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Primocane-Fruiting Blackberry: Optimum N-Fertilization

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and Period for Foliar Sampling

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ABSTRACT

This study was conducted in 2011 at the University of Arkansas, Fayetteville to determine the optimum rate and time of nitrogen (N) application for ‘Prime-Ark® 45’ primocane-fruiting (PF) blackberries under high tunnel conditions. There were four N treatments: Control (0), 10, 10-split, and 20 $kg{\cdot}ha^{-1}$ (Treatments 1, 2, 3, 4, respectively). In a randomized complete block (RCB) design, the following variables were compared: total and marketable yield, fresh weight of plant above ground, and cane diameter. Total fruit yields for Treatments 2 and 3 (2.5 and 2.5 kg, respectively) were highest and significantly different from the other treatments ($p < 0.05$). Marketable yield had a similar trend as total fruit yield, although not significantly different. Cane diameter and plant fresh weight were not significantly affected by fertilizer treatments. There were not significant differences in N content in leaves among treatments. Results indicated that either a single or split N application of 10 $kgN{\cdot}ha^{-1}$ could result in better yields.

Four experiments were conducted to determine the most stable period in foliar elemental concentration, in order to identify the best time for foliar fertilizer applications in ‘Prime-Ark® 45’ PF blackberry cultivar. The four experiments were conducted in five separate locations. In North Carolina (N.C.), ‘Prime-Ark® 45’ leaf samples were collected at three commercial farms; in Clarksville, Arkansas (Ark.), three cultivars ‘Prime-Ark® 45’, ‘Prime-Jan®’, and ‘Ouachita’ were sampled; and in Fayetteville, Ark., ‘Prime-Ark® 45’ blackberry plants were sampled from plantings managed under two cultural methods (high tunnel and ambient). For N fertilization trials, 0, 10, 10-split, and 20 $kg.ha^{-1}$ N rates were compared under high tunnel conditions. Rates were compared for cultural practices (mown, mown + tipped, and not pruned) under ambient conditions. Leaf samples were collected and analyzed every two weeks from June to Aug. 2011.

Sampling dates revealed variations in foliar elemental nutrient concentrations. In Fayetteville, Ark., in one-year-old ‘Prime-Ark® 45’ blackberry plants, under high tunnel conditions, the period with the highest level of elemental stability was between 11 July and 25 July. Under ambient conditions, the most stable period was from 7 July to 25 July. In Clarksville, Ark., the period of most stability in foliar nutrient concentration was from 30 June to 12 July. In N.C., the proper period with most stability in leaf nutrient content was between 5 July and 22 July. Also in N.C., the logarithm of variance means analysis indicated that the least variance in foliar elemental concentration occurred from 5 July and 22 July 2011.

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DEDICATION

This thesis is dedicated to my dear mother; for her guidance and supporting me spiritually throughout my life. I hope she will be both happy and proud of what I have accomplished.

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I. INTRODUCTION

The blackberry (*Rubus* L. subgenus *Rubus* Watson.) is a favorite fruit for many people not only in the U. S. but also around the world. Being a native fruit in many countries, it has been consumed as a favorite wild fruit for a long time. Blackberries are harvested for personal or commercial use, and they come not only from the wild but also from cultivated plants. Increasing production of this fruit and the development of new international markets are providing valuable opportunities for producers of blackberries. Development of new cultivars with superior fruit quality has created significant advantages for both farmers and consumers (Strik et al., 2007).

The most prominent area of blackberry production is the northern hemisphere; however, production worldwide is increasing (Moore and Skirvin, 1990; Clark, 2008). The number of wild blackberry genotypes is vast, and the amount of improved cultivars rises continuously. Indeed, various types of blackberries with particular characteristics have been developed by researchers and specific programs in the U.S. and overseas (Strik et al., 2007). The University of Arkansas has been developing new types of blackberry genotypes successfully over the last five decades. The latest type is the primocane-fruiting (PF) blackberry. The advantage of PF cultivars is the potential to expand the fresh market season annually through diverse management practices such as mowing and tipping, which makes PF blackberries an attractive crop to the wholesale trade (Thompson et al., 2007).

Commercial production is constantly increasing for PF blackberries, and guidelines for production of new cultivars of blackberries have not been fully developed. Farmers need specific information about PF cultivars such as macro- and micronutrient requirements to optimize yield and financial returns. The information about proper fertilization rates and timing for nitrogen (N) applications to the floricane-fruiting blackberries and raspberries is accessible to both researchers and farmers; however, nothing specific for PF blackberry genotype fertilization has been developed (Clark et al., 2005).

Soil elemental content and availability of these nutrients for plants are changing constantly due to diverse factors such as plant consumption, erosion, leaching, natural or artificial input of nutrients to soils, etc. Soil analysis provides information not only about the existing elemental nutritional status of soils but also information pertinent to sustaining an optimum fertility condition according to the crop needs (Mylavarapu, 2010). While soil analysis identifies the physical characteristics and amount of mineral nutrients contained in soils, foliar (leaf or petiole) analysis measures the amount of each mineral which is actually taken up by the plants and reveals the plant's nutritional status (Jones, 2001). According to Clark et al. (1989), the need to make nutritional corrections quickly and accurately makes leaf tissue analysis relevant. However, elemental nutrient content of the leaf changes according to sampling date. In some periods during the growing season due to plant, soil, and environmental factors (Clark et al., 1989), the elemental content fluctuates. Standardized elemental values in nutrient concentrations have been described to compare with the analysis results. If these elemental concentrations are within these standards, the plants are considered healthy (Troeh and Thompson, 2005).

This research on PF blackberry cultivars was conducted to address the need for information on management guidelines for proper application of N fertilizers. Also, it was performed to determine the optimum stage of plant development with less seasonal variation of elemental nutrient content to obtain reliable foliar tissue analysis for making the necessary recommendations to improve yield and fruit quality.

Objectives

The objectives of the following experiments are as follows:

1. To determine the optimum rate and time of application of N fertilization for 'Prime –Ark® 45' PF blackberry cultivar under high tunnel conditions in one-year-old plants.
2. To determine, at three separate geographical locations, the optimum period in which nutrient concentrations in PF blackberry leaves are the most stable.

Hypothesis

From these objectives, the following hypotheses (*Ho*: null hypotheses) have been developed:

1. *Ho:* *Yield of the 'Prime –Ark® 45' PF blackberry cultivar does not increase due to the application of increased levels of N fertilizer.*

   It is expected that different rates of N fertilizer applications will produce diverse responses on yield of 'Prime-Ark® 45' PF blackberry. The literature review indicates N fertilizers have a positive effect on yield, foliar nutrient levels, and fruit composition of thornless blackberry production (Alleyne and Clark, 1997).

2. *Ho:* *There is no seasonal variation of element nutrient content in PF blackberry leaves.*

   Researchers have studied changes to seasonal nutrient concentrations in leaves in diverse plant species. Clark et al. (1989) states that these changes are affected by several factors such as climate, plant age, soil, and time of sampling, etc. Leaf samples should be collected at an appropriate time when there is stability in leaf nutrient content and fluxes are at a minimum.

II. LITERATURE REVIEW

Brambles

Both blackberries (*Rubus* L. subgenus *Rubus* Watson) and red raspberries (*Rubus ideaus* L.) belong to a large set of species and hybrids which are usually called brambles or caneberries. They belong to the *Rosacea* family and the genus *Rubus* (Galletta and Violette, 1989; Clark and Moore, 2008; Clark and Perkins-Veazie, 2011). The genus *Rubus* consists of twelve subgenera. Blackberries and dewberries (*Rubus spp.*) belong to the subgenera *Eubatus*, red and yellow raspberries and black raspberries (*R. occidentalis* L.) belong to the subgenera *Idaeobatus* (Rieger, 2006).

Within this subgenus, the natural occurring ploidy level ranges from diploid (2x= 2n= 14 chromosomes) to dodecaploid (12x= 2n= 84 chromosomes) (Moore and Skirvin, 1990). Blackberries have been hybridized by researchers since the 1850s, when early cultivars such as ‘Lawton’ and ‘Dorchester’ were selected and introduced (Moore and Skirvin, 1990). According to Clark (2008), in 1909, the Texas A & M University was the first American public institution to develop a breeding program for blackberries. Many other researchers in the U.S. followed these efforts for several years. Through the activity of private and public breeding programs, 59 blackberry cultivars were released between 1985 and 2005 (Clark and Finn, 2008). The blackberry breeding program at the University of Arkansas (UA) was established in 1964. This program has developed several cultivars of blackberry including the most innovative and promising genotype, the primocane-fruiting (PF) blackberry. The program objectives are to improve plant and fruit characteristics, such as the quality of berries, including better taste, earlier ripening (to avoid summer heat), and later ripening (for extended harvest); pest resistance, thornlessness, and better shipping with improved handling capabilities (Clark, 1999; Clark and

Finn, 2008). During the last decade, the most remarkable achievement in blackberry production has been the development of the primocane-fruiting cultivars. Strik and Thompson (2009) describing the PF blackberry cultivars states that this plant type bears fruit on current-season canes (primocanes) and on second season canes (floricanes) while all other types of blackberry bear fruit only on the floricane. Although tested in various regions worldwide, these new types of PF blackberry were not grown commercially before 2005. According to Clark et al. (2005) the first commercial PF blackberry cultivars ever released were by the University of Arkansas, ‘Prime-Jan®’ (Cv. APF-8) and ‘Prime-Jim®’ (Cv. APF-12) in 2004, and ‘Prime-Ark® 45’ (Cv. APF-45) in 2009 (Clark, 2008; Clark and Perkins-Veazie, 2011). The development of new blackberry cultivars continues worldwide. Currently, breeding programs have important objectives to increase sweetness, soluble solids content, and flavor with lower levels of acidity and astringency to increase the consumption of blackberries (Clark and Finn, 2008).

The availability of old and new blackberry cultivars means that the amount of land dedicated to blackberry cultivation around the world has been increasing (Clark, 2008). In a survey conducted in 2005, Strik et al. (2007), found that 20,035 ha of blackberries were cultivated worldwide. From 1995 to 2005, the cultivated blackberry area increased 45%, and the entire production increased 154,603 tons worldwide. In 2005, 50% of the blackberry growing areas in the world were planted with semi-erect cultivars (for fresh market mostly), 25% with erect (for fresh market), and 25% with trailing types (for processing). There were a total of 2,528 ha of organic blackberry production globally in 2005. It is estimated that by 2015 there may be 27,032 ha of commercial blackberry production worldwide, which does not include wild plant production (Strik et al., 2007).

According to the same survey (Strik et al., 2007), the largest producing blackberry region in the world was Europe, where 7,692 ha were cultivated with a commercial target. Serbia had the largest blackberry cultivation area in both Europe and in the world with 5,300 ha (69% of European area). Hungary accounted for 1,600 ha (21%), the second-largest producer in Europe.

In North America, 7,159 ha of blackberries with a commercial target were cultivated in 2005. The area cultivated in the United States was the second highest in the world (4,818 ha), and it had the highest worldwide production (35,099 tons). The state of Oregon had the largest area of production in the United States (65%) where mostly trailing types were cultivated (‘Marion’ and ‘Boysen’). Ninety five percent of this production was for processing, and the remainder was marketed as fresh fruit. California was the second largest blackberry producing state. Mexico had 2,300 ha of commercially cultivated blackberry which was 32% of all North America’s area dedicated to blackberry production. This production area has been increasing steadily since 1995. In 2005, Central America, represented by Costa Rica and Guatemala, produced 1,753 tons of blackberries with a commercial target of 1,640 ha (94% and 6% of the blackberry area of each country, respectively). Ecuador, Chile, and Brazil were the largest blackberry producers in South America with 1,597 ha. Ecuador accounted for more than 50% of the production area (Strik et al., 2007).

Asia had 1,550 ha of commercially cultivated blackberries in 2005. All of the accounted area and production in this region was located in China. In Oceania, mostly in New Zealand, 259 ha of commercial blackberries were planted, and 3,690 tons were harvested during that same year. Africa reported 100 ha of commercial blackberry, which was grown in South Africa (Strik et al., 2007).

In the U.S., according to the U.S. Depart. of Agriculture (2012), 2,954 ha were harvested in 2011. During the same year, blackberries cultivated for the fresh market totaled $6,280,000 U.S., and for blackberries targeted for processing the amount was $36,503,000. Thus, the total U.S. blackberry production was $42,783,000.

The most recent data for Arkansas reported 209 ha of blackberry in 2007 (U.S. Dept. of Agriculture, 2009).

Vegetative growth

Blackberry plants grow as a small flowering shrub or a trailing vine with stems, commonly known as canes, arising from the root or buds on crowns (Westwood, 1993; Rieger, 2006). Blackberry canes are biennial, but the crown and root system are perennial (Moore and Skirvin, 1990; Crandall, 1995). According to Rieger (2006)**,** blackberry canes that develop the first year are vegetative and are called primocanes. During the first year, the primocane can grow to a length of 3-6 m on average, but it does not develop flowers (Crandall, 1995). Flower bud initiation occurs in the summer, then the cane overwinters and the next growing season the cane blooms, bears fruit, and new cane elongation usually occurs. This second year cane is called a floricane. The lateral buds break and produce flowers. Then after fruiting, the floricanes senesce. Except for the first year, during each season, mature plants have both primocanes and floricanes (Moore and Skirvin, 1990).

Primocane fruiting brambles have a modified habit. Fruit is produced on 1/3 to 1/2 tip portion of the cane at the end of its first growing season. This cane portion dies but not the rest of the cane, which remains in dormancy, overwinters, and produces fruits the next growing season. After this season, the cane dies (Crandall, 1995).

According to Rieger (2006), brambles can be thorny or thornless, and the canes can be erect, semi-erect, or trailing (either prostrate or arching down). Trailing cultivars include 'Silvan,' 'Marion,' and 'Thornless Evergreen' and the blackberry-raspberry hybrids 'Loganberry,' and 'Boysenberry.' semi-erect thorny types include 'Loch Ness, Thornfree,' 'Chester Thornless,' and 'Cacanska Bestrna.' Erect thorny types include 'Brazos,' 'Cherokee,' 'Choctaw,' 'Comanche,' 'Cheyenne,' and 'Tupy.' 'Arapaho,' and 'Navaho,' are erect, thornless cultivars. Trailing and semierect types such as 'Marion' and 'Kotata' only develop new primocanes from buds on the crown. Erect types develop new primocanes from buds on roots or from buds located at the floricane's root crown (Rieger, 2006; Strik et al., 2007).

Moore and Skirvin (1990) state that after emergence, primocanes grow quickly and generally develop palmately compound leaves which can have five or seven leaflets distributed alternately along the cane. The margins of the bright green colored leaves are fully toothed (Crandall, 1995; Westwood, 1993). In contrast, the leaves of floricanes have different shapes and smaller sizes, and the floricanes develop terminal inflorescences. They bloom in small clusters or racemes at the tip of the flowering lateral stems, usually during late spring and early summer (Moore and Skirvin, 1990). Each flower measures about 2–3 cm in diameter. The flower has five sepals and five white or pale pink petals with several stamens and pistils. All of these parts are arranged on a fleshy elongated receptacle. When the fruit is harvested, the receptacle remains on the fruit (Westwood, 1993; Crandall, 1995; Rieger, 2006). Floricanes produce several short lateral branches with some leaves, instead of developing lengthy canes. The fruit is not a true berry but is composed of small drupelets. Botanically it is named an aggregate fruit (Rieger, 2006). Berries change color from green to reddish, then to bright black (ripe) and finally to dull black. Blackberries can have a productive life span of 15 to 20 years. However, in commercial plantings, blackberries are kept for 5 to 10 years due to reduced productivity after this age (Pritts, 1991; Rieger, 2006; Clark et al., 2005; Clark and Perkins-Veazie, 2011).

Primocane-fruiting blackberry cultivars such as ‘Prime-Jan®’, ‘Prime-Jim®’, and ‘Prime-Ark® 45’ are erect, thorny types. The PF blackberry cultivars bear fruit on first-year canes (Strik et al., 2007; Clark and Perkins-Veazie, 2011). In general, PF blackberries produce two crops annually, for an extended off season production. ‘Prime-Ark® 45’ has two harvest periods: the floricane produces fruit from early to mid June, and the primocane produces fruit from August until the frost (Rieger, 2006; Strik et al., 2007; Clark and Perkins-Veazie, 2011).

Phenology of PF blackberries

Primocane year: During the first year, after planting in early spring, the primocane grows vegetatively until midsummer. The primocane forms lateral buds at the base of each leaf (Takeda et al., 2002). Only a few of these buds will be lateral branches the next growing season due to the strong apical dominance of the primocane. However, if the main cane is tipped in early summer, branching will be promoted, generating strong, fruitful laterals (Crandall, 1995; Takeda et al., 2002). Reaching a certain height, the apical meristem changes from a vegetative to a reproductive condition (Lopez-Medina et al., 1999), and if canes are not tipped, the top 10 to 12 buds on primocanes will flower. For ‘Prime-Ark® 45,’ in Clarksville Ark., the primocane’s first bloom date is 30 June, and the primocane’s first ripe fruit date is 8 Aug., but it continues until frost. Buds at and below ground level remain vegetative (Clark and Perkins-Veazie, 2011; J.R. Clark, personal commun.).

Floricane year: After overwintering, the primocane becomes a floricane and bears fruit on the lower part of the cane where prior-year fruiting occurred. After harvest, the complete cane dies. For ‘Prime-Ark® 45’ plants, located at Clarksville, Ark. (data collected during 2008 and

2009), phenological stages and dates are described by Clark and Perkins-Veazie (2011), who state the following: floricane bloom dates are: 1) 10% bloom, 28 Apr.; 2) 50% bloom, 4 May. Floricane harvest date is 24 June (peak), also in Clarksville.

Dormancy: The climate of the northern hemisphere is favorable for blackberry growth. This climate induces a period of dormancy due to short days and cool temperatures in fall (Moore and Skirvin, 1990). Canes become dormant due to freezing temperatures. Effective temperature to induce dormancy is around -2^{o} C. To break red raspberry bud dormancy, approximately 1,100 to 1,400 chill h of total accumulated temperature below 7^{o} C are needed. The chilling requirement for blackberries is variable and depends on the cultivar. For ‘Arapaho,’ dormancy occurs at between 400 and 500 h, whereas for ‘Navaho,’ from 800 to 900 h are required (Drake and Clark, 2000). After a trial with diverse blackberry cultivars, Clark and Carter (2006) reported a higher chilling requirement (>500 h) for ‘Apache' and ‘Ouachita’ cultivars. For ‘Prime-Jim®’, the chilling requirement was between 300 h and 400 h. For other PF selections, the chilling requirement was between 300 h and 500 h (Clark and Carter, 2006).

Cold hardiness: Blackberries cannot tolerate extremely low temperatures (Stanton et al., 2007). Canes can be damaged at temperatures below -17°C (Moore and Skirvin, 1990; Crandall, 1995). Blackberry canes can be severely injured or killed when temperatures are below -23^{0} C. Winter hardiness or resistance to winter injury to the canes is a crucial factor limiting the production of this crop in cold climates (Moore and Skirvin, 1990; Dana and Goulart, 1991). Genotypes from Arkansas are deficient in hardiness in the upper Midwest and northward (Moore and Skirvin, 1990). According to Christman (2008), blackberry flowers are vulnerable to late frosts, and are damaged when temperatures drop below -2^{o}C.

PF cultivars and fruiting characteristics

In 2004, the University of Arkansas released two PF cultivars, 'Prime-Jan®' and 'Prime-Jim®'. These cultivars have similar fruiting characteristics to primocane-fruiting red raspberry cultivars (Clark et al., 2005). In 2009, a third cultivar, 'Prime-Ark® 45' was released. 'Prime-Ark® 45' has significant commercial quality fruit features such as adequate fruit size (from 5 to 9 g on average) and high concentration of soluble solids (ranging from 8 to 10^{o} Brix). This means sweetness and superior flavor and postharvest capability for shipping. These features have been obtained specifically in moderate climates. The yield of the most productive cultivars of blackberries is higher in areas with mild climates, but not in Arkansas where yield is negatively affected by poor summer field-heat tolerance of PF blackberries. Excessive heat affects fruit-set and berry quality (Ruple, 2010; Clark and Perkins-Veazie, 2011). Primocane-fruiting blackberry cultivars which have been bred for a particular production region do not perform equally well in dissimilar regions (Clark and Perkins-Veazie, 2011).

Cultural practices

Pruning and trellising: Brambles are planted in rows for ease of cultural practices and increased yield (Pritts, 1991; Crandall, 1995; Morrison, 1998). Appropriate row spacing is approximately 2.7 to 3.0 m between rows. However, in this spacing plants do not develop efficient canopy architecture (Bushway et al., 2008). Although expensive, trellising is beneficial for blackberries because the plant canopy gets better air movement and improved pesticide efficacy due to a better spray penetration (Pritts, 1991; Rieger, 2006). Trellising is favorable for easier manipulation, such as access to fruit, cleaning, picking, and for providing a healthy environment for berry development. The trellis helps eliminate risk of cane breakage, and provides space for handling and picking. Proper pruning, training, and trellising depend on the cultivar grown (Moore and Skirvin, 1990). According to Vanden Heuvel et al., (2000), cane stabilization was beneficial to increase the amount and quality of Boyne' and 'Regency' red raspberry plants.

Mowing: For brambles, this practice is beneficial because removing excessive canes increases light penetration, disease and insect control, and provides space for manipulation (Pritts, 1991; Rieger, 2006). For primocane-fruiting raspberries, Pritts (1991) stated that to obtain a late-season crop, canes have to be cut to the ground annually in early spring. According to Bell et al., (1995) in floricane-fruiting blackberries, mowing has been tested and shown to increase yield. Mowing of primocanes of 'Marion' blackberries, not only enhanced yield but also produced other benefits such as simple training of canes, reduction in pests and diseases, increased percent of bud break, and closer spacing. Bell et al. (1995), compared mowed and not-mowed primocanes of 'Marion' trailing cultivar. Primocanes were cut off at ground level in four periods, late April, May, June, and July during 1991 and 1992. The authors found that yield of plants mowed (suppressed) in April was larger than unmowed plants in 1992. Yields of April-, May-, and June-mowed plants were larger than non-mowed plants in 1993.

An important reason to mow PF blackberry plants is the extension of the fresh market production season. These genotypes can be easily manipulated to adjust harvest time (Thompson et al., 2007; Strik and Thompsom, 2009). In 'Prime-Jan®' PF blackberry, Thompsom et al., (2009) found positive effects of mowing combined with soft-tipping.

Tipping or pinching: Tipping of primocanes is mostly practiced in commercial orchards. It is recommended so that plants can produce lateral buds on the upper portion of the cane in both floricane and PF blackberries (Moore and Skirvin, 1990). Thompson et al. (2007) stated that tipping is a relevant practice so that plants can interrupt the apical dominance and encourage branching of the upper portion of the primocane in early summer. A longer lateral branch develops more fruit but in smaller size, while shortened laterals will produce fewer but better quality fruit (Thompson et al., 2007). The main cane's growing tip is headed or removed carefully above an axillary bud (Rieger, 2006). Tipping the upper portion of plants is done in early summer (Moore and Skirvin, 1990; Thompson et al., 2007). According to Thompson et al. (2007) summer tipping of primocanes and winter pruning of floricanes are part of the typical cane management for erect, floricane-fruiting blackberries.

For PF blackberry cultivars, tipping is favorable to promote branching and to improve yield (Strick and Thompsom, 2009). It should be carried out in early summer when canes are 1 m tall. It consists of removing the upper 2 to 5 cm. According to Thompson et al. (2007), this practice encourages branching and increases the number of nodes per cane compared to untipped primocanes. For thorny, erect blackberries, tipping (also referred to as topping) is suggested when plants are 0.9 to 1.5 m high. In early spring, lateral branches are reduced to 0.30 to 0.45 m. Also, under high tunnel (HT) conditions, tipping and training are needed to enhance off-season production (Hanson, 2012).

General plant nutrition

Havlin et al. (2006) stated that a maximum yield in crops is accomplished only if two conditions occur simultaneously: First, the producer must be able to decrease or eliminate the negative impact of more than 50 factors which affect crop production, such as plant and soil factors. Secondly, the environmental conditions (climatic factors) during the growing season must be favorable to crop growth and development. Even though most climatic factors cannot be controlled, many crop and soil factors can be controlled and regulated to obtain increased yields.

All of these factors are interrelated; therefore, to maximize productivity they have to work harmoniously (Havlin et al., 2006).

The soil has organic and mineral parts and the diversity of soil types depends mostly on the parent material and climatic factors. Soils not only have physical, chemical, and biological properties, but also have solid, liquid, and gaseous phases which are normally in physical and chemical equilibrium (Kabata-Pendias and Pendias, 2000). The properties of soils come from these phases. Plants require 17 essential nutrient elements for proper development (Campbell et al., 2000, Kabata-Pendias and Pendias, 2000; Troeh and Thompson, 2005). An essential plant nutrient takes part in the total life cycle of plants. The classification of essential macro- and micronutrients is made according to their biochemical role and physiological function (Campbell et al., 2000; Taiz and Zeiger, 2006). Nine elements are referred to as macronutrients because plants utilize them in large quantities. Carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorous (P), and sulfur(S) comprise nearly 98% of a plant's dry weight, and they are the most important constituents of organic and inorganic compounds (Campbell et al., 2000). Potassium (K), magnesium (Mg), and calcium (Ca) are also considered macronutrients , but they are needed in smaller amounts, approximately 1.5% of a plant's dry weight. There are eight plant nutrient elements that plants need in very small quantities but are still vital for plant development. Those include iron (Fe), chlorine (Cl), copper (Cu), manganese (Mn), zinc (Zn), molybdenum (Mo), boron (B) and nickel (Ni) (Kabata-Pendias and Pendias, 2000; Campbell et al., 2000; Troeh and Thompson, 2005). Also, sodium, aluminum, cobalt, and silicon, and are considered as micronutrients for some plants (Havlin et al., 2006). These micronutrients have essential activities such as cofactors or components in enzymes (Campbell et al., 2000; Troeh and Thompson, 2005).

Nitrogen as a main plant nutrient: Nitrogen (N) is one of the most important macronutrients in crop nutrition. Nitrogen is a fundamental component for numerous biochemical substances which plants use to grow and develop such as hormones, coenzymes, nucleic acids, proteins, and ATP (Campbell et al., 2000). This nutrient element is a significant constituent of the plant's light-absorbing molecule, chlorophyll (Troeh and Thompson, 2005). Nitrogen is mostly taken up by roots and foliage. Plants can regulate absorption and assimilation of N or N compounds through diverse mechanisms (determined especially by the soil environment) (Krishna, 2002; Readman, 2004).

Nitrogen cycle: The process called the N cycle consists of the conversion of N to either molecules or substances necessary for human, animal, and plant existence. Most of the N is in earth's atmosphere, and approximately 80% of the molecules have two N atoms bonded together (N_2). The N_2 molecule is not a form that plants can use (Campbell et al., 2000; Havlin et al., 2006). In the N cycle, atmospheric N_2 is fixed by diverse processes that produce ammonium or nitrate (NH_4^+/NO_3^-) which are forms available for plant uptake (Janick, 1986). This transformation can be achieved by nonbiological or biological means. For instance, lightning and photochemical reactions are examples of non-biological fixation whereby N_2 molecules are converted into ammonium. Biological N fixation occurs when bacteria or cyanobacteria fix N_2 into ammonium (NH_4^+) in soils (Janick, 1986; Campbell et al., 2000; Havlin et al., 2006).

Several microorganisms such as N-fixing bacteria and mycorrhizal fungi assist roots in obtaining nutrients. Nitrogen is altered by bacteria into forms available to plants. The decomposition of these organisms causes the release of N into the soil or water. The transformation from amino acids to N forms available for plants is known as ammonification (decomposing organic N into NH_4) and nitrification (converting NH_4 to NO_2 and to NO_3)

(Janick, 1986; Troeh and Thompson, 2005). Some bacteria convert nitrates (NO_3) back to N_2 which is called denitrification, a process which reduces available N. Finally, to complete the cycle, through several chemical and microbial processes, N_2 is released into the atmosphere to begin the cycle again. All these processes occur not only naturally but also by industrial and combustion fixation (Janick, 1986; Taiz and Zeiger, 2006; Havlin et al., 2006). After plants complete their life cycle, absorbed nutrients from the soil that remain in plant debris go back to the soil or nearby water sources. As with the N cycle, each plant nutrient element has a particular cycle (Havlin et al., 2006).

