

ABSTRACT

ONOFRIETTI, MARIA. Propagation and Cultivation of Bloodroot (*Sanguinaria canadensis*). (Under the direction of David Danehower and Jeanine Davis.)

Bloodroot (*Sanguinaria canadensis* L. [Papaveraceae]) is a rhizomatous perennial, native throughout eastern North America (ITIS 2005). The species has a long history of medicinal use by Native Americans and early settlers. It is still used today in homeopathic medicine, but the major market for bloodroot is in Europe where it is being used as an alternative to antibiotic performance promoters in animal feed. The European market demand is met almost entirely by a supply coming from wild populations in eastern North America.

Research investigations with bloodroot were conducted in order to develop commercially feasible propagation and cultivation techniques. Agricultural production should reduce the impact on wild populations that comes from wild harvesting.

Studies were conducted to determine if chilling is required in order to break rhizome bud dormancy and, if so, to approximate how much chilling time is required. Different propagule types were studied for effects on plant emergence and growth.

Response to chilling was tested by storing rhizomes at 4° C for 28, 56, 84, and 112 days. In general, percent emergence increased with increased chilling time and days from planting to emergence decreased with increased chilling time. The optimal chilling time was between 56 and 84 days.

Studies were conducted to determine the best propagation methods to use for field production. Bloodroot rhizomes were cut and divided into three treatments consisting of different propagule types: 1) sections with a bud, 2) sections without a bud, and 3) whole rhizomes. Plots were established under a polypropylene shade structure and in woodland

tilled beds. Percent emergence, seed pod and flower production, rhizome weight, and rhizome number at harvest were measured. First year data showed emergence was significantly higher for whole rhizomes and rhizome sections with a bud than for rhizome sections without a bud. Second year data showed no difference between treatments. At harvest, there was no difference due to propagation treatments in rhizome weight and number, however, the rhizomes harvested from plots in the shade structure were greater in number and weight than those harvested from the woodland plots. In contrast, percent alkaloid content per unit dry mass was significantly greater in bloodroot grown in the woodland plots than under artificial shade.

PROPAGATION AND CULTIVATION OF BLOODROOT (*Sanguinaria canadensis*)

by
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DEDICATION

In loving memory of
George G. Pequignot
A beautiful soul who makes me smile
right down to my heart

BIOGRAPHY

Maria Felice Onofrietti was born and raised in upstate New York in the beautiful Hudson Valley. Her mother introduced her to the diversity of life in the woods near their home. Her love of plants, especially the plants of the Appalachians, comes from this early childhood experience.

Maria received her Associate degree in Political Science from Orange County Community College in New York. She has called North Carolina home since 1996 and has earned Bachelor of Science degrees in Biology and Botany from North Carolina State University. She will receive her Master of Science degree from North Carolina State University in 2007. Maria is currently employed at Bayer CropScience in their Plant Pathology lab in Research Triangle Park, NC.

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Propagation and Cultivation of Bloodroot (*Sanguinaria canadensis*).

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LITERATURE REVIEW

Background

Bloodroot (*Sanguinaria canadensis* L. [Papaveraceae]) is a woodland wildflower native to North America (ITIS 2005). *Sanguinaria* is a monotypic genus in the poppy family. The name is derived from the Latin *sanguis* meaning blood, which refers to the blood-red juice that exudes from the rhizomes and stems when wounded (Grey-Wilson 2000). Some other common names for this plant are Indian paint, tetterwort, red root, red puccoon, coon root, snakebite, and pauson (Gruenwald 2000).

Bloodroot has a long history of use by native North Americans and early European settlers. Fresh root was used in minute doses as an appetite stimulant. Larger doses were used as an arterial sedative. Root tea was used for rheumatism, asthma, bronchitis, lung ailments, pneumonia, fever and treatment of skin cancer. The root juice was applied to the skin to dissolve warts (Foster and Duke 2000; Hendershot 2002) and drops were added to maple sugar cubes for use in relieving sore throats and coughs (Herb Research Foundation 2001). Bloodroot was also used as a dye for clothing and basketry and as skin paint by some Native Americans. Bloodroot was listed in the *Pharmacopoeia of the United States* from 1820 to 1913 as a stimulating expectorant and emetic (Hayward 1982).

In its more recent history, bloodroot was used commercially as a plaque-inhibiting agent in toothpaste and mouthwash (Foster and Duke 2000). Currently there is a small demand for bloodroot in homeopathic medicine for such uses as headache remedy and poison ivy treatment (Insley 2004). Also there is a small demand from the ornamental garden industry where woodland wildflowers are a popular segment of the market in the native plant trade (Cullina 2000). Bloodroot is occasionally still used as a treatment for skin cancer in

some folk remedies and there has been current research in this area as well. One such study compared the antiproliferative and apoptotic potential of sanguinarine, derived from *Sanguinaria canadensis*, against human epidermoid carcinoma cells and normal epidermal keratinocytes. Sanguinarine dose-dependently decreased the viability of carcinoma cells and normal cells. However, sanguinarine-mediated loss of viability occurred at lower doses in the carcinoma cells. The researchers concluded that sanguinarine could be developed as an anticancer drug (Nihal 2000). Sanguinarine has also been found to be effective against multidrug resistance in human cervical cells where other drugs used for treating cervical cancer are often impeded by chemotherapy resistance (Ding et al. 2002). A 2003 study looked at the in vitro susceptibility of *Helicobacter pylori*, the bacteria associated with stomach ulcers, to isoquinoline alkaloids from *Sanguinaria canadensis* and found antibacterial activity against 15 strains (Mahady et al. 2003).

The predominant demand for bloodroot in 2001 came from Europe where it was used in an animal feed additive (Greenfield and Davis 2003). A German company, Phytobiotics, makes a product called Sangrovit in which bloodroot, at that time, was the critical ingredient. Sangrovit is used as a natural alternative to antibiotic performance promoters (Phytobiotics 2002). At the time this research project was initiated and the following studies were conducted, the demand for bloodroot was greater than could be supplied from wild harvesting. Because of the length of time and expense involved in cultivating bloodroot, Phytobiotics has looked into alternative sources of plants which contain the same bioactive alkaloids as bloodroot. This led to the use of *Macleaya cordata* (Willd.) R. Br. [Papaveraceae] as an alternative source of alkaloids (Danehower 2007).

Botany and Ecology

Bloodroot is native to the eastern half of North America. Its natural range is from Nova Scotia and Manitoba south to Florida and Texas. It is particularly prominent along the Appalachian mountain range (Hayward 1982; Cullina 2000; Grey-Wilson 2000). Bloodroot inhabits moist deciduous forests with a canopy consisting of such trees as *Liriodendron tulipifera* L. (Magnoliaceae), *Acer saccharum* Marshall (Sapindaceae), and *Fagus grandifolia* Ehrhart (Fagaceae). The soil is usually well drained and humus rich, with a pH that is slightly acidic to neutral (Hunter 2002; ITIS 2005). Bloodroot is prominent on wooded slopes in mesic coves, (Phillips 1985; Cullina 2000) open woodlands, semi-shaded roadsides, and meadow borders (Hayward 1982). Bloodroot is often found growing alongside other woodland wildflowers such as black cohosh (*Actaea racemosa* L. Nutt. [Ranunculaceae]), mayapple (*Podophyllum peltatum* L. [Berberidaceae]), trillium (*Trillium* L. [Liliaceae]), Solomon's seal (*Polygonatum biflorum* Walt. Ell. [Liliaceae]), and ginseng (*Panax quinquefolius* L. [Araliaceae] (ITIS 2005; Onofrietti Pers. Obs 2003).

Bloodroot is a spring ephemeral (Phillips 1985). Its flowers are short-lived, lasting only two or three days (Hayward 1982; Phillips 1985; Hendershot 2002). The flower comes up in late winter or early spring before the forest canopy has leafed out. Each flower bud is on a separate scape and is completely enclosed by the developing leaf as it first pushes up through the leaf litter (Phillips 1985). The flower scape reaches a mature height of 7 – 15 cm and produces a bloom 3 – 7 cm in diameter (Phillips 1985; Grey-Wilson 2000; Gruenwald 2000). Flowers have yellow stamens and 8 – 12 pure white petals (Hayward 1982; Phillips 1985; Hendershot 2002). The leaves continue to grow after flowering is finished. The petiole reaches a height of 15 – 31 cm and bears one basal palmately lobed leaf (Hayward 1982;

Phillips 1985; Grey-Wilson 2000). The leaf is slightly to deeply lobed, rounded, and can be up to 31 cm across.

Bloodroot is an herbaceous perennial that develops a thick fleshy rhizome. The rhizomes are dark red to brown, knobby, usually branching and lie horizontal close to the soil surface (Cullina 2000; Grey-Wilson 2000). Rhizomes have also been described as having strong apical dominance with little or no branching and can be found on the soil surface directly below the leaf litter (Danehower 2007). As the plant grows, rhizomes produce new growth and develop a large and lifeless pith section at the distal end. In early autumn, plants begin to send out new feeder rootlets that establish the plant for the next spring (Hayward 1982).

Bloodroot reproduces asexually by branching rhizomes and sexually by seed production. One to two weeks after the flower petals drop, a long slender light green capsule may be noticeable atop the scape. This narrowly elliptic capsule is the seedpod containing 25 or more seeds. It is slightly swollen but still green at maturity (Phillips 1985). When the seeds are fully mature, the capsule bursts open dispersing the seed. Seeds are glossy chestnut brown in color (Hayward 1982) and have a white fleshy appendage called an elaiosome. Elaiosomes are rich in lipids, proteins, sugars, and vitamins and are a major attractant to ants (Beattie 1982, 1990; Cullina 1999). Ants are therefore a major dispersal agent of bloodroot seed. They carry the seed back to their nest where the elaiosome is removed and eaten, leaving the rest of the seed intact and viable. There may be a selective advantage provided by the relocation of seeds to ant nests which are moist, richer in nutrients than surrounding soils, and where there is a greater chance that seeds will escape the attention of predators (Beattie 1982, 1990).

Based on pollen ovule ratios, Cruden (1977) classified bloodroot as xenogamous and Judd (1977) stated that the main pollinators are bees. In contrast, Schemske (1978) indicated that an Illinois population of bloodroot was autogamous with flowers emerging and self-pollinating in a matter of hours. Upon personal observation of greenhouse-grown bloodroot where no pollinators were seen, plants produced few seed pods. Of the seed pods produced, few contained seeds. This indicates that bloodroot may rely heavily, although not entirely, on insect pollinators or environmental conditions such as wind and rain for pollination. Perhaps the most likely description of bloodroot reproductive biology was made by Lyon (1992), who described bloodroot as facultatively xenogamous, being cross-pollinated by bees and self-pollinated when weather conditions are not favorable for pollinators.

Cultural Practices

Bloodroot is wild harvested in North America throughout its natural range, mostly along the Appalachian mountain range. Seed is not commercially available on a large scale and little is known about how to cultivate the plant on a commercial scale (Greenfield and Davis 2003). Bloodroot is one of more than 30 species of medicinal non-timber forest products collected from the National Forests of the Southern Appalachians (USDA 1999). The international supply of bloodroot is composed almost entirely of wild harvested material from the U.S. Bloodroot is difficult to harvest in a sustainable manner because the desired product is the plant's rhizome. With no large-scale production system in place, the rise in demand puts increasing pressure on wild populations. In addition to reducing the impact on native stands, cultivation could provide a good niche market for small scale North American farmers and enhance agricultural diversity and economic development in the Appalachian region, including rural western North Carolina (Davis 2004).

Bloodroot can be propagated by seed or rhizome. Seeds are intolerant of desiccation. Because they are hydrophilic, bloodroot seeds must be sown immediately upon ripening in the spring. (Hersey 1964; Phillips 1985; Cullina 2000; McCargo 2001). Cullina (2000) states that alternatively, seed can be stored after they are collected and cleaned if they are kept moist. They can be stored with dampened vermiculite in self-sealing plastic bags in the refrigerator for up to a year. Bloodroot seed is described by Cullina (1999) as requiring a 90-day moist stratification at 21° C (70°F) followed by a 90-day moist stratification at 4°C (40°F). Phillips (1985) cautioned that seeds stratified in moist sand tend to rot. Seed should be sown in a soil that is rich in organic matter and neutral or slightly acidic. Seed will usually germinate the following spring and flowering occurs two to three years from sowing. Various sources also state that seed should be sown in shade or partial shade with a covering of leaves (Hersey 1964; Phillips 1985; Grey-Wilson 2000).

Cullina (2000) says that while propagation is moderately difficult from seed, it is easy from rhizome division. He suggests that the rhizomes be handled in the fall when they are dormant. They can be dug and broken into 8 - 10 cm segments and replanted just below the soil surface in a moist but well-drained, lightly shaded location. Phillips (1985) and Hayward (1982) both state that the divided rhizomes sections should include one or more buds. Hayward (1982) also describes a lifeless pithy section that develops at the distal end of the rhizome and suggests that this portion should be broken off before the viable portions are replanted 20 – 25 cm (8 - 10 inches) apart.

Market.

There has been a steady growth in demand for bloodroot from the mid – 1990's. Demand currently exceeds supply, which has been met almost exclusively by wild harvested material.

A surge in demand for bloodroot that began in 2000 continues to put pressure on wild populations. The majority of material is used for animal or human consumption while a small amount is used for landscaping and gardening purposes. Currently, one major buyer dominates the market; Phytobiotics, a German company that produces the animal feed additive, Sangrovit, which contains bloodroot as a critical ingredient. One market analysis report estimated that bloodroot production for 2001 was approximately 18 metric tons (40,000 pounds) and demand was approximately 35 metric tons (77,000 pounds) (Herb Research Foundation 2001). Another market analysis report estimated that consumption of bloodroot in 2001 was approximately 61 metric tons (135,000 pounds) and the yearly projected demand from Phytobiotics alone was between 113 to 150 metric tons (250,000 to 330,000 pounds). From the mid-1990s up until 2000 the price of dried bloodroot rhizomes was \$11 - \$20 per kg. Currently, the price is \$20 - \$35 per dried kg. There is potential for the market to grow very aggressively. The Commission of European Communities stipulated that all synthetic antibiotic compounds incorporated into livestock feed had to be eliminated by the end of 2005 (Greenfield and Davis 2003). This ban coupled with the demonstrated safety and efficacy of bloodroot alkaloids as a replacement for antibiotics in poultry, swine, and cattle feeds could bring a rapid rise in demand (Danehower 2007). Alternately, commercial potential in the United States could drop drastically if bloodroot can be produced cheaper in other countries or if an alternative plant can be used for the feed additive (Greenfield and Davis 2003; Davis 2004). Most recently, the inability to meet growing demand for bloodroot rhizome from wild harvested supplies has led to the use of *Macleaya cordata* as an alternative source of the alkaloids for the Phytobiotics company (Danehower 2007).

