The mature *R. palustris* shrub that was removed was significantly taller than the nearby younger shrubs, had many more flowers, and had larger hips. Though some moderately sized shrubs remained in the area, none were as robust as the

shrub that was removed. These issues may have impacted the data when comparing the species and comparing the same species across the years o f the study. Additionally, *R. acicularis* did not flower in 2015 due to a prairie burn that spring. Therefore no data were collected. Finally, *R. arkansana* VC was heavily stricken by fungal diseases and did not produce enough flowers to sustain a harvest for the 2015 season (see Section 2.4.1)

Harvest Week	Start Date	End Date
1	8/22	8/31
2	9/1	9/7
3	9/8	9/14
4	9/15	9/21
5	9/22	9/30
6	10/1	10/7
7	10/8	10/14
8	10/15	10/21
9	10/22	10/31

Table 2.2: Date Ranges of Harvest Weeks

In order to avoid the problems with fruit distortion observed in 2014, hips were placed on black velvet and photographed individually by an Olympus DP70 camera attached to an Olympus SZX12stereo microscope. The hips were then rotated 180° and photographed a second time in order to capture both sides of the fruit. The camera was calibrated with the X-rite ColorChecker and the images were analyzed using the Tomato Analyzer program as described previously. Hips were weighed individually and subsequently a subset of 5 hips were separated to be processed for soluble solid analysis, with the rest being cut in half and frozen at 4° C before being lyophilized, weighed, deseeded, and weighed a final time. These hips (and the processed remnants from soluble solid analysis) were pooled by

date/species/location, pulverized, and analyzed in triplicate for the total phenolic analysis. Plant material from soluble solid analysis was grouped, pulverized, and analyzed separately in order to determine if there were differences in total phenolics between the two subsets. Figure 2.3 provides a diagram of the processing steps.

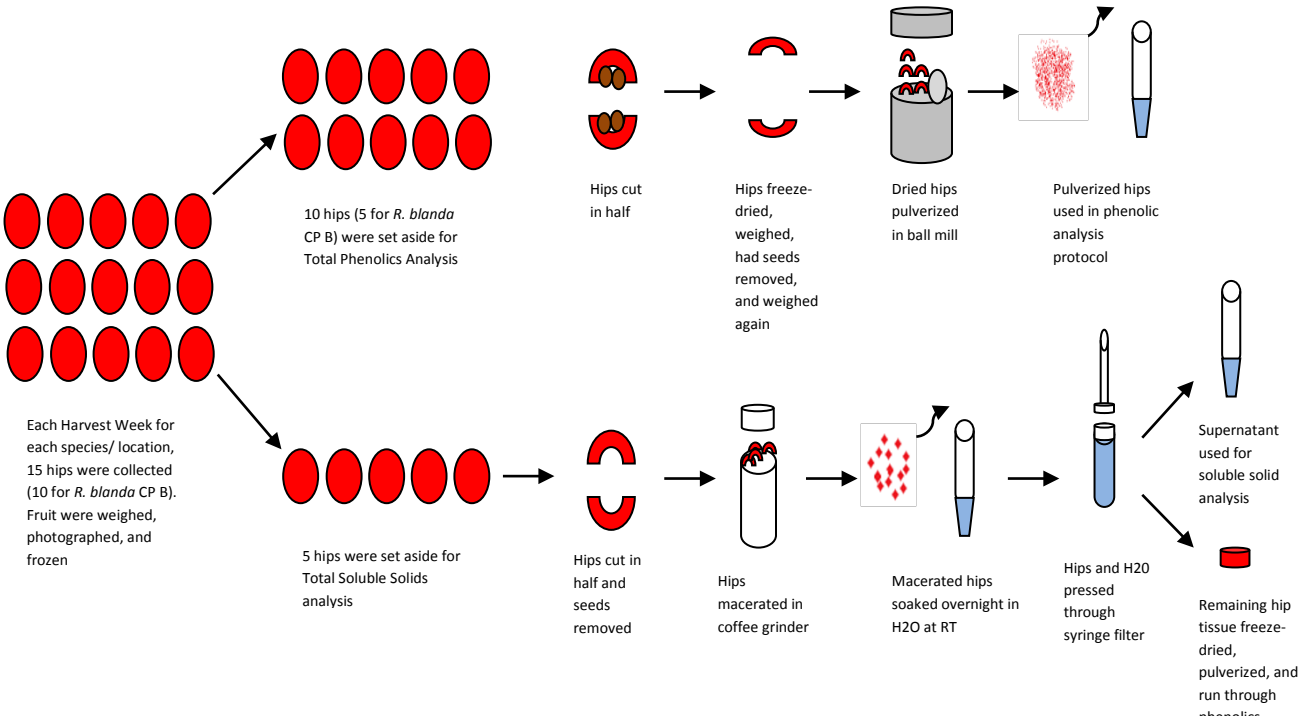


Figure 2.3: Diagram of harvest and processing steps used for the 2015 harvest season

2.3.3 Chemical Analysis

Soluble Solids

Soluble Solid concentration is typically used to assess the sugar content of fruits, so this analysis can help to pinpoint which species may be sweeter than others. This is valuable for the production of teas and edible products derived from rose hips. Due to the nature of rose hips as mostly dry fruit, particularly in later-season harvests, soluble solids were analyzed using a modified version of the protocol used by Carvalho and co-authors (2009).The method of extraction was a s follows: individual fruits were cut in half, deseeded, and weighed. They were then ground using a Proctor-Silex coffee

grinder to ≤1 mm diameter, placed into Sarstedt tubes, and suspended in 10 ml double-distilled water/gram of fruit. Suspended hips were pulverized with hand tools for ~5 seconds and left to shake overnight at 1500 rpm at room temperature to extract soluble solids. Water was added based on fruit weight in order to have a more accurate estimate of total soluble solids, rather than a simple comparison based on a standard volume of water as used by Carvalho et al. (2009). The next day, plant tissue and water were pushed through a syringe filter and the tissue caught by the filter was collected for freeze-drying and subsequent phenolic analysis (see Figure 2.3). The supernatant was vortexed for 30 seconds and then allowed to settle for 5 minutes before aliquoting 100 µl of liquid into the well of a Hannah Instruments (Woonsocket, Rhode Island) HI96811 refractometer to obtain soluble solid concentration in °Brix, which was reported as a percentage (1° Brix = 1% Soluble Solids). The refractometer was cleaned and recalibrated with distilled water between each sample.

Total Phenolics Assay

Many of the known bioactive compounds in rose hips are phenolics; and thus, the total concentration of phenolic compounds can serve as shorthand for the amount of useful and potentially medicinal phytochemicals in rose hips (Ercisli, 2007; Andersson, 2009; Barros et al., 2010; Günes, 2013; Smulders et al., 2011; Demir et al., 2014). Total phenolic concentrations were analyzed following a modified protocol of Demir et al. (2014). In brief, triplicates of the weekly harvests of each sample were freeze-dried, deseeded, pulverized, and suspended in dd water to a total concentration of 1 mg/ml. 100 µl of this solution was added to each cell in a 96-well plate. Subsequently, 100 µl of the Folin-Ciocalteau phenol reagent was added to each well and mixed by pipetting the solution. The reaction was allowed to proceed for 4 minutes and then 100 µl of 10%aqueous sodium carbonate was added. The resulting solution was allowed to incubate at room temperature for 2 hours, and the plates were then read on a Biotek Synergy HT plate reader (Winooski, Vermont) at 760 nm. A standard curve was generated by creating a series of solutions of gallic acid and water, with concentrations ranging from .01 mg/ml – .2

mg/ml. Results are given as mg Gallic Acid Equivalents (GAE) per gram of Dry Weight (DW). Some samples returned an overflow error in absorbance because the concentration of phenolics was too high for the plate reader to interpret. Diluting the samples and re-running them showed a nonlinear relationship between absorbance and concentration that could not be reliably defined, so any samples with the overflow error were assigned identical arbitrary high absorbance values for the purpose of calculating an estimate of total phenolic concentration.

2.4Results

2.4.1 Phenological Observations

Observed Differences in the Timing and Duration of Flowering

Flowering across species was observed from May through mid July during the 2015 growing season (Figure 2.4). Differences were observed between species as well as between the similar species at independent locations. For example, *R. blanda* CP A and PBC were the earliest flowering of all samples, beginning in late May and continuing through mid-June. In contrast, *R. blanda* CP B flowered only briefly in early-mid June and *R. blanda* VC didn't initiate flowering until mid-June, when the other samples of *R. blanda* had almost completed their bloom cycle. *R. blanda* VC did not complete flowering until mid-July. Another point of interest is how *R. arkansana* produced a second flush of flowering in mid-July, after a relatively long bloom period. As previously noted, *R. acicularis* did not flower. Therefore, no observations were made on that species. Figure 2.5 illustrates the development of the species' flowers and hips from the initial budding through the end of the harvest season. Differences in flower color, hip shape, and hip color throughout development can be observed.

Overall, *R. blanda* generally had the shortest bloom time,1-2 weeks from bud break to end of flowering depending on location, presenting a brief rush of flowers before quickly subsiding(Figure 2.4). In contrast, *R. carolina* (LSP A and B) had a week-long period of increasing flowering, followed by a week of full blooms and 2-3 weeks of slow decline. *R. palustris* PBC is noted in Figure 2.4 to also have a long

bloom period, though this could reflect that the large mature shrub flowered earlier than most of the surrounding younger shrubs (see also section 2.3.2). While the mature shrub had completed flowering

Species	5/20	5/28	5/31	6/3	6/8	6/12	6/15	6/19	6/24	6/29	7/2	7/8	7/14	7/21
R. arkansana PBC	NB													NF
R. blanda CP A							NF	NF	NF	NF	NF	NF	NF	NF
R. blanda CP B							NF	NF	NF	NF	NF	NF	NF	NF
R. blanda PBC							NF	NF	NF	NF	NF	NF	NF	NF
R. blanda VC	NB											NF	NF	NF
R. carolina LSP A														NF
R. carolina LSP B	NB												NF	NF
R. palustris PBC	NB													NF

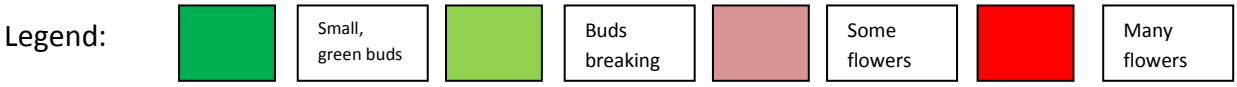


Figure 2.4: Observed buds, bud break, and flowering of *Rosa* spp. during the 2015 growing season. **NB:** No buds present/flowering not initiated, **NF:** No Flowers present/flowering complete

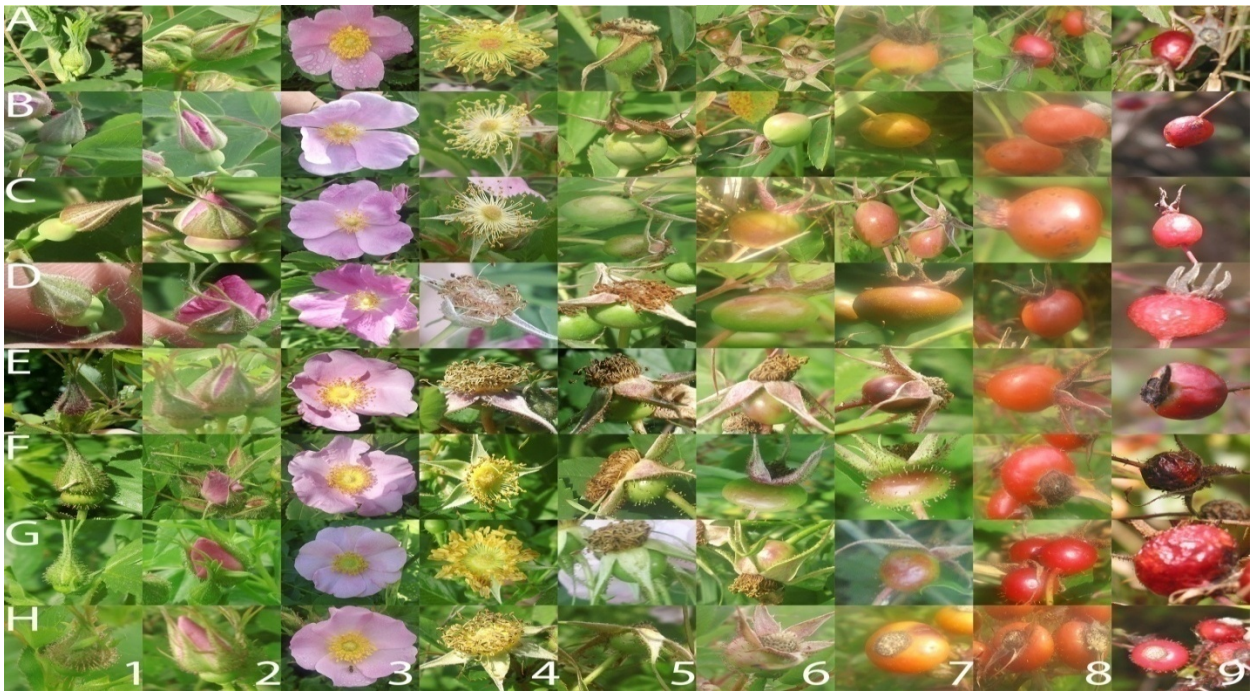


Figure 2.5: Developmental Stages of *Rosa* spp. in 2015**A**: *R. arkansana* PBC, **B**: *R. blanda* CP A, **C**: *R. blanda* CP B, **D**: *R. blanda* PBC, **E**: *R. blanda* VC, **F**: *R. carolina* LSP A, **G**: *R. carolina* LSP B, **H**: *R. palustris*. **1**: Unopened bud, **2**: Breaking bud, **3**: Full flower, **4**: Petals abscised, **5**: Stamens dying, hip developing, **6**: Hip beginning to redden, **7**: Hip mostly red, **8**: Hip fully red (mid-August), **9**: Hips during week 7 (mid October), towards the end of the harvest period

by 7/2/15, the younger shrubs were slower to initiate flowering and just completing their initial flush, thereby extending the bloom period. Also of interest may be the fact that the flowers on the younger shrubs were a much lighter shade of pink than those of the mature shrub.

Observed Differences in Flower Production and Plant Morphology

In addition to bloom time, the number and density of the flowers is important when considering their value as landscape plants as well as how many hips they produce and how efficiently they can be harvested. There were significant differences observed in plant height, with *R. arkansana* PBC the shortest (mean cane height: 8.30 inches) and *R. blanda* VC the tallest (mean cane height: 54.38 inches) (Figure 2.6, Table 2.3). Average flowers per cane were also measured with *R. arkansana* PBC having the least (3.5) and *R. palustris* having the most (80.1) (Figure 2.7, Table 2.3). Figure 2.8 shows the floral

density in flowers per inch of cane height, where *R. palustris* again has the highest value (1.90) and *R. arkansana* PBC and *R. carolina* LSP B have the lowest densities (.42 and .46, respectively).

There was significant overlap in the ranges of heights among all species aside from the distinctly small *R. arkansana* PBC (Figure 2.6, Table 2.3): *R. blanda* CP A (35-56 in.), *R. blanda* CP B (19-53 in.), *R. blanda* PBC (22-39 in.), *R. blanda* VC (45-61 in.), *R. carolina* LSP A (31-49 in.), *R. carolina* LSP B (26-50 in.), and *R. palustris* PBC (27-58 in). Many species were not significantly different from each other, though the species representing the extremes of the overall range of heights (*R. arkansana* PBC and *R. blanda* VC) were significantly different from most others (Table 2.4). This assessment is based on the Tukey Honest Significant Difference (HSD) test of an analysis of variance (ANOVA) in cane height and flowers per cane (Tukey, 1949). The Tukey test performs pairwise comparisons of means and identifies any difference in means greater than the standard error of the ANOVA.