Nitrogen in the soil and plant: According to Havlin et al. (2006), the surface of roots has the ability to absorb inorganic ions in small amounts from soil solution. The ion movement from soil to root surface requires contact between them, which can be achieved in three ways: root interception, mass flow, and diffusion. Havlin et al. (2006) stated that diffusion is slow in most soils and occurs in short distances on the surrounding root surface. If N demand is not fulfilled by mass flow, N need is supplied to the plant by diffusion or root interception**.** After root absorption, these inorganic ions are translocated to diverse plant parts to be utilized in their biological functions (Taiz and Zeiger, 2006; Havlin et al., 2006). Plants can take up soil N in two forms: dissolved nitrate ions (NO^-_3) and ammonium ions (NH^+_4). These forms of N must be present in sufficient amounts for satisfactory plant nutrition according to standardized values (Campbell et al., 2000; Krishna, 2002). Nitrogen is assimilated by plants via biochemical reactions such as oxidation and reduction to form covalent bonds with carbon to create organic compounds (Taiz and Zeiger, 2006). The lack of N as a nutrient is the single most common nutritional problem in plants (Campbell et al., 2000) because of either pH imbalance or insufficient amounts of available N in the soil. Another lesser factor is N depletion from non-legume crops, which demand large amounts of N for plant nutrition (Havlin et al., 2006).

Nitrate (NO^{-}_{3}) is mostly present in higher concentrations than ammonium (NH^{+}_{4}) in warm, moist, and well-aerated soils, and the movement of both ions (NO^{-}_{3} and NH^{+}_{4}) to plant roots is by mass flow and diffusion (Krishna, 2002). Ammonium is the major form of N that plants absorb (Havlin et al., 2006). Ammonium uptake increases at neutral pH values and decreases when acidity increases. Ammonium's absorption by roots decreases nutrient uptake of cations such as Ca^{2+}, Mg^{2+}, and K^{+} while absorption of NH^{+}_{4} by roots increases absorption of $H_2PO_4^{-}$, SO_4^{2-}, and Cl^{-}. When plants obtain NH^{+}_{4}, the pH of the rhizosphere decreases because of the exuded H^{+} by roots to conserve charge balance inside the plant. Both biological activity and nutrient availability can be affected by this acidification. Age and type of plant are two important factors that determine the plant's uptake of either NH_4^{+} or NO_3^{-} (Krishna, 2002; Havlin et al., 2006).

Plants respond quickly to the application of N, and usually annual applications are required (Campbell et al., 2000; Bordelon, 2001). Both stunted growth and yellow-green leaves, usually seen first in older leaves, are typical signs of N deficiency, (Janick, 1986; Campbell et al., 2000; Havlin et al., 2006). Nitrogen applied in excessive amounts can cause adverse effects on yield and enhance vigorous vegetative growth such as more elongated and weak primocanes in blackberries. These negative effects lead to increased breakage, extended internodes which can reduce yield per cane, and delayed maturity (Hart et al., 2006). High plant N content increased transpiration, and exacerbated weakness mostly in young plant tissues due to excessive N applications. A desiccated foliar effect is evidence of N toxicity under dry conditions (Pritts, 1991; Hart et al., 2006).

Nitrogen fertilizers: Fertilizers are materials that provide nutrient elements to plants (Janick, 1986). Fertilizers are added to soils not only to supply plant nutrients but also to replace utilized nutrients and recover its natural fertility (Troeh and Thompson, 2005). To obtain better production, higher levels of N are supplied to crops by using diverse chemical compounds. Amounts and proper method of application of N fertilizers depend on the crop and cultivar characteristics, environmental conditions, soil characteristics, and crop management practices (Janick, 1986). During the growing season, a specific plant growth pattern is shown by most plants as a function of accumulated nutrients. The shape of the growth curve depends on the plant. However, all plants have initial exponential growth and nutrient accumulation rates followed by a period of decreasing rates (Havlin et al., 2006).

According to Janick (1986), fertilizers can be classified as chemical or natural organics. Nitrogen chemical fertilizers are synthesized from inorganic sources such as ammonium nitrate, urea, and cyanamid. Most of the N fertilizers are synthesized by the Haber-Bosh process, in which N_2 reacts with hydrogen to form ammonia (Havlin et al., 2006). The ammonia can be utilized directly, but also manufactured in diverse forms of N fertilizers (Janick, 1986). Nitrogen-based fertilizers have been classified into NO^-_3-based or NH^+_4-based fertilizers (Krishna, 2002). The type and level of fertilizers applied and absorbed by plants depends on the crop nutrient needs, actual nutrient content in soil, and the availability of possible commercial nutrient supplies. The main challenge for fertilization programs is to predict a crop's nutrient needs (Janick, 1986). The availability of nutrients for plants in appropriate amounts is necessary information for farmers to obtain increased yields (Taiz and Zeiger, 2006; Havlin et al., 2006).

There are commercial fertilizers that provide immediately available N, such as calcium nitrate (15-0-0), sodium nitrate (16-0-0), and potassium nitrate (13-0-44), but also a less readily available form of N provided by ammonium N, which is available in monoammonium phosphate (11-52-0), diammonium phosphate (18-46-0), or ammonium sulphate (21-0-0). Nitrogen from organic sources is a third form of N that can be supplied to plants. First, N from organic sources is turned to ammonium N, and after that to nitrate N, but a complete transformation could be quick or take many years **(**Havlin et al., 2006; Troeh and Thompson, 2005**).**

The optimum time for fertilizer application depends on factors such as soil type, crop, nutrient, and climate. Nitrogen fertilizers are often a brief and limited source of nutrients because of the mobility of nitrates, which are mostly dissolved in soil water (Havlin et al., 2006). The same happens with the N provided by microorganisms feeding after high levels of organic matter are applied. Also, climate and temperature influence the N availability because microorganism populations increase nitrification in the spring (Janick, 1986). Therefore, there are positive responses in crops after N applications in the spring (Janick, 1986; Havlin et al., 2006).

Blackberry nitrogen requirement: Overall, brambles require large amounts of N for plant nutrition and they easily absorb the nitrate N form instead of the ammonium N form due to the simple solubility and rapid movement into the plant (Hart et al., 2006). According to Moore and Skirvin (1990), N is the nutrient element most required for blackberry cultivation to promote vegetative growth. Pritts (1991) states that the amount of N required for blackberries varies due to several factors such as physiological characteristics, water, soil characteristics, location, cultural management, etc. He recommends that pre-planting fertilizations should be based on soil test results. The results of laboratory analysis of nutrient content in soil are useful for farmers because this tool helps to predict the application rate of fertilizer and optimize yields (Taiz and Zeiger, 2006).

To encourage the highest growth early in the season, fertilizers should be applied during early spring, before the beginning of growth. Nitrogen fertilization is required for proper growth of primocanes during the spring (Hart et al., 2006). According to Strik (2003), in ‘Kotata,’ no N fertilizer was required or taken up until early April when the primocanes started to emerge. Also In ‘Kotata,’ it has been determined that around 5% of N applied in soils is stored in roots and crowns. This amount of N stored in crowns is used for early season primocane (and leaf) growth (Mohadjer et al., 2001). Usually N sources should be added to soils on a regular basis, typically each year; however, these applications should be done carefully to avoid burning plant roots or producing excessive vegetative growth instead of fruit buds. Applying N at inappropriate times can cause soft fruit in several species of fruit plants (Troeh and Thompson, 2005).

According to Eames-Sheavly et al., (1991), N applied in split applications is more efficient in brambles. Frequently, the first N split application is made in March and the following in May. Naraguma and Clark (1998) state that as an effect of N fertilization on 'Arapaho' thornless blackberry, split application allowed higher N concentrations in leaves; however, no more benefits were found from split applications. Moore and Skirvin (1990) state that if in the first application, one-year-old plants do not show an initial vigorous growth, an additional application should be done in the spring. For ‘Kotata,’ Strik (2003) stated that N is not necessary during the first year and N applications could cause winter injury as a result of N fertilizer applied later than June. Thus, annual applications of N fertilizer should be made during early spring, before growth begins.

Because PF blackberries have been recently developed, no rate of N fertilization has been determined for these new genotypes. Nitrogen fertilizer recommendations are based primarily on raspberry and floricane blackberry production practices.

According to Moore and Skirvin (1990), during the first year, prior to planting, fertilizer should be broadcast within the row area during land preparation, and the amount of fertilizer should be the minimal rates suggested but sufficient for plant growth. If initial applications are required, fertilizer applications should be postponed until growth begins. For brambles, recommended rates for N fertilization vary between 25 to 56 $kg \cdot ha^{-1}$ during the first year (Pritts, 1991; Crandall, 1995; Hart et al., 2006; Strik, 2008). For raspberry primocane nutrition, Hart et al. (2006) concluded that in the establishment year, summer-bearing red raspberries need 33.6 to 56 $kg \cdot ha^{-1}$ of N, and the following years 56 to 89.6 $kg \cdot ha^{-1}$ of N, using the higher rates for semi-erect types. According to Mahler and Barney (2000) raspberry production will require N application rates from 56 to 67.2 $kg \cdot ha^{-1}$, which should be applied shortly after planting. Primocane-fruiting or fall-bearing red raspberries need 22.4 $kg \cdot ha^{-1}$ of N additionally at bloom. For blackberries, recommended rates of N are 25 to 56 $kg \cdot ha^{-1}$ for the first year, 39.2 to 72.8 kg $N \cdot ha^{-1}$ for the second year, and 67.2 to 89.6 kg $N \cdot ha^{-1}$ the following years (Moore and Skirvin, 1990; Pritts, 1991; Spiers, et al., 1999; Hart et al., 2006; Kowalenko, 2006; Havlin et al., 2006; Strik, 2008).

Soil conditions for blackberries: In general, blackberries grow on a wide range of soil types, from sandy to clay loam, but not waterlogged soils. They prefer deep and fertile soils, and plenty of organic matter. Soils with proper drainage and moisture-retention properties are recommended. Blackberries are usually more drought tolerant and deeply rooted than raspberries (Moore and Skirvin, 1990). The soil pH should be between 5.8 to 6.5 for good performance (Gordon, 1991; Rieger M. 2006). According to Bordelon, (2001), a proper soil building program for blackberry production requires incorporating organic matter in soils for several important reasons, most important among them: Crop and microorganism residues are in constant decomposition, which leads to humus because both crop and microorganism residues have significant amounts of essential elements. These essential elements are released into soil solution, improving structure and physiochemical properties by significantly increasing the cation-exchange capacity (CEC) (Jones, 2001). Annual applications of large amounts of composted manure are suggested for preserving soil fertility in blackberry cultivation (Gordon, 1991).

Seasonal variation and plant nutrients

Leaf nutrient concentrations are affected by genotype, plant part, and sampling date. Clark et al. (1988) stated that the sampling date significantly influenced the elemental nutrient content of ‘Cherokee,’ ‘Cheyenne,’ and ‘Comanche’ blackberries**.** The elemental nutrient content in a plant can vary due to non-nutritional factors such as soil characteristics, type of plant, level of maturity, and environmental conditions (Huges et al., 1979). Variations in leaf nutrient concentrations are caused by the availability of nutrients in soil, whose values within standardized limits create positive impacts on yields. Variations above or below these standardized limits will impact yields negatively (Hart et al., 2006). Even though samples come from the same plant, specific plant parts can contain different amounts of elemental nutrients (Huges et al., 1979; Jones, 2001; Troeh and Thompson, 2005). In regard to the elemental content and its seasonal variation in brambles, Hughes et al. (1979) mentioned that in ‘Meeker’ red raspberries**,** it was found that the age of the plant did not significantly influence elemental nutrient concentrations, while there was considerable variation of element nutrient concentrations among genotypes and sampling date. In contrast, measurements of N content in leaves of ‘Chester Thornless’ blackberry were different within the sampling year (Malik, et al., 1991). Thus, the age of plants had effects on concentrations of N in leaves, which contradicts Hughes et al. (1979).

According to Hart et al. (2006), it is better to sample leaves when nutrient concentration in bramble leaves is stable. Also, the authors stated that tissue levels of N and K in some blackberries and raspberries become stable in late July and early August, giving a consistent analysis result. However, it may be difficult to achieve in some brambles because there are periods when rapid changes in elemental content occur; as a consequence, results of leaf samples collected at those times can have dissimilar results. In 'Willamette' raspberry, after N and B fertilization, Kowalenko (2006) found that leaf tissue N concentrations were extremely dynamic and had high variability which made it difficult and inappropriate to determine plant N status without comparative (standardized values) data (Kowalenko, 2006).

Clark et al. (1988) studied the seasonal variation of nutrient concentration in leaves (blade and petiole separately) of three blackberry cultivars: Cherokee, Cheyenne, and Comanche from May to Aug. Higher nutrient concentrations were found in blades but not in petioles. However, these blackberry leaf parts did not have significant differences in nutrient content. The authors found that concentrations of N, P, K, Zn, Cu, and Fe were highest in May and then decreased during each sampling date from June to August. Initially, Ca content increased (from 0.68 to 1.33%) and during the three following sampling dates the concentration was stable. Magnesium content in leaves fluctuated during the sampling dates. The concentration of Mg increased until the last date of sampling when it then decreased. All means of these Mg values were statistically non-significant. In the same experiment, it was found that cultivars had significantly different concentrations for P, Ca, Mg, Zn, Fe, and Mn. Also, Clark et al. (1988) found that blackberry nutrient concentrations varied seasonally, similar to the change in elemental concentration in red raspberry leaves during that same period. It was established that for these blackberry cultivars a proper time for collecting samples was between mid-July to mid-August for all of the elements because little or non-significant differences were observed during this period (Clark et al., 1988).

Nitrogen (N): Generally, plants contain 1 to 6% N by weight (Havlin et al., 2006). According to Pritts (1991), the N content of bramble plant dry matter is between 2 and 3%. In some blackberry genotypes, leaves are representative plant parts to determine the nutritional status, such as in the ‘Chester Thornless’ blackberry cultivar, whose leaves of primocanes and floricanes are the plant parts with higher N content than other tissues (Malik et al., 1991).

A bramble plant with less than 1.9% leaf N content is considered either N-deficient. (Pritts, 1991). A primary symptom of N deficiency in brambles is yellow leaf color, which may include older leaves with reddish tips. Nitrogen concentrations in leaves develop diverse relationships with other nutrients and substances (Pritts, 1991; Crandall, 1995). In the ‘Willamette’ red raspberry cultivar, N fertilizer applications resulted in higher leaf N content late in the growing season (Kowalenko, 2006). In ‘Dorman Red’ red raspberries, increased N application rates resulted in increased leaf Fe concentrations, in addition to decreased leaf contents of Ca and Mg (Spiers, et al., 1999).

Blackberries develop different types of responses regarding the amounts and time of application of N fertilizer. In an experiment with 'Arapaho' thornless blackberry, it was found that N, P, K, Ca, S, and Mn foliar nutrient concentrations were affected by both N application rate and time (Naraguma and Clark, 1998). In ‘Chester Thornless’ blackberries, it was found that N applied to the soil was transferred to the primocane tissues, fruit, and roots. The remaining N fertilizer was transferred to all plant parts such as roots and floricanes, and the unused portion was stored in the floricanes (Malik, et al., 1991). According to Naraguma and Clark (1998), Mn concentrations were greater when higher N rates were applied in an 'Arapaho' thornless blackberry. N levels greater than 3% in tissue tests result in N toxicity, and plants that are too vigorous with a reduced number of flower buds (Strik, 2003).

Phosphorus (P): This macronutrient has important functions in the metabolism of plants (Pritts, 1991). Through diverse chemical processes, P is an important component of organic substances, such as nucleic acids, phosphate compounds, phosphoproteins, phospholipids, enzymes, etc. (Janick, 1986; Troeh and Thompson, 2005; Havlin et al., 2006). Average P content in plants is between 0.10 and 0.5% (Westwood, 1993; Havlin et al., 2006). The common P deficiency symptoms in brambles are stunted plant growth and purple-colored older leaves (Pritts, 1991).

Phosphorus is an important macronutrient for commercial bramble production because P is required in relatively large amounts, and it often becomes deficient in commercial orchards. Compared with other crops, brambles require low levels of P (Crandall, 1995). Excessive P can obstruct micronutrient uptake. Thus, accurate P_2O_5 applications should be based on soil and leaf analysis, soil pH, and cation-exchange capacity (CEC). Either $H_2PO^{-}{}_4$ or $HPO^{2-}{}_4$ can be absorbed by plants. Soil pH impacts P availability directly. The molecule $HPO^{2-}{}_4$ is greatly absorbed at high soil pH values. Phosphate ions react with Ca and Mg in alkaline soils and with Al and Fe in acidic soils, generating few soluble substances. However, Mo is less available in acidic soils and more available in low alkaline soils. Phosphorous does not have good mobility in soil, so surface banding fertilizer applications of P is not as effective as subsurface banding applications. The development of a good root system is essential for P fertilization in brambles. Increased plant root mass will aid P uptake, which occurs basically through diffusion (Pritts, 1991; Crandall, 1995; Havlin et al., 2006). Phosphorous generates various types of interactions with other elements. For example, in ‘Dormanred’ raspberries, high P fertilizer rates increased leaf P, K, and Cu concentrations in leaves; however, Ca uptake was inhibited (Spiers, et al., 1999).

Potassium (K): Potassium, absorbed as K^+ ion, is required in large amounts for plant nutrition. The positive charge helps to regulate electrical neutrality in the soil and the plant. Potassium cations balance negative charges of anions such as nitrate, phosphate and others (Troeh and Thompson, 2005). Potassium is utilized to transport nitrates from roots to leaves and to regulate stomata for proper gas exchange (carbon dioxide, water vapor, and oxygen) with the atmosphere (Pritts, 1991; Havlin et al., 2006). In brambles, proper K_2O applications should be based on leaf and soil analysis, and soil parameters because excessive amounts of banded K may burn new roots, especially in sandy soils (Crandall, 1995). Uptake of K occurs essentially through diffusion, so root mass is needed to improve K plant uptake (Troeh and Thompson, 2005). Potassium becomes more effective when it is broadcast into soils before plants are established. Potassium is mostly required during fruit development (Pritts, 1991). Adequate K in the plant is usually reflected in appropriate fruit firmness (Hart et al., 2006). According to Pritts (1991), adequate foliar concentration range is from 0.6 to 2.5% in brambles. Contents of K less than 0.6% in leaves can mean low fruit quality, and more than 3% can reduce leaf Ca, Mg, Zn, and N (Pritts, 1991; Hart et al., 2006).

According to Hart et al. (2006), in brambles, no relationship has been found between K content in soil and K levels in leaves. Potassium fluctuates in leaves during the growing season, and it decreases as fruit load increases (Strik, 2003). Potassium fertilization can create nutritional imbalances. In ‘Dormanred’ raspberries, the highest K fertilizer rate augmented the leaf content of P, K, Fe, and Cu, but Ca and Mg uptake were reduced (Spiers, et al., 1999). In ‘Thornless

Evergreen' blackberries, K fertilization generated Ca and Mg deficiencies (Nelson and Martin, 1986).

Increased K fertilizer applications caused K concentration in leaves to rise. However, the increased K caused a decrease in Mg and Zn content in 'Shawnee' blackberry leaves. In the same experiment, K concentrations in 'Dormanred' raspberry leaves were negatively correlated with plant growth (Spiers, 1993). In contrast, Clark and Powers (1945) stated that increased rates of K applied in black raspberry and boysenberry plants resulted in increased yields with higher foliar K, larger canes, and bigger, firmer berries.

Calcium (Ca): This is an essential nutrient for cell wall membrane structure and permeability, as well as several physiological processes (Janick,1986; Pritts, 1991; Havlin et al., 2006). Calcium is present in sufficient amounts in soils and plant tissues, and it is rarely applied to blackberry plantings. In brambles, calcium content in plants is between 0.6 and 2.5%. Calcium deficiency does not commonly occur except in soils with high moisture fluctuation (Pritts, 1991). Foliar chelated Ca applications work well when corrections are needed by crops (Pritts, 1991; Havlin et al., 2006). To maintain a proper balance of Ca with P and K, these nutrients should be applied when blackberry plants are in dormancy, in late fall (Pritts, 1991).

In 'Cheyenne' blackberries, Ca fertilization increased plant growth after two growing seasons (Spiers, 1987). In 'Dormanred' raspberry plants, leaf Ca content was negatively correlated with plant growth. Also, leaf Ca and K increased linearly with Ca fertilization, but it had an opposite effect on leaf Mg (Spiers, 1993).

Magnesium (Mg): This element is essential for chlorophyll synthesis and N metabolism. Plant concentration of Mg in brambles ranges from 0.6 to 2.5% (Pritts, 1991). Rates from 4.5 to

27.2 $kg \cdot ha^{-1}$ of Mg are recommended for soil applications and 0.45 $kg \cdot ha^{-1}$ of Mg in 378.5 L of water for foliar applications (Havlin et al., 2006).

Kowalenko (2006) stated that leaf Mg was positively correlated with soil Mg. Through leaf analysis it has been observed that various types of relationships occur between Mg and other elements. In ‘Dormanred’ raspberry plants, the higher Mg fertilization rate positively influenced leaf Mg, but the leaf Ca decreased (Spiers, 1993; Spiers, et al., 1999). In ‘Cheyenne’ blackberries, Mg and Ca fertilization increased plant growth after two growing seasons (Spiers, 1987). Similarly, in ‘Dormanred’ raspberries, leaf Mg was positively correlated with plant growth (Spiers, 1993).

Sulfur (S): Both N and S are key components of proteins. Sulfur in the sulfate form, SO_4, is moderately mobile in soil. Deficiency symptoms are similar to N deficiency (Pritts, 1991; Janick, 1986; Havlin et al., 2006). Usually S applications are not required. If it is needed, 34 to 45 kg $S \cdot ha^{-1}$ could be sufficient for making nutritional corrections (Hart et al., 2006). Overall, proper amounts of plant S concentration are between 0.10 and 0.50 % with a common 15:1, N:S ratio (Pritts, 1991; Hart et al., 2006).

Boron (B): This element is important to auxin activity (Pritts, 1991). In brambles, B is necessary for bud break and fruit set in some cultivars (Hart et al., 2006). Boron promotes growth of tips and roots. When it is deficient in soils, roots do not grow properly, and this limits other nutrient uptake. This element is present in very small amounts and has high mobility in soils. Boron deficiencies can promote plant abnormalities such as reduced yields, small berries, deformed fruit, and, in extreme deficiencies, cane dieback (Hart et al., 2006). Dicot plants have B concentrations that range between 30 and 50 $mg \cdot kg^{-1}$ (Pritts, 1991; Havlin et al., 2006). For predicting B needs in fruit crops, tissue tests are more accurate than soil tests (Hart et al., 2006).

To correct nutritional problems in brambles, either broadcast or foliar sprays are recommended with application rates between 1.12 to 1.8 kg $B \cdot Ha^{-1}$ and 0.11 to 0.17 kg $B \cdot Ha^{-1}$, respectively (Pritts, 1991; Hart et al., 2006; Shaw, 2010).

Kowalenko (2006) observed that B concentrations had considerable variability in 'Willamette' red raspberry leaves. At the beginning of the growing season, primocane leaf B content was generally high. However, at the end of the growing season, it declined and became more stable.

Copper (Cu): Copper is required for carbohydrate and protein synthesis. It activates numerous enzymes and enhances respiration (Janick, 1986). General plant tissue concentration of Cu varies between 5 and 20 $mg \cdot kg^{-1}$ (Janick, 1986; Havlin et al., 2006), whereas for brambles it varies from 7 to 50 $mg \cdot kg^{-1}$ (Pritts, 1991). Copper is effective when applied to soil or leaves. Application rates between 1.12 and 22.4 $kg \cdot ha^{-1}$ of Cu were adequate to correct nutritional problems (Havlin et al., 2006). If deficiencies occur, foliar applications should be used only if needed. Constant applications of this element can produce excessive amounts of Cu concentrations in the soil (Janick, 1986; Havlin et al., 2006).

Manganese (Mn): Manganese is necessary for P and Mg uptake. Manganese deficiency in brambles is rarely observed. However, in soils with pH greater than 7.0, Mn deficiency can be present (Pritts, 1991; Hart et al., 2006). Instead of soil applications, several foliar sprays during the growing season at rates between 1.12 to 3.4 kg $Mn \cdot ha^{-1}$ can be effective (Crandall, 1995). Manganese content in plants varies from 20 to 500 $mg \cdot kg^{-1}$ (Pritts, 1991; Hart et al., 2006; Havlin et al., 2006). In 'Dormanred' raspberry, leaf Mn content was positively correlated with plant growth (Spiers, 1993).

Zinc (Zn): This element is regularly present in small quantities in fruit plants. It is a component of organic substances and complexes such as proteins and auxins (Janick, 1986; Pritts, 1991; Havlin et al., 2006). Zinc concentrations in bramble leaves are between 20 and 50 $mg \cdot kg^{-1}$ (Pritts, 1991). A common observable symptom of Zn deficiency is terminal leaves with rosette shape, and light green, yellow, or white intervenal chlorosis, mainly in older leaves (Janick, 1986; Pritts, 1991). Foliar applications are frequently used, but soil applications of this nutrient, either broadcast or banded, are more efficient because leaves can take up only small quantities of Zn. For rates between 5.6 and 22.4 kg $Zn \cdot ha^{-1}$, broadcast application is recommended (Pritts, 1991; Havlin et al., 2006).

Iron (Fe): Iron is a component of several organic substances, including chlorophyll and enzymes. It is involved in chlorophyll synthesis; thus, chlorosis is a typical symptom of Fe deficiency (Janick, 1986; Pritts, 1991; Havlin et al., 2006). In plant tissue analysis, adequate Fe content varies from 50 to 200 $mg \cdot kg^{-1}$ (Pritts, 1991). Foliar sprays are the best method for applying Fe (Crandall, 1995). Foliar rates of 2.24 kg $Fe \cdot ha^{-1}$ are recommended for effective Fe management (Pritts, 1991). Similar to Mn, Fe is strongly and easily tied-up, or fixed to the soil (Pritts, 1991; Havlin et al., 2006).

Diagnosis of plant nutrient status

Unhealthy plants will exhibit visual symptoms of nutrient imbalances (Campbell et al. 2000; Jones, 2001). Beyond this visual nutritional diagnosis, soil and plant analysis, which includes tissue testing, are relevant tools for decision making in agricultural production. According to Moore and Skirvin (1990), results of either soil or plant analysis are crucial to determining what elements are actually required. By using standard methods of analysis, laboratories generate reliable analytical data so that the information can be universally understood by farmers and the scientific community (Campbell et al., 2000; Jones, 2001).

Soil testing: Soil analysis, also referred to as soil testing, is a method for determining accurately what nutrient levels are present in the soil. Soil testing requires one to follow various necessary steps, beginning with field sampling, in which samples are collected using a probe that is pushed into the soil. Then, the representative sample is prepared for laboratory analysis. The soil test consists of mixing the soil sample with an extracting solution. The soil reacts with the extracting solution, releasing the nutrients (Troeh and Thompson, 2005; Havlin et al., 2006). The soil analysis indicates the amount of nutrient element potentially available to the root in the soil before planting. Soil tests provide significant information such as pH and CEC (Havlin et al., 2006; Taiz and Zeiger, 2006). A soil tests provide necessary information for lime, amendments, fertilizer, and cultural practices (Jones, 2001).

Plant tissue testing: Plant analysis, also referred to as leaf and tissue analysis, is the technique which measures the elemental content in tissue of a particular plant part. Complementary to the soil test, leaf analysis can tell us accurately about plant nutritional status. Likewise, the tissue elemental levels are reliable indicators of mineral nutrient sufficiency, deficiency, or toxicity. Plant analysis is not only useful to estimate the elemental mineral content of tissue, which is taken by a specific plant part, but it is also relevant for determining nutritional status by comparing with standardized values for a specific crop (Jones, 2001; Taiz and Zeiger, 2006; Havlin et al., 2006). Depending on the method used to determine the concentration of elemental nutrients in specific samples, there are corresponding standardized values for each nutrient. These values are used to compare plant test results and to obtain adequate information about plant nutritional status. The accuracy of these generalized limits of nutrient concentration can be affected by factors such as characteristics of the soil or growth medium, soil fertility management, crop genotype, plant growth characteristics, and specific factors related to each location (Krishna, 2002). This test is significant for both farmers and researchers when it is necessary to identify the mineral nutrition status of crops (Jones, 2001).

Increased tissue concentrations of a nutrient produce responses such as plant growth or fruit yield because these are often directly related to nutrient availability. If nutrient mineral elemental concentration in a tissue sample is low, in what is commonly named the deficiency zone, plant growth is usually also low. In this zone, if nutrient availability increases, growth or yield also increases. The maximum level of the deficiency zone is the critical concentration where the deficiency zone becomes the adequate zone. At this point, the minimum tissue content of a nutrient mineral produces maximum growth or yield. In the adequate zone nutrients are in sufficient amounts and may be increased to the level in which further increases do not stimulate growth or yield. However, the concentrations of the nutrients in the tissue may continue increasing. When the tissue content rises beyond the adequate zone, the nutrient mineral added drives plants to the toxicity zone, resulting in decreases in growth and yield (Taiz and Zeiger, 2006).

In brambles, N content is about 2-3% of plant dry matter. Plants with leaf elemental content less than 2% are in nutritional deficiency, show the common symptoms, and cannot yield or grow properly. Plants that have more than 3% leaf N content are in nutritional toxicity. Despite their vigorous appearance, they cannot harden sufficiently and develop low fruit firmness (Eames-Sheavly et al., 1991).

