EFFECT OF SHADE, IRRIGATION AND NUTRIENTS ON DRY MATTER YIELD

AND FLAVONOID CONTENT IN AMERICAN SKULLCAP

Except where reference is made to the work of other, the work described in this thesis is my own or was done in collaboration with my advisory committee. This thesis does not include proprietary or classified information.

_____

Arsène Similien

Certificate of Approval:

_____	_____
C. Wesley Wood Professor Agronomy and Soils	Dennis A. Shannon, Chair Professor Agronomy and Soils
_____	_____
Foshee G. Wheeler Assistant Professor Horticulture	Barbara W. Kemppainen Professor Anatomy, Physiology and Pharmacology
_____	_____
Nirmal Joshee Assistant Professor Fort Valley State University Fort Valley, GA	Agnes M. Rimando USDA University of Mississippi Oxford, MS

_____

Georges T. Flowers
Dean
Graduate School

EFFECT OF SHADE, IRRIGATION AND NUTRIENTS ON DRY MATTER YIELD AND FLAVONOID CONTENT IN AMERICAN SKULLCAP

Arsène Similien

A Thesis

Submitted to

the graduate Faculty of

Auburn University

in Partial Fulfillment of the

Requirement for the

Degree of

Master of Science

Auburn, Alabama
May 9, 2009

EFFECT OF SHADE, IRRIGATION AND NUTRIENTS ON DRY MATTER YIELD

AND FLAVONOID CONTENT IN AMERICAN SKULLCAP

Arsène Similien

_____

Signature of Author

_____

Date of Graduation

VITA

Arsène Similien, son of Joseph William Similien and Marie Solange Amazan was born and raised in Les Cayes, Haiti, W.I. He graduated from College Saint Jean High School in July 1986. He attended the American University of Les Cayes in Haiti in September 1986 and graduated with a bachelor of science in Agronomy in August 1991. From 1992 to date he worked for the Service in Evangelization, Education and Development Inc. in Les Cayes consecutively as Farm manager, teacher of agricultural production, Coordinator of Agricultural division and Assistant Director. He was also involved in many community works related to agricultural production as coordinator of the Agricultural Club for Research and Technical Assistance from 1991 to 2001, and broadcast a radio program on agricultural education. He also worked for Catholic Relief Services in 1994 as Food Crisis Program Manager. In August 2006, he entered graduate school at Auburn University where he was appointed as Graduate Research Assistant in the Agronomy and Soils Department. He received a Master degree in Agronomy and Soils (Crop Science) in May 2009. Arsène is married to Marie Dites Monise Fontaine and together they have two children, Axan Wesley and Anselle Monikha.

THESIS ABSTRACT

EFFECT OF SHADE, IRRIGATION AND NUTRIENTS ON DRY MATTER YIELD AND FLAVONOID CONTENT IN AMERICAN SKULLCAP

Arsène Similien

Master of Science, May 9, 2009
(B.S., American University of Les Cayes, August 1991)

140 Typed pages

Directed by Dennis A. Shannon

Growing interest in medicinal herbs results in a need to domesticate medicinal plants traditionally harvested in the wild. American skullcap *(Scutellaria lateriflora*), native to moist habitats in eastern North America, has sedative properties associated with the flavonoid, baicalin, and also contains baicalein, chrysin, wogonin and lateriflorin which have multiple uses. Information on how growing conditions affect dry matter yield, concentration and flavonoids yield is lacking. A field experiment was conducted at the EV Smith Research Center near Shorter Alabama in 2007 and 2008 to explore the effect of light, irrigation and nutrient application on dry matter yield and flavonoid concentration and yield of American skullcap. The field experiment was a 2 x 2 x 3 split

plot factorial in a randomized complete block design with shade as the main factor and irrigation and nutrients as subplots. Treatments were: shade (40% vs. no shade), irrigation (applied at 30 kPa vs. no irrigation and nutrients (no nutrients vs. fertilizer: 100 kg N, 68 kg P and 42 kg K ha^{-1}) vs. (chicken litter: 100 kg N, 50 kg P and 123 kg K ha^{-1}). Four harvests were carried out in 2007 and 2008 to determine dry matter yield and flavonoid content. Extraction of plant material was performed using the Accelerated Solvent Extraction method and extracts were analyzed by the HPLC method to determine flavonoid concentration.

All parameters considered in our study, except percent dry matter, performed better under shade than in full sun. Higher density was observed in 2008 due to spreading after removal of mulch fabric, however a decrease in stand was observed in the non-irrigated treatments in full sun. Powdery mildew was a problem encountered mainly under shade. Dry matter yield was 45% higher under shade, 61% higher with irrigation and 22% higher with added nutrients. Dry matter yield was not different according to nutrient source. A significant interaction of irrigation by nutrients was also observed. The highest dry matter yields were obtained with the irrigation + manure and irrigation + fertilizer treatments under shade and the lowest yield with fertilizer and the control treatments in full sun.

Shade decreased baicalin concentration but did not affect baicalein, wogonin and chrysin concentration. Irrigation increased baicalin, baicalein and wogonin concentration but had no effect on chrysin concentration. Nutrient application slightly increase baicalin and chrysin but did not affect baicalein and wogonin concentration. Total flavonoid concentration was 26% higher in full sun, 20 % higher with irrigation and 29% lower

with added nutrients. Significant interactions of shade by irrigation and shade by nutrient were observed for baicalin and baicalein concentrations. The highest concentrations were obtained with the irrigation + manure and irrigation in full sun and the lowest with manure under shade.

Shade, irrigation and nutrients increased yield of all four flavonoids. Total flavonoid yield was 26% higher under shade, 97% higher with irrigation and 44% higher with added nutrients. Significant interactions of shade by irrigation, shade by nutrients and irrigation by nutrients were also observed for flavonoid yield. The highest flavonoid yields were observed with the irrigation + manure and irrigation + fertilizer treatments under shade and the lowest with the control and fertilizer treatment in full sun. Higher dry matter and flavonoid yields were obtained with the same treatments, suggesting that increasing dry matter yield had a direct effect on flavonoid yield. Based on our results, we can recommend irrigation and added nutrients for higher dry matter and flavonoid yield and irrigation with added nutrients in full sun for higher flavonoid concentration.

ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude to Dr. Dennis Shannon for his uncommitted guidance throughout the course of this program. His appreciation is also extended to the members of his committee: Dr. C.Wesley Wood, Dr. Wheeler Foshee, Dr. Barbara Kemppainen, Dr. Nirmal Joshee and Dr. Agnes Rimando for their valuable advices and encouragement.

Special thanks to Dr. Edzard van Santen for his valuable help in statistical analysis, and Mrs. Brenda Wood for her assistance in conducting laboratory analysis. The author would like to express his greatest debt of gratitude to the board members of Service in Evangelization, Education and Development Inc. and specially Dr. and Mrs. Joe Kopec for their financial support advice and encouragement throughout the course of this study; Pastor Frantz Clotaire, and his family for their assistance to his family; Dr. and Mrs. Robert Beebe for their support and words of encouragement. Additionally, the author would like to thank Mr. Jason Burkett for his assistance in the field works, his fellow graduates, Anthony Kumi and Meili Wang for their encouragement. Finally, the author would like to express his deepest gratitude to his wife, Monise and children Axan and Anselle for their love, support and understanding throughout the course of this study period; his parents, William and Marie Solange Similien, brothers and sisters for their love and assistance. Last but not least, the author expresses his greatest thank to God for his guidance and protection throughout the course of this study period.

Style manual or journal used: Hort Science

Computer software used: Microsoft Word 2003, Microsoft Excel 2003, SAS v. 9.1

TABLE OF CONTENTS

LIST OF TABLES

Chapter II:

LIST OF FIGURES

Chapter II:

Chapter III

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

American skullcap, a medicinal herb native to North America, has been traditionally used by Native Americans for the treatment of many illnesses (Moerman, 1998; Wills and Stuart, 2004). Fossil records date human use of plants as medicines at least to the Middle Paleolithic age some 60,000 years ago (Solecki, R and Shanidar, I. V 1975). A resurgence of interest in American skullcap has been observed during the past few decades. Numerous studies have been conducted to identify and evaluate the chemical constituents and active ingredients of American Skullcap and many other medicinal species (Ref. Awad et al. 2003; Bergeron et al. 2005). Efforts to cultivate American skullcap and many other medicinal species have also been observed.

Cultivation of medicinal plants or concentration and composition of bioactive molecules is influenced by changes to their natural habitat which, according to the general belief would have an influence on their chemistry. Various models and theories (Satu, 2005) have been developed in search of an explanation of how environmental factors affect chemical constituents of plants. Such knowledge would not only contribute to a better assessment of medicinal material harvested from the wild, but also contribute to improve their therapeutic properties through proper management of their environment. Environmental factors such as light, humidity and nutrients are considered to be among

the most important affecting plant growth and yield. These factors are also believed to have great effects on chemical composition of plants.

Use of Herbal Medicine

Herbal medicine was considered the main source of natural therapies in ancient times. People of all ages and classes have made use of medicinal plants as a source of remedies, and van Wyk and Wink (2005) state that even today; many people rely on herbal medicine as their main source of remedies. With the advent of synthetic medicines, use of herbal therapies has considerably declined (Mannfried, 1993). However, in recent years, there has been resurgence in the use of herbal medicine (Azaizeh et al., 2005). Today, even developed countries such as United States and Japan consider natural medicine as an important alternative (McIntyre, 1995) and the World Health Organization reports that about 70 percent of the world population makes use of herbs as their main form of therapy (Wills et al 2000).

The resurgence of interest in phytomedicine is due to various factors. First, with the advent of new analytical procedures, knowledge on chemical constituents and therapeutic properties of various medicinal species are available and better documented. The systematic study of various medicinal species has contributed to improvement of the science of pharmacognosy, leading to better identification and study of chemical components of various herbal species and their therapeutic properties (Mannfried 1993). These studies have led to a better understanding of the mode of action and efficacy of many herbal products. Another explanation for renewed interest in herbal medicine is the high cost and failure of many synthetic drugs (Tyler, 1987). Knowledge of the active

ingredients and mode of action of many commonly used herbs results in a better appreciation and increased use of these products.

Concentration of active ingredients in the plant is not static; it is often affected by change in the environment (Tyler, 1987). To better understand and exploit medicinal plants, it is important to be knowledgeable not only about their chemical constituents, but also on how these components are affected by various environmental factors. Such knowledge may lead to a better assessment of these products and make it possible to optimize their concentration by proper manipulation of the environment which is generally associated with cultivation practices.

Cultivation of Medicinal Plants

Plant materials used for medicinal purpose are mostly harvested from wild sources (Sturdivant and Blakley, 1999). This type of harvest is considered to be advantageous for it requires almost no financial investment. Cultivation of medicinal plants and other species requires high investment along with some associated risks (Balunas, 2003). Wild plants are generally well adapted to their natural habitat. No investment in term of pest control, fertilization, irrigation and other cultural practices is required. To properly cultivate a medicinal species it is important to consider its natural habitat. Also, special management techniques such as shade structure, irrigation, and pest control often need to be provided for successfully production (Balunas, 2003). Another barrier to cultivation of medicinal plants is the belief that plant materials harvested from the wild may be more valuable in term of chemical content than cultivated ones due to their "natural" habitat. However, in spite of these aspects which work against cultivation

of medicinal species, there are important reasons for which cultivation of medicinal plants needs to be encouraged.

First, plant materials harvested from the wild are often not uniform (Azaizeh 2005). They come from various sources and were grown under various environmental conditions and sometimes mixed with other plant species through incorrect identification (Sturdivant 1999). As a result, many herbal products are found to be adulterated (Azaizeh, 2005). Cultivation of medicinal plants would prevent such problem and contribute to standardize or make uniform herbal products.

Another problem with wild harvest of medicinal plants is the risk of extinction for many species due to uncontrolled and excessive harvest. As interest in herbal medicine grows, demand for some species increases accordingly. As demand exceeds supply, this leads to declining populations of many species and increase in prices. American Ginseng (*Ginseng panax*) is one example of medicinal species that was under threat of extinction. Cultivation is one avenue that prevents such extinction and today, most ginsengs sold for medicinal purpose come from cultivated source (Sturtevant and Blakley, 1999).

Another advantage of cultivation is that knowing the active substances of medicinal species and the environmental factors affecting their production, proper cultivation practices may allow the grower to maximize these active substances, and enhance their value as medicine. Also, good management practices such as irrigation, fertilization, soil preparation, timing of planting and harvesting contribute to increased biomass production, which along with chemical composition, determines, the overall yield of medicinally active compounds (Zobayed et al., 2004),. Finally, cultivation of medicinal plants would increase supply and help to decrease high price of wild harvested

herbal materials (Azaizeh, 2005). Today, interest in cultivation of medicinal species is growing as the threat of extinction of many species seems to be understood. Many conservation groups have already suggested that wild species be brought under cultivation (Azaizeh 2005). However, the effect of cultivation on the phytochemistry and concentration of medicinally active ingredients of medicinal plants needs to be evaluated.

Plant phytochemistry: The flavonoids

Chemically, plants are composed of primary and secondary metabolites (Satu, 2005). Primary metabolites include large molecules of carbohydrates and proteins involved mainly in the primary metabolic processes such as respiration and photosynthesis (Satu 2005). They are the substrate for the synthesis of secondary metabolites, which constitute a wide variety of substances having different structures and functions in the plants. These two groups of compounds are inter-related and said to share a common substrate, the carbohydrates, for their synthesis (Stamp, 2004). However, primary metabolism is said to have priority over the secondary and in time of resource scarcity, synthesis of secondary metabolites are believed to suffer the most (Hamilton et al. 2001).

Medicinal plants synthesize various secondary metabolites. The most important include: the flavonoids, tannins, saponins, (Mannfried,1993), alkaloids polysaccharides (such as gums and mucilage), peptides (Wills et al. 2000) essential oils, vitamins and other trace elements (McIntyre 1995; Watson et al 2002; Azaizeh et al 2005). Many of these metabolites have been for long considered as worthless to the plant life process (Satu, 2005). Today, they are known to be responsible for various functions in the plant-environment relationship. These functions include: protection against environmental

stresses such as drought and excessive light radiation (Jaakola, 2004; Wills et al., 2000; Hernandez, 2004), herbivores and other pathogen attacks (Hernandez et al., 2004); allellopathy (Zobel et al 1999), metabolisms (Wills et al., 2000), and attractant to pollinators (Schreiner 2005). Several of these metabolites have therapeutic properties and their concentration in the plant tissues is considered as the main factor to evaluate the therapeutic value and quality of a given herb (Wills et al 2000). One of the most important groups of plant secondary metabolites having therapeutic properties is the flavonoid.

The Flavonoids

Flavonoids are an important class of phenolic secondary plant metabolites. They are distributed throughout the plant tissues where they are responsible for various functions (Jaakola et al, 2004). Flavonoids are considered to be one of the most powerful antioxidant groups of carbon-based phenolics synthesized by plants (Jaakola et al., 2004). Therapeutic properties of medicinal species are often associated with their antioxidant properties due to the presence of various types of flavonoids (Azaizeh, 2005). Different species of plants synthesize specific types of flavonoids with specific functions. Other therapeutic functions of various flavonoids include anti-inflammatory (Hernandez et al., 2004) anti allergenic, anti-viral, and anti-tumoral (Azaizeh et al 2005).

Flavonoids and other plant metabolites are not evenly distributed throughout the plant tissues. Their concentration and distribution in the plant are not only a function of genetics, but also are found to be influenced by various environmental factors such as light, humidity and soil fertility (Mannfried, 1993). Effects of these factors on the

concentration of plant metabolites are very important and need to be considered in assessing and evaluating medicinal plant materials.

Effects of Environmental factors on plants phytochemicals

Normal plant growth and chemical status are affected both by internal and external factors. Internal factors such as genetics play important roles in the composition and many characteristics, such as taste, shape, color and many other physical and chemical properties of a given species. Environmental factors such as light, humidity and nutrients play important roles in plant growth and metabolites synthesis and allocation (Robbers and Tylers, 1999). Effects of these factors on plant growth are readily observable. Water and nutrients are prerequisites for normal plant growth and yield. Under drought and low fertility, plant yield and biomass production is greatly reduced and in some instance the whole plant may die. Light is a prerequisite for the production of photosynthates required for synthesis of both primary and secondary metabolites. While the effect of environmental factors on physical appearances of a plant is obvious, it is not the case for its chemical composition. The effect of environmental factors on the chemical status of the plant is not clearly defined.

Various approaches and theories have been developed in search of an explanation of the effect of environmental factors on plant phytochemistry. The most well-known of these theories include: the carbon-nutrient balance hypothesis (CNB), the growth differentiation balance hypothesis (GDBH), the protein competition model (PCM) and the photo inhibition model (Satu, 2005). Common to each of these approaches, is the concept that there is a competitive relationship between primary and secondary metabolism, in which primary synthesis is prioritized over the secondary. Thus,

according to these models, the total photosyntate produced by a plant is primarily utilized by the growth process and reproduction before being allocated to secondary metabolism. According to the CNB hypothesis, lack of nutrients in the soil affects plant growth more than photosynthesis, while light reduction has a more negative impact on photosynthesis than on growth (Hamilton et al. 2001). Consequently, low nitrogen content of the soil leading to a decrease in plant growth would yield to an accumulation of extra carbohydrates that can be used to produce secondary metabolites. (Hamilton et al 2001). This hypothesis also suggests that shortage of light, limiting the photosynthetic process, will result in a decrease in carbohydrates production. Insufficient carbohydrate produced is used mainly by the growth process, which results in a decrease in carbon-based secondary metabolites. However, under shade conditions and adequate nitrogen, an increase in nitrogen containing metabolites such as the alkaloids and cyanogenic glycosides is observed (Hamilton et al 2001). In essence, the CNB concept states that an increase in sunlight or a decrease in nutrient leads to an increase in carbon-based metabolites such as the phenolics, while a decrease in light and an increase in nitrogen would produce an increase in the nitrogen based metabolites such as the alkaloids.

The growth differentiation hypothesis (GDBH) is closely related to the CNBH by giving priority to primary over secondary metabolites synthesis. However, this model is more generalist. According to this model, various environmental factors beside photosynthesis and nutrients, affect production and allocation of plant secondary metabolites (Koricheva, 2002). This hypothesis suggests that all factors contributing to a decrease in growth while not significantly affecting photosynthesis would result in an increase in photosyntate and consequently in secondary metabolites (Stamp, 2004). These factors, such as water and

nutrients, when moderately insufficient, have a negative impact more on growth than on photosynthesis and consequently would lead to an increase in carbohydrate available for secondary metabolites production (Stamp, 2004). However, according to this model, excessive shortage of nutrients and water would affect negatively both growth and secondary metabolite production (Stamp, 2004). Moderate supply of nutrients and water leading to moderate biomass production would lead to a higher concentration of carbohydrate and consequently an increase in secondary metabolites. Under high resource availability, growth would benefit over secondary metabolites production (Stamp, 2004).

According to the protein competition model (PCM), both proteins and phenolics use phenylalanine, an essential amino acid, for their synthesis (Satu, 2005). Consequently, any environmental factor that contributes to an increase in growth and protein synthesis would lead to a decrease in phenolics due to a decrease in phenylalanine available for their synthesis (Satu, 2005).

Finally, the photo inhibition model associates the production of phenolic metabolites with a response of the plant toward inhibiting oxidative damage caused by excess light intensity (Satu, 2005). In this case, increasing light intensity would contribute to an increase in phenolics production by the plant.

It is also believed that, along with the environment, genotypic factors play an important role in the synthesis of secondary metabolites, and Hamilton (2001) even argues that genotypic factors are far more important than environmental ones in determining concentration of secondary metabolites in plants. As a matter of fact, secondary metabolites synthesis is influenced both by genetics and environmental factors (Jeffery et

al. 2003) to be successful; any theory needs to take into account these two categories of factors. While some species respond readily to changes in their environment to produce extra phenolics others are more dependent upon their genetic make-up. In addition, response of a species to environmental change is believed to be influenced by their natural habitat (Stamp, 2004).

Among the environmental factors, light, moisture and nutrients are considered to have the most important impacts on plant growth and reproduction. Consequently, these factors should affect the secondary metabolic processes. Effect of light, moisture and nutrients have been tested and found to have great influences on the concentration and allocation of various secondary metabolites in many species, such as black cottonwood *(Populus tricoparta)* quaking aspen *(Populus tremuloides) and* Jonagold apple *(Malus domestica)* (Warren et al. 2003; Jocelyn et al. 1999; Awad et al. 2001).

Effect of light

As the main factor affecting photosynthesis, light intensity has a direct effect on primary metabolite production, which consists mainly of carbohydrates. Secondary metabolites production, especially the carbon-based phenols such as the flavonoids, depends on availability of primary photosynthate. Increasing light intensity increases primary photosynthate, which leads to an increase in phenolic concentration in the plant (Warren et al., 2003). Flavonoids are the most readily-produced phenolics in the epidermal cells of plants exposed to high light intensity. They are antioxidants, and their production is considered as a response toward protecting the plant against oxidative damage. Studies show an increase in flavonoid content of various plant species grown under high light condition compared to those in shade. In hemlock, the concentration of

various phenolics has been found to be lower in plants grown under shade than those found in full sun (Zobel et al., 1999). However, different plant species are found to have different levels of sensitivity to light intensity, which can be influenced by other environmental factors.

Effect of Humidity

Without adequate moisture, plant growth and development are seriously inhibited. Water is crucial to plant nutrition. Under drought, no nutrients can be made available for uptake by plants. Water stress affects plant growth and reproduction and alters plant physiological and biochemical properties (Zobayed et al., 2007). Drought stress results in increased formation of a reactive type of oxygen in the plant tissues (Hernandez et al 2004). These oxygen molecules are considered important for some plant functions such as cellular communication, however at high concentration, they are found to be very damaging to the plant (Hernandez et al 2004). To protect themselves against oxidative damage, most plant species under water stress condition react by producing secondary metabolites having anti-oxidant properties, mainly flavonoids (Zobayed, 2007). The survival of a plant under these stressful conditions is found to be associated with its ability to undergo physiological changes (Bohner et al 1996) leading to production and accumulation of the appropriate metabolites (Gulen and Eris, 2004). Many studies found an increase in concentrations of flavonoids and other antioxidant in plants found under drought condition compared to those grown under adequate moisture (Hernandez et al 2004). However, this observation is not always true; it varies sometimes with plant species and types of metabolites. In the medicinal plant species, St. John Wort, the concentration of the phenol, hypericin, decreases significantly under water stress, while

hyperforin, another phenol, increases by twofold under the same condition (Zobayed, 2007).

Effect of Nutrients

Compared to light and water, nutrients have little effect on photosynthesis but have great influence on growth (Glynn et al 2003). Increase in growth due to addition of nutrients results in higher consumption of available photosyntate (Jocelyn et al., 1999) which would otherwise be allocated to the production of secondary metabolites. Increasing growth by addition of nutrients while the photosynthetic rate stays the same, leads to a decrease in secondary metabolite production. (Glynn et al., 2003; Palm et al., 2006). Under very poor soil fertility, both growth and photosynthesis decrease. Under these conditions, little photosyntate is produced and it is used mainly by the growth process, resulting in a decrease in secondary metabolites (Azaizeh et al., 2005). Under such low fertility, addition of nutrients may contribute to an increase in secondary metabolites (Jocelyn et al., 1999). For some species, however, production of many metabolites is enhanced under shortage of nutrients and other adverse environmental conditions (Bruulsema, 2000). Better results in term of secondary metabolites production are obtained under conditions where intermediate amount of nutrient is provided. With an intermediate amount of nutrients, a slight decrease in growth may result, while the photosynthetic rate stays the same (Glynn et al., 2003). The net result is an increase in photosynthate available for secondary metabolites production. Since environmental factors such as light, humidity and nutrients affect chemical composition of plants, medicinal plants grown under environmental conditions different from their natural habitat would have their phytochemistry altered and consequently their therapeutic

properties. Wild plants and cultivated ones often differ in their content of secondary metabolites (Hassan, 2005). These differences lead to discrimination between medicinal plant harvested in the wild and those harvested from cultivated sources. Azaizeh (2005) states that it is important for ethno-pharmacologists to take into consideration the environment where an herb is harvested before considering its use as a remedies.

American Skullcap

American Skullcap (*Scutellaria lateriflora*), a medicinal plant used mainly for its sedative and anxiolytic properties, is one of these species for which an increased demand is expected as demand for medicinal materials with these properties has, according to (Brevoort, 1998), surpassed any other categories of herbal products these last years. Increase in demand for American skullcap may also be expected due to recent discovery in its tissues of the flavonoids baicalein, the active ingredient found in the root of Baikal skullcap (*Scutellaria baicalensis),* a Chinese species used for centuries in Asian natural medicine for its anti-inflammatory and anti-allergic properties. This discovery, according to (Hans Wohlmuth), suggests new therapeutic use for American skullcap similar to that of Baikal Skullcap.

American skullcap is a perennial herbaceous species native to temperate North America (Bergeron et al 2005), where it is distributed from Canada to Florida (Gafner et al 2003). Skullcap is a member of the mint family (Lamiaceae). The genus, *Scutellaria,* comprises about 300 species distributed around the world (Awad et al 2003). American skullcap is prevalent under moist habitat. It is found mainly in swampy woods (Awad et al 2003) and moist thickets (Foster and Duke, 2000). The species is classified by the United States Department of Agriculture either as facultative or obligate wetland species

depending on the region (USDA-NRCS Plant database, 2006). In Alabama it is classified as a facultative wetland species. American skullcap is commonly identified under various names such as: Mad-dog skullcap, mad dog weed, mad weed, hoodwort, helmet flower, Virginia skullcap, blue skullcap, and Quaker bonnet (Joshee et al 2002, Wills and Stuart 2004). The plant grows to a height up to three feet (Joshee et al 2002) and is characterized by a branched stem, opposite, serrate-crenate leaves and blue to violet-blue flowers turned to the side (explaining the epithet, "lateriflora" assigned to this species). American skullcap is also grown in Europe and commercially cultivated in Australia and New Zealand (Wills and Stuart, 2004).