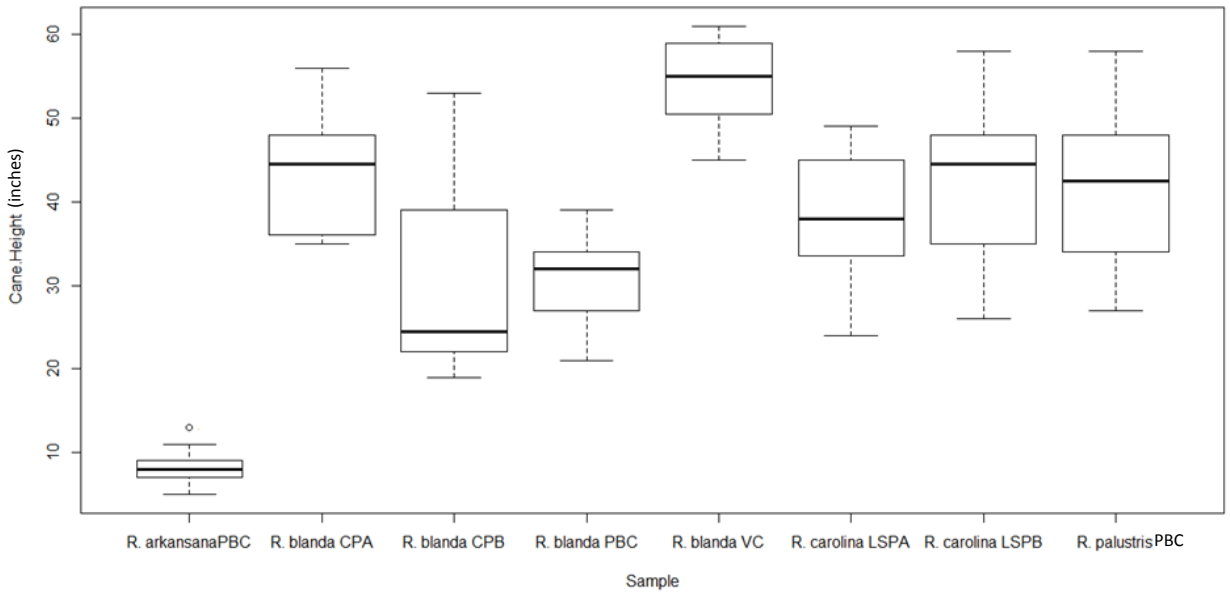


Figure 2.6: Boxplot of Cane Height, in inches, for *Rosa* spp.

In terms of flowers per cane, there was even more overlap among most species than there was for cane height (Figure 2.7). As illustrated in Table 2.3, the range in the number of flowers was quite broad in some species (i.e. 6 to 243 in *R. palustris* or 6 to 79 in *R. blanda* PBC), resulting in high variances and few significant differences (Table 2.4). However, *R. arkansana* clearly had the lowest average

number of flowers per cane (3.5) and *R. palustris* had the highest (80.1). Noted in Figure 2.7 are two outliers, which may have skewed the data. In particular, one cane of *R. palustris* was observed to have 243 flowers, nearly double the next-highest cane. Floral density measures the concentration of flowers on a plant based on the number of flowers and plant height. Of the species observed, *R. palustris* has the greatest density and *R. arkansana* the least, representing a range from .42 to 1.9 flowers per inch of height (Figure 2.8 and Table 2.3). *R. blanda* CP A and *R. blanda* PBC also have comparatively high floral densities compared to other samples, with densities of 1.03 and .89 respectively.

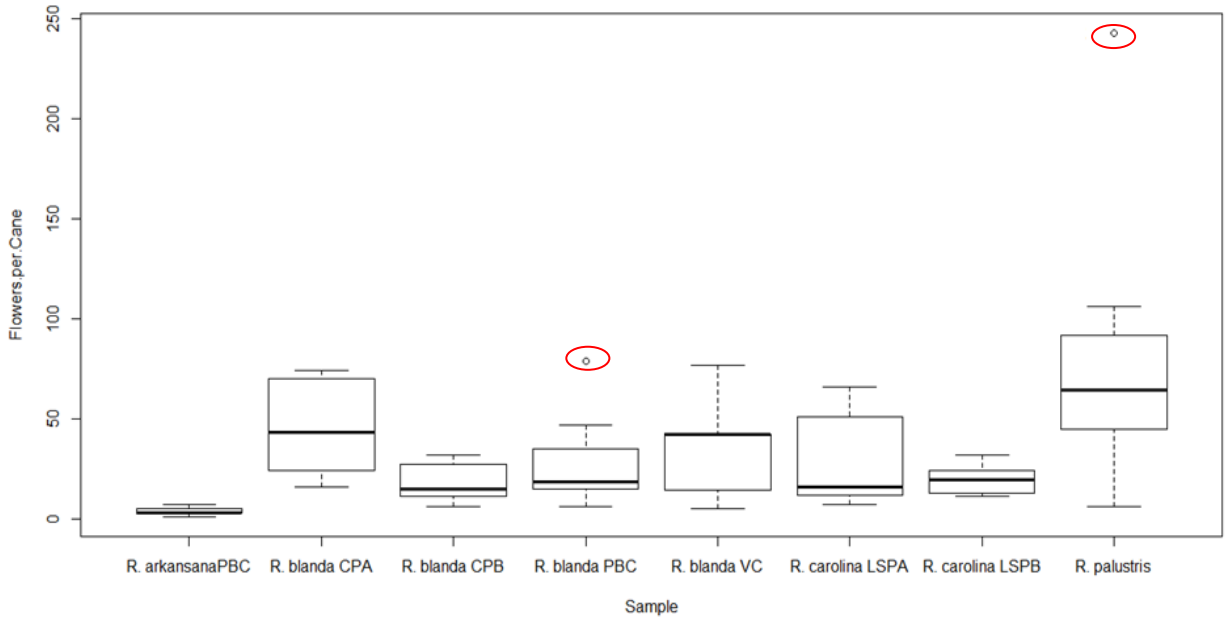


Figure 2.7: Boxplot of number of flowers per cane for *Rosa* spp. Outliers circled in red

Species	Minimum Cane Height (inches)	Maximum Cane Height (inches)	Mean Cane Height (inches)	Minimum Flowers per Cane	Maximum Flowers per Cane	Mean Flowers per Cane	Flower Density (mean # flowers per cane/mean cane height)
R. arkansana PBC	5	13	8.30 ± 2.26	1	7	3.50 ± 1.96	.42
R. blanda CP A	35	56	45.50 ± 21.61	16	74	44.20 ± 7.28	1.03
R. blanda CP B	19	53	29.9 ± 11.05	6	32	17.00 ± 8.81	.57
R. blanda PBC	22	39	30.60 ± 6.08	6	79	27.20 ± 21.81	.89
R. blanda VC	45	61	54.38 ± 5.68	5	77	33.86 ± 24.67	.62
R. carolina LSP A	31	49	38.27 ± 7.60	7	66	29.82 ± 24.15	.78
R. carolina	26	50	42.10 ±	11	29	19.50 ±	.46

LSP B			10.02			7.23	
R. palustris PBC	27	58	42.08 ± 9.66	6	243	80.10 ± 64.53	1.90

Table 2.3: Flower density, Minima, Maxima, and Mean for cane height and flowers per cane in *Rosa* spp.

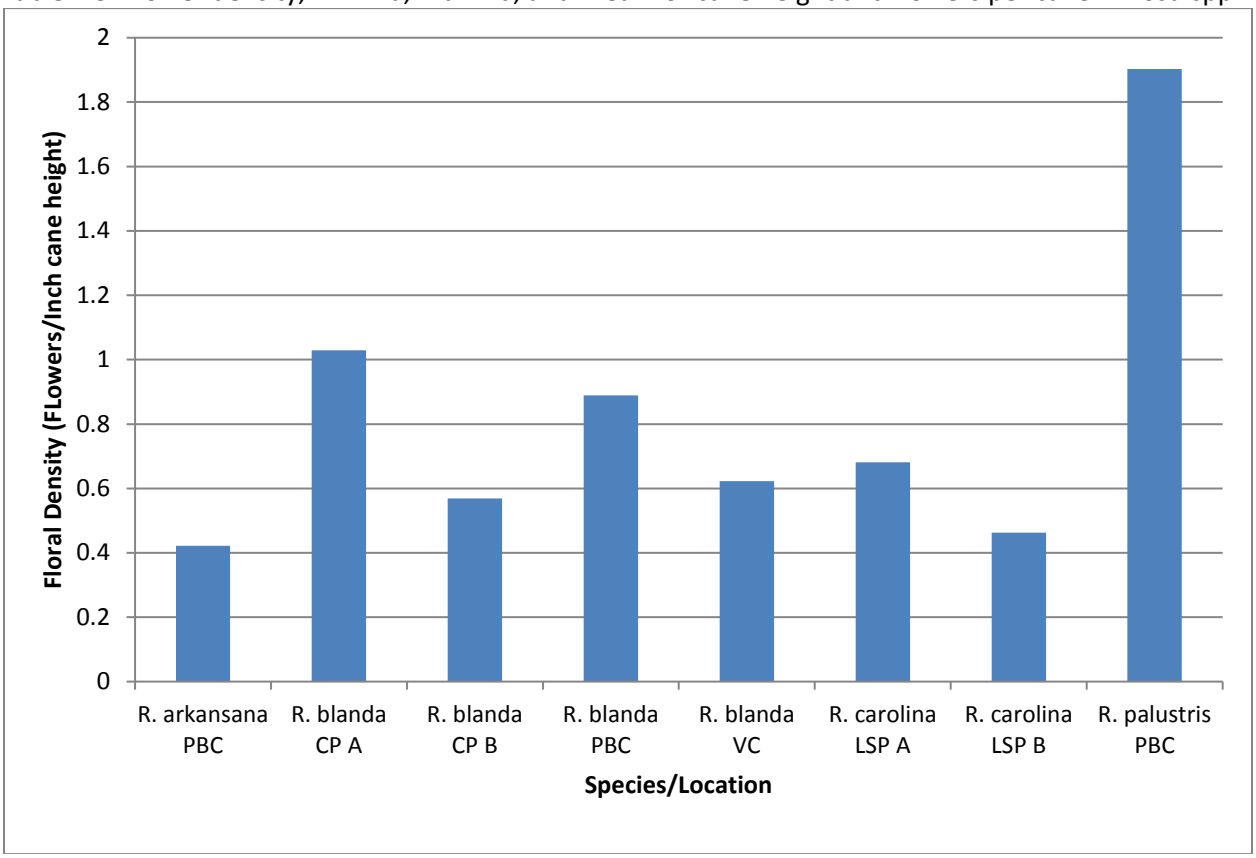


Figure 2.8: Estimated flower Density by species for *Rosa* spp. in flowers per inch of cane height.

Trait	**1 (smaller)**	**2**	**3**	**4**	**5**	**6 (larger)**
Cane Height	*R. arkansana* PBC[d]	*R. blanda* CP B[c] *R. blanda* PBC[c]	*R. carolina* LSP A[b, c]	*R. carolina* LSP B[b] *R.* palustris PBC[b]	*R. blanda* CP A[a, b]	*R. blanda* VC[a]
# Flowers per cane	*R. arkansana* PBC[c]	*R. blanda* CP B[b] *R. carolina* LSPA[b] *R. carolina* LSPB[b] *R. blanda* VC[b] *R. blanda* PBC[b]	*R. blanda* CP A[a, b]	*R. palustris* PBC[a]		

Table 2.4: Relative ranking of samples from smallest to largest in terms of height and number of flowers per cane by Tukey HSD post-hoc analysis of means. As column number increases, relative size increases, until all species are accounted for. Superscript letters represent different levels of significant difference from other species, with [a] representing the statistically largest sample(s), descending through [b], [c], [d], etc. Some species occupy multiple levels of significance, representing intermediate traits between the groups.

Observed Disease and Insect Pressure

In addition to bloom periods and flowering density, the presence and severity of disease and insect damage was observed. In 2014, little incidence of disease was observed other than some leaves of all species with black spot (*Diplocarpon rosae)*. In addition, a number of fruit (particularly of *R. blanda* CP A and CP B and *R. palustris*) had evidence of burrowing insects. In contrast, in 2015there were large outbreaks of disease, especially in the low-lying, wind-sheltered Arboretum. In that year, there had been over 8 inches of rain in the Madison area by the end of May, thus presenting a perfect growing climate for fungal diseases (NOAA, accessed 2016). Figure 2.9 provides examples of disease and pest damage. Diseases observed included black spot (Figure 2.9 A), rose rust (*Phragmidium tuberculatum,* 2.9 B and C), and an unknown fungus (2.9 D and E) that withered both individual and entire corymbs of hips into white husks. Disease was rampant throughout all colonies of roses in the Arboretum, rendering the *R. arkansana* sample near the Visitor's Center (*R. arkansana VC)* unharvestable in 2015. Fortunately, samples elsewhere were relatively less damaged. Some hips did see signs of insect damage (2.9 F) and Japanese Beetles damaged the foliage of all species (2.9 G). *R. palustris* flowers also attracted a large number of pollinating bees and flies (2.9 H), as well as a particular species of weevil that was readily found on the flowers (2.9 I).

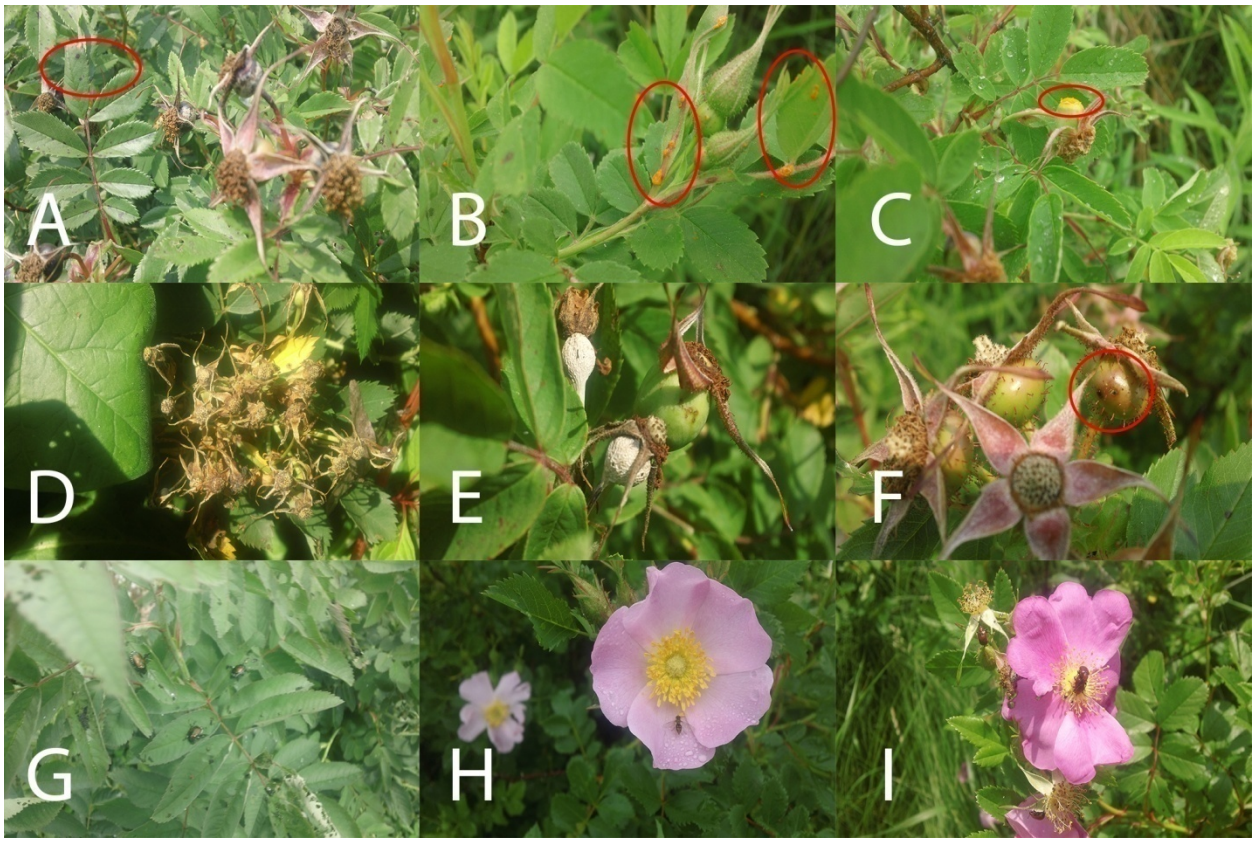


Figure 2.9: **A**. Rose black spot on *R. blanda* VC, with diseased hips in foreground **B**. Rose rust on *R. arkansana* VC **C**. Rose rust on the hip of *R. blanda* CP A **D**. A corymb of withered hips from *R. arkansana* EH **E**. 2 dead *R. blanda* CP A hips adjacent to healthy hip. **F**. Insect burrow on *R. palustris* hip **G**. Japanese Beetle foliar damage on *R. blanda* EH **H.** A pollinator approaching an *R. palustris* flower **I.** Weevils mating on an *R. palustris* flower, with others nearby

2.4.2 Physical Characteristics: Hip Perimeter, Mass, and Dry Matter Percentage

The five studied species showed significant differences from each other in terms of fruit perimeter, fruit mass (wet and dry), and percentage dry matter (Figures 2.10-2.15, Table 2.5). In addition, species present at multiple locations (*R. blanda* and *R. carolina*) showed significant differences between locations (Figures 2.10-2.15, Table 2.5). Overall, perimeter and dry mass remained constant throughout the season within each sample, though wet mass decreased in some samples and dry matter percentage increased over time in all samples as discussed below (Figures 2.12-2.14). Since most weekly data within a given sample were not significantly different from the other weeks, comparing the species and locations was done by performing an ANOVA using the weekly means as data points. The Tukey

HSD) test was conducted on this ANOVA model to determine which species were significantly different from each other, as described previously(Tukey, 1949) (Table 2.5). Between 2014 and 2015, there were no significant differences in fruit perimeter within any given species (Figures 2.10, 2.12). Since the 2014 data for mass and dry matter percentage each harvest week were bulk averages, I did not determine whether significant differences exist or not between years.