Leaf or plant analysis requires the following steps: sampling, sample preparation, laboratory analysis, and interpretation. Several variables contribute to misinterpretation of the results of a plant analysis including care of sampling, time of sampling, number of plants to sample, and the type and amount of tissue taken per plant. Environmental factors can affect the elemental nutrient content of plants. Elemental nutrient concentration varies according to the plant part. So, the results can differ because nutrient elements are not homogeneously distributed in the plant or within plant parts (Jones, 2001). Plant analysis is a better tool to manage the application and control of fertilization programs for blackberries and raspberries. By using plant analysis, it is possible to make nutritional corrections before nutritional problems occur. Through this method, it is feasible to improve yields and quality of fruit crops (Hart et al., 2006).

Foliar Sampling: Jones (2001) suggests the following procedure for foliar sampling of brambles: determining the specific plant part and location where samples will be taken, choosing the proper period for collecting samples, and determining the amount of samples per plant and the number of plant units per sampling batch. The suggested time for collecting leaves is the first week of August (Pritts, 1991). Young mature leaves, the ones that are located just below the growing tip on main branches or stems and exposed to full sunlight, should be collected randomly. Sample leaves have to be complete and healthy. These leaves should not be damaged by insects, mechanical contact, chemical sprays, infestations, dust, climatic or nutritional stress, shading, or death (Jones, 2001; Hart et al., 2006).

Clark et al. (1989) studying blueberries and their seasonal variation in foliar nutrient elemental content, stated that the period of sampling with stability is between mid-July to mid-August (following harvest). For raspberries, Huges et al. (1979) stated that the period with the least variation of nutrient level concentrations occurs during the last two weeks of August. Naraguma (1998) reported that blackberry leaf samples were collected in August. Clark et al. (1988) collected mature blackberry leaf samples from primocanes in the section six to ten nodes from the terminal between 15 and 20 May until roughly the first week of August. As a general suggestion for obtaining consistent results in raspberry and blackberry leaf samples, Hart et al. (2006) recommended that it is better to sample leaves when nutrient content is stable. For N and K, the period is late July and early August because variations of foliar elemental concentrations are at a minimum. Likewise, they suggest choosing only one fully expanded, clean, and healthy leaf per cane and taking fully expanded leaves from primocanes about 30.5 cm from the tip.

High tunnels and organic production of blackberries

High tunnels have the capability to protect plants from adverse weather conditions and to extend their period of growth and production (Thompsom et al., 2009). In 2005, under high tunnel (HT) conditions, 315 ha of commercial blackberries were grown worldwide. In the same year, under HT conditions, there were a total of 2,528 ha of organic blackberries were produced globally (Strik et al., 2007). According to Lamont et al. (2002), since 1993, HT crop production has been increasing as well as the types of crops cultivated. A HT is a type of greenhouse which has some specific characteristics such as a single polyethylene plastic layer which covers all the structural supports, no heating system. Ventilation is accomplished by two doors at opposite ends of the enclosure, and by raising the plastic on the both sides. Drip irrigation systems are commonly used. High tunnel dimensions vary and depend on the manufacturer. Typical structures are large enough to perform the best cultural practices including planting, monitoring, and harvesting. Bushway et al. (2008) stated that HT length and width can be variable, but dimensions should allow air circulation to reduce heat accumulation. It is recommended that the peak be a minimum of 2.4 to 2.7 m and post extensions about 1.2 to 1.5 m high. Wider HTs allow better management, but, totally dependent on materials. Long tunnels have poor cross ventilation. A standard HT size is 9 m wide and 29 m long. Thirty seven HTs of the standard size will cover the area of one hectare (Bushway et al., 2008; Demchak, 2009).

According to Lamont et al. (2002), HTs have advantages and disadvantages when compared to greenhouses. Advantages include: lower cost, semi-permanence, and portability. Additionally, HTs provide good crop protection against intense winds, heavy rains, low temperature, and frosts. Hanson et al., (2011) found earlier harvest and improved yields in HT production of PF and floricane-fruiting red raspberry cultivars as well as increased plant vigor, fruit quality, and fewer diseases. According to Giacomelli (2009), higher profits and product marketability are achieved due to reliability, increased yields, higher quality, and better crop timing. For small fruit production, HTs have many benefits for two main reasons: First, berry crops have better quality; second, fewer pest and pathogen attacks. Additional positive effects for PF blackberries are extended season and off-season fruit production (Heidenreich et al., 2008; Thompson et al., 2009). Rom et al. (2010) stated that when blackberry and raspberry plants were cultivated under HT conditions, the harvest period of those plants was extended for 3 weeks.

Heidenreich et al. (2008) stated that even though conventionally produced food is still less expensive than organic food, several advantages, such as lower input costs, high-value markets, and premium prices increase farm income and make organic bramble production more attractive. Rom et al., (2010) also mentioned that organic blackberry production presents fewer problems than other small fruits due to its special adjustability for this type of production. The authors determined that organic production of brambles can promote more revenues for farmers due to off season production, modified environmental conditions, reduced pest attack, and less need of pesticides.

Pest and disease control in high tunnels: Rom et al. (2010) stated that in organic, conventional, and HT bramble production, some insect and disease attacks may occur, and insect scouting should begin after planting and carried out weekly. Under HT production, a common bramble pest is the two-spotted spider mite (*Tetranychus urticae* Koch). This pest can be controlled with predatory mites. Another important pest is the Japanese beetle (JB) (*Popillia japonica* Newman) which can be controlled with traps, however, traps do not prevent neighboring JB from entering the area. The Organic Materials Review Institute (OMRI) has recommended using one of the following organic sprays in 10 to 14 day intervals: Surround particle film (Kaolin clay), Neem (vegetable oil), Pyganic (pesticide derived from chrysanthemums) or Pyrellin (Pyganic plus rotenone). The doors of the HT are opened to circulate air at ground level and eliminate humid spots to avoid fungal diseases. This air circulation can be enhanced by using household fans (Johnson and Lewis, 2005; Bushway et al., 2008).

Successful blackberry fruit production

To succeed in blackberry production in terms of amount and quality of fruit, producers need to consider cultivar selection (Perkins-Veazie and Collins, 1996), orchard cultural practices, and fruit plant nutrition (Pritts, 1991). The appropriate cultivar for environmental conditions influences fruit quality in erect blackberries. According to Perkins-Veazie and Collins (1996), blackberry cultivars have differences such as ripening date, fruit flavor, shape, size, and color. The cultivar can negatively affect marketable yield due to winter hardiness. According to Strik et al. (2012), proper cultural practices (tipping, mowing, and row covers) increase yield, extend fruiting season, and improve cane architecture of PF blackberries. Plant nutrition may be the most important factor in blackberry production because proper nutrient management can improve yield. In addition to N, other macro- and micronutrients are needed for adequate blackberry production (Pritts, 1991). Evaluating orchard returns, Rom (1994), found that gross returns depend on three factors: fruit quality, fruit quantity, and market value. Market value may not be controlled by fruit growers, however, farmers can control fruit quality and quantity.

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III. CHAPTER 1

OPTIMUM RATE AND TIME OF APPLICATION OF N-FERTILIZATION FOR PRIME–ARK® 45 PF BLACKBERRY CULTIVAR UNDER HIGH TUNNEL CONDITIONS

Abstract

Increasing production of blackberry (*Rubus* L. subgenus *Rubus* Watson.) and the development of new worldwide markets allows for important opportunities in the production of this fruit. New genotypes, such as primocane-fruiting (PF) blackberries, can extend the growing season and create benefits for farmers and consumers. As with new cultivars, there are some important cultural practices, including plant nutrition parameters, which need to be developed in order to optimize yield and financial returns. The objective of this research project was to determine the optimum rate and time of nitrogen (N) application to PF blackberry. This study was conducted in 2011 at the University of Arkansas, Agricultural Research and Ext. Center, Fayetteville. 'Prime-Ark® 45' blackberry plants were cultivated under high tunnel conditions. There were four N treatments: Treatment 1 (control - no N applied); Treatment 2 (10 $kg{\cdot}ha^{-1}$); Treatment 3 (10 $kg{\cdot}ha^{-1}$ - 50% split application); and Treatment 4 (20 $kg{\cdot}ha^{-1}$). Single applications of N were applied in mid May, and the split application of 50% in mid May (20 May), and 50% last week of July (27 July). Ammonium sulfate $(NH_4)_2SO_4$ was the source of N. A randomized complete block (RCB) design was used with four blocks and five plants per experimental unit. The following variables were measured: total and marketable yield, fresh weight of plant above ground, and cane diameter. Soil and foliar analysis for N concentration was conducted. Leaves for foliar analysis were collected on 11 July. Total fruit yields for Treatment 2 and Treatment 3 (2.5 and 2.4 kg, respectively) were the highest and significantly

different than the other two treatments (1 and 4). Although not significantly different, marketable yield had a similar trend as total fruit yield. Cane diameter and plant fresh weight were not significantly affected by the fertilizer treatments. Although there were no significant differences of N foliar content among treatments, Treatment 3 had the highest mean at the end of the sampling period.

Mn and B foliar concentrations were significantly different due to the N rates applied. Conversely, no significant differences among treatments were found for the following elements: P, K, Ca, Mg, S, Na, Fe, Zn, and Cu. Results indicated that either a single or split N application rate of 10 kg $N{\cdot}ha^{-1}$ did result in the highest yield.

Introduction

From 1995 to 2005, worldwide blackberry cultivation increased 45%. Approximately 20,035 ha of blackberries worldwide were cultivated, both conventionally and organically in 2005 (Strik et al., 2007; Clark, 2008). In 2009, the cultivar 'Prime-Ark® 45' (Cv. APF-45) was introduced by the University of Arkansas as a primocane-fruiting (PF) blackberry genotype (Clark, 2008). Primocane fruiting blackberries grow and flower on the first year growth. The currently available PF blackberry cultivars are erect, thorny types (Rieger, 2006; Strik et al., 2007). Blackberries are well adapted to diverse types of soils with pH values ranging from 4.5 to 7.5. However, for better plant development, soil pH should be between 6.0 to 6.5 (Pritts, 1991; Gordon, 1991; Crandall, 1995). At this pH, physiological disorders and nutritional problems can be avoided (Pritts, 1991). Some environmental factors are also important in blackberry cultivation. High or low temperatures can have adverse effects on fruit quality and quantity (Crandall, 1995). Also, the magnitude of light intercepted by the canopy of plants is a significant environmental factor for plant development. Canes that receive sunlight will bear more berries (Pritts, 1991). Lower light intensity, between 177 and 186 lux of radiation is needed for proper growth in brambles (Moore and Skirvin, 1990). Another factor is day length which can affect flower bud initiation. Wind is a crucial factor because canes can be desiccated by strong winds. Thus, it is necessary to reduce wind damage, such as cane breakage, through trellising and other cultural practices (Moore and Skirvin, 1990; Pritts, 1991; Rieger, 2006).

Irrigation is necessary throughout the growing season. It is required mainly during the period from bloom to harvest (Crandall, 1995). Drip irrigation is appropriate to brambles because it is not only efficient but also is an effective use of water when properly installed. Water constantly drips around the root zone, while raised rows and the area between rows stay dry and firm. Other advantages are even moisture surrounding the root zone and minimal water loss (Pritts, 1991; Stiles and Reid, 1991; Crandall, 1995; Rieger, 2006). Proper soil drainage reduces disease problems, such as Phytophthora root rot (*Phytophthora sp.)* (Pritts, 1991; Rieger, 2006).

Nitrogen is an essential macronutrient for plants, required in greatest amounts for proper growth and development of floricane-fruiting blackberries (Moore and Skirvin, 1990). According to Pritts (1991) nitrate N is mostly used by blackberries due to the solubility and the fast movement into plants. During establishment, for floricane-fruiting blackberry plantings, only manure applications, as a source of N, are suggested for plant fertilizing (Moore and Skirvin, 1990; Gordon, 1991). Pritts (1991), Hart et al. (2006), and Strik (2008) recommend N applications at rates from 22 to 56 $kg \cdot ha^{-1}$ in the first year. For the second year, suggested rates for N fertilizer vary from 45 to 112 $kg \cdot ha^{-1}$ (Pritts, 1991; Alleyne and Clark, 1997; Naraguma, 1998; Mahler and Barney, 2000; Kuepper et al., 2003; Hart et al., 2006; Strik 2008).

Nitrate- and urea-based fertilizer are the most commonly used for blackberry fertilization, Ammonium sulphate (21-0-0) is also an acceptable source of N because of its minimal hygroscopicity and leaching features (Havlin et al., 2006). The efficiency of N uptake in plants is improved by split applications of fertilizer because this kind of application synchronizes the N availability and the plant's growth process. In brambles, it is recommended for the first N split application to be in March and the second in May (Eames-Sheavly et al., 1991; Pritts, 1991; Taiz and Zeiger, 2006). Naraguma and Clark (1998) found that split applications promoted higher N concentrations in leaves in 'Arapaho' thornless blackberry. However, no other positive effects were reported.

Cultural practices on floricane-fruiting blackberries such as pruning, trellising, and annual mowing help to increase fruit production and yield quality (Morrison, 1998; Kuepper et al., 2003; Rieger, 2006; Thompson et al., 2007). Tipping of primocanes is recommended to promote branching and increase the number of fruiting nodes per cane (Moore and Skirvin, 1990; Thompson et al., 2007).

Phytophthora root rot (*Phytophthora spp*.), Japanese beetle (*Popillia japonica* Newman), Two-spotted mites *(Tetranychus urticae* Koch), and the Raspberry crown borer (RCB) *(Pennisetia marginata)* are frequently observed in commercial orchards (Johnson and Lewis, 2005; Rom et al., 2010). Blackberry pests and diseases can be controlled by planting clean stock and using adequate sanitary and pesticide measures (Moore and Skirvin, 1990).

The availability of appropriate amounts of nutrients in soils is necessary for increased yields and improve produce quality (Taiz and Zeiger, 2006; Havlin et al., 2006). Specific information about timing and rate of N applications for new PF blackberry genotypes is needed to increase production and financial returns (Clark et al., 2005; Clark and Perkins-Veazie, 2011).

High tunnel blackberry production is becoming popular in the U.S. (Demchak, 2009). High tunnel provides several advantages for PF blackberry growth and development such as off-season production, reduced incidence of disease, increased yield, and improved berry quality. (Demchak, 2009). High tunnel cultivation allows extension of the fruit production season (Thompson, et. al., 2009). High tunnels facilitate conventional and organic blackberry cultivation. Benefits of organic blackberries produced under HT include extended harvest season, and higher fruit quality with a reduction in pests and diseases (Rom et al., 2010).

Total yield: The goal of blackberry growers is to obtain a large yield of high quality berries (Pritts, 1991). Total yield of blackberries is the sum of all berries harvested and weighed periodically during the growing season. Diverse strategies to increase quality and yield of blackberries have succeeded during the last years, such as the use of HTs.

Marketable yield: Marketable yield consists of those berries chosen from the total fruit yield that meet market specifications. After harvest, the yield is selected and graded according to the market requirements. Berries should be harvested near ripe because eating quality does not improve after harvest (Mitcham et al., 1998). Marketable berry selection based on the grade standards. Berries should be black in color, turgid, properly shaped, and not overripe. Damaged or decayed fruit due to sun-scald, diseases, etc. should be rejected. Ranges of tolerance for mold are < 1% and < 5% depending on the grades. In any lot of berries, not more than 10%, by volume, should fail to meet the grades. For other type of defects and requirements are indicated by the standards for grades of dewberries and blackberries of the U.S. Dept. of Agr. (1928), Perkins-Veazie, et al. (1996), Mitcham et al. (1998), and Perkins-Veazie (2004).

Fresh weight: Fresh weight comprises the measurement of all plant parts that develop above ground (stems, leaves, laterals, etc.). Blackberry plants utilize nutrients in different amounts and periods during the growing season in order to develop a sufficient plant structure to bear fruit (Pritts, 1991). Plants utilize N throughout the growing season but the absorbed amounts of N vary according to plant age. Young plants absorb more nitrogen and N represents a greater percentage of the dry weight of a young plant than a mature plant. Nitrogen deficiency cause stunted plant growth and reproductive problems (Troeh and Thompson, 2005).

Cane diameter: Cane diameter is measured through the center of the perpendicular section of the main cane, cut at a specific height above ground. This parameter is linked to plant growth and development in most blackberry genotypes. Eyduran et al. (2008), while studying eight blackberry cultivars, found that fruit weight was positively affected by cane diameter in some cultivars with a negative effect in one cultivar. In raspberries, both cane diameter and cane height are strongly correlated (Crandall et al., 1974; Jennings and Dale, 1982). A large cane diameter means the production of multiple laterals at a node but a reduced number of lateral fruit-bearing nodes (Jennings, 1979).

Foliar nutrient concentration due to N fertilization: Proper use of soil and plant analyses is necessary to optimize yields. These tools help to provide balanced amounts of macro- and micronutrients (Mylavarapu, 2010). Plant analysis indicates the actual nutrient content in leaves and nutritional status (Pritts, 1991; Westwood, 1993; Hart et al., 2006). Leaf nutrient concentrations vary according to the plant part and season (Westwood, 1993). Less than 2% N in blackberry leaves causes nutritional disorders and plants cannot grow and develop optimally. In contrast, leaf N concentration greater than 3% produces plants with more vegetative growth (Pritts, 1991). The most reliable protocol to collect foliar samples is as follows: Primocane leaves exposed to full sunlight, and located just below the growing tip, six to ten nodes from the terminal are randomly collected. Complete, clean, healthy leaves, free of injuries are collected

(Clark et al., 1988; Clark et al., 1989; Pritts, 1991; Jones, 2001). For floricane-fruiting blackberries, the period for sampling with stability in elemental concentration is between mid July to mid August (Hart et al., 2006).

The objective of this study was to determine the optimum rate and time for N fertilization for the 'Prime-Ark® 45' PF blackberry cultivar under high tunnel conditions in one-year-old plants.

Materials and Methods

Experiment 1.

This study was conducted at the University of Arkansas Agricultural Research and Extension Center, Fayetteville, Ark. (lat. 36°5'4"N, long. 94°10'29"W). The soil type in the high tunnel (HT) was a Captina silt loam (Typic Fragiudult). The initial soil analysis (29 March, 2011), indicated low to moderate natural fertility, pH of 6.2, and a moderately low cation exchange capacity of 8.0 $cmolc.kg^{-1}$ (Appendix B, Table B.1). The soil pH was measured in a soil-water mixture extraction of 1:2 (weight:volume), according to the procedure indicated by Donahue (1983). Using the Specific Ion Electrode method (Donahue, 1992), NO_3-N concentration in soil was measured at 15 $mg.kg^{-1}$. The Mehlich-3 solution (Mehlich, 1984) and the Inductively Coupled Plasma (ICP) Emission Spectroscopy method was used to measure P and K, (78 and 136 $mg.kg^{-1}$, respectively), and Ca, Mg, S, Zn, Fe, Mn, Cu, and B, all of them were within optimum levels according to the University of Arkansas, Cooperative Extension Service, recommendations. These analyses were conducted at the Soil Testing and Research Laboratory, University of Arkansas, Marianna, Ark. All soil tests were analyzed following the same procedures and methods, with the exception of a complementary soil analysis for P, K, and microelements on 16 May (Appendix B, Tables B.2 to B.10). Soil samples were extracted by Mehlich-3 solution (Mehlich, 1984), and the concentrated solution was measured by using the Spectro ARCOS-SOP (side on plasma)-ICP method at the Agricultural Diagnostic Laboratory – Altheimer- Dept. of Crop Soil and Environmental Science, University of Arkansas, Fayetteville.

‘Prime-Ark® 45’ PF blackberry plants were cultivated under HT conditions, from spring to fall in 2011. Cuttings were rooted in 2010, and planted in rows in the high tunnel on 18 Mar. 2011. The HT (Haygrove solo series, Haygrove Ltd., U.K.) was constructed during 2010. The HT was a Quonset, (single-bay) with the following dimensions: 7.6 m wide, 58.8 m long, and 3.72 m high. The tunnel was oriented from north to south, and was covered with 6 mm transparent polyethylene plastic. Prior to planting, soil surface was leveled with a 0.5^{o} slope for proper drainage. The area of the high tunnel was divided into three main raised rows. These rows were 0.9 m wide, 0.2 m high, and 53.8 m long. Plants were set 0.6 m apart. Blackberry plants were established in two outside rows with a row spacing of 3.7 m. Each experimental unit consisted of five plants per treatment. Rows were divided in four blocks, each block contained all treatments. The west side row had only one block and east side row three blocks. Plants were drip irrigated as needed with in-line emitters spaced at 0.3 m intervals.

‘Prime-Ark® 45’ primocanes were mowed 10 cm above soil level 5 days after planting; then primocanes grew homogeneously. After weeding manually on 5 Apr., fresh rice hull mulch was applied, and the borders of the rows were covered with black plastic mulch leaving 0.3 m in the middle of row exposed, approximately. Manual weeding continued over the growing season on a regular basis. When plants were 1.0 m tall, on 14 June they were tipped. A ‘V’ double curtain trellis was installed on 27 June. Canes were trained between double sets of trellis wires spaced at 0.6 m and 1.2 m high and tied to the wires using plastic tape during the following 10 weeks, as they grew.

The following N fertilizer treatments were applied: 1) Control - no N applied; 2) 10 kg $N \cdot ha^{-1}$; 3) 10 kg $N \cdot ha^{-1}$ (split application); and 4) 20 kg $N \cdot ha^{-1}$. Single applications of N fertilizer were broadcast on 20 May. For Treatment 3, split application was broadcast 50% on 20 May and the 50% on 27 July. Actual NO_3-N in the soil was 33.6 $kg \cdot ha^{-1}$, according to the soil test (Appendix B, Table B.1). Ammonium sulfate (21-0-0) was the N fertilizer used. The fertilizer was broadcast uniformly on the surface of the row between canes in a 3.0 m x 0.2 m band. Fertilizer was not applied in areas between two different treatments. The mulch between plants was removed and fertilizer was applied directly to the soil. After the fertilizer application, the mulch was replaced.

The doors were closed to maintain proper temperature when day or night temperatures fell below 8 to 10^{o}C for extended off season production. The doors were opened when temperatures rose above 10^{o}C. Sidewalls were opened when necessary, during bloom and fruit bearing periods. High tunnel doors were closed as needed to encourage vegetative development until 15 Nov. After that date doors were opened to induce plant dormancy.

Signs and damage of Two-spotted spider mites *(Tetranychus urticae* Koch) were observed at very low and treatable levels of infestation. To control this pest, two species of beneficial predatory mites (*Galendromus occidentalis* Nesbitt and *Neoseiulus californicus* McGregor) were released in the high tunnel on 9 Jun and 4 Aug. 2011. On 24 June, Japanese beetles (*Popillia japonica* Newman) were observed attacking plants. To control Japanese beetles, kaolin clay (Surround WP®) was sprayed on leaves on 9 Jun and 1 July 2011 (Johnson and Lewis, 2005; Rom et al., 2010).

Parameters measured:

Total yield: The fruit yield of primocanes was measured as follows: All berries per each experimental unit were harvested and weighed using a precision scale (Sartorius, Acculab-Vicon, Elk Grove, Ill.), either once or twice a week, when they were totally blue or black colored. The total fruit weight per experimental unit was recorded. Total fruit yield was the sum of the weight of all berries which includes fruit with mechanical, insect, and disease damage. First fruits were harvested the first week of August and harvest was extended until the second week of November.

Marketable yield: Marketable fruit was graded and weighed. The following grading parameters were used to select marketable berries: size and shape, ripeness, color and physical condition. In general, fruit without defects unacceptable to the consumer in a direct sale was culled (U.S. Dept. of Agr., 1928; Perkins-Veazie, et al., 1996; Mitcham et al., 1998; Perkins-Veazie, 2004).

Fresh weight: Weight of green material (leaves and stems) per treatment was measured. All plants were cut 5 cm above ground after harvest. All material in each experimental unit was tied to avoid losing plant parts; then, it was weighed using a portable scale (VEIT Electronics – BAT1, Czech Rep.).

Cane diameter: This parameter was measured from the 10 widest stems from each experimental unit. The stem was measured at 10 cm above the ground. Two diameters per cane were measured. The 10 cane diameters were averaged.

Nutrient concentration: To determine macro- and micronutrient foliar concentration, leaf analyses were conducted approximately every two weeks, from June to August 2011. According to the protocol, six mature leaves from each experimental unit, located six to ten nodes below the growing tip, were collected randomly (Clark et al., 1988). After collection, leaves were rinsed in deionized water. In the laboratory, leaves were dried for 24 hr at 70^{o}C in a convection oven. Samples were subjected to total N analysis, which was conducted by combustion in an Elementar VarioMax analyzer instrument (Elementar Americas Inc., Mt. Laurel, NJ), and P, K, Ca, Mg, S, Na, Fe, Mn, Zn, Cu, and B by wet digestion using concentrated nitric acid and 30% hydrogen peroxide on a hot block. A 0.25 g sample was digested and brought to 25 ml volume. The digestate was analyzed using an inductively coupled plasma spectrophotometer (ARCOS-SOP, ICP - Spectro Analytical Instrument, Mahwah, NJ). This analysis was conducted at the Agricultural Diagnostic Laboratory- Altheimer – Dept. of C.S.E.S., University of Arkansas, Fayetteville.

The possible effects of N fertilization rates on the foliar macro- and micronutrient concentrations in 'Prime-Ark® 45' PF blackberries were determined for the sampling date with relative stability in elemental content. The sampling date was 11 July, because for floricane-fruiting blackberries, the period with most stability in elemental nutrient concentration and minimum variability is mid July (Clark et al., 1988).

All data were analyzed as a randomized complete block (RCB) design with four blocks, five plants per experimental unit, and four treatments (N fertilizer levels). Data were analyzed using SAS statistical program (SAS Institute Inc. 2008, Cary, N.C.), PROC MEANS and PROC MIXED. Means separated by Least Significant Difference (LSD) with 5% level of significance (p<0.05).

Results

The soil results (Appendix 2, Table B.2) indicated that all macro- and micronutrients were in adequate concentrations and were optimal for proper plant growth and development (Hart et al., 2006; Espinoza et al., 2007), (Appendix 2, Tables B.3 to B.10).

Total yield: Nitrogen fertilization rates significantly affected total fruit yield, $p = 0.048$ (Fig. 1). Through LSD comparison of means, at $p < 0.05$, Treatment 2 (631.1 g) and Treatment 3 (815.3 g) were statistically similar and resulted in the higher yields than Treatments 1 and 4. Treatment 4 (269.4 g), with the highest N rate applied, had a lower yield response, which was statistically similar to Treatment 1 (426.0 g). Harvest began on 12 Aug. and ended on 17 Nov. for all treatments, due to freezes during the following days, in which temperatures were below -5 °C. (See Appendix A. Table A.1 for descriptive statistics of this variable).

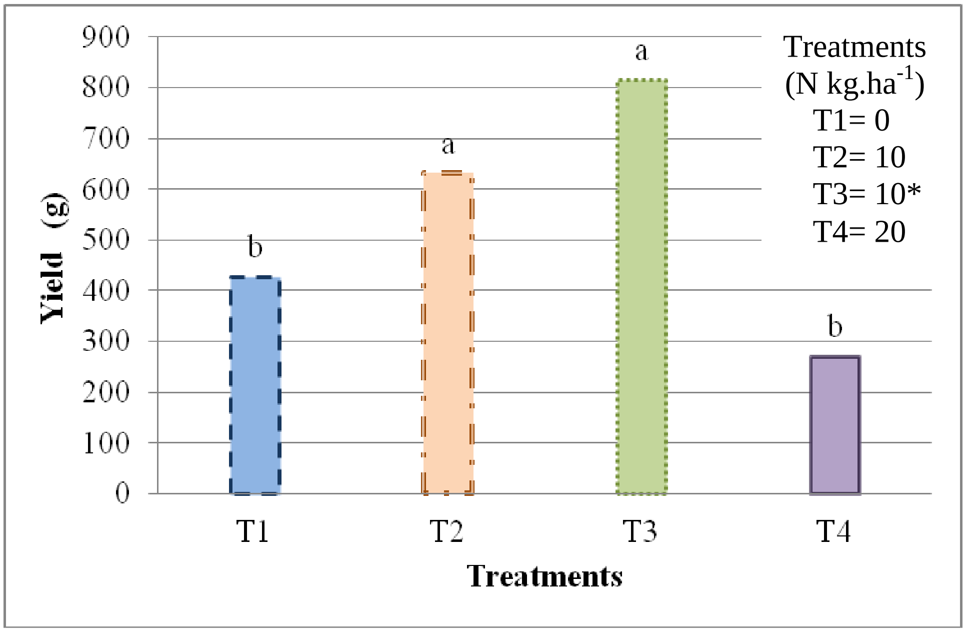

Fig. 1. Total fruit yield responses obtained from four N fertilizer rates applied on ‘Prime-Ark® 45’ PF blackberry cultivar. Mean separation was performed by LSD at *P* <0.05. n: 16 experimental units.
*Split application of N fertilization rate.

