ABSTRACT

BRALY, JILL MARIE. Bloodroot (*Sanguinaria canadensis* L.) Distribution and Supply on the Waynesville Watershed in Western North Carolina. (Under the direction of Frederick W. Cubbage and Erin Sills).

Inventory data from nine stands of bloodroot (*Sanguinaria canadensis* L.) on the Waynesville watershed in Western North Carolina are analyzed to determine distribution and supply of the resource. Inventory data analysis and a review of the relevant literature explore the potential for bloodroot to be harvested sustainably as a non-timber forest product in Southern Appalachia. Additional qualitative analyses of several forests in North Carolina provide a context for interpreting the data collected on the Waynesville watershed and a means for comparing harvested stands with protected stands. Statistical analysis of 174 bloodroot rhizomes reveals that stem height and stem diameter are good predictors of belowground biomass. Results may be used to guide management and monitoring of natural bloodroot populations.

Sampling took place in June through August of 2006 in Western North Carolina and data analysis examines the relationships between stand characteristics and characteristics of the tree overstory. There is a high degree of variability between the nine stands and mean bloodroot stand densities range from 2.1 to 35.417 plants per square meter. Stand sizes vary from 5.28 to 47,400 square meters. Projections based on the data and forest type maps estimate that only 0.5 to 1.8 percent of the Southern Appalachian region supports bloodroot growth. Comparisons between forests suggest that natural populations subject to harvesting face population decline.

Bloodroot (*Sanguinaria canadensis* L.) Distribution and Supply

on the Waynesville Watershed in Western North Carolina

by

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BIOGRAPHY

Jill Marie Braly is the daughter of Christine M. McManaman and Scott A. Braly, and the step-daughter of Eugene P. McManaman. She was born on October 13, 1981 in Lincoln, Nebraska. Upon graduating from Lincoln High School, Jill attended Eckerd College in St. Petersburg, Florida, where she earned a Bachelor of Science degree in biology. After graduation, the author returned to Nebraska to work on the prairie, before beginning graduate school at North Carolina State University the following year. At NCSU, she focused her studies on tropical forestry and non-timber forest products. A two month course in Costa Rica allowed Jill to take an ecological approach to studying tropical biology and live in the jungle as she had so often desired.

Throughout this period of formal education and work, the author continued to explore her additional avocations, including yoga, travel, reading and writing. These activities inspire her to combine her formal education and work with all of life’s experiences to continue to contribute to the conservation and responsible use of the environment.

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1. Introduction

The purpose of this study is to explore the potential for sustainable use of bloodroot (*Sanguinaria canadensis* L.) through a case study that examines the current status of the plant on the Waynesville watershed in Western North Carolina. As used in this document, sustainable use is defined as management of the resource that allows perpetual yields of the resource while maintaining viable natural populations. In order for sustainable harvesting to occur, sufficient regeneration of the resource is necessary. The inventory data presented in this study quantify the abundance of the resource in the forest and provide baseline data required for subsequent monitoring of bloodroot regeneration.

Bloodroot is harvested for its belowground rhizome, making the plant particularly vulnerable to population decline due to mortality following harvesting. In addition to providing inventory data, this study examines the relationship between aboveground plant characteristics and the belowground rhizome to guide sustainable harvesting practices.

An overview of the literature provides a thorough assessment of sustainable management of forests for timber (Davis et al. 2005), but as Wong (2000) describes, "NTFPs have been classified on many occasions though there is no consensus for either reporting, monitoring or as a basis for inventory and research". However, the author notes that most studies are focused on a small scale (less than 10,000 hectares) in order to address local management objectives, and should be designed to address the specific inventory objectives. In this case, a single resource inventory, the objectives are to quantify the abundance and distribution of the resource while examining relationships pertinent to sustainable harvesting.

The lack of definitive guidelines for sustainable management of NTFPs stems from the diversity present in this broad category of natural resources, which include a variety of products, from nuts to medicine. However, knowledge of some general characteristics of the resource should aid in the assessment of the potential for sustainable use of NTFPs. Knowledge of the total area of the species, harvestable volume, harvesting rates and growth rates will provide the information necessary to predict future growth trends and sustainable harvest limits (Cubbage pers. comm.), and according to Peters (1994), "Density and size-class structure data are the most fundamental pieces of information required for management".

A forest inventory is necessary to ascertain estimates of distribution and abundance and these quantitative inventories will provide baseline data for subsequent monitoring (Peters 1994). Establishing a reference point prior to the development of a harvesting regime will provide guidelines for maintaining sustainable levels of extraction. In this study, inventory data on plant sizes and population densities from the Waynesville watershed are used to provide baseline data on biological supply of bloodroot in natural stands. The entire Waynesville watershed is owned by the city and protected from both timber and non-timber forest product harvesting. The last timber harvest was over 70 years ago and there is no public access to the watershed. Thus, an inventory of the watershed provides a snapshot of an undisturbed population of the resource. Distribution patterns, plant sizes, population densities and forest type data are analyzed and the inventories could help with monitoring in other locations in Western North Carolina. In addition, the relationship between aboveground plant characteristics, such as stem height and number of lobes, and belowground biomass is examined to reveal trends to aid harvesters in effective and sustainable collection of the resource from natural stands.

1.1. Study Objectives

The general objective of this study is to examine the potential for bloodroot to be harvested sustainably as a non-timber forest product (NTFP). The specific research objectives, implemented through a case study on the Waynesville watershed and qualitative assessments of several forest communities in Western North Carolina, are to:

1) Describe the density, stem height, and stem diameter of natural bloodroot stands, and analyze (a) the relationship among these characteristics of the stand, and (b) the relationship of these stand characteristics with characteristics of the tree overstory.

2) Quantify the relationship between aboveground plant characteristics and belowground biomass.

3) Discuss the distribution and long term sustainability of bloodroot in Western North Carolina based on the Waynesville watershed case study.

2. Literature Review: *Sanguinaria canadensis*

Humans have utilized bloodroot (*Sanguinaria canadensis* L.) for centuries for both medicinal and artistic purposes. Native Americans used the plant to treat rheumatism, asthma, bronchitis, lung ailments, fevers, burns and warts, and to induce vomiting (Foster and Duke 2000; Krochmal and Krochmal 1984). European colonists continued the tradition of using the plant for medicinal purposes, and bloodroot was listed in pharmacopoeias by the 1800s (Predny and Chamberlain 2005). In modern times, the plant has been used in commercial toothpastes, mouth washes and as an anti-plaque agent. Bloodroot is currently used by the Eastern Band of Cherokee Indians as a dye plant for hand-woven baskets, a tradition that goes back for thousands of years. In recent years the plant has been considered as an alternative to synthetic antibiotics used in cattle feeds (Greenfield and Davis 2004) and as a component of cancer treatment medications (Danehower, personal communication).

For centuries, the many uses of bloodroot have led to its being intensively harvested from North American forests. Bloodroot is considered to be a non-timber forest product since nearly all harvesting is from natural stands, as opposed to cultivated sources. Non-timber forest products (NTFPs) are biological resources, such as medicinal plants, nuts, resins, dyes and ornamental plants, which can be harvested from the forest to provide income to local people (Peters 1994). NTFPs are an important source of income for millions of people around the world (Ticktin 2004; Jepma 1995) and have been proposed as an alternative to more destructive land uses such as clearing for agriculture and timber harvesting (Peters et al. 1989).

Despite the conservation potential of NTFPs, ecological assessment of harvesting implications is rare (Ticktin 2004). Peters (1994) highlights the myth that extraction and commercial harvesting have little or no environmental consequences on the surrounding forest and states that sustainable use requires controlled harvesting and consideration of forest ecology and management.

As Greenfield and Davis (2004) report, increasing demand for bloodroot is putting pressure on naturally occurring populations. Although growers are currently selling small quantities of dried bloodroot and some nurseries offer small volumes of cultivated stock, large volume harvests are not available from cultivated sources (Persons and Davis, 2005).

The USDA Forest Service Southern Research Station (SRS) has recognized an increase in the harvesting of special forest products in the southeastern United States and is aware of the threat to the long-term social, ecological, and economic sustainability of these forest resources (Chamberlain 2004). Chamberlain (2004) notes "Rural families of the South have a long history and deep cultural connections with special forest products. Many collectors learned from their ancestors, who, in turn, learned from Native Americans which plants to collect and how to use them."

The development of a management plan for a medicinal plant should incorporate various sources of knowledge and there are important cultural issues to be addressed. The traditional Cherokee culture of this region utilized and influenced forest resources long before the development of forest science. In order to most effectively manage forest resources such as bloodroot, collaboration between local communities, government agencies, and forest scientists is necessary. Understanding this traditional knowledge of forest practices is an integral part of developing sustainable management plans. But consumption patterns today differ from historical trends, and knowledge of the present state of the forests is important for guiding management plans. With a greater demand for forest products, it is necessary to combine traditional knowledge of distribution and growth patterns with monitoring efforts and inventory analysis. In addition to traditional knowledge, a forest inventory is necessary to ascertain estimates of current distribution and abundance, and these quantitative inventories will provide baseline data for subsequent monitoring (Peters, 1994).

Based on demonstrated historical uses and market trends, bloodroot should be considered as an additional source of income for landowners, growers and harvesters. Persons and Davis (2005) report that in 2003 the market for bloodroot was increasing as a European company explored the use of bloodroot in an animal feed product. However, there are possible substitutes for bloodroot that may prove more affordable and readily available, in which case the expected demand may not materialize. Based on prices for 2004, there is also a small market for organic bloodroot (Persons and Davis 2005). According to Greenfield et al. (2006) prices in 2006 for dried bloodroot were between $10 and $16 per pound, up from $5 to $9 per pound in the mid-1990's to 2000, and demand for dried bloodroot is expected to grow five to 10 percent over the next three to five years.

An assessment of the relevant literature reveals a multitude of uses and definitions of sustainability, as well as a variety of contexts (e.g. environmental, economic, development). As used here, sustainable defines the potential for bloodroot to be grown and harvested from natural forest stands for an indefinite period of time if proper management and harvesting regimes are utilized. An examination of the biology, ecology, and chemical makeup of bloodroot, with a focus on historical and medicinal uses and current markets, is necessary to explore the plant's potential as a non-timber forest product.

2.1. Botanical Description of Bloodroot

Bloodroot (*Sanguinaria canadensis*) is an herbaceous perennial that grows in rich, moist and well drained forest soils (Predny and Chamberlain 2005; Greenfield and Davis 2004). The plant is found in deciduous woods and woodland slopes (Krochmal and Krochmal 1984), semi-shaded roadsides and along borders of meadows (Hayword 1982). Bloodroot is adapted to soils with a high level of organic matter and a pH range from 5.5 - 6.5 (Greenfield and Davis 2004; Cech 2002).

Sanguinaria canadensis is the only species in its genus and is a member of the Papaveraceae family (Predny and Chamberlain 2005). Linnaeus is given credit for defining the genus in his *Species Plantarum*, following the first documented botanical mention of bloodroot by J.P. Cornuti in 1635, who placed the plant in the genus Chelidonium in his work *Historia Plantarum Canadensium*. The name sanguinaria was first used by the French botanist Pierre Morin in 1651, when he published a catalogue of garden plants, and by John Jacob Dillenius, an English botanist who recognized the plant as a unique genus (Felter and Lloyd 1898; Nieuwland 1910).

Bloodroot is the most common name for *Sanguinaria canadensis*. Additional common names include red puccoon, tetterwort, redroot, pauson, turmeric, sweet slumber and Indian paint (Millspaugh 1887; Predny and Chamberlain 2005; Krochmal and Krochmal 1984). The common name tetterwort comes from the Old English word tetter, meaning skin disease, and refers to the use of bloodroot to treat various skin ailments such as warts and eczema. The common names that refer to the use of the plant as a dye include puccoon, paucon, pauson, red puccoon, coonroot and Indian paint, and the name snakebite refers to the toxicity of the plant (Predny and Chamberlain 2005). In some parts of Canada the plant is known as Sang Dragon (Hayword 1982).

The historical range of bloodroot, as described by the physicians Felter and Lloyd in 1898, is from Quebec and Ontario south to the Gulf of Mexico, and east from the Atlantic to the states bordering the western bank of the Mississippi River. Current distributions are described from the Atlantic to the Rocky Mountains, and from southern Canada through the southern US (Cech 2002), with a general consensus that the plant grows throughout the eastern US reaching as far north as southern Canada (Hayword 1982; Grey-Wilson 1993).

Bloodroot plants grow to six to 14 inches (Krochmal and Krochmal 1984) with leaves up to eight to 12 inches across (Grey-Wilson 1993; Hayword 1982). The rhizome is between one and four inches long (Felter and Lloyd 1898) and is found parallel to the soil surface, where it will give rise to single-leaved buds (Grey-Wilson 1993). In spring, the young leaf arises from each bud of the rhizome, enclosing a flower within its folds. When the soil is warm enough, the flower and leaf will migrate together through the two to three inches of soil to emerge at the surface, with the leaf providing shelter to the delicate bloom throughout the journey (Hayword 1982). The leaf will unfurl a half inch above the forest floor as it is warmed by the sun and within a 24 hour period the flower will bloom, reaching its full height of three to six inches within a few days. In inclement weather and at night, the petals will fold inward in as little as ten minutes to protect the reproductive organs (Hayword 1982).

The flower, blooming on its individual stalk, has a yellow center surrounded by eight or more white petals and is one of the first spring flowers to appear (Greenfield and Davis 2004; Cech 2002). Schemske (1978) describes *Sanguinaria canadensis* as "one of the earliest-flowering species of the spring ephemeral flora".

The self-fertile flower of bloodroot is devoid of nectar and lasts only a few days (Cech 2002). Pollinating insects, such as bees and Syrphid-flies, will find an ample supply of pollen and insect visitation to the flower will usually result in cross-pollination if made in the two or three days the flower is in bloom. If such a visit fails to occur or does not result in pollination, the flower will close in on itself, forcing the anthers against the stigma. This action may result in self-pollination as a last resort (Graenicher 1906).

Schemske (1978) found that flowers in an Illinois population of bloodroot were open for only two days, closing during the night of the first day and shedding petals by the morning of the third day. The author describes how two hours following the first opening the anthers begin to dehisce centripetally, with the filaments bending towards the receptive stigma and pushing the anthers against it. By the time three hours have elapsed the stigma has been self-pollinated by the anther and unless outcrossing occurs, this will usually lead to autogamy (Schemske 1978).

In a study on the ecology of pollination, Motten (1986) documented that bloodroot bloomed for three to five days in the piedmont region of North Carolina before self-pollination occurred. The author notes that "In years when honey bees were active in the population, many anthers were stripped of pollen before contacting the stigma, and the flowers were presumably insect-pollinated" (Motten 1986).

Schemske (1978) documented that petal number varied from between 7 and 14 (mean = 9.4) for 71 flowers. These findings were similar to findings by Spencer (1944) who obtained a mean between 8 and 9 for 38,890 ramets.