In terms of fruit size (both perimeter and mass), *R. blanda* PBC and *R. carolina* LSP B were consistently the smallest hips among the studied species. I measured an average perimeter of 3.64 cm and 3.72 cm, wet mass of .59 g and .55 g, and a dry (deseeded) mass of .16 g and .14 g, respectively. *R. carolina* LSP A and *R. blanda* VC were consistently largest: perimeters of 4.45 cm and4.52 cm, wet masses of 1.07 g and 1.15 g, and dry (deseeded) masses of .27 g and .31 g, respectively. *R. arkansana* VC was statistically similar to *R. carolina* LSP A and *R. blanda* VC in terms of dry mass (.34 g) and *R. arkansana* PBC was statistically similar to the latter two samples in terms of wet mass (1.07 g). However, neither *R. arkansana* VC nor *R. arkansana* PBC were ranked consistently among the largest-sized samples in the other measures assessed in this section when the Tukey HSD test was applied (Table 2.5). In general, samples with larger perimeters were more massive, but for some samples, such as *R. blanda* CP A, a comparatively higher perimeter (4.35 cm) did not reflect higher mass (.23 g dry, deseeded mass).

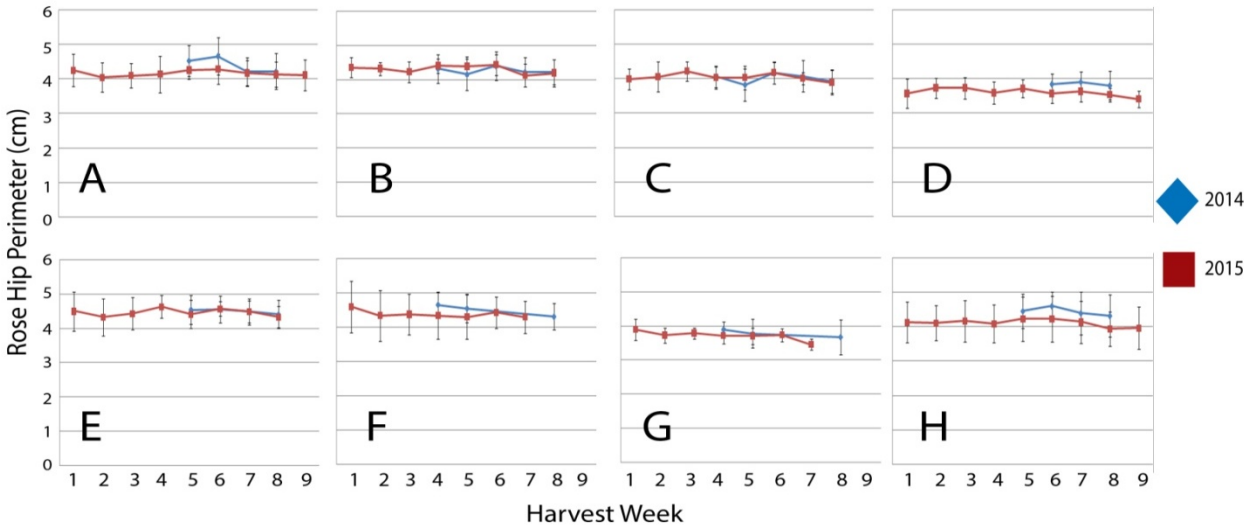


Figure 2.10: Perimeter of *Rosa* spp. over harvest period, 2014-2015. **A**: *R. arkansana* PBC, **B**: *R. blanda* CP A, **C**: *R. blanda* CP B, **D**: *R. blanda* PBC, **E**: *R. blanda* VC, **F**: *R. carolina* LSP A, **G**: *R. carolina* LSP B, **H**: *R. palustris* PBC

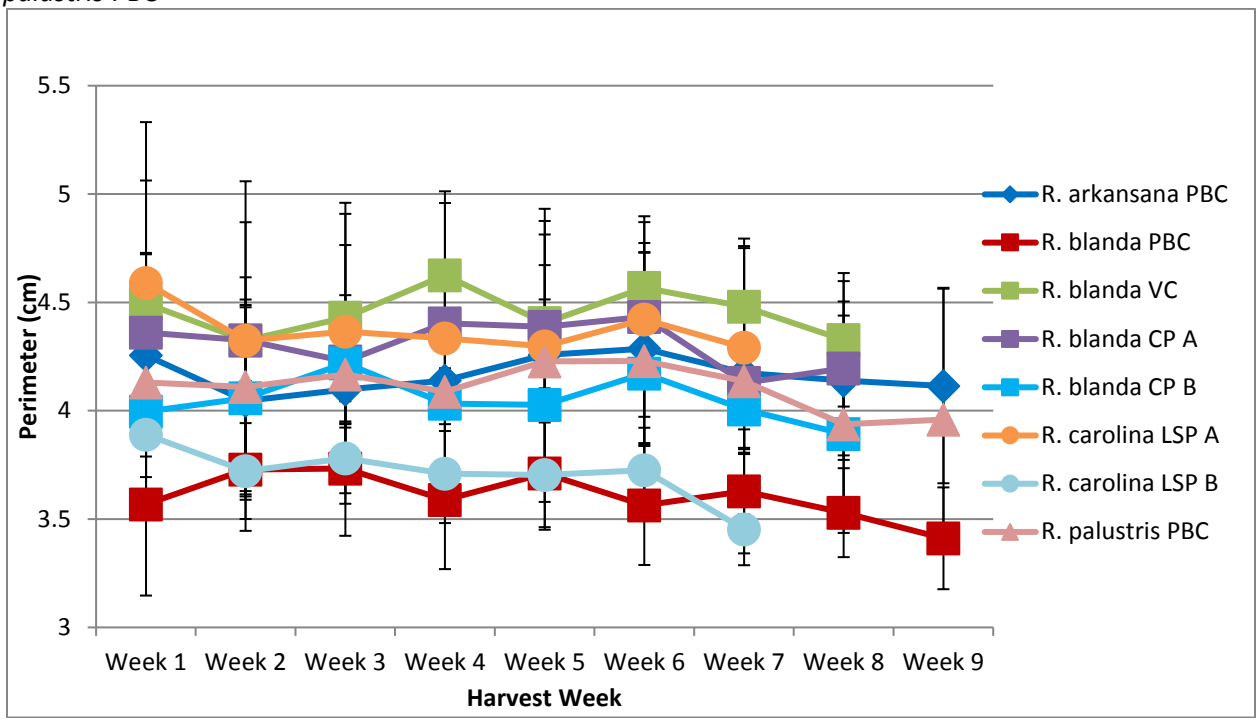


Figure 2.11: Comparison of Weekly Mean Perimeters of *Rosa spp.* for 2015 data

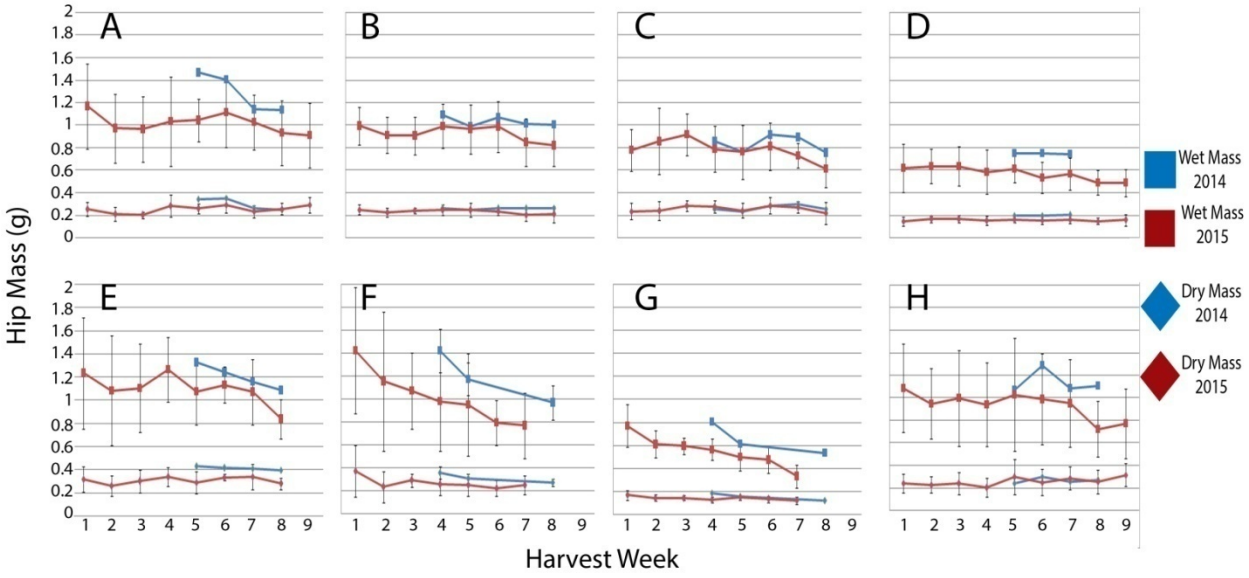


Figure 2.12: Fruit Mass (Wet and Dry, deseeded) in grams over harvest time, 2014-2015. 2014 data is bulk average per week. **A**: *R. arkansana* PBC, **B**: *R. blanda* CP A, **C**: *R. blanda* CP B, **D**: *R. blanda* PBC, **E**: *R. blanda* VC, **F**: *R. carolina* LSP A, **G**: *R. carolina* LSP B, **H**: *R. palustris* PBC

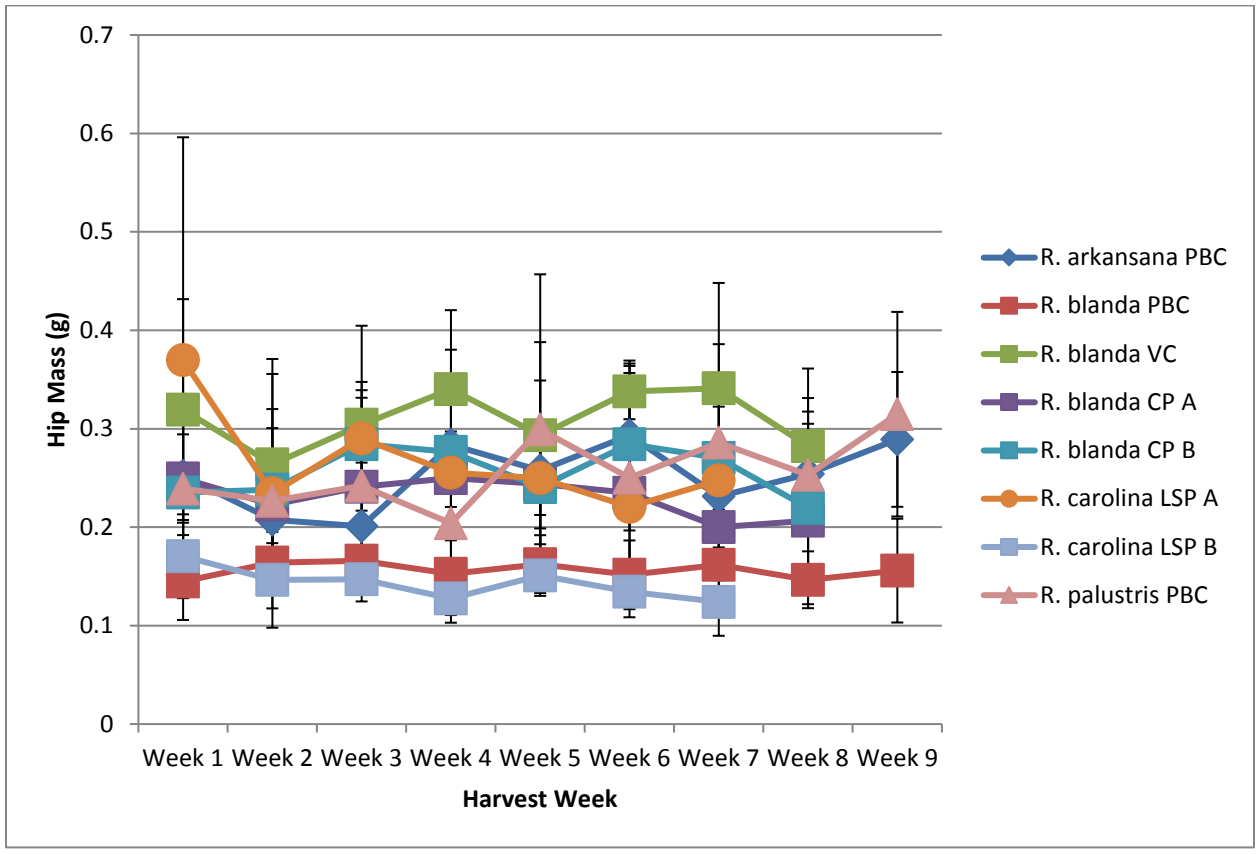


Figure 2.13: Weekly Mean Hip Dry Mass for *Rosa* spp. over the 2015 Harvest Season

Almost all species were statistically similar with regards to the percentage of dry matter. This was typically within the range of 20-35% across the whole season. Percentage dry matter was calculated by dividing total dry mass after deseeding the hips by the total wet mass (including seeds). *R. blanda* CP A and *R. arkansana* PBC had the least percentage dry matter across the entire harvest period (averaging 24.43% and 24.06%, respectively). *R. arkansana* VC had the highest percentage dry matter (36.09%), followed by *R. blanda* CP B (30.72%).