Marketable yield: Results indicated that all treatments produced similar responses on marketable berries**,** the estimated *p*=0.05 (data not shown). Although there were not significant differences among treatments, marketable yield had a similar trend as total fruit yield, where Treatment 3 had the highest marketable yield (725.2 g). Treatments 2, 1, and 4 had lower marketable yields (540.5 g, 354.4 g, and 221.2 g, respectively). Approximately 15% of the berries were eliminated per treatment based on quality according to the grading parameters recommended by the U.S. Dept. of Agriculture (1928), Perkins-Veazie, et al. (1996), Mitcham et al. (1998), and Perkins-Veazie (2004).

Fresh weight and Cane diameter: Plant fresh weight (branches and leaves) and cane diameter of these 'Prime-Ark® 45'plants were not significantly affected by any of the N treatment rates.

Nutrient concentration in leaves as affected by N fertilization: As was described at the beginning of this section, all leaf N concentration means were within the standardized values indicated by Clark, et al. (1988), Pritts (1991), and Garcia (2007). The total average of N concentrations measured during all sampling dates for all treatments combined (from June to August, 2011) was 2.6%. The minimum N concentration mean measured for the same sampling period was 1.9% and the maximum 3.3% (Appendix A. Table A.2).

Nitrogen concentration means were statistically similar on any given sampling date: 14 Jun= 2.88% (*p*= 0.16), 29 Jun= 2.89% (*p*= 0.49), 11 Jul= 2.66% (*p*= 0.06), 25 Jul= 2.66% (*p*= 0.88), 4 Aug.= 2.42% (*p*= 0.82), and 26 Aug= 2.31% (*p* = 0.30). However, there were significant differences in leaf N concentrations among sampling dates (*p*= <0.0001). Foliar N concentrations for all treatments (T), (T1: 0-control, T2: 10, T3: 10-split, and T4: 20 $kg.ha^{-1}$) displayed a downward trend for the sampling period (Fig. 2). However, Treatment 3 had the highest concentration and an ascending change of leaf N content at the end of the sampling period.

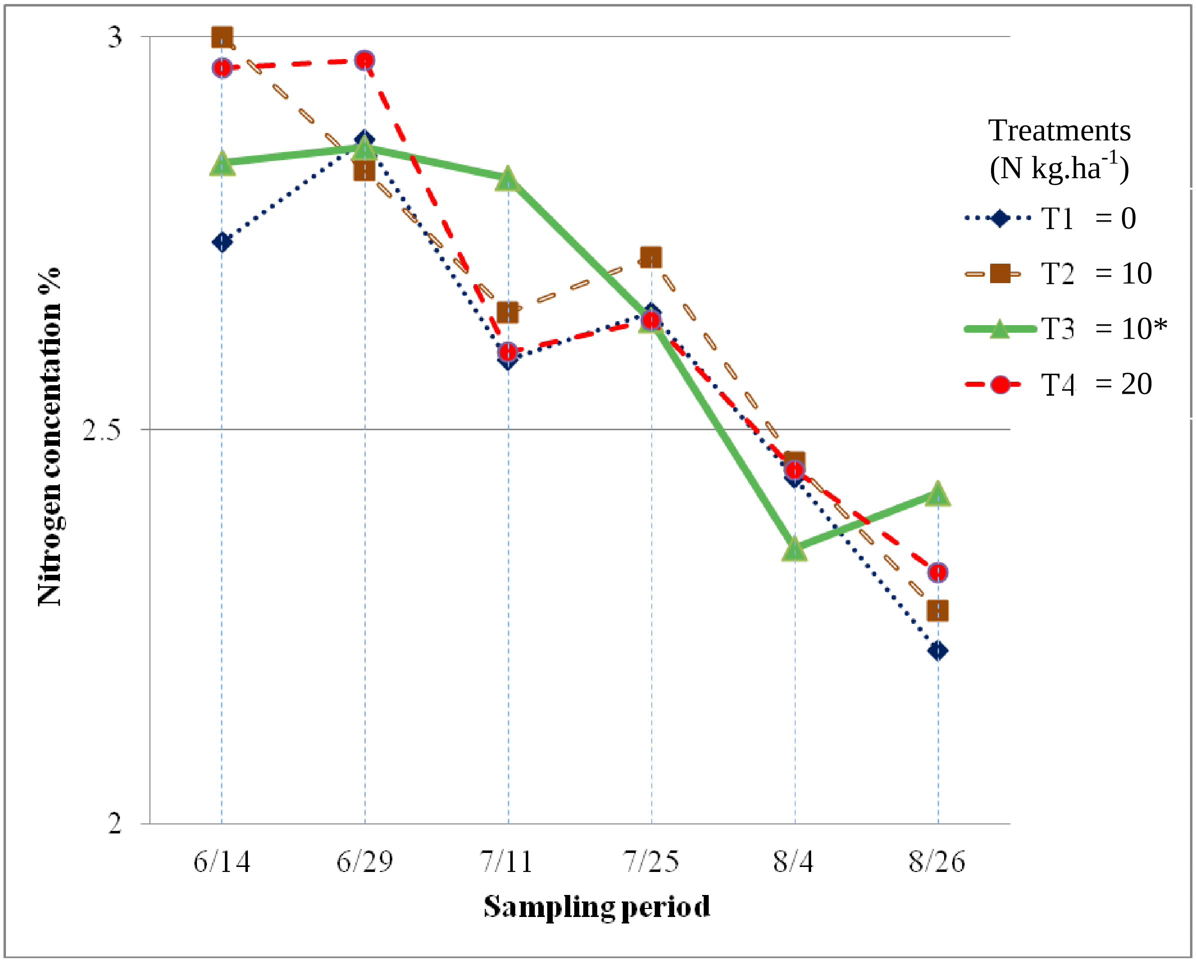

Fig. 2. Trends of N concentration means in leaves of ‘Prime-Ark® 45’ PF blackberry cultivar (from June to Aug. 2011), per treatment (kg·ha^{-1}).
*split application of N fertilization rate, Treatment 3 (10 kg·ha^{-1}).

Results indicated that there were no significant differences in N concentration in leaves of ‘Prime-Ark® 45’ on 11 July, 2011 [period of stability in nutrient concentrations during the growing season as stated by Clark et l. (1988) in floricane fruiting blackberries] due to the N fertilization rates applied (p= 0.06). In descending order, foliar N concentration was: Treatment 3 (10 $kg.ha^{-1}$, split application) 2.82% N, Treatment 2 (10 $kg.ha^{-1}$) 2.65% N, Treatment 4 (20 $kg.ha^{-1}$) 2.60% N, and Treatment 1 (0 $kg.ha^{-1}$) 2.60% N.

There were significant differences in elemental concentration in ‘Prime-Ark® 45’ leaves due to N fertilization rates for B (p = 0.003) and Mn (p = 0.01) on 11 July 2011 (Fig. 3). The N fertilization rates did not significantly influence concentrations of P (p= 0.44), K (p= 0.12), Ca (p= 0.47), Mg (p= 0.10), S (p= 0.27), Na (p= 0.48), Fe (p= 0.28), Zn (p= 0.36), or Cu (p= 0.36).

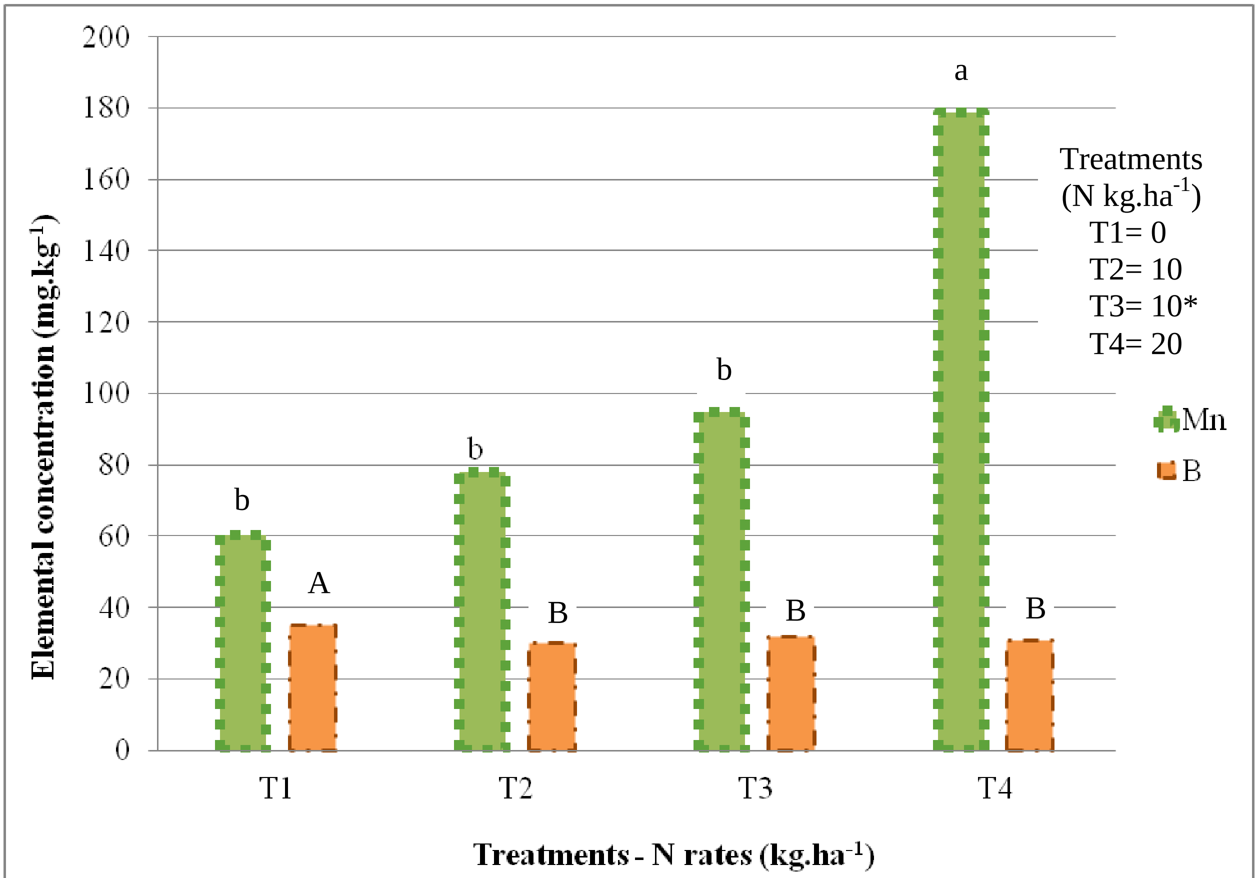

Fig. 3. Manganese and B content in 'Prime-Ark® 45' PF blackberry leaves ($mg\cdot kg^{-1}$), based on analysis of foliar samples collected on 11 July 2011.

*split application of N fertilization rate.

Discussion

According to Marx et al. (1999), NO_3^- -N and NH_4^+ -N concentrations in soil can be measured through soil tests at the time of sampling, only. However, future soil conditions for the same elements are not reflected (Marx et al., 1999). Thus, to determine the nutritional status over time, foliar analyses were conducted and the results indicated that N foliar concentrations were within standardized values suggested for brambles (Clark et al., 1988; Pritts, 1991; Garcia, 2007). During vegetative growth, foliar N concentrations were optimal, but decreased to 2.7% during blooming, and from 2.4 to 2.3% during fruiting, which is the low standard limit of the optimum range (Hart et al., 2006).

The ammonium sulfate (21-00-00) that was applied on 20 May (single application) and 27 July (split application). The analysis on 29 Aug. (Appendix 2, Tables B.3, 4, 5, and 6), indicated higher NO_3-N content in the soil than the previous report of sampling on 29 Mar. (Appendix 2, Tables B.1). Based on soil analysis on 4 Oct., per treatment, during the blooming and fruiting period (Appendix 2, Tables B.7, 8, 9, and 10), the NO_3-N content in soil was lower than before and after fertilizer applications. Hence, it was possible that some of the NO_3-N soil content was taken up by the plants.

There were no visual symptoms of N deficiency, such as yellow leaf color, older leaves with reddish tips, or evidence of excessive N applications such as vigorous vegetative growth, elongated and thinner primocanes or breakage, or extended internodes (Pritts, 1991; Hart et al., 2006).

Total yield: In this experiment, it was expected that different responses in fruit yield, according to each treatment, would be observed. Nitrogen contributes to increased fruit yields in brambles (Pritts, 1991; Hart et al., 2006). Both Treatments 2 and 3 had the higher yield responses while Treatments 1 and 4 were statistically similar and lower from the two others. Treatments 1 (0 kg $N \cdot ha^{-1}$ - control) and 3 (10 kg $N \cdot ha^{-1}$ - split) were significantly different and this result is consistent with Rempel et al. (2004), who stated that the unfertilized treatment had the lowest yield response, and the split-N treatment the highest yield in both years of experimentation (2001 and 2002) in 'Meeker' red raspberry. Hence, unfertilized blackberries plantings tend to produce the lowest response, and split applications to be more effective increasing yields.

Treatment 4, which had the highest amount of N applied, did not have the highest response in yield, as was expected. The yield of Treatment 4 might have been affected by an excessive N application, or some other unidentified factor(s). However, due to the observed vigorous growth in several plants, it is possible that the main cause was the excessive N application. According to Hart et al. (2006), minimum amounts of N have to be applied to soils because in brambles, less N is required in the planting year than in subsequent years. Nitrogen applied in higher levels than necessary negatively affect yield and promote excessive growth which leads to long and thin primocanes.

The significant differences in total yield, as responses to the N treatments, are the opposite of other results obtained in similar experiments (Naraguma and Clark, 1998; Rempel et al., 2004). In a three-year experiment with mature 'Arapaho' thornless blackberry plants, Naraguma and Clark (1998) found no significant responses in total yield after N fertilization at various levels and split applications in any years (1994, 95, and 96). Also in 'Meeker' red raspberry, Rempel et al. (2004) found no significant yield responses and these authors also reported that in other experiments in red raspberries a lack of response in yield has been observed.

Treatments 2 and 3 had the highest yields, which indicate that these N rates were adequate for the first year of cultivation. However, the amounts of harvests obtained in this experiment were less than 40% of the minimum typical harvest reported in Oregon by Clark et al. (2005) and Arkansas by Strik et al. (2008). In mature, field-grown 'Prime-Jan®' and 'Prime-Jim®' cultivars, yields of 1600 to 5900 $kg \cdot ha^{-1}$ (Clark et al., 2005) were obtained, and in Oregon 4000 to 6100 $kg \cdot ha^{-1}$ (Strik et al., 2008) were harvested. In 'Prime-Ark® 45,' yields of 14100 and 5600 $kg \cdot ha^{-1}$ were obtained in Arkansas in 2008 and 2009, respectively (Clark and Perkins-Veazie, 2011). The amounts of berries harvested also indicate that an economic analysis should be done in order to identify whether this amount harvested is cost-effective or not in the first year of 'Prime-Ark® 45'plants. One factor that could have produced these lower yields is the higher temperatures during the summer (Clark et al., 2005; Strik and Thompsom, 2009).

For the establishment year, either only manure applications (Moore and Skirvin, 1990; Gordon, 1991) or N fertilizer at rates greater than 20 kg $N \cdot ha^{-1}$, frequently are recommended in floricane-fruiting blackberries. Also, similar amounts of N fertilizer applications have been used in other experiments in PF raspberries and blackberries (Pritts, 1991; Spiers, et al., 1999; Hart et al., 2006; Kowalenko, 2006; Strik, 2008). In this experiment, rates of 10, 10 (split), and 20 kg $N \cdot ha^{-1}$ were applied and results indicate that 10 kg $N \cdot ha^{-1}$ is an appropriate fertilizer rate for 'Prime-Ark® 45' in either single or split application, if off-season production is desired under HT conditions during the first year of cultivation. During the following years, when the root system of plants is more developed, it will be possible to determine if the N concentrated in leaves is beneficial for extended production. Our findings indicate that for one-year old 'Prime-Ark® 45' PF blackberries, single or split applications of 10 $Kg \cdot ha^{-1}$ have the higher responses in yield.

Marketable yield: After grading and elimination of unmarketable fruit, selected berries were weighed. It was found that all treatment responses were statistically similar (*P* value= 0.051). Treatments 2 and Treatment 3 did not result in significant responses in marketable yield as opposed to the results for total yield. However, the numerical result is similar to the total fruit yield trend. Treatment 4 had a low response even though it was the highest N rate applied. Maybe this treatment was excessive for the plants and affected the berries. According to Hart et al. (2006), important amounts of N goes to the fruit, and excessive N applied in late-winter or early-spring could affect fruit firmness and quality in brambles. It was found that on average 15% of the berries were eliminated, which is consistent with Demchak and Clark, (2011) who reported that under high tunnel conditions from 29 Aug. to 21 Oct. 2011, the percentage of marketable berries in three-year-old plants of ‘Prime-Ark® 45’ PF was 85% of the total yield.

Cane diameter: All cane diameters were statistically similar regardless of N application rates. It was expected that different responses on cane diameter would occur due to the N fertilizer rates and the HT conditions because it has been shown in other studies that HTs promote plant and cane vigor (Demchak, 2009). Also, in ‘Willamette’ red raspberries, cane diameter was positively correlated with the amount of fruit per lateral and per cane; which is related to plant nutrition status (Crandall et al., 1974). This was also determined by Eyduran, et al. (2008) and Jennings (1979) who state that cane diameter is mostly linked to plant growth and development in most blackberry genotypes. Cane diameter measurements may represent a reasonable range of normal diameter for one-year-old plants of ‘Prime-Ark® 45’ PF blackberry cultivar in regard to the previous parameters results. On the other hand, Eyduran et al. (2008), studying eight blackberry cultivars, found that cane diameter significantly affected fruit weight in seven cultivars. Thus, ‘Prime-Ark® 45’ cane diameter may not only be related to nutritional status but also to another factor.

Total fresh weight: The weight of total leaves and branches per experimental unit were not significantly affected by any of the N treatments, (*P* value= 0.8525). However, as is explained in Naraguma (1998) root weight data was not collected and it could have presented a more complete picture of differences in total fresh weight due to the N rates applied in this experiment. Also Malik et al., (1991) in an experiment using ‘Chester Thornless’ stated that the roots and primocane plant parts are the major components of the plant biomass. For one-year-old ‘Prime-Ark® 45’ PF blackberry plants, cultivated under high tunnel conditions, fresh weight may not reflect differences due to N fertilization rates applied to these plants.

The results are consistent with results of experiments that used dry weight instead of fresh weight. According to Rempel et al. (2004), N application had no significant effect on plant dry weight in ‘Meeker’ red raspberry.

Nutrient concentration in leaves due to N fertilization: Based on the literature review, it was expected that the first weeks of July is a period with stability in elemental concentration in floricane-fruiting blackberry leaves (Clark et al., 1988). Thus, analysis was carried out in leaves collected on 11 July. Nitrogen fertilizer rates applied did not cause significant differences in the concentrations of most of the nutrient elements studied, except for Mn and B. This is consistent with Naraguma and Clark (1998), who found no significant responses in elemental foliar content after various N levels and split applications to the soil. All elements tested showed proper concentration within the standardized values suggested by Clark et al. (1988), Pritts (1991), and Garcia (2007). In experiments carried out in 1988, in young ‘Chester Thornless’ blackberry, N content in leaves was lower than when were two-year-old plants in 1989 (Malik, et al., 1991).

Thus, it is expected that in future years, the N concentrations may increase where the same N rates are used.

Means of N concentrations in leaves for the sampling period: N concentration decreased throughout the sampling period. This seasonal decline in N foliar content could be attributed to physiological reasons (Hart et al., 2006) but also declining N in the soil resulting from leaching and immobilization into the organic fraction (Havlin et al., 2006). Although statistically similar, the leaves from Treatment 3= 10 kg.ha^{-1} of N fertilizer (split application) had the highest concentration of N at the end of the sampling period (26 Aug.) (Fig. 2). Nitrogen concentrations were not measured in the following weeks. Determining the trend of these N concentrations may be important for off-season production because N will be required the rest of the extended harvest period. According to Naraguma and Clark (1998), N split application promoted higher N concentrations in leaves of plants cultivated under ambient conditions; however, no more benefits were found from split applications of N in 'Arapaho' thornless blackberry. Conversely, under high tunnel conditions, split application may be appropriate because the off-season production could require more N for increased yield. All means of N leaf concentration over time were within the standardized leaf nutrient content values (Garcia, 2007; Pritts, 1991) but also with those found by Clark et al. (1988).

Conclusion

For one-year-old ‘Prime-Ark® 45’ PF blackberry plants, under HT cultivation, results of this study indicated that either a single or split N application at rate of 10 kgN.ha^{-1} resulted in the best yield. The results suggested that both intermediate (moderate) rates of N produces the most desirable responses and the high rate may possibly be ineffectual for fruit production in one year old plants produced under HT conditions. Highest concentration of N at the end of the growing season remained in leaves which may be available for off-season production due to split application of N fertilizer. Nitrogen rates applied did not significantly affect the concentration of macro and micronutrient tested on July 11, with the exception of Mn and B.

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Appendix A. ***Statistical Data:***

Table A.1. Descriptive statistics of variables measured upon pomological traits in ‘Prime-Ark® 45’ blackberry cultivar.

Total yield (g)				
Treatment	Mean	SD	Minimum	Maximum
T1	426.00	159.23	217.90	601.30
T2	631.10	195.50	436.00	899.60
T3	815.33	343.83	505.60	1185.30
T4	269.38	82.03	163.50	363.30

Marketable yield (g)				
Treatment	Mean	SD	Minimum	Maximum
T1	354.35	139.93	189.10	530.70
T2	540.53	187.20	342.70	790.70
T3	725.23	343.79	413.90	1094.20
T4	221.15	79.14	128.10	315.90

Total fresh weight (Kg)				
Treatment	Mean	SD	Minimum	Maximum
T1	6.549	0.597	5.982	7.160
T2	6.778	1.205	5.768	8.516
T3	6.270	1.778	4.300	8.597
T4	6.518	0.738	5.590	7.200

Cane Diameter (mm)				
Treatment	Mean	SD	Minimum	Maximum
T1	11.27	0.86	10.30	12.31
T2	11.24	0.30	10.91	11.62
T3	11.55	0.89	10.30	12.39
T4	11.06	0.60	10.43	11.66

Table A.2. Descriptive statistics of variables measured upon elemental nutrient concentration in 'Prime-Ark® 45' blackberry cultivar, in Fayetteville, under high tunnel conditions.

		Leaf tissue concentration			
Variable*	N	Mean	SD	Minimum	Maximum
N	96	2.64	0.28	1.92	3.33
P	96	0.21	0.03	0.15	0.26
K	96	1.75	0.21	1.3	2.18
Ca	96	0.44	0.13	0.15	0.68
Mg	96	0.31	0.03	0.25	0.39
S	96	0.19	0.02	0.15	0.23
Na	95	23.34	30.27	1.3	125
Fe	96	65.11	32.96	40.8	359.2
Mn	96	127.01	82.36	16	419.8
Zn	96	34.47	4.20	25.8	43
Cu	96	10.18	2.21	6.2	20
B	96	33.70	4.93	25	43.8

* Units: N, P, K, Ca, Mg, and S: %;
Na, Fe, Mn, Zn, Cu, and B: $mg.kg^{-1}$.

Appendix B. ***Soil and foliar analysis:***

Table B.1. Soil analysis report for samples collected in Fayetteville on 29 Mar., under high tunnel conditions.

Date Processed:	3/29/2011
Field ID:	Fay. - High tunnel

1. Nutrient Availability Index

Nutrient	Concentration		Soil test level (Mehlich 3)
	ppm	**Kg·ha^{-1}**	
P	78	174.7	Above Optimum
K	136	304.6	Optimun
Ca	901	2018.2	-
Mg	68	152.3	-
SO4-S	17	38.1	-
Zn	3.4	7.6	-
Fe	237	530.9	-
Mn	133	297.9	-
Cu	2.1	4.7	-
B	0	0.0	-
NO3-N	15	33.6	Medium

2. Soil Properties

Property	**Value**	**Units**
Soil pH (1:2 soil-water)	6.2	--
Soil EC (1:2 soil-water)		μmhos/cm
Soil ECEC	7	cmolc/kg
Organic Matter (Loss on Ignition)		%
Estimated Soil Texture	Silt Loam	

Estimated base saturation (%)				
Total	Ca	Mg	K	Na
64.9	55.9	4.7	3.6	0.7

Table B.2. Soil analysis result for samples collected in Fayetteville on 16 May, under high tunnel conditions.

STUDY: Blackberry – High tunnel
ID: May 16
LOCATION: Fayetteville
ARRIVED: 5-24-2011
LOGGED: 5-26-2011
OUT: 6-01-2011
PROCEDURES : Mehlich 3 extractable (1:10 ratio), analysis by Spectro ARCOS ICP
PH: 6.4
EC: 459 umhos/cm

--------------------mg/kg--------------------											
P	K	Ca	Mg	S	Na	Fe	Mn	Zn	Cu	B	Total N
77.5	220.6	1076.3	86.1	111.5	13.1	260.2	178.2	3.6	2.0	0.6	1384

Table B.3. Soil analysis result for samples collected in Fayetteville on 29 Aug., under high tunnel conditions, Treatment 1.

Date Processed:	8/29/2011
Field ID:	Fay. – High tunnel – T1

1. Nutrient Availability Index

Nutrient	**Concentration**		**Soil test level (Mehlich 3)**
	ppm	**$kg \cdot ha^{-1}$**	
P	67	150.1	Above Optimum
K	101	226.2	Medium
Ca	935	2094.4	-
Mg	66	147.8	-
SO4-S	10	22.4	-
Zn	5.1	11.4	-
Fe	229	513.0	-
Mn	129	289.0	-
Cu	1.9	4.3	-
B	0.0	0.0	-
NO3-N	20	44.8	-

2. Soil Properties

Property	**Value**	**Units**
Soil pH (1:2 soil-water)	6.7	--
Soil EC (1:2 soil-water)		µmhos/cm
Soil ECEC	8	cmolc/kg
Organic Matter (Loss on Ignition)		%
Estimated Soil Texture	Silt Loam	

Estimated base saturation (%)				
Total	Ca	Mg	K	Na
68.8	58.1	6.8	3.2	0.8

Table B.4. Soil analysis result for samples collected in Fayetteville on 29 Aug., under high tunnel conditions, Treatment 2.

Date Processed:	8/29/2011
Field ID:	Fay. – High tunnel – T2

1. Nutrient Availability Index

Nutrient	**Concentration**		**Soil test level (Mehlich 3)**
	ppm	**kg·ha^{-1}**	
P	72	161.3	Above Optimum
K	109	244.2	medium
Ca	965	2161.6	-
Mg	73	163.5	-
SO4-S	15	33.6	-
Zn	3.9	8.7	-
Fe	222	497.3	-
Mn	136	304.6	-
Cu	2.0	4.5	-
B	0.0	0.0	-
NO3-N	28	62.7	-

2. Soil Properties

Property	**Value**	**Units**
Soil pH (1:2 soil-water)	6.3	--
Soil EC (1:2 soil-water)		µmhos/cm
Soil ECEC	8	cmolc/kg
Organic Matter (Loss on Ignition)		%
Estimated Soil Texture	Silt Loam	

Estimated base saturation (%)				
Total	Ca	Mg	K	Na
69.9	58.1	7.3	3.4	1.1

Table B.5. Soil analysis result for samples collected in Fayetteville on 29 Aug., under high tunnel conditions, Treatment 3.

Date Processed:	8/29/2011
Field ID:	Fay. – High tunnel – T3

1. Nutrient Availability Index

Nutrient	Concentration		Soil test level (Mehlich 3)
	ppm	$kg\cdot ha^{-1}$	
P	71	159.0	Above Optimum
K	101	226.2	Medium
Ca	923	2067.5	-
Mg	67	150.1	-
SO4-S	14	31.4	-
Zn	4.2	9.4	-
Fe	206	461.4	-
Mn	140	313.6	-
Cu	2.0	4.5	-
B	0.0	0.0	-
NO3-N	22	49.3	-

2. Soil Properties

Property	Value	Units
Soil pH (1:2 soil-water)	6.3	--
Soil EC (1:2 soil-water)		μmhos/cm
Soil ECEC	8	cmolc/kg
Organic Matter (Loss on Ignition)		%
Estimated Soil Texture	Silt Loam	

Estimated base saturation (%)				
Total	Ca	Mg	K	Na
68.8	57.5	7.0	3.2	1.1

Table B.6. Soil analysis result for samples collected in Fayetteville on 29 Aug., under high tunnel conditions, Treatment 4.

