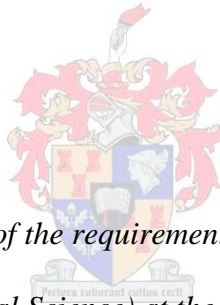


# **Seasonal patterns in carbohydrates and macro nutrients in southern highbush blueberry plants**

**By**

**Hannelize Kritzinger**



*Thesis presented in partial fulfilment of the requirements for the degree of Master of Science  
in Agriculture (Horticultural Science) at the University of Stellenbosch*

Supervisor: Prof K.I. Theron

Co-supervisor: Dr E. Louw

Dept. of Horticultural Science

Dept. of Horticultural Science

University of Stellenbosch

University of Stellenbosch

**December 2014**

## DECLARATION

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the authorship owner thereof and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: 03 October 2014

## ACKNOWLEDGEMENTS

I am sincerely grateful to...

My supervisor Prof Theron, for your support and input in this study and for always reassuring me that everything will work out fine.

My co-supervisor Dr. Louw, for your leadership and your faith in me and for having a different insight into this study.

Eurafruit SA (Pty) Ltd for your financial support and making this study possible.

Everyone at the nursery at Backsberg Wine Estate, especially Andries for your help and for always receiving me with a smile.

The national research foundation (NRF) for funding my bursary in 2013 and 2014.

The technical staff at the department, who showed more patience and perseverance than I'd ever think were possible. Thank you Tikkie, André, Mishela and Cecilia for all the hours you put into this study.

The administrative staff at the department, especially for the booking of vehicles.

Mpumelelo Nkomo for all the help and time in the lab.

Dr. Rohwer, for all the time and knowledge spent on my methodology and for always wanting to help and work out problems with me.

Renate Smit, for your endless support, both technical and emotional, without you I would not have been able to finish this thesis. I cherish your friendship more than you'd imagine.

Sean, for all the help, support, patience and love that kept me going through the rough times.

My parents for all your love, patience, advice and financial support and for making it possible  
for me to study at a world class university.

My God and Saviour that carried me through all my life.

## SUMMARY

Southern highbush (SHB) blueberries are relatively new to the blueberry industry and are adapted to grow in areas with low winter chilling and therefore can be grown in the Mediterranean-type climate of South Africa. The blueberry industry in South Africa is still relatively young, but it is quickly expanding and therefore appropriate cultivation practises are becoming more important. This study mainly focuses on the appropriate fertilization practises for SHB cultivars Snowchaser and Emerald grown in an ever-greening system.

Plants were cultivated in plant containers in a netted tunnel in the Paarl district of South Africa. All plants received continuous fertigation with a standard commercial nutrient solution containing nitrogen, phosphorus, potassium, magnesium, calcium and all micro-elements. Carbohydrate patterns were determined on newly established tissue culture plants at two-weekly intervals from April 2013 to June 2014 and macro nutrient patterns were determined at four-weekly intervals from May 2013 to June 2014. The phenology of these plants was also visually assessed during the sampling period. Macro nutrient content was determined for two-year old 'Snowchaser' and 'Emerald' plants at five phenological stages during the 2013/2014 season and nutrient losses due to harvest and pruning was recorded.

The phenology of evergreen 'Snowchaser' and 'Emerald' SHB blueberries were very different from deciduous blueberries, to such an extent that fruit could be harvested at the end of winter to early spring. Carbohydrate patterns differed between the first and the second season. Reserve carbohydrates were accumulated in the first season, but not in the second season possibly due to the difference in photosynthate production between the seasons. Plants were significantly bigger, with higher total leaf area, in the second season and it could be that carbohydrates from current photosynthesis were enough to supply new growth, thus

making reserves less important. Carbohydrates could also have been used to increase flower bud development instead of being stored as reserves in the second season.

Nutrient patterns also differed between the two seasons, but nutrient accumulation was apparent in the second season and not in the first. Nutrient uptake was highest when plants were growing rapidly, emphasizing the importance of fertilizer during periods of rapid growth. Huge fluctuations in the nutrient concentration patterns in the root, shoot and leaf tissues were observed over the sampling period and could have been a result of irregular fertigation and therefore it is uncertain whether flushes in nutrient uptake was a result of higher nutrient demand by the plant.

Nutrients are lost due to harvest and pruning and need to be replaced by applying the right amount of fertilizer. Nutrient uptake differs throughout the season as the demand for nutrients fluctuates and therefore fertilizers should be applied at different rates during the season. In the two-year old plants, the most nutrients were accumulated after summer pruning and before growth cessation and therefore most of the fertilization would occur during this stage. Recommendations for correcting nutrient losses due to harvest and pruning are made, together with recommendations for rate and timing of fertilizer application throughout the season.

## OPSOMMING

### **Seisoenale patrone van koolhidrate en makro-elemente in ‘southern highbush’ bloubessie plante**

‘Southern highbush’ (SHB) bloubessies is ’n relatiewe nuwe ontwikkeling in die bloubessie-industrie en is aangepas om in minder koue areas aangeplant te word en daarom kan hulle in die Mediterreëse-tipe klimaat van Suid-Afrika aangeplant word. Die bloubessie-industrie in Suid-Afrika is nog relatief jonk, maar dit is vinnig besig om uit te brei en daarom raak geskikte verbouingspraktyke al hoe belangriker. Hierdie studie fokus hoofsaaklik op die geskikte bemestingspraktyke vir SHB kultivars Snowchaser en Emerald wat in ’n immergroen sisteem verbou word.

Plante is in houers in ’n tunnel, wat met net bedek is, in die Paarl distrik van Suid-Afrika, aangeplant. Alle plante het dieselfde standaard kommersiële verreikte wateroplossing, teen ’n konstante vloei, ontvang. Die oplossing het stikstof, fosfor, kalium, magnesium, kalsium en al die mikro-elemente bevat. Koolhidraatpatrone is in twee-weeklikse intervalle, vanaf April 2013 tot Junie 2014, vir nuut gevestigde weefselkultuurplante bepaal en makro-element patrone is in vier-weeklikse intervalle, vanaf Mei 2013 tot Junie 2014, bepaal. Die fenologie van dié plante is visueel waargeneem tydens die monsternemingsperiode. Makro-elementinhoud is vir tweejarige ‘Snowchaser’ en ‘Emerald’ plante by vyf fenologiese stadiums tydens die 2013/2014 seisoen bepaal en die voedingstofverliese as gevolg van oes en snoei is bepaal.

Die fenologie van immergroen ‘Snowchaser’ en ‘Emerald’ SHB bloubessies het opmerklik verskil van bladwisselende bloubessies, tot so ’n mate dat vrugte al teen einde winter na vroeë lente geoes kon word. Koolhidraatpatrone van die eerste en tweede seisoen het verskil

deurdat reserwe koolhidrate in die eerste seisoen opgebou het, maar nie in die tweede seisoen nie, moontlik as gevolg van die verskil in fotosinteesproduksie tussen die twee seisoene. Plante was opmerklik groter, met groter blaaroppervlak, in die tweede seisoen en dit kon wees dat koolhidrate van huidige fotosintese genoeg was om die groei te onderhou en sodoende die afhanklikheid van reserwes te verminder. Koolhidrate kon ook vir verhoogde blomknopontwikkeling gebruik geword het, in plaas van om as reserwes vir die tweede seisoen gestoor te word.

Voedingstofpatrone het ook tussen seisoene verskil, maar voedingstofakkumulering was duidelik in die tweede seisoen en nie in die eerste nie. Voedingstofopname was die hoogste wanneer plante vinnig gegroei het en daarom is bemesting tydens periodes van vinnige groei uiters belangrik. Groot wisselinge in die voedingstofkonsentrasiepatrone van die wortels, lote en blare is tydens die monsternemingsperiode waargeneem en onreëlmatige verrykte watertoediening kon dit veroorsaak het. Daarom is dit onseker of fluktuasies in voedingstofopname 'n gevolg was van hoër voedingstofaanvraag deur die plant.

Voedingstowwe gaan verlore deur oes en snoei en moet deur die toediening van korrekte bemesting vervang word. Voedingstofopname verskil oor die verloop van die seisoen soos die aanvraag vir voedingstowwe deur die plant verander en daarom moet bemestingstowwe teen verskillende hoeveelhede deur die seisoen toegedien word. In die tweejarige plante is meeste van die voedingstowwe ná somersnoei en voor groeistaking opgeneem en daarom moet meeste van die bemesting tydens hierdie stadium toegedien word. Aanbevelings vir die korrigerende van voedingstofverliese as gevolg van oes en snoei, tesame met aanbevelings vir die hoeveelheid en tyd van bemestingstoediening deur die seisoen, word gemaak.



This thesis is a compilation of chapters, starting with a literature review, followed by three research papers. Each paper is prepared as a scientific paper for submission to *HortScience*. Repetition or duplication between papers might therefore be necessary.

# TABLE OF CONTENTS

<b>Declaration</b>	<b>i</b>
<b>Acknowledgements</b>	<b>ii</b>
<b>Summary</b>	<b>iv</b>
<b>Opsomming</b>	<b>vi</b>
<b>Explanation of style</b>	<b>viii</b>
<b>Table of contents</b>	<b>ix</b>
<b>General Introduction</b>	<b>1</b>
<b>Literature Review:</b> Carbohydrate and nutrient status of blueberries with reference to plant phenology	<b>5</b>
<b>Paper 1:</b> Seasonal change of carbohydrates in ‘Emerald’ and ‘Snowchaser’ southern highbush blueberries	<b>41</b>
<b>Paper 2:</b> Seasonal change in macro nutrients in ‘Emerald’ and ‘Snowchaser’ southern highbush blueberries	<b>73</b>
<b>Paper 3:</b> Seasonal changes and allocation of macro nutrients in ‘Emerald’ and ‘Snowchaser’ southern highbush blueberries	<b>109</b>
<b>General Discussion and Conclusions</b>	<b>148</b>
<b>Appendix A</b>	<b>152</b>
<b>Appendix B</b>	<b>173</b>

## GENERAL INTRODUCTION

The cultivation of blueberries in South Africa started in the 1970s and the industry is still relatively small (Retamales and Hancock, 2012). Blueberry production became wide-spread in the 1990s and production increased approximately tenfold in the last five years (Eurafruit SA Pty Ltd, personal communication; Retamales and Hancock, 2012). The expanding production of blueberries leads to a demand for appropriate cultivation practises, amongst others in the field of fertilization.

Southern highbush (SHB) is the predominant blueberry grown in South Africa (Eurafruit SA Pty Ltd, personal communication), but it is also the newest development in the blueberry industry (Braswell et al., 1997) and therefore little information regarding the cultivation of SHB is known. In order to provide insight into the nutritional management of blueberries, seasonal patterns of nutrients and carbohydrates in the plant tissues need to be studied. The best way to determine nutrient uptake and demand is to sample whole plants at predetermined intervals throughout the season and determining the dry mass and nutrient concentration of different tissue types (Weinbaum et al., 2001). If the pattern of nutrient uptake through the season could be established, fertilizers could be applied at times that coincide with the specific need of the plant (Tagliavini et al. 2005).

Besides the nutrient status of the plant, the carbohydrate content also has a significant effect on vegetative and reproductive growth. Deciduous fruit trees, such as blueberries, accumulate carbohydrates after harvest and store the carbohydrates during winter when the plant is endodormant. These stored carbohydrates are used during spring to sustain reproductive and vegetative growth (Darnell and Birkhold, 1996). The use of stored or reserve carbohydrates are especially important for the period before vegetative tissue from the present season starts

exporting photosynthetic assimilates that can sustain new growth (Darnell and Birkhold, 1996). The nutrient and carbohydrate contents of the various plant tissues vary throughout the season due to different demands as well as losses due to leaf drop, harvest and pruning (Retamales and Hancock, 2012). This study aimed to determine the seasonal changes of nutrients and carbohydrates throughout the season, with reference to plant phenology.

Firstly, a literature study was conducted to give the reader insight into the nutrient and carbohydrate status of blueberry plants in general and the phenology of blueberries plants is discussed with specific emphasis on northern highbush grown in the northern hemisphere, as little information on SHB is available.

The first aim of this thesis was to determine the carbohydrate patterns of the different plant tissue of SHB blueberry plants throughout the season. A detailed description of the starch and total sugar concentration and content is discussed in Paper 1. It should be noted that the phenol sulphuric acid assay (Buysse and Merckx, 1993; Dubois et al., 1956) was used to determine starch although it may not be as precise as other methods such as high performance liquid chromatography (HPLC). The main reason for this was that the phenol sulphuric acid assay was less expensive and less time consuming for the large number of samples we had. We were also only interested in the general pattern of starch throughout the season and not the precise starch values.

The second aim was to determine the macro nutrient concentration and content of the different plant tissues of SHB blueberry plants over a season. General patterns are discussed and attention is given to large changes over time, while smaller fluctuations were not rendered as very important. Data on micro nutrients were also gathered but will not be discussed due to the large amount of data, but the graphs are presented in Appendix A.

The third aim was to determine the uptake of macro nutrients and the extent of the nutrient losses throughout the season. Uptake and reallocation patterns are discussed in detail and the importance and quantity of fertilization at specific times during the season discussed. Micro nutrients are again not discussed due to the large amount of data, but the data are presented in Appendix B.

All plants were grown under 20% white net, which affected the micro-climate around the plants compared to open field conditions. This caused plants to retain their leaves during winter even though blueberries are naturally deciduous plants. Comparisons between deciduous fruit crops and evergreen 'Emerald' and 'Snowchaser' SHB blueberries were made in order to establish whether blueberries grown in an ever-greening system would differ in terms of their seasonal carbohydrate and nutrient patterns. Unfortunately no literature on the seasonal carbohydrate and nutrient patterns in deciduous blueberries are available and therefore comparisons could not be made to other blueberries.