Chemistry

The main bioactive components of bloodroot are alkaloids. Alkaloids are nitrogen-containing secondary plant products, so called because they are not essential for the primary metabolism of the plant. The purpose of alkaloids is most likely for plant defense against herbivores, fungi, bacteria and viruses. Alkaloids produced by bloodroot include the protopine alkaloids protopine and allocryptopine, the benzophananthridine alkaloids chelirubine, sanguinarine, chelerythrine, chelilutine, sanguilutine, (Salmore and Hunter 2001) and sanguirubine (Chauret et al.1990) and the protoberberine alkaloids berberine and palmatine (Schmeller et al. 1997). Sanguinarine is the predominant alkaloid, averaging up to 50% of the total. The orange-red color of the latex that oozes from wounded stems and rhizomes is due to the presence of sanguinarine. The Bennett et al. (1990) study of geographic variation in alkaloid content found that sanguinarine levels in bloodroot growing along the Appalachian mountain range were highest in the Southern portion of the range.

Sanguinarine has been found to inhibit choline acetyl-transferase and acetylcholine esterase, inhibit DNA synthesis and reverse transcriptase, and intercalate DNA (Schmeller et al. 1997). It has also been found to protect the aromatic amino acids, tryptophan and alanine, from decarboxylation to toxic biogene amines by inhibiting amino acid decarboxylase (Drasta et al.1996). The significance of this is its usefulness in the animal feed industry where it works as a performance promoter. In conventional feeding, intestinal flora that produce amino acid decarboxylase are minimized by antibiotics (Phytobiotics 2002).

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CHAPTER ONE

EFFECTS OF CHILLING TIME AND PROPAGULE TYPE ON EMERGENCE OF BLOODROOT.

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For submission to the Native Plants Journal.

Bloodroot (*Sanguinaria canadensis* L. [Papaveraceae]) is a rhizomatous perennial, native throughout eastern North America (ITIS 2005) where it has a long history of medicinal use. It is currently used in homeopathic medicine and as an animal feed additive in Europe. Bloodroot is almost exclusively wild harvested, raising concerns about over-harvesting of wild populations. Commercial cultivation of bloodroot could help reduce pressure on wild native stands. Studies were conducted to determine effects of chilling time and propagule type on dormancy and emergence of bloodroot rhizomes.

Response to chilling was tested by storing rhizomes at 4° C for 28, 56, 84, and 112 days before planting. Rhizomes were divided into pieces representing six different propagule types: 1) small lateral bud, 2) medium lateral bud, 3) large lateral bud, 4) rhizome piece with terminal bud only, 5) rhizome piece with no bud, and 6) whole rhizome. In general, percent emergence increased with increased chilling time and days from planting to emergence decreased with increased chilling time. The optimal chilling time was between 56 and 84 days.

Introduction

The U.S. market for medicinal herbs experienced enormous growth in popularity in the 1990s (Blumenthal 1998). Some herbs are cultivated to meet this demand while others, particularly woodland botanicals, are wild harvested. A large number of medicinal plant species are native to the Appalachian mountains of western North Carolina, which is one of the most bio-diverse regions in the world (Ricketts et al. 1999). More than 30 species of medicinal non-timber forest products are collected from the National Forests of the Southern Appalachians (USDA 1999).

Bloodroot (*Sanguinaria canadensis* L. [Papaveraceae]) (ITIS 2005) is a woodland botanical with a long history of medicinal use in North America by Native Americans and early European settlers (Foster and Duke 2000). Current research on bloodroot includes studies of its use as an anticancer agent and as an antibiotic (Nihal 2000; Ding et al. 2002; Mahady et al. 2003). There has been a steady growth in demand for bloodroot from the mid-1990s to the present. A small portion of the demand for bloodroot comes from its use in homeopathic medicine and folk remedies. The largest increase in demand and the largest market for bloodroot has come from Europe where it is a critical ingredient in an animal feed additive called Sangrovit, which is used as a natural alternative to antibiotic performance promoters (Greenfield and Davis 2003; Phytobiotics 2002).

The market demand for bloodroot is met almost exclusively by wild harvesting (Herb Research Foundation 2001; Greenfield and Davis 2003). When demand is high for wild harvested plants, native populations may be quickly overharvested, even to the point where extirpation is possible. Bloodroot is one of the medicinal plant species considered an “At Risk” plant by United Plant Savers, a non-profit organization dedicated to preserving native

medicinal plants (United Plant Savers 2005). Goldenseal (*Hydrastis canadensis* L. Batsch. [Ranunculaceae]) (ITIS 2005), a woodland botanical sharing similar habitat and range of bloodroot and also harvested for the medicinal properties of its roots and rhizome, is an example of a species that is endangered in some states due to over collection and habitat loss. Goldenseal is listed in CITES (Convention on Trade in Endangered Species of Wild Fauna and Flora) Appendix II and wild harvesting on public lands is now prohibited in North Carolina (Davis 2000, 2004).

Dormancy/Chilling Requirements. A plant physiologically changes, or acclimates, in response to its environment to ensure its continued survival (Rom 2003). Dormancy as defined by Lang (1987) is the temporary suspension of visible growth of any plant structure containing a meristem. Three terms used to describe the forms of dormancy are ecodormancy, paradormancy, and endodormancy (Lang 1987). Ecodormancy is regulated solely by environmental factors and occurs when environmental conditions are not suitable for growth. Growth resumes when an optimum growth environment returns. Paradormancy is regulated by physiological factors outside the plant structure that is dormant but originating from some other structure within the plant. The response may be induced by environmental factors or some inhibitory factor (Lang 1987; Rom 2003). Endodormancy is regulated by physiological factors inside a plant structure such as a bud. In response to environmental conditions, the plant structure enters a state of dormancy. Growth will not automatically resume when there is an appropriate growing environment unless certain requirements are met. One type of requirement is exposure to a specific period of cold temperatures. The period of exposure to cold temperature, required for buds to physiologically change and remove endodormancy, is referred to as the chilling requirement (Rom 2003).

The most familiar example of endodormancy occurs in temperate fruit trees where floral buds remain dormant until they accumulate enough exposure to cold temperature to complete their rest period. Studies have been conducted with temperate fruit trees to determine the minimum amount of chilling required for buds to be released from dormancy as well as observe the effects of chilling on different bud types (Rom 2003). Scalabrelli and Couvillon (1986) investigated requirements for bud break in 'Redhaven' peach by studying the influence of temperature level and length of chilling period on bud break of three different bud types; terminal vegetative, lateral vegetative and flower buds. Their data show that peach bud types vary in response to chilling temperature as well as length of chilling time.

There has been much attention given to dormancy studies of fruit trees but there are few reports available for woodland perennials similar to bloodroot. In a study with ginseng (*Panax quinquefolius* L. [Araliaceae]) (ITIS 2005) rhizomes, Konsler (1986) investigated the chilling time needed to fulfill rest requirements for bud break. Dormant ginseng roots were exposed to a range of stratification temperatures and times. Konsler found that effective storage temperatures for bud break from rest ranged from 0° to 9° C. Effective storage time for 100% bud break ranged from 60 to 90 days. The most effective chilling temperature, based on minimum chilling time required for 100% emergence, was 3°C. The number of days to emergence after planting decreased with increased time in stratification through the maximum storage time of 120 days.

Keever et al. (1999) investigated chilling requirements necessary to satisfy dormancy in Hosta (*Hosta sieboldiana* Lodd. Engl. [Liliaceae]) (ITIS 2005). Plant material was stored at 4°C. At two-week intervals (0-16 weeks), a group of plants was transferred to a greenhouse

with a controlled temperature range of 18° - 26°C. Results showed a rapid decrease in days to emergence followed by a more gradual decrease with increased chilling time. There was also less variation in emergence times with increased chilling. Minimum days to emergence was achieved with chilling periods of 6 – 7 weeks.

The Gracie et al 2000 study of rhizome dormancy and shoot growth in *Zingiber mioga* Rosc. (Zingiberaceae) (ITIS 2005) showed that chilling at 4°C promotes uniform shoot emergence and flower production. Their study showed no absolute chilling requirement for emergence. However, three-weeks chilling at 4°C significantly decreased the time to emergence when compared to plants without chilling treatment. The results also showed a trend towards reduced variability in emergence with increasing length of chilling time at 4°C.

In a 1996 study, Sanewski et al. (1996) investigated whether or not there is a dormancy requirement in ginger (*Zingiber officinale* Rosc. [Zingiberaceae]) (ITIS 2005) and, if so, the contributions made by endogenous and exogenous factors. Study results showed that ginger exhibits a form of dormancy. Length of dormancy was related to rhizome propagule type but not rhizome mass. Desiccation of rhizome pieces by seven days of pre-plant storage reduces the effects of dormancy. There may be a chilling time requirement, as the rhizomes lifted early in the spring have the slowest mean emergence rate despite favorable external growing conditions in the greenhouse.

In addition to chilling duration, the type of propagule used may have an effect on emergence and growth in rhizomatous plants. Size of rhizome section, presence or absence of a bud, or type of bud present may affect growth and emergence. Maqbool et al. (2002) investigated propagule types for suitable propagation material in a study of mayapple (*Podophyllum peltatum* L. [Berberidaceae] (ITIS 2005). They divided rhizomes into

segments to make up three different propagule types; two-node segments with a terminal and dormant node; one-node segments of a dormant node only; and terminal node only segments. All propagule segments were well rooted and were planted in raised beds. Maqbool et al. (2002) found that propagule types affected on shoot emergence. Propagules that included a terminal bud exhibit higher percentages of emergence compared to the propagule type that had only a dormant bud. The two-node propagule group with a terminal and dormant bud, the one-node propagule group with a terminal bud only, and the one-node propagule group with a dormant bud only showed mean percentage emergence of 93, 82, and 36% respectively.

Determination of chilling requirements is important to development of rhizome-based propagation systems for bloodroot. Upon visual examination, bloodroot rhizomes appear to exhibit at least two different bud types (apical and lateral buds). Bloodroot rhizomes are usually cut into pieces for propagation. If these buds have different chilling requirements, they may be affected differently by chilling treatments. The objectives of this study were to determine if bloodroot rhizomes had chilling requirements and the effects of different propagule types on rhizome bud emergence.

Materials and Methods

Greenhouse studies were conducted during the autumn and winter of 2002-2003 and 2003-2004 at North Carolina State University in Raleigh, North Carolina in the Horticultural Science Department greenhouses. This experiment utilized a split-plot statistical design with chilling time as the main plot and propagule type as the sub-plot with years as replications. Greenhouse 2002-2003 Bloodroot rhizomes were collected from wild native populations in Graham County, NC in October 2002. Rhizomes were washed, divided into four groups of

100 rhizomes each, and stored in flats filled with moist sphagnum peat at 4°C. Flats were loosely covered with plastic to prevent dessication but still allow for air flow (Figure 1.11). A group of rhizomes was taken out of cold storage at 4-week intervals up to sixteen weeks. Rhizomes were then divided into six propagule types; 1) small lateral bud, 2) medium lateral bud, 3) large lateral bud, 4) rhizome piece with no visible bud, 5) rhizome piece having a terminal bud only, and 6) whole rhizomes (undivided). When dividing rhizomes into treatments, lateral buds were snapped off by hand and only buds with roots were used (Figure 1.8 and 1.9). Treatment 5 consisted of rhizomes with only a terminal bud and no lateral buds or a rhizome in which all the lateral buds had been removed for use in treatments 1, 2, and 3. After chilling exposure rhizomes were planted in 10cm pots containing MetroMix 400 (purchased from Wyatt-Quarles, Garner, NC), watered, and placed on a greenhouse bench with 18°/24°C night/day temperatures (Figure 1.10). There were 25 rhizome sections per treatment, one rhizome per pot. Date of emergence and total emergence were recorded during daily visits to the greenhouse.

Greenhouse 2003-2004 Bloodroot rhizomes were collected from wild native populations in Graham County, NC in September 2003. Rhizomes were washed, divided into four groups of 100 rhizomes each, and stored in flats filled with moist sphagnum peat at 4°C, as described above, until planting. One group of rhizomes was planted in the greenhouse immediately, without any exposure to cold temperature. This no-chilling time group was only planted one year, and therefore not included in the statistical analysis. A group of rhizomes was then taken out of cold storage at four, eight, and twelve weeks for planting in the greenhouse. Rhizomes were divided into six propagule types; 1) small lateral bud, 2) medium lateral bud, 3) large lateral bud, 4) no-bud, 5) terminal bud only, and 6) whole rhizome. When dividing

rhizomes into treatments, only buds with roots were used. Lateral buds were snapped off by hand. Treatment 5 consisted of rhizomes with only a terminal bud and no lateral buds or a rhizome in which all the lateral buds had been removed for use in treatments 1, 2, and 3. Rhizomes were planted in MetroMix 400 in 10 cm pots, watered and set on a greenhouse bench with 18°/24°C night/day temperature. There were ten rhizome sections per treatment. Date of emergence and total emergence were recorded during daily visits to the greenhouse. At week 12, due to severe loss of planting material in cold storage because of mold growth, only treatments 4 (no-bud) and 6 (whole root) were planted in the greenhouse.

Statistics Data were analyzed using the GLM procedures of the SAS statistical package (SAS Institute, Cary, NC). Means were compared using the protected LSD procedure ($\alpha=0.05$). Although data were combined for analysis over years, results are discussed both together and separately in order to present additional information not included in the statistical analysis.

Results

Both Years: There were significant effects of length of chilling time on percent emergence and on days from planting until emergence. Percent emergence increased with increased length of chilling time for all propagule types (Figure 1.1) except the no bud treatment. Percent emergence of the no bud treatment increased from 4-week chilling to 8-week chilling then decreased from 8-week chilling to 12-week chilling (Table 1.1). The whole rhizome treatment and the terminal bud only treatment exhibited significantly higher percent emergence than the other four propagule types at each chilling time (Figure 1.2).