Chemical constituents and use

The chemical make up of the genus *Scutellaria* includes the flavonoids, volatile oils, iridoids, diterpenoids, waxes and tannins (Wills and Stuart, 2004). The flavonoids are considered to be responsible for therapeutic properties of the species. In *Scutellaria lateriflora*, different types of flavonoids have been identified. They include the flavonoid glycosides baicalin, dihydrobaicalin, ikonnikoside I, lateriflorin, scutellarin and oroxylin A-7-O-glucuronide and the aglycones baicalein, oroxylin A, wogonin, and 5,6,7-trihydroxy-2"-methoxyflavone.(Bergeron et al. 2005). Most herbalist literature report the flavonoids Scutellarin and its glycoside scutellarein as the major flavonoids component of American Skullcap (Wills and Stuart,2004). However, new studies based on more advanced techniques, found the flavonoids baicalein and its glycoside baicalin to be in greatest concentration in the plant. Bergeron (2005), in a recent study, found that the aerial part of American skullcap to contain baicalin as the major flavonoid glycosides and

oroxylin A, followed by baicalein as the major flavonoid aglycone. Lateriflorin and scutellarein were rather found to be less important components.

Skullcap was listed in the United States Pharmacopoeia from 1863 to 1916 and in the National Formulary until 1947 (Foster and Tyler, 1999). The herb was traditionally used by the Native Americans for the treatment of diseases including epilepsy, cholera, nervous tension state (Newall et al. 1996), insomnia, anxiety, neuralgia (Foster and Duke, 2000), rabies, diarrhea, digestive problem (Greenfield and Davis, 2004) promotion of menstruation and elimination of after birth (Wohlmuth, 2007). Skullcap was introduced as part of the American medicine in 1773 by the medical doctor Lawrence Van Derveer for the treatment of rabies where the name of "mad dog" is derived. Today, the herb is mainly used for its sedative and anti-spasmodic properties (Mills, 1985; Buntain, 1999) in the treatment of nervous condition, insomnia (van Wyk and Wink, 2005) and is believed to act as a nervous system restorative (Mills, 1985).

Cultivation Practices

Previous research on American skullcap published in refereed journals focused on identifying and extracting of various types of flavonoids and others chemicals constituents present in the plant tissues. (e.g. Awad et al., 2003; Bergeron et al., 2005). No agronomic experiments conducted in US on American skullcap are reported in the scientific literature. However, recommendations on its cultivation are available from Kansas State University (Rhonda, 2004) , North Carolina Consortium on Natural Medicines and public Health (Greenfield and Davis, 2004) and Saskatchewan Agriculture and Food (Porter, B. 2000). Skullcap can be propagated through direct seeding, transplanting or root divisions (Greenfield et al, 2004; Butain, 1999). A cold stratification

of 40 to 50 F for about a week is required for proper seed germination (Greenfield et al, 2004). Seedlings need to be grown in greenhouse 6 to 8 weeks before being transplanted to open field (Porter, 2000, Greenfield et al, 2004) during late spring or after danger of frost (Greenfield et al, 2004; Joshee et al 2002; Porter, 2000). Suggested plant spacings are 15-30 cm between plants in rows spaced up to 60 cm apart, which would yield a population density around 55,000 to 110,000 plants per hectare (Porter, 2000). Other suggested spacings are 20-30 cm between plants in rows spaced 45-90 cm apart. (Greenfield and Davis 2004). Skullcap responds well to added nitrogen (Jankee, 2004) which is particularly recommended once harvesting begins (Greenfield et al, 2004, Porter, 2000); however overfertilization must be avoided (Joshee et al 2002, Buntain, 1999). Skullcap grows successfully under dry conditions (Joshee et al 2002, Jankee 2004) and full sun (Faurot et al; Joshee 2002); However, under dry conditions, partial shade (Wills et al 2004) and irrigation (Greenfield et al, 2004) are recommended.

Diseases and Insects

Some diseases of American skullcap have been documented and reported in the Index of Plant diseases in the United States. These include the leaf spots: *Cercospora scutellariae*; the stem rot, *Botrytis cinerea*; the powdery mildews, *Erysiphe* rots, *Phymatotricum omnivorum* and *Rhizoctonia solani galeopsidis*, and *Microsphaera* sp (Greenfield et al, 2004); Insects such as Leaf beetles have been also reported in some places (Porter, 2000). In Auburn, a heavy infestation of powdery mildew was observed in a preliminary study conducted by the department of Agronomy and Soils at Auburn University (Shannon, 2007).

Harvesting, Storage, and Yield

American Skullcap can be harvested once it begins to flower (Greenfield and Davis, 2004, Porter, 2000). However, harvesting in late flowering or even at fruiting is also suggested (Porter, 2000). The above-ground part of the plant is cut about 3 inches from the base (Rhonda, 2004). A single cutting is recommended for the first year and two the following years (Greenfield et al, 2004) which can be done at 6 to 8 weeks intervals (Buntain, 1999). Once harvested, the plant material needs to be kept under shade and transferred as soon as possible to the drying area to prevent loss of flavonoids (Greenfield Davis, 2004). Physical damage of the leaves and stems and compaction must also be avoided during harvesting. Damage to the leaves and stems, such as a wound, can result in loss in flavonoids. Wills and Stuart (2004), in an experiment conducted for the Australian Government Rural Industries Research and Development Corporation, found that the flavonoid retention during drying of skullcap is 53.5 and 40.1 mg/g respectively under minimal and heavy damage and compression during harvesting. They also found no significant difference in flavonoid content under drying temperatures varying from 40 to 70 degrees Celsius. Porter (2000) recommended that full color be retained after drying. The dried materials need to be stored in a dark place under temperature from 5 to 30 C (Porter, 2000). Under optimum growing conditions, yields up to 2,275 kg of dry matter per hectare are possible (Jankee, 2004; Porter, 2000). Yield in flavonoid at harvesting stage, that is when the plant is at full bloom, varies with plant section harvested. In their experiment, Wills and Stuarts (2004) found that the concentration of flavonoid in mg/g to be 52.9 in leaves, 22.9 in stem and 32.4 in roots, which suggests that the leaf is the important plant part to be used for medicinal purpose.

RESEARCH GOAL AND OBJECTIVES

The goal of this research was to determine the appropriate growing conditions needed to cultivate American Skullcap commercially in order to optimize dry matter yield and flavonoid content in American skullcap. Under natural conditions, American skullcap is found in moist and shaded areas. Therefore, shade and irrigation was tested under shade and open field conditions. Also, based on the fact that vegetative growth and many plant metabolites are inhibited or enhanced by soil fertility level, the effect of chemical and organic fertilizers was studied. The specific objective of my research was to determine the effect of shade, irrigation and nutrients on dry matter yield and flavonoid concentration and yield in American skullcap.

REFERENCES

Awad, A. M., P. S., Wagenmakers, A.de Jager. 2001. Effects of light on flavonoid and chlorogenic acid levels in skin of Jonagold apples. Sciencia Horticulturae 88: 289-298.

Awad, R., J.T.Arnason, V.L.Trudeau, C. Bergeron, , J.W Budzinski,., B.C Foster, Z. Merali,. 2003. Phytochemical and biological analysis of skullcap (*Scutellaria lateriflora* L.): a medicinal plant with anxiolytic properties. Phytomedicine.10, 640-649.

Azaizeh, H, Predrag L., I. Portnaya, O. Said, U.Cogan and A.Bomzon. 2005. Fertilization induced changes in growth parameters and antioxidant activity of medicinal plants used in traditional Arab medicine. *ECam* 2005; 2(4) 549-556

Balunas, M. J. 2003. Ecological characteristics, harvesting impacts, and restoration potential of goldthread (*Coptis trifolia (L.)*, a medicinal plant. Master thesis, State University of New York, College of Environmental sciences and forestry, New York Col.

Bergeron C., S. Gafner, E. Clausen, J. D. Carrier. 2005. Comparison of the Chemical composition of extracts from *Scutellaria lateriflora* using accelerated solvent extraction and supercritical fluid extraction versus

standard hot water or supercritical fluid extraction versus standard hot water or 70% ethanol extraction . Journal of Agricultural and Food Chemistry 53, 3076 – 3080

Bohnert, H.J, R.J. Jensen. 1996. Strategies for engineering water-stress tolerance in plants. Trends Biotechnology. 14, 8997

Brevoort, P. 1998. The booming U.S botanical market: A new overview. Herbal Gram No 44, pp 33-48

Bruuselma, T.W. 2000. Phosphorus and Potassium for functional foods. Conference presented at the 29th North Central Extension-Industry Soil Fertility Conference, St Louis, MO, November 1999 and at Crops Update, Woodstock, ON, January 2000.

Buntain M.1999. Commercial production of medicinal herbs in Tasmania. Rural Industries Research and Development Corporation. Publication no. 99/149.

Foster S. and J. A. Duke.2000. Medicinal plants and herbs of Eastern and Central North America 2nd ed. Houston Mifflin Co. N.Y p. 211

Foster S., Tyler V.E. 1999. Tyler's Honest herbal. New York, Haworth Herbal Press.

Gafner,S.; C Bergeron, L. L. Batcha, J. E Burdette, J. Reich,; J. M Pezzuto, J.T. Arnason,. ; C.K Angerhofer. 2003. Inhibition of [3H]-LSD Binding to 5-HT7 Receptors by Flavonoids from Scutellaria lateriflora. J . Nat. Prod. 66,535-537

Glynn C., D. A. Herms, M.Egawa, R. Hansen, W. J. Mattson. 2003. Effects of nutrients availability on biomass allocation as well as constitutive and rapid induced herbivore resistance in poplar. OIKOS 101: 385-397

Greenfield, J., and M. J. Davis. 2004. Medicinal Herb Production Guide, Skullcap *(Scutellaria lateriflora),* North Carolina Consortium on Natural Medicines and public Health, funded by a grant from the Golden LEAF Foundation Inc., www.naturalmedicinesofnc.org

Gulen, H., A., Eris. 2004. Effect of heat stress on peroxidase activity and total protein content in strawberry plants. Plant Science. 166, 739-744.

Hamilton J. G., A. R. Zangerl, E. H. Delucia and May M. R. Berenbaum. 2001. The carbon-nutrient balance hypothesis: its rise and fall. Ecology Lettere, 4:86-95

Hernandez, I., L. Alegre and S.Munne-Bosch. 2004. Drought-Induced changes in Flavonoids and other low molecular weight antioxidants in *Cistus clusii* grown under Mediterranean field conditions. Tree Physiology 24, 1303-1311 http://plants.usda.gov/java/profile?symbol=SCLA2 ; 9/8/2006

Jaakola, L. M-R Kaisu, K.Sirpa, Anja Hohtola. 2004. Activation of flavonoid biosynthesis by solar radiation in bilberry leaves. Planta 218: 721-728

E. H. Jeffery, A. F. Brown, A. C. Kurilich, A. S. Keck, N. Matusheski, B. P. Klein and J. A. Juvik. 2003. Variation in content of bioactive components in broccoli**.** Journal of Food Composition and Analysis Volume 16, Issue 3:323-330

Jocelyn D.C. H. and R.L. Lindroth. 1999. Effects of light and nutrients availability on Aspen: growth, phytochemistry and insect performance. Journal of chemical ecology, vol. 25 no 7. 1687-1714

Joshee N, P.S.Thomas., S.M. Rao. and A. K. Yadav. Skullcap: Potential Medicinal Crop. Reprinted from: Janick and A. Whipkey (Eds.). American Society for Horticultural Science (ASHS) Press, Trends in New crops and new uses.2002. pp.580-586 J.

Alexandria, VA. Koricheva, J. 2002. The carbon-nutrient balance hypothesis is dead; long live the carbon-nutrient balance hypothesis. OIKOS 98:3

Mannfried, P. 1993. Healing plants. Barron's Educational Series, Inc. NY pp. 8-9

Matthew, J. P., F.E. Putz. and Stephen T. T. 2007. Nitrogen fertilizer and gender effects on the secondary metabolism of yaupon, caffeine-containing North American holly.Oecologia 151 no 1:1-9

McIntyre, A. The complete woman's herbal; Henry Holt and Co. 1995 pp.14, 16

Mills S. The Dictionary of Modern Herbalism, a comprehensive Guide to practical herbal therapy, Torsons Publishing group, Welling borough NY 1985 pp. 190-191

Moerman, D. E. 1998. Native American Ethnobotany. Timber Press., Portland, OR, p.927.

Newall A. C., A.A. Linda., J. D Phillison.1996. Herbal Medicine, A guide for health-care Professionals, The Pharmaceutical Press. London. p.239

Porter, B. 2000. Skullcap production in Saskatchewan. 2007. Saskatchewan Agriculture and Food. 3085 Albert Street, Regina, Saskatchewan, Canada S4S 0B1

www.agr.gov.sk.ca/docs/production/Skullcap.ASP 01/ 18/2007

Rhonda, J. May 2004. Farming a Few Acres of Herbs: Skullcap, Kansas State University Agricultural Experiment Station and Cooperative Extension Service: MF-2628

Robbers,E. J., Varro E. Tyler.1999. Tyler's Herbs of choice, the therapeutic use of Phytomedicinals, the Haworth Herbal Press Inc, NY. London. p.5

Satu, T. 2005. The effects of drought stress and enhanced UV-B radiation on growth and secondary chemistry of boreal conifer and willow seedlings. PhD dissertations in Biology, No: 39.ISSN 1457-2486. University of Joensuu, Finland

Schreiner, M. 2005. Vegetable crop management strategies to increase the quantity of

Shannon, D.A. and B.W. Kemppainen. 2007. Preliminary Findings of a New Medicinal Crop Research Program in East Alabama. p. 110 in A.K. Yadav, N. Joshee and A. Yadav (eds.) Program and Abstracts, International Symposium on Medicinal and Nutraceutical Plants, Held in Fort Valley Georgia, March 19-23, 2007. College of Agriculture, Home Economics and Allied Programs, Fort Valley State University, Fort Valley, Georgia. phytochemicals. Eur J. Nutr 44:85-94

Solecki, R and Shanidar, I. V 1975. A Neanderthal flower burial in northern Iraq. Science. 190:880-881.

Stamp N. 2004. Can the growth-differentiation balance hypothesis be tested rigorously? OIKOS 107:2

Sturdivant L. and T. Blakley 1999. Medicinal herbs in the Garden, field and marketplace. San Juan Naturals WA. Pp.116-117

SuperbHERBS: 10 medicinal herbs with economic promise. *(Scutellaria lateriflora L.*). www.ncarboretum.org/superb_herbs/superb_herbs.html, 9/8/2006

Tyler E. Varro. 1987. The New Honest Herbal; Georges F. Stickey Co., p. 1

United States Department of Agriculture Natural Resources Conservation Service http://plants.usda.gov/java/profile?symbo , 9/8/2006

Van Wyk B-E. and M. Wink. 2005. Medicinal plant of the World, An illustrated scientific guide to important medicinal plants and their uses .Briza Publications, Pretoria, South Africa. pp. 294- 295.

Warren M.J., J.Bassman, J. K. Fellman, D. S. Mattinson, S.Eigenbrode. 2003. Ultraviolet-B radiation of *Populus trichocarpata* leaves. Tree Physiology 23, 527-535

Wills R.B.H and D.L. Stuart. 2004. Generation of High Quality Australian Skullcap Products. A Report for the Rural Industries Research and Development Corporation, Australian Government. ISBN 0642587302, ISSN 1440-6845

Wills, R.B.H., K. Bone, and M. Morgan. 2000. Herbal Products: Active Active constituents, modes of action and quality control. Nutrition Research Review, 2000, 13: 47-77

Wohlmuth, H., Skullcap the herb with identity crisis .Botanical Pathways Issue 13 http://www.botanicalpathways.com/pdfs/13.pdf 08/12/07

Zobayed, S.M.A., F.Afreen, T. Kozai. 2007. Phytochemical and physiological changes in the leaves of St. John's wort plants under a water stress condition Environmental and Experimental Botany, 59: 109-116

Zobel, A.M., K. Glowniak, J.E. Lynch, S. Dukea and A. Alliota.1999. Phytochemistry of plants Associated with 400-year-old stand of Hemlock at clear Lake Reserve, Ontario. Proceedings symposium on sustainable Management of Hemlock ecosystem in Eastern North America. GTR-NE-267, 230-233.

CHAPTER II

EFFECT OF SHADE, IRRIGATION AND NUTRIENTS ON DRY MATTER YIELD IN AMERICAN SKULLCAP

ABSTRACT

American skullcap *(Scutellaria lateriflora),* a medicinal plant species valued for its sedative properties associated with flavonoids, is generally harvested from the wild. Information on how open field growing conditions affect dry matter yield is lacking. A 2X2X3 split plot factorial experiment was conducted at the EV Smith Research Center near Shorter Alabama to explore effects of light, irrigation and nutrient application on dry matter yield of American skullcap. Treatment factors were shade (40% shade vs. no shade), irrigation (applied at 30 kPa vs. no irrigation) and nutrients (no fertilizer vs. fertilizer (100 kg N, 68 kg P, 42 kg K ha^{-1}) and chicken litter (100 kg N, 50 kg P and 123 kg K ha^{-1}). Shade formed the main plot units; irrigation and nutrient factors were randomized within subplots. Seedlings were transplanted on April 30, 2007. Four harvests were carried out in 2007 and 2008. All growth parameters considered in this study, except percent dry matter, performed better under shade than in full sun. Dry matter yield was 45% higher under shade, 61% higher with irrigation and 22% higher with added nutrient. Significant interaction of irrigation X nutrients was observed at harvest 2 and 4. The highest dry matter yields were obtained with the irrigation + manure

and irrigation + fertilizer treatments under shade and the lowest with fertilizer and the control treatments in full sun.

INTRODUCTION

Herbal medicine, once the main source of natural therapies has considerably declined since the advent of synthetic medicines (Mannfried, 1993). However, in recent years, there has been resurgence in the use of herbal medicine (Azaizeh et al., 2005) and today, even developed countries such as United States and Japan consider natural medicine as an important alternative (McIntyre, 1995) and the World Health Organization (WHO) reports that about 70 percent of the world population makes use of herbs as their main form of therapy (Wills et al 2000).

Growing interest in medicinal herbs results in the need to domesticate medicinal plants that are, according to Sturdivant and Blakley (1999), traditionally harvested in the wild. Benefits of cultivation of medicinal plants include uniformity of herbal material (Azaizeh 2005), prevention of incorrect identification (Sturdivant 1999) and adulteration (Azaizeh, 2005). Increased interest in herbal medicine also produced an increase in demand for many species such as *Ginseng panax* that are now under threat of extinction. Increased demand, exceeding supply leads to an increase in prices of herbal material. To alleviate or prevent these problems, many conservation groups suggest that wild species be brought under cultivation (Azaizeh 2005). However, adaptation of these species to cultivation needs to be investigated. Various models and theories have been developed in search of an explanation of how environmental factors affect growth and chemical constituents of plants. Such knowledge would not only contribute to a better assessment

of medicinal material, but also contribute to increase total dry matter yield and improve therapeutic properties through proper management of their environment.

American Skullcap *(Scutellaria lateriflora)* is a medicinal species traditionally used by Native Americans in the treatment of many illnesses (Wills and Stuart, 2004). Today the herb is mainly used for its sedative properties. Increase in demand for American Skullcap is expected as demand for medicinal materials with sedative properties has, according to (Brevoort, 1998), surpassed any other categories of herbal products in recent times. American skullcap is a perennial herbaceous species native to temperate North America (Bergeron et al 2005), where it is distributed from Canada to Florida (Gafner et al 2003). Skullcap is naturally found in wet places (Awad et al 2003) and moist thickets (Foster and Duke, 2000); the plant is also reported to grow successfully in full sun and partial shade (Jankee and DeArmond, 2004; Joshee et al., 2002); Previous research published about skullcap in refereed journals focused on identifying and extracting of various types of flavonoids and others chemicals constituents present in the plant tissues (e.g. Awad et al., 2003; Bergeron et al., 2005). No agronomic experiments conducted in US are reported in the scientific literature.

Light, moisture and nutrients are among the most important factors affecting growth and chemistry of plants (Warren et al. 2003; Zobayed et al. 2007; Glynn et al. 2003). Knowledge of how these factors affect dry matter yield and flavonoid content could be used to improve yield and medicinal value of American skullcap through improved crop management practices. The goal of this research was to evaluate potential for American skullcap to be successfully grown under regular farming practices and determine the appropriate growing conditions needed to optimize total dry matter yield.

A field experiment was carried out to evaluate the effect of shade, irrigation and nutrient application on growth and total dry matter yield of American skullcap.

MATERIALS AND METHODS

Site Description and land preparation:

The experiment was conducted at the Horticulture Unit of the E.V Smith Research Center, near Shorter Alabama on a Marvyn loamy sand (fine-loamy, kaolinitic, Thermic Typic Kanhapludults), 2 – 5% slope. Soil pH measured in December 2006 before liming and March 2007 after liming, were respectively 5.1 and 5.8 with CEC 4.6 $cmol_c kg^{-1}$.

Prior to tillage, weeds were controlled using glyphosate herbicide (Round-up) at the rate of 2.1 kg a.i ha^{-1}. A preliminary tillage operation was done in March 2007 using a disk harrow. Following the first tillage and after liming, five soil samples were taken from each experimental block at a depth of 15 cm to determine pH and primary nutrients (N, P, and K) content. A second tillage operation was done on April 9 2007 using a RHINO SHV80 rotor tiller to loosen the soil. Dolomitic Limestone was applied using a truck spreader at the rate of 2500 kg ha^{-1} in March 2007 before second tillage and prior to bedding. Chemical fertilizer and chicken litter were hand broadcasted to respective plots on April 6 2007, prior to bedding. Bedding was done on April 10, 2007. A bedder 18 inches wide was used to prepare beds and place drip irrigation lines simultaneously. Beds were covered with FarmTek weed guard ground cover manufactured from UV-resistant black polyethylene to help control weeds while allowing air and water to reach the plant root system. Holes approximately 5 cm in diameter were cut at a spacing of 30 cm X 30

cm prior to pine bark application to allow transplantation of seedlings. Pine bark mulch was spread over the fabric to help control weeds between and on beds.

Experimental Design and treatments

The experiment was a 2x2x3 split plot factorial in a randomized complete block design with 4 replications. The shade factor formed the main plot units while irrigation and nutrients were randomized within subplots. The six treatments in the subplots were: 1) irrigation applied when soil moisture tension reached 30 kPa vs. no irrigation; 2) chemical fertilizer applied at the rate of 100 kg N, 68 kg P, and 42 kg K ha^{-1}; 3) chicken litter applied at the rate of 100 kg N, 50 kg P and 123 kg K ha^{-1}; 4) irrigation and chicken litter; 5) irrigation and chemical fertilizer; 6) control with no irrigation and no nutrients applied. Chemical fertilizer rates were based on commercial vegetable production. Plot size was 1.2 x 6.1 m (7.43 m^2). Each plot consisted of 40 plants. Seedlings were spaced 30 x 30 cm, yielding a population density of 53,000 plant ha^{-1} assuming a full stand. Single drip lines 16 mm inner diameter, 250 mm wall, 30 cm spacing between dripper, 340 L/H flow /100m @ .55 bars pressure were installed down the center of each bed. Sun Blocker Commercial Shade Houses measuring 7.3 m wide by 9.1 m long were assembled on site. Shade covers manufactured from knitted polyethylene fabric to provide 40 % shade were placed on top of a steel frame and around the South, West and East side of the frame. Shade houses were oriented North-South while plots were oriented East- West.

Seedling establishment and husbandry

Scutellaria lateriflora seed (lot # 4232, certified organic by Oregon Tilth) was obtained from Horizon Herbs LLC. William, OR 91544. Prior to seeding, seeds were

cold stratified in moist potting mix at 4.4 C˚ for 7 days (February 15 – 23, 2007). The flats were transferred to a Growth Chamber on February 23, 2007, where they were supplied mist irrigation from Flora-mist, running at the rate of 1 minute every hour from 6 AM to 4 PM. Mist was applied from six nozzles in H pattern. Four 400 watt sodium lamps provided 12 hours of light per day. Temperature was maintained at about 25.5 C˚. When seedlings reached 5 cm height, they were transferred to the greenhouse to harden for 2 days (March 7-9, 2007). Individual seedlings were transplanted to multicell trays between March 9 and 13. The potting mix, Sunshine Professional Peat-Lite Mixes # 8 / LC 8 - by Sun Gro Horticulture Canada Ltd., was used both in flats and multicell trays. The mix was formulated with Canadian sphagnum peat moss, coarse grade perlite, coarse grade vermiculite, dolomitic limestone, gypsum and long lasting wetting agent. Following transplantation, day and night temperatures were kept at 24.4 C˚ and 21.1 C˚. Seedlings were sprinkle irrigated on a daily basis. *Peter's* 20-10-20 Peat-Lite Special water soluble fertilizer by the Scotts Company Marysville, Ohio 43041, USA was applied twice at the rate of 250 mg L^{-1}. Seven days prior to transplantation, seedlings were placed in full sun to harden stems. Transplantation to the field was done on April 26, 2007 (Repetition I) and April 30 (Repetition II, III, and IV). At transplanting, seedlings averaged 12 cm tall and 10 true leaves based on random samples of 5 plants measured per tray. Soil moisture was low, the most recent precipitation consisting of 0.74 in. rainfall 10 days prior to transplantation, which provided little moisture. Meteorological data from Alabama Mesonet weather center showed that soil temperature at 10 cm and air temperature were respectively 19.7 ˚C and 16.6 ˚C.