### **Literature Cited**

- Braswell, J.H., J.M. Spiers, and F.B. Matta. 1997. Influence of N, P, K, Ca and Mg rates on leaf elemental concentration and plant growth of 'Gulf Coast' blueberry. *Acta Hort.* 446:363-368.
- Buyse, J. and R. Merckx. 1993. An improved colorimetric method to quantify sugar content of plant tissue. *J. Exp. Bot.* 44(10):1627-1629
- Darnell, R.L. and Birkhold, K.B. 1996. Carbohydrate contribution to fruit development in two phenologically distinct rabbiteye blueberry cultivars. *J. Amer. Soc. Hort. Sci.* 121(6):1132-1136.

- Dubois, M., K.A. Gilles, J.K. Hamilton, P. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28(3):350-356.
- Retamales, J.B. and J.F. Hancock. 2012. *Crop production science in horticulture: Blueberries*. Cabi, Wallingford, Oxfordshire (UK).
- Tagliavini, M., E. Baldi, P. Lucchi, M. Antonelli, G. Sorrenti, G. Baruzzi, and W. Faedi. 2005. Dynamics of nutrients uptake by strawberry plants (*Fragaria* × *Ananassa* Dutch.) grown in soil and soilless culture. *Eur. J. Agron.* 23(1):15-25.
- Weinbaum, S.A., P.H. Brown, R.C. Rosecrance, G.A. Picchioni, F.J.A. Niederholzer, F. Youseffi, and T.T. Muraoka. 2001. Necessity for whole tree excavations in determining patterns and magnitude of macronutrient uptake by mature deciduous fruit trees. *Acta Hort.* 564:41–49.

# LITERATURE REVIEW: Carbohydrate and nutrient status of blueberries with reference to plant phenology

## 1. Introduction

Blueberries belong to the genus *Vaccinium* and family Ericaceae and can be classified as woody perennial shrubs or undershrubs, which can either be deciduous or evergreen, depending on the climate. Blueberries are calcifuge (acid loving) plants and will commonly form a symbiosis with ericoid mycorrhizae. Cultivation is best in sandy loams with high organic matter content (Retamales and Hancock, 2012).

Rabbiteye (*Vaccinium ashei* Reade or *V. virgatum* Ait.), lowbush (*V. angustifolium* Ait.), northern highbush (NHB) (*V. corymbosum* L.) and southern highbush (SHB) (*V. corymbosum* L. interspecific hybrids) are the four blueberry species or types that are cultivated commercially (Darnell, 2006). The NHB and lowbush species require high winter chilling hours, with a minimum of 800 and 1000 chilling hours respectively (Eck, 1988; Retamales and Hancock, 2012), compared to rabbiteye and SHB which need a minimum of 300 and 150 chilling hours, respectively (Eck, 1988; Retamales and Hancock, 2012). A modified version of the Utah Chill Unit Model (UCUM) is used to calculate the required chilling hours for blueberries. For every hour NHB is exposed to 1 °C the chill units will equal 0.5, compared to 0 units for the UCUM (Eck, 1988).

The newest addition to the blueberry industry is the SHB (Braswell et al., 1997), which is a hybrid resulting from a cross between NHB (*Vaccinium corymbosum* L.) and species native to the south-eastern United States such as *V. darrowi*, *V. elliotii*, *V. angustifolium* and *V. ashei* (Lang and Parrie, 1992; Lyrene and Sherman, 1984, 2000). These species were bred to have a low winter chilling requirement to be cultivated in regions where the average

temperature of the three coldest months is as high as 15 °C (Lyrene and Sherman, 2000). SHB berries ripen early compared to other blueberry species (Makus and Spiers, 1995). This characteristic is an advantage to the grower, as higher prices are usually obtained in the early market (Lyrene and Sherman, 1984).

NHB fruit are harvested from mid-May to the end of September in the northern hemisphere and from mid-November to the end of March in the southern hemisphere (Lyrene and Sherman, 2000). These gaps in the harvest window can be filled with SHB blueberries, which are harvested from April to early May in the northern hemisphere and September to early November in the southern hemisphere, (Eurafruit SA Pty Ltd, personal communication; Lyrene and Sherman, 2000). The reason for this difference in harvest times, is that SHB cultivars can be grown in areas with relatively high temperatures, which occur in late winter and early spring, promoting early flowering and berry development, but where chilling is insufficient for NHB (Lyrene and Sherman, 2000).

Blueberries can be sold as a fresh or processed commodity. The fresh market is mainly supplied by highbush blueberries while lowbush blueberries are first frozen and then used in processed foods (Kalt et al., 2001). Processed blueberries can be stored for several years whereby early, midseason and late ripening blueberries are equal in value. Pricing is therefore the same irrespective of harvest date. The fresh market, however, gives the highest prices to the berries that enter the market early. Fresh berries should be marketed within two weeks after harvest. If supply is not evenly distributed over the season it will result in periods with large volumes in the market, leading to lower prices followed or preceded by periods of underproduction resulting in high prices (Lyrene and Sherman, 1984).



## **2. The blueberry industry in South Africa**

The South African blueberry industry is relatively young, with the first plantings only being established in the 1970s and extensive production starting in the 1990s (Retamales and Hancock, 2012). The majority of blueberries which have been planted are mainly rabbiteye and SHB due to their low winter chilling requirement, but the new focus for cultivation/plantings are predominately SHB (Eurafruit SA Pty Ltd, personal communication). NHB are planted in a few areas inland where the climate is cooler with sufficient winter chilling hours (Retamales and Hancock, 2012).

Blueberry production in South Africa has increased roughly tenfold in the last five years due to an increased local demand and higher international sales. According to leaders in the industry this growth rate will continue as global demand continues to increase (Eurafruit SA Pty Ltd, personal communication). A possible reason for the increase in blueberry consumption could be attributed to the increase in consumer awareness of nutritional value of food (Deng et al., 2013). It is commonly known that blueberries have health promoting effects and can delay the aging process in humans. Blueberries are high in antioxidants (Kalt et al., 2001; Sellappan et al., 2002) which fight damage from free radicals that cause aging, cancer, cardiovascular disease, immune system decline, brain dysfunction and cataracts (Ames et al., 1993; Lila, 2004; Wang, 2005). Antioxidants in blueberries are also helpful in preventing urinary tract infections, maintaining eye health and combating degenerative eye diseases (Ames et al., 1993; Kalt and Dufour, 1997).

The increase in production leads to a demand for knowledge on blueberry cultivation, especially in the area of nutritional management. Soils that are not optimal for growing blueberries are used as a result of the expanding industry, making nutrient management even more important (Retamales and Hancock, 2012). Blueberry crops benefit from fertilizer

application, but the amount that needs to be applied still needs to be defined to prevent over or under fertilization. The best way to determine fertilizer demands is to study the seasonal patterns of macro, micro and trace elements and carbohydrates in the plant.

Although blueberries are mainly deciduous, it is possible to manipulate SHB growing conditions to make them evergreen or non-dormant. Swain and Darnell (2001) studied the effects of dormant and non-dormant production systems on phenology and reserve carbohydrate concentrations of SHB. They observed higher carbohydrate concentrations and yield in the non-dormant system (Swain and Darnell, 2001). By executing a similar trial, the differences observed could be incorporated in the planning of fertilizer programs. Both production systems are currently used in South Africa and a detailed fertilizer program for both systems is therefore crucial.

### **3. Plant phenology**

#### **3.1. Growth stages**

According to Garcia-Salazar (2002) NHB has 17 growth phases (Table 1). During the dormant (1) phase there is no visible bud swell. Bud swell (2) follows when the reproductive buds start to swell. During vegetative bud break (3) the vegetative buds swell and green leaf tissue starts to come visible at the tip of the vegetative buds. The next stage, early green tip (4), is when leaves emerge from the vegetative buds. Bud burst or tight cluster (5) is the stage where reproductive buds open and individual flowers are visible between the bud scales. At the same time the 0.25" green phase (6) occurs where a quarter of an inch (0.64 cm) of leaf tissue can be seen. The next stage (7) is early pink bud. The flowers are separated and can be seen clearly, although only partially formed. When the majority of the flowers have almost completely developed, the late pink bud stage (8) is reached. This is followed by 25% bloom

(9) and full bloom (10) when most of the flowers are open. After full bloom the corolla tubes or petals drop and small green fruit is revealed. This stage (11) is called petal fall. Fruit size starts to increase due to cell division in the early green fruit stage (12) and cell expansion in the late green fruit stage (13). The fruit then turns colour (fruit colouring) (14) after which the first ripe fruit is harvested (first harvest) (15). This is followed by multiple harvests as fruit ripens (harvest) (16). During the last stage (postharvest) (17) blueberry plants will store reserves for the following season before going dormant again (Garcia-Salazar, 2002). The timeline for the different phases is summarized in Table 1.

### **3.2. Vegetative growth**

Vegetative buds are found on the proximal part of the current season's shoots. Buds are 3 mm to 5 mm long, have a tapered shape and are covered by two to four scales of the same length that end at the tip (Eck, 1988). Vegetative buds have up to six leaf primordia and additional leaves are initiated by the shoot apex every five days (Retamales and Hancock, 2012). Buds form in the leaf axils by the time leaves are fully expanded (Eck, 1988).

NHB buds begin to grow in early spring, beginning with the terminal buds showing activity first, after which lateral buds begin to develop and grow (Garcia-Salazar, 2002). Vegetative buds start to swell as the leaves start developing within the buds (Eck, 1988; Retamales and Hancock, 2012). The emerging shoot first looks like a leafy cluster, because the internodes are initially very short. Vegetative buds of NHB blueberries open about two weeks before reproductive buds break (Eck, 1988).

Blueberries have two to six vegetative growth flushes during the season (Bañados and Strik, 2006). Later ripening blueberries usually have fewer growth flushes than earlier ripening cultivars (Gough et al., 1976). Shoot growth is terminated by apical abortion of the growing tip. The distal portion of the shoot, about 2 mm of the leaf and stem tissue just below the

shoot apex, then turns chlorotic and necrotic. This phenomenon is known as “black tip” and gives rise to the episodic and sympodial extension growth pattern of blueberries. The “black tip” stays visible for one to two weeks, before the necrotic tissue falls off. A new shoot develops from the distal vegetative bud two to five weeks after black-tip abscission if conditions are favorable. This new shoot then becomes the second growth flush. Black-tip will occur a few times during the season, each time terminating a shoot growth flush. The number of growth flushes depends on the cultivar and growing conditions (Eck, 1988; Gough et al., 1978a).

### **3.3 Flower development**

Spann et al. (2003) determined that floral initiation of SHB is sensitive to photoperiod. Initiation is enhanced under short days, but flower bud differentiation is improved by long days (Spann et al., 2003). Flower bud initiation of SHB and NHB occur at 8 h photoperiods (short days), but not at 16 h photoperiods (long days) (Bañados and Strik, 2006; Spann et al., 2003). Darnell (1991) reported that the critical day length for ‘Beckyblue’ rabbiteye flower bud initiation is 12 h. She stated that similar results were obtained by Hall and Ludwig (1961) for lowbush and Hall et al. (1963) for NHB. In both these species and ‘Beckyblue’ the number of flower buds initiated increased as the day length decreased from 12 to 8 h and therefore Darnell (1991) concluded that the effect of photoperiod on flower bud initiation of blueberry is quantitative rather than qualitative.

Flower buds are 3.5 to 7 mm in length, large, ovoid in shape and occur on the distal part of the shoot (Eck, 1988). Budburst starts at the tip of the shoot and proceeds basipetally. The flower bud consist of six to 12 flowers, with the flowers at the tip of the bud opening first and potentially becoming the largest fruit. Fruit from proximal buds will always be smaller than

fruit from distally positioned buds. After full bloom, fruit starts to expand (Garcia-Salazar, 2002).

Reproductive buds for the next season are initiated on one-year-old wood during late summer and early autumn once the shoot flushes have terminated (Garcia-Salazar, 2002). Terminal buds will start to swell, as they change from vegetative to reproductive (Garcia-Salazar, 2002). As the buds start to enlarge the apex turns into the peduncle of the upcoming inflorescence. The apex aborts (black tip) after extension of the peduncle and axillary meristems that are initiated acropetally differentiate into flowers. Floral initiation of NHB in the northern hemisphere occurs in July and differentiation is finished by mid-April (Gough et al., 1978b). Bieniasz (2012) found that in NHB differentiation occurs during both autumn and spring, but not during the winter months (December to February in the northern hemisphere). In the northern hemisphere, SHB floral initiation starts in early September and by the end of January differentiation is completed. The difference in SHB is that floral differentiation commences through the winter. After the formation of microspore mother cells in November, NHB stops pollen development, but in SHB pollen grains and ovules continue to develop (Huang et al., 1997; Retamales and Hancock, 2012b). Knowing the time of flower differentiation is important for fertilizer programs, as fertilizers applied around flower differentiation and development could result in more viable flowers and ultimately higher yield (Bieniasz, 2012).

### **3.4 Fruit growth**

Fruit growth of blueberries (Fig. 1) displays a double sigmoidal curve (Godoy et al., 2008; Retamales and Hancock, 2012) with the first stage exhibiting rapid cell division and dry weight gain (Birkhold et al., 1992; Cano-Medrano and Darnell, 1997). The corolla and stamens fall off, the stigma turns brown and the style falls off after one or two days. The

calyx stays connected to the berry and ovary (Eck and Childers, 1966). The second stage shows little growth, but seeds are formed and the third stage exhibits rapid fruit growth due to cell enlargement (Birkhold et al., 1992; Cano-Medrano and Darnell, 1997; Eck, 1988; Godoy et al., 2008). Size increase is the highest during the third stage of fruit growth. During this stage the calyx becomes a purple colour and the green berry becomes semi-transparent. Berries then turn from semi-transparent to light purple to dark purple (Eck and Childers, 1966) and sugars and anthocyanins accumulate as berries mature (Retamales and Hancock, 2012). The first stage extends over 25 to 35 days, the second stage 30 to 40 days and the third stage lasts 30 to 60 days depending on the species, cultivar and environment (Darnell, 2006; Retamales and Hancock, 2012). Total fruit development ranges from 42 to 90 days for NHB, 55 to 60 days for SHB and 60 to 135 days for rabbiteye (Darnell, 2006). After the final fruit growth stage has been completed, the majority of the shoot and leaf growth also ceases.