After the petals die away, the leaf will reach its full height and the seeds will mature in a long seed capsule resembling a pea pod and forming atop the scape (Hayword 1982). At this time, the rhizome will begin to store nutrients, with feeder rootlets gathering nutrients from the soil as the leaf conducts photosynthesis (Hayword 1982).

The rhizome is dark brownish-red and contains an orange-red juice (Cech 2002; Greenfield and Davis 2004). It occurs in freely branching, jointed sections with amber-orange roots growing from the underside and scaled buds emerging from the growing tips (Cech 2002).

Leaves of bloodroot possess large, rounded lobes separated by rounded sinuses (Felter and Lloyd 1898). Each leaf has between one and nine lobes (personal observation). The underside of the leaf reveals orange-colored veins and is paler than the top side (Felter and Lloyd 1898).

The seeds, housed in oblong seedpods, mature in mid to late spring (Greenfield and Davis 2004). They are round and dark reddish-brown when ripe, and are between 3.5 and 4.5 mm in length (Cech 2002). At maturity, the pod bursts open, scattering the seeds up to ten feet (Hayword 1982) where they may be disseminated by seed-dispersing ants, who carry the seeds to underground nests. Conversely, they may be left to germinate near the parent or preyed upon. If successfully established, seeds will swell before splitting and produce a radicle by the following season (Cech 2002).

In 1869, J.W. Chickering provided a poetic description of the early blooms of spring in the *American Naturalist*: “Soon in the meadows the carpet of living green is embroidered with the golden flowers of *Caltha palustris* or English Marsh Marigold, improperly called Cowslip, and whether correctly or not, associated with creamy milk and yellow butter, while a little later are seen in the morning sun, the white stars of the Bloodroot (*Sanguinaria canadensis*), as fragile as they are beautiful, generally lasting but for a day. Its orange-colored juice is much used in medicine as an emetic, an expectorant, and a liniment. This plant readily bears transplanting, increases in size under cultivation, and becomes one of the most attractive ornaments of the early flower border”.

The common variety of bloodroot growing in the wild has a single-stalked flower with eight to 12 petals, but Hayword (1982) describes a second sterile variety propagated for ornamental purposes. This rare mutation of bloodroot was discovered in 1916 by Guido von Webern on his property near Dayton, Ohio. This variety has up to 60 petals, as opposed to the standard eight to 12 of the wild variety, and possesses a heavier rhizome and leaf. Guido von Webern had the foresight to allow the colony to grow to the point where he could safely divide the plants for propagation and analysis. He sent a sample to the Arnold Arboretum, the fate of which is unknown. The variety was named *Sanguinaria canadensis* var. *multiplex* (to distinguish it from the 14 to 16 petaled variety *floro pleno* mentioned by Dillenius in 1732). After von Webern died, his colony died as well, presumably due to the large and lifeless pith section at the end of the rhizome causing the colony to crowd itself towards the surface and dry out. The present rhizomes of this variety likely come from one of two friends with whom he shared them. One rhizome is known to have died, but the second was propagated and distributed by the recipient, Henry Teuscher, director emeritus of the Montreal Botanical Gardens (Hayword 1982).

2.2. Ecological Description of Bloodroot

In order for bloodroot to persist in the wild it requires a functioning forest ecosystem. Like most botanical species, bloodroot depends upon the action of microbes and other organisms vital to the decomposition of organic matter. These organisms facilitate the creation of the rich soils suitable for bloodroot growth. Tree species provide the partial shade of canopies, contributing to suitable habitats. Bloodroot depends directly upon other species, such as honey bees and ants, to aid in important life cycle stages including pollination and seed germination. It is clear from these interactions that protection of wild populations equates to protection of appropriate habitats.

In a study of herb distribution in a forest dominated by *Acer sacchuarum* and considered to have occupied the area undisturbed for several centuries, Struik and Curtis (1962) found that *Sanguinaria canadensis* had a nearly statistically significant non-homogenous distribution (P=0.05). The authors state that "The distributions of plants in a mesic forest is usually a complex blend of varying spatial arrangements" (Struik and Curtis 1962).

Bloodroot is patchily distributed throughout the landscape in the southern Appalachian region of North Carolina (personal observation) and Motten (1986) describes the patchy distribution of bloodroot in piedmont North Carolina. The mesic hardwoods-bloodroot ecosystem is relatively rare compared to other forest landscape ecosystems in the Southern Appalachians and often occurs on scales < 1 hectare (Abella et al. 2003). Marino et al. (1997) suggest that the rapid response of *Sanguinaria canadensis* to environmental variability may contribute to the patchy distribution of populations. The authors note that under-story perennials are subject to environmental heterogeneity in light intensity and chemical properties of the soil, variables that may affect the spatial distribution of bloodroot (Marino et al. 1997).

In addition to environmental variability (Marino et al. 1997), bloodroot distribution patterns are in part attributed to seed dispersal by ants. Ants that act as seed and fruit dispersers influence the organization of some temperate forest communities, such as bloodroot (Pudlo et al. 1980; Beattie and Culver 1980).

Bloodroot plants and ant species such as *Aphaenogaster rudis* carry out acts of myrmecochory, an ant-seed interaction. The seeds of bloodroot have a translucent elaisome approximately five mm in length and rich in nutrients (Cech 2002; Gammans et al. 2005). This elaisome attracts the ants, who carry the seed back to their nest where the elaisome is consumed. The intact seed is then discarded within the nest. This action is beneficial to the plant because it removes the seeds from areas where they may be found by predators and transports them to an area suitable for germination, seedling emergence and establishment. In addition, ant dispersal provides a favorable site for germination away from competition with other plants (Beattie and Culver 1980).

In a study of three populations of bloodroot with varying levels of disturbance, Pudlo et al. (1980) found that the "frequency of seed removal and the distances seeds were carried by ants were related to plant density, dispersion and the relative proportions of sexual and asexual reproduction in each population". In this study, the least disturbed site was 40-100 year old second growth forest. The intermediate site was 25 years old and the most disturbed site was 25 years old and subject to periodic flooding and frequent trampling by humans. The authors demonstrated that seeds in the least disturbed habitats were frequently removed and carried by a variety of ant species, for distances up to 12 meters, well beyond the range of the parent plants. In areas of high disturbance, removal rates and distances were greatly diminished and areas of intermediate disturbance resulted in intermediate rates and distances (Pudlo et al. 1980). Pudlo et al. (1980) conclude "Habitat disturbance, in disrupting the ant fauna and hence the ant-seed mutualism, may have profound effects upon population density, dispersion and patterns of reproduction". These findings are a warning of what could happen if suitable bloodroot habitats are significantly diminished or subjected to heavy levels of human activity.

The myrmecochorous activity is a mutualism between ants and plants and provides benefits to both species involved. Morales and Heithaus (1998) provide evidence that this seed dispersal activity benefits ants as well as plants. In an attempt to quantify the benefits to ants in such systems, they used seeds from *Sanguinaria canadensis* to supplement the basic food supply of *Aphaenogaster rudis* colonies. Colonies enhanced with bloodroot seeds with intact elaisomes, an external fleshy seed attachment that is consumed by ants, produced more gynes (reproductive females that may become queens) than did the control colonies and the authors conclude “that colonies that can feed elaisomes to offspring are more likely to produce at least some gynes, with the number of gynes being most influenced by colony size” (Morales and Heithaus 1998).

The main disadvantage to the ant-dispersing system is that the seeds are not usually carried very far from the parent plants (Cullina 2000). Although distances of 30 feet or more are not uncommon, a distance of one to three feet is more typical. Cullina (2000) concludes “plants like Wild Ginger, Trillium, Bloodroot, and Twinleaf are slow to recolonize areas from which they have been displaced by glaciation or agriculture”.

The effectiveness of seed or spore dispersal, the energy investment in clonal growth and the effectiveness of establishment in new sites influence the migration patterns of plants (Matlack 1994). In a study by Matlack (1994), *Sanguinaria canadensis* had a calculated rate of migration between 0.98 and 1.60 meters per year, which ranked 31 of 51 from lowest to highest rate of migration for the herbs present in the five successional stands contiguous with old growth forest.

Matlack (1994) describes stands that were disjunct from old regrowth as having relatively species-poor understories “due to a lack of species dispersed by ants and spores and those with no obvious dispersal vector” and states that “The extremely low migration rates of some species threaten their continued existence in the second-growth forest landscape”. Matlack (1994) suggests that species isolated due to limited forest preserves will be vulnerable to stochastic events and low-migration species may become rare or absent from the forest flora.

Pollination of bloodroot depends upon pollinating species such as honey bees and Syrphid flies (Motten 1986; Graenigher 1906; Schemske 1978). Motten (1986) describes the pollination ecology of three autogamous wildflowers (*Hepatica, Sanguinaria,* and *Thalictrum*) in piedmont North Carolina, noting that all three species lacked nectar and were visited less frequently than species which produced nectar. The author notes the three species lacking nectar bloomed early in spring, when fewer pollinators are available and interruptions of visitor activity due to inclement weather are most likely. Schemske (1978) noted self-pollination in *Sanguinaria* and his studies reveal that autogamy occurred within several hours of blooming. The flowers retained the ability to be cross-pollinated, but were ensured of fertilization in the absence of outcrossing. The author suggests that the lack of pollinators in early spring promotes autogamy in *Sanguinaria canadensis* (Schemske 1978).

Baker (1963) suggests that sharing an abundant supply of common visitors is an alternative strategy to utilizing specialized pollination vectors. The prevalence of white, open-bowl flowers in the spring wildflower community may be one manifestation of this strategy (Motten 1986).

Plant characteristics such as petal number (Schemske 1978; Spencer 1944), clone size, leaf size and allocation to sexual reproduction among local populations (Marino et al.

1997) exhibit a high level of variability. In a study by Marino et al. (1997) the researchers found that clonal growth, measured by leaf size and number, was variable and "responds vigorously to increased sunlight and to fertilization when under high-light conditions. In contrast, sexual reproduction exhibits relatively little plasticity". The authors conclude that local populations respond to disturbance, and the corresponding increases in sunlight and nutrients, through increased clonal growth (Marino et al. 1997).

In this study permanent, high-light plots had many multiple leafed plants (26.5%) compared to the many single or double leafed plants in the low-light site (99.9%). The high-light sites had an average light intensity 160 percent greater than the low-light sites. Plant densities were higher in the high-light sites than in the low-light sites (high-light: mean = 19.6 plants per square meter; low-light: mean = 4.0 plants per square meter) (Marino el al. 1997). Disturbance that results in tree fall gaps opens the canopy, increasing the light intensity reaching the forest floor. Bloodroot plants may respond with increased rates of vegetative propagation.

The plasticity of bloodroot plants is evidenced by the observations of Marino et al. (1997) that "Individual plants that have persisted at low densities in the shaded understory may respond through rapid vegetative spread and multiple branching of rhizomes when disturbances such as tree falls provide an elevated light environment. This may result in dense vigorous patches of *S . canadensis* in more open environments. Field observations also suggest that a patch population structure and variation in plant vigor among patches of *S. canadensis* may be a result of variation in the intensity of solar radiation reaching the forest floor".

2.3. Medicinal Uses and Chemical Constituents of Bloodroot

Bloodroot has an extensive history as a medicinal plant (Mahady et al. 1993; Foster and Duke 2000). In 1898, the physicians Felter and Lloyd describe the root being made into a powder which when boiled with water or alcohol is activated and used as a medicinal (Felter and Lloyd 1898). The first known users were the Native Americans, and remedies were made from the root to induce vomiting and to treat fever, rheumatism, skin burns and sore throat (Cech 2002). According to Foster and Duke (2000) bloodroot was used by Native Americans to treat rheumatism, asthma, bronchitis, lung ailments, fevers, and warts (Foster and Duke, 2000). European colonists used the plant for medicinal purposes as well, and bloodroot was well documented in pharmacopoeias by the early 1800's (Predny and Chamberlain, 2005). Felter and Lloyd (1898) describe how the Native Americans introduced the plant to the Virginians, "Strachey, who lived in Jamestown, in 1610, states that it was called by the natives '*Musquaspenne*'". They conclude that, "Perhaps no indigenous plant created greater interest among the early botanical physicians than the bloodroot". Indeed, physicians were experimenting and publishing on bloodroot's medicinal properties in the early 1800s (Felter and Lloyd, 1898).

Over-collection is an important factor of population decline for medicinal plants, and this is especially true of plants where the root, bark or whole plant is harvested (Hayden

2005). Since the rhizome of bloodroot contains the desired medicinal compounds, the plant is especially susceptible to mortality following harvesting.

The interest in bloodroot as a source of medicine likely led to its early chemical analysis in the 1800s. Mahady et al. (1993) site Krane et al. (1984) when they claim that the first isolation of the characteristic quaternary benzophenanthridine alkaloids was first reported in 1839 by Probst. According to Felter and Lloyd (1898), the main alkaloids present in bloodroot were isolated and presented by G. Konig in 1891 in the *American Journal of Pharmacology*. G. Konig isolated chelerythrine, sanguinarine, gamma-homochelidonine and protopine, which is also a constituent of opium and chelidonium.

Mahady et al. (1993) states that the group of alkaloids present in *Sanguinaria canadensis* have received intense biological investigation in recent years due to their cytotoxic, antitumor, antiplaque and antibacterial activities. Greenfield and Davis (2004) state that sanguinarine, one of the main alkaloids present in the plant, is used as an antiseptic and anti-inflammatory. Other alkaloids present include chelerythrine, berberine and oxysanguinarine. Berberine has demonstrated potential for combating brain tumors and other cancers (Greenfield and Davis 2004).

According to Greenfield and Davis (2004) modern medicinal uses include treatment of skin growths, such as warts, and utilizing the anti-cancer properties of the plant for treatment of skin cancer. Commercially, the plant has been used in toothpastes and mouthwashes as a deterrent of plaque.

Salmore and Hunter (2001) state that bloodroot produces several bioactive alkaloids which act as defense chemicals. Many of these chemicals are the same ones used medicinally, and to explore the environmental and genotypic influences on alkaloid content they conducted a study on the chemical defenses of *Sanguinaria canadensis*. Results revealed that “Total benzophenanthridine alkaloid concentrations, mainly sanguinarine and chelerytherine, decreased with increasing sunlight while the protopine alkaloids only weakly reflected this trend” and “Light intensity and mineral nutrition influence alkaloid concentration” (Salmore and Hunter 2001).

In a separate study, Salmore and Hunter (2001) examined trends in the chemical components of *Sanguinaria canadensis* and found that “In general, alkaloid content in bloodroot rhizomes declines with elevation, increases with rhizome water content, varies by site, and fluctuates seasonally with plant growth and reproduction. Alkaloid content was positively correlated with vegetative and reproductive effort with few exceptions”. They attribute these trends to biotic interactions along geographic gradients, noting that herbivore and pathogen pressure on plant populations increases as latitude decreases, and this trend is accompanied by a parallel increase in alkaloid content (Salmore and Hunter 2001). Salmore and Hunter (2001) refer to additional findings reported by Small et al. (1999) that show the highest alkaloid concentrations in bloodroot were detected during or immediately following flowering. Bennett et al. (1990) found that highest levels of sanguinarine occurred during flowering and fruiting stages.