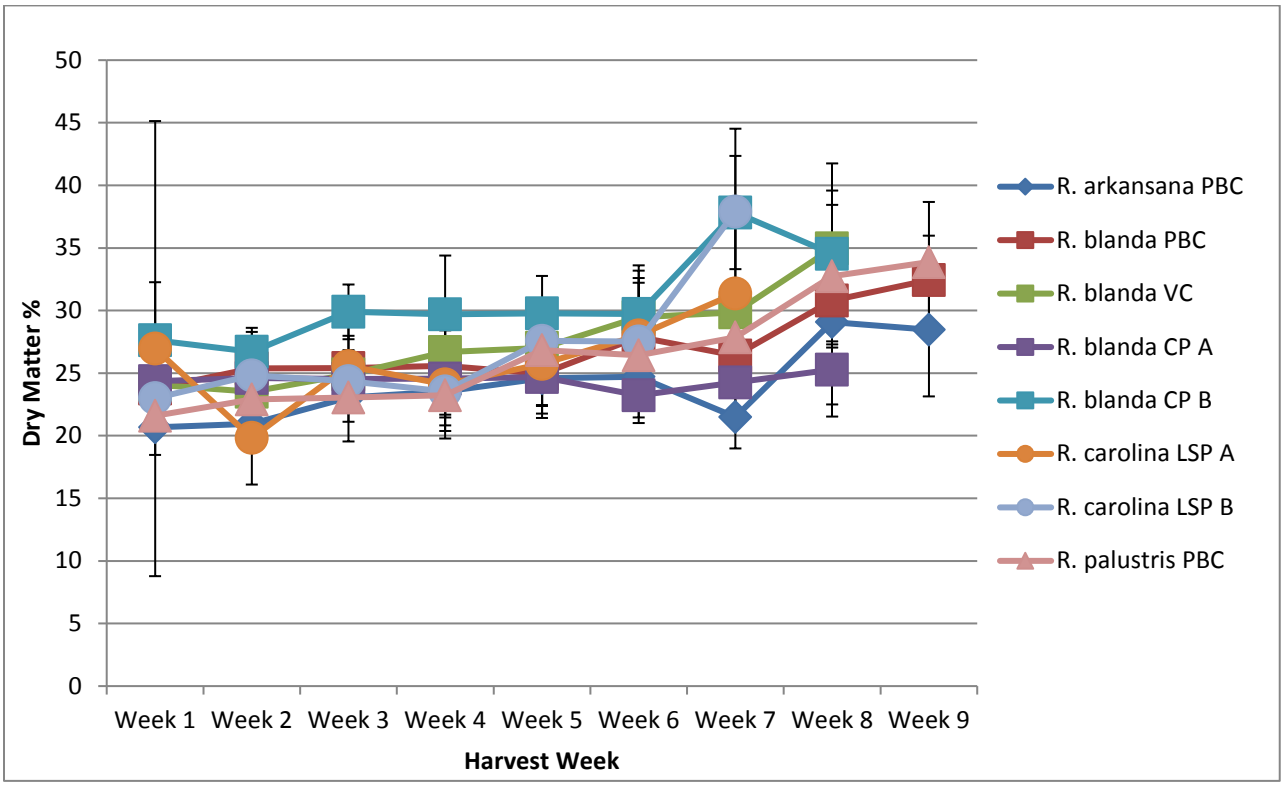


Figure 2.14: Weekly Mean Dry Matter Percentage for *Rosa* spp. over the 2015 harvest season

Unfortunately, *R. acicularis* and *R. arkansana* VC did not flower in 2015 due, respectively, to a controlled burn killing the flowering canes and to disease. In 2014, the previously mentioned issue with sample collection meant that these two species were studied only over a four and three week harvest period, respectively (Figure 2.15). The data for these two species was not included in Figures 2.10 and 2.12 due to concerns about comparability with the longer harvest periods of 2015. Their rankings compared to other samples within Table 2.5are only representative of this limited dataset from the previous year (see section 2.5.2 for an in-depth discussion). Overall, *R. acicularis* seemed to be similar to most other species studied, whereas *R. arkansana* VC often had comparatively larger hips (Table 2.5).

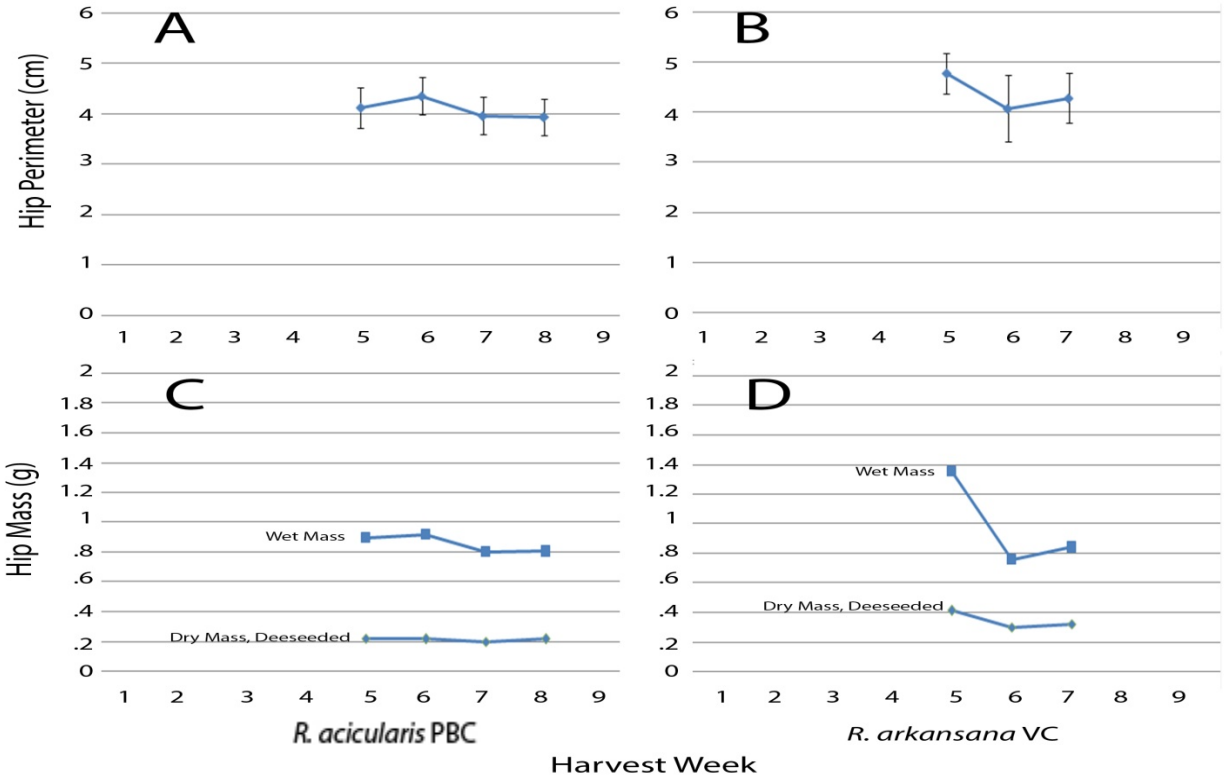


Figure 2.15: Weekly average perimeter and mass for rose hips of *R. acicularis* PBC and *R. arkansana* VC in 2014. **A**: *R. acicularis PBC* perimeter, **B**: *R. arkansana* VC perimeter, **C:** *R. acicularis* PBC wet and dry mass, **D**; *R. arkansana* VC wet and dry mass.

Trait	**1 (smaller)**	**2**	**3**	**4**	**5**	**6 (larger)**
Perimeter	*R. blanda* PBC^{d} *R. carolina* LSP B^{d}	*R.* palustris PBC^{c} *R. blanda* CP B^{c}	*R. arkansana* PBC$^{b, c}$ *R.* acicularis PBC$^{b, c}$	*R. arkansana* VC$^{a, b, c}$	*R. blanda* CPA^{a,b}	*R. carolina* LSP A^{a} *R. blanda* VC^{a}
Wet Mass	*R. carolina* LSP B^{c} *R. blanda* PBC^{c}	*R. blanda* CP B^{b}	*R. blanda* CP A$^{a, b,}$ *R. palustris* PBC^{a,b} *R. arkansana* VC$^{a, b}$ *R.* acicularis PBC ^{a,b}	*R. carolina* LSP A^{a} *R. arkansana* PBC^{a} *R. blanda* VC^{a}		
Dry Mass	*R. blanda* PBC^{d} *R. carolina* LSP B^{d}	*R. blanda* CP A^{c} *R. blanda* CP B^{c} *R.* palustris PBC^{c} *R. arkansana* PBC^{c} *R. acicularis* PBC^{c}	*R. carolina* LSP A$^{b, c}$	*R. blanda* VC$^{a, b}$	*R. arkansana* VC^{a}	
Total % Dry Matter	*R. blanda* CP A^{c} *R. arkansana* PBC^{c}	*R. palustris* PBC^{b,c} *R. carolina* LSP A^{b,c} *R. carolina* LSP B^{b,c} *R. blanda* VC^{b,c} *R. blanda* PBC^{b,c} *R.* acicularis PBC^{b,c}	*R. blanda* CP B^{a,b}	*R. arkansana* VC^{a}		

Table 2.5: *Rosa* spp. ranked from smallest to largest for 4 physical traits based on Tukey HSD post-hoc analysis. *R. acicularis* and *R. arkansana* VC data from 2014 averages. All others are from 2015. As column number increases, relative size increases, until all species are accounted for. Superscript letters represent different levels of significant difference from other species.

2.4.3 Color Analysis Data

Most species exhibited an increase in the average redness of the fruit over the harvest period, followed by a leveling off around approximately Weeks 4-5 of harvest. Curiously, *R. carolina* showed a decrease in redness towards the end of the season at both locations (Figure2.16) This may be due to the fact that the hips of that species tended to turn black at the end of the season. This can be seen in Figure 2.17, which depicts 50 hips from reddest to least red, and Figure 2.5, which shows the developmental stages of the rose flowers. The least-red fruit is a blackened *R. carolina* hip, so it is possible that black, or perhaps darker colors in general, do not reflect "redness" based on Tomato Analyzer's algorithms. Average greenness was also assessed. Andersson (2009) measured levels of chlorophylls over a long harvest period in *R. canina* and observed a decline in chlorophyll levels over time. It had been expected that greenness, as a proxy for green pigments such as chlorophyll, would also decline over the harvest period. However, we observed that greenness tended to either remain

Figure 2.16: Average Redness of *Rosa* spp. over the 2015 harvest period

relatively steady across harvest weeks or even increase before leveling off, in contrast to these predictions (Figure 2.18). There did not seem to be a consistent relationship between average redness and average greenness of the fruit (Figure 2.19). Figure 2.20 shows the range of hip greenness from highest greenness to lowest.

Figure 2.17: A spectrum of hip redness based on Tomato Analyzer calculations from most red to least

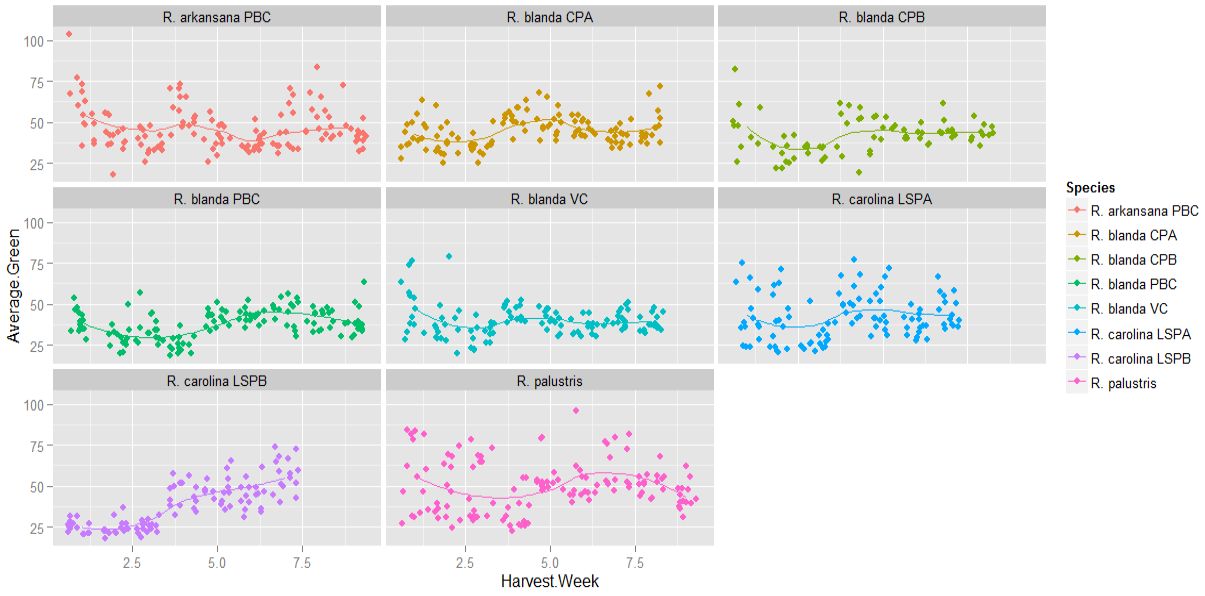


Figure 2.18: Average Greenness of *Rosa* spp. over the harvest period

Figure 2.19: Relationship of Average Red to Average Green across species and harvest week

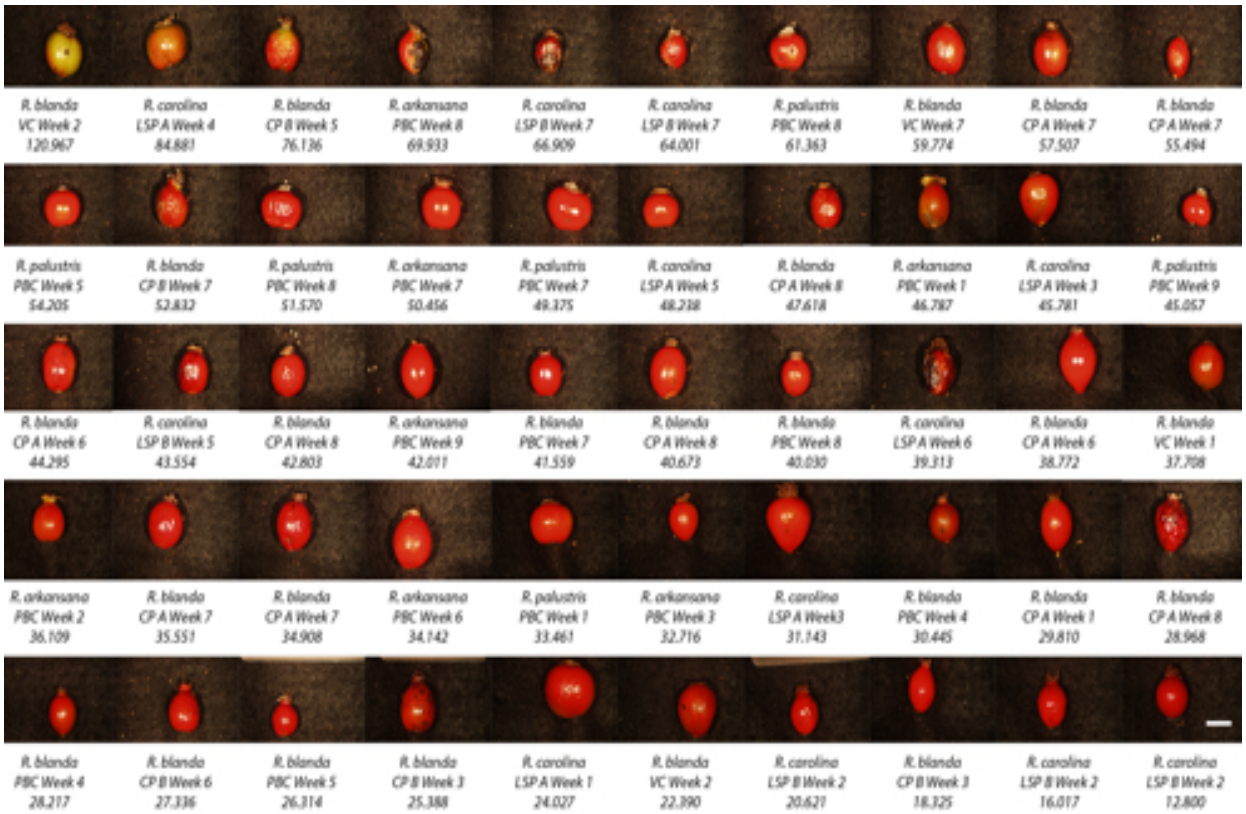


Figure 2.20: A spectrum of hip greenness based on Tomato Analyzer calculations from most green to least