Drip irrigation was applied to all treatments until complete establishment. Dead seedlings were replaced periodically until full stands were obtained. Twenty days after transplanting, on May 20, 2007, drip lines were cut from non irrigated treatments. Four (4) tensiometers, (Irrometer Co., Riverside, CA) were placed at 15 cm depth in fertilized irrigated and fertilized non-irrigated plots under shade and in full sun in repetitions 1 and 3. Tensiometer readings were taken only in irrigated plots in 2008 due to availability shortage of tensiometers. Soil moisture tension was recorded twice weekly and irrigation was provided to all irrigated plots when soil moisture tension reached 30 kPa in the irrigated treatments (Table 2.1a and 2.1 b).

Weeding control was done regularly by hand pulling on top and between beds. The herbicide sethoxydim (Poast) was applied twice during the growing period between and around the beds at the rate of 0.54 kg a.i /ha to control annual grasses. Powdery mildew was organically controlled in all affected plots with a mix of Sunspray Ultra horticultural fine oil at 3.1 ml L^{-1} and potassium bicarbonate (Millstop 85% potassium bicarbonate) at the rate of 3.97 mg L^{-1}. Spraying was done 4 times before the first harvest on June 1st, June 7, June 14 and June 19 2007. Neem oil extract (Trilogy) a certified organic insecticide, fungicide and miticide was applied at the rate of 1.25 to 1.5 % solutions (12.5-15 ml/L.) on August 20, 2007 for the first year. In 2008, spraying was done four times on May 18, May 27, June 10 and July 25 with Trilogy neem oil extract. At the beginning of year 2, right after emergence, mulch fabric was removed from all plots on April 7, 2008 to allow rhizomes, which had spread under the fabric, to grow shoots. Chemical fertilizer was applied at the rate of 136 kg ha^{-1} N, 125 kg ha^{-1} P and 110 kg/ha K and chicken litter at the rate of 136 kg/ha N, 68 kg/ha P and 102 kg/ha K.

Composted poultry litter organic pelletized fertilizer 4-2-3 from Longwood Plantation Newington GA was used instead of the dried poultry litter which was used in year 1. The pelletized poultry litter also provided 102 kg/ha Ca, 17 kg/ha Mg, 4.42 kg/ha Fe, 2.38 kg/ha Cu, Mn and Zn.

Harvesting, weighing and determination of plant stand and dry matter yield

Four harvests were carried out at full bloom on June 29 and September 5 in 2007 and on June 13 and July 25 in 2008. Plant height, based on average of 5 samples taken at random from each plot, was taken one day before each harvest, on June 28 and September 4, 2007 and on June 12 and July 24, 2008. The aboveground portion of each plant was cut 7.5 cm from the ground using pruning shears in 2007 and a gasoline-driven hand trimmer in 2008. The central 32 plants (5.96 m^2) of each plot were harvested and weighed to determine total fresh yield. A sample of about 250 grams was taken from each plot to determine percent dry matter and dry matter yield. Samples were placed in 30 x 60 cm paper bags perforated at the bottom and on the side to allow air circulation. In 2007, bags containing samples were placed with open tops in a forced-air dryer (Model AA-5460A, Parameter Generation and Control Inc., Black Mountain, N.C.) at 40˚ C for 3 days. In 2008, drying was done using forage dryer at 43 C˚ for 3 days at harvest 3 and a shed build on site at 38 C˚ for 4 days at harvest 4. Once removed from dryer, samples were weighted to determine percent dry matter. Total dry matter yield was calculated multiplying percent dry matter by the total fresh yield. Number of plants harvested for each plot was counted following each harvest to determine plant stand per treatment. Right before harvest 3, mulch fabric was removed making it impossible to count individual plant. Instead of individual plants, number of shoots was counted at

harvest 3 and 4. Table 2.3 presents a list of main field operations undertaken from February 24, 2007 to August 2008.

Soil testing and mineral uptake

At the beginning of the experiment, five soil samples were taken on March 23, 2007 from each experimental block right after the first plowing operation. Five samples were also taken from each plot on March 3, 2008 prior to second year emergence. Samples were taken at a depth of 15 cm and analyzed to determine pH level and nitrogen, phosphorus and potassium content using the Mehlich I method.

Plant samples were analyzed at the end of each harvest to determine Nitrogen uptake via dry combustion using a LECO TruSpec CN (Leco Corp., St. Joseph, MI). P, K, Mg, Ca, Fe, Na, ZN and Cu content was determined using a dry-ash method and dissolving the remaining ash in dilute acid (Hue and Evans, 1986), and analyzed via inductively coupled argon plasma spectroscopy (SPECTRO CIROS CCD, side on Plasma, GERMANY).

Data analysis

All data were analyzed using the mixed model procedure of SAS Version 9.1.3 (SAS Institute, Cary, NC) for a randomized complete block design with shade treatment as a split plot restriction on randomization. Shade, irrigation and nutrient treatments are fixed effects, while blocks and main error residuals are maintained as random effects

RESULTS

Rainfall, Air and Soil Temperature

Total rainfall for the first year of the experiment was 309 mm (41 mm for the first harvest period (April 26 - June 29, 2007), and 267.97 mm for the second harvest period

(June 29 - September 5, 2007). Total rainfall for the second year, starting April 2 at emergence, was 321.54 mm (164.59 mm at harvest 3 period and 156 mm at harvest 4 period) (Table 2.4). Total rainfall for the dormancy period going from September 1, 2007 to April 1, 2008 was 514.49 mm. Average air temperature for the growing period was 25.6 C° with average minimum and maximum respectively 18.9 C° and 32.3 C°. Average soil temperature was 29.5 C° with average minimum and maximum of 25.2 C° and 33.7 C° over the growing period.

Soil water tension measured during the growing period showed no water stress in the irrigated plots both in 2007 and 2008; However, the month of June and August in 2007 and May to the first week of June in 2008 were particularly dry and stressful for the non-irrigated plots(Table 2.1.a and table 2.1 b).

Soil test results and mineral content in plant tissues

In March 2007, prior to fertilizer application, soil pH was 5.8; Mehlich I available phosphorus (P) was 35 kg ha^{-1} and potassium (K) was 177 kg ha^{-1}. In March 2008, prior to plant emergence and application of nutrients for the second season, soil test results showed higher pH, P, K and Mg with application of fertilizer or manure than without soil amendment (Table 2.2).

Mineral concentration in shoots was higher under shade than in full sun under all experimental conditions both in 2007 and 2008. Irrigation decreased nitrogen and zinc uptake, increased phosphorus uptake but had no significant effect on potassium and copper uptake. Nutrient application slightly increased N, K, Zn and Cu uptake but had no significant effect on P, uptake. No nutrient deficiency was observed except for Zinc at harvest 1 (Table 2.5).

Powdery mildew, found mainly under shade, was the main problem encountered during production period; only a few plants were affected in full sun. However the pathogen was easily managed with the appropriate organic fungicides applied on a weekly basis during the period of infection.

Results for the analysis of variance of main effects and interactions of shade, irrigation and nutrient application on plant density, height, percent dry matter and dry matter yield are presented in Table 2.6.

Plant density

Plant density was higher under shade than in full sun at harvests 2, 3 and 4 (Table 2.7). At harvest 3 and 4, number of shoots was counted instead of individual plants. Stand loss was very high in 2008 especially in the non irrigated treatment in full sun. The highest densities were obtained with the control treatment under shade at harvest 1 in 2007 and with irrigation + manure under shade at harvest 3 in 2008.The lowest densities were obtained with fertilizer in full sun both in 2008 (Table 2.8). Irrigation did not affect plant stand at harvest 1 and 2 but, plant stands were higher with irrigation than without at harvest 3 and 4. Nutrient application did not significantly affect plant density (Table 2.6 and 2.8). No significant interactions were observed.

Plant height

Shade increased plant height for all 4 harvests respectively at $p=0.003$, $p<0.001$, $p=0.001$ and $p=0.002$ (Table 2.6 and 2.9). The tallest plants (60.7 cm) were found with irrigation+ manure under shade at harvest 3 and the shortest plants with fertilizer in full sun in harvest 4 (Table 2.7). Irrigation increased plant height significantly for the first 3 harvests at $p<0.001$, $p=0.001$ and $p=0.002$. The main effect of irrigation on plant height

was not significant at harvest 4 (Table 2.6 and 2.9). However, the interaction of shade by irrigation was significant at harvest 4 ($p=0.024$). Irrigation increased height in full sun by 99% ($p=0.032$) but had no significant effect under shade (Table 2.6 and 2.10). Nutrient application increased plant height at harvest 1, 3 and 4, respectively at $p=0.016$, $p<0.001$ and $p=0.083$ (Table 2.6). Fertilizer and manure increased plant height respective by 8 and 11 percent at harvest 1 ($p=0.045$ and $p=0.013$) and by 18 and 33% at harvest 3 ($p=0.014$ and $p< 0.001$) compared to the control plot (Table 2.9). There was no significant difference in height between fertilizer and manure application except at harvest 3, where plants receiving manure were 13 % taller than plants receiving fertilizer. Significant interactions of shade by nutrients were observed at all 4 harvests (Table 2.6 and 2.11). At harvest 1, the response to nutrients was significant only under shade but not in full sun (Table 2.10). At harvest 2, manure application slightly decreased plant height in full sun ($p=0.081$) and had no effect under shade. At harvest 3, manure increased plant height both in full sun and under shade ($p=0.007$ and $p<0.001$), however, fertilizer increased height only under shade but had no effect in full sun (Table 2.11).

Significant interactions of irrigation X nutrients were observed at harvest 2 and 3 ($p=0.019$ and $p=0.001$; Table 2.6). At harvest 2, nutrient application had no significant effect on plant height without irrigation but plants were 10% shorter with manure under irrigation (Table 2.12). At harvest 3, nutrient application had no effect on plant height without irrigation, but manure and fertilizer increased height respectively by 53% and 35 % (<0.001) with irrigation (Table 2.12).

Percent dry matter

Percent dry matter was higher in full sun than under shade under all experimental conditions at all four harvests (Table 2.6 and 2.13). The highest percent dry matter (36.3%) was obtained with the control treatment in full sun (Table 2.7). Irrigation decreased percent dry matter at harvest 1, 2 and 3 but had no significant effect at harvest 4 (Table 2.13). Significant interactions of shade X irrigation were also observed at harvest 2 ($p=0.007$) and harvest 3 ($p=0.023$). At harvest 2, irrigation decreased percent dry matter by 7% in full sun while having no significant effect under shade (Table 2.14). At harvest 3, the effect of irrigation was also higher in full sun than under shade (Table 2.14).

Nutrient application had no effect on percent dry matter at harvest 1 but decreased percent dry matter at harvest 2, 3, and 4 ($p=0.001$, $p=0.014$ and $p<0.001$, respectively) (Table 2.13 and 2.6). Both fertilizer and manure, decreased percent dry matter at harvest 2 and 3 but had no effect at harvest 1. At harvest 4, fertilizer had no effect, while manure application decreased dry matter yield significantly by 24% ($p<0.001$) (Table 2.13). An interaction of irrigation X nutrients was significant ($p=0.075$) at harvest 2 (Table2.15). Application of fertilizer or manure decreased percent dry matter without irrigation, but had no effect with irrigation.

Dry matter yield

Shade had no significant effect on dry matter yield at harvest 1, but increased yield by 64.4% ($p=0.017$) at harvest 2, 63% ($p=0.097$) at harvest 3 and 972% ($p=0.005$) at harvest 4 (Table 2.6 and 2.16 and Fig 2.1). Irrigation had no significant effect on dry matter yield at harvest 1, 2 and 4 but increased the yield by 294% ($p=0.002$) at harvest 3

(Table 2.16). Nutrient application increased yield significantly at harvest 1 and harvest 3 ($p<0.001$) Fertilizer and manure increased yield respectively by 32 % ($p=0.003$) and 47 % ($p<0.001$) at harvest 1 and by 107% ($p=0.009$) and 179% ($p<0.001$) at harvest 3 (Table 2.16). No significant effect was observed at harvest 2 and 4 (Table 2.6). The Interaction of irrigation X nutrients was significant at $p=0.019$ at harvest 2 and 3 (Table 2.6). At harvest 2, manure application increased dry matter yield by 36% ($p=0.011$) without irrigation but decreased the yield by 20% ($p=0.094$) with irrigation (Table 2.17 and Fig. 2.2). At harvest 3, manure and fertilizer had no effect on dry matter yield without irrigation but increased the yield respectively by 245% ($p<0.001$) and 147% ($p=0.001$) with irrigation (Table 2.17 and Fig. 2.2).

The highest dry matter yield for an individual harvest (1280 kg ha^{-1}) was obtained with the irrigation + manure treatment under shade at harvest 3 (Table 2.7). The highest total dry matter yield for the 4 harvests in 2007 and 2008 (2662 kg ha^{-1}) was also obtained with the irrigation + manure treatment under shade The highest yield for an individual harvest in full sun, 1162 kg ha^{-1} at harvest 3, and highest total yield, 1995 kg ha^{-1}, were also obtained with the irrigation + manure treatment. The lowest total yields for the 4 harvests (724.8 kg ha^{-1} and 771.4 kg ha^{-1}) were obtained with the fertilizer and control treatments in full sun (Table 2.7).

DISCUSSION

All growth parameters considered in this study, except for percent dry matter, gave better results under shade than in full sun. Higher plant survival was observed under shade for all treatments. The irrigated plots also had higher survival rates than the non irrigated ones. In 2008 plant stand was very low with the non irrigated treatments in

full sun especially at harvest 4; which can be considered as the main cause for lower dry matter yield. These results may be explained by the fact that plants under shade or with irrigation were subject to less stress than those exposed to full sun. Under shade, evapo-transpiration was lower, resulting in higher availability of moisture necessary for nutrient absorption while in full sun the soil was for the most part very dry resulting in lower nutrient availability and drought stress. These results were expected as that skullcap is classified as facultative wet land species.

Plants under shade were taller than those in full sun under all experimental treatments. This may be explained by the fact that growth hormones such as auxin and gibberellins that are responsible for plant cell growth and elongation are inhibited by direct sunlight (Ritchie and Carola 1983; Kingsley 1991). Under shade, the shortest plants were found in the control non-irrigated treatment and the tallest plants with irrigation and added nutrients. These results are partly in accordance with those obtained by Azaizeh et al. (2005) who observed substantial increase in growth and yield of Felty germander (*Teucrium polium* L.) and Eryngo (*Eryngium cretinum L.*), with moderate addition of nutrients. Alexievia et al. (2001) reported substantial decrease in height and dry matter yield of pea and wheat plants grown under drought stress and increased light intensity. They also observed little or no effect of irrigation or nutrients when applied independently but, when applied together, taller plants and higher yield were obtained. In our experiment, added nutrients without irrigation in full sun produced the shortest plants which suggest possible root injury due to higher salt content from fertilizer or manure or osmotic potential effect of dissolved salt in soil solution.

Higher percent dry matter in full sun than under shade for all treatments can be explained by higher photosynthetic and evapo-transpiration rates in full sun. Percent dry matter was higher at harvest 1 than at harvest 2 in 2007 and higher at harvest 3 than at harvest 4 in 2008. This can be explained by the fact that during the second harvest of each year the plants did not have enough time to attain full maturity and also because of the higher soil moisture resulting from higher rainfall during these periods.

Shade did not have a significant effect on dry matter yield at harvest 1 while the effect was significant at subsequent harvests. This result can be explained not only by the fact that plant survival was much higher under shade than in full sun at subsequent harvests, but also by taller and more vigorous plants yielding higher dry matter per individual plant under shade. These results seem to contradict those of Jocelyn (1999) who found higher total dry weight in Aspen trees grown under high light compared to partial shade conditions. Given that skullcap is naturally found in swampy woods (Awad et al 2003) where the temperature is cooler and the soil wetter, the plant is likely to be less tolerant to direct sunlight where the temperature is hotter and the soil dryer leading to the observed decrease in survival. Lower mineral concentration was also observed in full sun (Table 2.5) due possibly to low moisture and availability of nutrient in full sun. Irrigation and added nutrients increased total dry matter yield; however, irrigation may be more critical in full sun than under shade, due to higher moisture stress in full sun than under shade. The highest yields were obtained with irrigation and added nutrients and the lowest yield with the control and fertilized, non-irrigated plots. These results were expected given the importance of added nutrients along with adequate moisture to plant

growth and development. These results suggest that chemical fertilizers are not effective without adequate moisture.

At harvest 2, manure increased dry matter yield without irrigation while decreased the yield with irrigation. This can be explained not only by the fact that no nutrient was added at second harvest but also because without irrigation, nutrients were released slower and were still available at harvest 2, while with irrigation along with heavy rainfall, nutrient leached out faster and become less available at harvest 2. At harvest 3, nutrient application had no effect on dry matter yield without irrigation while it increased the yield significantly with irrigation. These results highlight the importance of water in nutrient availability and uptake by plants. Also without irrigation, manure application resulted in higher dry matter yield than did fertilizer both at harvest 1 and 2. This implies that manure works better without irrigation than do chemical fertilizers. Overall, the treatment combinations producing the highest dry matter yield are irrigation and added nutrients under shade.

CONCLUSION

American skullcap can be successfully cultivated in the Southeast. The main constraint encountered was powdery mildew, for which control methods are available. Highest dry matter yield can be expected with shade, irrigation and added nutrients. Irrigation is important to maintain plant stands and improve availability of nutrients. Without irrigation, survival and response to added nutrient was low, resulting in lower dry matter yield.

It is possible to grow American skullcap in full sun, however yield can be expected to be around 40% lower than under shade. Whether skullcap is to be grown

under shade or in full sun, irrigation and added nutrients (manure or fertilizer) can be considered as the best treatment combination to produce highest dry matter yield. However, it is important for a farmer to consider the costs and benefits before making any decision on inputs. Under shade, incidence and control of powdery mildew might require some additional investment in fungicides along with cost of shade structure; however irrigation may be less critical and total dry matter yield can be expected to be as much as 60% higher than in full sun.

REFERENCES

Alexieva, V, I Sergiev, S. Mapelli and E. Karanov. 2001. The Effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant, Cell and Environment (2001) 24, 1337-1344

Awad, R., J.T.Arnason, V.L.Trudeau, C. Bergeron, , J.W Budzinski,., B.C Foster, Z. Merali,. 2003. Phytochemical and biological analysis of skullcap (*Scutellaria lateriflora* L.): a medicinal plant with anxiolytic properties. Phytomedicine.10, 640-649.

Azaizeh, H, Predrag L., I. Portnaya, O. Said, U. Cogan and A. Bomzon. 2005. Fertilization induced changes in growth parameters and antioxidant activity of medicinal plants used in traditional Arab medicine. *ECam* 2005; 2(4) 549-556

Bergeron C., S. Gafner, E. Clausen, J. D. Carrier. 2005. Comparison of the Chemical composition of extracts from *Scutellaria lateriflora* using accelerated solvent extraction and supercritical fluid extraction versus standard hot water or supercritical fluid extraction versus standard hot water or 70% ethanol extraction . Journal of Agricultural and Food Chemistry 53, 3076-3080

Donald D. Ritchie and Robert Carola: Biology. Addison-Wesley Publishing Company 1983 pp. 360-361

Brevoort, P. 1998. The booming U.S botanical market: A new overview. Herbal Gram. Austin, TX : American Botanical Council and the Herb Research Foundation. Fall 1998. (44) p. 33-46.

Foster S. and J. A. Duke.2000. Medicinal plants and herbs of Eastern and Central North America 2nd ed. Houston Mifflin Co. N.Y p. 211

Gafner,S.; C Bergeron, L. L. Batcha, J. E Burdette, J. Reich,; J. M Pezzuto, J.T. Arnason,. ; C.K Angerhofer. 2003. Inhibition of [3H]-LSD Binding to 5-HT7 Receptors by Flavonoids from Scutellaria lateriflora. J . Nat. Prod. 66: 535-537

Glynn C., D. A. Herms, M.Egawa, R. Hansen, W. J. Mattson. 2003. Effects of nutrients availability on biomass allocation as well as constitutive and rapid induced herbivore resistance in poplar. OIKOS 101: 385-397

Joshee N., P.S.Thomas., S.M. Rao. and A. K. Yadav. Skullcap: Potential Medicinal Crop. pp.580-586 in J. Janick and A. Whipkey (Eds.). Trends in New crops and new uses.2002. ASHS Press, Alexandria, VA.

Kingsley R Stern. Introductory Plant Biology fifth edition. Wm. C. Brown Publishers, 1991. pp 184- 185

Mannfried, P. 1993. Healing plants. Barron's Educational Series, Inc. NY pp. 8-9

McIntyre, A. 1995. The complete woman's herbal; Henry Holt and Co. pp.14, 16

Sturdivant L. and T. Blakley 1999. Medicinal herbs in the Garden, field and marketplace. San Juan Naturals WA. pp.116-117

Van Wyk B.E. and M. Wink. 2005. Medicinal plant of the World, An illustrated scientific guide to important medicinal plants and their uses.Briza Publications, Pretoria, South Africa. pp. 294- 295.

Warren M.J., J.Bassman, J. K. Fellman, D. S. Mattinson, S.Eigenbrode. 2003. Ultraviolet-B radiation of *Populus trichocarpata* leaves. Tree Physiology 23: 527-535

Wills R.B.H and D.L. Stuart. 2004. Generation of High Quality Australian Skullcap Products. A Report for the Rural Industries Research and Development Corporation, Australian Government. ISBN 0642587302, ISSN 1440-6845

Zobayed, S.M.A., F.Afreen, T. Kozai. 2007. Phytochemical and physiological changes in the leaves of St. John's wort plants under a water stress condition Environmental and Experimental Botany, 59: 109-116

Zobel, A.M., K. Glowniak, J.E. Lynch, S. Dukea and A. Alliota.1999. Phytochemistry of plants Associated with 400-year-old stand of Hemlock at clear Lake Reserve, Ontario. Proceedings symposium on sustainable Management of Hemlock ecosystem in Eastern North America. GTR-NE-267: 230-233.

Table 2.1 a: Soil water tension in kPa at 15 cm depth in 2007

Dates	Rep 1				Rep 3			
	F	**IF**	**SF**	**SIF**	**F**	**IF**	**SF**	**SIF**
05/11	0	0	0	1	1	1	1	1
05/16	0	0	0	0	1	1	4	0
05/25	5.5	2	3.5	3.5	8	4	12	4
06/01	32	4	28	5	27	5	58	4
06/07	12	4	18	4	42	4	82	3
06/13	2	2	78	15	80	4	84	14
06/21	21	0	63	7	82	7	4	0
06/28	13	2	70	4	62	3	83	2
07/03	2	2	2	6	0	4	0	3
07/11	4	4	3	4	2	2	2	2
07/18	10	7.5	8	10	10	7.5	10	8
07/25	22	2	4	5	16	4	42	4
08/03	4	0	6	6	10	4	40	4
08/10	3	1	4	4	66	4	68	4
08/17	16	14	58	34	30	28	60	60
08/25	22	2	88	4	33	4	44	4
09/04	8	6	6	6	10	10	8	6
09/14	0	0	2	2	4	4	4	4
09/21	8	8	6	6	10	8	16	8
09/28	52	32	18	10	24	16	42	22
10/05	58	6	22	8	56	20	52	12
10/15	34	10	50	8	68	10	40	10
10/20	4	4	4	4	6	6	6	3
10/26	6	6	7	4	4	4	4	4
11/02	10	8	9	6	4	4	10	8
11/09	15	14	15	10	16	12	16	14
11/16	50	10	10	6	22	18	70	20
11/20	12	10	10	8	30	18	20	10
11/28	6	2	2	2	6	4	6	0
12/04	4	2	2	0	6	4	5	0
12/11	6	6	8	8	6	5	7	4

Note**:** F= fertilizer, IF= Irrigation + Fertilizer, SF= Shade + fertilizer, SIF= Shade + fertilizer + irrigation

Table 2.1 b: Soil water tension in kPa at 15 cm depth in 2008.