In a sampling of 100 eastern US populations, Bennett et al. (1990) found that older rhizomes have greater concentrations of sanguinarine than younger rhizomes, suggesting a net accumulation each year, and southern populations contain higher concentrations than northern populations. Concentrations varied from 0.6% to 6.2% (mean = 2.7%) of rhizome dry weight, but high concentrations were attributed to “mature, undisturbed, deciduous forests”.

According to Paratley (2002) bloodroot rhizomes have an alkaloid content of between four and seven percent. It is not clear whether this figure is for cultivated or wild plants, however, the author notes that this is a fairly high concentration.

Further examination of the trends in alkaloid content will aid in the development of the most effective management and harvesting plans. The medicinal market will depend upon secure, reliable sources of bloodroot, with consistent levels of the bioactive compounds.

2.4. Use of Bloodroot as a Dye Plant

Bloodroot has been utilized by Native American tribes across the Eastern United States and as far north as northern New England and Nova Scotia (Paratley 2002). As the early European settlers made contact with Native Americans they began to record their customary uses of plants. In 1612, John Smith described the use of bloodroot, or pocone as it was referred to locally, as a dye plant for skin and clothing (Paratley 2002). Since that early written report, several additional records have been made of use of the plant as a dye by various Native American tribes and the English colonists adopted the custom of dying clothing with bloodroot. Throughout the 1700's, the French imported the plant for this same purpose (Paratley 2002). The dye, created by boiling the rhizome, is still used today to color the traditional baskets of the Cherokee.

In a study of natural dye plants in Sierra Leone, MacFoy (2005) highlights the potential for sustainable utilization of renewable resources, such as dye plants, to create economic incentives for protection of local habitats. The author states that more destructive land uses, such as agriculture, timber harvesting, development and unsustainable harvesting of medicinal plants, can contribute to deforestation. Dye plants, however, can be collected in accordance with conservation efforts (MacFoy 2005).

2.5a. Non-timber Forest Products as a Source of Income

Non-timber forest products are defined by Peters (1994) as "biological resources other than timber which are harvested from either natural or managed forests. Examples include fruits, nuts, oil, seeds, latexes, resins, gums, medicinal plants, spices, wildlife and wildlife products, dyes, ornamental plans, and raw materials such as bamboo and rattan". The use of non-timber forest products (NTFPs) is increasing in the United States, and such products can now be found in mainstream drug and grocery stores (Jones et al. 2002). Increasing demand threatens ecosystem biodiversity as rising demand outpaces the knowledge necessary for sound management (Jones et al. 2002) and the loss of NTFP species due to over-harvesting has both economic and cultural repercussions beyond the ecological impacts.

According to Jones et al. (2002), Americans are hoping to ensure that forest ecosystems continue to function by asking public land managers to reduce timber harvests. The authors point to the many services land managers are now expected to provide: "Forest managers must now manage forests in ways that simultaneously allow for the production of commodities (fisheries, clean water, timber) and services (recreation opportunities, solitude) while conserving amenities (aesthetic landscapes, cultural heritage sites)" (Jones et al. 2002).

In order to meet an increasing variety of needs, alternative sources of income will be necessary to supplement the decline in revenue from timber harvesting. Jones et al. (2002) claim that in the past twenty years requests for commercial access to NTFPs have risen exponentially as timber harvesting declines. Wong (2000) points to technological substitution and timber harvesting as inevitable activities that take away from the income generating potential of the forests. As synthetics replace natural products, local communities harvesting from the forest face a decline in demand and the establishment of large plantations excludes local people. The author suggests that the rise of interest in NTFPs has been prompted by the rediscovery of the importance of supporting communities in meeting subsistence needs and improving livelihoods.

Among NTFPs, medicinal herbs such as bloodroot represent a significant source of potential income. The United States market for medicinal herbs is worth over three billion dollars and about 175 native plant species are for sale (PCA-MPWG website 2004).

Despite the positive outlook for NTFPs to serve as a means of both conservation and economic income, Belcher and Schreckenberg (2007) draw on a diverse group of 79 case studies from around the world to address some important concerns about the promotion of NTFP commercialization.

Market-related concerns include dispersed production and poorly developed markets. NTFPs are often harvested at low volumes from dispersed areas. Combined with the unreliability of quality, these uncertainties in the product makes establishing long-term market relationships difficult (Belcher and Schreckenberg 2007). For similar reasons, obtaining market access for NTFPs may be especially difficult.

Ecological and conservation implications of NTFP production include increased harvesting intensity, more extensive harvesting and intensified management (Belcher and Schreckenberg 2007). These activities can lead to over-exploitation of the product and detract from biodiversity conservation objectives.

If local NTFPs do become viable sources of economic income, a new set of potential challenges arise. Poor people may fail to benefit from increased commercialization as people with more resources are able to capitalize on the gains and may even be denied access to products that are important in meeting their subsistence and medicinal needs (Belcher and Schreckenberg 2007). Many NTFPs also play important roles in the traditional customs of communities and exploitation of these resources may adversely affect local culture.

Additional concerns arise from the complexities of using traditional knowledge as a base for generating income. It is a challenge to determine how to compensate people possessing indigenous knowledge and to avoid exploiting their culture and values. When NTFPs are associated with local ritual or medicinal uses, increasing commercialization may adversely effect cultural values or the health of local people (Belcher and Schreckenberg 2007).

In conclusion, it is important to carefully examine local markets and the potential for market development before promoting NTFP commercialization. The ecological viability and biodiversity impacts must also be addressed, along with the traditional values and uses associated with the resource proposed for commercialization.

2.5b. Bloodroot as an non-timber forest product

According to the United States NTFP species database, bloodroot is currently a commercially harvested plant in the United States (Institute for Culture and Ecology 1999). In Western North Carolina, bloodroot is harvested for both medicinal and artistic uses. Although non-timber forest products offer local communities in Southern Appalachia a supplemental income from the forest and are often important to local culture, over-harvesting of wild populations poses a threat to long-term population viability (Appalachian Voices Forestry Handbook 2006). The potential exists for bloodroot markets to expand (Appalachian Voices Forestry Handbook 2006), and with demand expected to increase (Persons and Davis 2005), bloodroot populations would certainly benefit from sustainable management plans for wild populations.

As early as 1898, Felter and Lloyd claimed that “Though extremely common throughout the eastern half of the union it [bloodroot] is rapidly becoming scarce in the New England states, where it formerly grew in abundance”. Cech (2002) cites “The continued increase in the incidence of all kinds of cancer, and the resurgence of interest in bloodroot as an anticancer herb” as sources of increasing pressure for wild stands. In the *Medicinal Plants Conservation Status Report* (2000) Boetsch describes the species as likely stable in some parts of its range, but declining locally through much of its range due to the destruction of suitable habitat and harvesting from wild populations”.

It is clear that there is a documented and demonstrated need for conservation efforts to be focused on bloodroot as demand and harvesting pressures continue to threaten natural populations of bloodroot. According to Persons and Davis (2005), the expected increase in demand for bloodroot will put pressure on native populations as large volume harvests are not available from cultivated sources.

Very limited information is known about the long-term sustainability of bloodroot in the wild or under cultivation and Wong (2000) highlights the lack of studies aimed specifically at NTFPs harvested for the root. As Hayden (2005) states, "Few records exist on collecting or over-collecting of plants for medicinal purposes" and historic ranges are often unknown. There is too little research to assess the current situation in the forest and to make sound decisions regarding management of the plant. The paucity of information necessitates current inventories for establishing baseline data for future research. The research presented in this thesis aims to contribute to the scientific body of literature documenting bloodroot and other harvestable forest perennial plants. Specifically, this thesis reports results from an inventory of bloodroot densities and sizes in the Waynesville watershed. Ferretti (1997) states that appropriate forest management plans should be based on knowledge of a forest's status and should be able to detect changes within that forest. By providing an inventory of the current state of bloodroot on a protected tract of forest, this study provides a snapshot of an undisturbed forest. This information may be useful in evaluating the health of bloodroot populations in forests open to harvesting and other uses.

2.5c Bloodroot Harvesting

The literature available on harvesting refers to the harvesting of cultivated stands. Harvesting usually takes place during the growing season before the leaves die back in late summer (Persons and Davis 2005). Once harvested, the roots are cleaned and dried down to about 25 percent of their fresh weight (Greenfield and Davis 2004). Persons and Davis (2005) project that one acre of planted bloodroot may yield as much as 1,462 pounds of dried root. Certainly this number would be much smaller for bloodroot collected in the wild.

Several studies have reported variation in the level of desired alkaloids present in bloodroot, related to elevational trends, light intensity and fertilization. A study by Salmore and Hunter (2001) found that increasing levels of fertilizer and light intensity reduced the level of alkaloids present. If the levels of sanguinarine, the most abundant alkaloid in the plant, are reduced this will affect the price paid for bloodroot intended to be used for medicinal purposes (Persons and Davis 2005). A separate study by Salmore and Hunter (2001) found that increasing elevations resulted in decreased levels of sanguinarine, while sanguinarine levels increased with increasing rhizome water content. Persons and Davis (2005) note that growing conditions can be manipulated to change the chemical composition of bloodroot, which has impacts for harvesting from both cultivated and wild stands.

Presently, bloodroot is harvested in Canada and the United States and sold to both domestic and international markets (Persons and Davis 2005). The United Plant Savers organization lists bloodroot as “At Risk”. This is a list of plants the organization believes to be most sensitive to human impacts. The plants on this list are often wild-harvested and then sold to markets both domestically and worldwide. The combined pressure due to over-collection, habitat loss and the sensitive nature of the plants is leading to the rapid decline of natural stands in their native range (Predny and Chamberlain 2005, United Plant Savers).

The rhizome is the desired part of the plant, both for medicinal and artistic uses, and harvesting bloodroot results in mortality of the individual unless preventative measures are taken. According to one wild harvester, it is important to break off a piece of the rhizome and give it back to the earth. This may allow the individual bloodroot plant to regrow the following spring. The same harvester reports that many harvesters will sit down and clear an entire patch of the resource, rather than selectively harvesting larger plants. Such practices will contribute to the rapid decline of populations. A comparison between mean stand densities of protected stands and stands subject to harvesting supports this conclusion. The stands sampled that are subject to harvesting are open to the community and permits for harvesting are not required. However, National Forests that allow harvesting do require permits for bloodroot collection.

3. Research Methods

This study was conducted on the Waynesville watershed in Western North Carolina during the summer of 2006. The watershed, located south of the town of Waynesville in Haywood County, consists of 8,030 acres of forestlands surrounding the town's drinking water supply. The town of Waynesville began acquiring tracts of land within the watershed around 1913 in order to create a reservoir for high quality drinking water and flood control, and between 1997 and 2002, the town purchased the few remaining pieces of privately owned land (NC Land Trusts 2005).

The 65 to 70 year old second-growth hardwood stands are located on slopes with primarily northern aspects at 3500-5000 feet. According to potential cover type maps of the Waynesville watershed, bloodroot stands were located in northern hardwood and cove hardwood forest types, with one stand in a mesic oak-hickory stand. Northern hardwoods in Southern Appalachia are composed of American beech, yellow buckeye, sugar maple and yellow birch and found between 3,000 and 5,000 feet elevation. Cove hardwoods are composed of primarily whit oak, northern red oak and yellow poplar species with other hardwood species, such as sugar maple and mountain silverbell, present in smaller numbers. Moist soils, a thick herbaceous layer of spring ephemerals and the presence of ferns are common cove forest components. Disturbance in the canopy helps maintain high diversity (Appalachian Voices Forestry Handbook 2006).

The watershed was logged heavily around the turn of the nineteenth century and again in the 1940s and 1950s. Some tracts were logged again in the 1980s, however, none of the bloodroot stands located appear to be in these tracts.

The Waynesville watershed encompasses hundreds of surface streams and underground springs that feed the town's water reservoir and the undeveloped land provides diverse habitat for wildlife (NC Land Trusts 2005). On September 22, 2005 the town of Waynesville signed a conservation agreement to preserve the watershed and protect the town's drinking water supply (NC Land Trusts 2005). This agreement ensures that the forestlands are protected from subdivision and development, making the watershed an ideal location for a baseline inventory of nontimber forest products to serve as a reference point for future monitoring efforts.

Before data collection began, meetings were held with key stakeholder groups to define research objectives, field methods and inventory techniques. The researchers met with Gary Kaufmann, a botanical expert with the United States Forest Service; Jeanine Davis, a North Carolina State University Professor and Extension Specialists; local artisans who utilize bloodroot for their traditional crafts; and Foresters with the Bureau of Indian Affairs.

Based on information shared and ideas generated during these meetings, a final methodology was decided upon by the principal research investigators. This methodology was revised in the field as necessary.

All statistical analyses were conducted using JMP 6.0 (SAS Institute 2006) and results were interpreted at the $\alpha=0.05$ significance level.

3.1a. Inventorying, Monitoring and Managing NTFP Resources

As interest in non-timber forest products (NTFPs) as a source of sustainable income increases, it is necessary to devise appropriate inventory methods for guiding management decisions and monitoring efforts. Broadly defined, a resource inventory is a way "to account quantitatively for goods on hand or provide a descriptive list of articles giving, at minimum, the quantity or quality of each" (Lund and Thomas 1989). In contrast, monitoring provides a method for detecting change and predicting trends over the long-term. Monitoring requires baseline data, often obtained from an initial inventory; subsequent re-measurements of the data characterized in the inventory; comparisons between repeated measurements; and statistical analysis of changes in the data (Kerns et al. 2002). Inventorying and monitoring provide a means to detect changes, such as population decline, and provide information for forest managers and policy makers (Lynch et al. 2004). The inventory techniques used for traditional forestry are not easily adapted for use with NTFPs and the often spaced and clumped nature of NTFPs poses an obstacle to the development of sound protocols (Wong 2000).

In a summary of the review paper by Wong (2000), the European Tropical Forest Research Network (ETFRN) identifies Wong's conclusions that a lack of information available on the appropriate NTFP measurement techniques and a similar lack of sampling design impairs monitoring of NTFP harvests. Determining the sustainability of harvesting is further made difficult by a lack of theoretical models. The ETFRN workshop on NTFPs held in Rome in May, 2000 concludes that "Further work by inventory specialists on the development of inventory methods and protocols for NTFPs is required, drawing on methods that currently exist in a variety of disciplines" (ETFRN 2000).

NTFPs are a diverse group of products and there is no single inventory method that will be applicable to all harvestable resources. Instead, an adaptable approach to inventory design can be utilized "that assists the practitioner to design a protocol that fits the peculiarities of the particular species, product, local capacity and objectives" (Wong 2001). Because conventional research designs do not address many of the characteristics of NTFPs (rarity, clumped distributions, imperfect detectability) and can be expensive, alternative sampling methods may offer advantages to NTFP analysis (Wong 2001).