### **3.5 Root growth**

Blueberry roots are devoid of any root hairs (Eck, 1988). Blueberry plants have two distinct root types, thick storage roots which also anchors the plant, and thin fibrous roots which absorb nutrients (Gough, 1994; Retamales and Hancock, 2012). The fibrous roots remain in the top soil, specifically in the area covered by the plant drip line (Abbott and Gough, 1987; Eck, 1988). These roots grow on average to depths of 23 cm from the soil surface, and spread in a horizontal fashion while the thicker roots can extend up to 80 cm deep (Gough, 1994).

Root growth occurs throughout the season, but two peaks can usually be observed (Abbott and Gough, 1987). The first peak is small and occurs in spring around fruit set, up until the early green stage of fruit. An increase in shoot growth occurs about two weeks after this first root growth peak. The second larger peak occurs after harvest and extends to the onset of dormancy (Abbott and Gough, 1987; Retamales and Hancock, 2012).

Water stress leads to reduced nutrient uptake resulting in an inability to build up reserves. If the soil is not moist after harvest, root growth is compromised and the plant will have weak and small roots. The plant cannot accumulate sugar and store it as starch reserves in the bark, wood and roots to sustain the next season's growth, resulting in poor spring budbreak (Garcia-Salazar, 2002). Furthermore, root growth is limited outside the 14 to 18 °C soil temperature range. Shoot and root growth of blueberries do not exhibit an antagonistic relationship. The growth trend is similar for roots and shoots, with both showing a decrease in growth during fruit maturation and harvest (Abbott and Gough, 1987).

### **3.6 Dormancy and the non-dormant production system**

As winter approaches blueberries become ecodormant with the onset of shorter photoperiods and lower temperatures (Retamales and Hancock, 2012). The plant stops growing due to the low temperatures (Gough, 1994) and then enters endodormancy. Endodormancy is defined by factors within the plant that keeps it from growing whereas ecodormancy results from external environmental factors. Once the plant has entered endodormancy it requires a minimum amount of chilling hours for normal floral and leaf growth in the spring (Gough, 1994; Retamales and Hancock, 2012). In the spring when growth starts, flower buds burst and open within three to four weeks. As discussed before, rabbiteye, NHB and SHB have different chilling requirements and the different cultivars of the same species also differ in the amount of chilling required. SHB cultivars such as Emerald, Jewel and Misty require less than 300 chilling hours (hours under 7.2 °C) while the SHB cultivars such as Arlen, Bladen and Reville require 800 to 900 chilling hours (Retamales and Hancock, 2012). It is possible however to grow blueberries in areas with insufficient chilling hours by growing plants in an evergreen (non-dormant) production system, enabling blueberry production in sub-tropical or even tropical regions. It is, however, important to evaluate cultivars to determine the

photoperiodic effects on flower bud initiation and whether it would tolerate higher temperatures. The evergreen system is thus limited to blueberry cultivars with a low chilling requirement (Darnell and Williamson, 1997). The SHB cultivar Sharpblue is grown successfully in an evergreen production system in Australia (Wright, 1993). Furthermore Reeder et al. (1994) proved that SHB ‘Gulf Coast’, ‘Sharpblue’ and ‘Wannabe’ could be grown successfully in Florida. SHB cultivars with a low chilling requirement are thus candidates for an evergreen production system. One of the advantages of growing blueberries in an evergreen system is the early ripening of the berries, which is achieved because flower buds do not enter endodormancy and starts blooming earlier than plants under a dormant system. The producer has little market competition at this stage and receives high prices (Reeder et al., 1994). A few aspects should be taken into consideration, however, before deciding on an evergreen system. An evergreen system can only be successful if the leaves are kept healthy and temperatures do not drop to such an extent that it causes defoliation. This limits an evergreen production system to regions that have mild winter temperatures (Darnell and Williamson, 1997). The system also requires N fertilization through autumn and winter (Reeder, 1998).

## **4. Nutrients**

### **4.1. Foliar nutrient concentrations**

Compared to other fruit crops blueberry plants have a low nutrient concentration in their leaves (Tamada, 2002). Foliar nutrient levels differ between blueberry species. Northern and southern highbush exhibit similar nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu) and aluminum (Al) foliar nutrient levels while rabbiteye blueberries have significantly lower N, P, K, Ca, S, Mn, Cu and Al levels (Clark et al., 1994). Factors such as crop load, time of



sampling and fertilizer rate will affect the foliar nutrient concentration. It is therefore necessary to consider these factors as well as differences in leaf nutrient concentration of different cultivars, when determining nutrient status and making fertilizer recommendations (Clark et al., 1994; Rivadeneira, 2012).

The dominant element in blueberry leaves is N, followed by Ca, K, and Mg (Clark et al., 1994). N concentration decreases in leaves as the season progresses (Smagula and Kreider, 2009) and Mg increases as leaves mature (Rivadeneira, 2012). High concentrations of N fertilizer decrease K and Mg uptake and result in high N, P, Ca, S and Mn, but lower boron (B) concentration in leaves (Braswell et al., 1997; Bryla et al., 2012).

If P fertilizer is increased, leaf P concentration increases, but the uptake of Cu, Zn and Fe decrease. High rates of Ca result in leaves with a decreased Mn and Zn content and toxicity symptoms appear during the first season. Increased Mg fertilizer causes an increase in Mg foliar concentration, but a decrease in Zn foliar concentration (Braswell et al., 1997).

Soil applied N, P and K results in increased foliar N, P and K concentrations. Soil applied K has a positive effect on leaf N levels and soil applied N has a positive effect on leaf P levels (Percival and Sanderson, 2004). Typical leaf mineral concentrations are presented in Table 2.

#### **4.2. Fertilization**

Blueberries have a low nutrient requirement compared to other fruit crops (Retamales and Hancock, 2012; Tamada, 2002). Absorption, use and transport of nutrients differ between blueberries and other fruit crops and the amount of fertilizer applied should be recommended according to the soil fertility as well as the nutrient status of the plant (Yadong et al., 2009). Fertilizer recommendations are currently made by determining leaf nutrient concentrations in

autumn, when levels are most stable in the season (Spiers, 1982), and comparing them to the set optimal leaf concentration range for the specific element (Bryla et al., 2012).

The best way to determine nutrient uptake and demand is to sample whole plants at predetermined intervals throughout the season and determining the dry weight and nutrient concentration of different tissue types (Weinbaum et al., 2001). Knowing the nutrient demand of the plant could lead to better nutrient management and more sustainable fertilization (Rivadeneira, 2012), because fertilizer uptake is the most efficient when the availability of nutrients in the soil is matched to the nutrient demand by the plant (Weinbaum et al., 2001). It is important to know the actual fertilizer demand of the plant throughout the season as well as the nutrient sources to plan a sustainable fertilizer program. Knowing the pattern of nutrient uptake through the season would allow for nutrient application that coincides with a specific need of the plant and installing a flexible fertilizer technique, such as fertigation, would make control more precise (Tagliavini et al., 2005). Applying the correct amount of fertilizer would lead to plants with an increased physiological status, increased yield and better fruit quality while over application affects the plant negatively (Yadong et al., 2009).

Fertilizers have a positive effect on blueberry plant growth, leaf area, chlorophyll content and the dry weight of leaves (Yadong et al., 2009). Nitrogen fertilizer is applied in commercial orchards at higher rates than any other fertilizer to increase growth and yield (Bryla et al., 2012). Optimum N fertilizer, which results in a leaf N concentration of 1.6%, delivers a good yield and superior fruit quality (Smagula and Kreider, 2008). Split applications of urea on mature NHB result in higher yields compared to single applications. If 38 kg N/ha urea is applied at budbreak and again at petal fall, higher yields are obtained compared to single applications of 76 kg N/ha at budbreak, 38 kg N/ha controlled-release fertilizer (CRF) with a 3-month release duration, 76 kg N/ha CRF with a 3-month release duration, 38 kg N/ha CRF with an 8-month release duration and 76 kg N/ha CRF with an 8-month release duration, all

applied at budbreak (Hanson and Retamales, 1992). Total leaf N content and cane diameter is higher in plants fertilized with N compared to non-fertilized plants (Hanson and Retamales, 1992). Soil applications of 448 kg/ha diammonium phosphate followed by foliar applications of 13.4 kg N/ha and 13.4 kg P<sub>2</sub>O<sub>5</sub>/ha increases branching, thus leading to more bearing positions and higher yields (Smagula and Kreider, 2009). In five-year-old NHB 'Bluecrop', the growth of shoots emerging from the crown of the plant as well as the leaf area and weight is higher with 28 g N, 14 g P and 14 g K per plant, compared to any combination of 14 g or 42 g N, 7 g or 21 g P and 7 or 21 g K per plant (Yadong et al., 2009). Individual stem length, individual stem dry weight, stem density and fruit set per stem increased significantly with N-P-K soil fertilizer applications (Percival and Sanderson, 2004). An excess of N fertilization decreases leaf weight and results in smaller fruit. Increasing P and K rates increase fruit set, while increasing K results in larger fruit (Percival and Sanderson, 2004; Yadong et al., 2009). Harvestable yield could be increased by fertilizing up to the leaf tissue level thresholds for N (1.6%), P (0.125%) and K (0.4%) (Percival and Sanderson, 2004). Although calcium (Ca) is also important for blueberry yield (Pannunzio et al., 2009), the Ca requirement, which is 0.3% to 0.8%, is much lower than the 1% to 3% indicated for other temperate fruit crops (Hanson and Berkheimer, 2004).

Blueberry species and cultivars differ in their optimum leaf nutrient concentrations, which make it difficult to determine when deficiencies or toxicities will appear. Table 2 lists the sufficient nutrient foliar levels published by various authors. We can assume that levels outside these ranges would result in either toxicity (if higher) or deficiency (if lower). N, Mg and Fe deficiencies have been found in commercial blueberries grown in the field (Fuqua et al., 2000). Nitrogen deficiency manifests on older leaves first. The whole leaf turns yellow then red and eventually dies. If the deficiency persists, all the leaves will be affected and the plant will be stunted. Magnesium deficiency results in interveinal chlorosis and older leaves

turns yellow or red first. Iron deficiency can be identified by interveinal chlorosis of the younger leaves and the veins that stay green (Eck, 1988). Low Mg fertilizer rates result in deficiency symptoms that become visible already during the first season while over application leads to toxicity that only becomes visible during the second year (Braswell et al., 1997). Nutrient deficiencies occur more commonly on sandy soils due to higher leaching and therefore blueberries grown in sandy soils benefit from more frequent fertilizer applications (Pannunzio et al., 2009).

### **4.3 Fruit nutrient content**

During the first stage of fruit growth N, P, K, Ca, Mg, Fe and Mn concentration in fruit, including the seeds, are the highest. Concentrations of all the mineral elements decrease through fruit development up to the middle of the second stage or beginning of the third stage where after concentrations stay constant. Mineral concentrations vary between different blueberry cultivars (Tamada, 2002). The nutrient composition of mature blueberry fruits is presented in Table 3.

### **4.4 Nutrient uptake**

Time of fertilizer application has a significant effect on the rate of uptake of NHB 'Bluecrop' due to demand and growth differences of the plant throughout the season (Throop and Hanson, 1997). Nutrient uptake is correlated to biomass accumulation and therefore the fastest nutrient uptake coincides with rapid growth or fruit development (Rose, 1999). In NHB 'Bluecrop', nutrient uptake of all elements except Cu increases from leaf budbreak to leaf abscission during the first year and from leaf budbreak to harvest in the succeeding years. Because nutrient uptake of most elements increases as the season progresses, most nutrients should be applied during the spring. Nutrients that are only taken up later in the season such

as K, Mg, Zn and Mn, should rather be applied during early or midsummer (Bryla et al., 2012).

The N content in mature NHB plants increases during the season with the highest level at the end of the season and larger plants taking up more N (Bañados et al., 2006a). The N uptake of NHB is slow at the beginning of the season when the leaf canopy has not expanded and transpiration is insufficient in driving uptake of water and thus nutrients. Due to the low accumulation of dry matter during this time, there is little need for N. During the early stages of petal fall until the end of harvest, N uptake is the highest as the plant accumulates dry matter and N at a fast rate in roots, stems and new growth. Nitrogen uptake is very slow just before the plant enters dormancy, when the leaves are senescing and plant dry weight stays constant. The highest demand for and uptake of N for blueberries is from late bloom to after harvest when active growth occurs. Therefore fertilizing should be done in multiple applications to maintain enough N in the soil (Throop and Hanson, 1997).

Due to N staying in NHB for successive seasons, increasing N reserves late in the season by fertilizer application could support growth for the next season (Throop and Hanson, 1997). Young NHB plants, however, do not have sufficient stored N reserves which can serve as a buffer, and are therefore more sensitive to under or over fertilization. Young roots are more prone to fertilizer burn therefore fertilizers should be applied at low rates in several applications in new plantings to prevent damage to young plants (Bañados et al., 2006a).

With the application of N fertilizer to NHB, the uptake of B, Ca and S are higher at the beginning of the first season. With no N fertilization, uptake of many nutrients such as N, P, K, Ca, S, and B decreases, while Cu uptake increases (Bryla et al., 2012). Nutrients in the plant are lost due to pruning, harvest and leaf abscission and therefore need to be replenished by the plant. Harvest results in a loss of half of the N, P, K, Cu, and Zn and leaf abscission

results in the loss of most of the Ca, Mg, S, Fe, Mn and B. In a study by Bryla et al (2012) only 21%, 3%, and 9% of N, P and K fertilizer, respectively were taken up by NHB plants (Bryla et al., 2012).