Dates	Rep 1				Rep 3			
	F	**IF**	**SF**	**SIF**	**F**	**IF**	**SF**	**SIF**
01/14	20	2	2	2	0	2	25	2
01/21	4	0	3	0	6	2	5	2
01/31	2	2	5	0	10	4	8	3
02/08	3	0	2	0	6	2	10	4
03/06	6	2	4	1	5	2	8	2
03/21	2	0	2	0	3	1	6	0
03/27	48	20	56	18	36	12	48	14
04/03	52	0	24	2	58	0	48	0
04/10	24	10	28	12	36	10	42	12
04/14	12	4	16	2	14	4	16	8
04/22	22	6	43	12	36	8	42	6
04/28	10	2	8	0	12	6	14	4
05/01	32	12	44	18	38	14	30	12
05/12	40	0	24	2	60	0	48	0
05/18	48	0	58	2	22	10	52	20
05/27	65	0	30	5	85	0	53	0
06/04	10	52	81	55	80	40	70	40
06/10	94	54	72	55	50	8	84	45
06/17	n/a	15	n/a	0	n/a	10	n/a	0
06/27	n/a	26	n/a	10	n/a	2	n/a	2
07/03	n/a	0	n/a	0	n/a	0	n/a	0
07/07	n/a	10	n/a	0	n/a	0	n/a	0
07/11	n/a	0	n/a	2	n/a	2	n/a	0
07/15	n/a	6	n/a	2	n/a	2	n/a	8
07/21	n/a	2	n/a	0	n/a	0	n/a	4

Note: F= fertilizer, IF= Irrigation + fertilizer, SF= Shade + fertilizer, SIF= Shade + fertilizer + irrigation

Table 2.2. Soil test results prior to plant emergence in March 2008

Treatments	pH	P	K	Mg	Ca
		<----------kg ha-1---------->			
F	6.4	75	157	159	1652
I	6.4	47	126	126	1312
M	6.3	62	184	161	1448
IM	6.6	57	147	158	1731
IF	6.5	67	153	140	1621
C	6.4	43	147	162	1593
SF	6.1	55	139	155	1389
SI	6.4	40	117	134	1371
SM	6.5	76	172	190	1686
SIM	6.5	55	122	144	1424
SIF	6.5	61	135	145	1576
SC	6.3	42	128	141	1327
	Average Manure or Fertilizer application Vs Control				
F	6.4	64	146	150	1560
M	6.5	63	156	162	1572
C	6	43	129	141	1401

Note: F=fertilizer, I= Irrigation, M= manure, IM= Irrigation+ manure, IF=Irrigation + Fertilizer , C= Control , S= Shade

Table 2.3. Main field operation from February 15, 2007 to August 4, 2008.

Dates	Activities
February 15, 2007	Cold stratification of skullcap seeds
February 23, 2007	Seeding in flat
March 9- 13, 2007	Transfer seedlings to root trainers
March 13, 2007	Land plowing (first operation) + lime application
March 14, 2007	Plots staking out
March 23, 2007	Soil sampling
April 6, 2007	Fertilizer and manure application
April 9, 2007	Second tillage operation, bedding and drip lines placement
April 11- 16, 2007	Layout mulch fabric and dig holes
April 18, 2007	Move seedlings to full sun
April 19- 24	Apply pine bark mulch
April 26, 2007	**Transplant Rep I**
April 30, 2007	**Transplant Rep II – IV**
April 30 –May 4, 2007	Build shade house
May 08, 2007	Place tensiometers
May 20, 2007	Cut drip lines from non- irrigated treatments
June 1, 2007	Spraying
June 7, 2007	Spraying
June 14, 2007	Spraying
June19,2007	Spraying
June 26, 2007	Height measurements
June 29, 2007	**Harvest 1**
June 29- July 3, 2007	Dry samples
August 20, 2007	Spraying
August 20-25, 2007	Cut mulch fabric (just the center line)
September 4, 2007	Height measurements
September 5, 2007	**Harvest 2**
September 5-10,2007	Dry samples
September 24, 2007	Ground harvest 1 samples
September 26,2007	Ground harvest 2 samples
October 8	Extraction and HPLC (Harvest 1 and 2)
November 28-Dec. 11 2007	Plants hibernate
March 3, 2008	Soil sampling
April 3 -7, 2008	Plants reemerge
April 7, 2008	Remove mulch fabric
April 10, 2008	Fertilizer and manure application
May 12, 2008	Spraying
May 18, 2008	Spraying
May 27, 2007	Spraying
June 10, 2008	Plant height measurement
June 13, 2008	**Harvest 3**
June 13-17, 2008	Dry samples
July 7,2008	Ground samples
July 11,2008	Spraying
July 24, 2008	Plant height measurement
July 25, 2008	**Harvest 4**
July 25-28	Drying
July 29, 2008	Grinding and packing
August 4, 2008	Extraction and HPLC (harvest 3 and 4)

Table 2.4 Rainfall record for E.V. Smith Research and Education Center, Shorter AL. April 2007 – July 2008

Date	2007									2008						
	April mm	May mm	June mm	July mm	Aug. mm	Sept mm	Oct mm	Nov mm	Dec mm	Jan mm	Feb mm	March mm	April mm	May mm	June mm	July Mm
1	X	0	0	0	0	0.25	0	0	0	0	29.72	0	0	0	0	0
2	X	0	0	36.8	0	0	0	0	0	0	0	0	20.57	0	0	0
3	X	0	0	46	0	11.43	0	0	10.67	0	0	0	0	0	0	0
4	X	0	2.29	0	0	0.25	0	0	0	0	0	25.91	0	9.14	0	0
5	X	0	0	0	0	0	0	0	0	0	0	1.78	39.88	0	0	0
6	X	0	2.29	0	0	0	0	0	0	0	0	0	18.8	0	0	0
7	X	0	0	27.2	0	0	0	0	0	0	10.41	18.54	1.52	0	0.51	0
8	X	0	4.57	13.7	0	0	0	0	0	0	0	0.76	0.25	0	0	0
9	X	0	0	0.25	0	0	0	0	0	1.02	0	0	0	6.86	0	6.35
10	X	0	0	28.5	0	0	0	0	0	0	0	0	0	0.25	1.52	0
11	X	0	0	4.83	0	0	0	0	0	41.4	0	0	0	7.62	0	42.67
12	X	0	0	2.03	0	17.27	0	0	0	0	0	2.03	6.1	0	0	8.64
13	X	5.59	5.84	0	0	0	0	0	0	0	12.7	0	3.3	0	9.91	1.016
14	X	0	0.25	0.25	0	7.87	0	0	0.51	0	0	0	0	0	14.22	19.81
15	X	0	1.52	0.25	0	18.03	0	5.33	0	0	0	3.56	0	0	1.52	0
16	X	0	0	0	0	0	0	0	10.92	0	0	0	0	40.13	3.3	0
17	X	6.35	0	0.25	0	0	0	0	0	25.91	0	0	0	0	0	0
18	X	0	0	0	9.4	0	1.52	0	0	0	8.38	0	0	0	4.318	0
19	X	0	0	0	0	0	25.91	3.56	0	3.3	0	0	6.86	0	0	0
20	X	0	8.64	0	0	0	0	0	0	12.19	0	23.88	0	0	0	0
21	X	0	0	0.25	0	0	0	0	1.52	0	0	0	0	0	0	0
22	X	0	0	0	0	0.76	0	1.27	0	0	17.53	0	0	0	0	0
23	X	0	0	0	0	0	31	0	5.84	2.55	2.54	0	0	0	0	9.14
24	X	0	0	0	0	0	16.26	0	0	0	0	0	0	0	0	19.05
25	X	0	0	1.53	0	0	0.51	1.78	0	1.52	0	0	0	0	0	0
26	0	0	0	0	31.24	0	0.25	29.7	7.87	6.6	16.5	0	0.51	0	0	X
27	0	0	0.76	0	0.76	0	0	12.95	0	0.76	4.06	0	0.25	0	0	X
28	0	0	0	6.86	4.32	0	0	0	2.79	0	0	0	2.54	0	0	X
29	0	0	3.05	0	0	0	0	0	3.56	0.25	0	0	0	0	8.64	X
30	0	0	0	0	0	0	0	0	34.54	15.2	x	0.76	0	0	6.33	X
31	0	0	X	0.06	37.08	X	0	X	16.26	0	x	0.25	x	0	x	X
Total	**0**	**11.94**	**29.21**	**169**	**82.8**	**55.86**	**75.45**	**54.59**	**94.48**	***111***	***102***	***77.47***	***100.58***	***64.01***	***50.27***	***106.7***
	Harvest 1 period			*Harvest 2 period*			*Dormancy period*						*Harvest 3 period*		*Harvest 4 period*	
Total	**41.15 mm**			**307.37 mm**			**514.49 mm**						**164.59**		**156.95**	

Table 2.5. Main effect of Shade, Irrigation and added Nutrient on Minerals concentrations of American skullcap in 2007 and 2008

	N				P				K				Zn				Cu			
	<----------%---------->												<----------ppm---------->							
	hvt 1	hvt 2	hvt 3	hvt 4	hvt 1	hvt 2	hvt 3	hvt 4	hvt 1	hvt 2	hvt 3	hvt 4	hvt1	hvt 2	hvt 3	hvt 4	hvt 1	hvt 2	hvt 3	hvt 4
Shade effect																				
Full sun	2.47	2.45	2.09	1.13	0.37	0.31	0.39	0.20	3.01	2.02	1.63	0.93	3.88	24.12	27.58	15.45	10.68	10.28	17.13	9.93
Shade	2.68	2.50	2.53	2.81	0.44	0.44	0.49	0.47	3.55	2.71	2.03	2.14	2.65	38.81	37.93	41.34	11.99	6.67	23.25	21.30
SE	0.09	0.07	0.14	0.14	0.02	0.01	0.02	0.01	0.14	0.11	0.07	0.12	2.84	2.48	3.29	2.05	1.08	1.49	1.78	1.26
P>F	**0.001**	0.591	**0.050**	**<0.001**	**0.090**	**0.004**	**0.001**	**<0.001**	**0.026**	**0.019**	**0.001**	**0.002**	0.77	**0.016**	**0.063**	**<0.001**	0.43	0.14	**0.010**	**0.001**
Irrigation effect																				
No irrigation	2.58	2.56	2.54	1.70	0.37	0.34	0.37	0.24	3.25	2.38	1.71	1.19	5.00	32.33	32.39	22.95	11.16	8.38	20.07	12.70
Irrigation	2.56	2.38	2.07	2.24	0.43	0.41	0.51	0.44	3.30	2.35	1.95	1.87	1.53	30.60	33.12	33.84	11.51	8.57	20.32	18.53
SE	0.09	0.06	0.14	0.12	0.02	0.01	0.02	0.01	0.13	0.09	0.07	0.09	2.32	2.12	3.12	1.69	0.96	1.23	1.78	1.06
P>F	0.759	**0.003**	**0.001**	**0.003**	**0.001**	**0.001**	**0.001**	**<0.001**	0.621	0.725	**0.003**	**0.001**	0.14	0.239	0.800	**<0.001**	0.76	0.88	0.912	**0.001**
Nutrient effect																				
None	2.66	2.31	2.22	1.92	0.43	0.36	0.53	0.38	3.23	2.18	1.80	1.55	3.65	29.71	35.90	27.22	10.66	9.95	20.16	14.84
Chemical	2.54	2.47	2.48	1.86	0.40	0.37	0.37	0.30	3.34	2.43	1.72	1.44	3.65	31.08	29.18	28.26	11.68	8.27	19.93	15.02
Manure	2.51	2.64	2.22	2.13	0.38	0.39	0.43	0.34	3.28	2.50	1.96	1.61	2.49	33.61	33.19	29.71	11.66	7.20	20.49	16.98
SE	0.10	0.07	0.15	0.14	0.02	0.01	0.02	0.01	0.14	0.10	0.07	0.10	2.59	2.24	3.35	1.96	1.12	1.39	2.01	1.27
Contrast[1]																				
Ctrl. Vs. chem.	0.201	**0.052**	0.214	0.938	0.131	0.914	**0.001**	**0.004**	0.567	**0.045**	0.566	0.416	1.00	0.658	0.111	0.914	0.698	0.463	0.995	0.994
Ctrl. Vs. man.	**0.092**	**<0.001**	1.000	0.411	**0.017**	0.154	**0.001**	0.100	0.885	**0.009**	0.186	0.740	0.89	**0.063**	0.658	0.568	0.708	0.153	0.989	0.422

[1]Multiple Pairwise comparisons were carried out using Dunnett-Hsu method
Bold numbers represent significant difference

Table 2.6. Significance levels for main effect and interactions for Dry matter yield Percent dry matter, plant Density and plant Height of American skullcap in 2007 and 2008

	2007		2008	
	Harvest 1	Harvest 2	Harvest 3	Harvest 4
Dry matter yield				
Shade	0.127	**0.017**	**0.097**	**0.005**
Irrigation	0.122	0.787	**0.002**	0.217
Shade*Irrigation	0.585	0.129	0.548	0.512
Nutrient	**0.000**	0.735	**0.000**	0.991
Shade*nutrient	0.124	0.422	0.173	0.261
Irrigation*nutrient	0.384	**0.001**	**0.001**	0.174
Percent dry matter				
Shade	**0.004**	**0.007**	**0.002**	**0.000**
Irrigation	**0.000**	**0.001**	**0.002**	0.335
Shade*Irrigation	0.790	**0.007**	**0.023**	0.943
Nutrient	0.837	**0.001**	**0.014**	**0.000**
Shade*Nutrient	0.486	0.880	0.868	0.154
Irrigation*Nutrient	0.705	**0.075**	0.316	0.268
Plant density				
Shade	0.807	0.211	0.151	**0.089**
Irrigation	0.940	0.253	**0.097**	0.208
Shade*Irrigation	0.677	0.384	0.510	0.706
Nutrient	0.511	0.289	0.247	0.466
Shade*Nutrient	0.870	0.315	0.815	0.735
Irrigation*Nutrient	0.235	0.496	0.247	0.424
Plant height				
Shade	**0.003**	**0.000**	**0.001**	**0.002**
Irrigation	**0.000**	**0.001**	**0.000**	0.136
Shade*Irrigation	0.633	0.703	0.458	**0.024**
Nutrient	0.016	0.803	**0.000**	**0.083**
Shade*Nutrient	**0.044**	**0.076**	**0.029**	**0.000**
Irrigation*Nutrient	0.820	**0.019**	**0.001**	0.150

Note: Bold numbers represent significant difference

Table 2.7. Treatments effect on plant Height, Density, Percent dry matter and Dry matter yield at 4 harvests in 2007 and 2008.

	Height				**Density**				**% dry matter**				**Dry mater yield (kg ha-1)**				
Treat	hvt1	hvt2	*hvt3*	*hvt4*	hvt1	hvt2	*hvt3*	*hvt4*	hvt1	hvt2	*hvt3*	*hvt4*	hvt1	Hvt2	*hvt3*	*hvt4*	TDMY
	<----------cm---------->				<--plants ha^{-1}--->		<--shoots ha^{-1}--->		<----------%---------->				<----------kg ha^{-1}---------->				
F	29	27	21	14	53300	37000	122100	23900	29	27	27	25	331	284	108	1	725
I	33	35	24	17	52100	44100	658000	383000	26	26	32	28	359	517	313	60	1249
M	30	28	20	15	50400	31500	323000	47800	29	27	36	21	348	497	102	1	948
IM	33	28	40	10	53400	38600	1375000	299000	27	25	29	21	477	336	1162	19	1995
IF	32	32	26	10	51000	41700	556000	143000	26	26	27	27	409	477	536	16	1438
C	28	29	19	13	53400	43800	347000	131000	29	29	36	33	283	347	111	30	771
SF	40	42	32	23	53000	50400	1088000	1244000	25	20	28	23	350	646	228	236	1461
SI	39	48	39	23	53000	51300	1351000	1064000	22	21	23	20	304	647	395	270	1616
SM	42	47	32	21	53400	50400	897000	909000	25	20	26	19	487	808	375	174	1843
SIM	45	46	61	22	52100	50400	2236000	1567000	23	20	22	17	486	593	1280	303	2662
SIF	46	49	57	31	53400	53000	1579000	1196000	23	21	22	20	527	711	1211	205	2654
SC	35	40	35	20	53800	52100	1351000	1064000	25	22	23	20	273	611	395	178	1457

Note: F=Fertilizer, I= Irrigation, M= Manure, IM= Irrigation + Manure, IF= Irrigation + Fertilizer, C= Control, hvt= harvest, TDM= total dry matter yield. Density for year 2007 was based upon counting discrete plants, while in 2008, number of stems was counted due to spreading after removal of mulch fabric

Table 2.8. Effect of Shade, Irrigation and Nutrient on Plant density of American skullcap in 2007 and 2008

Treatments	2007		2008	
	Harvest 1	Harvest 2	Harvest 3	Harvest 4
Shade effect	<-------plants ha^{-1}----→		←------shoots ha^{-1}------>	
Full sun	52500	40400	563000	171000
Shade	52300	48700	1329000	1132000
SE	1000	24000	133000	96000
Irrigation effect				
No Irrigation	52400	43200	600000	528000
Irrigation	52400	45800	1293000	775000
SE	988	1980	109000	80200
Nutrient effect				
Control	52700	46400	861000	70600
Chemical	52500	45100	770000	544000
Manure	52000	42100	1208000	706000
SE	995	2000	117000	88800
Contrast[1]	<----------P>F---------->			
Full sun vs. Shade	0.807	0.211	0.151	**0.089**
No Irrig. vs. Irrig.	0.940	0.253	**0.097**	0.208
Control vs. Chemical	0.920	0.645	0.741	0.464
Control vs. Manure	0.424	0.258	0.283	1.000

[1]Multiple pair wise comparisons were carried out using Dunnett-Hsu procedure
Bold numbers represent significant difference

Table 2.9. Effect of Shade, Irrigation and Nutrients on Plant height of American skullcap in 2007 and 2008

Treatments	2007		2008	
	Harvest 1	Harvest 2	Harvest 3	Harvest 4
Shade effect	<---------- cm ---------->			
No Shade	31.0	30.2	27.9	9.2
Shade	41.4	46.0	42.7	23.6
SE	0.97	0.91	1.56	1.48
Irrigation effect				
No Irrigation	34.0	36.1	26.7	14.6
Irrigation	38.4	40.2	43.9	18.3
SE	0.94	0.91	1.41	1.61
Nutrient effect				
Control	34.0	38.7	30.1	17.6
Chemical	37.0	37.9	35.6	16.8
Manure	37.7	37.9	40.1	14.8
SE	1.071	1.082	1.577	1.505
Contrast[1]	<----------P>F---------->			
Full sun vs. Shade	**0.003**	**<0.001**	**0.001**	**0.002**
No Irrig. vs. Irrig.	**<0.001**	**0.001**	**0.002**	0.335
Control vs. Chemical	**0.045**	0.797	**0.014**	0.756
Control vs. Manure	**0.013**	0.783	**<0.001**	**0.058**

[1]Multiple pair wise comparisons were carried out using Dunnett-Hsu procedure
Bold numbers represent significant difference

Table 2.10. Interaction of Shade X Irrigation on Plant Height of American skullcap in 2007 and 2008

	2007						2008					
	Harvest 1			Harvest 2			Harvest 3			Harvest 4		
	<---------- cm ---------->											
Treatments	Full sun	Shade	*P>F*	Full sun	shade	*P>F*	Full sun	Shade	P>F	Full sun	Shade	P>F
No Irrigation	29.1	39.0	**<0.001**	28.4	43.8	**<0.001**	19.9	33.5	**<0.001**	**6.2**	**23.0**	**<0.001**
Irrigation	33.0	43.8	**<0.001**	32.1	48.3	**<0.001**	35.9	51.9	**<0.001**	**12.3**	**24.3**	**<0.001**
SE	1.22			1.23			1.94			1.81		
P>F[1]	**0.015**	**0.003**		**0.035**	**0.010**		**<0.001**	**<0.001**		**0.032**	0.558	

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu procedure
Means shown in bold signify interaction is significant

Table 2.11. Interaction of Shade X Nutrient on plant height of American skullcap in 2007 and 2008

	2007						2008					
	Harvest 1			Harvest 2			Harvest 3			Harvest 4		
	<---------- cm ---------->											
Treatments	Full sun	Shade		Full sun	shade		Full sun	Shade		Full sun	Shade	
			P>F			*P>F*			P>F			P>F
Control	**30.8**	**37.2**	**0.003**	**32.4**	**44.9**	**<0.001**	**24.9**	**35.3**	**0.003**	**13.5**	**21.8**	**0.001**
Chemical	**30.9**	**43.1**	**<0.001**	**30.1**	**45.7**	**<0.001**	**25.2**	**46.1**	**<0.001**	**6.5**	**27.1**	**<0.001**
Manure	**31.5**	**43.8**	**<0.001**	**28.2**	**47.5**	**<0.001**	**33.5**	**46.7**	**<0.001**	**7.7**	**21.9**	**<0.001**
SE	1.42			1.48			2.21			1.79		
	<----------P>F---------->											
Ctrl vs. Chem.[1]	0.994	**0.005**		0.399	0.899		0.994	**0.001**		**0.001**	**0.009**	
Contrl vs. Man[1].	0.888	**0.002**		0.081	0.360		**0.007**	**<0.001**		**0.005**	0.994	

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu procedure
Means shown in bold signify interaction is significant

Table 2.12. Interaction Irrigation X nutrients on Plant Height of American skullcap in 2007 and 2008

	2007						2008					
	Harvest 1			Harvest 2			Harvest 3			Harvest 4		
	<---------- cm ---------->											
Treatments	No Irrig.	Irrig.		No Irrig.	Irrig.		No Irrig.	Irrig.		No Irrig.	Irrig.	
			P>F			*P>F*			P>F			P>F
Control	31.7	36.2	**0.019**	**35.4**	**42.0**	**0.002**	**26.3**	**33.9**	**0.012**	16.8	18.5	0.483
Chemical	34.5	39.5	**0.009**	**34.6**	**41.1**	**0.003**	**25.4**	**45.8**	**<0.001**	13.6	20.1	**0.025**
Manure	35.9	39.4	**0.066**	**38.3**	**37.4**	0.670	**28.3**	**51.9**	**<0.001**	13.4	16.3	0.247
SE	1.40			1.48			2.10			1.90		
	<----------P>F---------->											
Ctrl vs. Chem.[1]	0.236	0.137		0.907	0.875		0.924	**<0.001**		0.141	0.559	
Contrl vs. Man.[1]	**0.049**	0.158		0.272	**0.055**		0.698	**<0.001**		0.111	0.362	

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu procedure
Means shown in bold signify interaction is significant

Table 2.13. Main effects of Shade, Irrigation and Nutrient on Percent dry matter of American skullcap in 2007 and 2008.

Treatments	2007		2008	
	Harvest 1	Harvest 2	Harvest 3	Harvest 4
Shade effect	<---------- % ---------->			
No Shade	27.7	26.8	32.5	26.2
Shade	23.7	20.8	24.6	19.9
SE	0.65	1.39	0.98	1.07
Irrigation effect				
No Irrigation	26.8	24.3	31.3	23.9
Irrigation	24.6	23.2	25.8	22.2
SE	0.63	1.32	0.94	1.16
Nutrient effect				
Control	25.5	24.7	29.8	26.0
Chemical	25.7	23.5	27.7	23.4
Manure	25.8	23.2	28.1	19.8
SE	0.66	1.33	0.98	1.02
Contrast[1]	<----------P>F---------->			
Full sun vs. Shade	**0.004**	**0.007**	**0.002**	**<0.001**
No Irrig. vs. Irrig.	**<0.001**	**0.001**	**0.002**	0.335
Control vs. Chemical	0.927	**0.005**	**0.011**	0.136
Control vs. Manure	0.776	**0.001**	**0.042**	**<0.001**

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu procedure
Bold numbers represent significant difference

Table 2.14. Interaction of Shade X Irrigation on Percent Dry Matter of American skullcap in 2007 an 2008

	2007						2008					
	Harvest 1			Harvest 2			Harvest 3			Harvest 4		
	<----------Percent (%) ---------->											
Treatments	Full sun	Shade	*P>F*	Full sun	shade	*P>F*	Full sun	Shade	P>F	Full sun	Shade	P>F
No Irrigation	28.9	24.7	**<0.001**	**27.8**	**20.9**	**0.003**	**36.0**	**26.7**	**<0.001**	27.0	20.8	**0.003**
Irrigation	26.5	22.6	**<0.001**	**25.8**	**20.7**	**0.008**	**29.0**	**22.5**	**<0.001**	25.4	19.1	**<0.001**
SE	0.71			1.41			1.07			1.77		
P>F[1]	**<0.001**	**0.001**		**<0.001**	0.636		**<0.001**	**<0.001**		0.487	0.341	

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu procedure
Means shown in bold signify interaction is significant

Table 2.15. Interaction of Irrigation X Nutrients on Percent Dry Matter of American skullcap in 2007 and 2008

	2007						2008					
	Harvest 1			Harvest 2			Harvest 3			Harvest 4		
	<---------- Percent (%) ---------->											
Treatments	No Irrig.	Irrig.		No Irrig.	Irrig.		No Irrig.	Irrig.		No Irrig.	Irrig.	
			P>F			*P>F*			P>F			P>F
Control	26.7	24.3	**0.002**	**25.7**	**23.7**	**0.001**	32.1	27.6	**<0.001**	28.0	24.0	**0.063**
Chemical	27.0	24.4	**0.001**	**23.6**	**23.3**	0.599	31.0	24.4	**<0.001**	23.1	23.7	0.823
Manure	26.7	25.0	**0.021**	**23.7**	**22.8**	**0.083**	30.8	25.4	**<0.001**	20.4	19.1	0.551
SE	0.75			1.35			1.09			1.49		
	<----------P>F---------->											
Ctrl vs. Chem.[1]	0.927	0.987		**0.001**	0.741		0.524	**0.005**		**0.067**	0.977	
Contrl vs. Man.[1]	0.999	0.585		**0.001**	0.173		0.374	**0.069**		**0.001**	**0.018**	

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu procedure
Means shown in bold signify interaction is significant

Table 2.16. Main effects of Shade, Irrigation and Nutrients on Dry matter yield of American Skullcap in 2007 and 2008

Treatments	2007		2008	
	Harvest 1	Harvest 2	Harvest 3	Harvest 4
Shade effect	<----------kg ha^{-1}---------->			
Full sun	368.1	409.6	388.9	21.3
Shade	404.3	669.4	634.8	227.9
SE	24.5	54.9	89.1	23.3
Irrigation effect				
No Irrigation	345.3	532.1	207.5	107.8
Irrigation	427.1	546.9	816.2	141.3
SE	28.8	48.4	77.0	21.6
Nutrient effect				
Control	304.8	530.6	261.7	126.1
Chemical	404.4	529.7	543.8	123.1
Manure	449.4	558.3	730.0	124.5
SE	27.1	47.5	83.5	22.9
Contrast[1]:	<----------P>F---------->			
Full sun vs. Shade	0.127	**0.017**	**0.097**	**0.005**
No Irrig. vs. Irrig.	0.122	0.787	**0.002**	0.217
Control vs. Chemical	**0.003**	1.000	**0.009**	0.987
Control vs. Manure	**0.000**	0.727	**0.000**	0.996