Kerns et al. (2002) propose a variety of methods for inventorying NTFPs, including descriptive and nonstatistical approaches; presence/absence methods; photographic and remote sensing methods; and statistically based methods. Statistically based methods must account for both spatial and temporal variability. Spatial variability is especially important to consider given the clumped nature of many NTFP species (Wong 2001; Kerns et al. 2002).

Random sampling can be time-consuming and can leave large sections of an area unsampled, and is therefore not always appropriate for inventories and monitoring of NTFPs (Kerns et al. 2002). Instead stratified sampling methods may be used, which remove major sources of recognizable variation and divide “a study area into several homogenous regions based on factors such as soil type, elevation, seral stage, overstory cover type, or management application” (Kerns et al. 2002).

It is important to consider the objective of the inventory when designing the sampling methods. Since harvesting effects are likely to be critical in sustainable management of the resource, the monitoring of harvesting impacts should be considered in the design. The effects of harvesting are easily monitored at the plant population level. Peters (1994) describes plant populations as referring “to a group of plants of the same species growing together within a limited area of forest. In the ecological hierarchy, this level of resolution falls between the individual and the community”.

Once the sampling methods are devised, replication will be necessary for statistical hypothesis testing. Kerns et al. (2002) warn against pseudoreplication, which occurs when there is no replication at the population level. The authors state that replication at the stand level is required to make inferences between two generalized populations.

Sound management requires a sustainable system for exploiting non-timber forest products. Peters (1994) describes such a system as one in which NTFPs can be harvested indefinitely within designated forest areas with negligible impact on the populations being exploited. For such systems to be effective communities must be informed and capable of carrying out sound management and monitoring practices.

According to Janis Alcorn, in the foreword to a *Biodiversity Support Program* (Peters 1994), as demand for NTFPs increases, communities seek suitable forest management plans to mitigate the effects of increased harvesting pressures. These plans should include effective measures for guiding the allocation and management of the biological resource. Alcorn urges the project managers of such plans to consider all uses, both actual and potential, of the forest; who is making management decisions and who is enforcing those decisions; what are the labor requirements and investments; what are potential negative effects of sustainable management; and what are the cash and non-cash benefits of NTFPs and other services and products from the forest being considered.

The diversity of NTFPs available for harvesting necessitates management plans specific to the locale of the resource to be collected. Ticktin (2004) states that "The ways in which the responses to harvest of some NTFP vary significantly over space and time also suggests that, at least in these cases, harvest limits may have little meaning outside the specific conditions in which they were determined. Therefore adaptive management strategies, in which harvesters are actively involved in monitoring both harvest and feedback, may be important tools for regulating harvest".

In a report by the Institute for Culture and Ecology, Lynch et al. (2004) suggest that the lack of adequate information about NTFPs inhibits the implementation of sustainable forest management and that this knowledge gap can be addressed through participatory inventory and monitoring efforts which incorporate scientific, local and indigenous knowledge. The authors "argue that broadening participation in inventory and monitoring efforts can provide managers and policy makers with the data needed to develop and maintain sustainable NTFP management programs". The benefits of participatory methods include helping harvesters develop a sense of responsibility towards natural resources; promoting shared responsibility for management; capturing the geographic variability of NTFPs; and addressing important aspects of social and political processes related to natural resource use (Lynch et al. 2004).

3.1b. Collection of Bloodroot Inventory Data

This study evaluates inventory data collected in July and August of 2006 from nine stands of bloodroot located in the Waynesville watershed. These were the only bloodroot occurrences identified along the system of roads throughout the watershed. The inventory techniques used in traditional forest inventories are not easily adaptable to the often spaced and clumped nature of NTFPs (Wong 2000) and random sampling can be time-consuming and leave large areas of the resource unsampled (Kerns et al. 2002). Due to the relative homogeneity of the forest, the entire system of roads below 4,000 feet in elevation were cruised to be sure all accessible occurrences were sampled.

Field methods were devised to capture baseline data on bloodroot plant sizes and densities to be able to make landscape-level estimates of total bloodroot abundance. Data collection sheets for bloodroot and tree inventory data were developed for use in the field and modified after test-runs at North Carolina State University and Western North Carolina mountain sites.

Inventory data collection included locating bloodroot stands in the watershed; non-destructive sampling of bloodroot plants within those stands to obtain measurements of stem height, diameter and density; and collecting information about the tree species and sizes within the stand in order to characterize forest type and the relationships between overstory cover and bloodroot growth.

Bloodroot stands were located along the system of roads that are established throughout the Waynesville watershed. All bloodroot occurrences were identified within 50 meters of the 22,000 meters of roads cruised. In total, approximately 2,200,000 square meters were cruised along the roads. About 430,000 square meters of the total area cruised (mostly along streams) can be considered acidic coves and hence, not suitable bloodroot habitat. Another 18,000 square meters are considered unsuitable bloodroot habitat. In order to capture demographic data on the entire stand, bloodroot occurrences and sampling often continued more than 50 meters from the road. However, projections of total bloodroot growth area (5.1a) are based on the percentage of the 1,752,000 square meters of suitable habitat cruised.

Once a bloodroot occurrence was identified, the stand width (m) and length (m) were determined by measuring the entire bloodroot distribution at the location. This unit served as a stand. A transect line was laid across the entire length of the stand, mid-way between the plot-width boundaries. Along this transect, the diameter (cm) at soil surface and stem height (cm) of all individual bloodroot stems within a half-meter of either side of the transect line were measured using electronic calipers. Working with a field assistant, the principal research assistant would slowly walk the transect line, measuring all bloodroot individuals within the one-meter wide area. Measurements were called out to the field assistant who recorded the data.

Plant size data were recorded on data sheets, using a tally system based on size classes. The size classes for diameter increased in one-millimeter increments and the height size classes increased in two-inch increments, with the first class including all plants four inches in height or less. Tally sheets allowed data to be collected in 10 meter increments. Transect lengths varied according to the dimension of the bloodroot stand. Stem diameter measurements were taken in millimeters and stem height measurements were taken in inches, using a tally system to record the number of plants within each size class in each 10 meter section of the transect.

At the center of the stand, a single tree plot with a radius of 12.62 m (1/20 ha) was laid out and all tree stems greater than four inches at diameter breast height (dbh) were identified by species dbh was measured. The species and dbh of all trees greater than four inches were recorded (Appendix, Table 4).

Latitude and longitude were taken at each stand center. Additional variables measured at each stand center included elevation (m), aspect (degrees) and slope (degrees). Distinguishing stand characteristics, such as the presence of a stream or high-light conditions, were recorded.

In addition to the quantitative data collection on the Waynesville watershed, qualitative assessments of bloodroot stands were made near Lake Raleigh, on the Bryson City watershed, in Bent Creek Experimental Forest in Asheville and on public lands in Western North Carolina, where bloodroot stands are subjected to harvesting pressure. Including these additional stands in the case study helps to provide a context for interpreting the data collected on the Waynesville watershed and provides a means of comparing harvested stands with protected stands.

3.2. Belowground Biomass Sampling

The analyses of above- and belowground biomass relationships were based on 174 plants harvested from the Waynesville watershed between July 25 and August 4, 2006. One rhizome can support several stems so in order to look at the relationship between number of leaf lobes and rhizome mass, only rhizomes supporting one stem were used. The plants measured for belowground biomass sampling were located at the base of the slopes or at plot center of six of the stands used for inventory analysis. Plants were selected from a specified area in order to get a range of variability in aboveground plant characteristics that are recognizable to harvesters. The exact number of plants measured at each site varied, but included at least 20 individuals.

The rhizomes of bloodroot were found approximately 0 to 5 cm below ground. Following the base of the stem to the rhizome, the topsoil was carefully removed by hand and the rhizome extracted.

Rhizomes were measured for length and wet weight. The rhizomes were weighed wet in order to avoid destructive sampling and were replanted directly following measurement. Length measurements were made using electronic calipers and weights were measured using an electronic scale. Length measurements were made and recorded in centimeters and rhizome weights were made and recorded in grams.

The number of leaf lobes, stem diameter at the soil surface and stem height were recorded for the stems. Stem measurements were made with electronic calipers. Stem diameters were recorded in millimeters and stem heights were recorded in inches. Measurements were later converted to centimeters for analysis.

3.3. Inventory Data Analysis

Data were entered into Microsoft Excel (Microsoft 2002), and statistical analyses were performed using JMP (Sas Institute 2006).

From the inventory data collected, the following were calculated for each stand:

-mean stand density (number of plants per square meter),

-mean stem height (cm),

-mean stem diameter (cm),

-mean dbh of overstory trees

-species richness of overstory trees

-elevation (meters)

Stand density is the mean number of bloodroot plants per square meter sampled. Overstory tree species richness is the number of different tree species present in the 1/20 ha plot at the center of the bloodroot stand. The mean tree dbh is for all trees present in the 1/20 ha plot.

A one-way ANOVA test was performed to examine the variance in mean stand densities between bloodroot occurrences on the Waynesville watershed. Examination of the data reveals an obvious outlier (See Figure 1, Results section 4.1). This outlier was a valid data point in that it was not the result of a data collection error, but rather was a legitimate bloodroot stand. Numerical justification for labeling a data point as an outlier varies, but this study identified outliers based on the rule that an outlier was any data point more than 1.5 times the interquartile range (IQR) outside of the IQR. Following the advise of Ott and Longnecker (2001) that "If there is no identifiable reason to correct or omit the point, run the regression both with and without it to see which results are sensitive to that point", all analyses involving an outlier were run both with and without the identified data point The authors explain that undetected outliers, regardless of the source or reason for the outlier, can cause distortions of the regression equation and thus it is important that they are detected and identified. This is especially true for data sets with small sample sizes. The above ANOVA procedure was repeated, excluding the outlier from the data. A possible explanation, as well as justification for omitting the outlier in analyses, follows in the discussion section (5.1a).

To determine if there was a correlation between elevation and mean stand densities of the bloodroot stands simple linear regression (SLR) analyses were run both with and without the outlier. SLR analyses were run both with and without the outlier in order to examine the relationship between average stand densities and average stem height. The tests were repeated to analyze the relationship between average stand densities and average stem diameter. Additional SLRanalyses were conducted, both with and without the outlier, to examine the relationship between stand densities and overstory species richness and stand densities and average tree dbh.

The relationships between aboveground plant characteristics of stem height and diameter and tree dbh were analyzed with SLRstatistical procedures. The average stem height corresponding to the above mentioned stand density outlier was also identified as a numerical outlier (See Figure 3, Results section 4.1b). Thus, analyses including the variable of average stem height were run both with and without the outlier. The average stem diameter associated with the outlier stand density and stem height is noticeably larger than the other data points (See Figure 4, Results section 4.1b), but was not identified as a numerical outlier.

Regression models and nonlinear analyses were performed with the data but the SLRmodels proved to be the most appropriate. A multiple regression model involving the predictor variables used in the ANOVAs was not statistically significant and the nonlinear models did not increase the accuracy of the results.

3.4. Belowground Biomass Data Analysis

To examine the relationship between aboveground plant characteristics and belowground biomass a multiple linear regression was performed with rhizome biomass as a response variable, and stem height, stem diameter, and number of leaf lobes as indicator variables. After examining the parameter estimates, number of leaf lobes was excluded and the test was repeated using only stem height and stem diameter as indicator variables. Three individual simple linear regressions were run with rhizome biomass as the response variable and only stem height, stem diameter or number of leaf lobes as an indicator variable.

4 Results

Nine stands of bloodroot were located on the Waynesville watershed in Western North Carolina. In total, 2,790 plants were measured for inventory analysis. Bloodroot occurrences in the watershed are associated with elevations of 1050-1310 meters (3,445-4,298 feet), slopes of 30-40 degrees and aspects ranging from 345 to 90 degrees (Appendix, Table 3). These elevations and slopes were characteristic of much of the watershed area cruised, however, bloodroot occurrences were located on slopes with more northern aspects. According to potential forest cover type maps of the Waynesville watershed, all stands except one were located in northern hardwood slopes or poplar coves.

To examine the relationship between aboveground plant characteristics and belowground biomass, 174 rhizomes were analyzed from the Waynesville watershed. The rhizomes harvested for biomass analysis had wet weights of between 0.1 and 21.5 grams and were 0.6 to 13.81 cm in length (Figures 1 and 2). The corresponding stems had diameters at the soil surface of between 0.03 and 0.56 cm and heights of 6.99 to 37.47 cm (Figure 2). The number of lobes on the leaf ranged from one to nine (Figure 3).

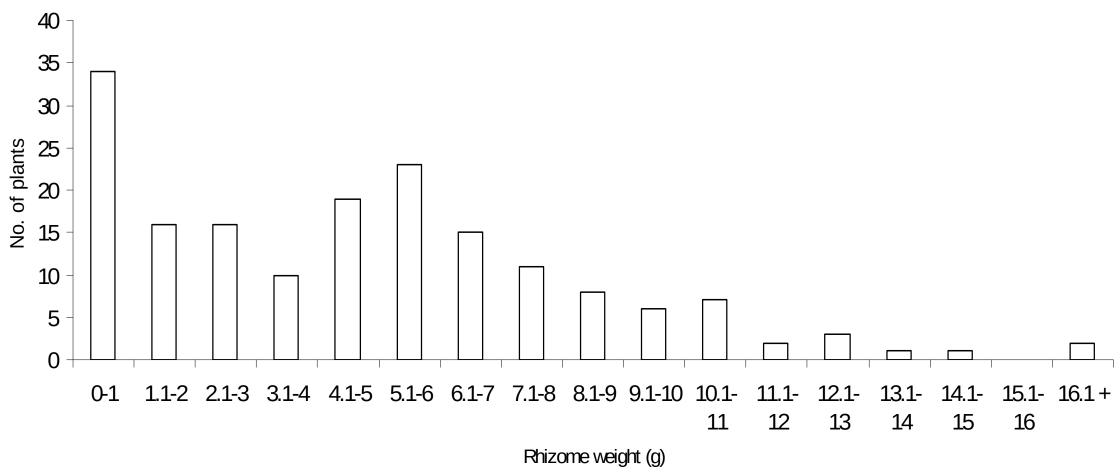

Figure 1. Wet weight (g) of 174 bloodroot (*Sanguinaria canadensis*) rhizomes on the Waynesville watershed in Western North Carolina.