Year One: The average percent emergence over all treatments in the 4-week, 8-week, and 12-week chilling groups was 26, 56, and 80%, respectively. At week 16, buds on the last group

of rhizomes were already growing when removed from cold storage. Rhizomes that exhibited bud break were counted and this group was not planted in the greenhouse to observe emergence. Because rhizomes were not divided into the six propagule types until the time of planting, it was simply noted that 88% of rhizomes in the 16-week chilling group had broken bud and these were not included in the statistical analysis. The rhizomes that were chilled for 4-weeks had a low percent emergence in the large lateral bud and no bud treatments and about 50% emergence in the terminal bud and whole rhizome treatments in year one. There was no emergence in the small and medium lateral bud treatments. Rhizomes chilled for 8-weeks showed much higher percent emergence in all treatment types when compared to the 4-week group. Rhizomes chilled for 12-weeks showed higher percent emergence than the 8-week group except in the no-bud treatment. Percent emergence increased for all treatments with increased chilling time except for the no bud treatment, which increased from 4 to 8 weeks then decreased from 8 to 12 weeks. (Table 1.1).

The length of chilling time also affected the number of days from planting until emergence and on the uniformity of emergence. The average days to emergence over all treatments in the 4-week, 8-week, and 12-week chilling groups was 64, 28, and 19. The average days to emergence for each propagule type decreased from 4-weeks chilling to 8-weeks chilling. The standard deviation for each propagule type was larger at 4-weeks chilling than at 8-weeks with the exception of the no bud treatment, which was slightly larger at 8 weeks. With this exception, there was less variation in the time it took for plants to emerge in the 8-week chilling group. When comparing the 8-week and 12-week chilling groups, the average days to emergence was shorter in the 12-week group with the exception of the no bud treatment. The average time to emergence for the no bud treatment in the 12-week group

was slightly longer than in the 8-week group. However, there was less variation in the emergence times of the no bud treatment in the 12-week chilling group (Table 1.2).

Year Two: The pattern of emergence in year two was the same as year one. Percent emergence increased with more chilling time, with the exception of the no bud treatment where percent emergence increased from 4 weeks to 8 weeks then decreased from 8 weeks to 12 weeks (Table 1.1). The average number of days from planting to emergence decreased with increased chilling time. The variation in days to emergence decreased with increased chilling time (Table 1.3). There was no emergence for the small and large lateral buds (treatments 1 and 3) at 4 weeks chilling time. In this year of the experiment, there was loss of plant material due to mold during the storage process, even though the plant material was stored in the same manner in which it was stored the previous year. There had been an extremely wet growing season preceding harvest of the material in 2003 and there was a much higher incidence of mold under the leaf litter than in the previous year. These conditions may have accounted for the high incidence of storage mold. Wildcrafters (people who collect plants in the wild) in the herb industry experienced similar problems when storing rhizomes dug in 2003 (Davis 2004). To accommodate the loss of material, the number of replications was reduced to ten. At 12 weeks of chilling time, there were not enough mold free rhizomes to produce all treatments (propagule types). Therefore, at 12 weeks only whole root and no bud propagules (treatments 4 and 5) were planted in the greenhouse. There was no emergence for the small and large lateral bud propagules (treatments 1 and 3) in year two at 4 weeks chilling time. There was no emergence for all rhizomes that received no chilling.

Discussion

These results suggest that bloodroot rhizomes require a period of exposure to low temperature to overcome dormancy. If the objective is to treat the rhizomes with enough exposure to low temperature to maximize the emergence but reduce the amount of time held in storage, then the optimum chilling time is approximately twelve weeks. By sixteen weeks, rhizomes began to emerge while still in the cooler. This is similar to the requirements of American ginseng observed by Konsler (1986), who found that ginseng rhizomes need a period of chilling to break bud dormancy and increased chilling time had a positive effect on emergence. In 1986, Scalabrelli and Couvillon studied the effects of various temperatures as well and chilling times on dormancy requirements for different bud types in 'Redhaven' peach. Their data show that bud type varies in response to chilling temperature as well as length of chilling time required for rest completion. For future studies, it would be beneficial to repeat rhizome dormancy studies with various chilling temperatures and to shorten the chilling times to 2-week intervals instead of 4-week intervals. There may be an optimum chilling time less than twelve weeks at a different temperature.

The number of days from planting to emergence was inversely proportional to the length of time the rhizomes received chilling. In addition, the variation in days from planting to emergence decreased with increased chilling times (as shown by decreasing standard deviation in tables 1.2 and 1.3). This has been shown with other rhizomatous plants as well. Gracie et al. (2000) found that increasing the duration of chilling treatment with *Zingiber mioga* rhizomes hastened shoot growth and decreased the variability in emergence time. Konsler (1986) found that when American ginseng rhizomes receive longer stratification times, the number of days to emergence after planting decreased.

Bloodroot seems to exhibit endodormancy in that it may respond initially to some environmental factor such as increasingly shorter day length to induce dormancy and then there is a minimum amount of chilling time required before rest is satisfied and the condition of dormancy ends. Observations made by dissecting rhizome buds in the fall, showed that the bud contains a formed leaf and flower primordia. This structure is already developed as the rhizome lies dormant over the winter. New white root growth was observed in rhizomes that received no chilling time or low chilling time and were planted in the greenhouse but the shoot did not emerge. This again suggests endodormancy because the bud structure had not received the cold exposure required for the physiological change to end dormancy within that structure and resume shoot growth.

The no-bud treatment had a different growth pattern than all the other propagule types. This propagule type reached its peak emergence after eight weeks chilling and then declined in percent emergence after twelve weeks of chilling. It is possible that there were no endodormancy requirements to be met in these rhizomes because there was no formed bud structure. Therefore the rhizome pieces started to form buds from unseen bud initials as soon as the environmental conditions were optimal (transfer from cooler to greenhouse). Perhaps there are axillary buds present on the no-bud sections but they are too small and undeveloped to have a rest requirement. If rhizome dormancy is regulated by the cold requirement of the structures in the bud (developed leaf and flower primordia) then the no-bud treatment may not have an intrinsic chilling requirement because these structures are not developed. Another possibility to explain the pattern of emergence of the no-bud treatment is that shoot growth was initiated adventitiously, therefore, there was no rest requirement (Werner 2007). This propagule type did have a significantly lower percent emergence than the whole

rhizomes and the rhizomes with terminal buds. Bloodroot forms its new buds toward one end while the older end of the rhizome eventually dies off. It could be possible that many of the no-bud pieces were the older rhizome sections that did not produce as many buds (therefore have as much emergence) as the younger end of the rhizome. It may be that when dividing rhizome sections for propagation, the older end of the rhizome would not be as successful in producing new plants as the younger portion of the rhizome. Future studies with comparisons of the older end of the rhizome and the young end of the rhizome, with any buds removed, to observe percent emergence may be useful.

These data show that exposure of bloodroot rhizomes to 4° C for twelve weeks results in optimal emergence for all propagules with a bud. Future research that includes a range of cold temperatures may determine a shorter cold period necessary for optimal emergence.

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Table 1.1 Average percent emergence for six propagule types at three chilling times

Propagule	Chilling Times					
	4 Weeks		8 Weeks		12 Weeks	
	year 1	year 2	year 1	year 2	year 1	year 2
Small lateral	0	0	32	11	80	---
Medium lateral	0	10	56	11	92	---
Large lateral	24	0	48	22	96	---
No bud	29	20	44	44	24	10
Whole rhizome**	48	30	92	67	96	70
Terminal bud only**	52	20	64	44	92	---

Percent emergence for each bloodroot propagule type was significantly different when comparing time exposed to 4° C prior to planting according to the protected LSD test ($P \leq 0.05$).

** Percent emergence was significantly different for propagules WR and T when compared to all other propagule types but not significantly different from each other according to the protected LSD test ($P \leq 0.05$).

--- Due to loss of plant material to storage mold damage, only treatments NB and WR were available for planting after the 12-week chilling time.

Table 1.2 Average days from planting until emergence -2002/2003

Propagule Type	Chilling Times					
	4 Weeks		8 Weeks		12 Weeks	
	Ave Days	St Dev	Ave Days	St Dev	Ave Days	St Dev
Small Lateral Bud	N/E	N/E	26.4	6.19	19.3	5.5
Medium Lateral Bud	N/E	N/E	29.5	15.8	15.7	5.8
Large Lateral Bud	82.5	19.5	28.5	12.26	15.7	8.7
No Bud	53.4	20.8	33.4	21.84	35.7	13.3
Whole Rhizome	56.9	25.4	25.1	10.96	12.0	4.4
Terminal Bud Only	61.2	26.6	24.4	16.18	13.1	3.7

N/E = No emergence

Bloodroot propagules were exposed to 4°C prior to planting

Table 1.3 Average days from planting until emergence - 2003/2004

Propagule Type	Chilling Times					
	4 Weeks		8 Weeks		12 Weeks	
	Ave Days	St Dev	Ave Days	St Dev	Ave Days	St Dev
Small Lateral Bud (1.0 - 1.9g)	N/E	N/E	26.0	1 plant	----	----
Medium Lateral Bud (2.0 - 2.9g)	70.0	1 plant	26.0	1 plant	----	----
Large Lateral Bud (4.0 - 4.9g)	N/E	N/E	21.0	1.4	----	----
No Bud	50.5	10.6	21.3	10.7	27	1 plant
Whole Rhizome	70.3	27.5	21.3	11.4	12	1.8
Terminal Bud Only	42.5	26.2	19.5	11.1	----	----

Bloodroot propagules were exposed to 4°C prior to planting.

1 plant = standard deviation could not be calculated because only one plant emerged

N/E = No emergence

--- = No data for 12 weeks due to loss of plant material to storage mold

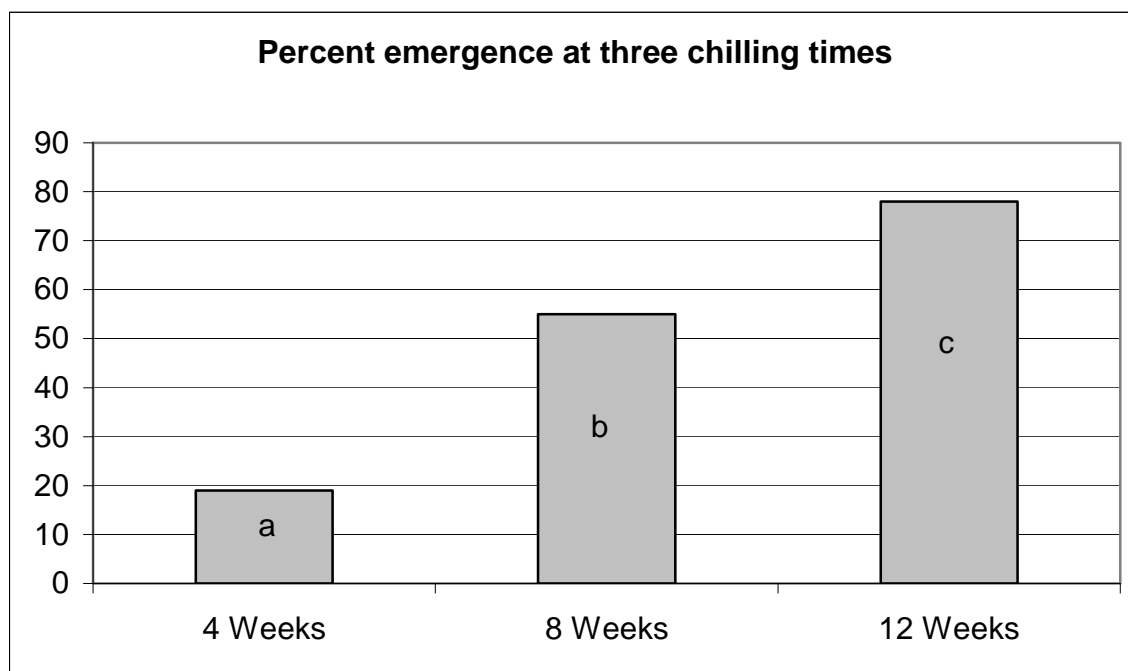


Figure 1.1 Percent emergence at each chilling time with exposure to 4° C is based on the average emergence of all bloodroot propagule types with years as replications. Different letters indicate a significant difference in emergence. Comparisons are significant at the alpha 0.05 level.

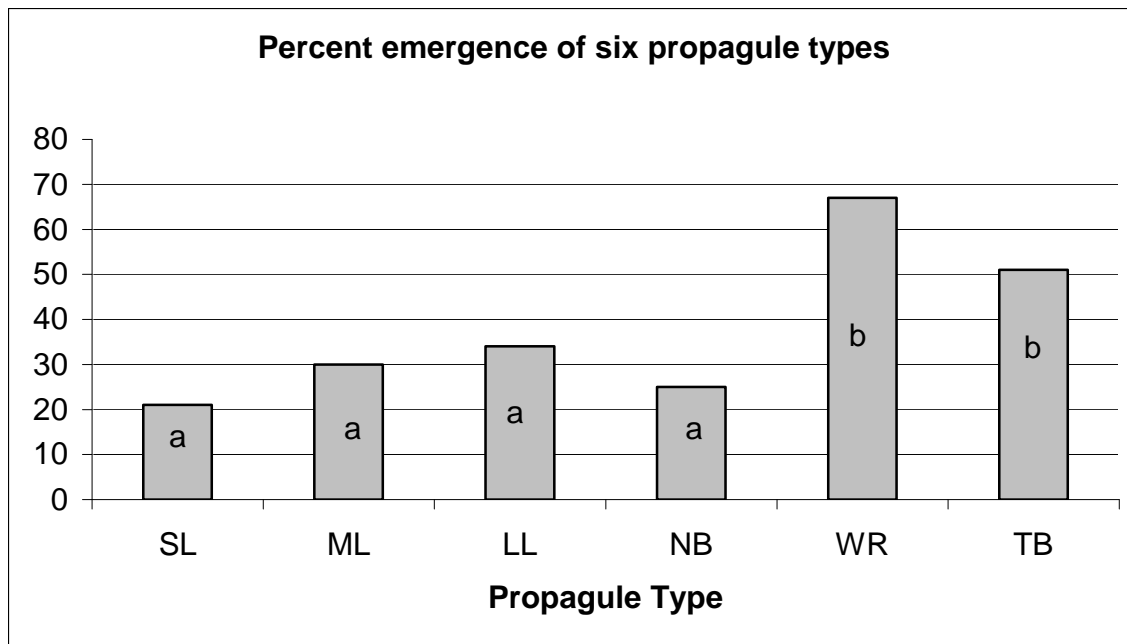


Figure 1.2 SL=small lateral bud, ML=medium lateral bud, LL=large lateral bud, NB=no bud, WR=whole rhizome, TB=terminal bud only. Percent emergence of each bloodroot propagule type is based on the average emergence across all chilling times with exposure to 4° C, with years as replications. Percent emergence was significantly different for propagules WR and T when compared to all other propagule types but not significantly different from each other according to the protected LSD test ($P \leq 0.05$). Different letters indicate significant difference.