#### **4.5 Nutrient allocation**

The highest nutrient concentrations in rabbiteye and NHB blueberries are found in the floral buds and then one-year-wood followed by fine roots. Younger tissue has higher concentrations of N, P and K than older tissue, thus N, P and K concentrations decline as tissue ages. The dry weight of different plant parts determines the amount of minerals allocated to it, with higher partitioning to parts with higher dry weight. The largest storage organs for N, P and K in the winter are the roots and crown, followed by old wood. Differences in nutrient concentrations exist between cultivars (Bañados et al., 2006b). The N, P, K, Ca and Mg content of NHB 'Bluecrop' increase in the woody stems from autumn to late winter possibly due to remobilization from senescing leaves or increased uptake of nutrients. The increase of Ca, however, cannot be ascribed to remobilization, as it is highly immobile in the plant (Bryla et al., 2012).

The majority, about 25%, of N is found in the leaves of blueberry plants while the rest is spread equally between the woody parts. The large and the small roots have the second highest N content and the trunk has the lowest N content. Although the highest concentration of P is also found in the leaves, distribution throughout the plant is more even than for N. Leaves have a higher K than P concentration. The larger roots have the second highest P concentration, followed by the root ball and the stem. The stem and leaves have the highest K, Ca and Mg concentrations and the second highest Mg concentration is found in the roots (Spiers and Marshall, 2012).

Organs, like buds, which are high in nutrient concentration could serve as vital sources for sustaining new growth. Higher nutrient concentrations are found in larger buds and therefore large buds could produce larger fruit, due to the increase in flower quality and early fruit growth (Bañados et al., 2006b).

## **5. Carbohydrates**

### **5.1. Reserve carbohydrates**

Deciduous fruit trees accumulate carbohydrates after harvest, before entering endodormancy in winter. During spring these stored carbohydrates are remobilized for use of reproductive and vegetative growth. This is especially important for the period before vegetative tissue from the present season starts exporting photosynthetic assimilates (Darnell and Birkhold, 1996). Because certain blueberry species exhibit simultaneous vegetative and reproductive budbreak and others flower before vegetative budbreak has occurred, they vary in dependency on reserves in spring. The flowering time of rabbiteye blueberries can occur simultaneously with vegetative bud break, making this stage in the phenology of the plant highly dependent on carbohydrates to sustain both reproductive and vegetative growth (Birkhold et al., 1992). In NHB, floral budbreak only occurs after new shoots have begun growing and therefore the newly expanded leaves are able to supply fruit of some carbohydrates, making reserve carbohydrates less important in these cultivars (Maust et al., 1999). In SHB reproductive budbreak occurs at the same time as vegetative budbreak or in some cases even four weeks before vegetative budbreak (Maust et al., 1999, 2000) and therefore fruit development is mainly supported by reserves as there is no net photosynthetic assimilation from leaves that can be exported to fruit (Darnell and Birkhold, 1996).

Some SHB cultivars, such as Sharpblue, that grow in a non-dormant (evergreen) system have higher carbohydrate availability. The higher carbohydrate availability is ascribed to photosynthates accumulated by photosynthetically active leaves during the winter. These plants, however, do not have increased carbohydrate reserve levels, but rather use the increased carbohydrate availability to increase flower bud initiation and development. Extending the time frame for photosynthesis by preventing leaf drop in autumn promotes reproductive growth with no effect on vegetative growth (Swain and Darnell, 2001). A heavy crop load can, however, result in fruit having a low soluble solid concentration if carbohydrate supply is insufficient to sustain the high sink demand (Maust et al., 1999, 2000).

Reserve carbohydrates are especially important during flower bud development of SHB cultivars Sharpblue and Misty. During this period (between dormancy and bloom) starch concentration in the roots and canes decreases rapidly. It keeps decreasing during the first four weeks of fruit development indicating the importance of reserves for fruit growth. Photosynthesis plays a lesser role in early development of blueberry fruit (Maust et al., 1999).

## **5.2. Carbohydrate allocation**

The main carbohydrate reserve in blueberry is starch (Darnell and Birkhold, 1996). Flore and Layne (1999) showed that cherry leaves are the main source of carbohydrates during the summer, while reserve tissues such as older shoots, buds and structural roots are the most important sources for budbreak and early leaf and flower development during spring. Maust et al. (1999) and Darnell and Birkhold (1996) reported similar findings for SHB and rabbiteye blueberries, respectively. They found that cane and root starch concentrations decreased rapidly during the first four weeks of fruit development indicating that roots and canes are the main carbohydrate sources during spring. In the summer leaves are fully expanded and serve as the main carbohydrate exporters (Darnell and Birkhold, 1996; Maust



et al., 1999). Carbohydrates are allocated according to the sink hierarchy of the organ, distance between the source and sink (receiver) and sink strength. Fruits, shoots, leaves and roots receive carbohydrates in descending order because of priority. Sinks that are closer to sources or have direct vascular connectivity to a source obtain more carbohydrates. The larger and the more active the organ, the bigger the sink strength and thus carbohydrate allocation to that organ (Flore and Lakso, 1986; Flore and Layne, 1999).

According to Retamales and Hancock (2012) the importance of a particular sink changes throughout the life cycle of a blueberry plant. In young non-bearing plants the majority of carbohydrates are allocated to vegetative growth such as roots, leaves and canes. As the plant matures and productivity increases, fruit becomes a greater sink and demands more carbohydrates until the end of the productive life of the orchard in 20 to 30 years where after carbohydrate allocation to fruits decreases. Mainly leaves, but also roots and canes then receive the carbohydrates (Retamales and Hancock, 2012). The sink hierarchy and whether a sink is present on the plant at a certain stage should however be an indication of the importance of a sink. In a bearing tree for example, the fruit will always be the most important sink, whereas shoots will be the most important sink in the absence of fruit. The total number of fruit will decrease as the plant's productivity declines after about 20 years and therefore the total amount of carbohydrates allocated to fruit will be less, but the importance of the fruit as a sink does not change. The ratio of reproductive growth to vegetative growth in very old orchards decreases and therefore more carbohydrates are allocated to leaves, roots and canes compared to younger bearing orchards (Retamales and Hancock, 2012d).

The photosynthetically active radiation (PAR) intercepted by leaves is responsible for most of the carbohydrate supply of fruit (80%). Therefore, factors such as plant density, early

cropping, rate of foliage expansion, duration of leaf area, rate of leaf area removal, and plant architecture influence fruit carbohydrate supply (Retamales and Hancock, 2012d).

Soluble sugars in leaves include glucose, sucrose and fructose with glucose and fructose in a 1:1 ratio (Darnell, 1991). Soluble sugar concentrations and total soluble sugar content in the roots and stems of rabbiteye blueberries decrease from dormancy to full bloom, but then stay constant throughout fruit development. Starch concentration decreases dramatically in the roots of rabbiteye blueberries with differences in the time period between cultivars. Although stem starch concentration also declines from dormancy to full bloom, the decrease is less drastic. The total starch content in both roots and stems display the same decrease as starch concentration. The loss of sugars and starch from the roots and stems during spring indicate reallocation to new growth (Darnell and Birkhold, 1996).

Root starch concentration in SHB decreases from dormancy to 28 days after full bloom (d.a.f.b.). Depending on the cultivar the root starch concentration will either continue to decrease or will increase as in the case of 'Misty' and 'Sharpblue'. In SHB cultivars Misty and Sharpblue the starch concentration in canes decreases until 28 d.a.f.b. and increases somewhat around fruit harvest (Maust et al., 1999).

When plants are grown in non-dormant systems which cause plants to retain their leaves for a longer period, carbohydrate allocation is affected. Carbohydrate concentrations in canes and roots are either the same or lower in non-dormant plants, but synthesis is higher due to longer photosynthetic capacity. This indicates that the increased carbohydrates are allocated to reproductive growth instead of being stored and can therefore result in higher flower bud initiation and yield (Swain and Darnell, 2001).

### 5.3. Fruit composition

Blueberry fruit consist of about 15.3% carbohydrates. The rest is made up of 83% water, 0.7% protein, 0.5% fat and 1.5% fiber (Hancock et al., 2003). Of the carbohydrates 3.5% is cellulose and 0.7% soluble pectin. The sum of all the sugars in blueberries add up to 10% of the fresh weight with the major reducing sugars being glucose and fructose which account for 2.4% of all the sugars (Ayaz et al., 2001; Cano-Medrano and Darnell, 1997; Retamales and Hancock, 2012). Accumulation of sucrose during fruit development is insignificant and sucrose makes up only a small part of the total soluble carbohydrate content of ripe fruit (Cano-Medrano and Darnell, 1997). Species and cultivars differ in their soluble sugar composition, but glucose and fructose are the main sugars in all blueberries (Ayaz et al., 2001).

Blueberries are quite acidic, having an acid range of 1 to 2% (Retamales and Hancock, 2012). The high acidity contributes to the keeping quality (Ballinger and Kushman, 1970; Eck, 1988). The organic acids found in blueberries are: citric, quinic, malic and succinic. Cultivars vary in their composition of organic acids. The predominant organic acids in rabbiteyes are succinic acid (50% of total organic acids) and malic acid (33% of total organic acids), whereas citric acid is the highest organic acid in NHB (75% of total organic acids) and cultivars derived from rabbiteyes (Ehlenfeldt et al., 1994; Wang et al., 2012). The different organic acid compositions of rabbiteyes and NHB as well as the acid content of lowbush blueberries can be used to distinguish between the different *Vaccinium* species (Ehlenfeldt et al., 1994; Kalt and McDonald, 1996). Other acids found in blueberry are the phenolic acid chlorogenic acid (Ayaz et al., 2001; Ehlenfeldt et al., 1994) and, in great quantities, ellagic acid which is said to reduce the risk of cancer (Hancock et al., 2003; Maas et al., 1991).

Starch together with some complex carbohydrates stay constant during berry maturation during stage three of fruit growth. Pectin methyl esterase activity increases during fruit maturation resulting in a continuous decrease in soluble pectin. The sugar-acid ratio increases throughout berry maturation, because the total acidity decreases and the sugar content increases (Ballinger and Kushman, 1970; Eck, 1988; Woodruff et al., 1960). The increase in sugar content is ascribed to an increase in specifically fructose and glucose while the acid decrease is due mainly to a decrease in citric acid, but also malic and quinic acid (Ayaz et al., 2001; Retamales and Hancock, 2012).

Vitamin, amino acid and mineral content of blueberries are in general low compared to other fruit and vegetables (Hancock et al., 2003). Blueberries have intermediate thiamine, riboflavin, niacin and ascorbic acid levels, but similar Ca, K, Mg and Fe levels when compared to other fruits and vegetables (Eck, 1988). Blueberry fruit comprise of 22.1 mg vitamin C per 100 g fresh weight and their most abundant amino acid is arginine. The aroma of blueberries is ascribed to *trans*-2-hexanol, *trans*-2-hexanal and linalool. Ethanol, hexanal, hexanol and limonene are volatiles that exist in greater than 1% concentrations. Acetaldehyde, methyl acetate, ethyl acetate, ethanol and ethylene are the predominant volatiles in blueberries (Parliment and Kolor, 1975).

Blueberries have a very high antioxidant capacity that range from 4.6 to 31.1  $\mu\text{mol}$  Trolox equivalent/g fresh weight (Ehlenfeld and Prior, 2001). The main anthocyanins in blueberries are delphinidin-monogalactoside, cyanidin-monogalactoside, petunidin- monogalactoside, malvidin-monogalactoside and malvidin-monoarabinoside. The total concentration of anthocyanins ranges from 85 to 270 mg per 100g (Retamales and Hancock, 2012). The anthocyanin content is correlated with the sugar-acid ratio and therefore total anthocyanins increase with berry ripening (Ballinger and Kushman, 1970). Phenolic acids are also found in very high quantities in blueberries. The major phenolic acid of *V. arctostaphylos*, a Turkish

blueberry, is caffeic acid in free and insoluble ester-bound forms and p-coumaric in soluble ester and glycoside forms (Ayaz et al., 2005).

## **6. Conclusion**

Blueberry production is increasing globally because of high demand. The demand can be ascribed to the health promoting effects of blueberries, such as preventing premature aging, cancer, cardiovascular disease, immune system decline, brain dysfunction and cataracts. The high antioxidant levels in blueberries are also helpful in preventing urinary tract infections, maintaining eye health and combating degenerative eye diseases.

The blueberry industry is still quite small in South Africa, but because of a rapid increase in plantings, a need for optimum farming practices arises. There is demand for knowledge on, amongst others, fertilizing practices under South African growing conditions. Providing plant organs with the required amount of carbohydrates at the required time will ensure long term plant productivity. Studying the seasonal patterns of nutrients and carbohydrates in blueberry plants could aid in developing a fertilizer program that will ensure optimum production.

It is necessary to gain understanding of the levels and types of nutrients taken up by the plant at certain phenological stages as well as the nutrients and amounts lost by pruning, harvest and leaf drop. This information gives an indication of the levels and types of nutrients that needs to be replenished by the plant to sustain maximum growth and development. The soil type needs to be considered when applying fertilizers to the soil, because it will determine the availability of the nutrients to the plant. It is also necessary to account for leaching and binding of nutrients and therefore the amount of each nutrient the plant requires should be applied in higher concentrations to the soil.

The storage and allocation of carbohydrates are largely correlated to nutrient availability. If the plant receives adequate nutrients, it is able to photosynthesize at a higher rate and for longer. Increased photosynthesis leads to higher production of carbohydrates and a capacity to store more carbohydrates before the plant enters endodormancy. Some cultivars highly depend on these stored carbohydrates to be remobilized in the spring to sustain new growth, while others use the carbohydrates to increase flower bud initiation and development. Ample carbohydrates are necessary for floral development and fruit set, ultimately affecting the productivity of the plant. A study of carbohydrate patterns compared to nutrient patterns and plant phenology would therefore be greatly beneficial to blueberry production.