Date Processed:	8/29/2011
Field ID:	Fay. – High tunnel – T4

1. Nutrient Availability Index

Nutrient	Concentration		Soil test level (Mehlich 3)
	ppm	$kg \cdot ha^{-1}$	
P	70	156.8	Above Optimum
K	97	217.3	medium
Ca	880	1971.2	-
Mg	64	143.4	-
SO4-S	31	69.4	-
Zn	3.4	7.6	-
Fe	212	474.9	-
Mn	124	318.1	-
Cu	1.8	4.0	-
B	0.0	0.0	-
NO3-N	26	58.2	-

2. Soil Properties

Property	Value	Units
Soil pH (1:2 soil-water)	6.3	--
Soil EC (1:2 soil-water)		µmhos/cm
Soil ECEC	8	cmolc/kg
Organic Matter (Loss on Ignition)		%
Estimated Soil Texture	Silt Loam	

Estimated base saturation (%)				
Total	Ca	Mg	K	Na
67.7	56.8	6.9	3.2	0.8

Table B.7. Soil analysis result for samples collected in Fayetteville on 4 Oct., under high tunnel conditions, Treatment 1.

Date Processed:	10/04/2011
Field ID:	Fay. – High tunnel – T1

1. Nutrient Availability Index

Nutrient	**Concentration**		**Soil test level (Mehlich 3)**
	ppm	**$kg \cdot ha^{-1}$**	
P	68	152.3	Above Optimum
K	96	215.0	medium
Ca	1113	2493.1	-
Mg	68	152.3	-
SO4-S	15	33.6	-
Zn	3.9	8.7	-
Fe	262	586.9	-
Mn	149	333.8	-
Cu	2.3	5.2	-
B	0.3	0.7	-
NO3-N	11	24.6	-

2. Soil Properties

Property	**Value**	**Units**
Soil pH (1:2 soil-water)	6.6	--
Soil EC (1:2 soil-water)		µmhos/cm
Soil ECEC	9	cmolc/kg
Organic Matter (Loss on Ignition)		%
Estimated Soil Texture	Silt Loam	

Estimated base saturation (%)				
Total	Ca	Mg	K	Na
72.2	62.0	6.3	2.7	1.1

Table B.8. Soil analysis result for samples collected in Fayetteville on 4 Oct., under high tunnel conditions, Treatment 2.

Date Processed:	10/04/2011
Field ID:	Fay. – High tunnel – T2

1. Nutrient Availability Index

Nutrient	Concentration		Soil test level (Mehlich 3)
	ppm	$kg\cdot ha^{-1}$	
P	71	159.0	Above Optimum
K	96	215.0	Medium
Ca	1126	2522.2	-
Mg	66	147.8	-
SO4-S	15	33.6	-
Zn	4.1	9.2	-
Fe	267	598.1	-
Mn	154	345.0	-
Cu	2.4	5.4	-
B	0.3	0.7	-
NO3-N	14	31.4	-

2. Soil Properties

Property	Value	Units
Soil pH (1:2 soil-water)	6.4	--
Soil EC (1:2 soil-water)		µmhos/cm
Soil ECEC	10	cmolc/kg
Organic Matter (Loss on Ignition)		%
Estimated Soil Texture	Silt Loam	

Estimated base saturation (%)				
Total	Ca	Mg	K	Na
68.5	59.1	5.8	2.6	1.1

Table B.9. Soil analysis result for samples collected in Fayetteville on 4 Oct., under high tunnel conditions, Treatment 3.

Date Processed:	10/04/2011
Field ID:	Fay. – High tunnel – T3

1. Nutrient Availability Index

Nutrient	**Concentration**		**Soil test level (Mehlich 3)**
	ppm	**$kg \cdot ha^{-1}$**	
P	74	165.8	Above Optimum
K	94	210.6	Medium
Ca	1065	2385.6	-
Mg	65	145.6	-
SO4-S	18	40.3	-
Zn	3.9	8.7	-
Fe	240	537.6	-
Mn	150	336.0	-
Cu	2.1	4.7	-
B	0.2	0.4	-
NO3-N	15	33.6	-

2. Soil Properties

Property	**Value**	**Units**
Soil pH (1:2 soil-water)	6.4	--
Soil EC (1:2 soil-water)		µmhos/cm
Soil ECEC	9	cmolc/kg
Organic Matter (Loss on Ignition)		%
Estimated Soil Texture	Silt Loam	

Estimated base saturation (%)				
Total	Ca	Mg	K	Na
71.3	61.1	6.2	2.8	1.2

Table B.10. Soil analysis result for samples collected in Fayetteville on 4 Oct., under high tunnel conditions, Treatment 4.

Date Processed:	10/04/2011
Field ID:	Fay. – High tunnel – T4

1. Nutrient Availability Index

Nutrient	**Concentration**		**Soil test level (Mehlich 3)**
	ppm	**kg·ha^{-1}**	
P	70	156.8	Above Optimum
K	93	208.3	Medium
Ca	1137	2546.9	-
Mg	66	147.8	-
SO4-S	18	40.3	-
Zn	3.9	8.7	-
Fe	243	544.3	-
Mn	140	313.6	-
Cu	2.1	4.7	-
B	0.3	0.7	-
NO3-N	9	20.2	-

2. Soil Properties

Property	**Value**	**Units**
Soil pH (1:2 soil-water)	6.4	--
Soil EC (1:2 soil-water)		µmhos/cm
Soil ECEC	10	cmolc/kg
Organic Matter (Loss on Ignition)		%
Estimated Soil Texture	Silt Loam	

Estimated base saturation (%)				
Total	Ca	Mg	K	Na
68.8	59.2	5.7	2.5	1.4

IV. CHAPTER 2

DETERMINATION OF THE OPTIMUM PERIOD FOR FOLIAR SAMPLING IN PRIME-ARK® 45 BLACKBERRY LEAVES.

Abstract

To determine the optimum period for foliar sampling in which elemental concentrations in ‘Prime-Ark® 45’ primocane-fruiting (PF) blackberry leaves are the most stable (the range of dates when elemental concentrations are similar), four studies were conducted at three locations, two in Arkansas (Fayetteville and Clarksville) and one in North Carolina (N.C.), from spring to fall of 2011. In Fayetteville, at the Univ. of Ark. Agr. Res. and Ext. Ctr., two experiments were conducted. In the first study, one-year-old ‘Prime-Ark® 45’ blackberry plants were cultivated under high tunnel conditions with conventional management practices and the effects of four N fertilization rates were evaluated (0- control, 10, 10-split, and 20 kg $N{\cdot}Ha^{-1}$). In the second study, under ambient conditions and organic management practices, three types of cultural practices were compared in three-years-old ‘Prime-Ark® 45’ blackberry plants: mowing of canes, (mown on 15 May), mowing and tipping (mown on 15 May and tip 15 June), and not pruned (control). In Clarksville, at the Univ. of Ark. Fruit Res. Sta., three blackberry cultivars, ‘Prime-Ark® 45,’ ‘Prime-Jan®,’ and ‘Ouachita,’ were used in order to compare the variation in elemental concentration of PF leaves to floricane-fruiting blackberries, because for this genotype the seasonal variation in elemental concentration has been more thoroughly studied. Plant phenology was noted in all experiments. In N.C., the period of stability in elemental concentration was determined over time for ‘Prime-Ark® 45’ blackberry plants, which were grown at three commercial orchards (‘SunnyRidge’ farms at Toluca, Faith, and Owl’s Den). All leaf samples were collected following the protocol described by Clark (1988), rinsed, ground, and analyzed.

Total N was analyzed by combustion and P, K, Ca, Mg, S, Na, Fe, Mn, Zn, Cu, and B by wet digestion. It was found that all elemental concentrations in ‘Prime-Ark® 45’ blackberry leaves varied according to sampling date for all locations, except in Clarksville, where sampling date did not significantly affect Fe concentration. The period of relative stability in foliar nutrient concentrations and the best time for leaf sampling were as follows: In Fayetteville under high tunnel and ambient conditions, from 11 July to 25 July (from 10% to 50% bloom) and from 7 July to 25 July (10% bloom to first ripe fruit), respectively. In Clarksville, the period with most stability in elemental nutrient content was from 30 June to 12 July (first harvest), and in N.C. from 5 July to 22 July (after 10% bloom).

In Fayetteville under high tunnel conditions, the N rate treatments affected only P and K concentrations, and under ambient conditions cultural practices treatment affected Mn and Zn concentrations. In Clarksville the cultivar treatment did not affect the elemental concentrations of any cultivar.

A complementary statistical analysis to determine when the lowest variance in foliar nutrient concentrations occurred was conducted only for N.C., and the results indicated that the lowest variance occurred from 5 July to 22 July for most of the elements.

Introduction

Increased concentrations of nutrient elements in plant tissues produce responses such as increased plant growth and fruit yield (Taiz and Zeiger, 2006). These concentrations can be affected by several factors such as environment, date of sampling, and genotype. Hughes et al. (1979) stated that in red raspberries cultivated in Oregon, the age of the plant did not significantly influence foliar elemental concentrations, while genotype and date of sampling resulted in wide variation in nutrient concentration. The authors determined that the period of minimum variance to nutrient concentrations occurred during the last two weeks of August. Clark et al. (1988) stated that sampling date significantly influenced elemental leaf content of floricane-fruiting blackberries. The authors concluded that in Arkansas, the period between mid-July to mid-August is the best for collecting leaf samples for floricane-fruiting blackberries due to the stability in foliar elemental concentrations.

Plant analysis is a technique to measure the elemental content of tissue of a particular plant part and to determine nutritional status (Jones, 2001). Depending on sampling time and the method used to determine the concentration of elemental nutrients in specific samples, there are corresponding standardized values exist for each nutrient and crop (Krishna, 2002).

For leaf sampling in brambles, the most recent mature and completely expanded leaves are randomly collected in midsummer (from mid-July to mid-August), taking only one leaf per cane (Clark et al., 1988; Jones, 2001; Domoto, 2007). Clark et al. (1988) suggested the lowest variation in elemental concentration occurs in mature leaves between the 6^{th} and 10^{th} node from the apex of primocanes. Studying the seasonal variation of nutrient concentration in leaves of three blackberry cultivars (‘Cherokee’, ‘Cheyenne’, and ‘Comanche’), Clark et al. (1988) determined that concentrations of N, P, K, Zn, Cu, and Fe were highest in May; then they decreased from June to August. Initial Ca content increased and then, from May, remained stable in all subsequent samplings. Magnesium content in leaves fluctuated during the period of sampling. It increased until July but then decreased until the last sampling date. Cultivars had significantly different concentrations for P, Ca, Mg, Zn, Fe, and Mn.

New consumer preferences for ‘healthy’ produce and the availability of organic cultivation guidelines and technologies, such as high tunnels, have increased the interest in organic production of blackberry under HT conditions.

Blackberries can be cultivated organically under HTs, with fewer problems than other small fruits due to enhanced environmental conditions, decreased pest and disease problems, and higher quality fruits (Rom et al., 2010). Yields of primocane-fruiting (PF) blackberries under high tunnel conditions are increased, and the growing season and off-season fruit production is extended (Thompson, et al., 2009). In addition, HT promote increased plant vigor, fruit quality, and fewer diseases in bramble production (Lamont et al., 2002; Hanson et al., 2011; Thompson, et al., 2009; Rom et al., 2010). According to Heidenreich et al., (2008), blackberry production season can be extended for a period of weeks by using HTs. In some summer-fruiting cultivars, the harvest can be extended beyond May and for some fall fruiting cultivars until November.

The phenological stages provide significant information than calendar dates for crop management. Phenology is important in plant testing because the nutrient status and the demand change throughout the growing season. The most critical stage for plant testing is the period from bloom to the early fruiting stage for most fruit plants (Havlin et al., 2006).

Experiments in other fruit species of the Rosaceous family can be used to obtain relevant information about blackberry nutrition. Some trends of elemental seasonal variation are described as follows: In apples, N, P, and K trends of nutrient concentration decreased throughout the growing season and as the leaves develop. Calcium, Mg, and B concentrations increased because plants demanded more of these nutrients as the growing season progressed (Rom, 1994). These trends are also consistent with those concentration trends found by Wright and Waister (1980) in red raspberries, except for Mg, which had a stable pattern. Clark et al. (1988) found in floricane-fruiting blackberry genotypes that the foliar elemental concentrations changed seasonally. The trends of these elements over time were similar to those for apples (Rom, 1994) and for red raspberries (Wright and Waister, 1980) described above.

The objective of this study was to determine, at three locations, the optimum period for collecting foliar samples in which nutrient element concentrations in ‘Prime-Ark® 45’ blackberry leaves are the most stable. The period of stability is defined as the interval of sampling dates for which the elemental concentrations, and aggregates, do not change or are statistically similar.

Materials and Methods

This study was conducted from May to Sept. 2011 in two states, Arkansas and North Carolina (N.C.). In Arkansas, in two locations: Fayetteville at the University of Arkansas Agricultural Research and Ext. Center, and Clarksville at the University of Arkansas Fruit Research Station. In N.C., in three ‘SunnyRidge’ farms (commercial orchards): Owl’s Den (Lincoln County), Toluca (Cleveland County), and Faith (Cleveland County).

Leaf analyses:

To conduct leaf analysis, six leaves were taken randomly from each experimental unit (EU). Each EU was a plot with five plants where a specific treatment was applied. Every two weeks, mature leaves, located six to ten nodes below the growing tip, were collected (Clark et al., 1988). Criteria for leaf selection included full solar exposure, no insect, disease, or mechanical damage, and no visible nutrition imbalances (Jones, 2001). When samples were collected, phenological stages were noted. After collection, leaves were rinsed in deionized water, labeled, placed in paper bags, and brought to the laboratory. In the laboratory, leaves were dried in a convection oven (24 hr, 70^{o}C), and passed through a 20 mesh sieve using the Intermediate Wiley Mill (A.H. Thomas Co., Philadelphia). Total N was analyzed by combustion in an Elementar VarioMax analyzer instrument (Elementar Americas Inc., Mt. Laurel, NJ) (Horneck and Miller, 1998), and P, K, Ca, Mg, S, Na, Fe, Mn, Zn, Cu, and B were analyzed by wet digestion using concentrated nitric acid and 30% hydrogen peroxide on a hot block. A 0.25 g sample was digested and brought to 25 ml volume by adding deionized water (Huang and Schulte, 1985). The digested solution was analyzed using an inductively coupled plasma spectrophotometer (ARCOS-SOP, ICP - Spectro Analytical Instrument, Mahwah, NJ) (Donohue and Aho, 1992). This analysis was conducted by the Agricultural Diagnostic Laboratory-Altheimer - Dept. of Crop, Soil, and Environ. Sci., University of Arkansas, Fayetteville.

Experiment 1. Fayetteville, Arkansas:

This study was conducted at the University of Arkansas Agricultural Research and Ext. Center, Fayetteville (lat. 36^{o}5’4” N, long. 94^{o}10’29”W), from spring to fall in 2011. This section of the study was conducted on plots in two environments: a) high tunnel and b) ambient.

Experiment 1.a. Fayetteville - High tunnel: ‘Prime-Ark® 45’ PF blackberry plants were cultivated under high tunnel (HT) conditions and leaf samples were collected from June to August, one sample from each treatment (five plants – per EU). The soil type was a Captina silt loam (typic fragiudult). Soil samples were collected using a probe and penetrating vertically 20 cm below ground level and removing a representative 1 kg of soil per treatment area as is suggested by Jones (2001). The soil analysis (soil sampled on 29 Mar., 2011) indicated a pH of 6.2, and a moderately low cation exchange capacity of 7.0 $cmolc.kg^{-1}$. The soil pH was measured according to the procedure indicated by Donahue (1983), in a soil-water mixture extraction of 1:2 (weight:volume). Soil NO_3-N was extracted with aluminum sulfate and measured with a specific-ion electrode (Donahue, 1992), according to procedures described by Baker and Thompson (1992). For the remaining macro- and microelements, the soil sample was processed and extracted by using the Mehlich-3 solution (Mehlich, 1984), and the concentrated solution was measured by using the Inductively Coupled Plasma (ICP) Emission Spectroscopy

(Donohue and Aho, 1992). These analyses were conducted at the Soil Testing and Research Laboratory, University of Arkansas, Marianna. Results were 15, 78, and 136 $mg.kg^{-1}$ for N, P, and K, respectively (Appendix D, Table D.1). All soil tests were analyzed following the same procedures and methods (Appendix D, Tables D.3 to D.10), with the exception of another soil analysis for P, K, Ca, Mg, S, Na, Fe, Mn, Zn, Cu, and B conducted on 16 May at the Agr. Diagnostic Laboratory - Altheimer - Dept. of Crop, Soil, and Environ. Sci., Univ. of Ark., Fayetteville. The soil sample was processed and extracted by Mehlich-3 solution (Mehlich, 1984), and the concentrated solution was measured by Spectro ARCOS-SOP (side on plasma) – ICP (Appendix D, Table D.2). The soil analysis report of a sample collected on 29 Mar. (Appendix D, Table D.1) indicated that NO_3-N level in soil before planting was medium (33.6 $kg \cdot ha^{-1}$), P was above optimum (174.7 $kg \cdot ha^{-1}$), and K was optimum (304.6 $kg \cdot ha^{-1}$) in the complete area of planting. Similar reports for samples per treatment collected on 29 Aug. (Appendix D, Tables D.3, 4, 5, and 6), indicated that for all treatments, P content in soil was above optimum (150.1, 161.3, 159, and 156.8 $kg \cdot ha^{-1}$; Treatments1, 2, 3, and 4, respectively) and K was medium (226.2, 244.2, 226.2, and 217.3 $kg \cdot ha^{-1}$; Treatments1, 2, 3, and 4, respectively). The NO_3-N concentration was medium (44.8, 62.7, 49, and 58 $kg \cdot ha^{-1}$; Treatments1, 2, 3, and 4, respectively). Based on the soil analyses, per treatment, sampled on 4 Oct. (Appendix D, Tables D.7, 8, 9, and 10) for all treatments, P concentration in soil was above optimum (152.3, 159, 165.8, and165.8 $kg \cdot ha^{-1}$; Treatments 1, 2, 3, and 4, respectively) and K was medium (215, 215, 210.6, and 208.3 $kg \cdot ha^{-1}$; Treatments 1, 2, 3, and 4, respectively). The NO_3-N soil concentration was medium (24.6, 31.4, 33.6, and 20.2 $kg \cdot ha^{-1}$; Treatments 1, 2, 3, and 4, respectively).

For the experiment, a Quonset (Single-bay) type of HT (7.6 m wide, 58.8 m long) was used. The HT (Haygrove solo series, Haygrove Ltd., U.K.) had been covered with 6 mm transparent plastic. Prior to planting, soil surface was leveled with a 0.5^{o} slope for proper drainage. The area of the high tunnel was divided into three rows. 'Prime-Ark® 45' plantlets were established in the two side rows. The rows were divided in four blocks. The west side row had only one block and the east side row three blocks. Each block contained all treatments. Raised beds were constructed (0.85 m wide, 0.15 m high) with drip irrigation, which consisted of in-line emitters spaced at 0.6 m. The rows were watered (3.8 $L \cdot h^{-1}$) for about 30 min (7.6 L) per day, from April to December. One-year-old cuttings were planted in rows in the high tunnel on 18 Mar. 2011. Five days after planting, primocanes were cut to 10 cm above soil level to invigorate the plants and induce branching. Plants were weeded manually during the growing season on a regular basis. On 5 Apr., fresh rice hulls mulch was applied to the whole planting area and the row borders were covered with black plastic mulch to prevent erosion and weed growth, leaving the planted row exposed 0.3 m wide. Plants were tipped on 14 June when they were 1.0 m tall. Plants were trellised on 27 June using a 'V' double curtain shape.

The effects of four N fertilizer rates were studied as follows: control- no N applied (Treatment 1), 10 kg $N \cdot ha^{-1}$ (Treatment 2), 10 kg $N \cdot ha^{-1}$ - split application (Treatment 3), and 20 kg $N \cdot ha^{-1}$ (Treatment 4). Ammonium sulfate (21-0-0) was the fertilizer N source due to its low leaching and hygroscopicity (Havlin et al., 2006). The fertilizer was broadcast uniformly on the surface of the row between the main canes in a 3.0 m x 0.25 m band. The first, N fertilizer single applications, and 50% of the split application were broadcast on 20 May, 63 days after planting. The remaining 50% of the split application was broadcast on 27 July, 131 days after planting. Two-spotted spider mites *(Tetranychus urticae* Koch) and Japanese beetles (*Popillia japonica* Newman) were observed in low and treatable levels of infestation. Two species of beneficial predatory mites (*Galendromus occidentalis* Nesbitt and *Neoseiulus californicus* McGregor) were released in the HT on 9 June and 4 Aug. to control the two-spotted spider mites. Kaolin clay (Surround WP®) was applied on 9 June and 1 July to control the Japanese beetles (Johnson and Lewis, 2005; Rom et al., 2010).

The statistical design was a randomized complete block (RCB) design. The data were analyzed as a split-plot in time for elemental concentration in ‘Prime-Ark® 45’ leaves, with N fertilization rates as the whole plot and the date as the subplot with four replicates. All analysis was carried out using Statistical Analysis System Program 9.2 (SAS Institute, Carry, N.C.). The ANOVA was conducted by the PROC MEANS and PROC MIXED procedures. Mean separation was by Least Significant Difference (LSD) at the 0.05 probability level ($p<0.05$).

Experiment 1.b. Fayetteville - Ambient: Two-year-old ‘Prime-Ark® 45’ PF blackberry plants were cultivated under ambient conditions and organic management practices. The blackberry ‘Prime-Ark® 45’ plants were established in six rows. Five plants per each EU were distributed on each row. Raised beds were constructed (0.85 m wide, 0.15 m high) with a drip irrigation system, which consisted of in-line emitters spaced at 0.60 m. The soil texture was silt loam- silt clay loam. To increase organic matter and soil fertility, poultry litter fertilizer was incorporated into the soil, 1 kg per experimental unit on 4 Apr. Nutrient concentration in poultry litter was total N= 2.8 %, NH_4-N= 2,291 $mg \cdot kg^{-1}$, NO_3-N= 2 $mg \cdot kg^{-1}$, Total P, 1.4 %, Total K, 1.8%. In addition, 0.74 $kg \cdot m^{-1}$ of an organic commercial fertilizer 3-1-5 (Bradfield Organics® - Lucious Lawn & Garden, PMI Nutrition International, Brentwood, MO) was applied. The soil analysis report indicated the soil had a pH of 6.2, and a cation exchange capacity of 14.0 $cmolc.kg^{-1}$. The soil had actual level content of NO_3-N, P, and K nutrients of 19, 76, and 253 $mg.kg^{-1}$ (above optimum), respectively (Apendix D, Table D.11). Weeds were controlled manually, and plants were mulched with aged hardwood bark. Plants were drip irrigated as needed. This experiment had 3 treatments, not pruned (control, neither mow nor tip - Treatment 1); mowing (Treatment 2); and mowing and tipping (Treatment 3), and these cultural practices were conducted as follows: First, for Treatment 1, plants did not receive any treatment (neither mow nor tip). For Treatment 2, plants were mowed 10 cm above ground on 15 May. Treatment 3, plants were mowed on 15 May 10 cm above ground and tipped by removing the main cane's growing tip when plants were 1 m high on 15 June. Leaf samples were collected from these plants from June to August. Phenological stages were noted for every sampling date.

The design of the experiment was a randomized complete block design with four replicates. The data were analyzed as a split-plot in time for elemental concentration, having three management practices as the whole plot and the date as the subplot. All analysis was conducted using Statistical Analysis System Program 9.2 (SAS Institute, Carry, N.C.), The ANOVA was calculated by the PROC MEANS and PROC MIXED procedures. Means separated by Least Significant Difference (LSD) at the 0.05 probability level.

Experiment 2. Clarksville, Arkansas:

This study was conducted at the University of Arkansas, Fruit Research Station at Clarksville, Ark. (lat. 35^{o}31'58" N, long. 93^{o}24'2" W) between spring and fall in 2011. The soil type was a Linker fine sandy loam (typic hapludults). Previously, a soil analysis indicated the soil had a pH of 6.2, and a moderately low cation exchange capacity of 7.0 cmolc/kg. The soil had a content of N, P, and K nutrients of 19, 44, and 101 mg.kg^{-1}, respectively (Appendix D, Table D.12). The area of the study had two rows and several EU (5 plants) with diverse blackberry cultivars. The rows in the field ran east to west, running perpendicular on a 25% slope. Plants were provided drip irrigation with in-line emitters, and water was applied as needed. The EUs were selected according to the cultivar required for this research. These plants were two-year-old, mulched with black plastic, and trellised in a 'V' type. Canes were trained between double sets of trellis wires located at 0.6 m and 1.2 m high.

Leaves from three blackberry cultivars were sampled from May to July. The treatments were two PF blackberry cultivars: 'Prime-Ark® 45'and 'Prime-Jan®,' and one floricane-fruiting blackberry cultivar: 'Ouachita.' This last cultivar was utilized as a control to assess differences in the seasonal variance of elemental concentration between PF versus floricane-fruiting blackberries. When samples were collected, phenological stages were noted. Plants were managed as follows. Compound fertilizer (19-19-19) was applied twice, once on 25 Mar. and again on 31 May. Per each EU 312 g were applied (five plants per plot). Plants were provided drip irrigation with in-line emitters spaced at 0.30 m intervals. Pruning was conducted in mid-November. No relevant pests or diseases were observed.

The design of the experiment was a randomized complete block. The data were analyzed as a split-plot in time, with three blackberry cultivars as the whole plot and the sampling date as the subplot. All analysis was carried out using Statistical Analysis System Program 9.2 (SAS Institute, Carry, N.C.). The ANOVA was calculated by the PROC MEANS and PROC MIXED procedures. Means separated by Least Significant Difference (LSD) with 5% level of significance ($p<0.05$).

Experiment 3. North Carolina:

This study was conducted on three farms: Owl's Den - Lincoln County (lat. 35°30'10" N, long.81°18'43" W), Toluca - Cleveland County (lat. 35°27'56" N, long.81°32'15" W), and Faith - Cleveland County (lat. 35°22'4" N, long.81°38'39" W). Five-year-old 'Prime-Ark® 45' PF blackberry plants were cultivated at those three commercial orchards. The soil type was clay loam at Toluca farm, clay loam at Owl's Den farm, and sandy clay loam at Faith farm. The soils had moderate natural fertility and moderate organic matter content. All farms had pH values of approximately 6.0, and a cation exchange capacity of 14.0 cmolc/kg. Periodic liquid fertilization was applied to maintain optimum soil-test levels (J. Beam, personal comm.). Fertilization, irrigation, and weed control followed standard commercial practices. Plants were provided drip irrigation with in-line emitters spaced at 0.3 m intervals. Plants were tipped at Toluca Farm on 12 May, at Owl's Den Farm on 10 May, and at Faith farm on 15 May. No insects or diseases were observed. Four plots per farm were established to collect samples.

To determine the most stable period of nutrient concentration over time, data of elemental foliar concentration for macro- and micronutrients were analyzed using a complete randomized block design with split-plot in time, having sampling dates as treatments and rows within farms as blocks. Leaves were collected from three farms (blocks) and four replicates. All analysis was carried out using Statistical Analysis System Program 9.2 (SAS Institute, Carry, N.C.). The ANOVA was calculated by the PROC MEANS and PROC MIXED procedures. Mean separation was by Least Significant Difference (LSD) at the 0.05 probability level. To support the statistical findings, a complementary observational description was conducted. It was assumed that the description of trends can help to confirm the statistical findings and accomplish the objective of this study, which consists of identifying periods of stability in elemental nutrient content.

Determining the lowest variance of elemental nutrient concentration in 'Prime-Ark 45®' leaves over time in North Carolina:

'Prime-Ark® 45' leaf concentrations data from North Carolina were appropriate for determining the date at which variances of nutrient concentrations among samples are the minimum. All data were transformed, and the logarithm of leaf elemental concentration variance for macro- and micronutrients were analyzed as a completely randomized block design, with dates of leaf sampling as treatments and farms (locations) as blocks. The analysis was carried out statistically by using SAS 9.2 (SAS Institute, Cary, N.C.), the ANOVA was calculated by the PROC MEANS and PROC MIXED. Means separated by Least Significant Difference (LSD) at $P<0.05$.

Results

Experiment 1. Fayetteville, Arkansas:

Experiment 1.a. Fayetteville - High tunnel:

Nitrogen fertilization rates applied in ‘Prime-Ark® 45’ blackberries significantly affected P and K foliar elemental concentrations ($p<0.05$) (Appendix C, Table C.5). For P, N fertilization rates had different responses ($p=0.016$). Treatments 1 and 4 (control and 20 Kg N$^{\cdot}$Ha^{-1}, respectively) were not significantly different from each other and showed the lowest foliar concentrations (0.20% for both treatments), while Treatments 2 and 3 (10 and 10-split Kg N$^{\cdot}$Ha^{-1}, respectively) were not significantly different and had the highest foliar concentrations (0.21% in both). Also for K, N fertilization treatment had different responses ($p=0.008$). Treatment 2 (10 Kg N$^{\cdot}$Ha^{-1}) had the highest K concentration (1.82%) and was significantly different from Treatments 1, 3, and 4 (Control, 10-split, and 20 Kg N$^{\cdot}$Ha^{-1}, respectively), whose concentrations were statistically similar (1.7%, 1.8%, and 1.7%, respectively). Nitrogen fertilization rates did not significantly affect foliar elemental concentration in N, Ca, Mg, S, Na, Fe, and B.