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu procedure
Bold numbers represent significant difference

Table 2.17. Interaction of Irrigation X Nutrients on Dry matter yield of American skullcap in 2007 and 2008

	2007						2008					
	Harvest 1			Harvest 2			Harvest 3			Harvest 4		
	<----------kg ha^{-1}---------->											
Treatments	No Irrig.	Irrig.		No Irrig.	Irrig.		No Irrig.	Irrig.		No Irrig.	Irrig.	
			P>F			*P>F*			P>F			P>F
Control	278.0	331.7	0.316	**479.1**	**582.0**	0.166	**169.7**	**353.8**	0.190	132.9	119.4	0.697
Chemical	340.7	468.0	**0.033**	**465.1**	**594.3**	**0.089**	**214.0**	**873.6**	**<0.001**	103.1	143.0	0.259
Manure	417.3	481.5	0.236	**652.1**	**464.4**	**0.020**	**238.9**	**1221.1**	**<0.001**	87.5	161.4	**0.046**
SE	37.0			58.9			107.6			28.5		
Contrasts[1]												
	<----------P>F---------->											
Ctrl vs. Che.	0.219	**0.003**		0.959	0.968		0.919	**0.001**		0.562	0.689	
Ctrl vs. Man.	**0.003**	**0.001**		**0.011**	**0.094**		0.817	**<0.001**		0.287	0.337	

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu procedure
Means shown in bold signify interaction is significant

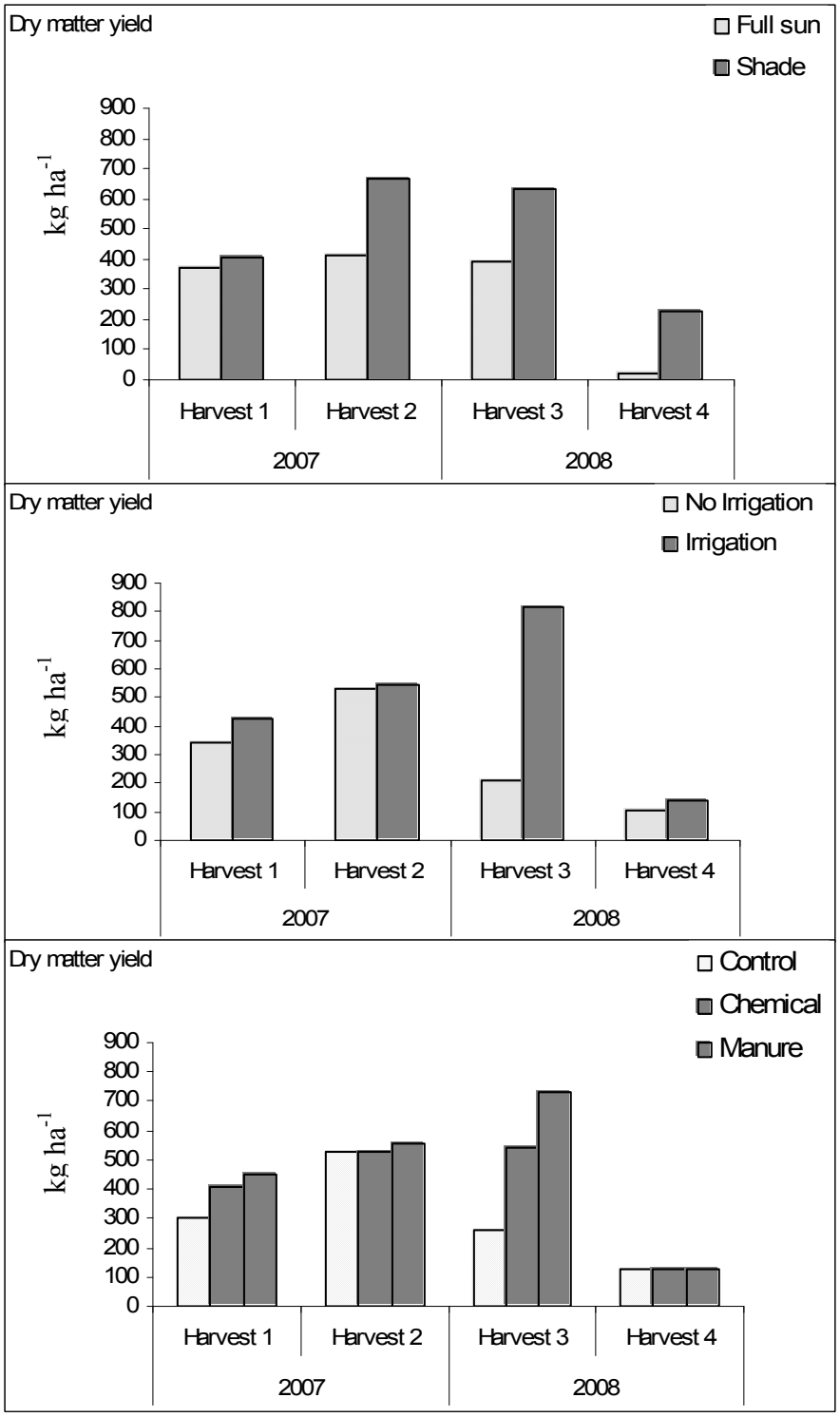


Fig. 1. Main effects of Shade, Irrigation and Nutrient on Dry matter yield of American skullcap in 2007 and 2008

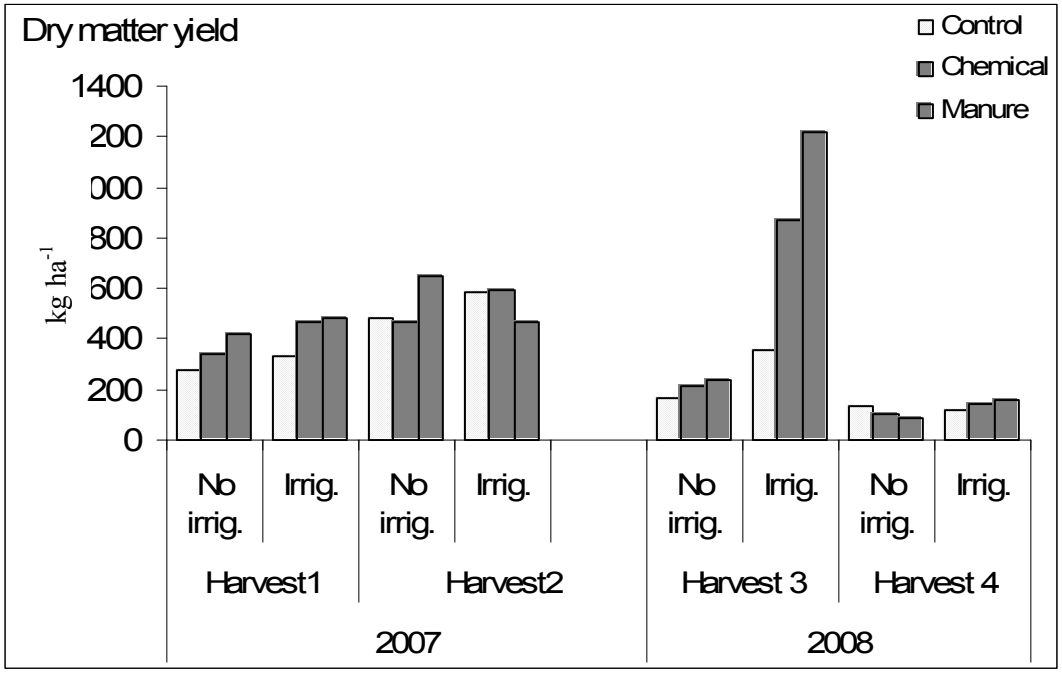


Figure 7. Interaction of irrigation by nutrient on dry matter yield in of American skullcap in 2007 and 2008

CHAPTER III

SHADE, IRRIGATION AND NUTRIENT EFFECTS ON FLAVONOID CONCENTRATION AND YIELD IN AMERICAN SKULLCAP

ABSTRACT

American skullcap *(Scutellaria lateriflora),* a medicinal species valued for its sedative properties associated with flavonoids, is generally harvested from the wild. Information on how open field growing conditions affect flavonoid content is lacking. A 2X2X3 split plot factorial experiment was conducted at the EV Smith Research Center near Shorter Alabama to explore effects of light, irrigation and nutrient application on flavonoid concentration and yield of American skullcap. Treatment factors were shade (40% shade vs. no shade), irrigation (applied at 30 kPa vs. no irrigation) and nutrients (no fertilizer vs. fertilizer (100 kg N, 68 kg P, 42 kg K ha^{-1}) and chicken litter (100 kg N, 50 kg P and 123 kg K ha^{-1}). Shade formed the main plot units; irrigation and nutrient factors were randomized within subplots. Seedlings were transplanted on April 30, 2007. Four harvests were carried out in 2007 and 2008. Dried and finely ground samples were extracted via accelerated solvent extractor and analyzed via HPLC for flavonoid concentration. Flavonoid yields were determined by multiplying concentration by dry matter yield. The flavonoid baicalin was found at higher concentration and yield,

followed by baicalein; wogonin and chrysin were found at very low concentration and yield. Flavonoid concentration was 26% higher in full sun, 20 % higher with irrigation and 29% lower with added nutrients. Significant interactions of shade X irrigation and shade X nutrients were also observed. The highest concentrations were obtained with the irrigation + manure and irrigation treatments in full sun and the lowest concentration with manure under shade. Shade, irrigation and nutrients increased the yield of all four flavonoids. Flavonoid yield was 26% higher under shade, 97% higher with irrigation and 44% higher with added nutrients. Significant interactions of shade X irrigation, shade X nutrients and irrigation X nutrients were also observed. The highest flavonoid yields were obtained with the irrigation + manure and irrigation + fertilizer treatments under shade and the lowest with the control and fertilizer treatments in full sun.

INTRODUCTION

Growing interest in medicinal herbs results in the need to domesticate medicinal plants that are, traditionally harvested in the wild (Sturdivant and Blakley 1999) .Plant materials harvested from the wild are often not uniform (Azaizeh, 2005), because they come from various sources and were grown under various environmental conditions Environmental factors such as light, humidity and nutrients play important roles in plant growth and metabolites synthesis and allocation (Robbers and Tylers, 1999). Also, change in their environment may affect therapeutic properties of cultivated medicinal species.

American Skullcap (*Scutellaria lateriflora*), a medicinal plant used mainly for its sedative properties, is one of these species for which an increased demand is expected. The demand for medicinal plants with sedative properties has surpassed any other

categories of herbal products in recent years (Brevoort, 1998). American skullcap is a perennial herbaceous species native to temperate North America (Bergeron et al 2005), where it is distributed from Canada to Florida (Gafner et al 2003). Skullcap is naturally found in wet places (Awad et al 2003) and moist thickets (Foster and Duke, 2000); the plant is also reported to grow successfully in full sun and partial shade (Jankee and DeArmond, 2004; Joshee et al., 2002). The herb was traditionally used by the Native Americans for the treatment of many diseases including epilepsy, cholera, nervous tension state (Newall et al. 1996), insomnia, anxiety, neuralgia (Foster and Duke, 2000), rabies, diarrhea, digestive problems (Greenfield and Davis, 2004), promotion of menstruation, and elimination of after birth (Wohlmuth, 2007).

The chemical make up of the genus *Scutellaria* includes the flavonoids, volatile oils, iridoids, diterpenoids, waxes and tannins (Wills and Stuart, 2004). The flavonoids are considered to be responsible for therapeutic properties of the species. In *Scutellaria lateriflora*, different types of flavonoids have been identified. They include the flavonoid glycosides baicalin, dihydrobaicalin, ikonnikoside I, lateriflorin, scutellarin and oroxylin A-7-O-glucuronide and the aglycones baicalein, oroxylin A, wogonin, and 5,6,7-trihydroxy-2'-methoxyflavone (Bergeron et al., 2005). Most herbalist literature reports the flavonoids scutellarin and its glycoside scutellarein as the major flavonoid component of American skullcap (Wills and Stuart, 2004). However, new studies based on more advanced techniques, found the flavonoids glycoside baicalin and its aglycone baicalein to be in greatest concentration in the plant tissues. Wills and Stuart (2004), in their study, found the flavonoid baicalin to be 40 to 50% of total flavonoid content in American skullcap. Bergeron (2005 found that the aerial part of American skullcap contains mainly

baicalin and oroxylin A as the major flavonoid glycoside followed by baicalein as the major flavonoid aglycone. Lateriflorin and scutellarein were found to be less important components.

Flavonoids are considered to be one of the most powerful antioxidant groups of carbon-based phenolics synthesized by plants (Jaakola et al., 2004). Therapeutic properties of medicinal species are often associated with their antioxidant properties due to the presence of various types of flavonoids (Azaizeh, 2005). Flavonoids and other plant metabolites are not evenly distributed throughout the plant tissues. Their concentration and distribution in the plant are not only a function of genetics, but also are found to be influenced by various environmental factors such as light, humidity and soil fertility (Mannfried, 1993).

Increasing light intensity is reported to increase phenolic concentration in plant tissues (Warren et al., 2003). Many studies found an increase in concentrations of flavonoids and other antioxidant in plants found under drought conditions compared to those grown under adequate moisture (Hernandez et al 2004). It is also reported that increase in growth due to addition of nutrients while the photosynthetic rate stays the same, leads to a decrease in secondary metabolite production (Glynn et al., 2003; Palm et al., 2006).

However, these observations are not always true; they vary sometimes with plant species and types of metabolites. For example in St. John Wort, a medicinal species, the concentration of the phenol hypericin, decreases significantly under water stress, while hyperforin, another phenol, increases by twofold under the same condition (Zobayed, 2007). Under very low soil fertility, addition of nutrients is reported to contribute to an

increase in secondary metabolites (Jocelyn et al., 1999). However, for some species, production of many metabolites is enhanced under shortage of nutrients and other adverse environmental conditions (Bruulsema, 2000).

An understanding of how these environmental factors affect growth and chemical content of medicinal plants would contribute to a better assessment of these species. Such knowledge would enable growers not only to increase total dry matter yield but also improve therapeutic properties through proper management of their environment. Previous research published about American skullcap in refereed journals focused on identifying and extracting of various types of flavonoids and others chemicals constituents present in the plant tissues (e.g. Awad et al., 2003; Bergeron et al., 2005). No agronomic experiments on American skullcap conducted in US are reported in the scientific literature.

The goal of our research was to determine the appropriate growing conditions needed to optimize flavonoid concentration and yield in American skullcap. A field experiment was carried out to evaluate the effect of shade, irrigation and nutrient application on flavonoid concentration and yield in American skullcap.

MATERIALS AND METHODS

Site Description and land preparation:

The field experiment was conducted at the Horticulture Unit of the E.V Smith Research Center, near Shorter Alabama on a Marvyn loamy sand (fine-loamy, kaolinitic, Thermic Typic Kanhapludults), 2 – 5% slope. Soil pH measured in December 2006 before liming and March 2007 after liming, were respectively 5.1 and 5.8 with CEC 4.6 $cmol_c kg^{-1}$.

Prior to tillage, weeds were controlled using glyphosate herbicides (Round-up) at the rate of 2.1 kg a.i ha^{-1}. A preliminary tillage operation was done in March 2007 using a disk harrow. Following the first tillage and after liming, five soil samples were taken from each experimental block at a depth of 15 cm to determine pH and primary nutrients (N, P, and K) content. Dolomitic Limestone was applied using a truck spreader at the rate of 2500 kg ha^{-1} in March 2007 before second tillage and prior to bedding. A second tillage operation was done on April 9 2007 using a RHINO SHV80 rotor tiller to loosen the soil. Chemical fertilizer and chicken litter treatments were hand broadcasted to respective plots on April 6 2007, prior to bedding. Bedding was done on April 10, 2007. A bedder 18 inches wide was used to prepare beds and place drip irrigation lines simultaneously. Beds were covered with weed guard ground cover manufactured from UV-resistant black polyethylene to help control weeds while allowing air and water to reach the plant root system. Holes approximately 5 cm in diameter were cut at a spacing of 30 X 30 cm prior to pine bark application to allow transplantation of seedlings. Pine bark mulch was spread to control weeds between and on beds.

Experimental Design and treatments

The experiment was of a 2x2x3 split plot factorial in a randomized complete block design with 4 replications. Shade formed the main plot units while irrigation and nutrients were randomized within subplots. The six treatments in the subplots were: 1) Irrigation applied when soil moisture tension reached 30 kPa vs. no irrigation; 2) Chemical fertilizer applied at the rate of 100 kg N, 68 kg P, and 42 kg K ha^{-1}. 3) Chicken litter applied at the rate of 100 kg N, 50 kg P and 123 kg K ha^{-1}. 4) Irrigation and chicken litter. 5) Irrigation and chemical fertilizer and 6) Control with no irrigation and no

nutrients applied. Chemical fertilizer rates were based on commercial vegetable production. Plots size was 1.2 x 6.1 m (7.43 m^2). Each plot consisted of 40 plants. Seedlings were spaced 30 x 30 cm yielding a population density of 53,000 plant ha^{-1} assuming a full stand. Single drip lines (16 mm inner diameter, 250 mm wall, 30 cm spacing between drippers, 340 L/H flow /100m @ .55 bars pressure) were installed down the center of each bed.

Sun Blocker Commercial Shade Houses measuring 7.3 m wide by 9.1 m long were assembled on site. Shade covers manufactured from knitted polyethylene fabric to provide 40 % shade were placed on top of a steel frame and around the South, West and east side of the frame. Shade houses were oriented North-South while plots were oriented East- West.

At the beginning of year 2, right after emergence, mulch fabric was removed from all plots on April 7, 2008 to allow shoots to grow from rhizomes which had spread across the beds underneath the fabric. Chemical fertilizer was applied to appropriate plots at the rate of 136kg ha^{-1} N, 125 kg/ha P_2O_5 and 110 kg/ha K2O and chicken litter at the rate of 136 kg/ha N, 68 kg/ha P and 102 kg/ha K. Organic pelletized composted poultry litter fertilizer (4-2-3) from Longwood Plantation Newington GA was used instead of the poultry litter used at year 1. The poultry litter also provided 102 kg/ha Ca, 17 kg/ha Mg, 4.42 kg/ha Fe, 2.38 kg/ha Cu, Mn and Zn

Harvesting, weighing and determination of dry matter yield

Four harvests were carried out at full bloom on June 29 and September 5 in 2007 and on June 13 and July 25 in 2008. Plant height, based on average of 5 samples taken at random from each plot, was taken one day before each harvest, on June 28 and September 4,

2007 and on June 12 and July 24, 2008. The aboveground portion of each plant was cut 7.5 cm from the ground using pruning shears in 2007 and a gasoline hand trimmer in 2008. The central 32 plants (5.96 m^2) of each plot were harvested in 2007 and weighed to determine total fresh yield. In 2008, 5.7 m from the 6 m were harvested from each plot. A sample of about 250 grams was taken from each plot to determine percent dry matter and dry matter yield. Samples were placed in paper bags 30 x 60 cm perforated at the bottom and on the side to allow air circulation. Bags containing samples were placed with open tops in a forced-air dryer (Model AA-5460A, Parameter Generation and Control Inc., Black Mountain, N.C.) at 40˚ C for 3 days. In 2008, drying was done using a grass drier at 43 C˚ for 3 days at harvest 3 and a shed build on site at 38 C˚ for 4 days at harvest 4. Once removed from dryer, samples were weighed to determine percent dry matter. Total dry matter yield was calculated by multiplying percent dry matter by total fresh yield. Samples were ground to pass through a 1mm mesh screen using the Thomas-Wiley Laboratory mill, Model 4 by Thomas Scientific, USA. Finely-ground samples were packed in Whirl-Pac air proof bag and stored at 25 C for chemical analysis.

Flavonoid determination

Analysis for flavonoid content was carried out by the reversed phase high performance liquid chromatography (RP-HPLC) procedure at the National Center for Natural Products Research at the University of Mississippi.

Extraction

Extraction of plant material was performed using an Accelerated Solvent Extraction (ASE®) apparatus (Dionex Corp., Sunnyvale, CA) at the USDA-Agricultural Research Service Natural Products Utilization Research Unit (USDA, ARS, NPURU).

Approximately 5 g of dried, powdered sample was mixed with purified sand (Fisher Scientific, Pittsburgh, PA) and loaded in extraction cartridges. Purified sand was added to prevent sample compaction, improve solvent movement and facilitate extraction. Extraction was carried out with the following parameters: heat , 5 min; static, 10 min; flush volume, 100 mL; purge, 90 sec; pressure, 6.9 MPa; temperature, 40 ºC; extraction solvent, methanol: water (80:20), four cycles for the plant samples of 2007, while 3 cycles was used for plant samples of 2008. The extracts were concentrated under vacuum using a Savant SpeedVac (Model SPD121P; Savant Instruments, Inc., Holbrook, NY). Dried extracts were weighed and an aliquot was dissolved in 0.5% HCl-methanol, and analyzed by high pressure liquid chromatography (HPLC) for levels of flavonoids.

Chemicals/ standards used

Six flavonoid standards were used: Apigerin, baicalin, baicalein, chrysin, scutellarein and wogonin. Apigerin, baicalein, baicalin and chrysin were purchased from Sigma Chemical Co. (St. Louis, MO). Scutellarein was purchased from Indofine Chemical Co. (Hillsborough, NJ) and wogonin was purchased from Wako Chemicals (Richmond, VA).

HPLC Analysis of extracts

The plant extracts were analyzed on a Hewlett-Packard 1100 HPLC using an Inertsil ODS-2 5 μ column and monitored for their content of Apigerin, baicalin, baicalein, chrysin, scutellarein and wogonin at λ270 nm. The mobile phase consisted of 0.005% phosphoric acid (solvent A) and acetonitrile (solvent B), eluted in a gradient manner starting from 36% to 100% B over a 37-min run at a flow rate of 1 $mL \cdot min^{-1}$.

The flavonoids were quantified from a calibration curve of the standards with 6-hydroxyflavone as internal standard.

Flavonoid yield is obtained by the product of flavonoid concentration (mg g^{-1}) and the total dry matter yield (kg ha^{-1}) and expressed in grams per hectare.

Data analysis

All data were analyzed using the mixed model procedure of SAS Version 9.1.3 (SAS Institute, Cary, NC) for a randomized complete block design with shade treatment as a split plot restriction on randomization. Shade, irrigation and nutrient treatments are fixed effects, while blocks and main error residuals are maintained as random effects.

RESULTS

Table 3.1 presents the results of the analysis of variance for main effects and interactions of shade, irrigation and nutrient application on flavonoid concentration and yield for four harvests in 2007 and 2008. The flavonoids apigerin and scutellarein were not detected in any of the four harvests. The flavonoid baicalin (Appendix 1) had the highest concentration and yield under all experimental conditions and represented about eighty percent of the total flavonoid, followed by baicalein (Appendix 2); the flavonoids wogonin (Appendix 3) and chrysin (Appendix 4) were found at very low concentration and yield under all experimental conditions (Table 3.2 and 3.3).

Baicalin concentration and yield

Baicalin concentration

Shade had no significant effect on baicalin concentration at harvest 2 and 3 (Table 3.1), but, decreased the concentration by 30% at harvest 1 in June 2007 ($p=0.03$) and 36% at harvest 4 in July 2008 ($p=0.065$) (Table 4). Irrigation increased the concentration

by113% at harvest 4 ($p<0.001$), but had no effect at the first 3 harvests (Table 3.4). Nutrient application decreased baicalin concentration at harvest 1 ($p=0.029$) and 4 ($p=0.001$) but had no significant effect at harvest 2 and 3. Fertilizer application had no significant effect at harvest 1 but decreased the concentration at harvest 4 ($p=0.001$). Manure decreased the concentration, both at harvest 1 ($p=0.026$) and 4 ($p=0.012$) (Table 4 and). The interactions of shade X irrigation were significant only at harvest 1 ($p=0.01$) and 4 ($p<0.001$) (Table 3.1 and Fig 1). At harvest 1, irrigation had no significant effect in full sun but decreased the concentration by 29% under shade. At harvest 4, irrigation increased the concentration by 347% in full sun but had no significant effect under shade (Table 3.5 and Fig. 3.1). In full sun without irrigation, baicalin concentration was very low. A significant interaction of shade X nutrient was observed only at harvest 4 ($p=0.002$, Table 1). Fertilizer application decreased baicalin concentration by 61% in full sun but had no effect under shade (Table 3.6 and Fig 3.2). Manure application decreased the concentration by 34% in full sun but had no effect under shade. The interaction of irrigation X nutrients was significant only at harvest 1 ($p=0.043$) (Table 3.1 and Fig. 3.3). Manure application decreased baicalin concentration by 30% without irrigation but had no effect in the irrigated plots (Table 3.7 and Fig 3.3); fertilizer application had no effect both with and without irrigation. Irrigation decreased baicalin concentration in fertilized plots but not in control or manure plots.

The highest baicalin concentration for an individual harvest (2.57mg g^{-1}) was found with the irrigation treatment in full sun at harvest 4 (Table 3.2). The highest average concentration over the four harvests (1.66 mg g^{-1}) was also found with the

irrigation treatment in full sun. The lowest average concentration for the four harvests (0.86 mg g^{-1}) was found with the manure treatment under shade (Table 3.2).