2.4.4 Phytochemical Data

Total Soluble Solids

Across all species and harvest weeks, total soluble solids ranged from 11.4% to 30.4%. For comparison, the ideal concentration of soluble solids for winemaking is 22% (Bisson, 2001). Of the samples studied, only 3 were found to have significant differences in total soluble solid concentrations across the harvest period: *R. blanda* CP A, *R. blanda* VC, and *R. carolina* LSP A. Figure 2.21 shows the total soluble solid concentration over the harvest period for all 2015 samples and Table 2.6 displays the maximum and minimum observed total soluble solid concentration in comparison to existing data. Of the studied species, *R. blanda* CP A and *R. blanda* CP B had the highest average total soluble solids for the harvest period (22.28% and 22.75%, respectively) and *R. carolina* LSP B had the lowest concentration (13.23%). In general, it appears that the North American species are similar to Eurasian species, though they tend to be on the low end of reported ranges in the existing literature. Ercisli (2007) in particular reports hips with much higher total soluble solid levels (29.42-37.33%). The North American species

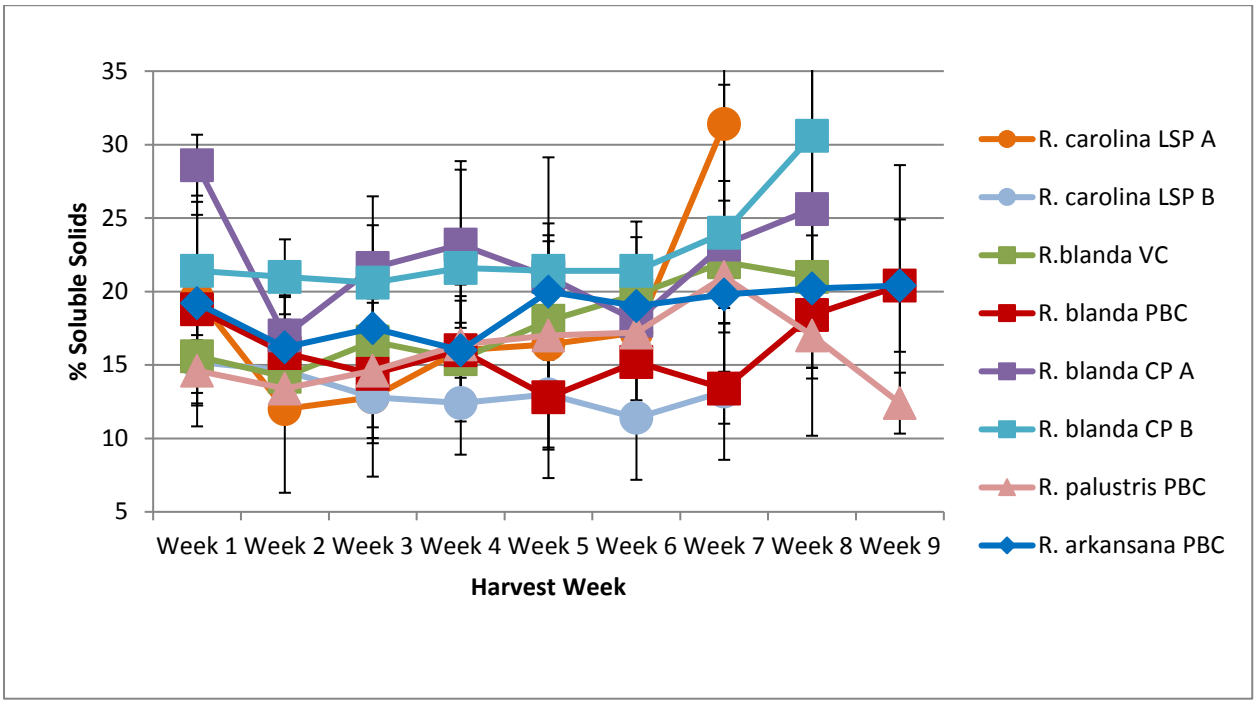


Figure 2.21: Total Soluble Solids over the harvest period for North American *Rosa* spp.

Species	Year or Author	Minimum Total Soluble Solids (Percent)	Maximum Total Soluble Solids (Percent)
R. arkansana PBC	2015	16.00 ± 4.47	20.40 ± 4.51
R. blanda CP A	2015	17.00 ± 2.74	28.60 ± 2.07
R. blanda CP B	2015	20.60 ± 3.91	30.60 ± 8.68
R. blanda PBC	2015	12.80 ± 3.56	20.40 ± 8.20
R. blanda VC	2015	14.20 ± 2.17	22.00 ± 4.18
R. carolina LSP A	2015	12.00 ± 5.70	31.40 ± 11.35
R. carolina LSP B	2015	11.40 ± 4.22	15.20 ± 1.48
R. palustris PBC	2015	13.40 ± 1.34	21.00 ± 10.00
R. canina	Ercisli (2007)	--	32.26
R. dumalis ssp. *boissieri*	Ercisli (2007)	--	37.33
R. dumalis ssp. *antalyensis*	Ercisli (2007)	--	34.01
R. villosa	Ercisli (2007)	--	29.42
R. pisifomis	Ercisli (2007)	--	31.89
R. pulverulenta	Ercisli (2007)	--	35.44
R. dumalis	Günes (2013)	20.7 ± 2.15	24.6 ± 3.12
R. dumalis	Günes (2013)	18.8 ± 1.11	20.4 ± 2.00
R. canina	Günes (2013)	24.6 ± 2.01	27.4 ± 1.73
R. canina	Günes (2013)	21.0 ± 1.14	27.3 ± 1.30
R. dumalis	Günes (2013)	18.6 ± 2.85	23.0 ± 1.73
R. jundzillii	Günes (2013)	13.2 ± 4.40	22.7 ± 2.24
R. villosa	Günes (2013)	25.3 ± 2.34	25.3 ± 1.92
R. jundzillii	Günes (2013)	16.2 ± 1.22	23.7 ± .33
R. jundzillii	Günes (2013)	20.1 ± .05	22.9 ± .95
R. hirtissima	Günes (2013)	14.4 ± 2.08	16.2 ± 1.88
R. dumalis	Günes (2013)	22.2 ± 2.19	24.7 ± 2.63
R. dumalis	Uggla (2004)	15.2	24.4
R. rubiginosa	Uggla (2004)	13.1	25.7
R. spinosissima	Uggla (2004)	10.2	16.3

Table 2.6**:** Comparison of the range of Total Soluble Solid concentrations of North American and Eurasian species

were more variable than those in the existing literature. Table 2.8 ranks our studied species from higher to lower concentrations of soluble solids based on Tukey HSD analysis.

Total Phenolics

Overall, total phenolic concentrations appeared to be much higher in North American species than in Eurasian species that were previously studied with similar methodologies (Table 2.7). The highest concentrations reported were found by Yilmaz and Ercisli (2011), in a specimen of *R. canina* that

Species	Year or Author	Min. Mean Total Phenolics (mg GAE/g DW)	Max Mean Total Phenolics (mg GAE/g DW)
R. acicularis	2014	151.83 ± 8.60	157.77 ± 1.22
R. arkansana VC	2014	143.43 ± 4.45	156.75 ± 7.91
R. arkansana PBC	2014	142.42 ± 2.60	152.48 ± 2.94
R. blanda CP A	2014	158.81 ± 5.389	168.95 ± 3.09
R. blanda CP B	2014	181.33 ± 3.93**	185.87 ± 0 **
R. blanda PBC	2014	160.69 ± 3.77	165.36 ± 11.87**
R. blanda VC	2014	127.84 ± 8.50	136.91 ± 4.47
R. carolina LSP A	2014	144.65 ± 9.03	149.29 ± 7.50
R. carolina LSP B	2014	109.25 ± 11.79	126.83 ± 9.02
R. palustris	2014	121.71 ± 7.00	134.97 ± 7.79
R. arkansana PBC	2015	114.36 ± 2.90	141.75 ± 2.64
R. blanda CP A	2015	135.83 ± 6.52	163.15 ± 1.62
R. blanda CP B	2015	136.92 ± 6.96	183.60 ± 3.93**
R. blanda PBC	2015	143.44 ± 14.37	175.13 ± 13.15**
R. blanda VC	2015	110.28 ± 5.45	131.191 ± 5.48
R. carolina LSP A	2015	108.424 ± .48	128.75 ± 11.71
R. carolina LSP B	2015	107.08 ± 2.34	116.44 ± .63
R. palustris	2015	106.67 ± 3.11	134.42 ± 10.88
R. canina	Demir et al. (2014)	--	31.08 ± .19
R. dumalis	Demir et al. (2014)	--	36.86 ± 3.88
R. gallica	Demir et al. (2014)	--	31.51 ± .22
R. dumalis ssp. *boissieri*	Demir et al. (2014)	--	52.94 ± .47
R. hirtissima	Demir et al. (2014)	--	35.73 ± 2.36
R. canina	Barros et al. (2010)	--	143.17 ± 5.25*
R. canina	Ercisli (2007)	--	96
R. dumalis ssp. *boissieri*	Ercisli (2007)	--	84
R. dumalis ssp. *Antalyensis*	Ercisli (2007)	--	85
R. villosa	Ercisli (2007)	--	73
R. pisifomis	Ercisli (2007)	--	79
R. pulverulenta	Ercisli (2007)	--	94
R. pisiformis	Yilmaz & Ercisli (2011)	--	83
R. canina	Yilmaz & Ercisli (2011)	--	102
R. villosa	Yilmaz & Ercisli (2011)	--	78
R. dumalis ssp. *Antalyensis*	Yilmaz & Ercisli (2011)	--	91

Table 2.7: Observed minima and maxima of Total Phenolics analyses in this study and existing literature. *: Denotes extraction protocol using methanol as opposed to water. **: Denotes a sample period where at least one instance of running the analysis caused an "overflow" error, and an arbitrary absorbance higher than the machine's maximum (4.15) was assigned. Actual values may therefore be higher or slightly lower. See Section 2.1.3 for more details.

showed concentrations of 102 mg Gallic Acid Equivalents / g dry weight. In addition, a study by Barros et al. (2010) showed higher levels (143.17 mg GAE/g DW), but they used a methanol extraction method that is much more efficient than the aqueous extraction used in this and other studies (Maeda; 2016 personal communication). Comparatively, the lowest levels observed in this study in a given week were 106.67 mg GAE/g DW (*R. palustris)* and the highest were 185.87 mg GAE/g DW (*R. blanda* CP B).

As they were analyzed similarly, data on total phenolics from 2015 and 2014 were considered comparable. In all species, total phenolic levels were significantly higher in 2014 (Figure 36). Figure 2.22 shows a comparison of all species in 2015 across the harvest period. In 2015, all species, aside from *R. carolina* LSP B, showed some significant differences at p =.05over the harvest period (*R. arkansana* PBC: $p=4.9x10^{-5}$; *R. blanda* CP A: p=.028; *R. blanda* CP B: $p=1.75x10^{-7}$; *R. blanda* PBC: p.001; *R. blanda* VC: $p=3.2\ x10^{-5}$; *R. carolina* LSP A: p=.015; and *R. palustris* PBC: p=.032) Generally, levels of total phenolics decline until ~ Weeks 4-5, whereupon they stabilize (Figure 2.22). Andersson (2009) observed specific phenolic compounds over time and also saw a decline followed by stabilization in concentrations of particular compounds, so this is consistent with existing research. Table 2.8 ranks species from lowest to highest concentration of total phenolics. As can be seen in Table 2.8 and Figures2.22-2.23, *R. blanda* samples (except location VC) have the highest levels of total phenolics. *R. blanda* CP B has the highest concentration overall, with an average of 166.76 mg GAE/g DW across the entire harvest period. These data were technical replicates, so variability was low, as can be seen in Figure 2.23.

However, while concentrations might be higher in some samples, overall fruit mass should be considered when determining which species and location produces the greatest amount of phenolic compounds. Weekly mean fruit dry mass and mean total phenolics were multiplied to give a weekly estimated value for total phenolics per hip for each sample (Figure 2.24). The samples were then ranked from lowest to highest concentration of phenolics per hip (Table 2.7). In the 2015 data, we can see that though *R. blanda* VC was not among the highest concentrations of total phenolics (121.85 mg GAE/ g

DW), its larger mass meant that it was comparable to the sample with the overall highest concentrations of phenolics in that year, *R. blanda* CP B (37.78 mg GAE/hip for *R. blanda* VC compared to 42.83 mg GAE/hip for *R. blanda* CP B). Conversely, we see that the tiny size of the fruit of *R. blanda* PBC gives that sample the second lowest concentration per hip (24.92 mg GAE/hip) despite having the some of the highest concentrations of phenolics.

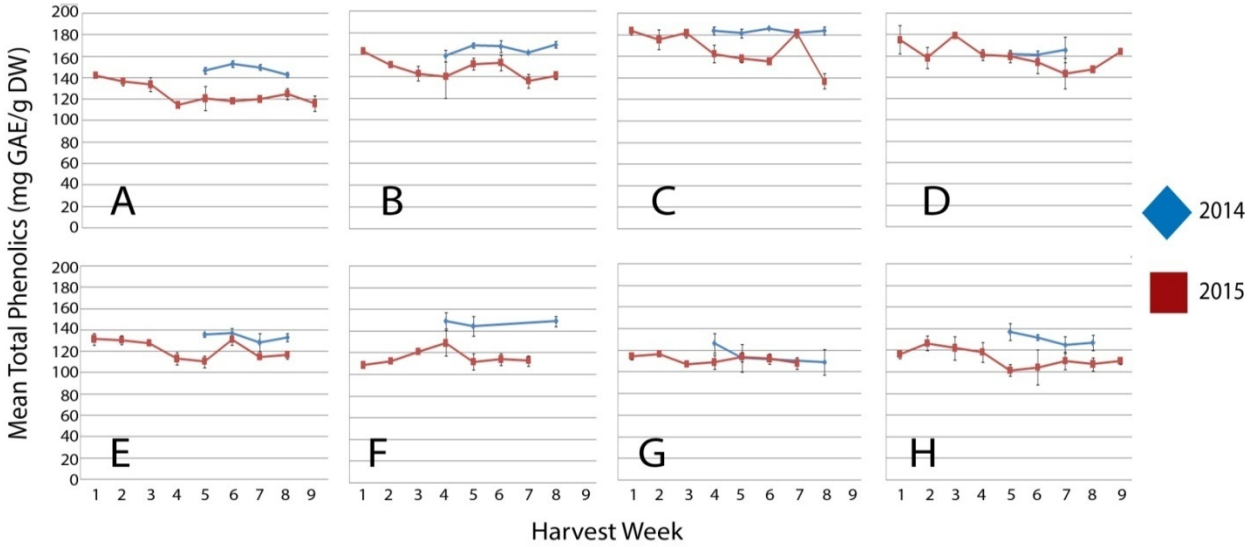


Figure 2.22: Comparison of Total Phenolics for 2014 and 2015 for all samples taken during both years. **A**: *R. arkansana* PBC, **B**: *R. blanda* CP A, **C**: *R. blanda* CP B, **D**: *R. blanda* PBC, **E**: *R. blanda* VC, **F**: *R. carolina* LSP A, **G**: *R. carolina* LSP B, **H**: *R. palustris* PBC