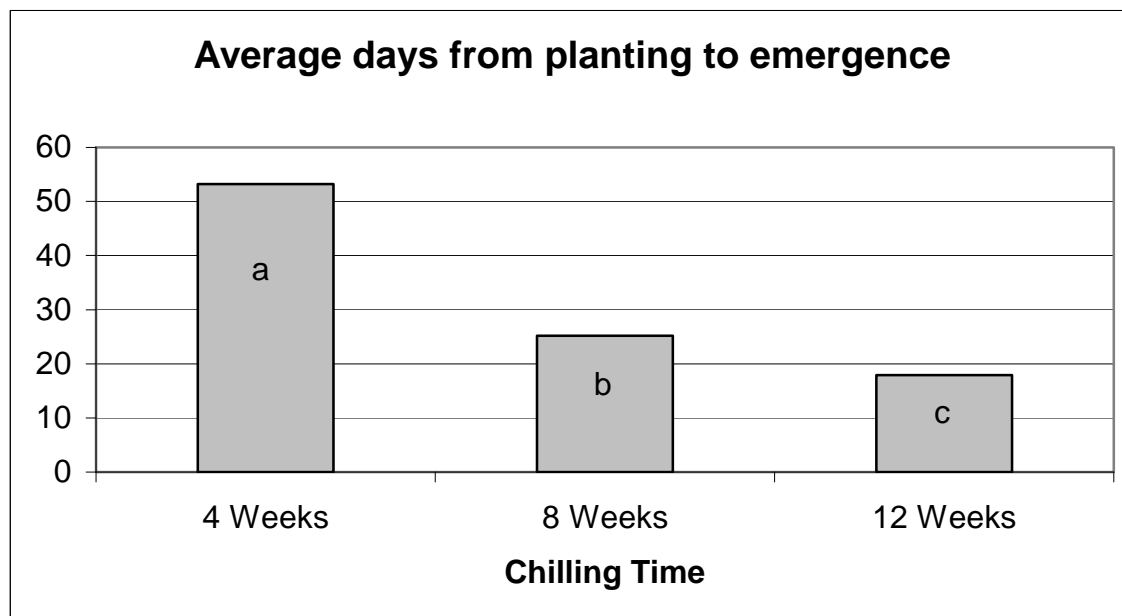


Figure 1.3 Number of days at each chilling time at 4° C is based on the average days from planting to emergence of all bloodroot propagule types with years as replications. Different letters indicate a significant difference in number of days to emergence. Comparisons are significant at the alpha 0.05 level.

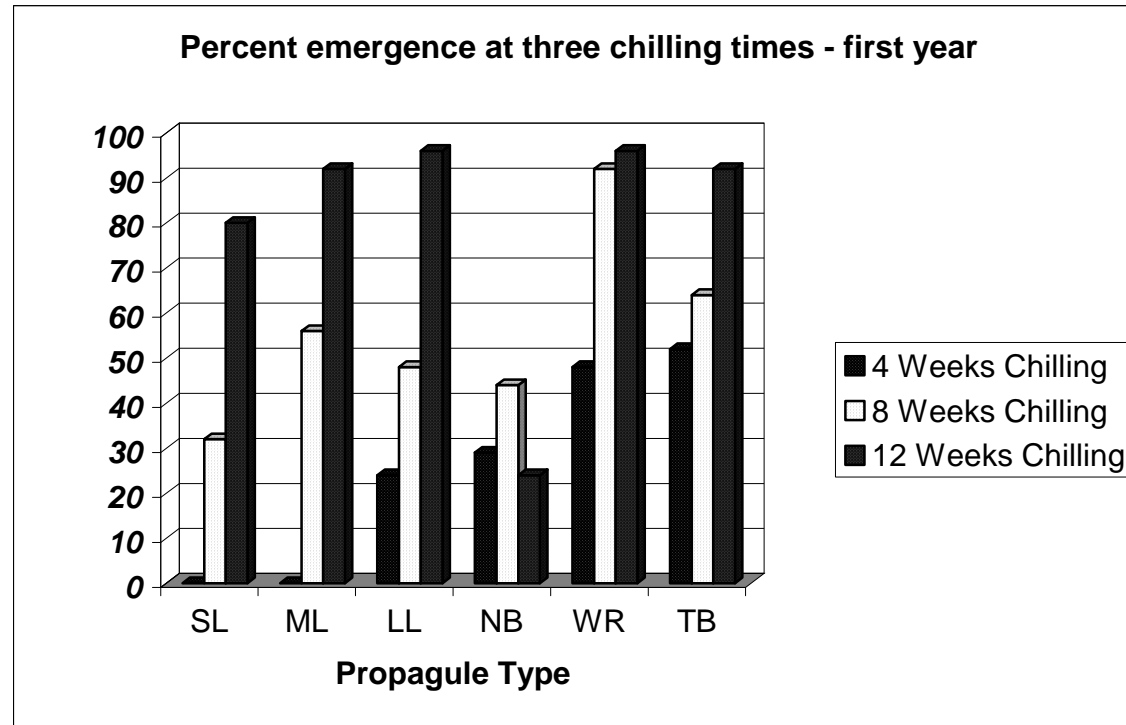


Figure 1.4 Percent emergence of six bloodroot propagule types following three periods of chilling at 4°C (2002/2003 trial).

Propagule types: SL = small lateral bud , ML = medium lateral bud , LL = large lateral bud, NB = no-bud, WR = whole rhizome, TB = terminal bud only.

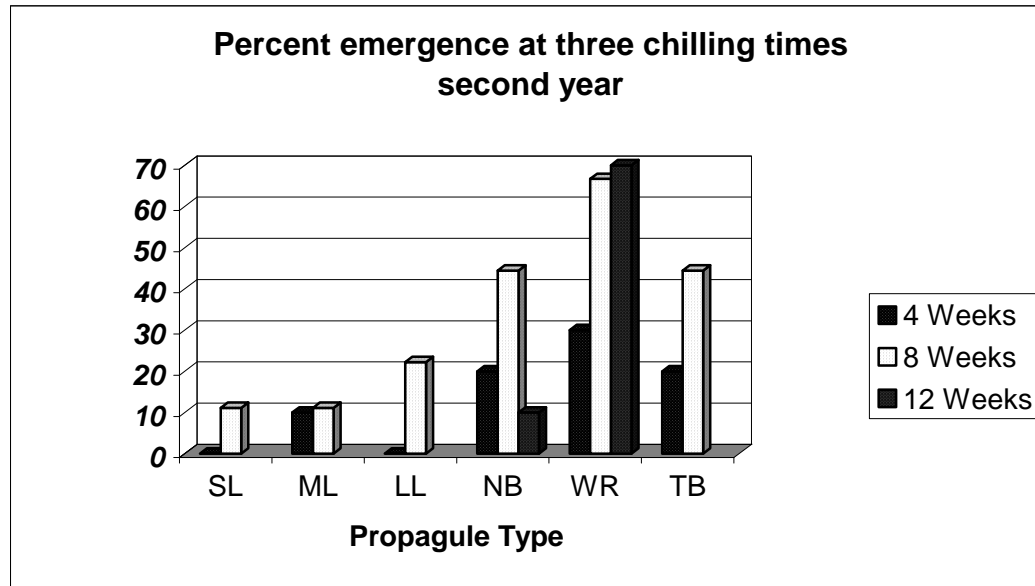


Figure 1.5 Percent emergence of six bloodroot propagule types following three periods of chilling at 4°C (2003/2004 trial). Propagule types: SL = small lateral bud, ML = medium lateral bud, LL = large lateral bud, NB = no-bud, WR = whole rhizome, TB = terminal bud only. At 12 weeks chilling, only no-bud and whole root treatments are present SL, ML, LL, and TB are missing but not zero.

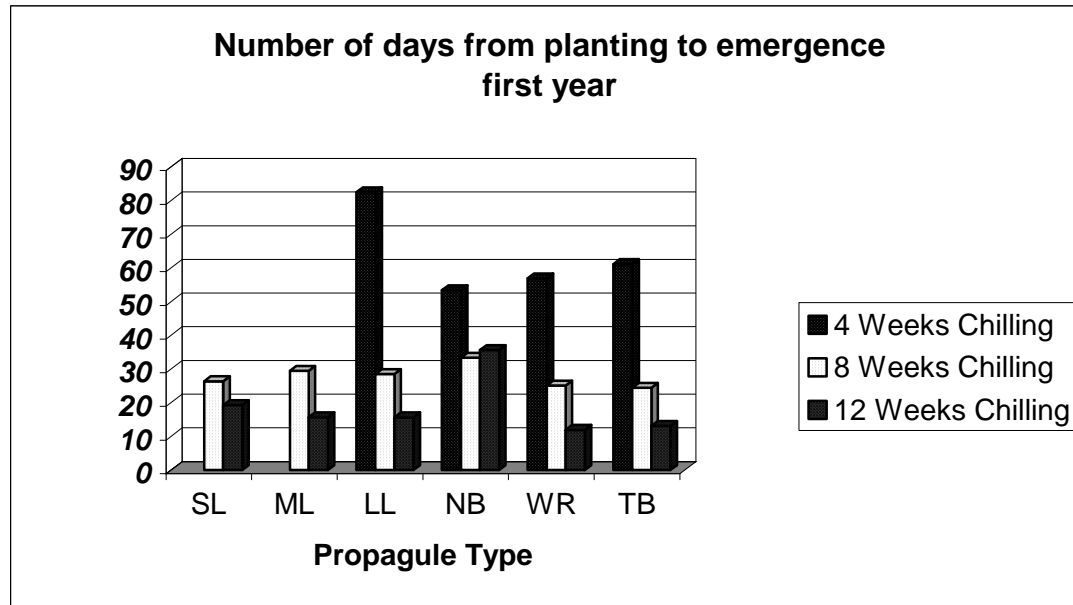


Figure 1.6 Average number of days from planting to emergence of bloodroot after chilling at 4°C (2002/2003 trial).

Propagule types: SL = small lateral bud, ML = medium lateral bud, LL = large lateral bud, NB = no-bud, WR = whole rhizome, TB = terminal bud only

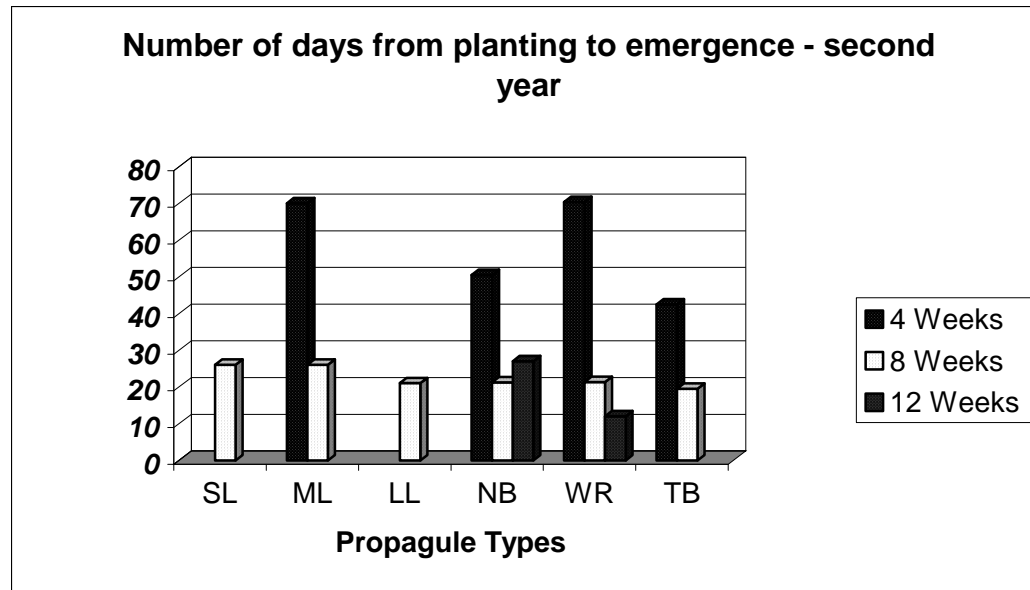


Figure 1.7 Average number of days from planting to emergence of bloodroot after chilling at 4°C (2003/2004 trial).

Propagule types: SL = small lateral bud, ML = medium lateral bud, LL = large lateral bud, NB = no-bud, WR = whole rhizome, TB = terminal bud only. At 12 weeks chilling, only no-bud and whole root treatments are present.



Figure 1.8 Bloodroot rhizome with terminal bud (right) and lateral buds.



Figure 1.9 Medium to large lateral buds with roots. Buds were snapped off of a whole rhizome.



Figure 1.10 Pots on the greenhouse bench.



Figure 1.11 Trays for rhizome cold storage. Trays have perforated bottoms filled with slightly moistened peat and rhizomes and loosely covered with plastic.

CHAPTER TWO

PROPAGATION AND CULTIVATION OF BLOODROOT IN A WOODED SETTING AND UNDER POLYPROPYLENE SHADE STRUCTURE

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Bloodroot (*Sanguinaria canadensis* L.[Papaveraceae]) is a rhizomatous perennial, native throughout eastern North America (ITIS 2005). The species has a long history of medicinal use by American Indians and early European settlers. It is still used today in homeopathic medicine but the major market for bloodroot is in Europe where it is being used as an alternative to antibiotic performance promoters in animal feed.