Baicalin yield

Shade decreased baicalin yield by 28% at harvest 1 ($p=0.042$) but increased the yield by 31% ($p=0.014$) at harvest 2 and by 323% ($p<0.001$) at harvest 4 (Table 3.8). Irrigation increased baicalin yield by 21% at harvest 1 ($p=0.030$), 21% at harvest 2 ($p=0.087$), 465% at harvest 3 ($p<0.001$) and 94.7% at harvest 4 ($p=0.007$) (Table 8). Nutrient application had no significant effect on baicalin yield at harvest 1, 2 and 4 but increased the yield significantly at harvest 3 ($p=0.23$). Manure application produced a significant increase ($p=0.012$) while fertilizer produced no effect (Table 3.8). An interaction of shade X irrigation was significant only at harvest 1 ($p=0.001$) (Table 1 and Fig. 3.1). Irrigation increased the yield by 55% in full sun but had no effect under shade (Table 3.9 and Fig. 3.1). A similar trend was evident at harvest 2, but was not significant, while at harvests 3 and 4, irrigation increased baicalin yield both in full sun and under shade. Significant interactions of irrigation by nutrients were observed at harvests 2 ($p=0.019$) and 3 ($p=0.019$) (Table 3.1 and Fig. 3.3). At harvest 2, manure application increased the baicalin yield by 37.6% without irrigation, but decreased the yield by 23% with irrigation. At harvest 3, application of either fertilizer or manure without irrigation had no effect on baicalin yield, but with irrigation, manure increased the yield by 285% and fertilizer by 163% (Table 3.10 and Fig. 3.3).

The highest baicalin yield for the four harvests (3312 and 3212 g ha^{-1}) were found with irrigation + manure and irrigation + fertilizer under shade (Table 3.3).The highest yield for an individual harvest (2006 g ha^{-1}) was also found with irrigation + manure

under shade at harvest 3 (Table 3.3). The lowest yields for the 4 harvests were found with the fertilizer treatment (935 g ha^{-1}) and the control plot (964 g ha^{-1}) in full sun (Table 3.3).

Baicalein concentration and yield

Baicalein concentration

The main effect of shade on baicalein concentration was not significant at any harvest (Table 3.1). Irrigation had no effect at the first three harvests but increased the concentration by 119% at harvest 4 ($p=0.001$) (Table 3.11). An interaction of shade X irrigation was significant at harvest 4 ($p=0.014$); irrigation increased the concentration by 347% in full sun but had no significant effect under shade (Table 3.12). Nutrient application had no significant effect on baicalein concentration (Tables 3.1, 3.11).

The highest baicalein concentration for an individual harvest (0.34 mg g^{-1}) was found with fertilizer in full sun at harvest 1 (Table 3.2). The highest average concentrations for the four harvests (0.20 mg g^{-1}) were found with irrigation + manure in full sun and irrigation + fertilizer under shade. The lowest concentrations for the four harvests (0.14 mg g^{-1}) were found with the control treatment in full sun, fertilizer, irrigation and control under shade (Table 3.2).

Baicalein yield

Shade had no effect on baicalein yield at harvest 1 and 3 but increased the yield by 59% at harvest 2 ($p<0.001$) and 791% at harvest 4 ($p<0.001$) (Table 3.13). Irrigation had no effect at harvest 2, but increased the yield by 33% at harvest 1 ($p=0.01$), 372% at harvest 3 ($p<0.001$) and 68.7% at harvest 4 ($p=0.026$) (Table 3.13). Nutrient application had no significant effect at harvest 4, but increased the yield at harvest 1 ($p=0.004$)

(Table 3.1), harvest 2 ($p=0.01$) (Table 3.1) and harvest 3 ($p<0.001$) (Table 3.1). At harvest 1, fertilizer had no effect, but manure application increased the yield by 61%. At harvest 2, manure and fertilizer increased the yield by 57 % ($p=0.005$) and 37% ($p=0.08$), respectively. A harvest 3, manure and fertilizer increased the yield by 267% ($p<0.001$) and 159% ($p=0.024$), respectively (Table 3.13). An interaction of shade X nutrients was observed at harvest 2 ($p=0.021$) and harvest 3 ($p=0.083$) (Table 3.1). At harvest 2, application of nutrients did not significantly affect baicalein yield in full sun, but under shade application of fertilizer or manure increased baicalein yield by 61 and 106 %, respectively. At harvest 3, manure application increased the yield in full sun and under shade, by 314% and 226%, respectively; fertilizer application had no effect in full sun but increased the yield by 248% under shade (Table 3.14). At harvest 2, shade increased baicalein yield by 56% with application of fertilizer and by 112 % with manure, but did not affect baicalein yield without added nutrients. At harvest 3, shade increased baicalein yield when chemical fertilizer was applied, but not where manure or no nutrients were applied.

A significant interaction of irrigation X nutrients was observed at harvest 3 ($p=0.007$) (Table 1). Without irrigation, nutrient application (manure or fertilizer) had no effect on baicalein yield, but with irrigation, manure and fertilizer application increased yield by 22 % and 16%, respectively (Table 3.15). The highest baicalein yield for an individual harvest (208 g ha^{-1}) was found with irrigation + manure in full sun at harvest 3 (Table 3.3). The highest yields for the four harvests (449.3 g ha^{-1} and 448 g ha^{-1}) were found respectively with irrigation + manure and irrigation + fertilizer under shade. The

lowest yield for the four harvests (112.5 g ha^{-1}) was found with the control treatment in full sun (Table 3.3).

Wogonin concentration and yield

Wogonin concentration

The main effects of shade on wogonin concentration did not test significant at any harvest (Table 3.1). Irrigation increased wogonin concentration by 124% at harvest 4 ($p=0.004$) but the main effect of irrigation did not test significant at the first 3 harvests (Table 3.16). A significant interaction of shade X irrigation was observed at harvest 1 ($p=0.074$) and harvest 4 ($p=0.011$) (Table 3.1). At harvest 1, irrigation decreased wogonin concentration by 17% in full sun, while irrigation increased wogonin concentration by 38% under shade (Table 3.17). At harvest 4, irrigation increased the concentration by 587% in full, sun but had no significant effect under shade (Table 3.17). Nutrient application had no significant effect on wogonin concentration (Table 3.1). The highest wogonin concentration for an individual harvest (0.08 mg g^{-1}) was found with irrigation + fertilizer in full sun at harvest 1 (Table 3.2). The highest average concentration for the four harvests (0. 06 mg g^{-1}) was also found with irrigation + fertilizer and irrigation + manure in full sun. The lowest average concentrations (0. 03 mg g^{-1}) was found with fertilizer in full sun (Table 3.2).

Wogonin yield

The main effect of shade on wogonin yield did not test significant at harvest 1 and 3, but shade increased the yield by 114% at harvest 2 ($p<0.001$) and 1006% at harvest 4 ($p=0.015$) (Table 3.18). Irrigation had no significant effect at harvest 1, 2 and 4, but increased the yield by 214% at harvest 3 ($p<0.001$) (Table 3.18). Nutrient application

increased wogonin yield at harvest 1 (p=0.036) and harvest 3 (p=0.008), but had no significant effect at harvest 2 and 4 (Table 3.18). Manure and fertilizer application increased the wogonin yield by 92% and 55%, respectively, at harvest 2 and, by 46% and 32% at harvest 3 (Table 3.18). An interaction of shade X nutrients was observed at harvest 3 (p=0.094) (Table 1). At harvest 3, manure increased the yield by 298% in full sun and by 209% under shade while fertilizer had no significant effect in full sun but increased the wogonin yield by 306% under shade (Table 3.19). An interaction of irrigation X nutrients was significant at harvest 3 (p=0.042) (Table 3.1). Nutrient application had no significant effect without irrigation while both manure and fertilizer application increased the wogonin yield respectively by 329% and 346% with irrigation (Table 3.20). Similarly, irrigation increased wogonin yield in presence of fertilizer or manure, but had no effect without nutrient application.

The highest wogonin yield for an individual harvest (76.8 g ha^{-1}) was found with fertilizer + irrigation under shade. The highest yield for the sum of the four harvests (156.2 g ha^{-1}) was also found with irrigation + fertilizer under shade (Table 3.3). The lowest yield for the four harvests (31.1 g ha^{-1}) was found with the control treatment in full sun (Table 3.3).

Chrysin concentration and yield

Chrysin concentration

The main effects for shade, irrigation and nutrient application effects on chrysin concentration did not test significant. An interaction of shade X irrigation was significant at harvest 1 (p=0.009) (Table 1); irrigation had no effect in full sun but increased the concentration by 53% under shade (Table 3.21). Similarly, shade had no effect on chrysin

concentration without irrigation, but with irrigation, shade increased chrysin concentration by 67%. A significant interaction of irrigation X nutrients was observed at harvest 2 ($p=0.022$) (Table 3.1). Fertilizer increased chrysin concentration by 100% ($p=0.011$) without irrigation and had no effect with irrigation, while manure had no effect without irrigation and increased the concentration by 100% ($p=0.028$) with irrigation (Table 3.22). Irrigation decreased chrysin concentration with fertilizer, but had no effect without nutrient application.

The highest chrysin concentration for an individual harvest (0.07 mg g^{-1}) was found with irrigation + manure at harvest 2 and irrigation + fertilizer at harvest 4 (Table 3.2). The highest average concentration for the four harvests (0.21 mg g^{-1}) was also found with the same treatments in full sun. The lowest concentration for the four harvests (0.10 mg g^{-1}) was found with fertilizer in full sun (Table 3.2).

Chrysin yield

The main effect of shade on chrysin yield did not test significant at harvest 1 but increased the yield by 84% at harvest 2 ($p=0.013$), 115% at harvest 3 ($p=0.035$) and 779% at harvest 4 ($p=0.018$) (Table 3.23). The main effect of irrigation was not significant at harvest 2, but irrigation increased the yield by 35% at harvest 1 ($p=0.042$), 372% at harvest 3 ($p<0.001$) and 104% at harvest 4 ($p=0.019$) (Table 3.23). Nutrient application had no effect at harvest 4 but increased the yield at harvest 1 ($p=0.006$), harvest 2 ($p=0.047$ and harvest 3 ($p=0.001$). Fertilizer and manure increased the yield respectively by 87% and 64% at harvest 1, 54% and 59% at harvest 2 and 104% and 227% at harvest 3 (Table 3.23). A significant interaction of shade X irrigation was observed at harvest 1 ($p=0.026$) (Table 3.1); irrigation had no significant effect on

chrysin yield in full sun but increased the yield by 76% under shade (p=0.019) (Table 3.24). Shade had no effect on chrysin yield without irrigation but increased chrysin yield by 72.6 with irrigation. The interaction of shade X nutrients on chrysin yield was significant at harvest 2 (p=0.030) and harvest 3 (p=0.043) (Table 3.1). At harvest 2, nutrient application had no significant effect in full sun while manure and fertilizer increased the yield, respectively, by 114% (p=0.005) and 118% (p=0.004) under shade. At harvest 3, nutrient application had no effect in full sun, while manure and fertilizer increased the yield respectively by 357% (p<0.001) and 220% (p=0.001) (Table 3.25). Shade had no effect on chrysin yield without nutrients, but increased yield in presence of fertilizer or manure. An interaction of irrigation X nutrients was significant at harvest 3 (p=0.006) (Table 3.1). Nutrient application had no significant effect without irrigation, while manure and fertilizer increased the yield, respectively, by 284% (p<0.001) and 141% (p=0.018) (Table 3.26). Shade increased chrysin yield in presence of fertilizer or manure, but not without nutrient application.

The highest chrysin yield for an individual harvest (61.6 g ha^{-1}) was observed with irrigation + manure under shade at harvest 3 (Table 3.3). The highest chrysin yield for the sum of the four harvests (136.9 g ha^{-1}) was also observed with irrigation + manure under shade. The lowest yield for the four harvests (25.4 g ha^{-1}) was found with the control treatment in full sun (Table 3.3).

Overall highest total flavonoid concentrations were found with irrigation + manure (1.94 mg g^{-1}) and irrigation (1.90 mg g^{-1}), both under shade. The lowest total concentration (1 mg g^{-1}) was found with manure in full sun (Table 3.2). Highest total flavonoid yields were found with irrigation + manure (7904 g ha^{-1}) and irrigation +

fertilizer (7745 g ha^{-1}) both under shade. The lowest yields were found with the control treatment (2260.6 g ha^{-1}) and fertilizer (2283.6 g ha^{-1}) both in full sun (Table 3.3)

DISCUSSION

Baicalin was the flavonoid with the highest concentration and yield under all experimental conditions for all 4 harvests, followed by baicalein. These results are in accordance with results reported by Wills and Stuart (2004) Bergeron and Gafner (2005) and Awad (2003); these reports also found wogonin and chrysin at very low concentration and yield. However, other flavonoids such as lateriflorin, scutellarin, ikonnikoside and dihydrobaicalin reported by Bergeron and Gafner (2005) were not considered in our analysis. Total flavonoid concentrations obtained in our trials (Table 2) were much lower than average flavonoid concentrations (36 mg g^{-1}) reported by Wills and Stuart (2004) in stems and leaves of American skullcap. Awad et al (2003) reported 40 mg g^{-1} of baicalin when extracted with 50% EtOH and 33 mg g^{-1} of baicalein when extracted with 95% EtOH which are also higher than our results. These differences may be due to growth environment and extraction methods. In our study, higher concentrations of baicalin and baicalein were obtained at the first harvest of each year, suggesting a seasonal effect on there concentration. Wogonin and chrysin concentration were not affect by seasonal change. At harvest 4, total flavonoid yields were lower in full sun and without irrigation due to poor survival.

Mohamed et al.(2001), studying the effect of light on flavonoid concentration in Jonagold apples, reported higher concentration of flavonoid in fruit skin grown in full sun compared to those grown under shade. Similarly, higher concentrations were obtained in full sun than under shade in our study. Results from our experiment are also in

accordance with the photo-inhibition theory, suggesting that plants exposed to direct sunlight react by producing antioxidants such as flavonoids, as sun screen protectants Satu (2005), Zobel et al. (1999). Lower flavonoid concentration under shade may also be explained by the fact that nitrogen concentration in plant tissues tends to be higher under shade which, according to the carbon nitrogen balance hypothesis (CNB) lead to a decrease in phenolic concentration (Matthew et al. 2006). Finally, as suggested by Zobayed (2007) higher flavonoid concentration in full sun may be due to the fact that in full sun, plants were more likely to be under drought stress and react by producing extra antioxidants to protect themselves against oxidative effect. However, although flavonoid concentrations were higher in full sun, the total flavonoid yield was much higher under shade due to higher dry matter yields harvested under shade than in full sun.

Irrigation did not have a significant effect on flavonoid concentration under shade. However irrigation increased the concentration significantly in full sun. These results are not in accordance with findings by Alexievia et al, (2001); Zobayed et al (2007); and Khalid, (2006), who reported higher concentration of flavonoids in plants grown under water stress. Irrigation applied alone significantly increased flavonoid yield in full sun while having no significant effect under shade; which suggests that irrigation is more critical in full sun than under shade not only for higher concentration but also for higher flavonoid yields.

Addition of nutrients alone slightly decreased baicalin concentration both under shade and in full sun but had no effect on baicalein, wogonin and chrysin. According to the CNB hypothesis (Matthew et al., 2006), increased nutrients, especially nitrogen, increase alkaloid concentrations but decrease phenolics such as flavonoids. Our results

for baicalin are thus in agreement with the CNB hypothesis. Glynn et al., (2003) suggest that an increase in growth due to added nutrients, while photosynthetic rate stays the same, leads to a decrease in available photosyntate which would otherwise be allocated to production of secondary metabolites such as flavonoids. In our experiment, decrease in baicalin concentration with added nutrients may be due to the increase in growth resulting from addition of nutrients. Our results for baicalin are also in accordance with Anttonen et al (2006) who found higher flavonoid concentration in strawberry fruits grown under lower fertilization level compared to those grown under higher level. Addition of nutrients did not have a significant effect on flavonoid yield without irrigation. Fertilizer in full sun without irrigation tends to decrease flavonoid yield. However, with irrigation, both manure and fertilizer increased flavonoid yield significantly either under shade or in full sun, suggesting that irrigation must be applied with nutrients in order to increase flavonoid yield. Higher flavonoid yield when irrigation is applied also highlights the importance of water in mineral uptake by plants. Overall, the best treatment combinations for higher flavonoid yield were irrigation + manure and irrigation + fertilizer under shade. There was no significant difference in yield between manure and fertilizer when irrigation is applied, however, when irrigation was not applied, manure produced a higher yield than fertilizer, suggesting that irrigation may be more critical when chemical fertilizer is applied.

CONCLUSION

Higher baicalin, baicalein and total flavonoid concentration was obtained in full sun, while higher yield under shade. Shade did not affect wogonin and chrysin concentration, but increased their yield due to an increase in dry matter yield.. Irrigation

tended to increase flavonoid concentration and yields greater in full sun than under shade, which suggests that irrigation is more critical in full sun than under shade. Application of nutrients decreased baicalin concentration and had no significant effect on baicalin yield, while they had minimal effect on baicalein concentration but significantly increased baicalein yield. When irrigation was provided, both manure and chemical fertilizer increased flavonoid yield but did not affect flavonoid concentration. Overall, shade and irrigation, with either manure or fertilizer, produced the highest flavonoid yield and can be considered as the best treatment combinations if the objective is to increase total flavonoid yield. However, if the objective is to obtain the highest concentration of baicalin and total flavonoid in the plant tissue, irrigation + manure or irrigation alone in full sun should be recommended.

Any decision of a farmer on how to grow skullcap must be based on cost effectiveness. Although highest flavonoid yields were obtained under shade, cost of shade structure and irrigation must be considered in order to determine if the returns merit the additional investment.

REFERENCES

Alexievia, V., I Sergiev, S. Mapelli and E. Karanov. 2001. The Effect of drought and Ultraviolet radiation on growth and stress markers in pea and wheat. Plant, Cell and Environment (2001) 24, 1337-1344

Awad, R., J.T.Arnason, V.L.Trudeau, C. Bergeron, J.W Budzinski,., B.C Foster, Z. Merali, 2003. Phytochemical and biological analysis of skullcap (*Scutellaria lateriflora* L.): a medicinal plant with anxiolytic properties. Phytomedicine.10, 640-649.

Azaizeh, H, Predrag L., I. Portnaya, O. Said, U. Cogan and A. Bomzon. 2005. Fertilization induced changes in growth parameters and antioxidant activity of medicinal plants used in traditional Arab medicine. *eCam* 2005; 2(4) 549-556

Bergeron C., S. Gafner, E. Clausen, J. D. Carrier. 2005. Comparison of the chemical composition of extracts from *Scutellaria lateriflora* using accelerated solvent extraction and supercritical fluid extraction versus standard hot water or supercritical fluid extraction versus standard hot water or 70% ethanol extraction . Journal of Agricultural and Food Chemistry 53, 3076- 3080

Brevoort, P. 1998. The booming U.S botanical market: A new overview. HerbalGram No. 44, pp 33-48

Bruuselma, T.W. 2000. Phosphorus and Potassium for functional foods. Proc. Conference presented at the 29th North Central Extension-Industry Soil Fertility Conference, St Louis, MO, November 1999 and at Crops Update, Woodstock, ON, January 1999 and 2000. pp. 2 and 3.

Donald D. Ritchie and Robert Carola. 1983: Biology. Addison-Wesley Publishing Company pp. 360-361

Foster S. and J. A. Duke.2000. Medicinal plants and herbs of Eastern and Central North America 2nd ed. Houston Mifflin Co. N.Y p. 211

Gafner,S.; C Bergeron, L. L. Batcha, J. E Burdette, J. Reich,; J. M Pezzuto, J.T. Arnason, C.K Angerhofer. 2003. Inhibition of [3H]-LSD Binding to 5-HT7 Receptors by Flavonoids from Scutellaria lateriflora. J . Nat. Prod. 66: 535-537

Glynn C., D. A. Herms, M.Egawa, R. Hansen, W. J. Mattson. 2003. Effects of nutrient availability on biomass allocation as well as constitutive and rapid induced herbivore resistance in poplar. OIKOS 101: 385-397

Hernandez, I., L. Alegre and S.Munne-Bosch. 2004. Drought-Induced changes in flavonoids and other low molecular weight antioxidants in *Cistus clusii* grown under Mediterranean field conditions. Tree Physiology 24, 1303- 1311

Jaakola, L. M-R Kaisu, K.Sirpa, Anja Hohtola. 2004. Activation of flavonoid biosynthesis by solar radiation in bilberry leaves. Planta 218: 721-728

Joshee N., P.S.Thomas. S.M. Rao and A. K. Yadav.2002 Skullcap: Potential Medicinal Crop. Reprinted from: J. Janick and A. Whipkey (eds.). Trends in New crops and new uses. pp.580-586 American Society for Horticultural Science (ASHS) Press, Alexandria, VA.

Kingsley R Stern. 1991 Introductory Plant Biology fifth edition. Wm. C. Brown Publishers. pp 184- 185

Khalid Kh. A. 2006. Influence of water stress on growth, essential oil and chemical composition of herbs (Ocimum sp.) Int. Agrophysics 20: 289-296

Mannfried, P. 1993. Healing plants. Barron's Educational Series, Inc. NY pp. 8-9

McIntyre, A. 1995 The complete woman's herbal; Henry Holt and Co. pp.14, 16

Mohamed A. A., Wagenmakers S. P. Jager de A. 2000. Effects of light on flavonoid and chlorogenic acid levels in the skin of Jonagold apples. Scientia Horticulturae 88: 289-298

Satu, T. 2005. The effects of drought stress and enhanced UV-B radiation on growth and secondary chemistry of boreal conifer and willow seedlings. PhD dissertations in Biology, No: 39.ISSN 1457-2486. University of Joensuu, Finland

Sturdivant L. and T. Blakley 1999. Medicinal herbs in the Garden, field and marketplace. San Juan Naturals WA. pp.116-117

Van Wyk B-E. and M. Wink. 2005. Medicinal plant of the World, An illustrated scientific guide to important medicinal plants and their uses.Briza Publications, Pretoria, South Africa. pp. 294- 295.

Warren M.J., J.Bassman, J. K. Fellman, D. S. Mattinson, S.Eigenbrode. 2003. Ultraviolet-B radiation of *Populus trichocarpata* leaves. Tree Physiology 23: 527-535

Wills R.B.H and D.L. Stuart. 2004. Generation of High Quality Australian Skullcap Products. A Report for the Rural Industries Research and Development Corporation, Australian Government. ISBN 0642587302, ISSN 1440-6845

Zobayed, S.M.A., F.Afreen, T. Kozai. 2007. Phytochemical and physiological changes in the leaves of St. John's wort plants under a water stress condition Environmental and Experimental Botany, 59: 109-116

Zobel, A.M., K. Glowniak, J.E. Lynch, S. Dukea and A. Alliota.1999. Phytochemistry of plants Associated with 400-year-old stand of Hemlock at clear Lake Reserve, Ontario. Proceedings Symposium on Sustainable Management of Hemlock Ecosystem in Eastern North America. General Technical Report GTR-NE 267: 230-233.

Table 3.1. Significance levels for main effect and interactions for baicalin, baicalein, wogonin and chrysin concentration and yield of American skullcap in 2007 and 2008

	Concentration				**Yield**			
	2007		2008		2007		2008	
	Harvest 1	Harvest 2	Harvest 3	Harvest 4	Harvest 1	Harvest 2	Harvest 3	Harvest 4
Baicalin								
Shade	**0.03**	0.478	0.372	**0.065**	**0.042**	**0.014**	0.291	**<0.001**
Irrigation	0.58	0.112	0.12	**<0.001**	**0.03**	**0.087**	**<0.001**	**0.007**
Shade*Irrigation	**0.01**	0.431	0.772	**<0.001**	**0.001**	0.229	0.634	0.923
Nutrients	**0.03**	0.517	0.703	**0.001**	0.297	0.876	**0.023**	0.258
Shade*nutrients	0.49	0.432	0.618	**0.002**	0.278	0.323	0.375	0.222
Irrigation*nutrients	**0.04**	0.752	0.496	0.196	0.157	**0.019**	**0.019**	0.883
Baicalein								
Shade	0.17	0.346	0.316	0.937	0.2	**<0.001**	0.327	**<0.001**
Irrigation	0.39	0.722	0.333	**0.001**	**0.01**	0.111	**<0.001**	**0.026**
Shade*Irrigation	0.69	0.862	0.887	**0.014**	0.898	0.139	0.773	0.383
Nutrients	0.58	0.577	0.113	0.725	**0.004**	**0.01**	**<0.001**	0.654
Shade*Nutrients	0.51	0.591	0.433	0.864	0.971	**0.021**	**0.083**	0.115
Irrigation*Nutrients	0.27	0.21	0.732	0.612	0.37	0.168	**0.007**	0.783
Wogonin								
Shade	0.69	0.258	0.795	0.75	0.417	**<0.001**	0.256	**0.015**
Irrigation	0.62	0.873	0.296	**0.004**	0.164	0.657	**<0.001**	0.243
Shade*Irrigation	**0.07**	0.661	0.756	**0.011**	0.201	0.323	0.767	0.735
Nutrients	0.49	0.295	0.415	0.565	**0.036**	0.17	**0.008**	0.798
Shade*Nutrients	0.19	0.827	0.629	0.792	0.23	0.439	**0.094**	0.428
Irrigation*Nutrients	0.77	0.292	0.12	0.454	0.699	0.784	**0.042**	0.553
Chrysin								
Shade	0.28	0.538	0.355	0.812	0.148	**0.013**	0.035	**0.018**
Irrigation	0.48	0.902	0.296	0.13	**0.042**	0.858	**<0.001**	**0.019**
Shade*Irrigation	**0.01**	0.173	0.974	0.121	**0.026**	0.106	**0.098**	0.17
Nutrients	0.14	**0.03**	0.457	0.58	**0.006**	**0.047**	**0.001**	0.471
Shade*Nutrients	0.27	0.16	0.193	0.956	0.114	**0.03**	**0.043**	0.251
Irrigation*Nutrients	0.17	**0.022**	0.618	0.486	0.322	0.465	**0.006**	0.187

Note: Multiple pair wise comparisons were carried out using Dunnett-Hsu procedure
Bold numbers represent significant difference

Table 3.2. Treatments effect on baicalin, baicalein, wogonin and chrysin concentration of American skullcap at 4 harvests in 2007 and 2008.