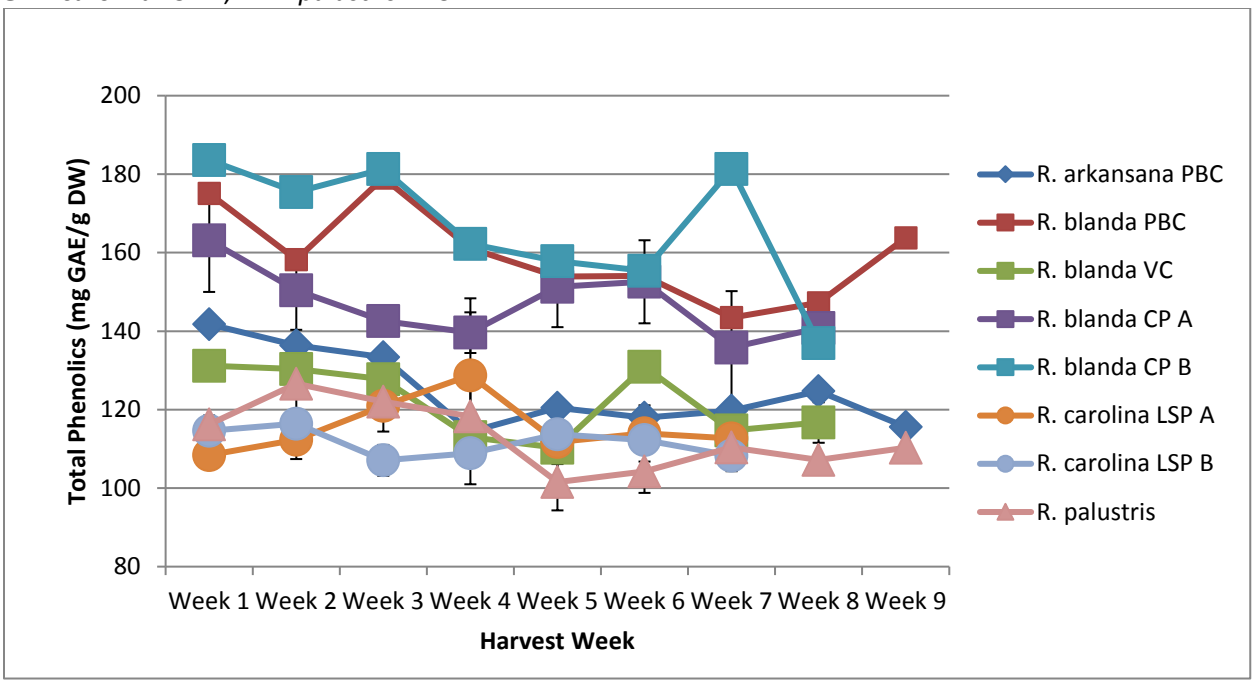


Figure 2.23: Comparison of total phenolics concentration over the harvest period for all 2015 samples

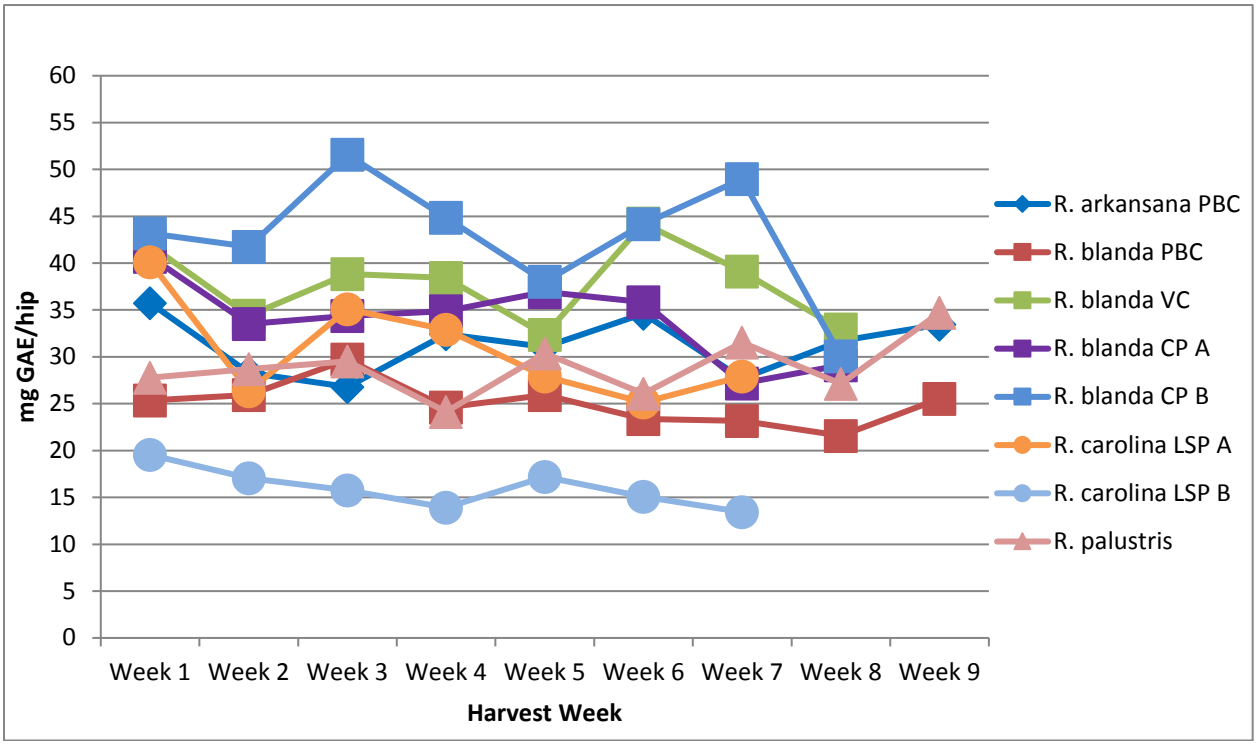


Figure 2.24: Comparison of total phenolics per Hip for *Rosa* spp. in 2015

Using the data on the concentration of phenolics per hip and the average number of flowers per cane, a rough estimate of which samples would produce the most possible phenolic compounds per cane was obtained (Figure 2.25). This allows us to assess which species might be most valuable to plant for maximizing the production of bioactive compounds. *R. palustris* PBC had the greatest potential, with 2313.93 mg GAE per cane, followed by *R. blanda* CP A (1549.87 mg GAE/cane) and *R. blanda* VC (1279.12 mg GAE/cane). *R. arkansana* PBC had the lowest potential (109.561 mg GAE/cane). Despite their lower concentrations of phenolics relative to other samples (Table 2.8), *R. palustris* PBC and *R. blanda* VC had the highest potential thanks to large hips and a high number of flowers per cane. Conversely, *R. arkansana* PBC and *R. blanda* CP B, despite having high concentrations of phenolics per hip, did not have high concentrations of phenolics per cane due to lower numbers of flowers.

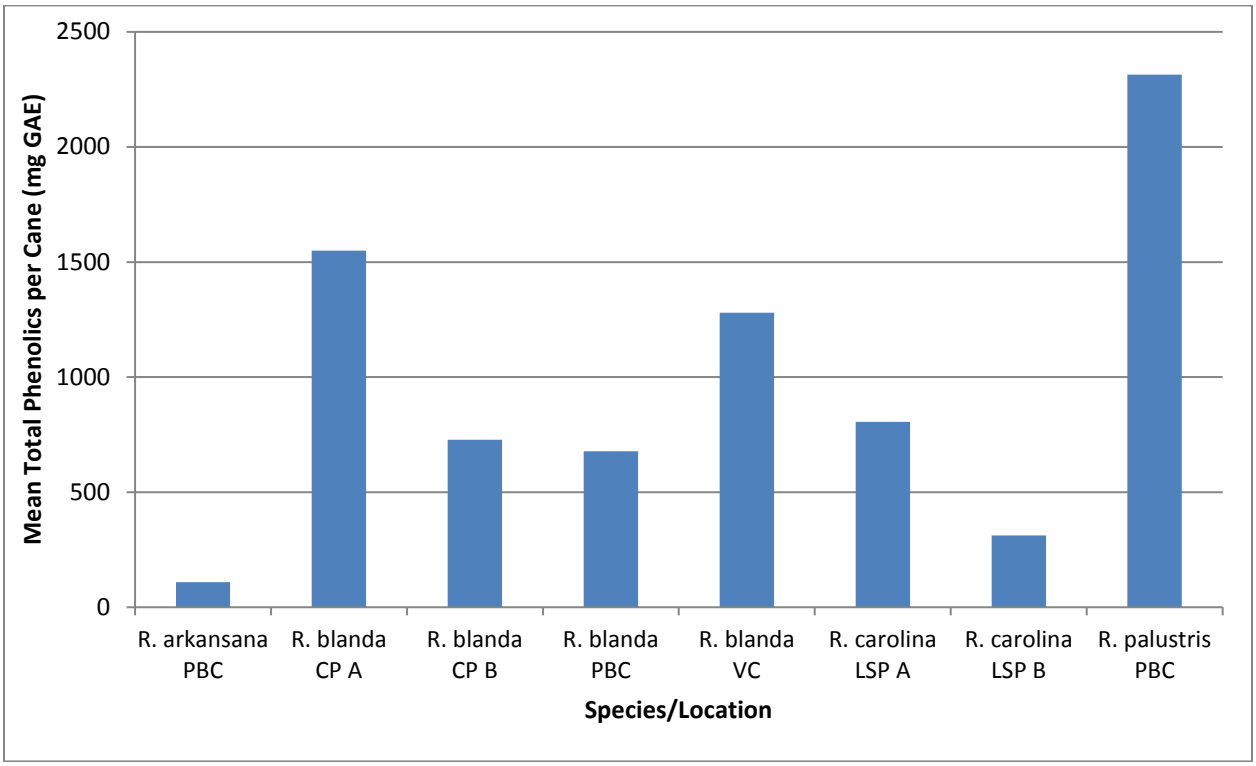


Figure 2.25: A comparison of estimated mean total phenolics per cane for *Rosa* spp. in the 2015 harvest season based on average concentrations of phenolics per hip and average flowers per cane

Due to the issues with harvest mentioned above, *R. acicularis* and *R. arkansana* VC were measured in 2014 only. Additionally, as was noted previously, most samples had significantly higher levels of total phenolics in 2014.This fact must be taken into account when considering their relative concentration of total phenolics when compared to the other samples (Table 9). Compared to the 2015 data, these two species had some of the highest concentrations of total phenolics on both per gram of dry weight and per hip measures (Table 9). In fact, the large size of the *R. arkansana* VC hips makes it the highest concentration per hip of any of the other samples. In 2014, *R. arkansana* VC contained51.72 mg GAE per hip on average, compared to the highest average from 2015, *R. blanda* CP B's 42.83 mg GAE/hip. *R. acicularis* was average in concentration of phenolics per hip, despite a high concentration of phenolics per gram of dry weight.

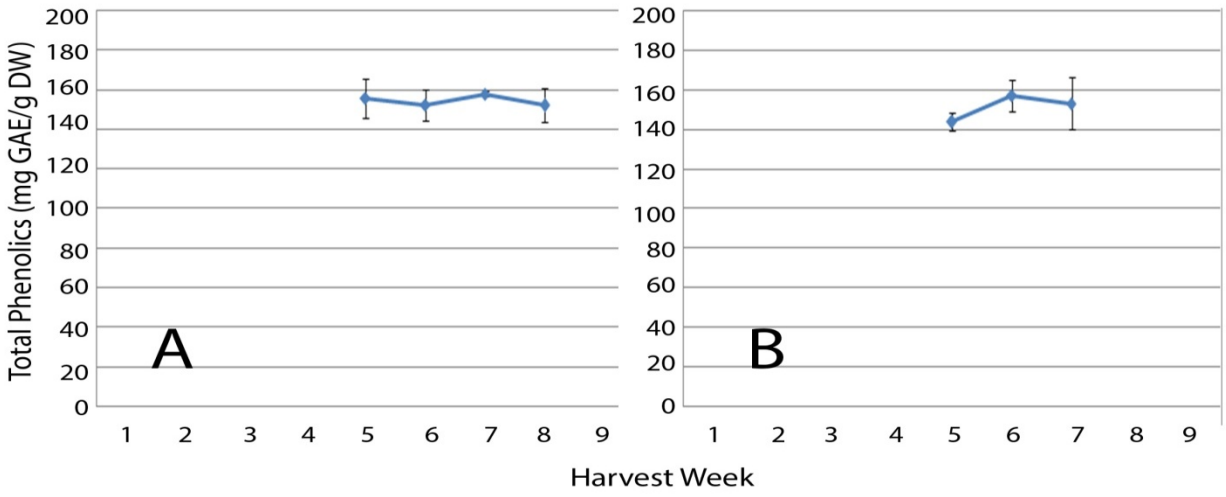


Figure 2.26: Total phenolics of *R. acicularis* (A) and *R. arkansana* VC (B). Values are for the 2014 harvest season only.

Total Phenolics: Analysis of Tissue Post-Soluble Solids Extraction

In an effort to verify phenolic concentrations, we used an alternative method to correct for interference in the total phenolics analysis. It is known that Vitamin C (ascorbic acid), a substance found in abundance in rose hips, can interfere with the Folin-Ciocalteau reagent's capacity to accurately determine total phenolics by reacting with the reagent in the same way phenolic compounds do, potentially inflating observations (Prior et al., 2005). Ascorbic acid is known to be highly water soluble. Therefore, by analyzing hip tissue after extracting soluble solids in an aqueous solution, it may be possible to get a more conservative estimate of actual phenolic compounds present (Figure 2.27, Table 2.8). After soluble solids were extracted from macerated tissue, this tissue was collected from the filter, freeze-dried and analyzed in triplicate using the same procedure as the unadulterated hips. In general, rankings of species in terms of concentration of phenolics remained the same. Compared to the , base total phenolics *R. blanda* CP B and PBC still had the highest overall concentration, averaged across the harvest period (99.68 and 96.34 mg GAE/g DW, respectively). In this case however, *R. carolina* LSP B was also among the highest concentrations (89.76 mg GAE/g DW) and *R. palustris* PBC had the lowest concentration (76.47 mg GAE / g DW) It should be noted that different phenolic compounds have

different levels of solubility in aqueous solutions (Mota et al.; 2008), so the shifts in relative ranks compared to the base total phenolic concentration may be caused by either different ascorbic acid concentrations or different profiles of phytochemicals. It is also worth noting that even after the extraction of a confounding factor and possibly some phenolic compounds, the concentration of total phenolics in the samples studied here are comparable to or higher than the concentrations reported in Eurasian species in the existing literature despite the Eurasian species' analyses not controlling for the confounding impact of ascorbic acid. Though there are significant differences between the weeks, there does not appear to be a clear pattern of total phenolics across the harvest period in this case.

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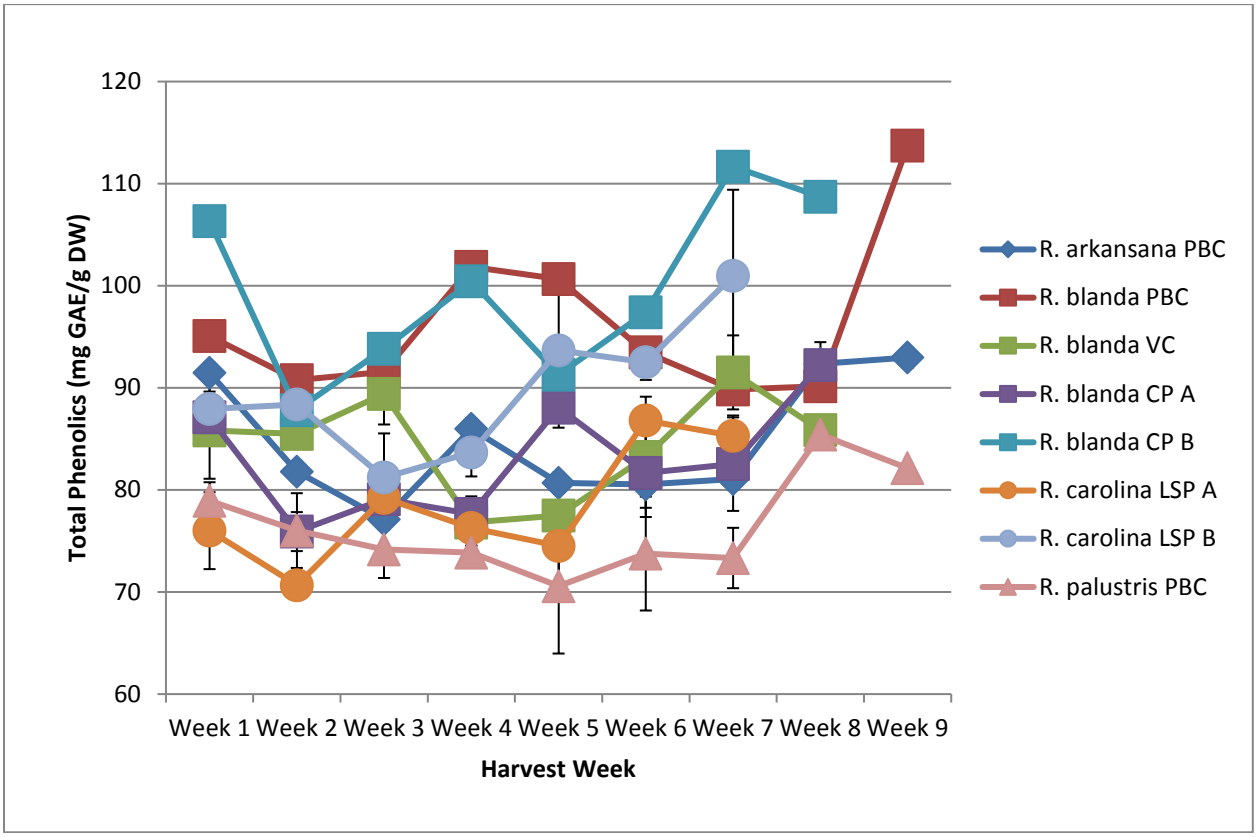

Figure 2.27: Post Soluble-Solid Extraction Total Phenolics for *Rosa* spp.