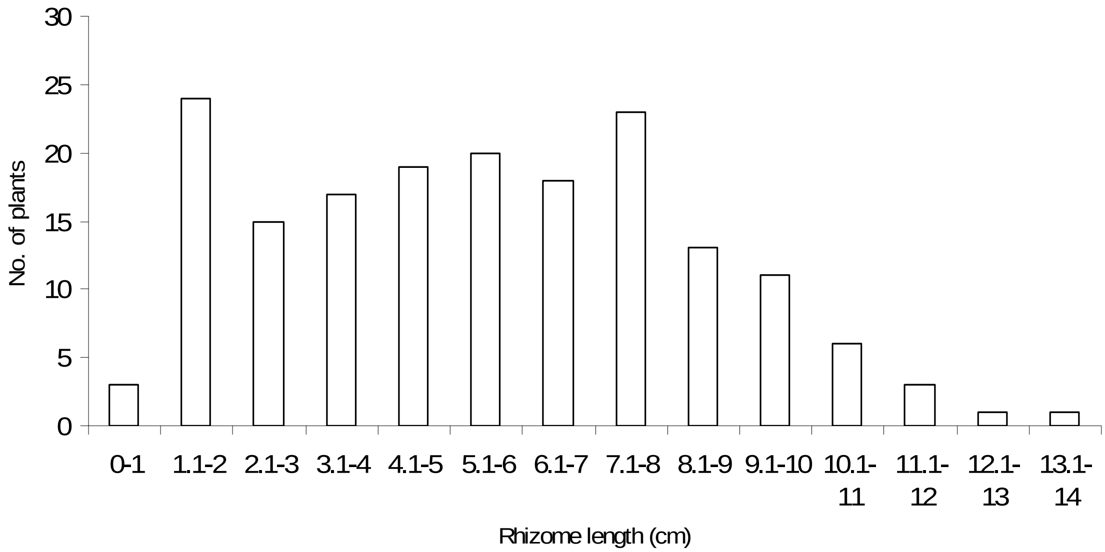

Figure 2. Rhizome length (cm) of 174 bloodroot (*Sanguinaria canadensis*) plants on the Waynesville watershed.

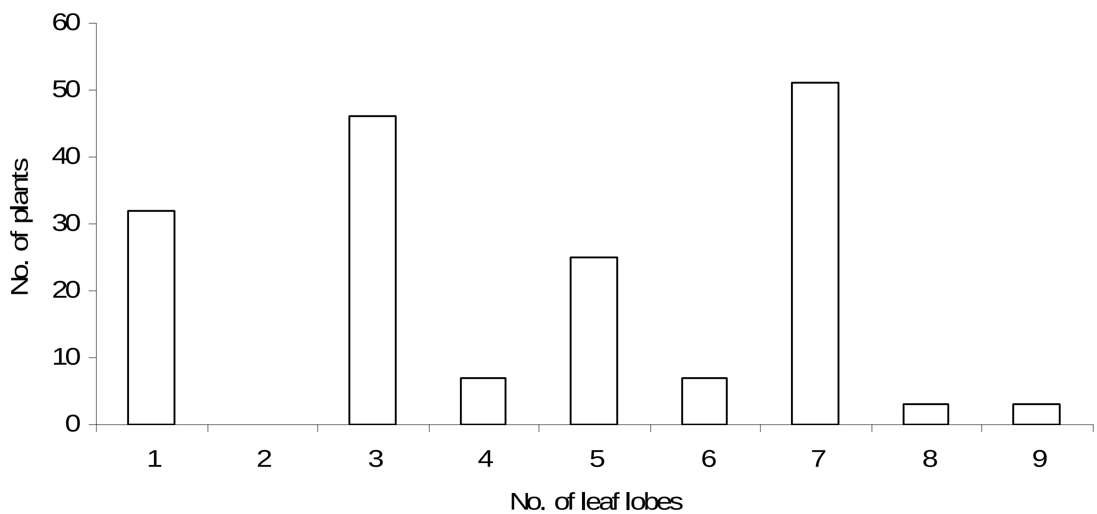

Figure 3. The number of leaf lobes on 174 bloodroot (*Sanguinaria canadensis*) plants on the Waynesville watershed in Western North Carolina.

4.1a. Analysis of Inventory Data

Average bloodroot densities for the nine stands on the Waynesville watershed varied from 1.68 to 35.42 plants per square meter. However, eight out of nine of the stands had average densities of 6.77 plants per square meter or lower (Table 1). The stand with a density of 35.42 plants per square meter was unusual when compared to all other stands sampled for this study and seen at other Western North Carolina locations. As described in the Methods chapter (3.3) of this document, this high density stand was classified as an outlier and following the methods of Ott and Longnecker (2001), statistical analyses were run both with and without the outlier.

The stem height at the soil surface of bloodroot plants in the inventory stands varied from 7.62 cm to 33.02 cm and stem diameter ranged from 0.08 cm to 0.75 cm. Average stem heights at the stand level varied from 13.94 cm to 26.386 cm and average stem diameters at the stand level varied from 0.233 cm to 0.402 cm (Table 1).

The average tree diameter at breast height (dbh) for the trees within the 12.62 m (1/20 ha) radius located at each stand center varied from 6.054 in to 22.857 in (Table 1). However, 7 out of 9 stands had average tree dbh measurements between 10.78 in and 14.235 in. The tree species richness, defined as the number of tree species within the stand center radius of 12.62 m, ranged from four to eight species (Appendix, Table 4).

Table 1. Bloodroot (*Sanguinaria canadensis*) inventory data for the Waynesville watershed in Western North Carolina.

Stand No.	Avg. density (plants per square meter)	Average stem height (cm)	Average stem diameter (cm)	Average tree DBH (in)	Tree species richness (No. of species at plot center)
1	35.417	26.386	0.402	6.054	5
2	5.986	16.18	0.262	14.235	4
3	2.092	19.61	0.335	11.02	8
4	2.1	18.143	0.293	11.244	5
5	1.683	18.132	0.293	10.78	7
6	6.767	16.329	0.246	11	5
7	1.821	16.928	0.259	11.667	8
8	3.308	15.579	0.233	22.857	5
9	5.793	13.94	0.29	12.525	6

The stands varied in both length and width, with widths ranging between 2.2 – 395 meters and lengths ranging between 2.4 – 140 meters (Table 2). It is important to note that eight of the nine stands had widths between 80 and 395 meters and lengths between 60 and 140 meters. The outlier stand had notably small dimensions, which lend further support to treating these data points with caution in analyses. The extremely small size of the stand is likely a result of unusual stand characteristics, such as a possible treefall gap, and such high-density stands were not seen over large expanses of space.

Table 2. Stand dimensions of the nine stands of bloodroot (*Sanguinaria canadensis*) on the Waynesville watershed in Western North Carolina.

Stand No.	Width (m)	Length (m)	Total Area (m^2)	Stand No.	Width (m)	Length (m)	Total Area (m^2)
1	2.2	2.4	5.28	6	170	60	10,200
2	177	70	12,390	7	210	95	19,950
3	150	130	19,500	8	396	120	47,400
4	150	60	9,000	9	130	140	18,200
5	80	60	4,800				

4.1b. Analysis of Bloodroot Stand Densities

To analyze the mean stand density data for the nine bloodroot stands, a one-way SLRwas performed. Mean stand density is defined as the number of plants per square meter. The results of this analysis reveal that there is no significant statistical difference ($\alpha=0.05$) in mean stand densities between bloodroot occurrences on the Waynesville watershed (SLR with $F=2.583$; $df=1,7$; $P=0.152$; Figure 4). The analysis was repeated with the outlier removed. Again, there was no statistical difference detected between mean stand densities (SLR with $F=0.092$; $df=1,6$; $P=0.772$; Figure 5).

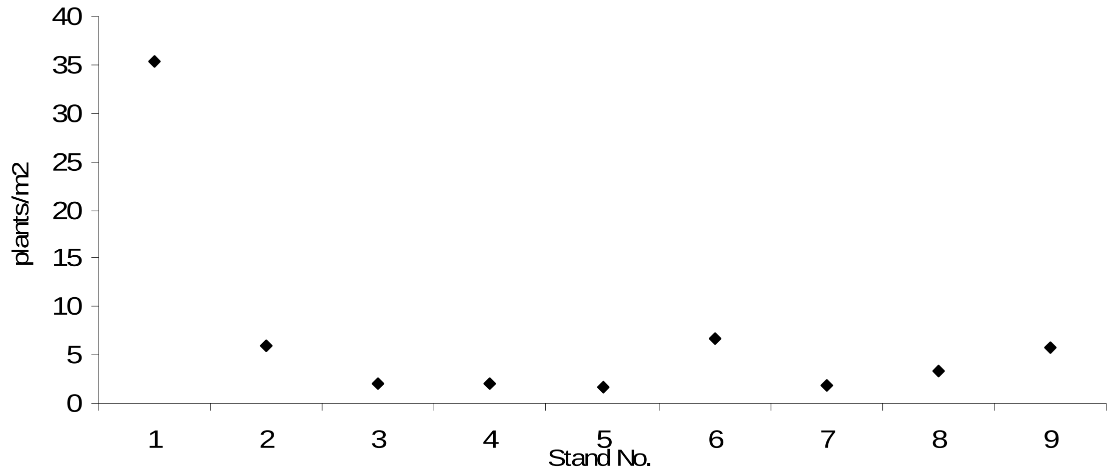

Figure 4. Bloodroot (*Sanguinaria canadensis*) mean stand densities (plants/m^2) on the Waynesville watershed in Western North Carolina (outlier stand included).

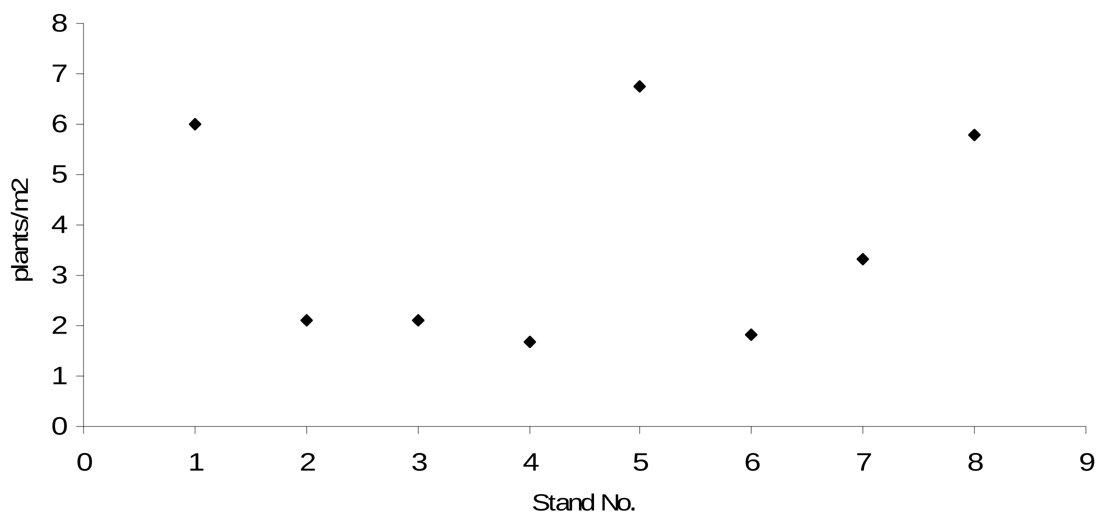

Figure 5. Bloodroot (*Sanguinaria canadensis*) mean stand densities (plants/m^2) on the Waynesville watershed in Western North Carolina (outlier stand excluded).

SLR analysis of the elevation and stand density data revealed no significant relationship between the two variables on the Waynesville watershed. To look at the relationship between average stand densities and stem characteristics, four separate SLRanalyses were run. The first two SLRs examine the relationship between average stand densities and average stem height of each stand. When the analysis was conducted including the outlier, average stem height is an accurate predictor of average stand density (SLR with F=13.737; df=1,7; P=0.0076, Figure 6). The results indicate an inverse relationship between stand density and average stem height, with an increase in average stem height leading to a decrease in average stand density. When the outlier is removed and the analysis is re-run, the P-value is no longer statistically significant at the α=0.05 level (SLR with F=5.2138; df=1,6; P=0.0625, Figure 7). However, the P-value remains low, suggesting that there is a significant biological relationship between stem height and mean stand densities at the α=0.10 level. It is important to note that the correlation between average stem height and average stand density changes from a positive correlation to a negative correlation when the outlier is removed (See Figures 6 and 7).

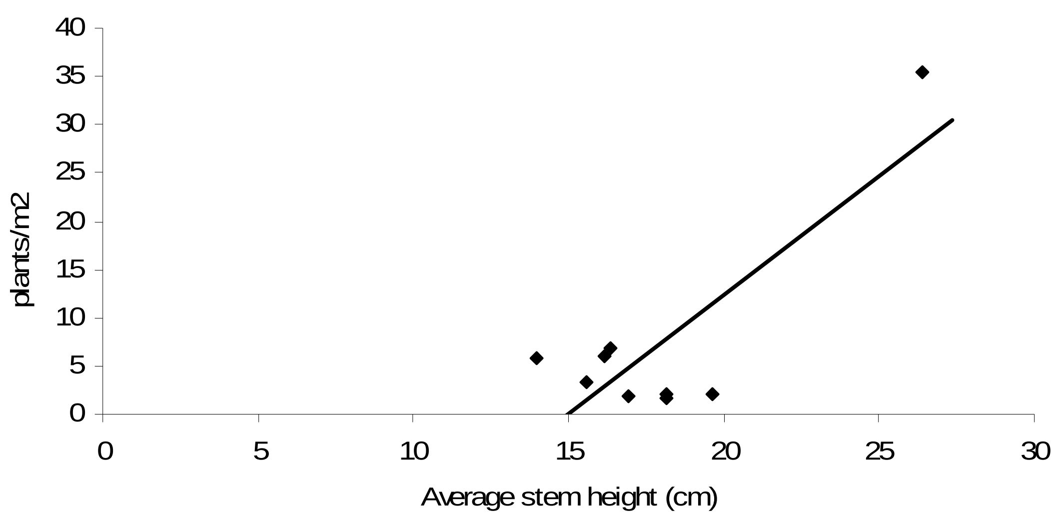

Figure 6. Regression analysis of stem height (cm) as a predictor of bloodroot (*Sanguinaria canadensis*) stand density (plants/m^2) on the Waynesville watershed in Western North Carolina (outlier included). P=0.0076; R^2=0.66. Mean stand density (plants/m^2) =-36.6188 + 2.4471(average stem height (cm)).

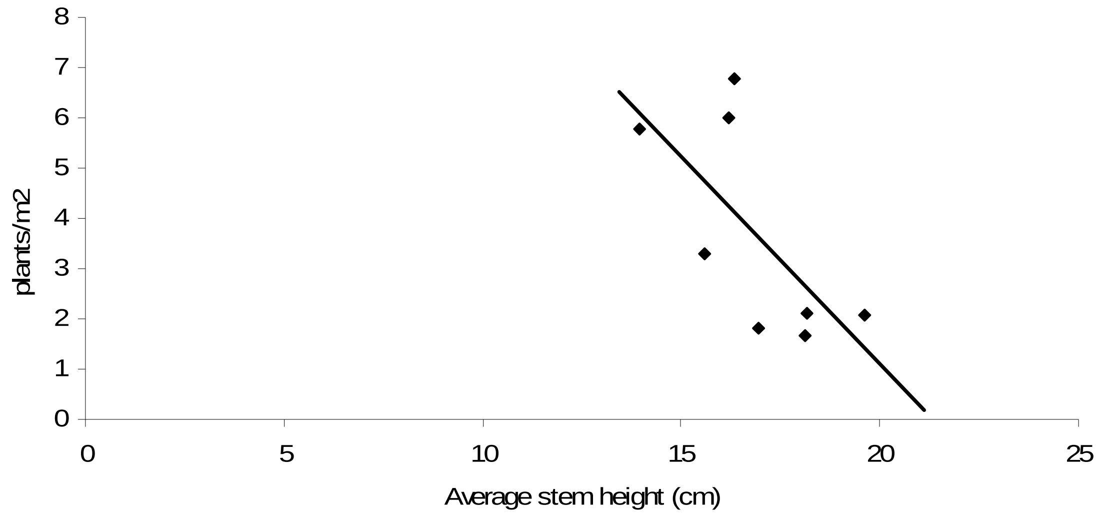

Figure 7. Regression analysis of stem height (cm) as a predictor of bloodroot (*Sanguinaria canadensis*) stand density (plants/m2) on the Waynesville watershed in Western North Carolina (outlier excluded). P=0.0625; R^2=0.46. Mean stand density (plants/m^2) = 17.6172 – 0.8261 (average stem height (cm)).