## 7. Literature Cited

- Abbott, J.D. and R.E. Gough. 1987. Seasonal development of highbush blueberry roots under sawdust mulch. *J. Amer. Soc. Hort. Sci.* 112(1):60-62.
- Ames, B.N., M.K. Shigenaga, and T.M. Hagen. 1993. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. of the Natl. Acad. of Sci.* 90(17):7915-7922.
- Ayaz, F.A., A. Kadioglu, E. Bertoft, C. Acar, and I. Turna. 2001. Effect of fruit maturation on sugar and organic acid composition in two blueberries (*Vaccinium arctostaphylos* and *V. myrtillus*) native to Turkey. *N. Z. J. Crop Hort. Sci.* 29(2):137-141.
- Ayaz, F.A., S. Hayirlioglu-Ayaz, J. Gruz, O. Novak, and M. Strnad. 2005. Separation, characterization, and quantitation of phenolic acids in a little-known blueberry (*Vaccinium arctostaphylos* L.) fruit by HPLC-MS. *J. Agric. Food Chem.* 53(21):8116-8122.
- Ballinger, W.E. and L.J. Kushman. 1970. Relationship of stage of ripeness to composition and keeping quality of highbush blueberries. *J. Amer. Soc. Hort. Sci.* 95(2):239-242.

- Bañados, M.P. and B. Strik. 2006. Manipulation of the annual growth cycle of blueberry using photoperiod. *Acta Hort.* 715:65-71.
- Bañados, M.P., B. Strik, and T. Righetti. 2006a. The uptake and use of <sup>15</sup>N-nitrogen in young and mature field-grown highbush blueberries. *Acta Hort.* 715:357-364.
- Bañados, M.P., C. Bonomelli, J. González, and F. Jiullerat. 2006b. Dry matter, nitrogen, potassium and phosphorus partitioning in blueberry plants during winter. *Acta Hort.* 715:443-448.
- Bieniasz, M. 2012. The differentiation of highbush blueberry flower buds. *Acta Hort.* 932:117-122.
- Birkhold, K.T., K.E. Koch, and R.L. Darnell. 1992. Carbon and nitrogen economy of developing rabbiteye blueberry fruit. *J. Am. Soc. Hort. Sci.* 117(1):139-145.
- Braswell, J.H., J.M. Spiers, and F.B. Matta. 1997. Influence of N, P, K, Ca and Mg rates on leaf elemental concentration and plant growth of 'Gulf Coast' blueberry. *Acta Hort.* 446:363-368.
- Bryla, D.R., B.C. Strik, M.P. Bañados, and T.L. Righetti. 2012. Response of highbush blueberry to nitrogen fertilizer during field establishment-II. Plant nutrient requirements in relation to nitrogen fertilizer supply. *HortScience* 47(7):917-926.
- Cano-Medrano, R. and R.L. Darnell. 1997. Sucrose metabolism and fruit growth in parthenocarpic vs seeded blueberry (*Vaccinium ashei*) fruits. *Physiol. Plantarum* 99(3):439-446.

- Clark, J.R., D. Creech, M.E. Austin, M.E. Ferree, P. Lyrene, M. Mainland, D. Makus, L. Neuendorff, K. Patten, and J.M. Spiers. 1994. Foliar elemental analysis of southern highbush, rabbiteye, and highbush blueberries in the southern United States. *HortTechnology* 4(4):351-355.
- Darnell, R.L. 1991. Photoperiod, carbon partitioning, and reproductive development in rabbiteye blueberry. *J. Am. Soc. Hort. Sci.* 116(5):856-860.
- Darnell, R.L. 2006. Blueberry botany/environmental physiology. *Blueberries. Hort. Publ., Florida* 5-13.
- Darnell, R.L. and K.B. Birkhold. 1996. Carbohydrate contribution to fruit development in two phenologically distinct rabbiteye blueberry cultivars. *J. Amer. Soc. Hort. Sci.* 121(6):1132-1136.
- Darnell, R.L. and J.F. Williamson. 1997. Feasibility of blueberry production in warm climates. *Acta Hort.* 446:251-256.
- Deng, J., Z. Shi, X. Li, and H. Liu. 2013. Soluble polysaccharides isolation and characterization from rabbiteye blueberry (*Vaccinium ashei*) fruits. *Bioresources* 8(1):405-419.
- Eck, P. 1988. *Blueberry*. CRC Press, Boca Raton, Florida, USA.
- Eck, P. and N.F. Childers. 1966. *Blueberry culture*. Rutgers University Press, New Brunswick, N.J.
- Ehlenfeldt, M.K., I.M. Filmore, and J.R. Ballington. 1994. Unique organic acid profile of rabbiteye vs. highbush blueberries. *HortScience* 29(4):321-323.



- Ehlenfeldt, M.K. and R.L. Prior. 2001. Oxygen radical absorbance capacity (ORAC) and phenolic and anthocyanin concentrations in fruit and leaf tissues of highbush blueberry. *J. Agric. Food Chem.* 49(5):2222-2227.
- Flore, J.A. and A.N. Lakso. 1986. Environmental and physiological regulation of photosynthesis in fruit crops. *Hort. Rev.* 11:111-157.
- Flore, J. and D.R. Layne. 1999. Photoassimilate production and distribution in cherry. *HortScience* 34(6):1016.
- Fuqua, B., P. Byers, M. Kaps, L. Kovacs, and D. Waldstein. 2000. Growing blueberries in Missouri. *Missouri State University Bulletin*. 23 April 2013. <<http://mtngrv.missouristate.edu/assets/publications/B44GrowingBlueberries.pdf>>
- Garcia-Salazar, C. 2002. Crop timeline for blueberries in Michigan and Indiana. Prepared for the US Environmental Protection Agency. 23 April 2013. <<http://www.cipm.info/croptimelines/pdf/RCblueberry.pdf>>
- Godoy, C., G. Monterubbianesi, and J. Tognetti. 2008. Analysis of highbush blueberry (*Vaccinium corymbosum* L.) fruit growth with exponential mixed models. *Scientia Hort.* 115(4):368-376.
- Gough, R., V. Shutak, and N. Windus. 1976. Observations on vegetative and reproductive growth in blueberry. *HortScience* 11(3):260-261.
- Gough, R., V. Shutak, and R. Hauke. 1978a. Growth and development of [cultivars of] highbush blueberry. I. Vegetative growth [and its reduction by daminozide]. *J. Amer. Soc. Hort. Sci.* 103(1):94-97.

- Gough, R., V. Shutak, and R. Hauke. 1978b. Growth and development of highbush blueberry. II. Reproductive growth, histological studies. *J. Amer. Soc. Hort. Sci.* 103:476-479.
- Gough, R.E. 1994. *The highbush blueberry and its management*. The Haworth Press, Inc., Binghampton, N.Y.
- Hancock, J.F., J.J. Luby, and R. Beaudry. 2003. Fruits of the Ericaceae, p. 2762-2768. In: Trugo, L.,P. Fingas, and B. Cabalaero (eds). *Encyclopedia of food science, food technology and nutrition*. Academic press, London.
- Hanson, E.J. and J.B. Retamales. 1992. Effect of nitrogen source and timing on highbush blueberry performance. *HortScience* 27(12):1265-1267.
- Hanson, E.J. and S.F. Berkheimer. 2004. Effect of soil calcium applications on blueberry yield and quality. *Small Fruits Rev.* 3:133-139.
- Huang, Y., C. Johnson, and M. Sundberg. 1997. Floral morphology and development of 'Sharpblue' southern highbush blueberry in Louisiana. *J. Am. Soc. Hort. Sci.* 122(5):630-633.
- Kalt, W. and J.E. McDonald. 1996. Chemical composition of lowbush blueberry cultivars. *J. Am. Soc. Hort. Sci.* 121(1):142-146.
- Kalt, W. and D. Dufour. 1997. Health functionality of blueberries. *HortTechnology* 7(3):216-221.
- Kalt, W., D.A. Ryan, J.C. Duy, R.L. Prior, M.K. Ehlenfeldt, and S. Van der Kloet. 2001. Interspecific variation in anthocyanins, phenolics, and antioxidant capacity among genotypes of highbush and lowbush blueberries (*Vaccinium* section *cyanococcus* spp.). *J. Agric. Food Chem.* 49(10):4761-4767.

- Lang, G.A. and E.J. Parrie. 1992. Pollen viability and vigor in hybrid southern highbush blueberries (*Vaccinium corymbosum* L.× spp.). *HortScience* 27(5):425-427.
- Lila, M.A. 2004. Anthocyanins and human health: an in vitro investigative approach. *BioMed Research International* 2004(5):306-313.
- Lyrene, P.M. and W.B. Sherman. 1984. Breeding early-ripening blueberries for Florida. *Proc. of the Florida State Hort. Soc.* 97:322-325.
- Lyrene, P.M. and W.B. Sherman. 2000. 'Star' southern highbush blueberry. *HortScience* 35(5):956-957.
- Maas, J.L., G.J. Galletta, and G.D. Stoner. 1991. Ellagic acid, an anticarcinogen in fruits, especially in strawberries: a review. *HortScience* 26(1):10-14.
- Makus, D. and J. Spiers. 1995. Preliminary study on the effect of root zone temperatures on vegetative growth and leaf mineral nutrients of 'Gulf Coast' blueberry plants. *HortScience* 30(3):436-436.
- Maust, B., J. Williamson, and R. Darnell. 1999. Flower bud density affects vegetative and fruit development in field-grown southern highbush blueberry. *HortScience* 34(4):607-610.
- Maust, B., J. Williamson, and R. Darnell. 2000. Carbohydrate reserve concentrations and flower bud density effects on vegetative and reproductive development in southern highbush blueberry. *J. Am. Soc. Hort. Sci.* 125(4):413-419.
- Pannunzio, A., P. Texeira, F. Vilella, and L. Puhl. 2009. Fertigation in blueberries in Concordia, Argentina. *Acta Hort.* 810:771-776.

- Parliment, T.H. and M.G. Kolor. 1975. Identification of the major volatile compounds of blueberry. *J. of Food Sci.* 40:762-763.
- Percival, D. and K. Sanderson. 2004. Main and interactive effects of vegetative-year applications of nitrogen, phosphorus, and potassium fertilizers on the wild blueberry. *Small Fruits Rev.* 3(1-2):105-121.
- Reeder, R.K., R.L. Darnell, and T.A. Obreza. 1994. Establishment of an evergreen high density blueberry planting in southwest Florida. *Proc. of the Florida State Hort. Soc.*107:326-326.
- Reeder, R.K. 1998. Establishment of a non-dormant blueberry (*Vaccinium corymbosum* hybrid) production system in a warm winter climate. *J. of Hort. Sci. & Biotech.*73:655-663.
- Retamales, J.B. and J.F. Hancock. 2012. *Crop production science in horticulture: Blueberries.* Cabi, Wallingford, Oxfordshire (UK).
- Rivadeneira, M. 2012. Nutrient concentration in leaves of different developmental stages in blueberry. 23 April 2013. <<http://ria.inta.gov.ar/english/wp-content/uploads/2013/02/Bu-10013-Rivadeneira-ingles-2.pdf>>
- Rose, M.A. 1999. Nutrient use patterns in woody perennials: implications for increasing fertilizer efficiency in field-grown and landscape ornamentals. *HortTechnology* 9(4):613-617.
- Sellappan, S., C.C. Akoh, and G. Krewer. 2002. Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. *J. Agric. Food Chem.* 50(8):2432-2438.

- Smagula, J. and L. Kreider. 2008. Effects of timing of N and phi foliar sprays on lowbush blueberry leaf nutrient concentrations, growth, and yield. *Acta Hort.* 810:733-740.
- Spann, T.M., J.G. Williamson, and R.L. Darnell. 2003. Photoperiodic effects on vegetative and reproduction growth of *Vaccinium darrowi* and *V. Corymbosum* interspecific hybrids. *HortScience* 38:192-195.
- Spiers, J. 1982. Seasonal variation of leaf nutrient composition in 'Tifblue' rabbiteye blueberry (*Vaccinium ashei*). *J. Amer. Soc. Hort. Sci.* 107(2):255-257.
- Spiers, J.M. and D.A. Marshall. 2012. Macronutrient distribution in 'Tifblue' rabbiteye blueberry. *Intl. J. of Fruit Sci.* 12(1-3):48-53.
- Swain, P. and R. Darnell. 2001. Differences in phenology and reserve carbohydrate concentrations between dormant and nondormant production systems in southern highbush blueberry. *J. Am. Soc. Hort. Sci.* 126(4):386-393.
- Tagliavini, M., E. Baldi, P. Lucchi, M. Antonelli, G. Sorrenti, G. Baruzzi, and W. Faedi. 2005. Dynamics of nutrients uptake by strawberry plants (*Fragaria* × *Ananassa* Dutch.) grown in soil and soilless culture. *Eur. J. Agron.* 23(1):15-25.
- Tamada, T. 2002. Stages of rabbiteye and highbush blueberry fruit development and the associated changes in mineral elements. *Acta Hort.* 574:129-137.
- Throop, P.A. and E.J. Hanson. 1997. Effect of application date on absorption of <sup>15</sup>N by highbush blueberry. *J. Am. Soc. Hort. Sci.* 122(3):422-426.
- Wang, Y., C. Chang, J. Chou, H. Chen, X. Deng, B.K. Harvey, J.L. Cadet, and P.C. Bickford. 2005. Dietary supplementation with blueberries, spinach, or spirulina reduces ischemic brain damage. *Exp. Neurol.* 193(1):75-84.

- Wang, S.Y., C. Hangjun, M.J. Camp, and M.K. Ehlenfeldt. 2012. Genotype and growing season influence blueberry antioxidant capacity and other quality attributes. *Intl. J. of Food Sci. and Techn.* 47:1540–1549.
- Weinbaum, S.A., P.H. Brown, R.C. Rosecrance, G.A. Picchioni, F.J.A. Niederholzer, F. Youseffi, and T.T. Muraoka. 2001. Necessity for whole tree excavations in determining patterns and magnitude of macronutrient uptake by mature deciduous fruit trees. *Acta Hort.* 564:41–49.
- Woodruff, R., D. Dewey, and H. Sell. 1960. Chemical changes of ‘Jersey’ and ‘Rubel’ blueberry fruit associated with ripening and deterioration. *Proc. of the Amer. Soc. for Hort. Sci.* 75:387-401.
- Wright, G. 1993. Performance of southern highbush and rabbiteye blueberries on the Corindi Plateau N.S.W. Australia. *Acta Hort.* 346:141-148.
- Yadong, L., Z. Shuang, D. HanPing, G. XiuWu, K. Hummer, B. Strik, and C. Finn. 2009. Effects of nitrogen, phosphorus and potassium on growth, fruit production and leaf physiology in blueberry. *Acta Hort.* 810:759-764.