Trait	1 (lower)	2	3	4	5	6	7 (higher)
Total Soluble Solids (%)	*R. carolina* LSP B[c]	*R. blanda* PBC[b,c] *R.* palustris PBC[b,c]	*R. blanda* VC[a,b,c] *R. carolina* LSP A[a,b,c]	*R. arkansana* PBC[a,b]	*R. blanda* CP A[a] *R. blanda* CP B[a]		
Mean Total Phenolics (mg GAE/g DW)	*R. palustris* PBC[c] *R. carolina* LSP A[c] *R. arkansana* PBC[c] *R. carolina* LSP B[c] *R. blanda* VC[c]	*R. blanda* CP A[b]	*R. blanda* PBC[a,b] *R. arkansana* VC [a,b] *R.* acicularis PBC[a,b]	*R. blanda* CP B[a]			
Total Phenolics per Hip	*R. carolina* LSP B[f]	*R. blanda* PBC[e]	*R. palustris* PBC[d,e] *R. carolina* LSP A[d,e] *R. arkansana* PBC[d,e] *R. acicularis* PBC[d,e]	*R. blanda* CP A[c,d]	*R. blanda* VC[b,c]	*R. blanda* CP B[b]	*R. arkansana* VC[a]
Mean Total Phenolics: Post-Soluble Solid Extraction	*R. palustris* PBCi[c]	*R. blanda* VC[b,c] *R. carolina* LSP A[b,c] *R. arkansana* PBC[b,c] *R. blanda* CP A[b,c]	*R. carolina* LSP B[a,b]	*R. blanda* CP B[a] *R. blanda* PBC[a]			

Table 2.8: Relative ranking of phytochemical concentrations of samples of *Rosa* spp. from low to high based on Tukey HSD analysis. *R. acicularis* and *R. arkansana* VC placements based on 2014 data. All other data from 2015. As column number increases, relative concentration increases, until all species are accounted for. Superscript letters represent different levels of significant difference from other species.

2.5Discussion

2.5.1 Use by Growers

Regarding landscape use, it is clear that those gardeners wishing to use native roses in their landscapes would benefit from using an array of species, given their different flowering times, growth form, and hip morphology. Growers may wish to avoid *R. carolina* due to the hips tending to become discolored at the end of the season. Poor color detracts from use as an ornamental for winter interest, as they are lacking the bright red hips that would contrast with duller surroundings. The diversity of these species in terms of phenology and morphology also allow for a wide array of potential uses. For example, the densely flowered shrub *R. palustris* may be best for specimen or border plantings, whereas the more colonial *R. blanda* would be ideal for a wide hillside. Varieties which flower less densely may be appropriate for creating splashes of background color without overwhelming other plants in a

garden. *R. arkansana* VC was observed to have rapidly colonized disturbed ground after the removal of invasive shrubs. This rapid growth, coupled with *R. arkansana*'s short statue suggests potential as a groundcover. The cultivation and breeding of these species for landscape use could be a valuable proposition given how popular roses are as shrub plants and the growing interest in using natives in landscape designs.

All of these species may be useful for hip production on the home scale; however different individuals may prefer different species based on their growth habits. For some growers, *R. arkansana*'*s* dwarf stature may make harvesting too inconvenient. While *R. blanda*'s spineless flowering stems may make harvesting less potentially painful, its colonizing growth habit would require a large area, unless shrub forms like *R. blanda* VC could be developed. Alternatively *R. palustris* has the greatest density of flowers and fruits, but its' extremely prickly stems may make harvesting for the individual, or families with small children, undesirable. It will be up to the gardener to determine their needs, but it is evident that native roses can fulfill a number of roles.

Larger-scale growing operations will require different considerations than the home-scale gardener or herbalist. For example, they will most likely want the highest amount of bioactive chemicals in the least amount of production space possible. *R. blanda* generally had the highest concentration of total phenolics, but given its colonial nature, this may not be ideal for eventual mechanization of harvest. It could potentially be trellised like other cane fruit in Rosaceae (i.e. raspberries, blackberries, etc.), but its rather loose arrangement of canes (roughly 1-3 canes every 6-8 inches based on visual estimation) may make such plantings not dense enough to justify production. With that in mind, *R. palustris* would likely make the best possible option for large-scale production. Its dense, shrubby growth form with large numbers of flowers makes up for relatively low concentrations of phenolics per fruit by sheer volume of fruit produced. However, since *R. blanda* CP A also had a relatively large number of flowers per cane as well as higher concentrations of phenolics, a long-term breeding program

could focus on hybridizing high-phenolic genotypes of *R. blanda* with shrubbier, more heavily flowered species. This is known to occur between *R. blanda* and *R. palustris*. Additionally, *R. blanda* VC was much shrubbier, had larger fruit than other *R. blanda* specimens, and had a greater density of fruit per cane as well as high levels of phenolics. If the supposition presented earlier that that specimen is a hybrid with *R. rugosa* is true, perhaps hybrids would make mechanization easier and boost overall yields of bioactive compounds. Since *R. blanda* VC and *R. carolina* (which has a hybrid origin between *R. blanda* and *R. palustris* as previously reported here) have generally lower concentrations of phenolics despite having some of the largest fruit overall, selection of high-phenolic genotypes of both parental species would be important.

Also important for both industry and home-scale landscape use is pest and disease presence. Black spot seemed to be relatively common amongst all samples in both years, but it never seemed to overwhelm any individual plant. This may be due to low disease pressure or resistance. More study is necessary. However, in 2015, rose rust was a large problem in the UW Arboretum, striking all samples of *R. blanda* and *R. arkansana* there, particularly *R. arkansana* VC. These samples, along with the plantings at Eagle Heights, were also struck by an unknown fungus that withered the hips into white husks (See Figure 24). Samples at other sites were only minimally affected by these diseases despite the wet weather. This may be due to inherent resistance, or site characteristics such as the fact that the Arboretum is relatively sheltered by forest, whereas much of Pheasant Branch Creek Conservancy and the particular area of the Lakeshore Preserve where the roses were located were upland areas mostly clear of trees. More study of the impacts of genetic variability and site selection on disease resistance in native roses is needed.

Additionally, *R. palustris* also seemed to attract a larger amount of insect life than other species, at least when it was observed. Many pollinators were seen flying around the plants, and the species of weevil mentioned in Figure 2.9 appeared to use the plant as a location for mating. Discovery of small

grubs inside the hips when bisecting them for study suggests that those weevils may have contributed significantly to the observed insect damage on that species.

2.5.2 Physical Traits of Rose Hips

Overall, there was almost no significant change in perimeter or dry mass from week to week or from year to year. This suggests that the hips of the studied species had reached full size and physical development by the time harvest had begun at the end of August and that environment had a minimal effect on the physical traits of rose hips. Therefore, where physical traits are concerned, the hips provide a consistent landscape feature and a long harvest season for foragers. In general, it appears that in these species', fruit size is consistent within a given sample. Since significant differences were observed between a single species at different locations, we can presume that there is some variability by location, and possibly by genotype. This is further supported by the fact that there is existing research documenting considerable variation by location and genotype in dog-roses.

Surprisingly, fruit size remained consistent in *R. palustris* PBC, where the most robust shrub, with the largest fruit, was removed before the 2015 harvest season. Given the differences observed in that species between the youngest shrubs and the mature shrub, (e.g. different floral color and number/size of hips) it is possible that the age of a given cane, or whole plant, has impacts on the characteristics of the hips and flowers. However, as only once location of *R. palustris* was studied containing multiple ages of canes, the lack of difference between harvest years may be impacted by genetic drift or environmental factors. Despite that fact, an assessment of how long a given species takes to reach peak production, their overall productive lifespan, and how the environment affects fruit production and characteristics would be valuable. It is known that management practices can impact overall yield in roses, so assessing which species respond best to which practices is important (Sanderson & Fillmore, 2012)

In contrast to dry mass and perimeter, the other physical traits did not follow a stable pattern across the harvest period. Wet mass generally tended to trend downwards as the season progressed, while dry matter percent trended upwards. As dry mass remained constant across the harvest period, this makes sense: fruits tend to dry out on their own as the season progresses. This could be valuable to growers looking to make medicinal products, most of which are based off of dried hip tissues. Waiting until the end of the harvest period, when hips are drier, would require less energy expended to dry the hips for creating products. Conversely, growers wishing to make edible products such as jellies or soups may wish to harvest earlier in the season, where wetter fruits may be easier to process in food processors.

Comparing the 2014-only data from *R. acicularis* and *R. arkansana* VC to the 2015 harvest data as was done here may be somewhat problematic. For the purposes of ranking the species from smallest to largest, the weekly averages were used as data points for an analysis of variance and subsequent post-hoc analysis by the Tukey HSD method. *R. acicularis* and *R. arkansana* VC were only harvested over3-4 weeks, compared to the 7-9 weeks for all the 2015 data. The smaller sample sizes may make the averages for these two species less representative. In particular, the first week of harvest for *R. arkansana* VC (Week 5 of 2014) had much, much larger fruit than the subsequent two weeks. This may have been an outlier, so there may have been some skew in terms of the physical traits analyzed (perimeter, mass, and dry matter percent). Though mass and perimeter did tend to remain stable over the season within other species, without a longer harvest period, comparable to the 2015 data, *R. acicularis*' and *R. arkansana* VC's purported relative size should be viewed with skepticism.

2.5.3 Chemical Characteristics

In terms of potential for medicinal purposes, North American rose species are clearly worth studying further. Overall, the concentrations of total phenolics in these specieswere2-6 times greater than the levels seen in Eurasian dog roses that are reported in the existing literature. Even the samples

that were analyzed after soluble solid extraction had removed confounding ascorbic acid and soluble phenolics were roughly equivalent to the concentrations found in dog roses. These high levels of bioactive compounds could be due to novel phytochemicals, which may have novel medicinal properties. Since the existing literature studying medicinal use of rose hips all report on the use of extracts from dog roses, the chemical composition and medicinal effects of North American species needs to be further assessed. Given the high levels of bioactive compounds present species in this study, all are worth further investigation. There is some evidence that *R. blanda* has the most potential for medicinal purposes, with the highest concentrations of phenolics (soluble and insoluble), high levels of total soluble solids, modestly large hips, relatively high floral density, and high percentage dry matter (making them cheaper to process).

However, due to the observed variability in *R. blanda* based on location and/or genotype, more research must be performed to ascertain the differences caused by the environment and by genetics. The variability of *R. blanda* is typified in this study by *R. blanda* VC, which was significantly different from the other *R. blanda* samples. It had a total phenolic concentration lower than the other three, as well as a different growth habit and larger hips. These differences may be due to its' potentially hybrid nature, or reflective of large impacts of genotype and/or environment on the production of phenolic compounds. Given that there were significant differences between years within a given sample for total phenolics, as opposed to physical traits, the differences may be due to environmental impacts on total phenolic concentrations. This could be due to different microclimates, disease pressure, site management (i.e. prairie burns), or epigenetic effects. When compared to the other *R. blanda* samples, the larger mass of *R. blanda* VC made up for the lesser concentration of phenolics in terms of total phenolics per hip, so fruit size must also be considered if these species are to be developed further. This further reinforces the fact that the intersection of physical traits and chemical profile is extremely important. Even if *R. blanda* CP A were to be found to have the most potent medicinal properties, the

productive capabilities of samples such as *R. blanda* VC and *R. palustris* PBC would outweigh that impact assuming the latter two species had at least somewhat comparable medicinal properties. More species than simply dog roses need to be studied to determine medicinal effectiveness of all *Rosa* species.

For determining when to harvest for the maximum concentration of phenolics, the data are unclear. Though most species' concentrations of phenolics were significantly different across the harvest period, the practical effects of those differences are, as yet, unknown. The weeks may even only be significantly different because the weekly averages were based 3 technical replicates analyzing the bulked hip tissue each week. This likely led to low variability observed for most weekly samples. Overall, it appears unlikely that a range of 10-30 mg GAE/g DW (see Table 2.7) for a given species from week to week would be significant, especially if phenolic concentrations are subject to significant environmental factors beyond the control of growers. Identifying optimum harvest time in terms of concentration of phenolics may not be readily feasible for growers in the field, but given the relatively long harvest period studied here and relatively small differences across that period, it may mean that harvest period is flexible, which would be highly beneficial to growers and foragers.

While we had predicted that redness and total phenolics would peak together, this does not appear to be the case. Total average redness peaked around harvest week 4-5, whereas this was when levels of total phenolics generally finished declining and leveled off. Thus, color may not be the best way to estimate ripeness in rose hips, though this may have been a factor of the imaging program. Other methods of measuring color and comparing it to levels of phytochemicals should be investigated, as this may be a way for growers to pick an ideal harvest time if further research suggests that such methods would be valuable.

As with physical traits, comparing *R. acicularis* and *R. arkansana* VC to the 2015 samples suffers from sampling over a shorter harvest period and potential for skew. Additionally, the fact that the 2014 total phenolic concentrations for almost all other samples were significantly higher than 2015

concentrations increases the chances of inaccuracy in comparing these species for this trait. Considering also that *R. arkansana* VC's high average mass may be due to an outlier, that species may not actually have the highest concentration of phenolics per hip as depicted in Table 2.8.

Measurements for total soluble solids and phenolic concentrations of tissue after soluble solid extraction betrayed no clear pattern across the harvest period. Total soluble solids in particular seemed highly variable. This may be an effect of small sample size, as only 5 hips were used in soluble solid analysis. It may also be an effect of inconsistencies in the maceration and extraction process as much as *in vivo* differences. It is not clear what effect, if any, the total soluble solids concentration would have on taste for roses. Physically tasting the rose hips did not reveal any particular sweetness; likely many of the soluble solids observed in this study were soluble phenolics that refract light and not sucrose. Depending on what proportion of soluble solids were phenolic compounds vs. sugars, soluble solid concentration may be an easy and cheap way to estimate phytochemical diversity. Once species' phytochemical profiles are explored, known chemicals' solubility can be researched, which can allow us to see if changes in soluble solid concentration reflect changes in the phytochemical profile. If certain compounds are determined to be particularly desirable, and their solubility is known, growers can use handheld refractometers to assess harvest for the highest concentrations of desirable chemicals. Further study is warranted.

2.6 Chapter 2 References

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Chapter 3: Reflection and Future Studies

In conducting this study, I ran into a number of problems during the first harvest season in 2014. Hasty need to find samples after greenhouse-grown roses did not produce flowers, lack of knowledge about the subject matter, and haphazard sample collection led me to take poor measures of data (i.e. bulk averages as opposed to individual observations) and inconsistent harvest times sample sizes. However, I was able to rectify much of this in the second year of the study. In 2015, consistent harvest times and sample sizes, as well as more thorough measurements gave me a much more comprehensive set of data. Unfortunately, *R. acicularis* only flowered in 2014 due to a prairie burn at the Pheasant Branch Creek Conservancy site which killed the flowering growth of that sample. Thankfully, despite challenges, problems with specimen destruction, and disease, the better-designed parameters of the 2015 harvest season contributed to much better data. Still, knowing more of what phenological variables to track and when would have been beneficial, as flower density here is only an estimate based upon the measurements for cane height and total number of flowers from different canes. Overall, I think I have obtained a valuable education in designing my own scientific study by pursuing a novel project and making many mistakes. I certainly know better how I would go about pursuing a follow-up study.