Research investigations with *Sanguinaria canadensis* were conducted in order to develop propagation and cultivation techniques. Currently, most of the bloodroot marketed is gathered from wild populations. With an understanding of how to effectively propagate and cultivate bloodroot, stress on native stands caused by over harvesting can be reduced. By providing this information to growers, bloodroot supply can be met by cultivated material instead of wild harvested material.

Field studies were conducted in order to observe plant growth in a cultivated setting and determine if the type of propagation material affects growth. Bloodroot rhizomes were cut and divided into three treatment groups consisting of different propagule types: 1) rhizome sections with a bud, 2) rhizome sections without a visible bud and 3) whole rhizomes. Plots were established under a polypropylene shade structure and in a wooded setting. Growth performance was evaluated by measuring percent emergence, seed pod production, leaf size, plant height, and rhizome weight and number at harvest. First year data

showed emergence was significantly higher for whole rhizomes and rhizome sections with a bud than for rhizome sections without a bud. There was a trend for growth performance of whole rhizomes to be slightly better than that of rhizome pieces with a bud. Second year data showed no difference between treatments. Although there was no difference between treatments in rhizome weight and number, the rhizomes harvested from plots in the shade structure were greater in number and weight than those harvested from the woods plots. Percent alkaloid content was significantly greater in bloodroot grown in the woods plots.

Introduction

The U.S. market for medicinal herbs has experienced enormous growth in popularity through the 1990s to present (Blumenthal 1998). Some herbs are cultivated to meet this demand while others, particularly woodland botanicals, are wild harvested. A large number of medicinal plant species can be found growing in the Appalachian mountains of western North Carolina, which are situated in one of the most bio-diverse regions in the world (Ricketts et al.1999). Bloodroot (*Sanguinaria canadensis* L. [Papaveraceae] (ITIS 2005) is one out of more than 30 species of medicinal non-timber forest products collected from the National Forests of the Southern Appalachians (USDA 1999).

Bloodroot is a woodland wildflower and medicinal herb native to the eastern half of North America. Its natural range is from Nova Scotia and Manitoba south to Florida and Texas. It is particularly prominent along the Appalachian Mountain Range (Hayward 1982; Cullina 2000; Grey-Wilson 2000). *Sanguinaria* is a monotypic genus in the poppy family (Papaveraceae) and like other species in this family, bloodroot is mainly sought after for its useful alkaloids. The plant parts most often harvested are the roots and rhizome, which have the highest alkaloid concentration. Alkaloids are nitrogen-containing secondary plant products, so called because they are not essential for the primary metabolism of the plant. Usually they are synthesized from amino acids. The purpose of these alkaloids is most likely for plant defense against herbivores, fungi, bacteria, and viruses.

Sanguinarine and chelerythrine are the dominant alkaloids, with sanguinarine averaging up to 50% of the total (Bennett et al. 1990). The orange-red color of the latex that oozes from wounded stems and rhizomes is largely due to the presence of sanguinarine. Sanguinarine has been found to inhibit choline acetyl-transferase, acetylcholine esterase,

DNA synthesis, reverse transcriptase, and intercalate DNA (Schmeller et al., 1997). It has also been found to protect the aromatic amino acids, tryptophan and alanine, from decarboxylation to toxic biogenic amines by inhibiting amino acid decarboxylase (Drasta et al., 1996). The significance of this is its usefulness in the animal feed industry where it works as a performance promoter. In conventional feeding, intestinal flora that produce amino acid decarboxylase are minimized by antibiotics (Phytobiotics 2002).

Bloodroot has a long history of medicinal use by Native Americans and European settlers. Currently, researchers are investigating the use of bloodroot alkaloids as an anti-cancer agent and for antibiotic usage. There is a small demand for bloodroot in homeopathic medicine and it is still used in some folk remedies. However, the largest market for bloodroot is in Europe where it is the critical ingredient in an animal feed additive called Sangrovit. This product is used as a natural alternative to antibiotic performance promoters (Grey-Wilson 2000; Herb Research Foundation 2001; Hendershot 2002). Bloodroot supply is met almost entirely by wild harvesting from U.S. forests. It is difficult to harvest in a sustainable manner because the desired product is the plant's rhizome. With no large-scale production system in place, this puts stress on wild populations. Bloodroot is one of the medicinal plant species considered a priority for conservation in North America by the U.S. Forest Service and is considered an "At Risk" plant by United Plant Savers, a non profit organization dedicated to preserving native medicinal plants (United Plant Savers 2005). Cultivation of bloodroot on a commercial scale could provide a good niche market for North Carolina farmers and enhance agricultural diversity and economic development in rural western North Carolina.

Few studies have been conducted concerning the propagation and cultivation of bloodroot. Bloodroot, however, shares a similar habitat and range with other woodland botanicals that have been cultivated for the medicinal properties of their rhizomes. Goldenseal (*Hydrastis canadensis* L. [Ranunculaceae]) (ITIS 2005) and ginseng (*Panax quinquefolius* L. [Araliaceae]) (ITIS 2005) are two such herbs.

The majority of market supply of these herbs was or still is met by wild harvesting. Because the desired medicinal portions of the plants are the roots and rhizome, the whole plant is dug up when harvesting, leaving behind no portion to sustain the wild population. Due to over collection and habitat loss, goldenseal is listed in the Conservation on International Trade of Endangered Species Appendix II, as an endangered species in North Carolina, and is considered an “at risk” plant by United Plant Savers (Gagnon 1999; Davis and McCoy 2000; United Plant Savers 2005). If bloodroot cannot be harvested or cultivated in a sustainable manner, it may follow the same path as goldenseal in the wild. Some literature is available concerning the propagation and cultivation of woodland botanicals that share a common wild habitat with bloodroot. These provide a good starting point for the investigations of propagation and cultivation of bloodroot.

Rhizome cuttings were chosen for the propagation material in this study. Propagating with rhizomes allows for a shorter time between planting and harvest and simplifies the propagation process compared to production from seed. It can also allow for future selection of clonal populations with high alkaloid content. Bloodroot seed requires stratification, a period of warm followed by a period of cold to germinate. Rhizomes require only a single cold period to break bud dormancy. Bloodroot is similar to ginseng and goldenseal in that the rhizomes are slow growing. If starting from seed, it would take two to three years longer to

reach the time when rhizomes are large enough to harvest. Numerous sources suggest that conditions needed for cultivation are growing conditions that closely resemble those where these herbs grow in the wild (Hersey 1964; Phillips 1985; Cech 2002). Persons and Davis says that bloodroot will grow under a wide range of shade levels but suggests growing it under 70% – 80% shade in raised beds with a soil pH of 5.5 – 6.5. Persons and Davis also suggest following the basic growing recommendations for goldenseal (2005).

Goldenseal has been successfully propagated by rhizome division. Rhizomes pieces should be at least one half inch in size, have healthy fibrous roots and preferably have a visible bud (Davis and McCoy 2000; Persons and Davis 2005). Veninga and Zaricor (1976) state that each division should have two or more buds and a few good roots. Rhizome pieces should be planted 5 – 8cm (2 – 3in) deep in raised beds (Veninga and Zaricor 1976; Davis and McCoy 2000) with 15 – 30cm (6 – 12in) spacing (Cech 2002) and covered with mulch. The growing climate must be such that the rhizome buds will be exposed to sufficient cold to break dormancy.. For goldenseal, this is three months of cold temperatures at 4°C (40° F) or less (Cech). Goldenseal grows well in moist, loamy, well-drained soil that is rich in organic matter and slightly acidic with a pH of 5.5 – 6.5 (Veninga and Zaricor 1976; Cech 2002). If goldenseal is grown in a forested location, a good site will have a stand of hardwood trees and a northern or northeastern facing slope with good drainage (Veninga and Zaricor 1976; Persons and Davis 2005). Soil should be tilled, tree roots and weeds removed, and raised beds constructed with a walk space in between. If goldenseal is to be field planted, artificial shade will be necessary (Cech 2002; Persons and Davis 2005). Veninga and Zaricor (1976) suggest using 80% shade in southern states, Cech (2002) states that 70% shade is ideal, and Persons and Davis (2005) report that 63% shade is best. Shade can be provided by a natural

forest canopy or a shade structure can be made from a variety of materials, most commonly wood lath or polypropylene cloth. At least two ends should be open for sufficient air circulation (Veninga and Zaricor 1976; Persons and Davis 2005). Time from planting until harvest is usually three to five years when starting with rhizome pieces/divisions (Davis 2000), although Veninga and Zaricor (1976) and Cech (2002) state that harvestable material can be obtained after only two to three years. Harvested rhizome and roots are washed with a high pressure hose or with a tumbling style root washer. The rhizomes and roots are then spread out on screens to dry in a well-ventilated area or in forced air drier at 35°C (95° F) (Persons and Davis 2005).

Ginseng is another commonly cultivated woodland botanical. It is a slow-maturing rhizomatous crop (Hofstetter 1992; Davis 1997). The growing climate must have sufficient cold temperatures to satisfy bud chilling requirements. Ginseng requires a moist but not wet soil that drains well and is rich in organic matter (Thompson 1987; Hofstetter 1992; Long 1996; Persons and Davis 2005) with a slightly acidic pH of 5.5-6.5 (Cech 2002). Natural or artificial shade must be provided. Thompson (1987) and Long (1996) suggest that it should be at least 65% shade although 75 to 80% is best. Davis states that shade should be between 75% and 85%. Artificial shade can be provided by structures such as wooden lath or polypropylene cloth (Thompson 1987; Davis 1997). Natural shade should be provided by hardwood trees such as poplar, oak, and basswood. Sites selected for planting need to have good drainage and preferably a north or northeastern facing slope. The soil should be plowed and tilled for preparation of beds. Raised beds help to provide good drainage and can make harvesting easier. Beds should be mulched with organic matter such as hardwood bark, straw, or leaves from deciduous trees (Davis 1997). Ginseng is harvested in autumn (Thompson

1987) with spades, forks, or large mechanical root diggers. Roots are washed with water, usually in a tumbling style washer, and spread evenly in a single layer to dry on screen trays in a forced air dryer.

The type of propagule used may have an effect on emergence and growth in rhizomatous plants. Size of rhizome section, presence or absence of a bud, or type of bud present may affect growth and emergence. Maqbool et al. (2002) investigated propagule types for suitable propagation material in a study of Mayapple (*Podophyllum peltatum* L. [Berberidaceae]) (ITIS 2005). They divided rhizomes into segments to make up three different propagule types; two-node segments with a terminal and dormant node; one-node segments of a dormant node only; and terminal node only segments. All propagule segments were well rooted and planted in raised beds. Maqbool et al. (2002) found that propagule types that included a terminal bud exhibited higher percentages of emergence compared to the propagule type that had only a dormant bud. The two-node propagule group with a terminal and dormant bud, the one-node propagule group with a terminal bud only, and the one-node propagule group with a dormant bud only exhibited mean percentages emergence of 93%, 82%, and 36% respectively.

The objective of this study was to investigate rhizome-based bloodroot propagation and cultivation methods through experiments observing growth in both woodland and shade cloth cultivated settings and to determine effects of different propagule types on emergence, growth, and root and alkaloid yield.

Materials and Methods

Experiments were established in the woods and under artificial shade. Each experiment consisted of three trials scheduled to be harvested at 2, 3, and 4 years after planting. Each trial was a randomized complete block design with three propagule treatments and four replications in the wooded site and five replications in the artificial shade site. For the scope of this master's research, growth data was collected and analyzed across all three studies while rhizome yield data was collected and analyzed from one study (harvested two years from planting). Bloodroot rhizomes were harvested from wild populations in Graham County, North Carolina in October of 2001. Whole rhizomes were then cut to produce the following treatments: (1) 5 cm sections with a bud, (2) 5 cm sections without a bud, and (3) uncut whole rhizomes. The average weight of a rhizome piece was 3.1g. These were planted in raised beds in which soil was tilled and hilled into beds. Plots were located under a lath weave polypropylene shade structure (80% shade) and in plots under a forest canopy at the Mountain Horticultural Crops Research Station in Fletcher, North Carolina. Each 1 x 1.5 meter plot consisted of seven rows of seven rhizome pieces planted using a 15 cm by 15 cm spacing.

Soil Samples Soil samples were collected in November 2001. The North Carolina Department of Agriculture soil testing lab performed analysis for P, K, Ca, Mg, Mn, Zn, Cu, S, percent humic matter, and pH (NCDA 2007)

Plant Growth Observations Time of emergence, total emergence, flowering, and seed pod number were recorded approximately once each week from March to July. Overall vigor was also recorded. Vigor was rated using a scale of 0 – 5 where 0 = plants were completely missing or dead and 5 = no apparent animal browsing, plot was full lush and green.

Statistical Analysis Data were subjected to analysis of variance and means were separated using the protected LSD procedure (MSTAT-C, Michigan State University).

Harvest Plots were harvested by hand with use of a pitchfork to turn the soil. Rhizomes were washed with a hose over mesh screens then allowed to dry sufficiently for the surface water to evaporate. The number of rhizomes dug from each plot and their fresh weight was recorded. A sample of ten rhizomes from each plot was chosen randomly and the number of buds on each rhizome was recorded. Rhizomes were spread on mesh screens and placed in a forced air drier at 35°C (95°F) for two days before dry weight was recorded.

Statistical Analysis Data were subjected to Mixed Models analysis and means were separated using the protected LSD procedure using the SAS statistical package (SAS Institute, Cary, NC).

HPLC analysis Quantitative high-performance liquid chromatography analyses were performed to determine percent sanguinarine content. Alkaloid analysis was performed at the Natural Products Laboratories of Research Triangle Institute (RTI), Research Triangle Park, North Carolina using a procedure for extraction and analysis developed by Tyler Graf and Nicholas Oberlies as described in the Journal of Agricultural and Food Chemistry (Graf et. al. 2007) and summarized below.

Dried rhizomes were chopped with a Cuisinart Mini-Mate Plus grinder (East Windsor, NJ) then milled into a fine powder using a Glenmills MicroHammerMill IV with a 2.0mm sieve (Clifton, NJ). The grinder and the mill were cleaned thoroughly and dried between samples, and all subsequent steps were performed in low-light conditions. A 500mg sample of ground material was extracted with 100mL MeOH in an amber flask overnight while stirring. The MeOH extract was filtered and dried under vacuum in an amber flask.