Sampling date significantly affected the leaf concentration of N, P, K, Ca, Mg, S, Na, Fe, and B in ‘Prime-Ark® 45’ blackberries at $p<0.05$ (Appendix C, Table C.5).

Significant interactions of N fertilization rates x sampling dates (Appendix C, Table C.5) were found for Mn (Table 2.1), Zn (Table 2.2), and Cu (Table 2.3) leaf concentrations in ‘Prime-Ark® 45’ for the sampling period ($p<0.05$).

Through the LSD mean comparison for each element, it was found that N, K, Ca, Mg, Na, and Fe foliar concentrations were not significantly different between 11 July and 25 July sampling dates for all treatments, and shared a period of stability for these elements. Independently, per each element, foliar concentration of P, S, and B was significantly different during the sampling period. These concentrations fluctuated, which indicates no period of stability for these elements between 11 July and 25 July (Table 2.4). Also, the independent analysis of interactions by using the LSD test for Mn (Table 2.1), Zn (Table 2.2), and Cu (Table 2.3) foliar concentrations indicated that all of these elemental mean contents had a shared period of relative stability in elemental concentrations between 11 July and 25 July sampling dates. During these intervals, the foliar concentrations were statistically similar.

In a qualitative description, elemental concentrations in ‘Prime-Ark® 45’ leaves changed during the sampling period as follows: Foliar N content was the highest in June (2.88%). Then, the N concentration decreased during the subsequent sampling dates to 2.31% (Table 2.4). Phosphorous and K decreased during for the sampling dates (from 0.22 to 0.19% and 1.99 to 1.57%, respectively). Conversely, B concentration increased over time, from the first sampling date (28.14 $mg.kg^{-1}$) to the final sampling dates (39.09 $mg \cdot kg^{-1}$), exhibiting some minor fluctuation in the last sampling date (Table 2.4). However, a period of relative stability was observed from 29 June to 11 July. Calcium, Mg, S, Na, and Fe concentrations (Table 2.4) fluctuated over the entire sampling period. All these elements, except S, had a period of relative stability during 11 July to 25 July (Table 2.4). For all N rate treatments, Mn (Table 2.1), Zn (Table 2.2), and Copper (Table 2.3) concentrations fluctuated during the sampling period. All these elements had a period of relative stability during 11 July to 25 July.

Plant phenology: In Fayetteville under high tunnel conditions, 10% blooming was observed on 4 July, 50% of plants were in bloom on 25 July. First ripe fruit was observed on 4 Aug. Harvest ended on 17 Nov. with a freeze event.

Table 2.1. Experiment 1.a: Interaction of N fertilization rates and sampling date on Mn concentration in leaves of ‘Prime-Ark® 45’ PF blackberry cultivars grown in Fayetteville, Ark. under high tunnel conditions from June to August 2011.

N rates ($Kg.ha^{-1}$)	Sampling dates					
	14 Jun	29 Jun	11 Jul	25 Jul	4 Aug.	26 Aug.
			$mg.kg^{-1}$			
Control	67.88	57.55	60.45	66.63	45.50	122.77
10	224.72	177.12	77.75	72.10	48.50	185.72
10-split	155.07	96.40	94.63	85.43	60.75	230.92
20	229.60	188.35	178.90	141.52	99.25	280.70

LSD (α=0.05) to compare means at same date: 70.94
LSD (α=0.05) to compare means at different dates: 59.96
Shaded area represents similarities of means within elements and dates of stability in nutrient concentrations for most of the elements.

Table 2.2. Experiment 1.a: Interaction of N fertilization rates and sampling date on Zn concentration in leaves of ‘Prime-Ark® 45’ PF blackberry cultivars grown in Fayetteville, Ark. under high tunnel conditions from June to August 2011.

N rates ($Kg.ha^{-1}$)	Sampling dates					
	14 Jun	29 Jun	11 Jul	25 Jul	4 Aug.	26 Aug.
			$mg.kg^{-1}$			
Control	30.33	37.28	34.85	36.63	28.25	31.68
10	35.53	37.00	32.75	35.03	33.43	32.83
10-split	31.15	37.95	30.45	38.73	37.28	33.38
20	31.50	41.30	35.38	37.40	31.08	36.10

LSD (α=0.05) to compare means at same date: 4.40
LSD (α=0.05) to compare means at different dates: 4.68
Shaded area represents similarities of means within elements and dates of stability in nutrient concentrations for most of the elements.

Table 2.3. Experiment 1.a: Interaction of N fertilization rates and sampling date on Cu concentration in leaves of 'Prime-Ark® 45' PF blackberry cultivars grown in Fayetteville, Ark. under high tunnel conditions.

N rates	Sampling dates					
($Kg.ha^{-1}$)	14 Jun	29 Jun	11 Jul	25 Jul	4 Aug.	26 Aug.
			$mg.kg^{-1}$			
Control	7.98	13.43	10.98	10.75	9.20	7.80
10	8.70	10.75	10.68	11.55	12.73	7.50
10-split	8.10	11.18	9.73	11.70	13.63	7.25
20	7.83	11.05	10.58	11.98	11.10	8.08

LSD (α=0.05) to compare means at same date: 1.867
LSD (α=0.05) to compare means at different dates: 1.869
Shaded area represents similarities of means within elements and dates of stability in nutrient concentrations for most of the elements.

Table 2.4. Experiment 1.a: Mean nutrient concentrations of macro and micronutrients in one-year-old 'Prime-Ark® 45' blackberry leaves collected in Fayetteville, Ark. under high tunnel conditions, and sampled from June to August 2011.

	Sampling Date					
Element	14-Jun	29-Jun	11-Jul	25-Jul	4 Aug.	26 Aug.
			%			
N	2.88 c[z]	2.88 c	2.67 b	2.66 b	2.43 a	2.31 a
P	0.22 b	0.24 c	0.19 a	0.21 b	0.19 a	0.19 a
K	1.99 c	1.91 c	1.78 b	1.73 b	1.55 a	1.57 a
Ca	0.47 b	0.44 b	0.47 bc	0.52 dc	0.21 a	0.56 d
Mg	0.29 b	0.32 d	0.30 bc	0.30 bc	0.27 a	0.36 e
S	0.20 d	0.22 e	0.18 b	0.20 d	0.17 ab	0.18 b
			$mg.kg^{-1}$			
Na	14.88 b	9.95 ab	9.12 ab	14.78 ab	87.19 c	2.73 a
Fe	55.39 a	89.29 b	51.44 a	66.13 a	55.88 a	72.54 ab
B	28.14 a	30.53 b	31.82 b	34.07 c	39.09 d	38.56 d

[z]Means separated by LSD, P= 0.05. Each value is a mean of 16 sample concentrations.
Means within elements with the same letter are statistically similar.
Shaded area represents similarities of means within elements and dates of stability in nutrient concentrations for most of the elements, based on the period that includes statistical similarities in all of the elements.

Experiment 1.b. Fayetteville - Ambient:

The cultural practice treatments resulted in significant effects on Mn and Zn concentrations (p<0.05), (Appendix C, Table C.6). For Mn, cultural practice treatments had significantly different responses (p=0.02). The Treatment 1, (not pruned - control) and Treatment 2 (mown) were not significantly different from each other and showed higher foliar concentrations (169.85 and 149.92 $mg \cdot kg^{-1}$, respectively) than Treatment 3. Conversely, Treatment 3 (mown and tip) had the lowest concentration (112.50 $mg \cdot kg^{-1}$), and was significantly different from the others. Also, cultural practice treatment affected significantly Zn concentrations in leaves (p=0.03). The higher foliar concentration responses were observed in the Treatment 1 (not pruned - control, 38.29 $mg \cdot kg^{-1}$) and Treatment 2 (mown, 37.63 $mg \cdot kg^{-1}$), which were not significantly different from each other. The lowest concentration was observed in Treatment 3 (mown and tip, 33.71 $mg \cdot kg^{-1}$), which was significantly different from the other two treatments. Cultural practice did not significantly affect elemental concentration in N, P, K, Mg, S, and Fe (Appendix C, Table C.6).

Sampling dates significantly affected the foliar concentrations of N, P, K, Mg, S, Fe, Mn, Zn in ‘Prime-Ark® 45’ blackberries (p<0.05), (Appendix C, Table C.6).

Significant differences were found for the interaction of cultural practices x sampling dates at p<0.05 (Appendix C, Table C.6), for Ca (Table 2.5), Na (Table 2.6), Cu (Table 2.7), and B (Table 2.8) in leaf concentrations of ‘Prime-Ark® 45’blackberries for the sampling period.

The results of the LSD mean comparison for each element indicated that N, P, K, Mg, S, Fe, and Mn foliar concentrations were statistically similar between 7 July to 25 July sampling dates, having a similar a period of stability during that time. Zinc did not have similar means over the same sampling dates (Table 2.9). Additionally, the independent analysis of interactions for Ca (Table 2.5), Na (Table 2.6), Cu (Table 2.7), and B (Table 2.8) foliar concentrations by using the same test indicated that all of these means had a common period of relative stability between 7 July and 25 July sampling dates.

Qualitatively, the 'Prime-Ark® 45' foliar concentration trends for the sampling period were as follows: N and P concentration means decreased from 10 Jun to 24 Jun. Then, they exhibit a trend of relative stability (Table 2.9). Potassium fluctuated during the sampling period and displayed a period of stability from 7 July to 25 July (Table 2.9). Magnesium and S concentrations increased over time and these elemental concentrations had a similar period of stability from 7 July to 25 July (Table 2.9). Iron and Mn varied overtime, these foliar elemental concentrations had a common period of stability during 7 July to 25 July (Table 2.9). Zinc concentration fluctuated during the sampling period and it had a similar period of stability with the rest of the elements from 10 Jun to 7 July (Table 2.9). For all cultural practices, Na leaf concentration was low during June and July, but increased during the first week of August and then decreased at the final sampling date. Despite those fluctuations, Na concentration showed a period of stability between 7 July and 25 July (Table 2.6). Calcium, Cu, and B concentrations varied according to the sampling dates (Tables 2.5, 2.7, and 2.8, respectively) and these elements displayed a period of stability from 7 July to 25 July.

Plant phenology: In Fayetteville under ambient conditions, 10% bloom of primocanes was observed on 24 June and first ripe fruit was on 14 July.

Table 2.5. Experiment 1.b: Interaction of cultural practice and sampling date on Ca concentration in leaves of 'Prime-Ark® 45' PF blackberry cultivars grown in Fayetteville, Ark. under ambient conditions from June to August 2011.

Cultural practices	Sampling dates					
	10 Jun	24 Jun	7 July	25 July	4 Aug.	27 Aug.
			%			
Control	0.52	0.38	0.49	0.49	0.38	0.40
Mowing	0.55	0.45	0.47	0.62	0.45	0.49
Mowing + Tipping	0.54	0.62	0.52	0.55	0.30	0.50

LSD (α=0.05) to compare means at same date: 0.13
LSD (α=0.05) to compare means at different dates: 0.10
Shaded area represents similarities of means within elements and dates of stability in nutrient concentrations for most of the elements.

Table 2.6. Experiment 1.b: Interaction of cultural practice and sampling date on Na concentration in leaves of 'Prime-Ark® 45' PF blackberry cultivars grown in Fayetteville, Ark. under ambient conditions from June to August 2011.

Cultural practices	Sampling dates					
	10 Jun	24 Jun	7 July	25 July	4 Aug.	27 Aug.
			$mg.kg^{-1}$			
Control	16.33	19.00	23.38	19.08	101.00	3.93
Mowing	22.03	20.65	18.45	18.55	116.25	5.55
Mowing + Tipping	8.88	13.03	14.68	20.98	86.75	6.50

LSD (α=0.05) to compare means at same date: 10.62
LSD (α=0.05) to compare means at different dates: 10.58
Shaded area represents similarities of means within elements and dates of stability in nutrient concentrations for most of the elements.

Table 2.7. Experiment 1.b: Interaction of cultural practice and sampling date on Cu concentration in leaves of 'Prime-Ark® 45' PF blackberry cultivars grown in Fayetteville, Ark. under ambient conditions from June to August 2011.

Cultural practices	Sampling dates					
	10 Jun	24 Jun	7 July	25 July	4 Aug.	27 Aug.
			$mg.kg^{-1}$			
Control	10.18	12.58	12.53	15.78	11.48	14.80
Mowing	9.85	11.03	11.85	13.35	8.98	15.03
Mowing + Tipping	10.30	9.80	11.20	12.10	10.58	18.53

LSD (α=0.05) to compare means at same date: 2.64
LSD (α=0.05) to compare means at different dates: 2.37
Shaded area represents similarities of means within elements and dates of stability in nutrient concentrations for most of the elements.

Table 2.8. Experiment 1.b: Interaction of cultural practice and sampling date on B concentration in leaves of 'Prime-Ark® 45' PF blackberry cultivars grown in Fayetteville under ambient conditions from June to August 2011.

Cultural practices	Sampling dates					
	10 Jun	24 Jun	7 July	25 July	4 Aug.	27 Aug.
			$mg.kg^{-1}$			
Control	40.13	31.75	34.15	38.50	36.90	36.58
Mowing	41.03	32.23	34.18	42.75	43.25	38.35
Mowing + Tipping	36.55	35.70	34.43	35.73	41.90	39.65

LSD (α=0.05) to compare means at same date: 6.59
LSD (α=0.05) to compare means at different dates: 4.59
Shaded area represents similarities of means within elements and dates of stability in nutrient concentrations for most of the elements.

Table 2.9. Experiment 1.b: Mean nutrient concentrations of macro and micronutrients in 'Prime-Ark® 45' blackberry leaves collected in Fayetteville, Ark. under ambient conditions, sampled from June to August 2011.

Element	Sampling Date					
	10-Jun	24-Jun	7-Jul	25-Jul	4 Aug.	27 Aug.
			%			
N	2.73 b[z]	2.29 a	2.40 a	2.40 a	2.19 a	2.36 a
P	0.28 d	0.23 b	0.22 b	0.24 bc	0.19 a	0.24 bc
K	1.56 b	1.52 b	1.64 bc	1.69 c	1.63 bc	1.42 a
Mg	0.28 a	0.28 a	0.30 b	0.30 b	0.32 c	0.34 d
S	0.15 a	0.17 b	0.18 c	0.18 c	0.19 c	0.20 d
			$mg.kg^{-1}$			
Fe	60.69 a	63.70 ab	61.62 ab	57.10 a	59.83 a	66.87 c
Mn	177.20 c	133.00 a	126.80 a	147.20 ab	118.40 a	161.90 b
Zn	33.09 a	33.21 a	34.68 a	42.86 b	33.71 a	41.72 b

[z]Means separated by LSD, *P*= 0.05. Each value is a mean of 12 sample concentrations.
Means within elements with the same letter are statistically similar.
Shaded area represents similarities of means within elements and dates of stability in nutrient concentrations for most of the elements, based on the period that includes statistical similarities in all of the elements.

Experiment 2. Clarksville, Arkansas:

Cultivar did not significantly affect Mg, Fe, Mn, Zn, and B foliar concentrations at $p<0.05$ (Appendix C, Table C.7).

Sampling date significantly affected Mg, Mn, Zn, and B foliar concentrations ($p<0.05$). However, Fe concentrations were not affected by sampling date (Appendix C, Table C.7).

Significant interactions for blackberry cultivar x sampling date (Appendix C, Table C.7) were found for N (Table 2.10), P (Table 2.11), K (Table 2.12), Ca (Table 2.13), S (Table 2.14), Na (Table 2.15), and Cu (Table 2.16) in 'Prime-Ark® 45' leaf concentrations at $p<0.05$ for the sampling period.

The LSD mean comparison per each element showed that Mg, Mn, Zn, and B foliar concentrations were statistically similar between 20 June and 12 July sampling dates, exhibiting a similar period of stability (Table 2.17). Also, the independent analysis of interactions by using the LSD test, indicated that these means of foliar concentrations had a common period of relative stability mostly located between 20 June and 12 July sampling dates, however, some exceptions were observed as follows: for N (Table 2.10), 'Ouachita' and 'Prime-Ark® 45' had statistically different means on 12 Jul. For P (Table 2.11), 'Ouachita' had a statistically different mean from other dates on 12 Jul and 'Prime-Ark® 45' on 20 Jun. For K (Table 2.12), 'Prime Jan®' had a statistically different mean from other dates on 12 Jul. For Ca (Table 2.13), 'Ouachita' had a statistically different mean from other dates on 20 Jun and 'Prime-Ark® 45' on 12 Jul. For S (Table 2.14), 'Ouachita' and 'Prime-Ark® 45' on 12 Jul. For Na (Table 2.15), 'Prime-Ark® 45' and 'Prime Jan®' on 12 Jul. Finally, for Cu (Table 2.16), in 'Prime Jan®' on 12 Jul.

Qualitatively, the 'Prime-Ark® 45' foliar concentrations described trends during the sampling period as follows: N foliar concentrations increased from May to June, and then these elemental concentrations decreased during the later sampling dates until 12 Jul. The last sampling date on 28 Jul the N leaf content increased (Table 2.10). These N concentrations showed a relative period of stability from 20 June to 30 Jun sampling dates. Phosphorous, K, and S concentrations increased from May to June and then these elemental concentrations decreased during the later sampling dates (Tables 2.11, 2.12, and 2.14, respectively), and these elemental concentrations displayed a relative period of stability from 20 June to 12 July sampling dates. Conversely, Mg, Fe, and B leaf concentrations increased during the sampling period, and they had a period of relative stability from 20 June to 12 July sampling dates (Table 2.17). Iron increased from 31.44 $mg^{.}kg^{-1}$ on 19 May to 73.07 $mg^{.}kg^{-1}$ for the last sampling date (28 Jul), and B from 17.00 to 26.12 $mg.kg^{-1}$. Manganese, and Ca leaf content decreased from May to June, and then these elemental concentrations increased during the later sampling dates (Table 2.17 and table 2.13, respectively). Manganese and Ca concentrations displayed a period of relative stability from 20 June to 30 June. Copper foliar concentrations (Table 2.16), fluctuated from the beginning to the end of the sampling period; also, Na (Table 2.15) and Zn (Table 2.17) foliar concentrations fluctuated during the same sampling period and they had a period of stability between 20 June to 12 July sampling dates.

Plant phenology: In Clarksville, 10% bloom of floricanes was observed on 25 April in 'Ouachita', 16 April in 'Prime-Jan®', and on 17 April in 'Prime-Ark® 45.' First harvest was on 13 June in 'Ouachita', on 5 June in 'Prime-Jan®', and on 6 June in ''Prime-Ark® 45.' Last harvest was on 25 July in 'Ouachita,' 9 July in 'Prime-Jan®', and 8 July for 'Prime-Ark® 45.'

Table 2.10. Experiment 2: Interaction of cultivar and sampling date on N concentrations in leaves of three blackberry cultivars grown in Clarksville, Ark. from June to August 2011.

Cultivar	Sampling dates					
	19 May	3 Jun	20 Jun	30 Jun	12 Jul	28 Jul
			%			
Ouachita	2.99	2.95	2.28	2.24	1.98	2.06
Prime Jan®	2.68	2.21	1.80	1.72	1.78	1.77
Prime-Ark 45®	3.05	3.09	1.95	1.80	1.67	1.84

LSD (α=0.05) to compare means at same date: 0.25
LSD (α=0.05) to compare means at different dates: 0.24
Shaded area represents similarities of means within elements and dates of stability in nutrient concentrations for most of the elements.

Table 2.11. Experiment 2: Interaction of cultivar and sampling date on P concentrations in leaves of three blackberry cultivars grown in Clarksville, Ark. from June to August 2011.

Cultivar	Sampling dates					
	19 May	3 Jun	20 Jun	30 Jun	12 Jul	28 Jul
			%			
Ouachita	0.23[z]	0.25	0.19	0.16	0.15	0.15
Prime Jan®	0.22	0.19	0.19	0.17	0.19	0.17
Prime-Ark 45®	0.25	0.27	0.22	0.18	0.17	0.13

LSD (α=0.05) to compare means at same date: 0.04
LSD (α=0.05) to compare means at different dates: 0.03
Shaded area represents similarities of means within elements and dates of stability in nutrient concentrations for most of the elements.

Table 2.12. Experiment 2: Interaction of cultivar and sampling date on K concentrations in leaves of three blackberry cultivars grown in Clarksville, Ark. from June to August 2011.

Cultivar	Sampling dates					
	19 May	3 Jun	20 Jun	30 Jun	12 Jul	28 Jul
			%			
Ouachita	1.44[z]	1.77	1.18	1.15	1.21	1.08
Prime Jan®	1.23	1.32	1.21	1.33	1.55	1.33
Prime-Ark 45®	1.44	1.83	1.54	1.49	1.47	1.15

LSD (α=0.05) to compare means at same date: 0.21
LSD (α=0.05) to compare means at different dates: 0.17
Shaded area represents similarities of means within elements and dates of stability in nutrient concentrations for most of the elements.

Table 2.13. Experiment 2: Interaction of cultivar and sampling date on Ca concentrations in leaves of three blackberry cultivars grown in Clarksville, Ark. from June to August 2011.

Cultivars	Sampling dates					
	19 May	3 Jun	20 Jun	30 Jun	12 Jul	28 Jul
			%			
Ouachita	0.57	0.58	0.67	0.84	0.86	0.96
Prime Jan®	0.59	0.70	0.70	0.71	0.62	0.68
Prime-Ark 45®	0.56	0.38	0.41	0.44	0.58	0.87

LSD (α=0.05) to compare means at same date: 0.12
LSD (α=0.05) to compare means at different dates: 0.13
Shaded area represents similarities of means within elements and dates of stability in nutrient concentrations for most of the elements.

Table 2.14. Experiment 2: Interaction of cultivar and sampling date on S concentrations in leaves of three blackberry cultivars grown in Clarksville, Ark. from June to August 2011.

Cultivar	Sampling dates					
	19 May	3 Jun	20 Jun	30 Jun	12 Jul	28 Jul
			%			
Ouachita	0.19	0.22	0.19	0.19	0.14	0.13
Prime Jan®	0.15	0.15	0.13	0.13	0.12	0.12
Prime-Ark 45®	0.19	0.23	0.17	0.16	0.13	0.12

LSD (α=0.05) to compare means at same date: 0.023
LSD (α=0.05) to compare means at different dates: 0.022
Shaded area represents similarities of means within elements and dates of stability in nutrient concentrations for most of the elements.

Table 2.15. Experiment 2: Interaction of cultivar and sampling date on Na concentrations in leaves of three blackberry cultivars grown in Clarksville, Ark. from June to August 2011.

Cultivar	Sampling dates					
	19 May	3 Jun	20 Jun	30 Jun	12 Jul	28 Jul
			$mg.kg^{-1}$			
Ouachita	21.87	19.17	8.97	9.26	6.23	17.23
Prime Jan®	15.50	19.10	11.40	12.10	4.60	19.13
Prime-Ark 45®	19.53	10.50	13.97	14.53	5.30	23.07

LSD (α=0.05) to compare means at same date: 5.69
LSD (α=0.05) to compare means at different dates: 5.31
Shaded area represents similarities of means within elements and dates of stability in nutrient concentrations for most of the elements.

Table 2.16. Experiment 2: Interaction of cultivar and sampling date on Cu concentration in leaves of three blackberry cultivars grown in Clarksville, Ark. from June to August 2011.

Cultivar	Sampling dates					
	19 May	3 Jun	20 Jun	30 Jun	12 Jul	28 Jul
			$mg.kg^{-1}$			
Ouachita	6.20[z]	7.17	7.90	6.79	6.77	7.10
Prime Jan®	8.13	7.77	11.93	10.13	12.70	10.50
Prime-Ark 45®	7.97	8.33	11.63	11.97	10.97	6.90

LSD (α=0.05) to compare means at same date: 2.35
LSD (α=0.05) to compare means at different dates: 1.95
Shaded area represents similarities of means within elements and dates of stability in nutrient concentrations for most of the elements.

Table 2.17. Experiment 2: Nutrient concentrations of macro and micronutrients in leaves of three blackberry cultivars collected in Clarksville, Ark., sampled from May to July 2011.

Element	Sampling Date					
	19-May	3-Jun	20-Jun	30-Jun	12-Jul	28-Jul
			%			
Mg	0.23 a[z]	0.30 b	0.31 b	0.32 b	0.29 b	0.32 b
			$mg{\cdot}kg^{-1}$			
Fe	31.44[NS]	51.14[NS]	66.62[NS]	55.56[NS]	69.14[NS]	73.07[NS]
Mn	132.98 a	118.46 a	122.87 a	148.86 ab	188.04 b	250.28 c
Zn	30.68 c	29.51 bc	32.52 d	29.45 bc	26.61 b	22.24 a
B	17.00 a	17.19 a	15.36 a	18.15 ab	19.62 b	26.12 c

[z]Means separated by LSD, *P*= 0.05. Each value is a mean of 9 sample concentrations. Means within elements with the same letter are statistically similar.
Shaded area represents similarities of means within elements and dates of stability in nutrient concentrations for most of the elements, based on the period that includes statistical similarities in all of the elements.
[NS]Nonsignificant.

Experiment 3. North Carolina:

There were significant differences for P, K, Ca, Mg, Na, Fe, and B foliar concentrations of 'Prime-Ark® 45' across the six sampling dates (p<0.05). However, there were no significant differences in sampling dates for N, S, Mn, Zn, and Cu foliar concentrations (Appendix C, Table C.8).

The LSD test for mean comparison for P, K, Ca, and Mg, foliar concentrations indicated that they were statistically similar between 5 July and 22 July sampling dates, showing a period of stability for all of these elements (Tables 2.18). Nitrogen and S concentrations were statistically similar throughout the sampling period. For the microelements, the LSD test indicated that for Na, Fe, and B foliar concentrations there were statistically similar foliar mean content between 5 July and 22 July sampling dates, showing a period of stability for all of them (Tables 2.19). Manganese, Zn, and Cu concentrations were statistically similar throughout the sampling period.

Qualitatively, the data indicated that mean concentrations had specific trends for each element tested over time as follows: N, P, K and S concentrations decreased during the sampling period. Nitrogen and S content were higher in May (3.26 and 0.22%, respectively) than in later sampling dates. Nitrogen and S concentrations decreased until the last week of July (2.36 and 0.15%, respectively), but exhibited a small increase during the last sampling dates in August (2.48 and 0.16%, respectively) (Table 2.18). Also, P and K foliar concentrations decreased from the beginning until the final sampling period (from 0.29 and 1.91 to 0.17 and 1.22%, respectively) (Table 2.18). Calcium concentrations fluctuated over time. Calcium concentrations decreased from the first date of sampling (last week of May to first week of June). Then, the concentration increased until the third week of July when the concentration decreased until the last date (Table 2.18).

Magnesium, Na, Fe, Mn, and B foliar concentrations increased during the sampling period (Tables 2.18 and 2.19). With the exception of an initial fluctuation during the second and third sampling dates, Na and Mn foliar content increased in the remaining sampling dates. Iron had a significantly lower foliar concentration in May (37.7 $mg.kg^{-1}$), then values were higher and remained consistent for the remainder of the sampling period. Magnesium and B concentrations increased from the first sampling date (0.31% and 33.14 $mg.kg^{-1}$, respectively) throughout the sampling period with only a minor decrease between the last two sampling dates (Tables 2.18 and 2.19). Sodium and Mn concentrations fluctuated from the first sampling date until the end of June, then they increased in subsequent sampling dates (Table 2.19).

Zinc and Cu concentrations decreased over time. All of these elements showed a period of relative stability in elemental concentrations between 5 July to 22 July sampling dates (Tables 2.18 and 2.19).

Plant phenology: In North Carolina, 10% bloom of primocanes was observed as follows: at Toluca Farm on 15 June, at Owl’s Den Farm on 13 June, and at Faith Farm on 14 June. First harvest was: at Toluca Farm on 1 Aug., at Owl’s Den Farm on 1 Aug., at Faith Farm on 31 July. Last harvest was on 21 Oct. at all North Carolina plantings.

Table 2.18. Experiment 3: Mean nutrient concentrations of macronutrients in 'Prime-Ark 45®' blackberry leaves collected in North Carolina from May to August 2011.

Element	Sampling Date						
	20-May	4-Jun	22-Jun	5-Jul	22-Jul	4 Aug.	22 Aug.
				%			
N	3.26^{NS}	2.91^{NS}	2.63^{NS}	2.51^{NS}	2.36^{NS}	2.50^{NS}	2.48^{NS}
P	0.29 b	0.28 b	0.23 ba	0.19 a	0.18 a	0.18 a	0.17 a
K	1.91 c	1.97 c	1.59 b	1.65 b	1.40 ba	1.37 ba	1.22 a
Ca	0.44 a	0.38 a	0.48 ab	0.62 bc	0.75 c	0.67 c	0.66 c
Mg	0.31 a	0.34 ab	0.37 b	0.40 c	0.41 c	0.44 d	0.41 c
S	0.22^{NS}	0.22^{NS}	0.19^{NS}	0.17^{NS}	0.15^{NS}	0.17^{NS}	0.16^{NS}

^{z} means separated by LSD, *P*<0.05. Each value is a mean of 12 sample concentrations.
Means within elements with the same letter are statistically similar.
Shaded area represents similarities of means within elements and dates of stability in nutrient concentrations for most of the elements, based on the period that includes statistical similarities in all of the elements.
^{NS}Nonsignificant.