The relationship between mean stand density and average stem diameter was examined with two additional ANOVAs. When run with the outlier, average stem diameter is an accurate predictor of mean stand density (SLR with F=8.898; df=1,7; P=0.0204; Figure 8), with an increase in average stem diameter corresponding to an increase in stand density. When the analysis is re-run excluding the outlier, the significance does not remain (SLR with F=1.239; df=1,6; P=0.3082).

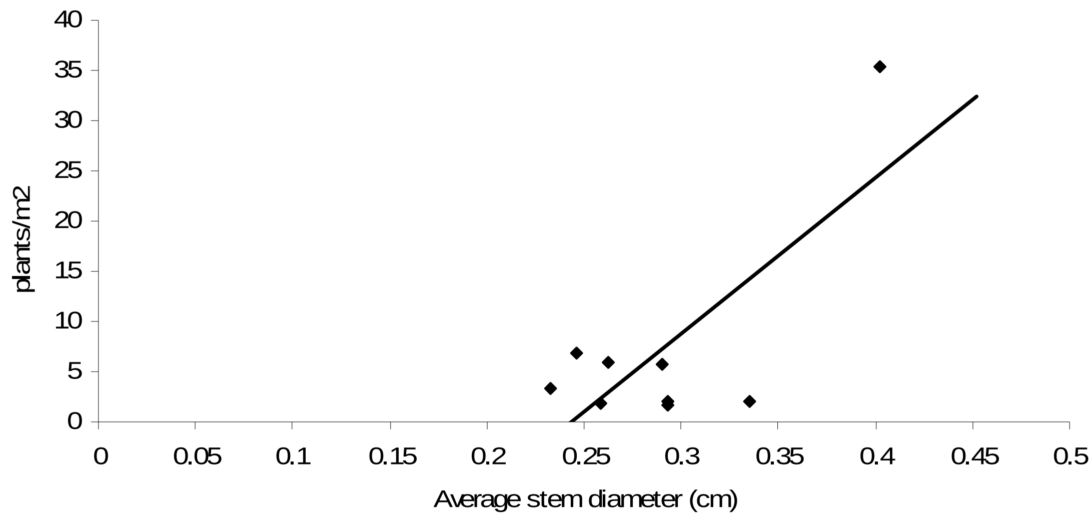

Figure 8. Regression analysis of average stem diameter (cm) as a predictor of bloodroot (*Sanguinaria canadensis*) stand density on the Waynesville watershed in Western North Carolina (outlier included). P=0.0204; R^2=0.78. Mean stand density (plants/m^2) = -37.8532 + 155.2414(average stem diameter (cm)).

Statistical analyses of the relationship between mean stand density and overstory tree species richness at plot center were run both with and without the outlier stand. When the outlier is included, there is no statistical significance (SLR with F=0.93; df=1,7; P=0.3670). Without the outlier, there is still no statistically significant relationship at the α=0.05 level between stand density and overstory tree species richness, however, the low P-value suggests there may be a biologically significant relationship (SLRtest with F=4.339; df=1,6; P=0.0824). The inverse relationship suggests that as tree species richness increases, the average density decreases. See Appendix, Table 4 for a list of tree species.

The final statistical analyses of stand densities examine the relationship between mean stand densities and the average tree diameter at breast height (dbh) of the overstory trees at stand center. The SLR analyses were run both with and without the outlier. Including the outlier stand, there is a statistically significant relationship between the variables, with an increase in average tree dbh corresponding with a decrease in mean bloodroot stand density (SLR with F=13.395; df=1,7; P=0.0081; Figure 9). This relationship is no longer statistically significant when the outlier is excluded (SLR with F=2.373; df=1,6; P=0.1744; Figure 10). However, it is important to note that the trend reverses, with an increase in average tree dbh corresponding to an increase in stand density.

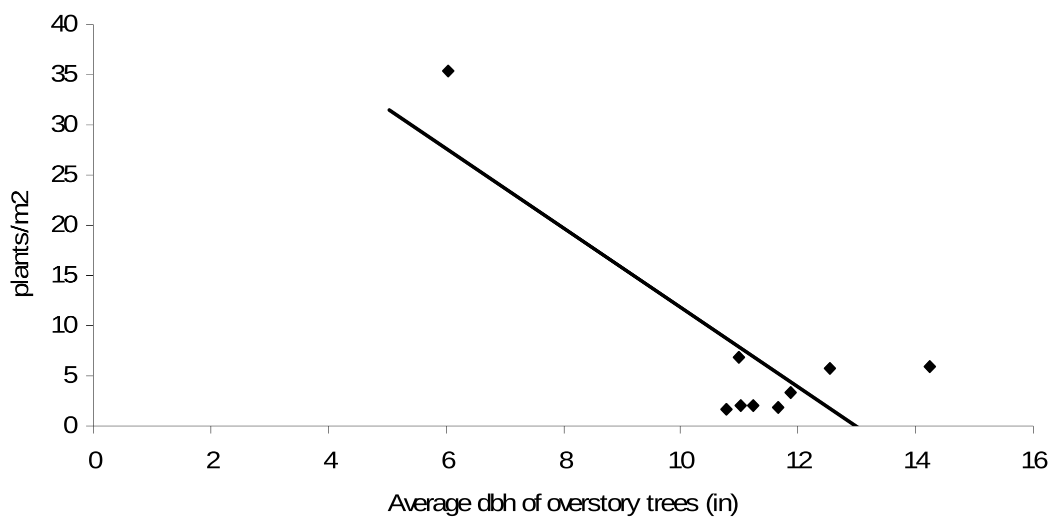

Figure 9. Regression analysis of average overstory tree dbh as a predictor of bloodroot (*Sanguinaria canadensis*) stand density on the Waynesville watershed in Western North Carolina (outlier included). P=0.0081; R^2=0.66. Mean stand density (plants/m^2) = 51.6579 – 3.9843(average tree dbh).

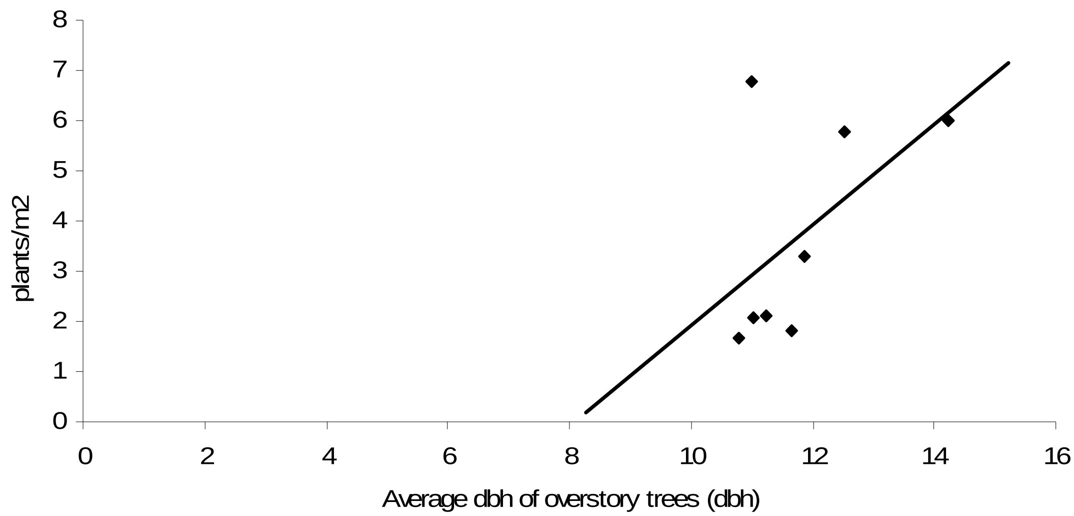

Figure 10. Regression analysis of average ovestory tree dbh as a predictor of bloodroot (*Sanguinaria canadensis*) stand density on the Waynesville watershed in Western North Carolina (outlier excluded). P=0.1744; R2=0.28. Mean stand density (plants/m^2) = -8.0783 + 0.9984(average tree dbh).

4.1c. Analysis of aboveground plant characteristics

Average stem heights of the nine stands of bloodroot ranged from 13.94 to 26.386 cm (Figure 11). As described in the Methods chapter of this document, the highest average stem height associated with the stands was classified as an outlier. Following Ott and Longnecker (2001), statistical analyses were run both with and without the outlier.

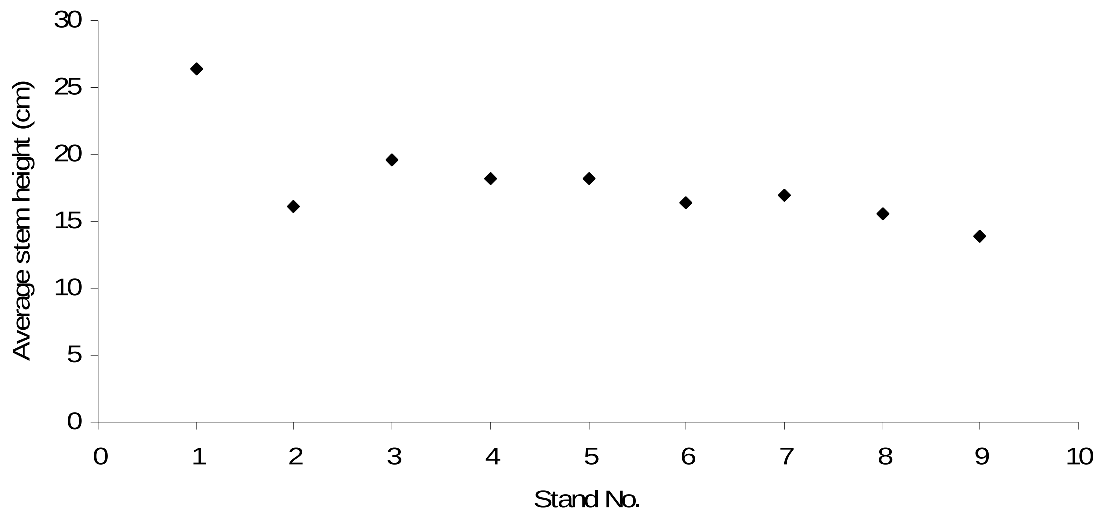

Figure 11. Average stem heights (cm) of nine stands of bloodroot (*Sanguinaria canadensis*) on the Waynesville watershed in Western North Carolina.

Average stem diameters of nine stands of bloodroot ranged from 0.233 cm to 0.402 cm (Figure 12). As described in the Methods chapter, the stand associated with the outlier density had a higher average stem height than the other stands, but this stand was not classified as a numerical outlier.

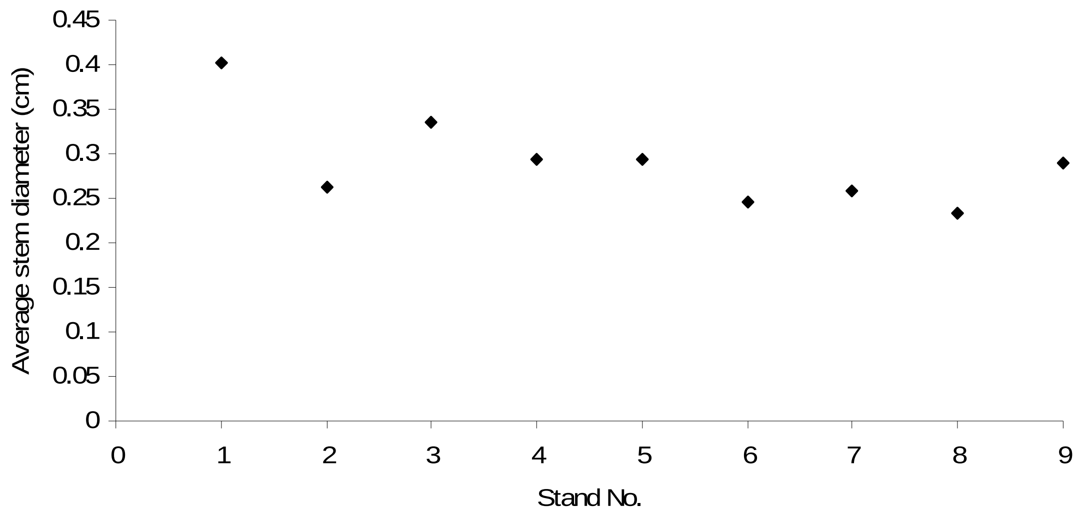

Figure 12. Average stem diameters (cm) of nine stands of bloodroot (*Sanguinaria candensis*) on the Waynesville watershed in Western North Carolina.

The aboveground plant characteristics of stem height (cm) and stem diameter (cm) were analyzed for a correlation with the average diameter at breast height (dbh) of the overstory cover trees located at stand center. When analyzed with the outlier, average stem height is significantly negatively correlated with average dbh (SLR with F=28.665; df=1,7; P=0.0011; Figure 13). When the outlier is excluded, the correlation no longer remains significant (SLR with F=2.471; df=1,6; P=0.1670). However, the trend for increasing average dbh to correlate with a decrease in average stem height is still evident.

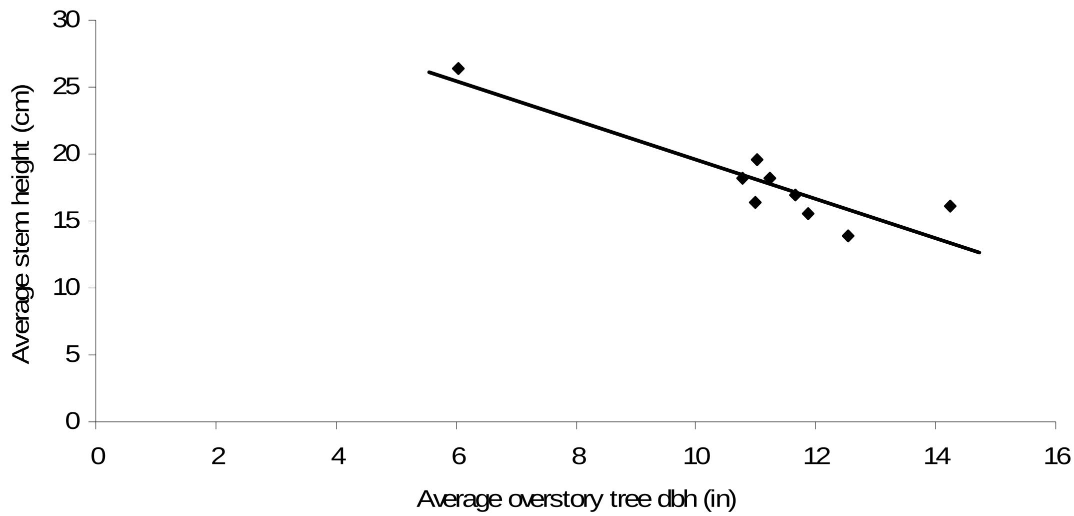

Figure 13. Regression analysis of average overstory tree dbh (in) as a predictor of average stem height (cm) for nine bloodroot (*Sanguinaria canadensis*) stands on the Waynesville watershed in Western North Carolina (outlier included). P=0.0011; R^2=0.80. Average stem height (cm) = 34.2649 - 1.466(average tree dbh (in)).