The dried sample was resuspended with MeOH using sonication, and transferred into a scintillation vial to be dried under nitrogen in the dark.

Fractionation of the extract was performed to remove sugary materials. The extract was dissolved in 0.5mL DMSO and then diluted to 10mL with 10:90 acetonitrile:water. Each sample was loaded on to a pre-equilibrated C18 column and washed with 3mL of 10:90 acetonitrile:water. The alkaloid fraction was eluted slowly using 28mL of 75:25 acetonitrile:water, and then dried under nitrogen in the dark.

The alkaloid fraction was analyzed by HPLC using a multi-step gradient as follows: 10:90 acetonitrile:water (0.01% TFA) to 50:50 acetonitrile:water (0.01%) over 20 minutes; then to 75:25 acetonitrile:water (0.01% TFA) over 5 minutes; then isocratic at 75:25 acetonitrile:water (0.01% TFA) for 10 minutes. Elution profiles were observed at 335nm. All chromatograms were 10µL injections by an autosampler. Sanguinarine standard was purchased from Chromadex. Chelerythrine standard was purchased from Sigma-Aldrich (Graf et al. 2007).

Statistical Analysis Data were subjected to Mixed Models Analysis using the SAS statistical package (SAS Institute, Cary, NC).

Results and Discussion

First Year Emergence During the first spring (2002) following planting, maximum emergence of 94%, 64%, and 96% were obtained with the rhizome section with a bud treatment (bud), the rhizome section with no bud treatment (no bud), and the whole rhizome treatment (whole), respectively, in the polypropylene shade house plots. The bud and whole treatments had significantly greater emergence than the no bud treatment. In the woods,

maximum emergence of 89%, 44% and 86% was obtained for the bud, no bud, and whole treatments, respectively, with the bud and whole treatments being significantly greater than the no bud treatment (Fig 2.1 and 2.2). This may be an indication that, although the no-bud sections had no visible buds, there may have been small axillary buds that developed at rhizomal nodes after the rhizome was cut and planted. Dividing the rhizomes into treatments by cutting may have eliminated apical dominance by removing the terminal bud on the no-bud treatment allowing axillary buds to develop. Alternately, cutting the rhizomes may have stimulated adventitious growth and emergence resulted from non-axillary tissue.

Observations made during rhizome chilling trials (Onofrietti 2007) lend support to the possibility of adventitious growth; in the greenhouse, leaves that developed from no-bud treatments resembled seedlings (first year plants grown from a seed as opposed to a rhizome with a developed bud) in that they were small and lobeless, while leaves that developed from all the treatments with a visible bud were lobed. Also, all treatments with a bud responded to increased chilling with increased emergence as is typical in species where exposure of buds to low temperature is required to overcome bud dormancy (Scalabrelli and Couvillon 1985). However, the no-bud treatment did not respond to increased chilling with increased emergence indicating that a bud structure was not formed in the no-bud treatment (Onofrietti 2007). In the shade house, emergence in the whole treatment increased over a period of six weeks from the first date of emergence on 3/25/02 until reaching maximum emergence on 5/6/02. Emergence in the bud treatment increased over a period of seven weeks from the 3/25/02 until 5/14/02. Compared to the other treatments, emergence in the no bud treatment was delayed one week and increased at a slower rate, reaching maximum emergence on

5/21/06. At each date that emergence was recorded, the bud and whole treatments had significantly higher percent stand than the no bud treatment (Figure 2.1).

In the woods, plants in the bud and whole treatments emerged faster and earlier than under the shade house (Figure 2.1 and 2.2). As in the shade house, emergence was slower for the no bud treatment than the other two treatments. Emergence in the whole treatment increased over a period of six weeks from 3/25/02 until reaching maximum emergence on 5/06/02. Emergence in the bud and no bud treatments increased from 3/25/02 until reaching maximum emergence on 5/14/02. At each date that emergence was recorded, the whole and bud treatments had significantly higher percent stand than the no bud treatment (Figure 2.3).

Second Year Emergence Maximum emergence in the shade house in 2003 was 91%, 84%, and 94% for the bud, no bud, and whole treatments, respectively (Figure 2.3). There was, however, no significant difference between treatments. In the woods, maximum emergence was 61%, 58%, and 51% for the bud, no bud, and whole treatments, respectively (Figure 2.4). There was no significant difference between treatments. These data suggest that the rhizome sections in the no bud treatment set buds for the second growing season, starting out with buds that already had a developed leaf and flower primordia, as opposed to the first year where they did not, allowing the no bud treatment to “catch-up” with the other two treatments in emergence by the second spring. In the shade house plots, emergence in the whole and bud treatments increased over a period of three and a half weeks from the first date of emergence on 3/21/03 until reaching maximum emergence on 4/14/03. The no bud treatment was slightly behind the other two treatments, reaching maximum emergence one week later on 4/22/03. In early spring the bud and whole treatments had a significantly higher percent stand than the no bud treatment, however, by late spring there was no

significant difference in percent stand between treatments (Figure 2.4). In the woods, maximum emergence was reached by all three treatments between 4/22/03 and 5/7/03. There was, however, a decrease in percent emergence recorded between April 4 and April 14. This was due to plant loss by animal browsing. Percent emergence dropped drastically instead of slowly after peak emergence was reached. This was also due to plant loss from animal browsing. In the early spring, the bud and whole treatments had significantly higher percent stand than the no bud treatment. By late spring, however, there was no significant difference in percent stand between treatments (Figure 2.4).

Additional Emergence Observations There were almost no flowers or seed pods observed in the no bud treatment during 2002 while the bud and whole treatments produced similar numbers of flowers and seed pods (Figure 2.5 and 2.6). The plants that emerged from the rhizome sections without a bud during the first year resembled seedlings in that they had small leaves lacking lobes. Due to plant browsing and hail damage in 2003, accurate seed pod and flower production observations were difficult to record. Therefore an overall vigor rating for each plot was recorded. The rating scale was 0 – 5, with 5 being a plot that was lush, full, and healthy and 0 being a plot where the plants were all dead or dying. In the polypropylene shade house site, bud and whole treatment plots looked better than no bud plots and received an average rating of 4.7 and 4.6 respectively. The no bud received an average rating of 3.9. In the woods site, the bud, no bud, and whole treatments received ratings of 1.6, 1.7, and 1.6 respectively.

Harvest Results Treatment had no effect on the number of rhizomes harvested. There was, however, a greater number of rhizomes harvested from the polypropylene shade structure plots than from the woods plots (Figure 2.7). The average number of rhizomes harvested per

plot in the shade was 38.7, i.e., 79% of the number of rhizomes planted. The average number of rhizomes harvested per plot in the woods was 8.6, i.e., 18% of the number planted. There were no significant differences at the $p = 0.05$ level in fresh weight or dry weight yields when comparing propagule types. When comparing sites, fresh weight and dry weight yields were significantly greater from the shade structure plots than from the woods plots (Figure 2.8 and 2.9). Average dry weight was 94.5 grams per square meter for shade house grown bloodroot and 4.5 grams per square meter for woods grown bloodroot. Average fresh weight was 359.2 and 26.4 grams per square meter for shade house and woods grown bloodroot respectively. (Table 2.1). The average fresh weight and dry weight of the rhizomes harvested from the shade plots was 501 and 134 grams per plot, respectively, with a 61% gain in fresh weight from the time of planting to harvest. The average fresh weight and dry weight of the woods plots was 37 and 6 grams per plot, respectively, with an 89% loss in fresh weight from planting to harvest.

The average number of buds per rhizome over both sites was 3.0 and 3.2 for the bud and whole treatment, respectively, but was significantly less for the no bud treatment at 2.0. When comparing sites, the average number of buds per rhizome over all the treatments was greater under the polypropylene shade structure than in the woods (Figure 2.10 and 2.11).

These results suggest that environment had a large effect on bloodroot growth. However, the difference between sites may not necessarily mean that growing bloodroot in a field under an artificial shade structure is always better than a woods site. It does point to the possibility that, at least in this situation, the woods site was probably not ideal for bloodroot cultivation. Unpublished data from a woodland cultivation study in Waynesville, NC showed that woodland sites produced good bloodroot yield (Danehower 2007). Various sources state

that bloodroot thrives in humus rich soils with a neutral to slightly acid pH (Hersey 1964; Phillips 1985; Grey-Wilson 2000). The woods site for these research plots had a soil pH of 4.4, while the polypropylene shade structure site had a soil pH of 6.4. The pH of the latter site is within the range considered ideal for bloodroot to thrive (Hersey 1964). Although there may have been other factors contributing to the poor performance of bloodroot in the woods plots, it is possible that soil pH was not ideal for bloodroot growth. Another possible explanation for the difference in bloodroot growth between the two sites is a difference in available soil nutrients. Soil test results showed that the percent of cation exchange capacity occupied by calcium was much higher in the shade structure plots than the woods plots. Calcium plays a crucial role in plant health in ginseng cultivation (Persons and Davis 2005). It is possible that low calcium levels negatively affects bloodroot plant health as well. Phosphorus and potassium levels were also higher in the shade structure plots than the woods plots. A consideration for future studies in bloodroot cultivation would be to include trials with varying rates of soil pH, calcium, and other soil nutrients.

HPLC Results There were no significant differences in percent sanguinarine or chelerythrine content between propagule treatments (Figures 2.12 and 2.13). There was a significant difference in percent sanguinarine and chelerythrine content between sites. The bloodroot grown in the woods site showed a higher percentage of sanguinarine and chelerythrine content than the bloodroot grown under the polypropylene shade cloth (Figure 2.12 and 2.13). Rhizome material harvested from the shade house had a sanguinarine content ranging from 1.5% to 3.0% with an average content of 2.2%. Rhizome material harvested from the woods had a percent sanguinarine content ranging from 2.2% to 4.1% with the average content being 3.2%. Based on dry weight mass, average sanguinarine yield was 2.1g

per square meter from the polypropylene shade plots and 0.1g per square meter from the woods plots. There was slightly more variation in the woods than the shade house. The site x treatment interaction was not significant ($Pr > F = 0.6775$). Chelerythrine ranged from 0.9% to 1.7% with average content being 1.3% for shade house material and 1.7% to 3.2% with average content being 2.4% for woods grown material. Based on average dry weights, chelerythrine yield was 1.2g per square meter from the polypropylene shade plots and 0.1g per square meter from the woods plots. There was more variation in the woods than the shade house. The site x treatment interaction was not significant ($Pr > F = 0.1924$).

The number and size of rhizomes harvested from the shade site was much greater than those harvested from the woods but the percent alkaloid content per unit weight was higher in the rhizomes from the woods. There was a problem with animal browsing in the woods site that was not a condition at the artificial shade site. Woods plots sustained severe damage from deer (*Odocoileus virginianus*), turkey (*Meleagris gallopavo*), and, particularly, groundhogs (*Marmota monax*) eating bloodroot leaves off the plants. There was also less competition from tree roots and other plant species in the polyhouse. Animal browsing and plant competition could have produced a higher level of stress on the woods grown plants and could have induced the bloodroot growing there to produce higher amounts of alkaloid as a survival response. Animal browsing could have also been the main factor in lower rhizome yields from the woods. Rockwood and Lobstein (1994) observed that extensive defoliation by deer and groundhogs nearly eliminated reproduction of bloodroot in current and following seasons. Growing bloodroot under a polypropylene shade house is more expensive than woods grown but may result in harvest of far more rhizomes, outweighing the lower alkaloid yield. Currently, most medicinal plants are bought on a dry weight basis. Growers and

wildcrafters (people who collect plants from the wild) can expect to be paid \$13 - \$26 per dried kilogram (\$6 -\$12 per pound) for bloodroot. Estimated cost of production for 680 kg over a three-year period is \$12,290. That would yield an estimated profit of \$1,210 if the cost of a shade structure is not included (Persons and Davis 2005). A natural shade planting of bloodroot can be made more economical by utilizing a wooded hillside where other crops cannot be planted. Although the cost of woods grown bloodroot is lower and the percent alkaloid per unit dry mass is higher, artificial shade is easier to cultivate and easier to harvest because there are no tree roots to contend with. Also, according to data from this study, rhizomes harvested from artificial shade cultivation are greater in number and size and alkaloid yield is greater because there is so much more rhizome mass than from woods cultivated bloodroot.

These studies suggest that cultivation of bloodroot under artificial shade is more productive than woods cultivation. Graf et al (2007) found that artificial shade cultivated bloodroot produced a more consistent product with less variation in alkaloid yield than wild simulated bloodroot. Determining optimal conditions for woods grown bloodroot could result in higher root and alkaloid yields. Although medicinal plants are generally bought on a dry weight basis, manipulating conditions to produce a consistent high alkaloid yield could provide a market advantage if buyers should come to value the combined dry mass and alkaloid yield above the dry mass yield only. Future studies to help determine what locations bloodroot performs best in might concentrate on multiple woods locations with particular attention to soil pH and surrounding vegetation or soil treatments to adjust pH as well as trials to include varying rates of calcium or other soil nutrients. If sanguinarine content is the main goal of production, then more information is needed before determining that field

grown bloodroot under artificial shade is more productive than woods grown bloodroot.

When biomass yield is the main concern, according to data from these studies, artificial shade grown bloodroot is more productive than woods grown bloodroot.