Based on the results described here, further research is warranted on all the species studied as their high levels of bioactive compounds make them potentially valuable for a number of products. This includes pharmaceuticals, herbal supplements, nursery plants, and teas. Due to the high variability in observations, new trials should be evaluated for multiple seasons. Specifically, a variety of genotypes of each species should be grown in controlled trials in a number of locations as a way to determine the roles of genetic and environmental variability on phenolic content. This could also help to control for

factors that impacted this study, such as disease and insect damage. It may also be possible to observe how much environment impacts traits such as flowering time, cane height and flower density. Longer trials would also help to evaluate climatic and environmental stress factors on production and fruit quality; important for any perennial crop.

I would begin weekly observations starting from pollination, tracking fruit physical characteristics and concentrations of phenolic compounds until into the winter. This would allow me to determine changes across the entire developmental period of the hips. A detailed assessment identifying what phenolic compounds are present, and at which developmental stage, would be valuable for tracking medicinally valuable compounds for optimal harvest time and identifying potentially novel phytochemicals. Samples would be run though HPLC to quantify and identify the chemical profile within each species. In addition, an assessment of total vitamin C content and antioxidative capacity would be useful for determining a species' value as a vitamin supplement. Subsequently creating preparations of rose hips to use in studies of medicinal properties such have already been done with dog roses would be the next step towards determining whether or not North American species have the same potential for medicinal use. Additionally, given the fact that ethnobotanical reports suggest medicinal uses for many parts of these rose species, it may be useful to study the phytochemical properties of the roots, stems, leaves, and petals as well as the hips. When planting specimens at the Eagle Heights plots, I noticed that the roots of these species were a dark red color. As was noted previously, many red pigments are phenolic compounds, so the roots of those species may have significant levels of phenolics as well. A thorough assessment of potential medicinal value should study all potential sources of beneficial compounds, in case there are varying concentrations of bioactive compounds in different plant tissues.

In terms of color and overall shape analysis, it may be beneficial to use a program other than Tomato Analyzer. In particular, the different sources of imaging (scanner in 2014 vs. microscope and camera in 2015) led to dramatic differences in observed color between the years according to this

program. A field colorimeter may be valuable for confirming whether or not color is a useful way of tracking ripeness and overall phenolic concentrations.

Roses also have a relatively small genome (n=7) (Smulders et al.; 2011), so the diploid species may be useful for genetic studies of phytochemical biosynthesis pathways. This could lead to genomic improvements of other crops in the family Rosaceae, as well as other North American species that might possess novel compounds. Roses may also be useful as models for woody perennial crops in the Rosaceae family. Genetic analysis of the species studied here will also help delineate species boundaries and may be useful for identifying traits that could be valuable to ornamental rose breeders. In particular, the colonial rose species studied here fulfill a landscape niche not commonly occupied by roses. For example, a *Rosa arkansana* cultivar bred for filling in space as a groundcover might be a highly desirable new product given its growth habit and long bloom period. Crossing different species to try to create new varieties may also be valuable for both medicinal and landscaping uses, providing yet another avenue to pursue. Overall, this study's characterization of basic traits of these five rose species provides a glimpse of their potential. Having not been well studied previously, there is now evidence to suggest that further study is warranted and that these species may be more useful to the growing natural products and medicinal plants industries than the more well-studied Eurasian roses.

3.1 Chapter 3 References

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Chapter 4: Addendum

4.1 Climatological Data for Madison, WI

This addendum provides graphs showing the temperature and precipitation in Madison, Wisconsin for the years 2014-2015 (Figures 4.1-4.4). Temperatures were above average more often in 2015, particularly in the spring (Figures 4.1-4.2). Though there was more rain overall in 2014, in the spring of 2015 the rain fell more as continuous small showers (reflected by a gradual increase over time), whereas rainfall in the spring of 2015 tended to be more in large, singular downpours (Figures 4.3-4.4). This likely means that 2015 was generally cloudier, which, combined with constant moisture and warmer temperatures, likely influenced to the increased disease presence on roses described in Chapter 2. The climate may also have had additional impacts on fruit size and chemical concentrations.

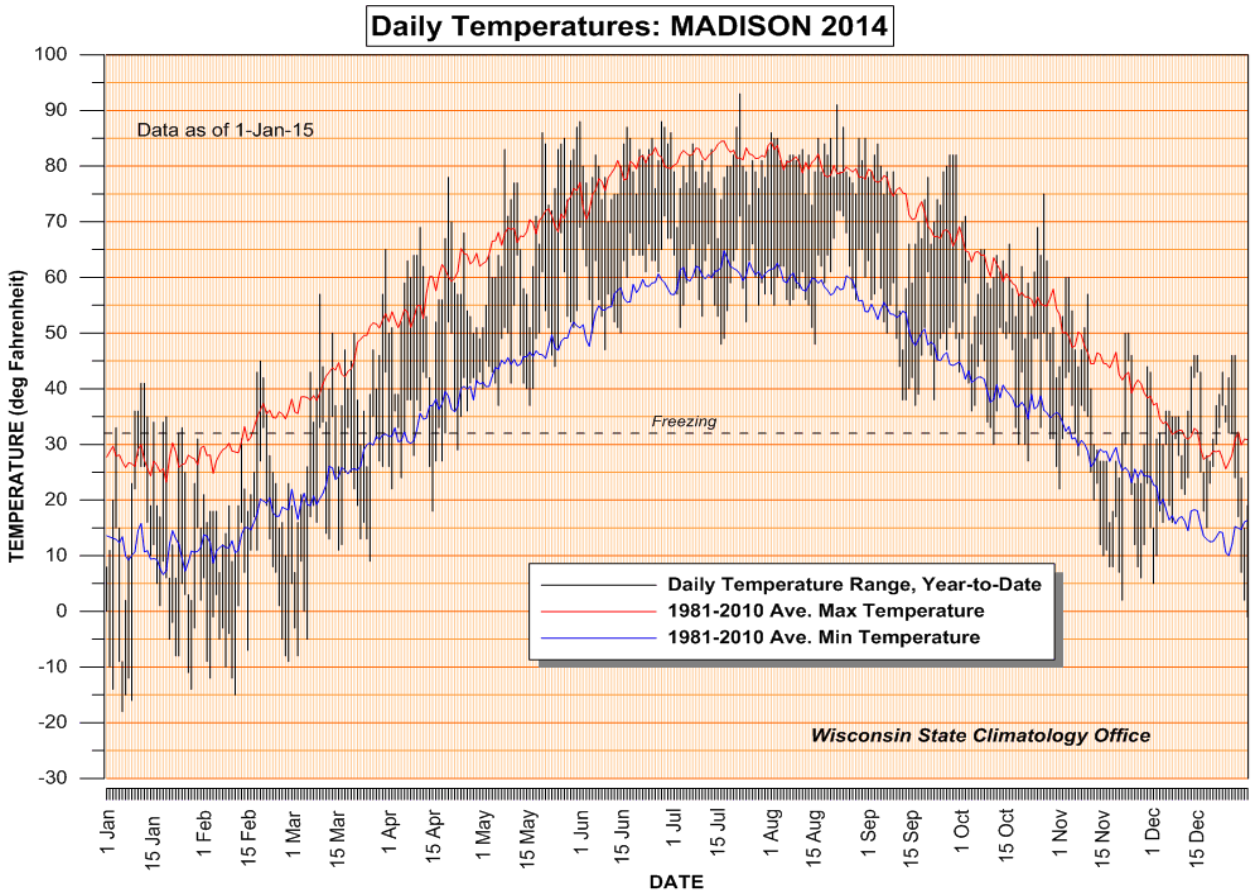

Figure 4.1: Daily high and low temperatures in the area of Madison, WI during 2014 (Young, 2016)

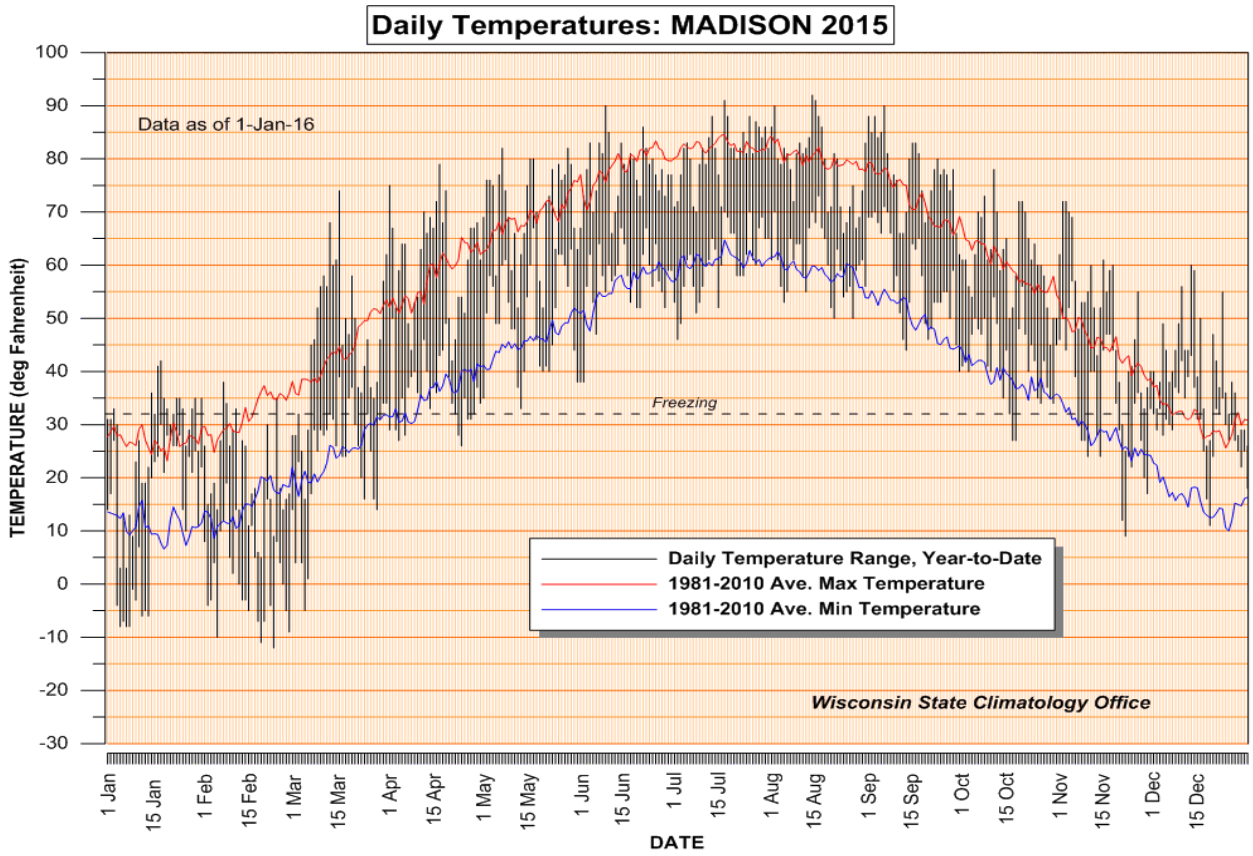

Figure 4.2: Daily high and low temperatures in the area of Madison, WI during 2015 (Young, 2016)

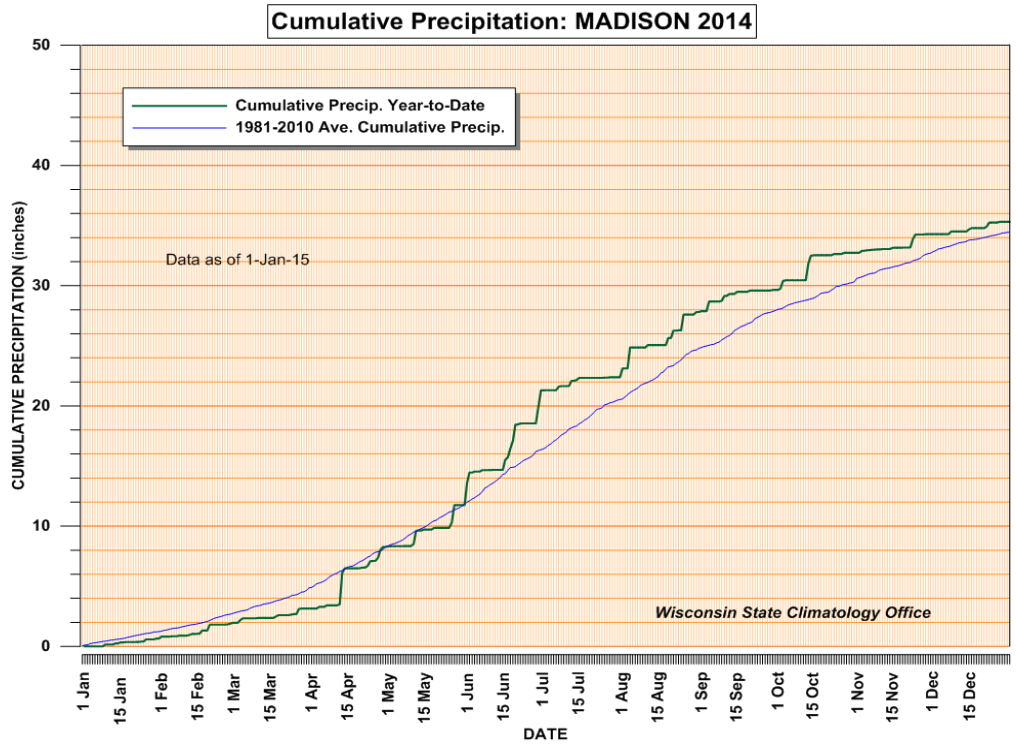

Figure 4.3: Cumulative Precipitation for Madison, WI during 2014 (Young, 2016)

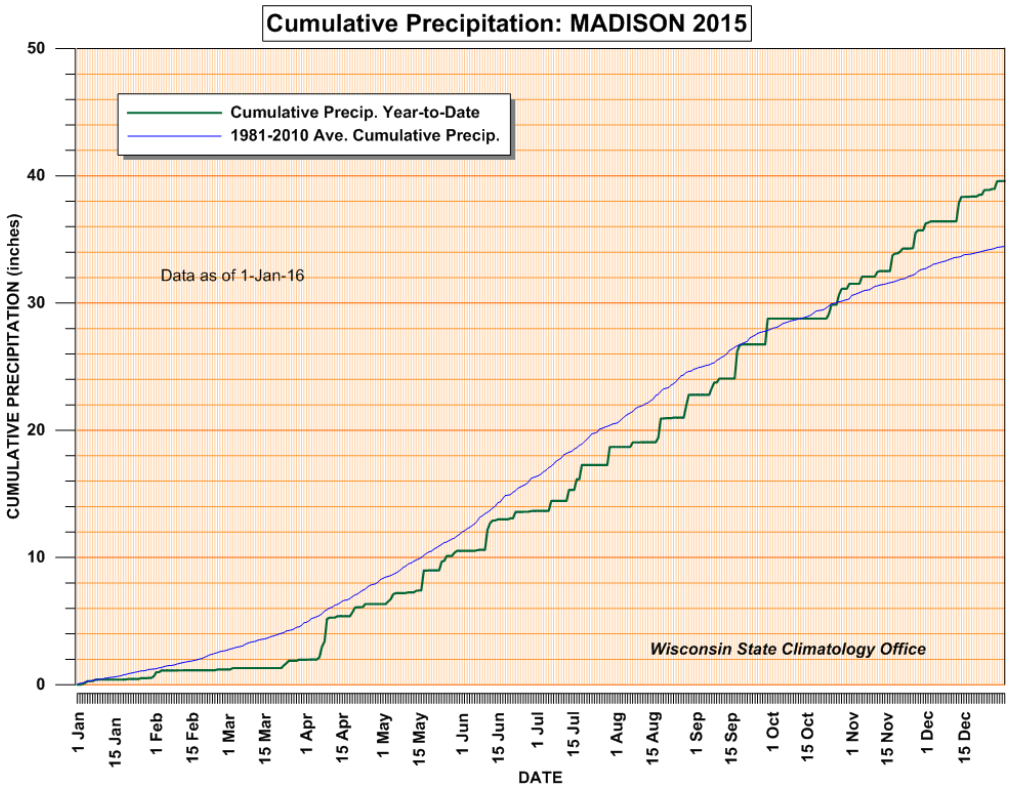

Figure 4.4: Cumulative Precipitation for Madison, WI during 2015 (Young, 2016)

4.2 Chapter 4 References

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