Table 2.19. Experiment 3: Mean nutrient concentrations of micronutrients in 'Prime-Ark 45®' blackberry leaves collected in North Carolina, sampled from May to August 2011.

Element	Sampling Date						
	20-May	4-Jun	22-Jun	5-Jul	22-Jul	4 Aug.	22 Aug.
				$mg \cdot kg^{-1}$			
Na	16.82 a^{z}	12.50 a	6.05 a	17.51 a	25.37 a	114.42 b	119.92 b
Fe	37.71 a	63.08 b	62.79 b	55.08 b	63.81 b	68.58 b	63.92 b
Mn	221.04^{NS}	169.81^{NS}	190.28^{NS}	239.79^{NS}	339.20^{NS}	355.67^{NS}	392.92^{NS}
Zn	35.30^{NS}	37.67^{NS}	31.82^{NS}	29.39^{NS}	29.87^{NS}	28.54^{NS}	28.58^{NS}
Cu	9.38^{NS}	11.45^{NS}	9.73^{NS}	9.35^{NS}	9.20^{NS}	8.83^{NS}	8.02^{NS}
B	33.14 a	39.58 ab	36.38 ab	41.61 bc	47.90 c	60.08 d	58.80 d

^{z} means separated by LSD, *P*<0.05. Each value is a mean of 12 sample concentrations.
Means within elements with the same letter are statistically similar.
Shaded area represents similarities of means within elements and dates of stability in nutrient concentrations, based on the period that includes statistical similarities in all of the elements.
^{NS}Nonsignificant.

Determining the lowest variance of elemental nutrient concentration in 'Prime-Ark 45®' leaves over time in North Carolina:

The analysis of the means of logarithm (log) variances of foliar concentrations showed that the variance among leaf samples during the sampling period were not significantly different for N, P, K, Mg, S, Fe, Mn, Zn, and Cu content in 'Prime-Ark® 45' leaves. The foliar concentrations of Ca, Na, and B, however, were significantly different (Appendix C, Table C.9). The means of log variances of Ca concentrations fluctuated during the sampling period. At the beginning, higher values were observed. Then, the lowest and statistically similar variances were observed on 22 June, 5 July, and 22 July sampling dates. On the subsequent sampling dates the variances of the concentrations were statistically different and larger (Table 2.20). The means of log variances of Na concentrations increased over time. Due to the statistical similarities, this element had the lowest concentration from 20 May to 22 July (Table 2.21). Boron variances, within element, increased over time. The lowest variances were observed on three sampling dates, (20 May, 4 June, and 22 July) (Table 2.21).

Table 2.20. Experiment 3: Mean of log variances of macronutrient concentrations of 'Prime-Ark 45®' in North Carolina, sampled from May to August 2011, to identify when the minimum variance period occurs.

Element	Sampling Date						
	20-May	4-Jun	22-Jun	5-Jul	22-Jul	4 Aug.	22 Aug.
N	-2.96[z]NS	-3.56NS	-4.03NS	-3.63NS	-4.66NS	-3.50NS	-2.84NS
P	-7.71NS	-7.66NS	-6.98NS	-7.99NS	-7.79NS	-8.39NS	-8.86NS
K	-4.25NS	-4.10NS	-3.25NS	-4.99NS	-4.87NS	-4.23NS	-5.07NS
Ca	-6.80 c	-6.50 bc	-4.49 a	-3.97 a	-5.13 ab	-6.28 bc	-4.26 a
Mg	-8.30NS	-8.00NS	-6.55NS	-6.80NS	-7.19NS	-7.26NS	-7.38NS
S	-8.28NS	-8.73NS	-9.34NS	-9.45NS	-9.14NS	-9.19NS	-8.06NS

[z] mean of log variances separated by LSD, P<0.05.
Mean of log variances within elements with the same letter are statistically similar.
Shaded area represents similarities of mean of log variances within elements and sampling dates of lowest variance in nutrient concentrations.
NSNonsignificant.

Table 2.21. Experiment 3: Mean of log variances of micronutrient concentrations of 'Prime-Ark 45®' in North Carolina, sampled from May to August 2011, to identify when the minimum variance period occurs.

Element	Sampling Date						
	20-May	4-Jun	22-Jun	5-Jul	22-Jul	4 Aug.	22 Aug.
Na	1.40[z] a	2.23 ab	1.46 ab	1.98 ab	2.94 ab	4.58 bc	6.05 c
Fe	2.94NS	5.25NS	4.04NS	3.66NS	4.10NS	4.40NS	3.26NS
Mn	8.12NS	7.27NS	7.78NS	7.41NS	7.69NS	8.04NS	8.92NS
Zn	1.53NS	1.94NS	2.40NS	1.89NS	2.46NS	2.59NS	1.18NS
Cu	-0.85NS	-0.73NS	-0.49NS	0.21NS	0.49NS	0.05NS	-1.22NS
B	0.58 a	1.11 a	3.17 b	3.09 b	1.23 a	3.50 b	3.76 b

[z] mean of log variances separated by LSD, $p<0.05$.
Means of log variances within elements with the same letter are statistically similar.
Shaded area represents similarities of mean of log variances within elements and sampling dates of lowest variance.
NSNonsignificant.

Discussion

In all locations, the average of N, P, K, Ca, Mg, S, Na, Fe, Mn, Zn, Cu and B means of foliar concentration (foliar analyses conducted from May to August) were within the ranges stated by Pritts (1991), Havlin et al. (2006), and Garcia (2007), but also within the concentration values for floricane-fruiting blackberries described by Clark et al. (1988). On average, N concentrations values were below 3%, which is inside the range required for plant growth (Pritts, 1991; Havlin et al., 2006; Garcia, 2007; Clark et al., 1988). Nitrogen levels greater than 3% in tissue test result in N toxicity to plants, excessive growth, and small number of flower buds as indicated by Strik (2003). Hence, the N supply from soil in those plantings was sufficient to meet plant demand. Nutritional imbalances were not observed or noticed through leaf analyses on these plants, and yield decreased at high N rate.

Experiment 1. Fayetteville, Ark.:

Experiment 1.a. Fayetteville - High tunnel: Under these conditions, N fertilization rates had diverse effects on elemental nutrient content in leaves of 'Prime - Ark® 45' blackberry.

Nitrogen fertilization rates provided significantly different responses for P and K leaf concentrations. The effects of N fertilization treatments on 'Prime - Ark® 45' blackberry leaf elemental concentrations for the sampling period can be attributed directly to the N fertilization levels used in this experiment because this was the major nutrient contained in the soil in different amounts while the reminder macro- and microelement were not modified and at optimum levels. According to Naraguma and Clark (1998) N, P, K, Ca, S, and Mn leaf content were affected by both N rate and application time treatments in 'Arapaho' thornless (floricane-fruiting) blackberry, which is consistent with our results for P and K, only.

For P, results indicated that Treatments 2 and 3 (10 and 10-split kg $N^{.}ha^{-1}$, respectively) had the higher responses in leaf nutrient content, while Treatments 1 and 4 (0-control and 20 kg $N^{.}ha^{-1}$, respectively) had the lowest responses and were similar to each other, but significantly different from Treatments 2 and 3. Both concentrations values 0.21% (Treatment 2 and 3) and 0.20% (Treatments 1 and 4) were within the limits of the standardized for N foliar concentrations.

Also for K, Treatment 2 (10 kg $N^{.}ha^{-1}$) had the highest response in leaf nutrient content (1.82%), while Treatments 1 (1.7%), 3 (1.76%), and 4 (1.74%) had the lowest responses. These three treatments were statistically similar to each other (0-control, 10-split, and 20 kg $N^{.}ha^{-1}$, respectively) but significantly different from Treatment 2. Treatment 2 appeared to be more effective for increased K foliar content due to N fertilization in 'Prime - Ark® 45' blackberries for plant growth and development. Sufficiency range for K foliar content is between 1.5 and 2.5% (Pritts, 1991; Garcia, 2007). Even though these foliar concentrations were significantly different, they may not be biologically significant. In like manner, Naraguma and Clark, (1998) found that N fertilization rates affected P and K foliar concentrations. However, some concentrations were significantly different but too small and not relevant for plant nutrition.

Increased amounts of N are found in leaves after N fertilization. According to Malik et al. (1991) and Naraguma at al. (1999), recently N fertilizer applied is allocated first in new plant parts such as primocane leaves and berries and these N tissue concentrations remain until the end of the season. Even though N fertilization rates did not produce significant differences in N foliar concentration, there are numerical differences in foliar concentrations that reflects the different N levels applied.

Sampling date affected N, P, K, Ca, Mg, S, Na, Fe, and B foliar concentrations of ‘Prime-Ark® 45’ blackberries, showing significantly different concentrations for the sampling period. For N and B, this is consistent with Kowalenko (2006) who found that leaf tissue N concentrations in ‘Willamette’ raspberry, after N and B fertilization, were too dynamic and had high variance. This made it difficult and unsuitable to determine plant N status without comparative (standardized values) data. Also in this experiment, elemental nutrient concentrations of P and Mn were variable according to sampling dates. The seasonal variation in leaf nutrient concentrations, according to sampling dates found by Clark et al. (1988), was consistent with our results for N, P, K, Ca, Mg, and Fe concentrations (S, Na, and B were not studied by those authors).

The interaction of N fertilization rate x sampling date significantly affected Mn, Zn, and Cu concentrations, which means that the trends in N concentrations over time were different for different fertilization treatments. Naraguma and Clark, (1998) stated that 'Arapaho' thornless blackberries develop different types of responses in leaf elemental content in regard to the amount of and time when N fertilizer is applied. So, these types of interactions were expected. For these interactions, independent analyses per element were conducted to identify periods of stability through the LSD test, which is described below.

According to our objective, it was assumed that a detailed analysis of interactions were not relevant in this case. The central purpose of this study is to identify the periods of stability of elemental nutrient content, if any. The qualitative description of mean concentration trends helped to support the statistical finding of this study.

Based on the LSD comparison and the statistical similarities, there is a period of relative stability of nutrient concentration means between 11 July and 25 July sampling periods (approximately) in one-year-old 'Prime-Ark® 45' blackberry leaves collected in Fayetteville under high tunnel conditions.

The shared period of stability, found statistically, was supported by the qualitative description of mean concentration trends over time. Numerically, N content was the highest in June and remained stable from 11 July to 25 July. The N concentration decreased during the subsequent sampling dates. Also, P and K decreased during the sampling period with a period of stability from 11 July to 25 July. This N tendency is consistent with those described for apples, raspberries and blackberries by Wright and Waister (1980), Clark et al. (1988) and Rom (1994).

Calcium, Mg, Na, Fe, Cu, Mn, and Zn concentrations fluctuated over the entire sampling period, but had a period of relative stability between 11 July and 25 July. Calcium trend was not consistent with the trend described for apples (Rom, 1994), but consistent with the Ca trend indicated by Hughes et al. (1979) for red raspberries and Clark et al. (1988) for floricane-fruiting blackberries. In apples, Mg concentrations increased during the sampling period (Rom, 1994). However, our result is consistent with Hughes et al. (1979), which found that concentrations fluctuated over time for red raspberries. Also, Wright and Waister (1980) found the same concentration trend in the same type of raspberry. Similarly, for floricane-fruiting blackberries, Clark et al. (1988) found that Mg concentrations fluctuated for the sampling dates. In red raspberries, Hughes et al. (1979) found that Mn concentration decreased over time, which is not consistent with our result. Conversely, Fe concentrations decreased during the sampling period in floricane-fruiting blackberries (Clark et al., 1988). Copper concentrations over time fluctuated, which is consistent for the red raspberries (Hughes et al., 1979), but not for the three cultivars of floricane-fruiting blackberries studied by Clark et al. (1988). In our study, Zn concentrations fluctuated during the sampling period, while Zn concentrations decreased in the three cultivars of floricane-fruiting blackberries studied by Clark et al. (1988). Sodium concentrations fluctuated during the sampling period, but descriptions of concentrations over time were not found in the literature for this element. Also, Mn, Na, and S concentration trends were not reported by Clark et al., (1988) for blackberries, nor in Wright and Waister, (1980) for raspberries, or Rom (1994) for apples.

Boron concentrations increased over time, from the first sampling date to the end sampling dates, exhibiting some minor fluctuations for the last sampling date. This concentration trend is consistent with the trend for apples described by Rom (1994) and for red raspberries by Wright and Waister (1980). However, in red raspberries, leaf B concentrations fluctuated during the growing season (Huges et al., 1979; Kowalenko, 2006).

As a result, a common period of relative stability of elemental content in leaves was observed from 11 July to 25 July, approximately, due to the N fertilization rates applied to 'Prime-Ark® 45' under high tunnel conditions for most of the elements, which mostly coincides with the trends found for apples, raspberries, and floricane-fruiting blackberries.

It was found, statistically, that there are periods of relative stability in foliar elemental concentration means caused by N fertilization rate effects for N, Ca, Mg, S, Na, Fe, Cu, and B. The description of most of the elemental trends helped to confirm the statistical findings. These periods of relative stability of foliar nutrient concentration means mostly occurred between 11 July and 25 July in 'Prime-Ark® 45' blackberry leaves collected in Fayetteville in high tunnel conditions. Likewise, this period coincides with the phenology stage of 10% to 50 % bloom.

Experiment 1.b. Fayetteville - Ambient: Plants of 'Prime-Ark® 45' cultivated under organic management were subjected to the following cultural practice treatments: not pruned - control, mown, and mown and tip (Treatment 1, 2, and 3, respectively).

Cultural practices significantly affected the concentration of Mn and Zn. Drake and Clark (2003), studying the effects of some cultural practices, in ‘Prime-Jan®,’ and ‘Prime-Jim®,’ such as tipping and cane management, found significant effects on yield and berry weight. This indicates that those cultural practices altered the physiological and nutritional activity of these plants so that they can produce more fruit, requiring more nutrients. Independent analysis for Mn indicated that Treatment 1, (not pruned - control) and Treatment 2 (mown) were statistically similar from each other and showed the higher foliar concentrations (169.85 and 149.92 $mg \cdot kg^{-1}$, respectively) while Treatment 3 (mown and tip) had the lowest concentration (112.50 $mg \cdot kg^{-1}$), and was significantly different from the others. Manganese foliar concentrations were high at the beginning of June and decreased during the subsequent months until the end of August. This fact could imply that Mn was required during the blooming and initial fruiting periods, because as is explained in Havlin et al. (2006), the role of Mn in photosynthesis and other organic processes, and promoting enzyme reactions is well known. Also, cultural practice treatment significantly affected Zn concentrations in leaves. The higher foliar concentration responses were observed in the Treatment 1 (not pruned - control, 38.29 $mg \cdot kg^{-1}$) and Treatment 2 (mown, 37.63 $mg \cdot kg^{-1}$), which were not significantly different from each other. The lowest concentration was observed in Treatment 3 (mown and tip, 33.71 $mg \cdot kg^{-1}$), which was significantly different from the other two previous treatments. The Zn concentration in leaves increased from the blooming period to the end of the growing season. Zinc is an important cofactor and participates in several enzymatic functions (Havlin et al. (2006). The availability of Zn is positively correlated with the availability of auxins, which is important to promote cell elongation (Pritts, 1999). Thus, the increased Zn foliar concentrations observed during the last weeks of July and August could mean that this nutrient was present in sufficient amounts for plant development (Table 2.9). Sufficiency range for Zn foliar content is between 20 and 50 $mg \cdot kg^{-1}$ (Pritts, 1991; Garcia, 2007). Cultural practices did not significantly affect the concentrations of N, P, K, Mg, S, and Fe.

Sampling date significantly affected the concentration of N, P, K, Mg, S, Fe, Mn, and Zn, for the sampling period possibly due to physiological, phenological, and environmental factors. Most of these effects may be attributed to changes in elemental concentration according to the phenological stages, as the levels were changing for fruit production. According to Edwards and Asher (1974), for periods of exponential development, nutrient requirement increases in regard to plant age and size. Thus, for optimum plant growth, nutrients supply and uptake have to increase. Not only in red raspberries but also in floricane-fruiting blackberries, sampling date affected foliar nutrient concentrations generating the seasonal variation in leaf nutrient concentrations, according to sampling dates (Clark et al., 1988; John and Daubeny, 1972).

The cultural practices x sampling date interactions were only significant for Ca, Na, Cu, and B concentrations. These interactions required independent description in order to understand the pattern through both factors affected the foliar concentrations. Also, these analyses were performed per element so that periods of stability of elemental concentration through the LSD test can be determined, which is described below.

As a result of the LSD mean comparison, a stable period in foliar concentration was observed between 7 July to 25 July sampling dates, for most of the elements in ‘Prime-Ark® 45’ blackberry leaves collected in Fayetteville under ambient conditions. Also independent analysis for N, P, K, Ca, S, Na, and Cu due to interactions by using the same test, revealed the same period of relative stability.

Statistical findings were supported by the qualitative description of elemental foliar concentration trends for the sampling period as follows: In a numerical basis, N and P leaf content decreased during the sampling period. These trends were consistent with the N and P foliar concentration trends for apples, red raspberries, and the three floricane-fruiting blackberry cultivars described by Rom, (1994), Wright and Waister (1980), and Clark et al. (1988), respectively. Potassium foliar concentrations fluctuated over time. This finding contradicts the foliar content trends found by Rom (1994) for apples, Wright and Waister (1980) for red raspberries, and Clark et al. (1988) for three floricane-fruiting blackberry cultivars. A reason for this fluctuation might be need of this element for fruit development due to the increased concentrations observed during the fruiting period (7 July to 4 Aug.). These elements showed a period of stability between 7 July and 25 July sampling dates.

Calcium, Na, Cu, and B foliar concentrations varied according to the sampling dates and these elements described a period of stability between 7 July to 25 July sampling dates. Calcium trend was not consistent with the foliar concentration trend described for apples (Rom, 1994) for red raspberries, or for floricane-fruiting blackberries (Clark et al., 1988), but consistent with Hughes et al. (1979). Copper foliar concentrations fluctuated over the sampling period, which is consistent for the trend of red raspberries (Hughes et al., 1979), but not for the three cultivars of floricane-fruiting blackberries studied by Clark et al. (1988). Boron foliar concentration trend is consistent with the trend for red raspberries (Huges et al., 1979; Kowalenko, 2006).

Manganese fluctuated during the sampling period. In red raspberries, Hughes et al. (1979) found that Mn concentrations decreased over time, which is not consistent with our findings. Sulfur foliar concentration increased during the sampling period. Manganese, Na, and S foliar concentration trends were not reported in Clark et al., (1988) for blackberries, nor in Wright and Waister, (1980) for raspberries, or Rom (1994) for apples.

Magnesium foliar concentrations increased for the sampling period. In apples, Mg foliar content increased over time (Rom, 1994). Iron concentrations varied over time. This trend is not consistent for the three floricane-fruiting blackberry cultivars reported by Clark et al. (1988). Also, Zn concentrations fluctuated during the sampling period in this experiment while Zn concentrations decreased in the three cultivars of floricane-fruiting blackberries studied by Clark et al. (1988). All of these elements had a period of relative stability during the interval of 7 July to 25 July sampling dates, which mostly coincides with those found for apples, raspberries, and floricane-fruiting blackberries.

Statistically, there are intervals of dates for which elemental concentrations did not change or were similar for N, P, K, Mg, S, Fe, Mn, and Zn obtained through LSD mean comparisons and independent analysis of interactions. These periods of relative stability of nutrient concentrations means mostly occurred between 7 July and 25 July sampling dates, in 'Prime-Ark® 45' blackberry leaves collected in Fayetteville in ambient conditions. This period matches with the phenology stage of 10% bloom and first ripe fruit.

Experiment 2. Clarksville, Ark.:

Fully productive plants of 'Prime-Jan®,' 'Prime-Ark® 45,' and 'Ouachita' blackberry cultivars have been previously cultivated for experimental purposes with sufficient fertilization and proper management.

In this experiment, none of the elemental concentrations were significantly affected by the cultivar treatment ('Prime-Jan®,' 'Prime-Ark® 45,' and 'Ouachita'). Studying three floricane-fruiting blackberries cultivars ('Cherokee,' 'Cheyenne,' and 'Comanche') Clark et al. (1988) found significant differences in foliar concentrations, among cultivars for P, Ca, Mg, Zn, Fe, and Mn, which is not consistent with our results. Also, John and Daubeny (1972), in fourteen cultivars and selections of red raspberries found significant differences for N, P, K, Ca, Mg, S, Na, Fe, Zn, and B, and particular variations for B, Ca, and S were higher among some of these red raspberries while Mn and Cu foliar concentrations were not significantly different.

Sampling date significantly influenced elemental leaf concentrations of Mg, Mn, Zn, and B. Thus, these concentrations varied according to each sampling date. This finding was supported by John and Daubeny (1972) who stated that foliar macro- and microelements concentrations were affected by sampling date in red raspberries with the exception of Mg and Mo. Also, Clark et al. (1988) found significant differences due to sampling dates for N, P, K, Ca, Mg, Zn, Fe, and Cu foliar concentrations in three floricane-fruiting blackberries which is partially consistent with our results.

The cultivar x sampling date interaction significantly affected N, P, K, Ca, S, Na, and Cu foliar concentrations for the sampling period. In three floricane blackberries, Clark et al. (1988) found significant interaction of cultivar x sampling date only for Mg. Thus, these interactions are not consistent with our results. The cultivar x sampling interaction did not significantly affect Mg, Fe, Mn, Zn and B concentrations. Independent statistical analyses per element were conducted to determine periods of stability, for all of these interactions, through the LSD test.

By using the LSD mean comparison within elements, it was found that Mg, Fe, Mn, Zn, and B concentrations were statistically similar during 30 June to 12 July, showing a period of stability for most of these elements at that time. Also, in independent analyses through the same test for the interactions of N, P, K, Ca, S, Na, and Cu was found the same interval of sampling dates as a period of relative stability.

Qualitatively, each elemental mean concentration described trends for the sampling period as follows: N, P and K concentrations in leaves decreased over time and they showed a period of stability from 20 June to 12 July. These trends are consistent with those found for apples (Rom, 1994), red raspberries (Wright and Waister, 1980), and blackberries (Clark et al., 1988). Calcium and Cu concentrations fluctuated from the beginning to the end of the sampling period. This Ca trend was not consistent with the trend described for apples (Rom, 1994), but consistent with Hughes et al. (1979) for red raspberries and Clark et al. (1988) for floricane-fruiting blackberries. Copper concentrations fluctuated over time, which is consistent for the red raspberries (Hughes et al., 1979), but not for the three cultivars of floricane-fruiting blackberries studied by Clark et al. (1988).

Magnesium, Fe, Mn, and B leaf concentration increased during the sampling period, and they had a period of relative stability from the 20 June to 12 July sampling dates, approximately. In apples, Mg, concentrations increased during the sampling period (Rom, 1994). Conversely, concentrations fluctuated over time for red raspberries (Hughes et al., 1979; Wright and Waister, 1980) and for floricane-fruiting blackberries (Clark et al., 1988). Fe concentrations decreased during the sampling date in floricane-fruiting blackberries Clark et al. (1988). Also, in red raspberries Mn concentrations decreased over time (Hughes et al., 1979), which is not consistent with our findings. Boron concentration increased over time. This concentration trend is consistent with the trend for apples described by Rom (1994) and for red raspberries by Wright and Waister (1980).

Sodium and Zn concentrations fluctuated during the same sampling period and they had a period of stability between the 20 June and 12 July sampling dates. Sulfur and Zinc concentrations fluctuated during the sampling period. Contrarily, Zn concentrations decreased in the three cultivars of floricane-fruiting blackberries studied by Clark et al. (1988). Manganese, Na, and S concentration trends were not reported in Clark et al., (1988) for blackberries, nor in

Wright and Waister, (1980) for raspberries, or Rom (1994) for apples. These description of trends of elemental concentration means over time indicated that the best period with the most stability in elemental nutrient content is from the last week of June to the second week of July, which mostly coincide with those found for apples, raspberries, and floricane-fruiting blackberries.

These periods of relative stability of mean nutrient concentrations are mostly located between the 30 June to 12 July sampling dates in leaves of ‘Prime-Ark® 45’ collected in Clarksville. This period overlaps with the plant phenology stage of first ripe fruit.

Experiment 3. North Carolina:

There were significant differences among sampling dates for P, K, Ca, Mg, Na, Fe, and B. However, it was found that sampling date did not significantly influence elemental concentration of N, S, Mn, Zn, and Cu. For these elements there is a period of total stability from the beginning to the end of the sampling period. For red raspberries, John and Daubeny (1972) stated that foliar elemental concentrations were affected by sampling dates for most of the elements tested: N, P, K, Ca, S, Na, Fe, Mn, Zn, Cu, and B with the exception of Mg. In three floricane-fruiting blackberries, Clark et al. (1988) found significant differences for N, P, K, Ca, Mg, Zn, Fe, and Cu foliar concentrations, which corroborates the results of this study. The seasonal variation in elemental concentration also occurs for ‘Prime-Ark® 45’ PF blackberry cultivar.

The LSD test for mean comparison within elements indicated that P, K, Ca, Mg, Na, Fe, and B, had statistically similar concentrations during the 5 July to 22 July sampling dates, showing a period of stability for most of these elements.

Qualitatively, N, P, K, S, and Zn decreased during the sampling period and had a phase of stability from 22 June to 22 July, but P and K had a period of stability between 5 July to 22 July. These N, P, and K trends are consistent with the trends described for apples, raspberries and blackberries by Rom, (1994), Wright and Waister (1980), and Clark et al. (1988), respectively. Zinc concentrations fluctuated during the sampling period which is consistent with the study of three cultivars of floricane-fruiting blackberries by Clark et al. (1988). Calcium, Mg, Na, Mn, Fe, and Cu concentrations fluctuated during the sampling period. The Ca trend was totally different from the trends stated by Rom (1994) in apples, Wright and Waister (1980) for red raspberries, and Clark et al. (1988) for blackberries. It is only consistent with Hughes et al. (1979). In apples, Mg, concentrations increased during the sampling period (Rom, 1994). In red raspberries concentrations of Mg decrease over time (Hughes et al., 1979), but in blackberries the trend fluctuated (Clark et al., 1988). According to Clark et al. (1988), Fe decreased during the sampling period, this is not consistent with the result obtained in this experiment. Copper concentrations over time decreased and this is consistent with the three cultivars of floricane-fruiting blackberries trends studied by Clark et al. (1988). Manganese, Na, and S concentration trends were not reported in Clark et al. (1988) for blackberries, nor in Wright and Waister, (1980) for raspberries, or Rom (1994) for apples. Boron concentration increased over time. This concentration trend is consistent with the trend for apples described by Rom (1994) and for red raspberries by Wright and Waister (1980). Thus, the description of elemental concentration means showed that the period with the most stability in elemental nutrient content was between

05 July to 22 July sampling dates for most of the macro- and microelements tested in ‘Prime-Ark® 45’ cultivar cultivated in North Carolina.

It was found statistically that there were no differences in elemental concentration means for the sampling period for N, S, Mn, Zn, and Cu. Additionally, the LSD mean comparison test indicated periods of relative stability of nutrient concentration means, which were mostly located between 5 July and 22 July sampling dates in ‘Prime-Ark® 45’ blackberry leaves collected in three commercial orchards in North Carolina. These findings were supported by the trends of mean concentration over time which describe periods of stability during the same period for most of the elements. This period of stability coincided with the plant phenology stage of after 10% bloom of primocanes (on 15 June 10% was observed).

Determining the lowest variance of elemental nutrient concentration in ‘Prime-Ark 45®’ leaves over time in North Carolina:

Due to the variations within sampling date, the analysis of the mean of logarithm (log) variances was used to identify periods with minimum variance. N, P, K, Mg, S, Fe, Mn, Zn, and Cu variances in foliar concentrations were statistically not different for the sampling period, which represents a period of relative stability. Conversely, Ca, Na, and B were significantly different. According to the LSD mean comparison test, the log variances of Ca concentrations showed the lowest variances were observed between 22 June to 22 July sampling dates. The means of log variances of Na concentrations increased over time. This element had the lowest concentration between 20 May to 22 July sampling dates. Boron means of log variances increased over time. The lowest variances were observed on 20 May, 4 June, and 22 July sampling dates (Table 2.26).

As a result, the period of minimum variance among samples taken at the same date was found between the 5 July and 22 July sampling dates, approximately, for all of the macro- and micro-elements tested.