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Table 2.1 Bloodroot rhizome yield in grams per square meter

Treatment	Shade House		Woods	
	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight
Bud	352.4	89.8	14.1	2.8
No Bud	214.6	56.4	48.2	7.9
Whole	510.5	137.2	16.9	2.9
Mean	359.2	94.5	26.4	4.5

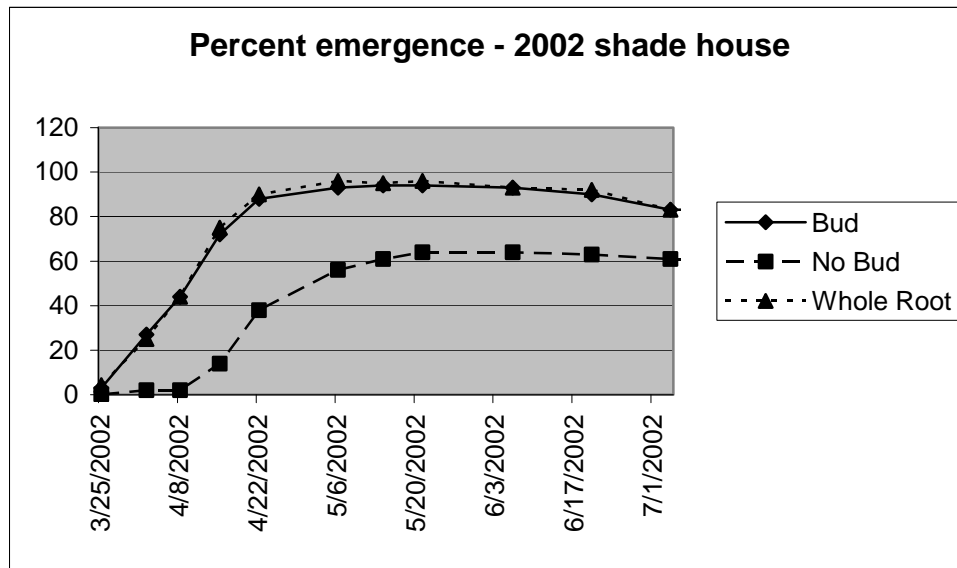


Fig 2.1 Total bloodroot emergence recorded at 12 dates from 3/25/02 – 7/4/02 expressed as a percent of total rhizomes planted. Treatments consist of three propagule types: 1) Bud = 5 cm rhizome piece with a bud, 2) No Bud = 5 cm rhizome piece without a bud, 3) Whole = Whole rhizome (uncut). Bud and Whole Rhizome treatments were significantly greater than the No Bud treatment but not different from each other according to the protected LSD test ($P \leq 0.05$).

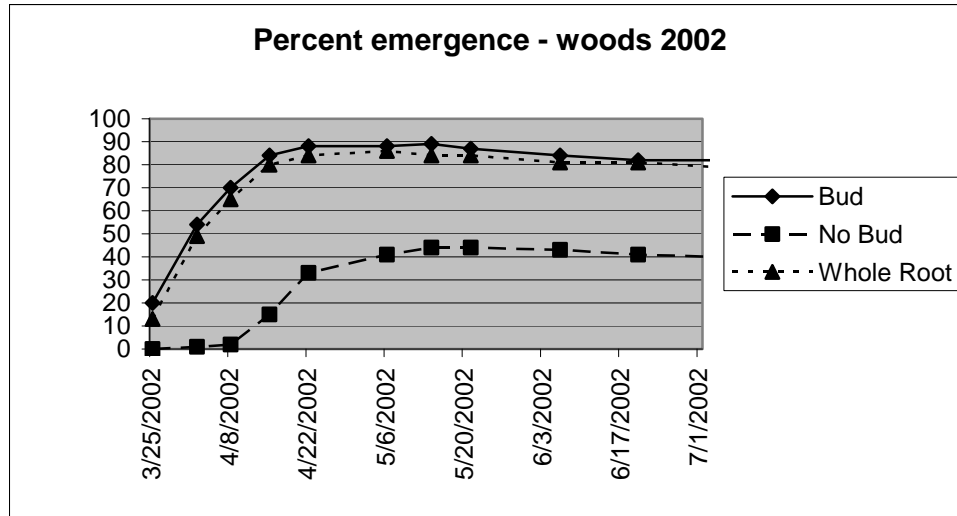


Fig 2.2 Total bloodroot emergence recorded at 12 dates from 3/25/02 – 7/4/02 expressed as a percent of total rhizomes planted. Treatments consist of three propagule types: 1) Bud = 5 cm rhizome piece with a bud, 2) No Bud = 5 cm rhizome piece without a bud, 3) Whole = Whole rhizome (uncut). Bud and Whole Rhizome treatments were significantly greater than the No Bud treatment but not different from each other according to the protected LSD test ($P \leq 0.05$).

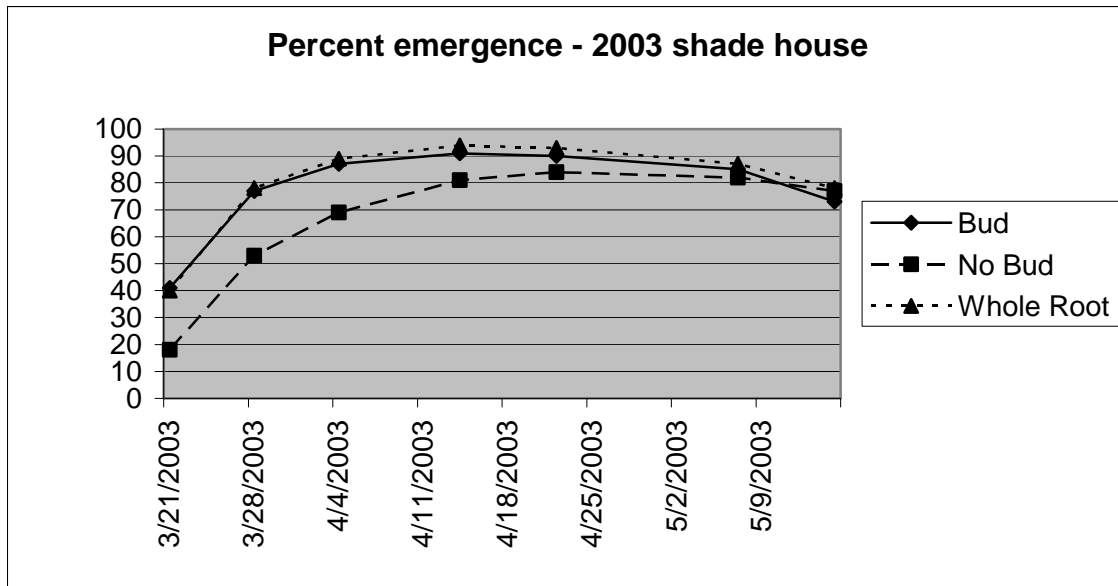


Fig 2.3 Total bloodroot emergence recorded at 12 dates from 3/21/03 – 5/15/03 expressed as a percent of total rhizomes planted. Treatments consist of three propagule types: 1) Bud = 5 cm rhizome piece with a bud, 2) No Bud = 5 cm rhizome piece without a bud, 3) Whole = Whole rhizome (uncut). Treatment differences were not significant.

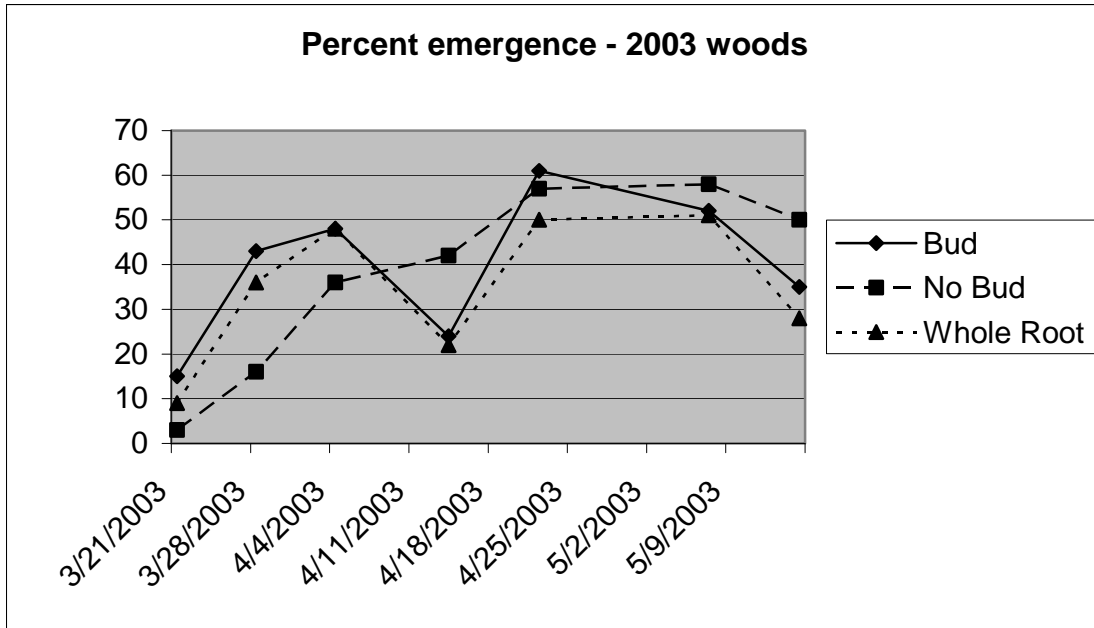


Fig 2.4 Total bloodroot emergence recorded at 12 dates from 3/21/03 – 5/15/03 expressed as a percent of total rhizomes planted. Treatments consist of three propagule types: 1)Bud = 5 cm rhizome piece with a bud, 2)No Bud = 5 cm rhizome piece without a bud, 3)Whole = Whole rhizome (uncut). Treatment differences were not significant.

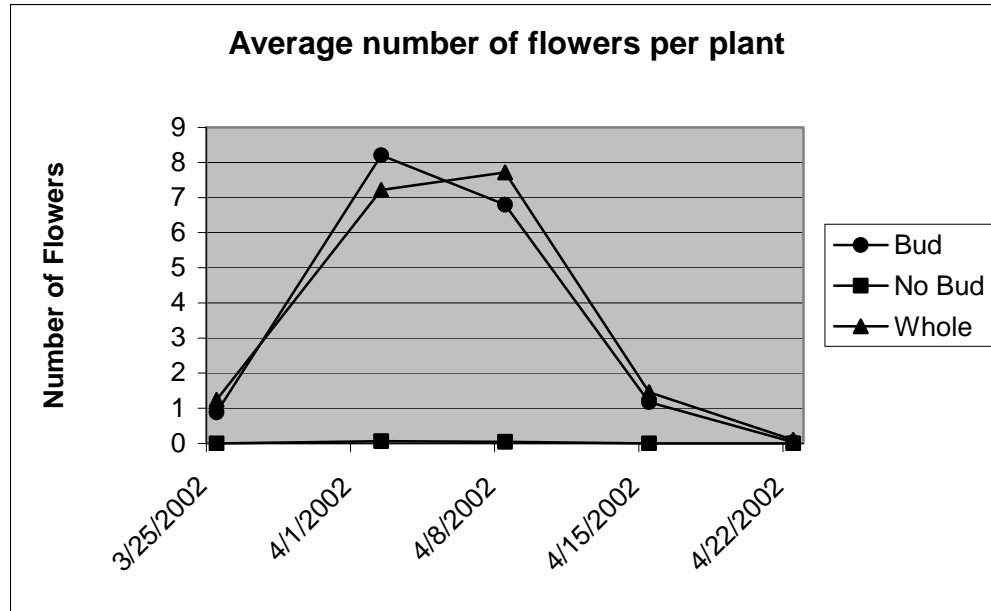


Fig 2.5 Average number of bloodroot flowers per plot in each treatment: 1) Rhizome piece with a bud, 2) Rhizome piece without a bud, and 3) Whole Rhizome. Bud and Whole Rhizome treatments were significantly greater than the No Bud treatment but not different from each other according to the protected LSD test ($P \leq 0.05$).

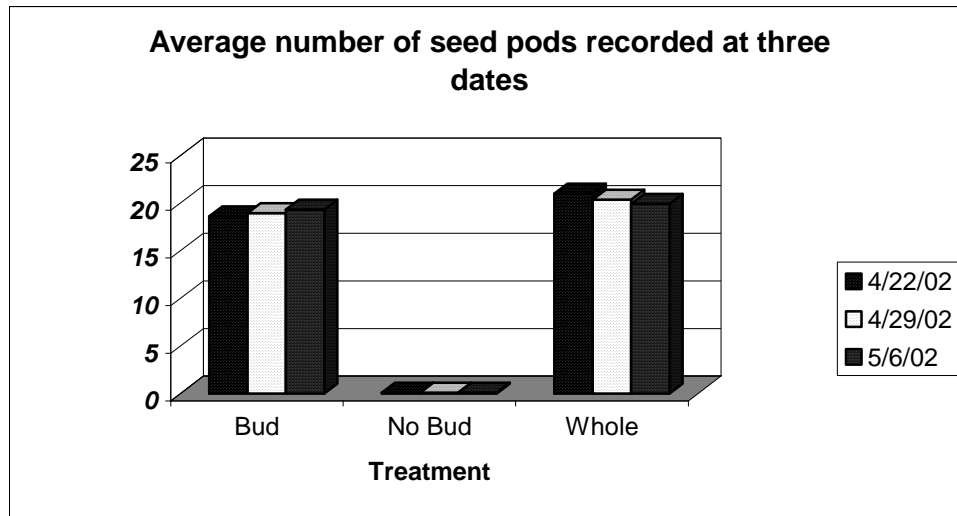


Fig 2.6 Average number of bloodroot seed pods per plot for 2002. Bud and whole rhizome treatments were significantly greater than the No Bud treatment but not different from each other according to the protected LSD test ($P \leq 0.05$).

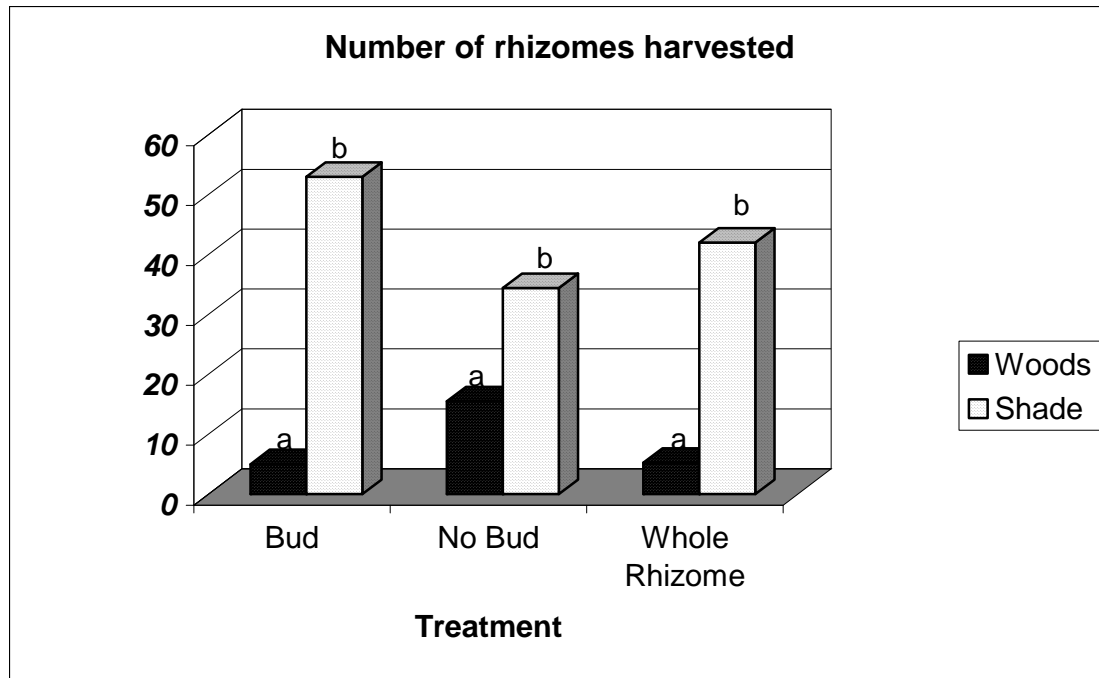


Fig 2.7 Average number of bloodroot rhizomes harvested from two field sites; woods and polypropylene shade structure. There was no significant difference between treatments however there was significant difference between field sites according to the protected LSD test ($P \leq 0.05$). Different letters indicate statistical significance.