Table 1. Growth phases calendar for NHB in Michigan and Indiana. (Adapted from Garcia-Salazar, 2002).

Crop stage	Description of crop stage	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1	Dormancy	■	■							■	■
2	Bud swell		■								
3	Budbreak		■								
4	Early green tip			■							
5	0.25" green			■							
6	Early pink bud			■	■						
7	Late pink bud			■	■						
8	25% bloom			■	■						
9	Full bloom			■	■						
10	Petal fall				■	■					
11	Early green fruit				■	■					
12	Late green fruit					■	■				
13	Fruit colouring					■	■				
14	1 <sup>st</sup> harvest						■	■			
15	2 <sup>nd</sup> harvest							■	■		
16	3 <sup>rd</sup> harvest							■	■		
17	Postharvest								■	■	■

Table 2. Sufficient foliar nutrient levels for blueberries.

Nutrient	Rabbiteye	NHB	SHB	Source
Macro-elements (%)				
N	1.16	1.44	1.53	Clark et al 1994
	1.84	NA	NA	Spiers and Marshall 2012
	1.20-1.70	1.80-2.10	NA	Eck 1988
	NA	1.70-2.10	NA	Hanson and Hancock 1996
	NA	1.50-2.10	NA	Fuqua et al 2000
P	0.08	0.11	0.10	Clark et al 1994
	0.08-0.17	0.12-0.40	NA	Eck 1988
	NA	0.08-0.40	NA	Hanson and Hancock 1996
	NA	0.07-0.12		Fuqua et al 2000
K	0.36	0.49	0.55	Clark et al 1994
	0.79	NA	NA	Spiers and Marshall 2012
	0.28-0.60	0.35-0.65	NA	Eck 1988
	NA	0.40-0.65	NA	Hanson and Hancock 1996
	NA	0.40-0.80	NA	Fuqua et al 2000
Ca	0.47	0.60	0.65	Clark et al 1994
	0.42	NA	NA	Spiers and Marshall 2012
	0.24-0.70	0.40-0.80	NA	Eck 1988
	NA	0.30-0.80	NA	Hanson and Hancock 1996
	NA	0.40-0.90	NA	Fuqua et al 2000
Mg	0.16	0.20	0.18	Clark et al 1994
	0.163	NA	NA	Spiers and Marshall 2012
	0.14-0.20	0.12-0.25	NA	Eck 1988
	NA	0.15-0.30	NA	Hanson and Hancock 1996
	NA	0.10-0.30	NA	Fuqua et al 2000



S	0.13	0.15	0.15	Clark et al 1994
	NA	0.12-0.20	NA	Eck 1988
	NA	0.12-0.20	NA	Hanson and Hancock 1996
	NA	0.10-0.20	NA	Fuqua et al 2000
Micro-elements (ppm)				
Mn	81	234	229	Clark et al 1994
	25-100	50-350	NA	Eck 1988
	NA	50-350	NA	Hanson and Hancock 1996
	NA	40-250	NA	Fuqua et al 2000
Fe	69	106	97	Clark et al 1994
	25-70	60-200	NA	Eck 1988
	NA	60-200	NA	Hanson and Hancock 1996
	NA	40-70	NA	Fuqua et al 2000
Zn	13	14	15	Clark et al 1994
	10-25	8-30	NA	Eck 1988
	NA	8-30	NA	Hanson and Hancock 1996
Cu	6	8	8	Clark et al 1994
	2-10	5-20	NA	Eck 1988
	NA	5-20	NA	Hanson and Hancock 1996
B	12-35	30-70	NA	Eck 1988
	NA	20-50	NA	Fuqua et al 2000
	NA	25-70	NA	Hanson and Hancock 1996

---

NHB = Northern highbush, SHB = Southern highbush, NA = not available

Table 3. Nutrient composition of mature blueberry fruits.

Element	Unit	Rabbiteye	NHB	Source
N	% DW	0.36	0.53	Tamada 2002
P	% DW	0.018	0.03	Tamada 2002
	% FW	0.0097	0.013	Eck 1988
K	% DW	0.45	0.57	Tamada 2002
	% FW	0.093	0.081	Eck 1988
Ca	% DW	0.055	0.04	Tamada 2002
	% FW	0.006	0.015	Eck 1988
Mg	% DW	0.028	0.033	Tamada 2002
	% FW	0.0056	NA	Eck 1988
Mn	ppm	4	13	Tamada 2002
Fe	% DW	9	14	Tamada 2002
	% FW	0.0002	0.001	Eck 1988
Na	% FW	0.0011	0.001	Eck 1988

DW= Dry weight, FW= Fresh weight, NA = not available

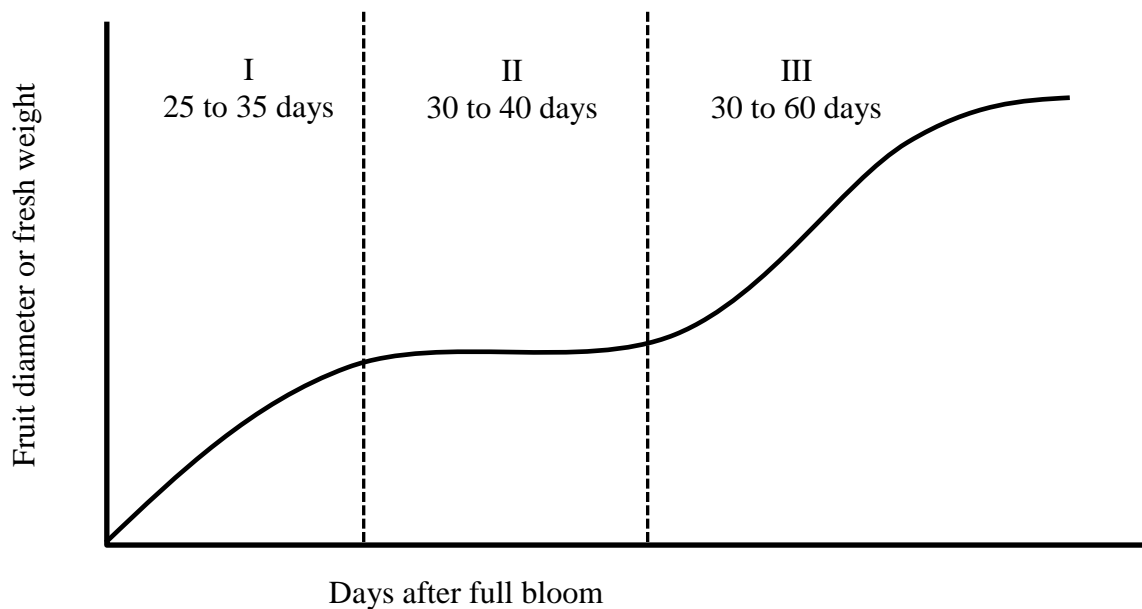


Fig. 1. Growth curve of blueberry fruit. (Adapted from Retamales and Hanson, 2012). During stage I rapid cell division occurs, during stage II seeds develop and during stage III cells enlarge and fruit growth is rapid.

## **PAPER 1: Seasonal change of carbohydrates in ‘Emerald’ and ‘Snowchaser’ southern highbush blueberries**

---

**Abstract.** Five-month old tissue culture plants of the southern highbush blueberry cultivars Snowchaser and Emerald were sampled in two-weekly intervals from 30 Apr. 2013 to 24 June 2014 from a netted tunnel. Carbohydrate analysis was performed on the roots, leaves and shoots in the first season and in the second season the canes and flowers were included. Both cultivars accumulated total sugars and starch before reproductive budbreak in the first season, but during the second season this was not clear. For both cultivars the highest starch concentration and content was found in the roots and the highest sugar concentration and content was found in the leaves. All plants retained their leaves during the 14 month sampling period. The carbohydrate patterns of ‘Snowchaser’ and ‘Emerald’ are indicative of southern highbush blueberries grown in an evergreening system.

---

The carbohydrate content of deciduous plants changes during the season as the demand fluctuates. Carbohydrate content typically increases before winter and carbohydrates are stored as reserves for the following season. These stored carbohydrates are used in the spring for new growth, when photosynthetic tissue from the present season has not yet started exporting photosynthetic assimilates (Darnell and Birkhold, 1996; Kramer and Kozlowski, 1979; Spann et al., 2008).

Different blueberry species differ in their dependency on reserves during spring due to different budbreak patterns (Birkhold et al., 1992). Rabbit-eye blueberries exhibit concomitant reproductive and vegetative budbreak and, therefore, are highly dependent on reserve

carbohydrates in order to sustain both reproductive and vegetative growth (Birkhold et al., 1992). Northern highbush (NHB) blueberries, however, do not depend as highly on reserve carbohydrates, as new shoots have already expanded and started exporting assimilates before floral budbreak occurs (Maust et al., 1999). Reserve carbohydrates play an important role in fruit development of southern highbush (SHB) blueberries. In most cases reproductive and vegetative budbreak occur at the same time, but reproductive budbreak can occur up to four weeks before vegetative budbreak (Maust et al., 1999, 2000). When this happens the plant has no leaves to produce photosynthetic assimilates to support fruit growth (Darnell and Birkhold, 1996).

Maust et al. (1999) found that reserve carbohydrates are important during flower bud development of SHB cultivars Sharpblue and Misty, similar to what Darnell and Birkhold (1996) found on rabbiteye blueberries. They also determined that the starch concentration in the roots and canes decreased rapidly between dormancy and bloom and continued decreasing during the first four weeks of fruit development (Maust et al., 1999). Decreasing starch indicates the importance of reserve carbohydrates and proves that photosynthates are less important in the early development of blueberry fruit (Maust et al., 1999). SHB blueberries can, however, be manipulated to stay evergreen and therefore carbohydrate patterns could differ from those reported by Maust et al. (1999).

Swain and Darnell (2001) reported that SHB blueberry plants in an evergreening system had higher carbohydrate availability due to leaf retention and longer active photosynthesis than dormant plants, but not increased reserve levels. The higher availability of carbohydrates between flower bud initiation and differentiation and 50% bloom was used to increase the amount of flower buds. An evergreening system is thus positive for reproductive development (Swain and Darnell, 2001), but if crop load is already high and carbohydrate

supply is insufficient, an increased load could result in lower soluble sugar content in the berries (Maust et al., 1999, 2000).

In South Africa SHB blueberry plants tend to stay evergreen due to the warm conditions and the seasonal pattern of storage and utilisation of carbohydrate reserves are unknown. The aim of this study was to quantify the carbohydrate reserves of young SHB blueberry plants with regards to the phenology of the plants and to subsequently determine the relative importance of different tissues for carbohydrate storage.

### **Material and Methods**

*Plant material.* The trial was conducted on ‘Emerald’ and ‘Snowchaser’ at Backsberg Wine Estate (S 33° 49, 684’ E 18° 54, 917’) in the Paarl district in the Western Cape of South Africa during the 2013/2014 growing season. Plants derived from tissue culture were planted into 1 L pots in Dec. 2012. In Apr. 2013 these plants were transplanted into 20 L black plastic plant bags. A mixture of peat moss, coir and perlite in a 7:2:11 ratio was used as planting medium. All plants were grown in an evergreen system under 20% white net.

*Treatments and trial design.* The trial comprised of two cultivars in a completely randomised design. A continuous fertigation system with single dripper lines was installed in the tunnel. All plants received the same standard commercial nursery nutrient solution containing nitrogen, phosphorus, potassium, magnesium, calcium and all the micro-elements. Air temperature, relative humidity and soil temperature was measured using Tinytag Plus 2 TGP 4500 and 4020 loggers with a thermistor probe PB-5001-1M5 (Gemini Data Loggers, UK).

Flowers were removed during the first season in order to stimulate vegetative growth. Plants were sampled for 14 months at two-weekly intervals from 30 Apr. 2013 to 24 June 2014. Six replications, consisting of two plants per replication (in order to obtain enough dry material

for analysis), were sampled for each cultivar for six months, where after only one plant was sampled per replication. On 20 Dec. 2013 summer pruning was performed and strong, thick shoots were pruned to about 20 cm above the ground, while all thin, weak shoots were removed completely. Between two and four strong shoots were left, depending on how many strong shoots were present. The following stages were assessed visually for the sampled plants: root growth, growth cessation, reproductive budbreak, vegetative budbreak and full bloom.

*Preparation for analysis.* Initially plants were separated into shoots, leaves and roots, but after 10 months they were separated into canes (thickened shoots older than one year), new shoots, leaves and roots. Flowers were also sampled from 13 May 2014. Fresh weight of each plant part was determined and plant material was stored at -80 °C before being lyophilized. Dried plant material was weighed and ground to pass through a 500 µm sieve. Ground material was stored in 15 mL plastic tubes, vacuum sealed and stored at room temperature until analysis.

*Total soluble sugars extraction.* 0.10 g of dried sample was weighed off in duplicate using a Precisa XB 320M balance (Precisa Gravimetrics AG, Dietikon, Switzerland). 5 mL 80% ethanol was added to the dried sample and mixed using a vortex mixer (Vortex-Genie 2 model G560E, Scientific Industries, Inc., New York, USA) before it was placed in a heating block (Grant QBD4, Grant Instruments Cambridge Ltd, Shepreth, England) at 80 °C for 30 min. Samples were centrifuged (Eppendorf Centifuge 5810 R, Eppendorf, Hamburg, Germany) at 3000 rpm for 5 min. at 20 °C. The supernatant was decanted and the pellet re-extracted twice. The supernatants were pooled and the volume was filled up to 15 mL with 80% ethanol. The ethanol extract was filtered using 0.45 µm syringe filters (Sartorius, Goettingen, Germany) and stored at ± 6 °C until analysis.