Average stem diameters for the nine stands of bloodroot are negatively correlated with an increase in average tree dbh (SLR with F=11.295; df=1,7; P=0.0121; Figure 14). As the average dbh of the overstory trees at stand center increases, there is a decrease in average stem diameter.

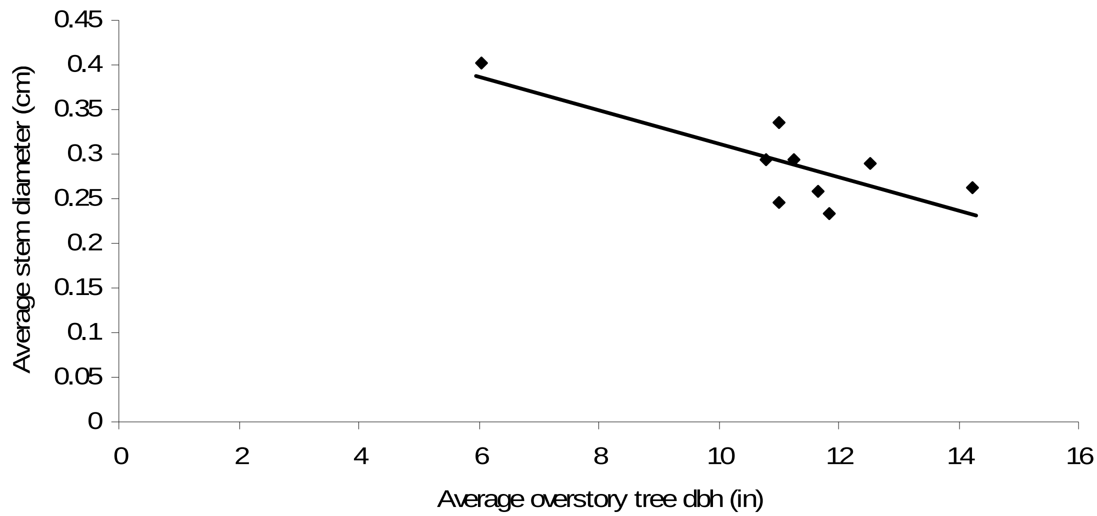

Figure 14. Regression analysis of average overstory tree dbh (in) as a predictor of average stem diameter (cm) for nine stands of bloodroot (*Sanguinaria canadensis*) on the Waynesville watershed in Western North Carolina. P=0.0121; R=0.62. Average stem diameter (cm) = 0.498 – 0.0186(average tree dbh (in)).

4.2. Analysis of Belowground Biomass Data

Site-specific allometric equations were developed relating the aboveground plant characteristics of stem height, stem diameter and number of leaf lobes to the belowground biomass of rhizomes. The relationship between the predictor variables (stem height, stem diameter, number of lobes) and the response variable (rhizome weight) was determined using multiple linear regression analyses. The results of a multiple linear regression demonstrate that the model is statistically a good fit for the data (R^2=0.77, F_{model}=189.145, P_{model}<0.0001). The allometric equation for predicting belowground biomass with the multiple linear regression model including all three predictor variables is:

Rhizome weight (g) = -4.4807 + 0.1063(number of lobes) + 0.1623(stem height) + 17.912 (stem diameter).

The parameter estimates of stem height and stem diameter are good predictors of belowground biomass ($P_{stem\ diameter}$<0.0001, $P_{stem\ height}$<0.0001), but the variable of number of lobes is not a statistically significant predictor of belowground biomass (P_{lobes}=0.3042). However, a simple linear regression model with rhizome weight as the response variable and number of lobes as the predictor variable has an R^2 value of 0.54 and a statistically significant P value of <0.0001 (Figure 15).

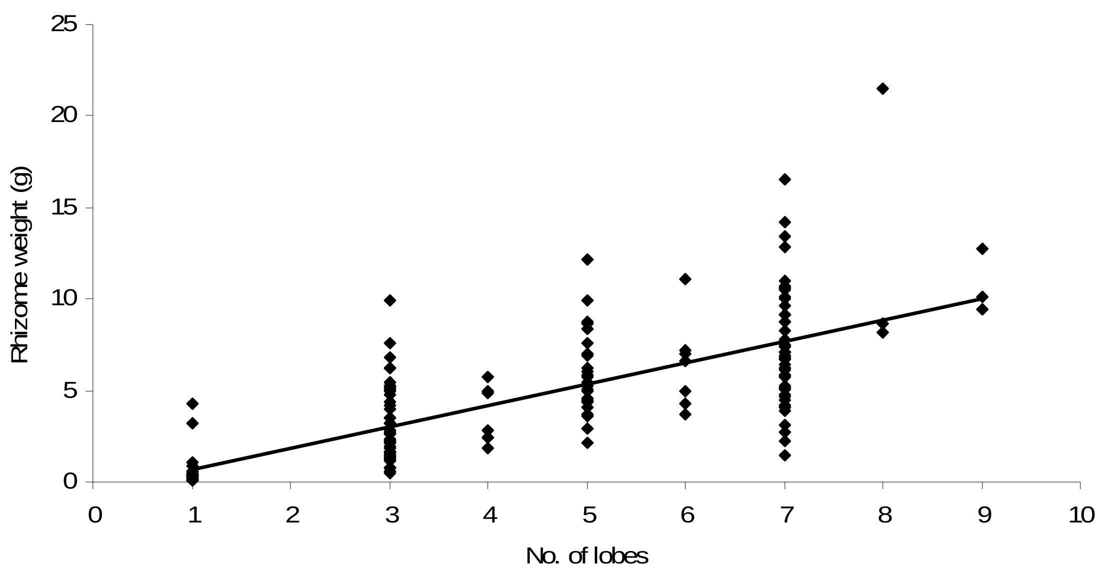

Figure 15. Regression analysis of the number of leaf lobes as a predictor of rhizome weight for 174 bloodroot (*Sanguinaria canadensis*) plants on the Waynesville watershed in Western North Carolina. Rhizome weight (g) = -0.0516 + 1.1748(number of lobes); R^2=0.54; P<0.0001.

Following the methods of Bond-Lamberty et al. (2002), the intercept was included in all regression equations to avoid inflated R^2 values. However, it is recognized that root weight would equal zero for a zero stem diameter or stem height.

To further examine the relationship between each of the predictors of belowground biomass individually, two additional simple linear regressions were run – one with stem height as a predictor variable of rhizome weight and one with stem diameter as a predictor of rhizome weight. Both variables are good predictors of belowground biomass ($R^2_{stem\ height}$=0.653; P_{model}<0.0001; $R^2_{stem\ diameter}$=0.729; P_{model}<0.0001, Figures 16 and 17).

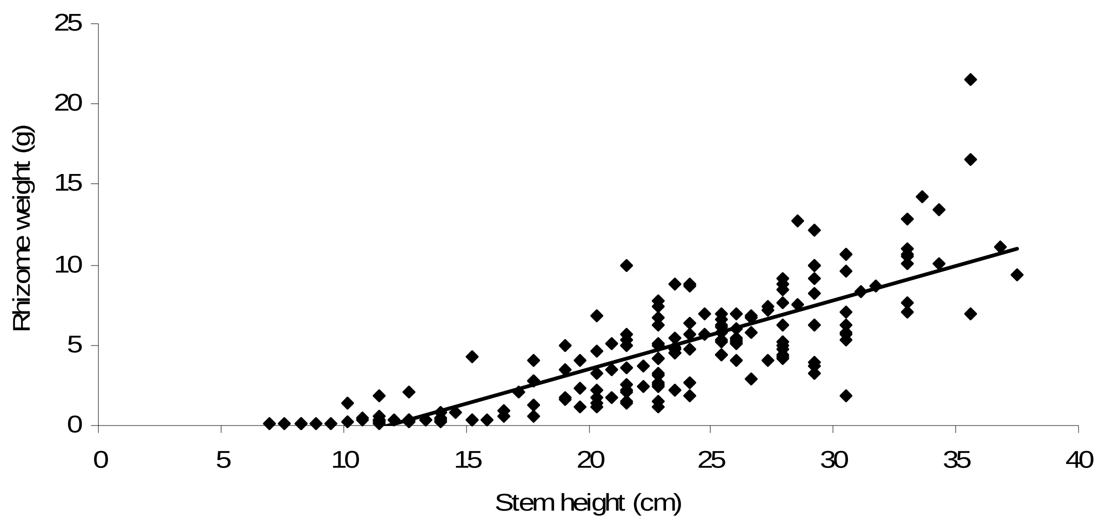

Figure 16. Regression analysis of stem height (cm) as a predictor of rhizome weight (g) for 174 bloodroot (*Sanguinaria canadensis*) plants on the Waynesville watershed in Western North Carolina. Rhizome weight (g) = -5.1387 + 0.4319(stem height); R^2=0.65; P<0.0001.

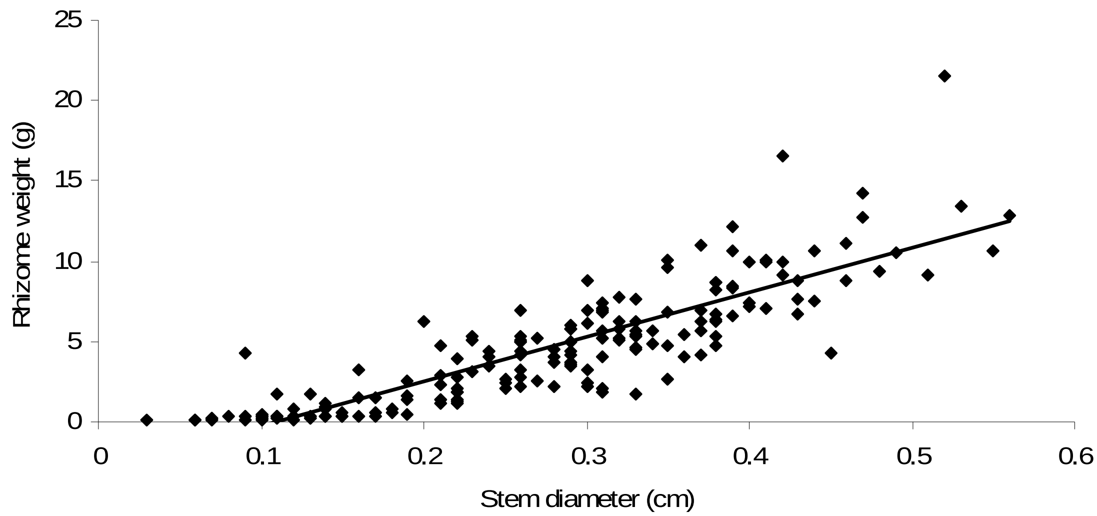

Figure 17. Regression analysis of stem diameter (cm) as a predictor of rhizome weight (g) for 174 bloodroot (*Sanguinaria canadensis*) plants on the Waynesville watershed in Western North Carolina. Rhizome weight (g) = -2.9825 + 27.5459(stem diameter); R^2=0.73; P<0.0001.

When the multiple regression analysis was repeated excluding number of lobes as a predictor variable the model continued to provide a good fit for the data (R^2=0.77, P_{model}<0.0001). The allometric equation determined for predicting belowground rhizome biomass with only the statistically significant predictor variables of stem height and stem diameter is:

Rhizome weight (g) = -4.6284 + 0.1802(stem height) + 18.7254(stem diameter) .

5.0 Discussion

This study examines the current supply of bloodroot, *Sanguinaria canadensis*, on the Waynesville watershed in Western North Carolina in order to explore the potential for sustainable use of the plant as a non-timber forest product. Sustainable, as used in this study, has both biological and managerial components. Sustainable use should allow the continuation of viable natural populations and should incorporate sound management practices that allow indefinite yields of the resource. Secondary goals include maintenance of biodiversity within natural stands, preservation of traditional knowledge and providing a source of income for local communities.

In order for sustainable use to occur, density and size-class structure data are required fundamentals for establishing sound management practices (Peters 1994). Forest inventories, completed before the development of a harvesting regime, establish a reference point for guiding future management. Knowledge of bloodroot's current status in natural stands will aid in exploring the potential of this resource to be sustainably utilized.

Throughout the process of reviewing relevant literature, it became apparent that there is a scarcity of literature relating to the sustainable use of non-timber forest products (Wong 2000), especially for bloodroot. In order to address this gap in the scientific literature, this study provides plant size and stand density information of nine stands of bloodroot in the Waynesville watershed as a basis for a forest inventory. A separate analysis of aboveground plant characteristics and belowground biomass may contribute to guidelines for sustainable harvest of the resource. A brief discussion of the distribution and long term sustainability of bloodroot in Western North Carolina provides a conclusion and suggestions for future research.

5.1a Inventory Data

Eight of the nine bloodroot stands inventoried are located on northern hardwood slopes or poplar coves in the Waynesville watershed. The ninth stand was located in a mesic oak-hickory stand. The primarily north-facing slopes are some of the most productive in the region (Peter Bates, personal communication) and it is important to note that the Waynesville watershed is a protected tract of forest, where timber harvesting is not permitted. In this sense, it is not a typical representation of Southern Appalachian forests and results should be interpreted in light of this fact.

The 65 to 70 year old hardwoods stands are typical of stands growing on high quality sites in Southern Appalachia (Peter Bates, personal communication), and bloodroot is often associated with rich coves and northern aspects.

Compared to a continuous distribution, bloodroot stands on the Waynesville watershed are clumped within the suitable habitat types (northern hardwood slopes, poplar coves and mesic oak-hickory). Excluding the outlier stand with a small area of only 5.28 square meters, the total area of the other eight stands ranged from approximately 4,800 to 47,400 square meters (4.1a, Table 2). In total, 34.95 acres of bloodroot were located and sampled on the Waynesville watershed.

Eight of the nine bloodroot stands inventoried for this study had average stand densities of 6.77 plants per square meter or less. The outlier stand had an average stand density of 35.42 plants per square meter and an average stem height of 26.386 cm (Table 1, Section 4.1a). Both of these values were numerical outliers according to the definition that an outlier is any data point more than 1.5 times the interquartile range (IQR) outside of the IQR of the data set. These data points are valid in that they are not the result of sampling or equipment error, and should therefore not be simply thrown out. However, the stand was unusual when compared to all other stands seen in Western North Carolina. Following Ott and Longnecker (2001), analyses were run both with and without the outlier to determine which results were sensitive to the outlier data points.