	Baicalin					**Baicalein**					**Wogonin**					**Chrysin**					**TFC**
Treat	hvt1	hvt2	hvt3	hvt4	**Avg**	hvt1	hvt2	hvt3	hvt4	**Avg**	hvt1	hvt2	hvt3	hvt4	**Avg**	hvt1	hvt2	hvt3	hvt4	**Avg**	
<----------mg g-1---------->																					
F	1.76	0.98	1.14	0.35	**1.06**	0.34	0.2	0.14	0.02	**0.18**	0.04	0.05	0.04	0.00	**0.03**	0.04	0.03	0.03	0.01	**0.03**	**1.29**
I	1.28	1.01	1.77	2.57	**1.66**	0.3	0.14	0.14	0.13	**0.18**	0.04	0.06	0.04	0.03	**0.04**	0.02	0.03	0.05	0.03	**0.03**	**1.91**
M	1.95	1.44	1.12	0.17	**1.17**	0.26	0.21	0.17	0.01	**0.16**	0.05	0.06	0.06	0.00	**0.04**	0.06	0.04	0.03	0.00	**0.03**	**1.41**
IM	1.59	1.11	1.56	2.13	**1.60**	0.28	0.16	0.18	0.18	**0.20**	0.03	0.07	0.05	0.07	**0.06**	0.05	0.07	0.03	0.06	**0.05**	**1.91**
IF	1.54	1	1.41	1.11	**1.26**	0.23	0.13	0.13	0.18	**0.17**	0.08	0.06	0.05	0.05	**0.06**	0.05	0.06	0.03	0.07	**0.05**	**1.54**
C	1.97	1.43	1.43	1.08	**1.48**	0.25	0.18	0.08	0.05	**0.14**	0.05	0.06	0.05	0.01	**0.04**	0.03	0.04	0.02	0.02	**0.03**	**1.69**
SF	1.3	1	0.85	0.82	**0.99**	0.24	0.13	0.11	0.09	**0.14**	0.05	0.07	0.04	0.03	**0.05**	0.04	0.05	0.03	0.04	**0.04**	**1.22**
SI	1.26	1.09	0.96	0.98	**1.07**	0.23	0.12	0.11	0.11	**0.14**	0.04	0.05	0.03	0.03	**0.04**	0.03	0.04	0.03	0.03	**0.03**	**1.28**
SM	0.76	1.08	1.03	0.57	**0.86**	0.2	0.17	0.13	0.13	**0.16**	0.03	0.04	0.07	0.04	**0.05**	0.03	0.03	0.04	0.03	**0.03**	**1.09**
SIM	2.01	0.88	1.59	0.70	**1.29**	0.22	0.16	0.12	0.11	**0.15**	0.04	0.05	0.03	0.03	**0.04**	0.05	0.04	0.05	0.05	**0.05**	**1.53**
SIF	0.92	1.09	1.36	0.96	**1.08**	0.27	0.22	0.16	0.14	**0.20**	0.04	0.04	0.06	0.04	**0.05**	0.03	0.04	0.04	0.03	**0.04**	**1.36**
SC	0.91	0.94	1.32	0.84	**1.00**	0.19	0.19	0.10	0.07	**0.14**	0.04	0.04	0.04	0.03	**0.04**	0.03	0.04	0.03	0.02	**0.03**	**1.21**

Note: F=Fertilizer, I= Irrigation, M= Manure, IM= Irrigation + Manure, IF= Irrigation + Fertilizer, C= Control, hvt= harvest, TFC= Total flavonoid concentration.
S=shade, tot= total

Table 3.3. Treatments effect on baicalin, baicalein, wogonin and chrysin yield of American skullcap at 4 harvests in 2007 and 2008.

	Baicalin					Baicalein					Wogonin					Chrysin					TFY
Treat	hvt1	hvt2	*hvt3*	*hvt4*	***tot***	hvt1	hvt2	hvt3	hvt4	**tot**	hvt1	hvt2	*hvt3*	*hvt4*	***tot***	*hvt1*	*hvt2*	*hvt3*	*hvt4*	***tot***	
	<----------g ha-1---------->																				
F	502	340	90	2.0	**93**	93.7	47.6	9.9	0.1	**151**	22.2	15.2	2.5	0.0	**40**	16.4	12.8	2.5	0.0	**32**	**2284**
I	717	704	614	170	**2205**	92.0	84.9	44.7	8.8	**230**	13.8	18.6	10.3	2.1	**45**	8.9	23.5	14.5	2.1	**49**	**5010**
M	443	442	120	0.5	**1006**	111.5	55.2	19.1	0.0	**186**	18.0	18.1	6.5	0.0	**43**	9.6	15.3	3.2	0.0	**28**	**2500**
IM	831	347	1697	49	**2924**	163.0	70.3	208.0	2.9	**444**	27.1	20.3	61.9	0.9	**110**	17.0	16.2	31.5	1.0	**66**	**7023**
IF	794	685	759	16	**2255**	109.7	97.1	76.1	2.2	**285**	20.8	18.9	25.3	0.7	**66**	10.3	16.9	13.2	0.8	**41**	**5252**
C	437	349	170	8	**964**	67.0	45.2	10.1	0.2	**122**	10.3	13.9	6.8	0.1	**31**	9.3	13.3	2.6	0.1	**25**	**2261**
SF	680	552	268	150	**1650**	77.6	103.4	32.5	20.7	**234**	11.0	44.9	13.4	6.5	**76**	16.9	44.7	7.9	7.0	**76**	**3996**
SI	393	574	348	176	**1492**	74.8	74.9	39.2	18.5	**207**	13.3	27.2	12.6	5.0	**58**	11.9	15.0	9.7	6.1	**43**	**3560**
SM	438	761	319	86	**1604**	92.0	156.9	53.9	20.0	**323**	25.2	50.5	32.2	6.7	**115**	13.7	36.2	14.1	4.4	**68**	**4151**
SIM	454	636	2006	215	**3312**	135.5	132.0	149.7	32.0	**449**	37.3	38.8	36.4	9.6	**122**	23.8	36.4	61.6	15.2	**137**	**7900**
SIF	410	772	1773	257	**3212**	104.2	122.5	185.2	36.2	**448**	27.7	39.9	76.8	11.9	**156**	28.4	29.2	45.2	9.2	**112**	**7745**
SC	344	572	304	195	**1415**	62.7	65.7	23.2	17.6	**169**	12.1	30.0	9.6	7.3	**59**	5.8	18.8	6.9	5.0	**36**	**3323**

Note: F=Fertilizer, I= Irrigation, M= Manure, IM= Irrigation + Manure, IF= Irrigation + Fertilizer, C= Control, hvt= harvest, S= Shade, TFY= Total flavonoid concentration.

Table 3.4. Main effects of Shade, Irrigation and Nutrient on baicalin concentration of American skullcap in 2007 and 2008

Treatments	2007		2008	
	Harvest 1	Harvest 2	Harvest 3	Harvest 4
Shade effect	<----------mg g^{-1}---------->			
Full sun	1.72	1.16	1.41	1.27
Shade	1.19	1.01	1.18	0.81
SE	0.10	0.13	0.16	0.14
Irrigation effect				
No Irrigation	1.49	1.00	1.15	0.66
Irrigation	1.42	1.17	1.44	1.41
SE	0.10	0.11	0.15	0.12
Nutrient effect				
Control	1.60	1.13	1.37	1.38
Chemical	1.55	1.13	1.19	0.81
Manure	1.22	1.00	1.32	0.92
SE	0.11	0.12	0.17	0.13
Contrast[1]	<----------P>F---------->			
Full sun vs. Shade	**0.030**	0.478	0.372	**0.065**
No Irrig. vs. Irrig.	0.581	0.112	0.120	**<0.001**
Control vs. Chemical	0.899	1.000	0.633	**0.001**
Control vs. Manure	**0.026**	0.503	0.968	**0.012**

[1]Multiple Pairwise comparisons were carried out using Dunnett-Hsu method
Bold numbers represent significant difference

Table 3.5. Interaction of Shade X Irrigation on baicalin concentration of American skullcap in 2007 and 2008

	2007						2008					
	Harvest 1			Harvest 2			Harvest 3			Harvest 4		
	<----------mg g^{-1}---------->											
Treatments	Full sun	Shade	*P>F*	Full sun	shade	*P>F*	Full sun	Shade	P>F	Full sun	Shade	P>F
No Irrigation	**1.6**	**1.4**	0.317	1.0	1.0	0.779	1.2	1.1	0.576	**0.038**	**0.093**	0.208
Irrigation	**1.8**	**1.0**	**0.001**	1.3	1.1	0.319	1.6	1.3	0.364	**0.168**	**0.118**	0.239
SE	0.13			0.15			0.21			0.03		
P>F[1]	0.130	**0.026**		**0.095**	0.558		0.191	0.363		**<0.001**	0.37	

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu method
Means shown in bold signify interaction is significant

Table 3. 6. Interaction of Shade X Nutrients on baicalin concentration of American skullcap in 2007 and 2008

	2007						2008					
	Harvest 1			Harvest 2			Harvest 3			Harvest 4		
	<----------mg g^{-1}----------											
Treatments	Full sun	Shade		Full sun	shade		Full sun	Shade		Full sun	Shade	
			P>F			*P>F*			P>F			P>F
Control	1.93	1.28	0.009	1.21	1.04	0.483	1.60	1.14	0.192	**1.86**	**0.91**	**0.002**
Chemical	1.70	1.39	0.162	1.28	0.98	0.235	1.27	1.11	0.630	**0.73**	**0.89**	0.537
Manure	1.52	0.91	0.014	0.98	1.02	0.886	1.34	1.31	0.930	**1.22**	**0.62**	**0.047**
SE	0.16			0.17			0.24			0.19		
Contrasts [1]	<----------P>F----------											
Ctrl vs. Chem.	0.469	0.823		0.917	0.910		0.484	0.991		**<0.001**	0.995	
Contrl vs. Man.	0.106	0.163		0.345	0.984		0.619	0.816		**0.018**	0.317	

[1] Multiple pairwise comparisons were carried out using Dunnett-Hsu method
Means shown in bold signify interaction is significant

Table 3. 7. Interaction of Irrigation X Nutrients on baicalin concentration of American skullcap in 2007 and 2008

	2007						2008					
	Harvest 1			Harvest 2			Harvest 3			Harvest 4		
	<----------g mg^{-1}---------->											
Treatments	No Irrig.	Irrig.		No Irrig.	Irrig.		No Irrig.	Irrig.		No Irrig.	Irrig.	
			P>F			*P>F*			P>F			P>F
Control	**1.6**	**1.6**	0.768	1.0	1.2	0.341	1.4	1.4	0.972	1.0	1.8	**0.001**
Chemical	**1.8**	**1.3**	0.021	1.0	1.3	0.152	1.0	1.4	0.227	0.6	1.0	**0.034**
Manure	**1.1**	**1.3**	0.256	1.0	1.0	0.693	1.1	1.6	0.126	0.4	1.4	**0.000**
SE	0.2			0.1			0.2			0.2		
Contrasts[1]	<----------P>F---------->											
Ctrl vs. Chem.	0.455	0.195		0.953	0.957		0.384	0.997		0.112	**0.002**	
Contrl vs. Man.	0.**055**	0.281		0.875	0.510		0.540	0.739		**0.032**	**0.205**	

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu method
Means shown in bold signify interaction is significant

Table 3. 8. Main effects of Shade, Irrigation and Nutrient on baicalin yield of American skullcap in 2007 and 2008

Treatments	2007		2008	
	Harvest 1	Harvest 2	Harvest 3	Harvest 4
Shade effect	<----------g ha^{-1}---------->			
Full sun	628.3	488.6	575.1	42.9
Shade	453.1	644.5	836.6	181.5
SE	49.9	64.4	159.9	23.1
Irrigation effect				
No Irrigation	489.8	513.3	212.1	76.1
Irrigation	591.6	619.8	1199.6	148.2
SE	48.3	64.4	147.6	22.8
Nutrient effect				
Control	496.7	549.7	359.1	140.6
Chemical	584.0	587.3	722.7	106.5
Manure	541.5	562.6	1035.7	89.5
SE	53.3	71.2	175.4	25.4
Contrast[1]:	<----------P>F---------->			
Full sun vs. Shade	**0.042**	**0.014**	0.291	**0.000**
No Irrig. vs. Irrig.	**0.030**	**0.087**	**0.000**	**0.007**
Control vs. Chemical	0.210	0.831	0.219	0.418
Control vs. Manure	0.631	0.978	**0.012**	0.196

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu method
Bold numbers represent significant difference

Table 3.9. Interaction of Shade X Irrigation on baicalin yield of American skullcap in 2007 and 2008

	2007						2008					
	Harvest 1			Harvest 2			Harvest 3			Harvest 4		
	<----------mg g^{-1}---------->											
Treatments	Full sun	Shade		Full sun	shade		Full sun	Shade		Full sun	Shade	
			P>F			*P>F*			P>F			P>F
No Irrigation	**492.6**	**487.1**	0.938	398.3	628.3	**0.011**	126.9	297.3	0.572	5.6	146.7	**0.001**
Irrigation	**764.1**	**419.2**	**0.001**	578.9	660.7	0.346	1023.3	1375.9	0.250	80.1	216.3	**<0.001**
SE	59.1			77.3			208.7			30.0		
P>F[1]	**<0.001**	0.293		**0.042**	0.707		**0.002**	**<0.001**		**0.047**	**0.051**	

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu method
Means shown in bold signify interaction is significant

Table 3.10. Interaction of Irrigation X Nutrients on baicalin yield of American skullcap in 2007 and 2008

	2007						2008					
	Harvest 1			Harvest 2			Harvest 3			Harvest 4		
	<----------g ha^{-1}---------->											
Treatments	No Irrig.	Irrig.	*P>F*	No Irrig.	Irrig.	*P>F*	No Irrig.	Irrig.	P>F	No Irrig.	Irrig.	P>F
Control	438.0	555.3	0.141	**460.5**	**638.9**	**0.098**	**237.1**	**481.0**	0.463	107.9	173.3	0.136
Chemical	591.0	577.0	0.858	**445.8**	**728.9**	**0.011**	**179.3**	**1266.0**	**0.002**	76.0	136.9	0.147
Manure	440.5	642.6	**0.014**	**633.6**	**491.7**	0.184	**219.7**	**1851.7**	**0.000**	44.5	134.5	**0.059**
SE	66.0			88.4			240.3			34.3		
Contrasts[1]	<----------P>F---------->											
Contrl vs. Chem.	0.103	0.944		0.986	0.602		0.978	**0.042**		0.678	0.586	
Contrl vs. Man.	0.999	0.434		0.187	0.284		0.998	**<0.001**		0.296	0.573	

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu method
Means shown in bold signify interaction is significant

Table 3.11. Main effects of Shade, Irrigation and Nutrients on baicalein concentration of American skullcap in 2007 and 2008

Treatments	2007		2008	
	Harvest 1	Harvest 2	Harvest 3	Harvest 4
Shade effect	<----------mg g^{-1}---------->			
Full sun	0.29	0.33	0.14	0.10
Shade	0.22	0.17	0.12	0.11
SE	0.03	0.11	0.01	0.02
Irrigation effect				
No Irrigation	0.25	0.23	0.12	0.07
Irrigation	0.27	0.26	0.14	0.14
SE	0.03	0.09	0.01	0.02
Nutrient effect				
Control	0.25	0.26	0.11	0.09
Chemical	0.25	0.18	0.13	0.11
Manure	0.27	0.30	0.15	0.11
SE	0.03	0.11	0.02	0.02
Contrast[1]	<----------P>F---------->			
Full sun vs. Shade	0.174	0.346	0.316	0.937
No Irrig. vs. Irrig.	0.394	0.722	0.333	**0.001**
Control vs. Chemical	0.962	0.741	0.356	0.790
Control vs. Manure	0.655	0.893	**0.071**	0.669

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu method
Bold numbers represent significant difference

Table 3.12. Interaction of Shade X Irrigation on baicalein concentration of American skullcap in 2007 and 2008

	2007						2008					
	Harvest 1			Harvest 2			Harvest 3			Harvest 4		
	<----------mg g^{-1}---------->											
Treatments	Full sun	Shade		Full sun	shade		Full sun	Shade		Full sun	Shade	
			P>F			*P>F*			P>F			P>F
No Irrigation	0.289	0.212	0.141	0.300	0.156	0.457	0.132	0.114	0.46	**0.038**	**0.093**	0.208
Irrigation	0.298	0.237	0.224	0.353	0.175	0.358	0.151	0.128	0.36	**0.168**	**0.118**	0.239
SE	0.04			0.13			0.02			0.03		
P>F[1]	0.75	0.38		0.71	0.90		0.43	0.56		**<0.001**	0.37	

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu method
Means shown in bold signify interaction is significant

Table 3.13. Main effects of Shade, Irrigation and Nutrients on baicalein yield of American skullcap in 2007 and 2008

Treatments	2007		2008	
	Harvest 1	Harvest 2	Harvest 3	Harvest 4
Shade effect	<----------g ha^{-1}---------->			
Full sun	111.1	68.5	61.3	2.7
Shade	91.1	109.2	80.6	24.2
SE	15.8	7.0	12.8	2.7
Irrigation effect				
No Irrigation	86.7	80.8	24.8	10.0
Irrigation	115.5	97.0	117.1	16.9
SE	15.5	7.0	11.6	2.6
Nutrient effect				
Control	78.1	67.7	29.3	11.6
Chemical	99.7	92.7	75.9	14.8
Manure	125.5	106.3	107.7	13.9
SE	16.4	8.6	13.6	3.0
Contrast[1]				
	<----------P>F---------->			
Full sun vs. Shade	0.200	**<0.001**	0.327	**<0.001**
No Irrig. vs. Irrig.	**0.010**	0.111	**<0.001**	**0.026**
Control vs. Chemical	0.180	**0.083**	**0.024**	0.572
Control vs. Manure	**0.002**	**0.005**	**0.000**	0.767

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu method
Bold numbers represent significant difference

Table 3.14. Interaction of Shade X Nutrients on baicalein yield of American skullcap in 2007 and 2008

	2007						2008					
	Harvest 1			Harvest 2			Harvest 3			Harvest 4		
	<----------g ha^{-1}---------->											
Treatments	Full sun	Shade		Full sun	shade		Full sun	Shade		Full sun	Shade	
			P>F			*P>F*			P>F			P>F
Control	87.4	12.7	0.346	**65.0**	**70.3**	0.759	**27.4**	**31.2**	0.891	5.2	18.1	**0.016**
Chemical	108.6	19.3	0.373	**72.3**	**113.0**	**0.023**	**43.0**	**108.8**	**0.024**	1.1	28.4	**<0.001**
Manure	137.2	31.3	0.241	**68.2**	**144.5**	**<0.001**	**113.5**	**101.8**	0.671	1.8	26.0	**<0.001**
SE	19.0			12.1			19.3			4.0		
Contrasts[1]	<----------P>F----------											
Ctrl vs. Chem.	0.414	0.481		0.877	**0.032**		0.760	**0.008**		0.643	**0.072**	
Ctrl vs. Man.	**0.019**	**0.011**		0.975	**<0.001**		**0.003**	**0.015**		0.761	0.218	

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu method
Means shown in bold signify interaction is significant

Table 3.15. Interaction of Irrigation X Nutrients on baicalein yield of American skullcap in 2007 and 2008

	2007						2008					
	Harvest 1			Harvest 2			Harvest 3			Harvest 4		
	<----------g ha^{-1}---------->											
Treatments	No irrig.	Irrig.		No irrig.	Irrig.		No irrig.	Irrig.		No irrig.	Irrig.	
			P>F			*P>F*			P>F			P>F
Control	72.8	13.6	0.564	55.4	79.9	0.162	**16.6**	**42.0**	0.319	9.6	13.6	0.428
Chemical	85.6	24.2	0.132	75.5	109.8	**0.053**	**21.2**	**130.6**	**<0.001**	10.4	19.2	**0.079**
Manure	101.7	28.8	**0.014**	111.5	101.2	0.550	**36.5**	**178.8**	**<0.001**	10.0	17.8	0.161
SE	18.8			12.1			18.5			4.0		
Contrasts[1]	<----------P>F---------->											
Ctrl vs. Chem.	0.706	0.177		0.404	0.156		0.976	**0.002**		0.981	0.425	
Contrl vs. Man.	0.211	**0.040**		**0.004**	0.366		0.646	**<0.001**		0.996	0.632	

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu method
Means shown in bold signify interaction is significant

Table 3.16. Main effects of Shade, Irrigation and Nutrients on wogonin concentration of American skullcap in 2007 and 2008

Treatments	2007		2008	
	Harvest 1	Harvest 2	Harvest 3	Harvest 4
Shade effect	<----------mg g^{-1}---------->			
Full sun	0.05	0.05	0.05	0.03
Shade	0.05	0.06	0.05	0.03
SE	0.01	0.01	0.01	0.01
Irrigation effect				
No Irrigation	0.05	0.05	0.05	0.02
Irrigation	0.05	0.05	0.04	0.04
SE	0.01	0.01	0.01	0.01
Nutrient effect				
Control	0.04	0.04	0.04	0.03
Chemical	0.05	0.05	0.05	0.03
Manure	0.05	0.06	0.05	0.04
SE	0.01	0.01	0.01	0.01
Contrast[1]	<----------P>F---------->			
Full sun vs. Shade	0.691	0.258	0.795	0.750
No Irrig. vs. Irrig.	0.617	0.873	0.296	**0.004**
Control vs. Chemical	0.845	0.444	0.524	0.758
Control vs. Manure	0.385	0.226	0.342	0.467

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu method
Bold numbers represent significant difference

Table 3.17. Interaction of Shade X Irrigation on wogonin concentration of American skullcap in 2007 and 2008

	2007						2008					
	Harvest 1			Harvest 2			Harvest 3			Harvest 4		
	<----------mg g^{-1}---------->											
Treatments	Full sun	Shade		Full sun	shade		Full sun	Shade		Full sun	Shade	
			P>F			*P>F*			P>F			P>F
No Irrigation	**0.05**	**0.04**	0.316	0.05	0.06	0.210	0.05	0.05	0.988	**0.01**	**0.03**	**0.058**
Irrigation	**0.04**	**0.06**	0.122	0.05	0.06	0.432	0.05	0.04	0.682	**0.05**	**0.03**	0.120
SE	0.01			0.01			0.01			0.01		
P>F[1]	0.351	0.106		0.843	0.672		0.600	0.338		**<0.001**	0.757	

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu method
Means shown in bold signify interaction is significant

Table 3.18. Main effect of Shade, Irrigation and Nutrients on wogonin yield of American skullcap in 2007 and 2008

Treatments	2007		2008	
	Harvest 1	Harvest 2	Harvest 3	Harvest 4
Shade effect	<----------g ha^{-1}---------->			
Full sun	18.1	18.0	18.9	0.7
Shade	21.1	38.6	30.2	7.8
SE	4.4	4.6	6.3	1.3
Irrigation effect				
No Irrigation	17.0	29.3	11.9	3.5
Irrigation	22.2	27.3	37.2	5.0
SE	4.4	4.6	5.5	1.2
Nutrient effect				
Control	13.2	22.4	9.8	3.7
Chemical	20.4	29.7	29.5	4.8
Manure	25.2	32.7	34.3	4.3
SE	4.8	5.1	6.3	1.3
Contrast[1]	<----------P>F---------->			
Full sun vs. Shade	0.417	**<0.001**	0.256	**0.015**
No Irrig. vs. Irrig.	0.164	0.657	**<0.001**	0.243
Control vs. Chemical	0.199	0.320	**0.028**	0.730
Control vs. Manure	**0.020**	0.123	**0.006**	0.920

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu method
Bold numbers represent significant difference

Table 3.19. Interaction of Shade X Nutrients on wogonin yield of American skullcap in 2007 and 2008

	2007						2008					
	Harvest 1			Harvest 2			Harvest 3			Harvest 4		
	<----------g ha^{-1}---------->											
Treatments	Full sun	Shade		Full sun	shade		Full sun	Shade		Full sun	Shade	
			P>F			*P>F*			P>F			P>F
Control	13.6	12.7	0.888	16.3	28.6	0.120	**8.6**	**11.1**	0.844	1.3	6.2	**0.059**
Chemical	21.5	19.3	0.739	17.1	42.4	**0.002**	**13.9**	**45.1**	**0.023**	0.4	9.2	**0.002**
Manure	19.2	31.3	**0.066**	20.8	44.7	**0.004**	**34.2**	**34.3**	0.994	0.4	8.1	**0.009**
SE	5.7			6.4			8.9			1.8		
Contrasts1	<----------P>F---------->											
Ctrl vs. Chem.	0.367	0.481		0.992	0.148		0.838	**0.007**		0.880	0.281	
Contrl vs. Man.	0.587	**0.011**		0.786	**0.083**		**0.045**	**0.072**		0.912	0.585	