*Starch extraction.* 5 mL deionised water was added to the pellet (left from the ethanol extract), mixed using a vortex mixer and centrifuged at 3000 rpm for 5 min. at 20 °C. The water was decanted and 2 mL acetate buffer (5mM, pH 4.8) was added to the pellet. The sample was mixed using a vortex mixer and placed in a heating block at 100 °C for 30 min. After the sample was mixed using a vortex mixer it was replaced in the heating block for 30 min. The sample was removed from the heating block and left to cool down for 10 min. after which 2 mL amyloglucosidase (Sigma-Aldrich, Steinheim, Germany) was added and placed in a heating block at 60 °C overnight. The duration of the overnight step was kept constant for all samples (18 h 50 min). After removal from the heating block, samples were centrifuged at 3000 rpm for 5 min. at 20 °C. The supernatant was decanted and 3 mL deionised water was added to the pellet. Samples were mixed using a vortex mixer and centrifuged at 3000 rpm for 5 min. at 20 °C. The supernatant was decanted and 3 mL deionised water was added to the pellet (for the second time), where after samples were mixed using a vortex mixer and centrifuged at 3000 rpm for 5 min. at 20 °C. The pooled supernatant was filled up to 11 mL with deionised water. The starch extract was filtered using 0.45 µm syringe filters and stored at ± 6 °C until analysis.

*Phenol sulphuric acid assay.* Total soluble sugars and starch (as glucose equivalents) were determined using the phenol sulphuric acid assay (Buisse and Merckx, 1993; Dubois et al., 1956). Extracts were diluted in order to obtain absorbance values smaller than 1.2 au in order to avoid spectrophotometer accuracy limitations. 200 µL phenol (Merck, Darmstadt, Germany) was added to 200 µL of diluted sample and mixed using a vortex mixer. 1 mL concentrated sulphuric acid (Merck, Darmstadt, Germany) was added, mixed using a vortex mixer and left to cool down for 10 min. The assay was done in triplicate and solutions of glucose (Sigma-Aldrich, Steinheim, Germany) were used as standards. The samples were

analysed using a Cary 50, Conc UV-visible spectrometer (Varian Australia Pty Ltd., Victoria, Australia).

*Statistical analysis.* Comparisons were made within cultivars. Data were subjected to analysis of variance (ANOVA) and least significant difference (LSD) values were determined when the significance level was  $<0.05$  using Fisher's least significant difference test in the SAS enterprise guide 5.1 program (SAS Institute Inc., Cary, NC, USA).

## **Results and Discussion**

*General plant phenology.* Growth cessation in 'Snowchaser' and 'Emerald' occurred around 14 May 2013. Reproductive budbreak occurred on 23 July 2013 in 'Snowchaser' and on 6 Aug. 2013 in 'Emerald'. Flowers that formed during the first growing season were removed, but as the initiation and differentiation of these flowers would have required carbohydrates, reproductive budbreak in the first season is indicated on the graphs. Vegetative budbreak occurred on 20 Aug. 2013 in both cultivars. Roots were active during the whole sampling period, but rapid DW gain (data not shown) started 29 Oct. 2013 and lasted until the end of sampling (24 June 2014). According to Abbott and Gough (1987) root growth is limited outside the 14 to 18 °C soil temperature range, but as can be seen in Fig. 1, soil temperatures were above 18 °C after 29 Oct. 2013.

In the second season plants did not stop growing, possibly due to high enough temperatures (Fig. 2) and adequate water and fertilization. No vegetative budbreak is shown for season two, as both cultivars retained their leaves and new expanding leaves were present throughout the full sampling period. Reproductive budbreak in the second season occurred in autumn. In 'Snowchaser' it occurred on 1 Apr. 2014 and in 'Emerald' on 15 Apr. 2014. Full bloom for both cultivars occurred in the beginning of winter (13 May 2014). In 'Emerald' the period



between reproductive budbreak and full bloom was two weeks less than in 'Snowchaser' and for both cultivars flowering in the second season commenced over an extended period and full bloom was noted as the time that most flowers were open. New flowers formed in the month after full bloom and green berries were already formed by the end of sampling which was mid-winter (24 June 2014).

The phenology of SHB grown under nets in the Paarl district of South Africa differs greatly from NHB and SHB grown under natural conditions and SHB grown in a non-dormant system in the northern hemisphere (Bañados, 2006; Garcia-Salazar, 2002; Swain and Darnell, 2001). According to Bañados (2006) budbreak occurred in the beginning of March, bloom in the beginning of April and the first ripe fruit were seen between the end of June and the end of July in NHB blueberries in the northern hemisphere. Garcia-Salazar (2002) documented similar phenological dates for NHB in the northern hemisphere. According to him dormancy occurred from November to mid-April, budbreak occurred during April, full bloom during May and green fruit was seen during June (Garcia-Salazar, 2002). For SHB in a non-dormant system in the northern hemisphere, 50% bloom was in middle January, 50% fruit set was in the beginning of March and harvest was in mid-June (Swain and Darnell, 2001). Swain and Darnell (2001) compared the phenology of SHB in a dormant and a non-dormant system and although slight differences were found, harvest occurred at the same time in both systems. According to the above mentioned literature, budbreak in the northern hemisphere thus occurs at the beginning of spring after a period of endodormancy, bloom occurs towards the end of spring and harvest is in mid-summer, irrespective of the production system. SHB cultivars 'Snowchaser' and 'Emerald' grown under net in the Paarl district of South Africa do not enter endodormancy and are adapted to flower in the colder months and subsequently be harvested in late winter to early spring. Soil temperature, air temperature and relative humidity were logged and are presented in Figs. 1 to 3. These data could be used to explain

differences from blueberry plants grown under open field conditions, but as no such data is currently available, comparisons could not be made.

*Starch.* A significant increase in root starch concentration and content in ‘Snowchaser’ occurred from growth cessation in May 2013 to reproductive budbreak approximately two months later (23 July 2013) (Fig. 4a and b). The root starch concentration and content then decreased significantly up to two weeks after reproductive budbreak (6 Aug. 2013). The same pattern was found in ‘Emerald’ root starch concentration and content (Fig. 5a and b). The increase indicates a build-up of reserves in the roots and the decrease is probably a result of the reallocation of the starch to sustain reproductive development and other growth (Darnell and Birkhold, 1996; Kramer and Kozlowski, 1979; Loescher et al., 1990). The root starch concentration in ‘Snowchaser’ and ‘Emerald’ increased from two weeks before vegetative budbreak (6 Aug. 2013) until vegetative budbreak (20 Aug. 2013). The removal of the flowers thus probably increased carbohydrate availability for vegetative growth and the starch concentration would probably have decreased further if fruit were allowed to develop. The root starch concentration in ‘Snowchaser’ decreased from two weeks after vegetative budbreak (3 Sept. 2013) until four weeks before summer pruning (26 Nov. 2013) and the starch content in the roots declined steadily from reproductive budbreak (23 July 2013) until four weeks before summer pruning in Dec. 2013 (Fig. 4a and b). In ‘Emerald’ the starch concentration in the roots declined already from vegetative budbreak (20 Aug. 2013) until two weeks before summer pruning (24 Dec. 2013) (Fig. 5a). The starch content in ‘Emerald’ roots displayed the same decrease although it was not significant (Fig. 5b). Starch reserves were therefore utilized during the spring and early summer development of both cultivars. There was a steady increase in root starch concentration of ‘Snowchaser’ from four weeks before pruning (26 Nov. 2013) until reproductive budbreak the next season (1 Apr. 2014) where after the starch concentration stayed relatively constant (Fig. 4a). Root starch content

of ‘Snowchaser’ increased from four weeks before pruning until two weeks after full bloom (early May) (Fig. 4a) due to a rapid increase in dry weight (DW) (data not shown). The starch concentration and content of ‘Emerald’ increased steadily from pruning until full bloom (13 May 2014). This increase in starch concentration before full bloom is different from rabbiteye ‘Bonita’, where a significant decrease was observed from 31 days before full bloom until full bloom (Darnell and Birkhold, 1996) and from SHB ‘Misty’ and ‘Sharpblue’ where the decrease occurred from 18 days before full bloom (d.b.f.b) until full bloom (Maust et al., 1999).

‘Snowchaser’ and ‘Emerald’ roots did not reach the starch concentration of the previous season probably because reproductive development started earlier (Figs. 4a and 5a). For both cultivars, the starch concentration in the roots, however, did not decrease significantly after reproductive budbreak as seen in the first season, possibly because at this stage the photosynthetic capacity of the plant was much higher and therefore the carbohydrate supply from current leaves was sufficient to sustain reproductive growth. Root starch is therefore not as important for reproductive budbreak in older, larger plants. The latter is supported by the increase in root starch content between reproductive budbreak and full bloom, which suggests that there was excess carbohydrates during this stage which was stored in the roots as starch (Figs. 4b and 5b).

Although the slight build up of root starch in the second season was not used prior to reproductive budbreak, it was utilized after full bloom. ‘Snowchaser’ root starch was reallocated two weeks after full bloom (27 May 2014), indicated by a significant drop in root starch concentration and content at this stage (Fig. 4a and b). In ‘Emerald’ a significant decrease in starch concentration and content in the roots was observed from full bloom (13 May 2014) until two weeks after full bloom. Evergreen ‘Snowchaser’ and ‘Emerald’ are therefore different from deciduous rabbiteye blueberries ‘Bonita’ and ‘Climax’ and deciduous

SHB ‘Misty’ and ‘Sharpblue’, which reallocates starch from the roots before full bloom to sustain reproductive development (Darnell and Birkhold, 1996; Maust et al., 1999). It could be that the total carbohydrate availability in ‘Snowchaser’ and ‘Emerald’ was higher than in the plants studied by Darnell and Birkhold (1996) and Maust et al. (1999) and therefore ‘Snowchaser’ and ‘Emerald’ required reserves from the roots only at a later stage. It seems that the dependency on root starch reserves is of short duration, because in ‘Snowchaser’ the starch concentration and content increased again only two weeks later and four weeks later in ‘Emerald’. This is similar to SHB ‘Sharpblue’, which had an increase in root starch concentration 28 days after full bloom (d.a.f.b.) (Maust et al., 1999).

A decrease in leaf starch concentration was observed from the beginning of sampling (30 Apr. 2013) until two weeks after growth cessation early May in ‘Snowchaser’ where after it increased significantly up to two weeks before reproductive budbreak (9 July 2013) and then decreased again until two weeks after reproductive budbreak (6 Aug. 2013) (Fig. 4c). The increase in leaf starch concentration started two weeks later than the increase in root starch concentration and the decrease in leaf starch concentration started two weeks earlier than the decrease in root starch concentration. Leaf starch is remobilized first, possibly because it is nearer to the sink (the new growth) (Kramer and Kozlowski, 1979). In ‘Snowchaser’ the leaf starch concentration increased from vegetative budbreak until two weeks thereafter. The starch concentration then decreased significantly until middle November. The sudden increase at vegetative budbreak can be explained by increased carbohydrate availability after the removal of flowers, but the starch concentration decreased again when new growth demanded the carbohydrates, until the stage that the new growth could sustain itself in middle November (Kramer and Kozlowski, 1979). In ‘Emerald’ the leaf starch concentration also displayed a peak two weeks before reproductive budbreak, but the second peak occurred at vegetative budbreak and not two weeks thereafter as seen with ‘Snowchaser’ (Figs. 4c and

3c). Although peaks in ‘Emerald’ were temporally different from ‘Snowchaser’, the same conclusions about the increases and decreases can be made. In both cultivars peaks in leaf starch concentration were observed in middle December and early February (Figs. 4c and 5c). These peaks were also visible in the leaf content, but in ‘Emerald’ the second peak in leaf starch content occurred earlier than the peak in concentration (Figs. 4d and 5d). These peaks can be explained by the growth habit of SHB blueberries. SHB blueberries have several growth flushes which range from two to five weeks apart. During a growth flush, shoots grow rapidly and then stop growing until the next growth flush starts (Gough, 1994). The increase in concentration will then coincide with the period of no growth and the decrease will coincide with a period of rapid growth. The plant thus prepares for rapid growth by increasing carbohydrates and then uses these carbohydrates for rapid growth. When growth stops, the plant accumulates carbohydrates again until the next growth flush. The exhaustion of carbohydrates with every growth flush, which is followed by replacement of carbohydrates, is characteristic of species that have periodic growth flushes (Kramer and Kozlowski, 1979).

The leaf starch content in ‘Snowchaser’ increased steadily from the end of April season one until summer pruning (24 Dec. 2013) and then kept increasing until reproductive budbreak in the next season (1 Apr. 2014) (Fig. 4d) due to an increase in leaf DW. The leaf starch content decreased from reproductive budbreak (1 Apr. 2014) until two weeks after reproductive budbreak (15 Apr. 2014) and then increased again to two weeks before full bloom (29 Apr. 2014). The starch was probably used to sustain reproductive budbreak and then increased again after the demand no longer persisted or carbohydrates from current photosynthesis were enough to supply the sink. There was a rapid decrease in leaf starch content and concentration starting two weeks before full bloom (Fig. 4c and d), indicating that leaf starch is also used during flowering and not just preceding flowering. The decrease in leaf starch content after

full bloom can also be ascribed to remobilization to the roots and shoots. In ‘Emerald’ the leaf starch content stayed almost unchanged until three weeks after pruning where after it increased rapidly for four weeks. The leaf starch content was inconsistent thereafter, with significant increases and decreases (Fig. 5d). The rapid increase in starch content can be ascribed to rapid DW gain. The decreases and increases thereafter could be a result of competition for carbohydrates between vegetative and reproductive growth as new leaves were still forming by the time flower buds formed, thus explaining the decreases in leaf starch content around floral development. When the new leaves started to produce carbohydrates, the starch content in the leaves increased again, until the next growth flush when new leaves expanded and demanded carbohydrates.