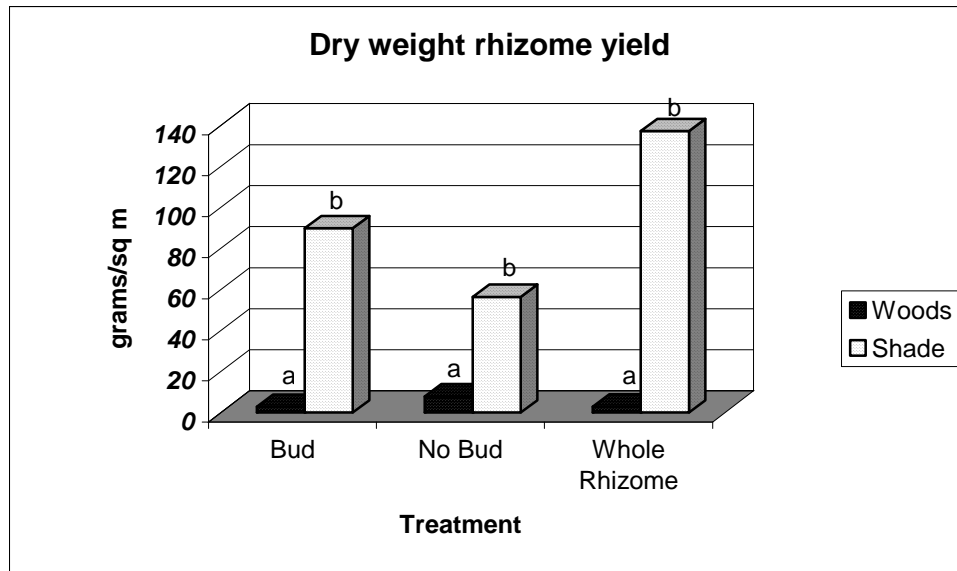


Fig 2.8 Average dry weight yield of bloodroot rhizomes per square meter. Different letters indicate statistical significance at the $p = 0.05$ level.

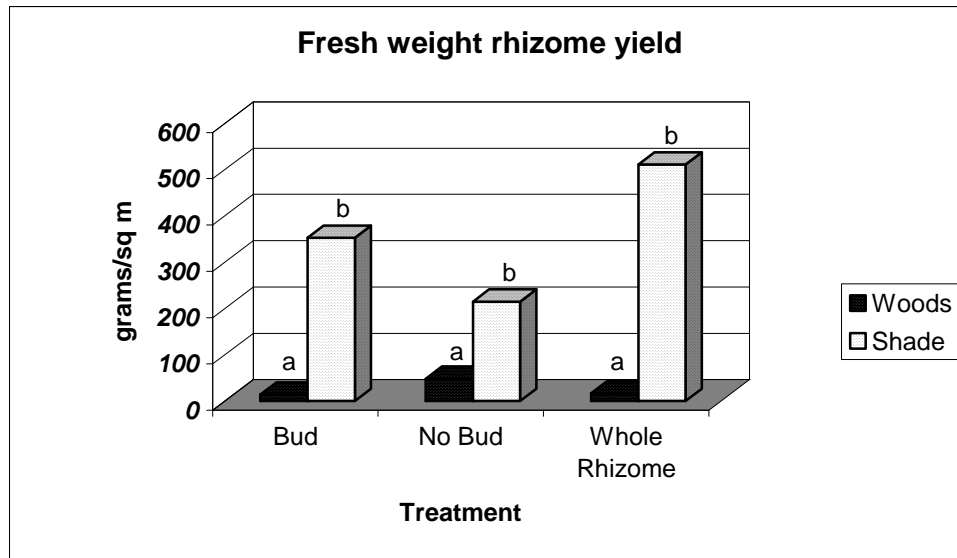


Fig 2.9 Average fresh weight yield of bloodroot rhizomes per square meter. Different letters indicate statistical significance at the $p = 0.05$ level.

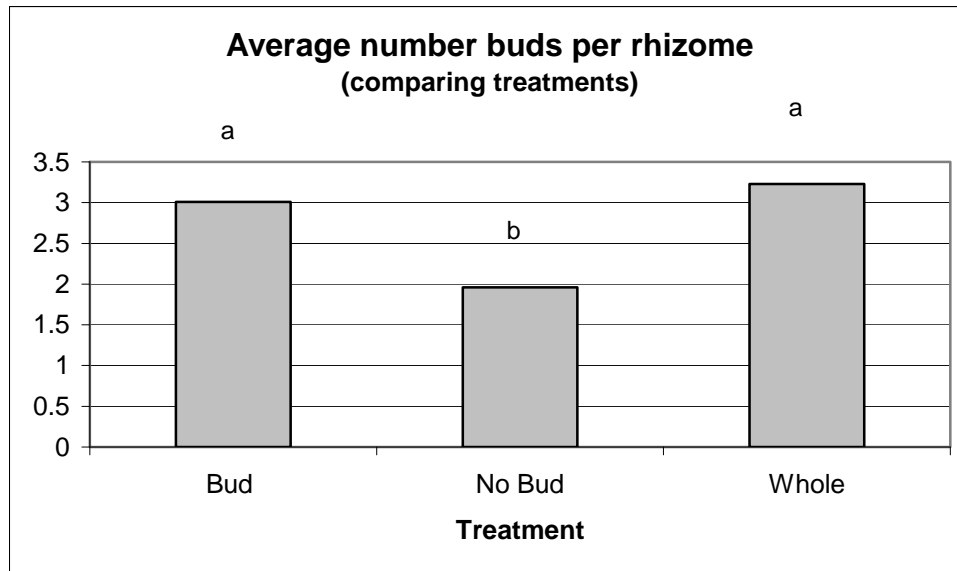


Figure 2.10 Average number of buds per bloodroot rhizome. Different letters indicate significant difference according to the protected LSD test ($P \leq 0.05$).

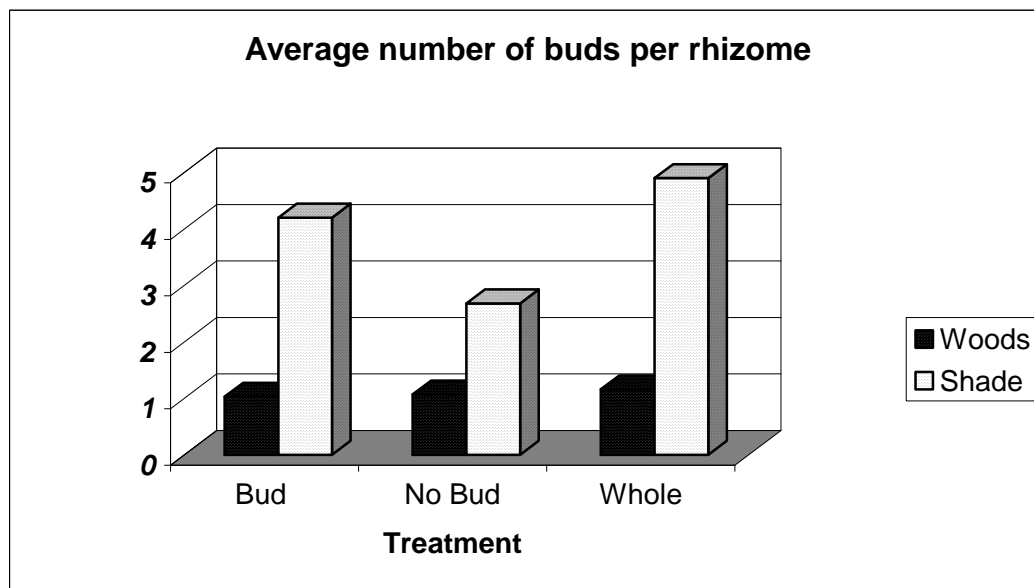


Figure 2.11 Average number of buds per bloodroot rhizome is based on a sample of ten rhizomes from each plot.

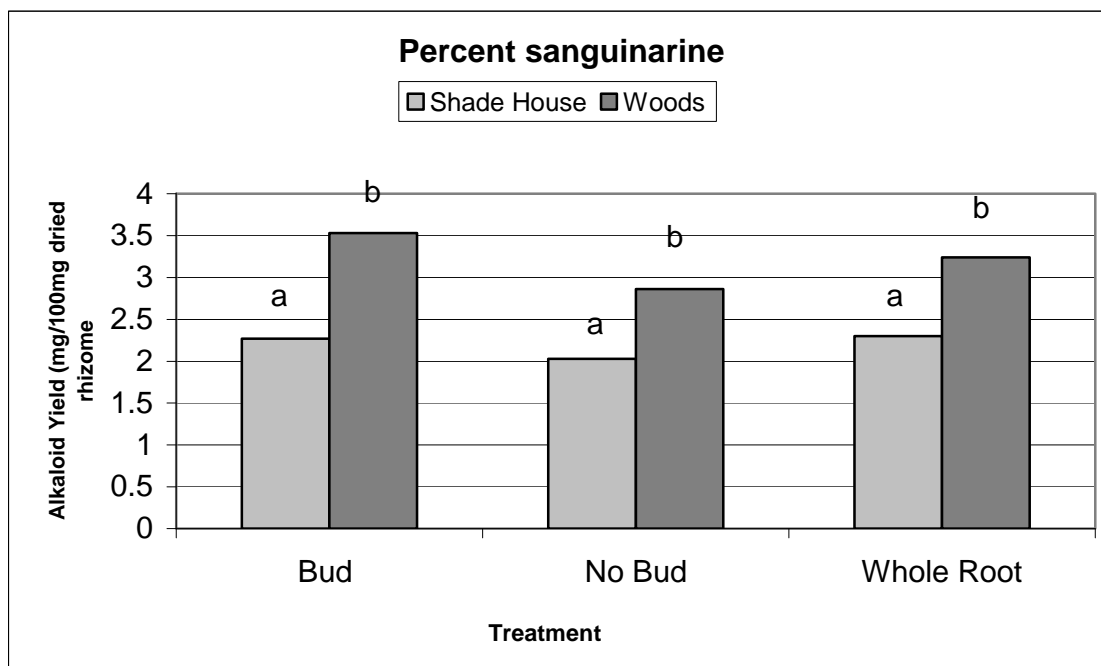


Figure 2.12 Percent of the dried bloodroot rhizome mass as sanguinarine. Different letters indicate statistical significance at the $p = 0.05$ level.

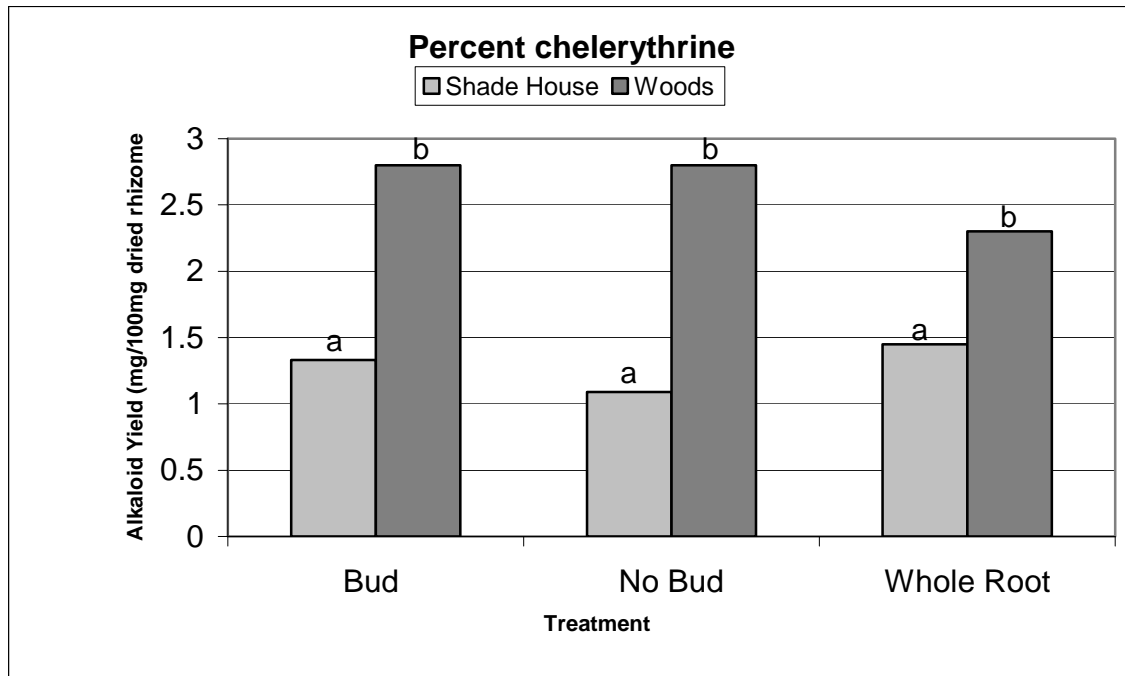


Figure 2.13 Percent of the dried bloodroot rhizome mass as chelerythrine. Different letters indicate statistical significance at the $p = 0.05$ level.