Effects of sampling date in elemental concentrations for all locations:

It has been reported that sampling date significantly affected foliar nutrient concentrations in red raspberries (John and Daubeny, 1972; Huges et al., 1979) and in floricane-fruiting blackberries; (Clark et al., 1988). In this experiment, also sampling date effects were observed in all locales (Table 2.22). The significance of this effect per element is described as follows: Nitrogen foliar concentrations depended upon sampling date in Fayetteville under both HT and ambient conditions. In Clarksville, N foliar content was also affected by the interaction of cultivar x sampling date, while in N.C. nitrogen foliar concentration did not depend upon sampling date. Phosphorous leaf content depended upon sampling date in Fayetteville, under HT and ambient conditions and in N.C. In Clarksville, P foliar content was also affected by the interaction of cultivar x sampling date. Potassium foliar concentrations depended upon sampling date in Fayetteville under HT conditions, under ambient conditions, and in N.C. In Clarksville, K foliar content was also affected by the interaction cultivar x sampling date. Calcium leaf concentrations depended upon sampling date in Fayetteville under HT conditions and in N.C. In Fayetteville, under ambient conditions and in Clarksville, Ca foliar concentrations depended upon sampling date but also the interactions of cultural practice x sampling date and cultivar x sampling date, respectively. In all locations (Fayetteville- HT and ambient conditions, Clarksville, and N.C.), Mg foliar concentrations depended upon sampling date. Sulfur foliar concentrations depended upon sampling date in Fayetteville under both HT and ambient conditions. In Clarksville, S foliar content was also affected by the interaction of cultivar x date, while in N.C., sampling date did not affected S leaf content. Sodium foliar concentrations depended upon sampling date in Fayetteville under high tunnel conditions and in N.C. In Fayetteville, under ambient conditions and in Clarksville, Na leaf content was also affected by the interaction of cultural practice x sampling date and cultivar x date, respectively. Iron foliar concentration depended upon sampling date in Fayetteville under HT and ambient conditions and in N.C. In Clarksville, sampling date did not significantly affect Fe concentrations. Manganese leaf concentrations were significantly affected by sampling date in Fayetteville under ambient conditions and in Clarskville. In Fayetteville under HT conditions, Mn leaf content was also affected by the interaction of N rate x sampling date. In N.C., sampling date did not affect Mn leaf concentrations. In Fayetteville under ambient conditions and in Clarksville, sampling date significantly affected Zn leaf content. In Fayetteville under HT conditions, Zn foliar concentration was also significantly affected by the interaction of N rate x sampling date. In N.C., sampling date did not affect Zn foliar concentrations. The interactions of N rates x sampling date, cultural practice x sampling date, and cultivar x sampling date, also significantly affected Cu foliar concentrations in Fayetteville under HT, and under ambient conditions, and in Clarksville, respectively. In N.C., sampling date did not significantly affect Cu leaf concentrations. In Fayetteville under HT conditions, in Clarksville, and in N.C., sampling date significantly affected B foliar concentrations, while in Fayetteville under ambient conditions, the interaction of cultural practice x sampling date also significantly affected B foliar concentrations.

In 'Willamette' raspberry, For N and B, Kowalenko (2006) found that leaf tissue N concentrations were too dynamic and had high variance, after N and B fertilization. Likewise, P and Mn were variable according to sampling dates. Also for red raspberries, John and Daubeny (1972) stated that foliar elemental concentrations were affected by sampling dates for most of the elements tested: N, P, K, Ca, S, Na, Fe, Mn, Zn, Cu, and B with the exception of Mg. Conversely, N foliar concentration in N.C. and Mg for all locations contradicts this report.

In three floricane-fruiting blackberries, Clark et al. (1988) found significant differences due to sampling dates for N, P, K, Ca, Mg, Zn, Fe, and Cu foliar concentrations, which corroborates the results of this study (Sulfur, Na, and B were not studied by those authors), with the exception of Fe foliar concentration in Clarksville, and Zn and Cu in N.C. that contradict their findings (Table 2.22). Thus, sampling date significantly affected foliar N, P, K, Ca, Mg, S, Na, Fe, Mn, Zn, Cu and B elemental concentrations for all locations, with the exception of N, S, Mn, Zn, and Cu in N.C. and Fe in Clarksville. Even though these elements were not significantly affected, there was a tendency for N in N.C. to vary with sampling date because the *p*-value (0.094) was close to 0.05. The high variability that creates these levels of significance can be attributed to the variable environment and the leaf sampling process, which was conducted by different technicians.

Table 2.22: Significance due to sampling date across locations.

Element	Sampling date / locations			
	Fayetteville - High tunnel	Fayetteville - Ambient	Clarksville	North Carolina
N	S	S	S*	NS
P	S	S	S*	S
K	S	S	S*	S
Ca	S	S*	S*	S
Mg	S	S	S	S
S	S	S	S*	NS
Na	S	S*	S*	S
Fe	S	S	NS	S
Mn	S*	S	S	NS
Zn	S*	S	S	NS
Cu	S*	S*	S*	NS
B	S	S*	S	S

S Sampling date is significant
NS Sampling date is not significant
* Interaction is significant (different sites had different 2^{nd} factor).

Conclusions

Experiment 1. Fayetteville, Arkansas:

Experiment 1.a. Fayetteville - High tunnel:

Foliar elemental concentrations in one-year-old 'Prime-Ark® 45' PF blackberry varied seasonally for the macro- and micronutrients tested. Despite this variation, a period of relative stability, between 11 July and 25 July sampling dates were determined for the most of elements tested, approximately, after 10% bloom occurs. Treatment 2 (10 $kg \cdot ha^{-1}$) resulted in highest concentration for P and K after the N applications while in the reminder elements tested N fertilizer applications did not affect the foliar content for the remaining of elements tested.

Experiment 1.b. Fayetteville - Ambient:

In leaves of 'Prime-Ark® 45' PF blackberries collected in Fayetteville, grown under ambient conditions, seasonal variation of foliar elemental content was found for the macro- and micronutrients tested. Conversely, periods of relative stability of nutrient concentration means were determined between 7 July and 25 July sampling dates, between 10% and 50 % blooming, approximately. Cultural practices (mowing and tipping) affected Mn and Zn foliar elemental concentrations. Treatments not pruned and mown had the highest responses for these elements.

Experiment 2. Clarksville, Arkansas:

'Prime-Ark® 45' PF blackberry grown in Clarksville, sampling date caused ample variation in foliar elemental concentrations. However, periods of relative stability of nutrient concentration means were located between the 30 June and 12 July sampling dates, during the phenological stage of first ripe fruit. Cultivar ('Prime-Jan®,' 'Prime-Ark® 45,' and 'Ouachita' blackberry cultivars) did not affect the foliar elemental concentrations for the sampling period.

Experiment 3. North Carolina:

In North Carolina, based on the analysis for the period of stability over time and the minimum variances, the best period for collecting leaf samples of ‘Prime-Ark® 45’ is from 5 July to 22 July approximately, after 10% bloom occurs. This founding was supported first, qualitatively, where trends of elemental concentration means indicated that the period with most stability in elemental nutrient content was the same period.

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Appendix C. ***Statistical Data:***

Table C.1. Descriptive statistics of variables measured for Chapter II upon elemental nutrient concentration in ‘Prime-Ark® 45’ blackberry cultivar, in Fayetteville, under high tunnel conditions.

		Leaf tissue concentration			
Variable*	N	Mean	SD	Minimum	Maximum
N	96	2.64	0.28	1.92	3.33
P	96	0.21	0.03	0.15	0.26
K	96	1.75	0.21	1.3	2.18
Ca	96	0.44	0.13	0.15	0.68
Mg	96	0.31	0.03	0.25	0.39
S	96	0.19	0.02	0.15	0.23
Na	95	23.34	30.27	1.3	125
Fe	96	65.11	32.96	40.8	359.2
Mn	96	127.01	82.36	16	419.8
Zn	96	34.47	4.20	25.8	43
Cu	96	10.18	2.21	6.2	20
B	96	33.70	4.93	25	43.8

* Units: N, P, K, Ca, Mg, and S: %;
Na, Fe, Mn, Zn, Cu, and B: $mg.kg^{-1}$.

Table C.2. Descriptive statistics of variables measured for Chapter II upon elemental nutrient concentration in 'Prime-Ark® 45' blackberry cultivar in Fayetteville under ambient conditions.

		Leaf tissue concentration			
Variable*	N	Mean	SD	Minimum	Maximum
N	72	2.39	0.24	1.94	3.12
P	72	0.23	0.04	0.17	0.34
K	72	1.58	0.18	1.26	2
Ca	72	0.48	0.11	0.22	0.84
Mg	72	0.30	0.03	0.24	0.38
S	72	0.18	0.02	0.14	0.23
Na	71	30.04	33.89	2.7	123
Fe	72	61.63	8.80	47.7	97
Mn	72	144.09	46.64	39	265.6
Zn	72	36.54	5.90	23.1	53.5
Cu	72	12.22	2.89	7.9	22.1
B	72	37.43	5.03	27.4	55.1

* Units: N, P, K, Ca, Mg, and S: %;
Na, Fe, Mn, Zn, Cu, and B: $mg.kg^{-1}$.

Table C.3. Descriptive statistics of variables measured for Chapter II upon elemental nutrient concentration in 'Prime-Ark® 45,' 'Prime-Jan®,' and 'Ouachita' blackberry cultivars in Clarksville.

		Leaf tissue concentration			
Variable*	N	Mean	SD	Minimum	Maximum
N	54	2.22	0.52	1.56	3.29
P	54	0.19	0.04	0.13	0.31
K	54	1.37	0.24	0.98	2.02
Ca	54	0.65	0.17	0.35	1.04
Mg	54	0.30	0.05	0.19	0.4
S	54	0.16	0.04	0.1	0.26
Na	54	13.94	6.31	3.9	32.1
Fe	54	57.78	33.12	23.1	257.3
Mn	54	159.09	78.96	55.4	406.9
Zn	54	28.51	5.15	19.4	40.9
Cu	54	8.94	2.40	5.6	17.1
B	54	18.85	4.63	10.5	35.6

* Units: N, P, K, Ca, Mg, and S: %;
Na, Fe, Mn, Zn, Cu, and B: $mg.kg^{-1}$.

Table C.4. Descriptive statistics of variables measured for Chapter II upon elemental nutrient concentration in ‘Prime-Ark® 45’ blackberry cultivar in North Carolina.

		Leaf tissue concentration			
Variable*	N	Mean	SD	Minimum	Maximum
N	84	2.68	0.57	1.64	4.64
P	84	0.22	0.07	0.11	0.41
K	84	1.60	0.32	1.04	2.56
Ca	84	0.57	0.18	0.29	0.95
Mg	84	0.38	0.05	0.27	0.52
S	84	0.18	0.04	0.12	0.32
Na	84	44.65	48.24	2.7	158
Fe	84	59.28	15.18	22.3	98
Mn	84	272.67	221.85	30	1001
Zn	84	31.87	7.11	20.2	55.5
Cu	84	9.49	2.10	5.5	15.8
B	84	45.36	11.24	29.2	71.3

* Units: N, P, K, Ca, Mg, and S: %;
Na, Fe, Mn, Zn, Cu, and B: $mg.kg^{-1}$.

Table C.5. Experiment 1.a: *p*-values from the ANOVAs for the elemental content in ‘Prime-Ark® 45’ leaves collected in Fayetteville, Ark. under high tunnel conditions from June to August 2011.

Element	N treatment	Sampling date	N x Date interaction
N	0.417[z]	<.0001	0.624
P	0.016	<.0001	0.532
K	0.008	<.0001	0.996
Ca	0.917	<.0001	0.845
Mg	0.174	<.0001	0.270
S	0.105	<.0001	0.307
Na	0.356	<.0001	0.994
Fe	0.657	0.006	0.597
Mn	0.004	<.0001	0.012
Zn	0.013	<.0001	0.020
Cu	0.845	<.0001	0.003
B	0.134	<.0001	0.689

[z] *P*-value smaller than 0.05 indicates statistical significance at 5% (*P*< 0.05). Shaded areas indicate significant differences.

Table C.6. Experiment 1.b: *p*-values from the ANOVAs for the elemental content in 'Prime-Ark® 45' leaves collected in Fayetteville, Ark. under ambient conditions from June to August 2011.

Element	Cultural practice	Sampling date	Cultural practice x Date interaction
N	0.580[z]	<.0001	0.539
P	0.700	<.0001	0.784
K	0.158	<.0001	0.060
Ca	0.248	<.0001	0.001
Mg	0.910	<.0001	0.083
S	0.166	<.0001	0.529
Na	0.032	<.0001	0.012
Fe	0.163	0.0085	0.249
Mn	0.022	0.0015	0.166
Zn	0.033	<.0001	0.130
Cu	0.317	<.0001	0.009
B	0.639	<.0001	0.036

[z] P-value smaller than 0.05 indicates statistically significant at 5% (P< 0.05). Shaded areas indicate significant differences.

Table C.7. Experiment 2: *p*-values from the ANOVAs for the elemental content in 'Prime-Ark® 45' leaves collected in Clarksville, Ark. from June to August 2011.

Element	Cultivar	Sampling date	Cultivar x Date interaction
N	0.005	<.0001	0.0002 [z]
P	0.419	<.0001	<.0001
K	0.099	<.0001	<.0001
Ca	0.001	<.0001	<.0001
Mg	0.086	<.0001	0.239
S	0.003	<.0001	0.002
Na	0.831	<.0001	0.005
Fe	0.152	0.078	0.476
Mn	0.488	<.0001	0.059
Zn	0.069	<.0001	0.289
Cu	0.022	<.0001	0.001
B	0.2589	<.0001	0.1372

[z] P-value smaller than 0.05 indicates statistically significant at 5% ($P < 0.05$). Shaded areas indicate significant differences.

Table C.8. Experiment 3: Table of *P*-values for each element concentration over time in 'Prime-Ark 45®' blackberry leaves collected in North Carolina from May to August 2011.

Element	*P*-value
N	0.094[z]
P	0.027
K	0.0001
Ca	0.002
Mg	<.0001
S	0.183
Na	<.0001
Fe	0.023
Mn	0.319
Zn	0.201
Cu	0.301
B	<.0001

[z] P-value smaller than 0.05 indicates statistically significant at 5% ($P < 0.05$). Shaded areas indicate significant differences.

Table C.9. *p*-values from the ANOVAs of mean of log variances of elemental concentrations over time of 'Prime-Ark 45®' blackberry leaves collected in North Carolina from May to August 2011.

Element	Date [Z]
N	0.526
P	0.175
K	0.083
Ca	0.005
Mg	0.411
S	0.345
Na	0.009
Fe	0.300
Mn	0.367
Zn	0.427
Cu	0.556
B	0.007

[Z] P-value smaller than 0.05 indicates statistically significant at 5% ($P < 0.05$).
Shaded areas indicate significant differences.

Appendix D. ***Soil and foliar analysis:***

Table D.1. Soil analysis report for samples collected in Fayetteville on 29 Mar., under high tunnel conditions.

Date Processed:	3/29/2011
Field ID:	Fay. - High tunnel

1. Nutrient Availability Index

Nutrient	Concentration		Soil test level (Mehlich 3)
	ppm	**$Kg \cdot ha^{-1}$**	
P	78	174.7	Above Optimum
K	136	304.6	Optimun
Ca	901	2018.2	-
Mg	68	152.3	-
SO4-S	17	38.1	-
Zn	3.4	7.6	-
Fe	237	530.9	-
Mn	133	297.9	-
Cu	2.1	4.7	-
B	0	0.0	-
NO3-N	15	33.6	Medium

2. Soil Properties

Property	**Value**	**Units**
Soil pH (1:2 soil-water)	6.2	--
Soil EC (1:2 soil-water)		µmhos/cm
Soil ECEC	7	cmolc/kg
Organic Matter (Loss on Ignition)		%
Estimated Soil Texture	Silt Loam	

Estimated base saturation (%)				
Total	Ca	Mg	K	Na
64.9	55.9	4.7	3.6	0.7

Table D.2. Soil analysis result for samples collected in Fayetteville on 16 May, under high tunnel conditions.

STUDY: Blackberry – High tunnel ARRIVED: 5-24-2011
ID: May 16 LOGGED: 5-26-2011
LOCATION: Fayetteville OUT: 6-01-2011
PROCEDURES : Mehlich 3 extractable (1:10 ratio), analysis by Spectro ARCOS ICP
PH: 6.4
EC: 459 umhos/cm

---mg/kg---											
P	K	Ca	Mg	S	Na	Fe	Mn	Zn	Cu	B	Total N
77.5	220.6	1076.3	86.1	111.5	13.1	260.2	178.2	3.6	2.0	0.6	1384

Table D.3. Soil analysis result for samples collected in Fayetteville on 29 Aug., under high tunnel conditions, Treatment 1.

Date Processed:	8/29/2011
Field ID:	Fay. – High tunnel – T1

1. Nutrient Availability Index

Nutrient	**Concentration**		**Soil test level (Mehlich 3)**
	ppm	**kg·ha^{-1}**	
P	67	150.1	Above Optimum
K	101	226.2	Medium
Ca	935	2094.4	-
Mg	66	147.8	-
SO4-S	10	22.4	-
Zn	5.1	11.4	-
Fe	229	513.0	-
Mn	129	289.0	-
Cu	1.9	4.3	-
B	0.0	0.0	-
NO3-N	20	44.8	-

2. Soil Properties

Property	**Value**	**Units**
Soil pH (1:2 soil-water)	6.7	--
Soil EC (1:2 soil-water)		µmhos/cm
Soil ECEC	8	cmolc/kg
Organic Matter (Loss on Ignition)		%
Estimated Soil Texture	Silt Loam	

Estimated base saturation (%)				
Total	Ca	Mg	K	Na
68.8	58.1	6.8	3.2	0.8

Table D.4. Soil analysis result for samples collected in Fayetteville on 29 Aug., under high tunnel conditions, Treatment 2.

Date Processed:	8/29/2011
Field ID:	Fay. – High tunnel – T2

1. Nutrient Availability Index

Nutrient	**Concentration**		**Soil test level (Mehlich 3)**
	ppm	**kg·ha^{-1}**	
P	72	161.3	Above Optimum
K	109	244.2	medium
Ca	965	2161.6	-
Mg	73	163.5	-
SO4-S	15	33.6	-
Zn	3.9	8.7	-
Fe	222	497.3	-
Mn	136	304.6	-
Cu	2.0	4.5	-
B	0.0	0.0	-
NO3-N	28	62.7	-

2. Soil Properties

Property	**Value**	**Units**
Soil pH (1:2 soil-water)	6.3	--
Soil EC (1:2 soil-water)		µmhos/cm
Soil ECEC	8	cmolc/kg
Organic Matter (Loss on Ignition)		%
Estimated Soil Texture	Silt Loam	

Estimated base saturation (%)				
Total	Ca	Mg	K	Na
69.9	58.1	7.3	3.4	1.1

Table D.5. Soil analysis result for samples collected in Fayetteville on 29 Aug., under high tunnel conditions, Treatment 3.

Date Processed:	8/29/2011
Field ID:	Fay. – High tunnel – T3

1. Nutrient Availability Index

Nutrient	**Concentration**		**Soil test level (Mehlich 3)**
	ppm	**kg·ha^{-1}**	
P	71	159.0	Above Optimum
K	101	226.2	Medium
Ca	923	2067.5	-
Mg	67	150.1	-
SO4-S	14	31.4	-
Zn	4.2	9.4	-
Fe	206	461.4	-
Mn	140	313.6	-
Cu	2.0	4.5	-
B	0.0	0.0	-
NO3-N	22	49.3	-

2. Soil Properties

Property	**Value**	**Units**
Soil pH (1:2 soil-water)	6.3	--
Soil EC (1:2 soil-water)		µmhos/cm
Soil ECEC	8	cmolc/kg
Organic Matter (Loss on Ignition)		%
Estimated Soil Texture	Silt Loam	

Estimated base saturation (%)				
Total	Ca	Mg	K	Na
68.8	57.5	7.0	3.2	1.1

Table D.6. Soil analysis result for samples collected in Fayetteville on 29 Aug., under high tunnel conditions, Treatment 4.

Date Processed:	8/29/2011
Field ID:	Fay. – High tunnel – T4

1. Nutrient Availability Index

Nutrient	**Concentration**		**Soil test level (Mehlich 3)**
	ppm	**kg·ha^{-1}**	
P	70	156.8	Above Optimum
K	97	217.3	medium
Ca	880	1971.2	-
Mg	64	143.4	-
SO4-S	31	69.4	-
Zn	3.4	7.6	-
Fe	212	474.9	-
Mn	124	318.1	-
Cu	1.8	4.0	-
B	0.0	0.0	-
NO3-N	26	58.2	-

2. Soil Properties

Property	**Value**	**Units**
Soil pH (1:2 soil-water)	6.3	--
Soil EC (1:2 soil-water)		µmhos/cm
Soil ECEC	8	cmolc/kg
Organic Matter (Loss on Ignition)		%
Estimated Soil Texture	Silt Loam	

Estimated base saturation (%)				
Total	Ca	Mg	K	Na
67.7	56.8	6.9	3.2	0.8

Table D.7. Soil analysis result for samples collected in Fayetteville on 4 Oct., under high tunnel conditions, Treatment 1.

Date Processed:	10/04/2011
Field ID:	Fay. – High tunnel – T1

1. Nutrient Availability Index

Nutrient	**Concentration**		**Soil test level (Mehlich 3)**
	ppm	**kg·ha^{-1}**	
P	68	152.3	Above Optimum
K	96	215.0	medium
Ca	1113	2493.1	-
Mg	68	152.3	-
SO4-S	15	33.6	-
Zn	3.9	8.7	-
Fe	262	586.9	-
Mn	149	333.8	-
Cu	2.3	5.2	-
B	0.3	0.7	-
NO3-N	11	24.6	-

2. Soil Properties

Property	**Value**	**Units**
Soil pH (1:2 soil-water)	6.6	--
Soil EC (1:2 soil-water)		µmhos/cm
Soil ECEC	9	cmolc/kg
Organic Matter (Loss on Ignition)		%
Estimated Soil Texture	Silt Loam	

Estimated base saturation (%)				
Total	Ca	Mg	K	Na
72.2	62.0	6.3	2.7	1.1

Table D.8. Soil analysis result for samples collected in Fayetteville on 4 Oct., under high tunnel conditions, Treatment 2.

Date Processed:	10/04/2011
Field ID:	Fay. – High tunnel – T2

1. Nutrient Availability Index

Nutrient	**Concentration**		**Soil test level (Mehlich 3)**
	ppm	**kg·ha⁻¹**	
P	71	159.0	Above Optimum
K	96	215.0	Medium
Ca	1126	2522.2	-
Mg	66	147.8	-
SO4-S	15	33.6	-
Zn	4.1	9.2	-
Fe	267	598.1	-
Mn	154	345.0	-
Cu	2.4	5.4	-
B	0.3	0.7	-
NO3-N	14	31.4	-

2. Soil Properties

Property	**Value**	**Units**
Soil pH (1:2 soil-water)	6.4	--
Soil EC (1:2 soil-water)		µmhos/cm
Soil ECEC	10	cmolc/kg
Organic Matter (Loss on Ignition)		%
Estimated Soil Texture	Silt Loam	

Estimated base saturation (%)				
Total	Ca	Mg	K	Na
68.5	59.1	5.8	2.6	1.1

Table D.9. Soil analysis result for samples collected in Fayetteville on 4 Oct., under high tunnel conditions, Treatment 3.

Date Processed:	10/04/2011
Field ID:	Fay. – High tunnel – T3

1. Nutrient Availability Index

Nutrient	**Concentration**		**Soil test level (Mehlich 3)**
	ppm	**kg·ha⁻¹**	
P	74	165.8	Above Optimum
K	94	210.6	Medium
Ca	1065	2385.6	-
Mg	65	145.6	-
SO4-S	18	40.3	-
Zn	3.9	8.7	-
Fe	240	537.6	-
Mn	150	336.0	-
Cu	2.1	4.7	-
B	0.2	0.4	-
NO3-N	15	33.6	-

2. Soil Properties

Property	**Value**	**Units**
Soil pH (1:2 soil-water)	6.4	--
Soil EC (1:2 soil-water)		µmhos/cm
Soil ECEC	9	cmolc/kg
Organic Matter (Loss on Ignition)		%
Estimated Soil Texture	Silt Loam	

Estimated base saturation (%)				
Total	Ca	Mg	K	Na
71.3	61.1	6.2	2.8	1.2

Table D.10. Soil analysis result for samples collected in Fayetteville on 4 Oct., under high tunnel conditions, Treatment 4.

Date Processed:	10/04/2011
Field ID:	Fay. – High tunnel – T4

1. Nutrient Availability Index

Nutrient	**Concentration**		**Soil test level (Mehlich 3)**
	ppm	**kg·ha^{-1}**	
P	70	156.8	Above Optimum
K	93	208.3	Medium
Ca	1137	2546.9	-
Mg	66	147.8	-
SO4-S	18	40.3	-
Zn	3.9	8.7	-
Fe	243	544.3	-
Mn	140	313.6	-
Cu	2.1	4.7	-
B	0.3	0.7	-
NO3-N	9	20.2	-

2. Soil Properties

Property	**Value**	**Units**
Soil pH (1:2 soil-water)	6.4	--
Soil EC (1:2 soil-water)		μmhos/cm
Soil ECEC	10	cmolc/kg
Organic Matter (Loss on Ignition)		%
Estimated Soil Texture	Silt Loam	

Estimated base saturation (%)				
Total	Ca	Mg	K	Na
68.8	59.2	5.7	2.5	1.4

Table D.11. Soil analysis result for samples collected in Fayetteville on 4 Oct., under ambient conditions.

Date Processed:	3/29/2011
Field ID:	Fay. - Amb.

1. Nutrient Availability Index

Nutrient	**Concentration**		**Soil test level (Mehlich 3)**
	ppm	**kg·ha^{-1}**	
P	76	170.2	Above Optimum
K	253	566.7	above Optimum
Ca	1683	3769.9	-
Mg	148	331.5	-
SO4-S	19	42.6	-
Zn	7.6	17.0	-
Fe	164	367.4	-
Mn	123	275.5	-
Cu	4.7	10.5	-
B	0	0.0	-
NO3-N	19	42.6	-

2. Soil Properties

Property	**Value**	**Units**
Soil pH (1:2 soil-water)	6.2	--
Soil EC (1:2 soil-water)		µmhos/cm
Soil ECEC	14	cmolc/kg
Organic Matter (Loss on Ignition)		%
Estimated Soil Texture	Silt Loam	
	Silty Clay Loam	

Estimated base saturation (%)				
Total	Ca	Mg	K	Na
74.8	60.7	8.9	4.7	0.5

Table D.12. Soil analysis result for samples collected in Clarksville on 9 Aug. in Clarksville.

Date Processed:	8/9/2011
Field ID:	Clarksville

1. Nutrient Availability Index

Nutrient	**Concentration**		**Soil test level (Mehlich 3)**
	ppm	**kg·ha^{-1}**	
P	44	98.6	Optimum
K	101	226.2	Optimun
Ca	795	1780.8	-
Mg	40	89.6	-
SO4-S	9	20.2	-
Zn	9.6	21.5	-
Fe	81	181.4	-
Mn	110	246.4	-
Cu	4.7	10.5	-
B	0.2	0.4	-
NO3-N	19	42.6	-

2. Soil Properties

Property	**Value**	**Units**
Soil pH (1:2 soil-water)	6.2	--
Soil EC (1:2 soil-water)		µmhos/cm
Soil ECEC	8	cmolc/kg
Organic Matter (Loss on Ignition)		%
Estimated Soil Texture	Silt Loam	

Estimated base saturation (%)				
Total	Ca	Mg	K	Na
68.7	56.4	7.1	4.4	0.8

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V. CONCLUSIONS

For one-year-old 'Prime-Ark® 45' blackberry plants cultivated under HT conditions, either a single or split N application at a rate of 10 $kg \cdot ha^{-1}$ using ammonium sulfate fertilizer resulted in a higher fruit yield. Under the same conditions, marketable yield, cane diameter, and fresh weight were not significantly affected by the applied N fertilizer rates. Applied N treatments did not cause significant differences on foliar elemental concentration, except for Mn and B.

Under HT conditions, N fertilizer rates affected P and K foliar concentrations of one-year old 'Prime-Ark® 45'plants; however, the differences were small and probably not important for plant nutrition. The best period of relative stability of nutrient concentrations and for collecting samples was between 11 July and 25 July sampling dates, approximately, when 10% and 50% bloom occurs.

Under ambient conditions when mowing and tipping were practiced, the 7 July to 25 July sampling dates, approximately, were a relatively stable period for nutrient concentration and the best period for collecting samples, during the 10% bloom and the first ripe fruit. The cultural practices used as treatments did not result in a significant impact on foliar elemental concentrations.

In Clarksville, for 'Prime-Ark® 45' blackberry cultivar, the period with most stability in foliar elemental nutrient content was between 30 June and 12 July sampling dates, approximately, during the first ripe fruit. This period is the most appropriate for collecting samples, during the first ripe fruit. Independent analyses for interactions indicates this period of stability for Prime ark 45, which is not necessarily the same for the remaining cultivars tested.

In North Carolina, the period with most stability in elemental nutrient content was between 5 July and 22 July sampling dates. In the same location, the logarithm of variance means indicated that the least variance occurs from 5 July to 22 July sampling dates for most of the elements, after 10% bloom of primocanes.