Five statistical analyses were run involving the outlier data points. In four of these analyses, removing the outlier significantly changed the results from statistically significant at the $\alpha=0.05$ level to statistically insignificant. Simple linear regressions run to examine the mean stand density data for the nine stands (4.1a) are statistically insignificant both with and without the outlier, indicating that there is no statistical difference in mean stand densities of the bloodroot stands.

Large differences in the P-values of all five analyses run with and without the outlier data points indicate that the outlier data points associated with the high density stand (mean average stand density and average stem height) have a strong influence over the data set, likely made more pronounced due to the small sample size. Results should be considered in light of this fact.

Biological justification for considering the high density and high average stem height as outliers is found through personal observation and the literature. The high density stand was the smallest stand in total area and located in a patch of bright sunlight with very little overstory cover. It is known that bloodroot responds to increased sunlight through increased clonal growth "as measured by leaf size and leaf number" (Marino et al. 1997).

Marino et al. (1997) suggest that "local populations of *S. canadensis* rapidly respond to increases in light and nutrients associated with disturbance through increased clonal growth. This rapid response to environmental variability may partly explain the patchy spatial distribution of *S. canadensis* populations." As disturbance conditions (increased light and nutrients) are temporary, it is possible this stand of bloodroot will thin out as time and succession progress. It is important to note this stand was very small (5.28 m^2) compared to the other stands, whose mean area is 17,679 m^2, and bloodroot would not be found growing naturally in large open fields or in very young stands with a low mean tree dbh and lots of competitive herbaceous cover. Too much sunlight and not enough shade are not favorable bloodroot conditions and this site still had shade cover for at least part of the day.

5.1b Bloodroot Stand Densities

Analysis of the relationship between mean stand densities (plants per square meter) and the aboveground plant characteristics of stem height (cm) and stem diameter (cm) reveals a positive correlation between the variables when the outlier stand density and corresponding mean stem height are included in analysis. These results suggest that as the individual plant sizes increase mean stand density increases as well. However, when the outlier is excluded - and as noted in the above section, both statistical and biological reasoning exists for excluding the outlier stand – the relationship between the variables changes, with a decrease in plant size associated with an increase in mean stand density.

These results makes intuitive sense as well – as more plants become established in a limited area with limited nutrients, growth rates will decline.

When the outlier is included, regression analysis of the relationship between mean stand densities and the average dbh of overstory trees suggests a negative correlation between mean stand density and the average diameter at breast height (dbh) of the overstory trees at stand center. As the average dbh of overstory trees increases, the mean stand density declines. Bloodroot clonal growth has been shown to respond vigorously to increased sunlight (Marino et al. 1997) and the smaller average diameter of overstory trees may equate with more sunlight reaching the forest floor, which may result in denser stands of bloodroot. Marino et al. (1997) found that high-light sites had a much higher mean stand density (19.6 plants per square meter) than low-light sites (4.0 plants per square meters) and states that "Field observations also suggest that a patchy population structure and variation in plant vigor among patches of *S. canadensis* may be a result of variation in the intensity of solar radiation reaching the forest floor." It is important to interpret these results within the range of the data (dbh range of 6.054 to 22.857 in); an extremely low average tree dbh would not necessarily correlate with an increase in mean bloodroot stand density, and too little overstory cover will prohibit bloodroot growth.

When the outlier is excluded, the relationship is no longer statistically significant, and the opposite trend becomes apparent – as average dbh increases there is an increase in mean stand density (4.9b, Figures 9 and 10). Although this relationship is not statistically In significant, it suggests that the relationship between bloodroot sizes and overstory tree dbh needs further exploration.

addition to the quantitative measurements made on the Waynesville watershed, both qualitative and quantitative assessments were conducted on several differently managed tracts of forest in North Carolina. A bloodroot stand bordering lake Raleigh in Raleigh, North Carolina consisted of small, single-lobed plants rarely exceeding seven or eight cm in height. In comparison, the stands on the Waynesville watershed often contained up to nine lobes and had a mean height of 17.9 cm, including the outlier stand. Excluding the outlier, the mean average stem height for the watershed is 16.9, still much greater than the Lake Raleigh location. The Lake Raleigh site is at a lower elevation, around 130 meters, contains a different tree composition and is relatively flat compared to the slopes of the Waynesville watershed. Poor drainage and periodic flooding may have resulted in the short-stature of the bloodroot plants, which prefer well-drained soils.

The stands at Bent Creek experimental forest had an approximate mean stem height of 14 cm. This value is closer to the Waynesville watershed average stem height, but still notably decreased. The Bent Creek forest is managed by the United States Forest Service for recreation and four of the five stands studied had experienced timber harvests within the last 45 years, one as recently as 1983. Several of the stands had large amounts of leaf litter, herbaceous groundcover and competing vegetation. In addition, the sites had high levels of light compared to the Waynesville watershed. These site conditions may contribute to the diminished bloodroot sizes and densities. The stands were distinctly clumped in nature. In contrast, the bloodroot growing on the Bryson City watershed appears to be more continuous in distribution. However, the densities still appeared to be notably less than on the Waynesville watershed.

The final bloodroot site for comparison is located in Western North Carolina near the Waynesville watershed and is open to harvesting. In addition, there are periodic timber harvests and the forests are managed for a variety of uses. Likely due to the similarity in forest type, these plants had comparable mean stem heights to those on the Waynesville watershed, but stand densities were notably reduced. The mean stand density for this location, based on sampling of six stands, is 0.9 plants per square meter compared to 7.2 plants per square meter on the Waynesville watershed. If the Waynesville high density stand outlier is removed, the mean stand density drops to 3.7 plants per square meter, still significantly larger than the harvested stands. Due to the similarity in forest types and bloodroot plant sizes, it is likely that this discrepancy in stand density can be attributed to the increased pressure from harvesting. If this harvesting is indeed the source of the diminished bloodroot densities, sustainable management of the resource is required to prevent further decline of bloodroot populations.

5.1c Aboveground plant characteristics

The mean stem height associated with the outlier high density stand is also a numerical outlier. With a calculated average of 26.386 cm, the mean stand height is 8.243 cm greater than the next tallest stand. As with the high density value, the tall stems may be a result of the high light and open canopy associated with the stand. If located in a recent gap, the disturbance conditions may have created an abundance of light and nutrients leading to high bloodroot clonal growth. The mean stem diameter of 0.402 cm associated with the outlier high density stand is 0.77 cm greater than the next highest value, but is not considered a numerical outlier.

Mean stem height (cm) is significantly negatively correlated with average overstory tree dbh (in) when the outlier stand is included (4.1c, Figure 13). When the outlier is excluded the results are no longer significant at the $\alpha=0.05$ limit, but the trend for increasing dbh to correspond with a decreasing stem height remains. If average overstory dbh can reasonably be associated with shade cover, one possible explanation is that as less light reaches the forest floor bloodroot stem height growth declines.

Mean stem diameter (cm) of the bloodroot stands is also significantly negatively correlated with average overstory tree dbh (in) (4.1c, Figure 14), further supporting the hypothesis that if average tree dbh can be associated with shade cover, increasing average overstory tree dbh may cause a decrease in plant growth due to limited sunlight, within the range of the data. It is also possible that increased tree growth results in limited nutrients, which may in turn lead to a decline in bloodroot growth rates.

These result indicate that the largest bloodroot plants will be found in medium-sized hardwood stands on northern aspects. As the mean dbh of mature stands increases, bloodroot growth may decline. Small disturbances that create gaps in the canopy likely facilitate the growth of more robust bloodroot populations.

5.2 Belowground Biomass

Plants that are harvested for their root or rhizome are especially susceptible to population decline due to harvesting since mortality rates are high if careful collection practices are not followed (Hayden 2005). Members of the Eastern Band of Cherokee Indians recommend re-planting a piece of the rhizome back into the earth while harvesting bloodroot in order to maintain viable populations. This practice is in accordance with recommended propagation methods, which suggest breaking the rhizome into small pieces (approximately one to two inches) for successful clonal propagation.

The analyses of the relationship between easily recognized aboveground characteristics (stem height, stem diameter and number of leaf lobes) and belowground biomass of rhizomes were performed to aid in the development of sustainable harvesting regimes. If a harvester can make a prediction of belowground biomass without actually harvesting the plant, sustainable harvesting practices are more feasible. If harvesters can practice selective harvesting, plant mortality rates may decline.

Regression analysis of the 174 harvested rhizomes and their corresponding aboveground plant parts reveal that a model including the three predictor variables - stem height (cm), stem diameter (cm) and number of leaf lobes – is statistically a good fit (R^2=0.77; P<0.0001). An R^2 value of 0.77 means that 77 percent of the variation in response can be attributed to the aboveground plant characteristics of stem diameter, stem height and number of leaf lobes. However, the parameter estimate of number of lobes was not statistically significant at the α=0.05 level and separate regression analyses of each of the predictor variables were run.

Both stem diameter and stem height were good predictors of belowground biomass (4.2, Figures 16 and 17), although with an R^2 value of 0.73, the predictor variable of stem diameter had a higher R^2 value than the predictor variable of stem height, with a R^2 value of 0.65. A final model including both stem characteristics as predictor variables resulted in an R^2 value of 0.77. Adding stem height to the model results in an increase in R^2 of only 0.04, but it is included as a predictor in the final allometric equation because of its usefulness as an easily recognizable variable in the field. Harvesters can quickly estimate stem height when collecting bloodroot in the wild, and use this estimate as a predictor of harvestable material belowground.

5.3 Bloodroot Supply

Bloodroot growth areas made up approximately 34.95 acres (141,445 m^2) of the 432.9 acres (1,752,000 m^2) of suitable habitat cruised, or about 4.2 percent. According to work on the ecological zones of the Southern Appalachian Mountains by Simon et al. (2005), 695,000 of the 5,640,700 total acres of Southern Appalachian Mountains can be considered Northern Hardwood or Rich Cove ecological zones. If the Waynesville forest types can be considered representative of Southern Appalachia, these figures suggest that only 29,190 acres of bloodroot are located in the region. This number represents only 0.5 percent of the total area. If the 1,772,000 acres of Mesic Oak-Hickory (Simon et al. 2005) are added to the total area of suitable habitat, the total number of bloodroot acres is still only 103,614, or 1.8 percent, and this is likely over-estimating the total acres of bloodroot growth. Furthermore, as described above (5.1b), it appears that bloodroot growth on the Waynesville watershed may not be representative of the region as a whole. Other areas studied had smaller plants and lower mean stand densities of bloodroot, suggesting that total bloodroot areas in Southern Appalachia are even less than projected above.

5.4 Future Research

This study attempts to evaluate the current biological supply of bloodroot (*Sanguinaria canadensis*) on the Waynesville watershed in Western North Carolina. Such a study is necessary for providing baseline data for subsequent harvesting of the resource, and provide a context for making comparisons between protected stands of the resource and areas subject to harvesting and mixed-use management.

Based on the research and data collection involved in this study, several directions for future research efforts became apparent. A study directly comparing various harvesting rates would be beneficial in elucidating the effects of harvesting on bloodroot growth and reproduction. In addition, ethnographic studies involving harvesters and users of bloodroot would be helpful in determining if harvesting rates are increasing and if harvested stands are experiencing an increased rate of decline. As Hayden (2005) notes, historic ranges of medicinal plants are often unknown, contributing to the difficulty in interpreting current supply. Traditional knowledge of previous distributions will aid in assessing current populations.

A detailed analysis of stand types and bloodroot distribution across stand types would help identify suitable habitat in Western North Carolina. Research on suitable stand types would be beneficial in establishing natural stands for harvesting within diminished bloodroot stands and may help harvesters secure a reliable source of wild-grown bloodroot.

Finally, based on the qualitative studies of a variety of sites it does not appear that the Waynesville watershed is representative of bloodroot stands. Sampling of more stands under a variety of management types will shed further light on how representative the Waynesville stands are and will help put the diminished stands on the harvested public lands in context.

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Appendix

Appendix

Table 3. Bloodroot (*Sanguinaria canadensis*) stand location and site description on the Waynesville watershed in Western North Carolina.

Stand No.	Geographic coordinates	Altitude (m)	Aspect (degrees)	Slope (degrees)	Plot Length (m)	Plot width (m)
1	35°24.327' N 82°24.327'W	1133.858	90	10	2.4	2.2
2	35°23.958'N 82°59.718'W	1302.715	40	30	177	70
3	35°23.876'N 82°59.613'W	1308.811	90	40	150	130
4	35°24.329'N 83°00.885'W	1251.814	65	35	150	70
5	35°25.510'N 83°01.075'W	1154.278	50	40	80	60
6	35°24.955'N 83°00.904'W	1051.865	45	30	170	70
7	35°24.607'N 83°00.266'W	1162.202	0	40	210	95
8	35°24.546'N 83°00.253'W	1162.202	345	30	395	120
9	35°24.508'N 83°00.602'W	1170.432	15	35	130	140

Table 4. Tree species and quantities located at the stand center (1/20 hectare) of the nine stands of bloodroot (*Sanguinaria canadensis*) on the Waynesville watershed in Western North Carolina.

Stand No.	Species Richness	Tree Species	Quantity
1	5	*Acer saccharum*	3
		Acer rubrum	13
		Robinia pseudoacaia	13
		Liriodendron tulipifera	3
		Halesia diptera	14
2	4	*Acer rubrum*	11
		Robinia pseudoacacia	2
		Tiliacea	1
		Prunus serotina	3
3	8	*Acer pennsylvanicum*	2
		Acer rubrum	1
		Robinia pseudoacacia	1
		Betula	1
		Hippocastanaceae	2
		Tiliacea	5
		Prunus serotina	2
		Magnolia acuminate	1
4	5	*Acer rubrum*	9
		Robinia pseudoacacia	2
		Quercus 1	2
		Quercus 2	2
		Magnolia acuminata	19
5	7	*Acer rubrum*	1
		Robinia pseudoacacia	1
		Betula	1
		Tiliaceae	3
		Tsuga	1
		Magnolia acuminate	12
		Magnolia fraseri	1
6	5	*Acer rubrum*	6
		Robinia pseudoacacia	4
		Quercus sp.	1
		Liriodendron tulipifera	1
		Magnolia acuminata	7

Table 4 (continued).

Stand No.	Species Richness	Species	Quantity
7	8	*Unknown*	1
		Acer	1
		Acer rubrum	1
		Liriodendron tulipifera	1
		Betula	1
		Tiliaceae	3
		Prunus serotina	1
		Magnolia acuminata	6
8	5	*Acer pennsylvanicum*	1
		Acer rubrum	3
		Quercus sp.	1
		Liriodendron tulipifera	3
		Magnolia acuminate	6
9	6	*Acer rubrum*	6
		Robinia pseudoacacia	1
		Liriodendron tulipifera	2
		Tiliaceae	5
		Prunus serotina	2
		Magnolia acuminate	4