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu method
Means shown in bold signify interaction is significant

Table 3.20. Interaction of Irrigation X Nutrients on wogonin yield of American skullcap in 2007 and 2008

	2007						2008					
	Harvest 1			Harvest 2			Harvest 3			Harvest 4		
	<----------g ha^{-1}---------->											
Treatments	No irrig.	Irrig.		No irrig.	Irrig.		No irrig.	Irrig.		No irrig.	Irrig.	
			P>F			*P>F*			P>F			P>F
Control	12.7	13.6	0.899	21.9	22.9	0.898	**8.2**	**11.4**	0.767	3.9	3.6	0.890
Chemical	16.6	24.2	0.237	30.1	29.4	0.930	**8.0**	**51.1**	**<0.001**	3.3	6.3	0.164
Manure	21.6	28.8	0.265	35.9	29.6	0.420	**19.4**	**49.1**	**0.010**	3.3	5.3	0.426
SE	5.7			6.4			8.3			1.8		
Contrasts[1]	<----------P>F---------->											
Ctrl vs. Chem.	0.772	0.177		0.477	0.620		1.000	**0.002**		0.943	0.353	
Contrl vs. Man.	0.287	**0.040**		0.142	0.606		0.491	**0.003**		0.961	0.676	

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu method
Means shown in bold signify interaction is significant

Table 3.21. Interaction of Shade X Irrigation on chrysin concentration of American skullcap in 2007 and 2008

	2007						2008					
	Harvest 1			Harvest 2			Harvest 3			Harvest 4		
	<----------mg g^{-1}---------->											
Treatments	Full sun	Shade		Full sun	shade		Full sun	Shade		Full sun	Shade	
			P>F			*P>F*			P>F			P>F
No Irrigation	**0.04**	**0.03**	0.248	0.04	0.05	0.225	0.03	0.03	0.527	0.01	0.03	0.430
Irrigation	**0.03**	**0.05**	**0.010**	0.04	0.04	0.867	0.03	0.04	0.497	0.06	0.04	0.224
SE	0.005			0.007			0.005			0.014		
P>F[1]	0.160	**0.019**		0.376	0.291		0.388	0.366		**0.026**	0.405	

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu method
Means shown in bold signify interaction is significant

Table 3.22. Interaction of Irrigation X Nutrients on chrysin concentration of American skullcap in 2007 and 2008

	2007						2008					
	Harvest 1			Harvest 2			Harvest 3			Harvest 4		
	<----------mg g^{-1}----------											
Treatments	No irrig.	Irrig.		No irrig.	Irrig.		No irrig.	Irrig.		No irrig.	Irrig.	
			P>F			*P>F*			P>F			P>F
Control	0.03	0.03	0.884	**0.03**	**0.03**	0.772	0.03	0.04	0.140	0.02	0.03	0.495
Chemical	0.05	0.04	0.561	**0.06**	**0.04**	**0.028**	0.03	0.03	0.660	0.02	0.05	**0.075**
Manure	0.03	0.04	**0.055**	**0.04**	**0.06**	**0.082**	0.04	0.04	0.765	0.02	0.06	**0.048**
SE	0.01			0.01			0.01			0.02		
Contrasts[1]	<----------P>F----------											
Ctrl vs. Chem.	0.197	0.398		**0.011**	0.917		0.872	0.714		1.000	0.348	
Contrl vs. Man.	0.728	0.259		0.463	**0.028**		0.314	0.997		0.977	0.242	

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu method
Means shown in bold signify interaction is significant

Table3. 23. Main effects of Shade, Irrigation and Nutrients on chrysin yield of American skullcap in 2007 and 2008

Treatments	2007		2008	
	Harvest 1	Harvest 2	Harvest 3	Harvest 4
Shade effect	<----------g ha^{-1}---------->			
Full sun	12.6	16.3	11.3	0.9
Shade	16.8	30.0	24.2	7.8
SE	1.8	2.8	3.4	1.3
Irrigation effect				
No Irrigation	12.5	23.5	6.2	2.9
Irrigation	16.9	22.9	29.3	5.9
SE	1.8	2.6	3.0	1.2
Nutrient effect				
Control	9.8	16.9	8.4	3.5
Chemical	18.2	25.9	17.2	4.2
Manure	16.0	26.8	27.6	5.4
SE	2.1	3.1	3.5	1.3
Contrast[1]	<----------P>F---------->			
Full sun vs. Shade	0.148	**0.013**	**0.035**	**0.018**
No Irrig. vs. Irrig.	**0.042**	0.858	**0.000**	**0.019**
Control vs. Chemical	**0.004**	**0.073**	**0.096**	0.814
Control vs. Manure	**0.035**	**0.047**	**<0.001**	0.370

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu method
Bold numbers represent significant difference

Table 3.24. Interaction of Shade X Irrigation on chrysin yield of American skullcap in 2007 and 2008

	2007						2008					
	Harvest 1			Harvest 2			Harvest 3			Harvest 4		
	<----------g ha^{-1}---------->											
Treatments	Full sun	Shade		Full sun	shade		Full sun	Shade		Full sun	Shade	
			P>F			*P>F*			P>F			P>F
No Irrigation	**12.8**	**12.1**	0.826	13.7	33.2	**0.002**	**2.8**	**9.7**	0.269	0.2	5.5	**0.024**
Irrigation	**12.4**	**21.4**	**0.012**	18.9	26.9	0.146	**19.8**	**38.8**	**0.007**	1.5	10.2	**0.002**
SE	2.4			3.7			4.2			1.6		
P>F[1]	0.876	**0.003**		0.302	0.202		**0.002**	**<0.001**		0.458	**0.008**	

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu method
Means shown in bold signify interaction is significant

Table 3.25. Interaction of Shade X Nutrients on chrysin yield of American skullcap in 2007 and 2008

	2007						2008					
	Harvest 1			Harvest 2			Harvest 3			Harvest 4		
	<----------g ha^{-1}---------->											
Treatments	Full sun	Shade		Full sun	shade		Full sun	Shade		Full sun	Shade	
			P>F			*P>F*			P>F			P>F
Control	10.7	8.8	0.619	**16.8**	**16.9**	0.982	**8.6**	**8.3**	0.968	1.4	5.6	**0.079**
Chemical	13.8	22.7	**0.025**	**14.9**	**36.9**	**0.002**	**7.8**	**26.6**	**0.014**	0.4	8.1	**0.003**
Manure	13.3	18.8	0.147	**17.3**	**36.3**	**0.005**	**17.4**	**37.9**	**0.008**	0.9	9.8	**0.002**
SE	2.8			4.4			4.9			1.8		
Contrasts[1]	<----------P>F---------->											
Ctrl vs. Chem.	0.591	**0.001**		0.929	**0.004**		0.990	**0.011**		0.844	0.339	
Contrl vs. Man.	0.692	**0.017**		0.995	**0.005**		0.278	**<0.001**		0.957	**0.082**	

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu method
Means shown in bold signify interaction is significant

Table 3.26. Interaction of Irrigation X Nutrients on chrysin yield of American skullcap in 2007 and 2008

	2007						2008					
	Harvest 1			Harvest 2			Harvest 3			Harvest 4		
	<----------g ha^{-1}---------->											
Treatments	No Irrig.	Irrig.		No Irrig.	Irrig.		No Irrig.	Irrig.		No Irrig.	Irrig.	
			P>F			*P>F*			P>F			P>F
Control	9.1	10.4	0.726	14.4	19.3	0.422	**4.7**	**12.1**	0.243	2.9	4.1	0.560
Chemical	16.7	19.8	0.385	28.8	23.0	0.346	**5.2**	**29.2**	**<0.001**	3.5	5.0	0.462
Manure	11.7	20.4	**0.021**	27.3	26.3	0.867	**8.7**	**46.6**	**<0.001**	2.2	8.5	**0.008**
SE	2.8			4.3			4.7			1.7		
Contrasts[1]	<----------P>F---------->											
Ctrl vs. Chem.	**0.078**	**0.024**		**0.042**	0.757		0.996	**0.018**		0.932	0.863	
Contrl vs. Man.	0.704	**0.017**		**0.072**	0.409		0.752	**<0.001**		0.937	**0.074**	

[1]Multiple pairwise comparisons were carried out using Dunnett-Hsu method
Means shown in bold signify interaction is significant

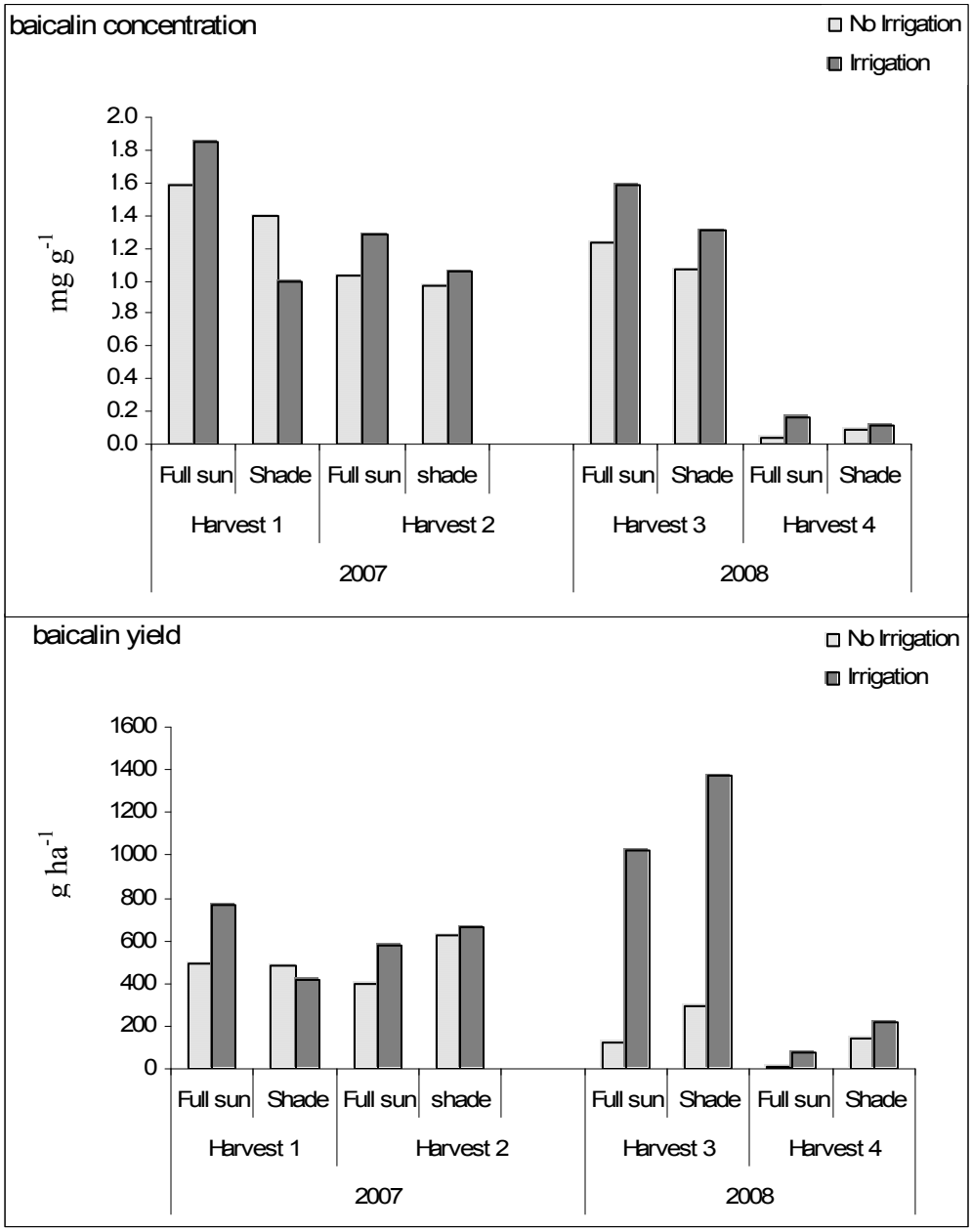


Figure 3.1 Interaction of Shade X Irrigation on baicalin concentration and yield of American skullcap in 2007 and 2008

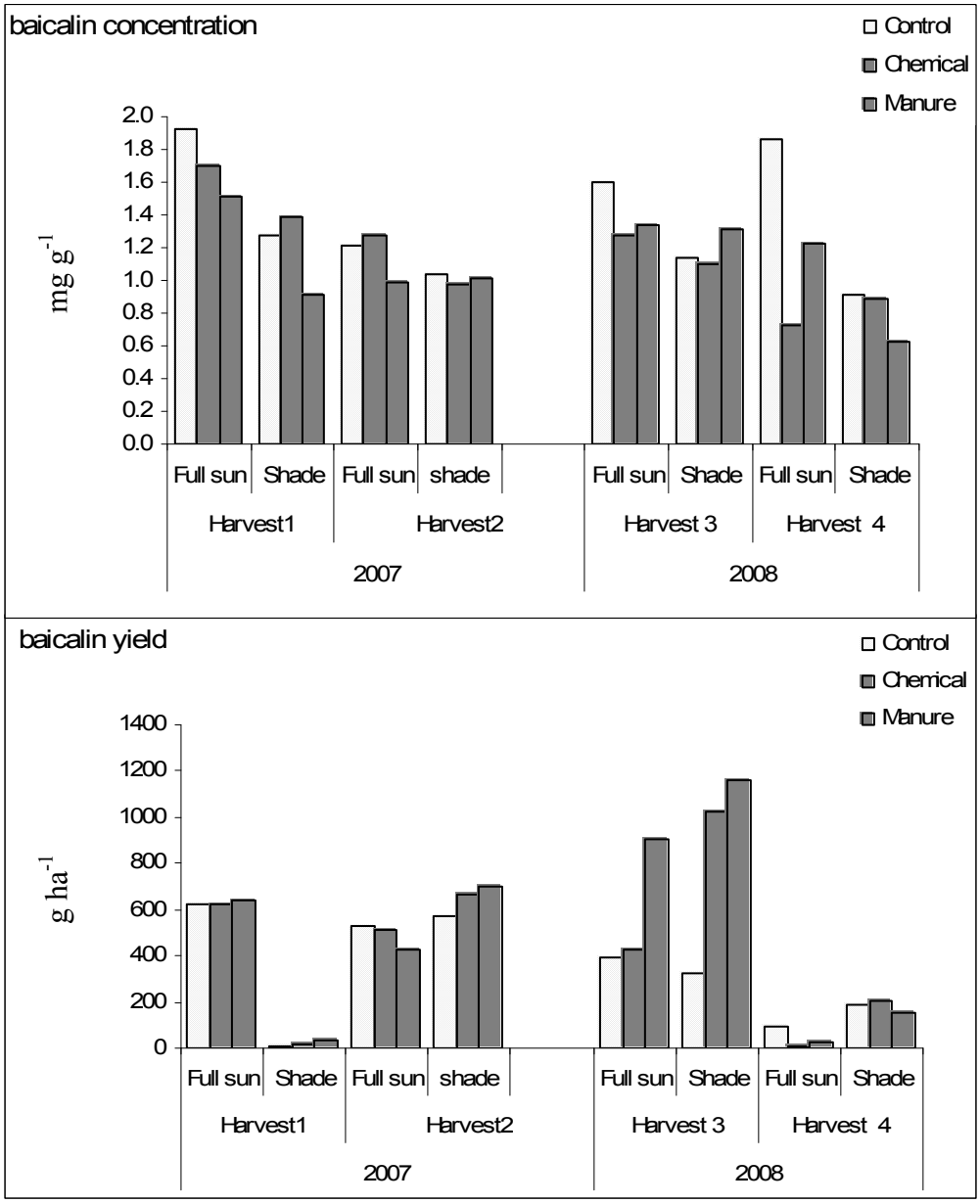


Figure 3.2. Interaction of Shade X Nutrient on baicalin concentration and yield of American skullcap in 2007 and 2008

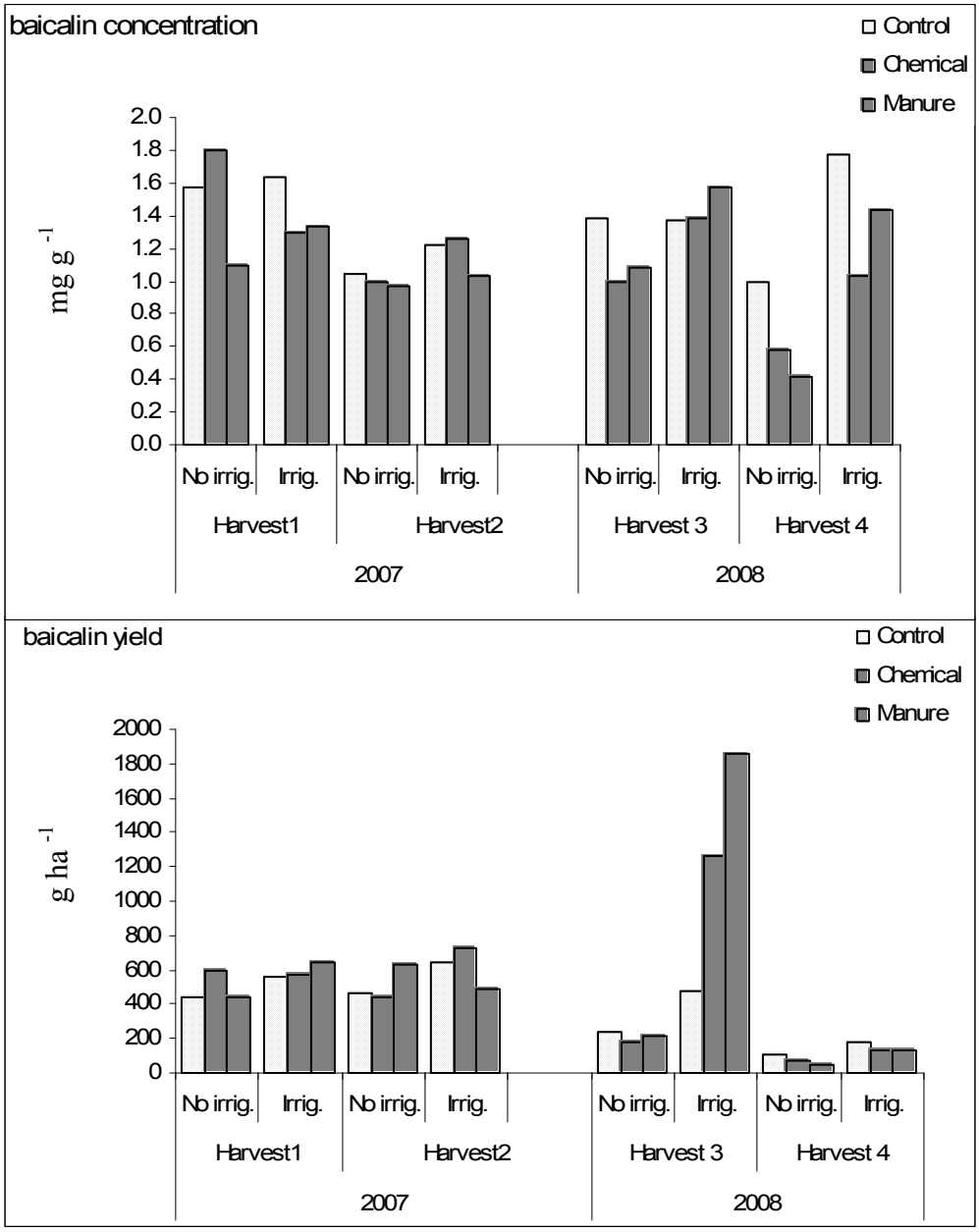


Figure 3.3. Interaction of Irrigation X Nutrient on Baicalin concentration and yield of American skullcap in 2007 and 2008

CHAPTER IV

SUMMARY AND CONCLUSIONS

A field experiment was established at Shorter Alabama in order to determine the most appropriate growing condition needed to optimize dry matter yield and flavonoid content of American skullcap. A 2x2x3 split plot factorial design where established to study the effect of shade, irrigation and nutrient application on dry matter yield, flavonoid concentration and flavonoid yield in American skullcap at 4 harvests in 2007 and 2008. All growth parameters considered in this study, except percent dry matter performed better under shade, with irrigation and added nutrients. Shade increased dry matter by 45%, decreased flavonoid concentration by 26% and increased flavonoid yield by 26%. Irrigation increased dry matter yield by 61%, flavonoid concentration by 20% and flavonoid yield by 97%. However, the increase due to irrigation is higher in full sun than under shade, suggesting that irrigation is more critical in full sun. Nutrient application increased dry matter yield by 22%, decreased flavonoid concentration by 29 % and increased flavonoid yield by 44%. Without irrigation the effect of nutrient was not significant on dry matter and flavonoid yield; but with irrigation, nutrient application produced the highest dry matter and flavonoid yield, suggesting that irrigation is required when nutrient is added. The highest dry matter (2662 kg ha^{-1} and (2654 kg ha^{-1}) and flavonoid yields (7903.9 g ha-1, and 7745 gha^{-1}) for the four harvests were obtained with

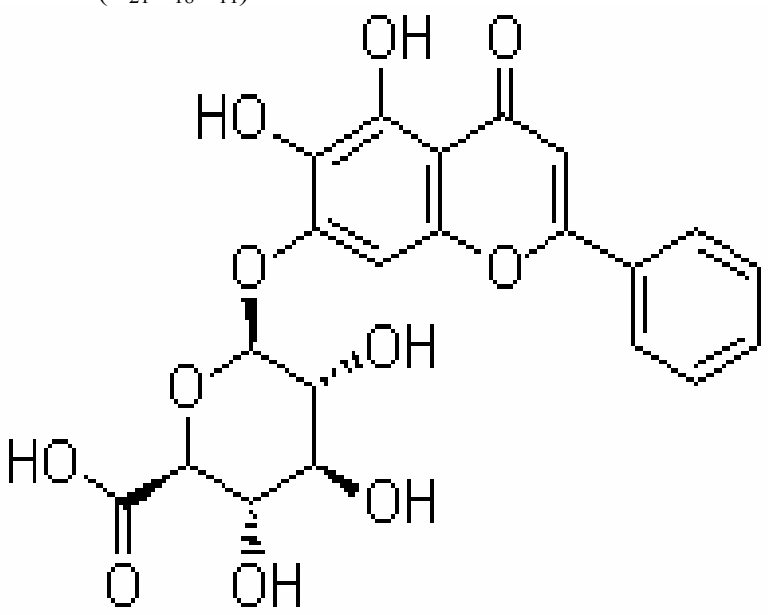
the irrigation + manure and irrigation + fertilizer treatments under shade, while the highest Flavonoid concentrations (1.94 mg g^{-1} and 1.90 mg g^{-1}) were obtained with irrigation + manure and irrigation treatments in full sun. Irrigation + manure and irrigation + fertilizer also produced higher dry matter and flavonoid yield in full sun and the fertilizer and control treatments producing the lowest dry matter and flavonoid yields in full sun also produced the lowest yield under shade.

There is thus a correlation between dry matter and flavonoid yield either in full sun or under shade. Any treatment aiming at increasing dry matter yield will also increase flavonoid yield. Depending on the objective, if a farmer aim at producing higher total flavonoid yield, irrigation + manure and irrigation + fertilizer under shade would be recommended, however, if the objective is to produce plant material with high concentration of flavonoid, irrigation + manure in full sun would be the best choice. However any final decision must be based on cost effectiveness. Although highest flavonoid yields can be obtained under shade, cost of shade structure and irrigation must be considered in order to determine if the returns merit the additional investment. Based on our results, irrigation seems to have the highest impact on dry matter and flavonoid yield in American skullcap. These results were expected given that skullcap is classified as a facultative wetland plant. Further investigation is needed to determine the best irrigation rate and nutrient dosage to produce the highest flavonoid concentration and yield economically under cultivation.

APPENDIX

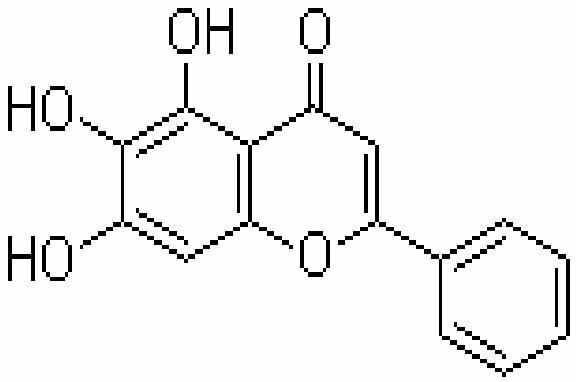
1- Baicalin molecular and structural formula

($C_{21}H_{18}O_{11}$)



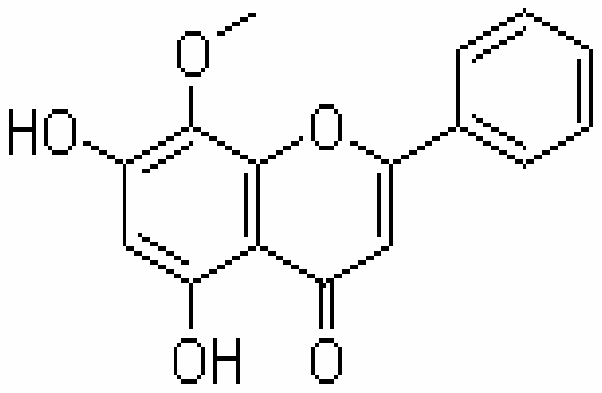
2- Baicalein molecular and structural formula

($C_{15}H_{10}O_5$)



3- Wogonin molecular and structural formula

($C_{16}H_{12}O_5$)



4- Chrysin molecular and structural formula

($C_{15}H_{10}O_4$)