Shoot starch concentration in ‘Snowchaser’ increased from the end of Apr. 2013 up to two weeks before reproductive budbreak (9 July 2013) (Fig. 4e) and until vegetative budbreak in ‘Emerald’ (20 Aug. 2013) (Fig. 5e). In ‘Snowchaser’ the shoots started building up starch reserves at the same time as roots, but started reallocating starch at the same time as leaves, because shoots are also closer to the sinks than roots. In ‘Emerald’ reallocation occurred at the same time for roots, leaves and shoots (Fig. 5a, c and e). There was a decrease in shoot starch concentration in ‘Snowchaser’ from two weeks before reproductive budbreak season one (9 July 2013) until vegetative budbreak (20 Aug. 2013) (Fig. 4e). Shoot starch was therefore also reallocated for reproductive development. There was a rapid increase in shoot starch concentration in ‘Snowchaser’ for two weeks after vegetative budbreak and then it declined rapidly until four weeks before pruning (26 Nov. 2013) (Fig. 4e). This decline also occurred in ‘Emerald’, but it lasted from vegetative budbreak (20 Aug. 2013) until two weeks before pruning (10 Dec. 2013) (Fig. 5e). Just as in the leaves and roots, the increase in starch around vegetative budbreak could be due to the removal of the flowers, because starch increased in the other plant organs, because the main sink (flowers/fruit) was removed. When

vegetative growth started, the starch declined again, because a new sink was formed. The shoot starch concentration in both cultivars remained fairly constant after pruning, with only a slight increase from pruning until the middle of June 2014 (Figs. 4e and 5e). In ‘Snowchaser’ the shoot starch concentration exhibited a similar pattern to the root starch concentration (Fig. 4a and e), but in ‘Emerald’ the root concentration had more peaks than the shoot concentration (Fig. 5a and e). In both cultivars the shoot starch content increased slightly from growth cessation (14 May 2013) until pruning (24 Dec. 2013). In ‘Snowchaser’ the shoot starch content increased steadily from four weeks after pruning and in ‘Emerald’ it increased from two weeks after pruning (Figs. 4f and 5f). The increase lasted until mid-June and was due to an increase in DW. In ‘Emerald’ shoot starch content increased from two weeks after pruning. The shoot starch reserves do not play a significant role in sustaining reproductive development in larger, older plants as seen from Fig. 4e and f and Fig. 5e and f. Darnell and Birkhold (1996) found the opposite for rabbiteye blueberries ‘Bonita’ and ‘Climax’, where shoot starch is important for the period prior to full bloom.

The starch concentration and content in the ‘Snowchaser’ canes increased until reproductive budbreak (1 Apr. 2014) then decreased until two weeks before full bloom (w.b.f.b.) (29 Apr. 2014) (Fig. 6a and b), indicating that the starch reserves in the canes were utilized for reproductive budbreak. The starch concentration in the ‘Emerald’ canes increased from the beginning of March until two weeks before full bloom (29 Apr. 2014) and then decreased until full bloom (13 May 2014) (Fig. 7a). In SHB ‘Sharpblue’ and ‘Misty’ cane starch concentration decreased from 18 d.b.f.b. until 28 d.a.f.b. (Maust et al., 1999). So although ‘Emerald’ cane starch was depleted during the same time, the duration was shorter, because after full bloom the starch concentration in ‘Emerald’ canes increased again (Fig. 7a). An increase in the starch concentration and content of the ‘Snowchaser’ canes occurred after two w.b.f.b. (29 Apr. 2014), but where the starch content kept increasing the concentration

levelled off two w.a.f.b. (27 May 2014) (Fig. 6a and b). This is different from SHB ‘Sharpblue’ and ‘Misty’, which, according to Maust et al. (1999), exhibited a decrease in cane starch concentration during this period and only had a slight increase in starch concentration around fruit harvest.

The starch content of the ‘Emerald’ canes increased until reproductive budbreak (15 Apr. 2014), stayed steady until the middle of June and then increased again (Fig. 7b). There was no increase in ‘Emerald’ cane starch during flowering, possibly because all the carbohydrates were allocated to the flowers. The starch concentration of the flowers of both cultivars did not change significantly (Figs. 6c and 7c). The starch content in the flowers of ‘Snowchaser’ increased significantly over time, due to an increase in DW (Fig. 6d) while no change occurred in ‘Emerald’ flowers (Fig. 7d).

Although leaves were present and photosynthetically functional during reproductive development, carbohydrates from photosynthesis were not enough to sustain flowering and starch reserves were therefore remobilized for a short period during reproductive development in the second season. In both cultivars the roots had the highest starch concentrations followed by the leaves and then the shoots. The majority of the starch is thus situated in the roots. This is common in many plant species (Loescher et al., 1990) as roots are the main storage organs in species such as apple (Yoshioka et al., 1988) and peach (Stassen et al., 1981).

*Total sugars.* The total sugar concentration and content in the roots of ‘Snowchaser’ increased from growth cessation (14 May 2013) until two weeks before reproductive budbreak (9 July 2013) (Fig. 8a and b) and in ‘Emerald’ it increased from four weeks prior to reproductive budbreak (9 July 2013) to reproductive budbreak (6 Aug. 2013) (Fig. 9a and b). Both cultivars thus accumulated sugars before visible reproductive development. In



‘Snowchaser’ the sugars decreased until two weeks before vegetative budbreak (6 Aug. 2013) (Fig. 8a and b) and in ‘Emerald’ the sugars decreased until two weeks after vegetative budbreak (3 Sept. 2013) (Fig. 9a and b). In ‘Snowchaser’ two peaks followed, the first at vegetative budbreak (20 Aug. 2013) and the second smaller one six weeks later (1 Oct. 2013). These peaks in ‘Snowchaser’ sugar concentration were similar to peaks in starch. In ‘Emerald’ reallocation of sugars from the roots continued through vegetative budbreak, while in ‘Snowchaser’ the roots accumulated sugars two weeks before vegetative budbreak and then started reallocating the sugars at vegetative budbreak. The total sugar concentration in ‘Snowchaser’ roots increased from middle October to reproductive budbreak (1 Apr. 2014) and then decreased towards the end of June (Fig. 8a). In ‘Emerald’ the increase was less pronounced and the decrease started four weeks prior to reproductive budbreak (15 Apr. 2014) (Fig. 9a). Both cultivars thus had a slight build-up of sugars in the roots preceding reproductive development and then a slight decrease indicating the reallocation of sugars from the roots to the reproductive organs. Darnell and Birkhold (1996) observed a significant decrease in root and shoot sugar concentration from 31 d.b.f.b. until full bloom in rabbiteye blueberries. Although there was a slight decrease in sugar concentration in ‘Emerald’ and ‘Snowchaser’ roots (Figs. 8a and 9a), it was not significant and in ‘Emerald’ and ‘Snowchaser’ shoots there was no change at this stage (Figs. 8e and 9e). The total sugar content in the roots increased rapidly from middle October until full bloom (13 May 2014) in ‘Snowchaser’ and until four weeks before reproductive budbreak (18 Mar. 2014) in ‘Emerald’, due to a rapid increase in DW (Figs. 8b and 9b). In ‘Snowchaser’ the sugar content in the roots then decreased for four weeks, but increased again similar to the root starch content, verifying the short reallocation of carbohydrates from the roots to the reproductive organs. The sugar content of ‘Emerald’ fluctuated from middle March possibly due to competition between vegetative and reproductive growth.

The total sugar concentration of the leaves and the shoots in both cultivars exhibited two peaks early in the season (Figs. 8c and e and 9c and e). In ‘Snowchaser’ the first peak occurred two weeks before reproductive budbreak (9 July 2013) while in ‘Emerald’ it occurred at reproductive budbreak (6 Aug. 2013). The second peak occurred at vegetative budbreak (20 Aug. 2013) in ‘Snowchaser’ (Fig. 9c and e) and six weeks after vegetative budbreak in ‘Emerald’ (1 Oct. 2013) (Fig. 9c and e). The leaf sugar concentration increased slightly from the end of Oct. 2013 until reproductive budbreak (1 Apr. 2014) in ‘Snowchaser’ (Fig. 8c) and until four weeks before reproductive budbreak (18 Mar. 2014) in ‘Emerald’ (Fig. 9c). The production of carbohydrates was thus greater than the demand, starting from the end of October and therefore there was a slight accumulation of sugars in the leaves of both cultivars preceding reproductive budbreak the following season. After this build-up of sugar, the leaf sugar concentration decreased slowly, probably due to demand by reproductive development (Figs. 8c and 9c). Shoot total sugar concentration in both cultivars increased from the end of Oct. 2013 until four weeks before reproductive budbreak (Figs. 8e and 9e). In ‘Snowchaser’ the sugar concentration then dropped for two weeks where after it increased again until reproductive budbreak (1 Apr. 2014) (Fig. 8e). ‘Emerald’ shoots had a slight decrease in sugar concentration four weeks prior to reproductive budbreak (18 Mar. 2014) (Fig. 8e). In both cultivars the shoot sugar concentration stayed unchanged after reproductive budbreak (Figs. 8c and 9c).

The total sugar content of the leaves increased steadily up to pruning (24 Dec. 2013) where after it increased rapidly until full bloom (13 May 2014) for ‘Snowchaser’ (Fig. 8d) and until mid-March for ‘Emerald’ (Fig. 9d), due to an increase in DW. In ‘Snowchaser’ a decrease in leaf sugar content occurred after full bloom (13 May 2014) (Fig. 8d) suggesting reallocation to flowers, while in ‘Emerald’ the leaf sugar content was inconsistent from mid-March (Fig. 9d), as was observed in the roots (Fig. 9b). The total sugar content of the shoots of both

cultivars increased over time as DW increased (Figs. 8f and 9f). In ‘Snowchaser’, however, a small drop (also observed for shoot sugar concentration) occurred in mid-March (Fig. 8f). This drop cannot be ascribed to reallocation, since there was no related change in leaf or root concentration or content. The total sugar concentration in the shoots of both cultivars stayed constant during floral development in contrast to the rabbiteye blueberries ‘Climax’ and ‘Bonita’, which showed sugar depletion from 31 days before full bloom until full bloom (Darnell and Birkhold, 1996).

The total sugar concentration in the canes of ‘Snowchaser’ increased until four weeks before reproductive budbreak (4 Mar. 2014) and then decreased until two weeks before reproductive budbreak (18 Mar. 2014) (Fig. 10a), as was observed in the shoots (Fig. 8e). It could be that reallocation to reproductive growth started earlier for these two organs than for leaves and roots. The total sugar concentration in the canes then increased until reproductive budbreak (1 Apr. 2014) where after the sugar concentration in the canes stayed relatively constant (Fig. 7a), similar to shoot total sugar concentration (Fig. 8e). The total sugar concentration and content in ‘Emerald’ canes increased until two weeks before reproductive budbreak (1 Apr. 2014) where after it declined until reproductive budbreak (15 Apr. 2014) and then stayed relatively constant until sampling was terminated on 24 June 2014 (Fig. 11a and b). The canes and shoots quickly replenished their own sugar concentrations up to the point they were at before reallocation. The total sugar content in the canes of both cultivars peaked twice (Figs. 10b and 11b). In both cultivars a peak occurred at full bloom (13 May 2014), but in ‘Snowchaser’ a peak occurred at reproductive budbreak (1 Apr. 2014) (Fig. 10b) while in ‘Emerald’ it occurred two weeks before reproductive budbreak (1 Apr. 2014) (Fig. 11b). There was a slight decrease in the total sugar concentration of ‘Snowchaser’ flowers until two w.a.f.b. (Fig. 10c). The concentration then increased significantly over two weeks and then stayed constant (Fig. 10c). The total sugar content in the flowers of ‘Snowchaser’ increased

over time as DW increased (Fig. 10d). There was no significant difference in the concentration or content of ‘Emerald’ flowers (Fig. 11c and d).

The increase in total sugar content in the roots, leaves and shoots of ‘Emerald’ and ‘Snowchaser’ were due to rapid DW gain and thus an increase in total sugars in the plant. This is also the time when air temperatures were higher (Fig. 2), which would result in increased photosynthesis that would lead to more carbohydrates being produced and incorporated into the plant structure as DW. The leaves contained the most sugars, followed by the shoots and then the roots. In summer, leaves were fully expanded and served as the main carbohydrate source (Darnell and Birkhold, 1996; Maust et al., 1999) and therefore the leaves had higher sugar levels than the shoots and roots. The total sugar concentration was also the highest in the leaves. This is expected, because photosynthetically active leaves are the sites of carbohydrate synthesis (Retamales and Hancock, 2012).

In ‘Snowchaser’ two significant peaks in root, leaf and shoot starch concentration occurred; the first occurring near reproductive budbreak in the first season and the second two weeks after vegetative budbreak (Fig. 4a, c and e), but no significant differences were seen in the starch content (Fig. 4b, d and f). Similar peaks were observed in ‘Emerald’ roots and leaves (Fig. 5a and c). The peaks in starch concentration coincide with peaks in total sugar concentration of the corresponding plant parts (Figs. 8a, c and e and 9a, c and e). Stephenson et al. (1989) reported similar findings for macadamia and stated that growth flushes cause carbohydrates to fluctuate (Spann et al., 2008). This is common in species exhibiting recurring growth flushes (Kramer and Kozlowski, 1979).

In both ‘Snowchaser’ and ‘Emerald’ carbohydrate (starch and total sugars) concentrations in the roots, leaves and shoots increased between growth cessation and reproductive budbreak during the first season indicating that although the plants remained evergreen, they still

tended to build-up reserves for spring growth. In the second season, however, a build-up of reserves did not occur in both cultivars possibly due to adequate photosynthates produced by current growth. Stephenson et al. (1989) found that, in evergreen macadamias, the absence of a crop the previous season suppressed the build-up of reserves, because carbohydrates were used to increase flowering and was therefore not stored, possibly explaining why no reserves were accumulated in ‘Snowchaser’ and ‘Emerald’ during the second season. In the second season plants had greater leaf area for photosynthesis and could also explain why plants were not as dependant on reserves in the second season. The carbohydrate patterns thus differed between the non-bearing and the bearing plants. The removal of flowers in the first season resulted in higher carbohydrate availability for vegetative growth.

Both cultivars retained their leaves for the whole period of sampling and active growth occurred for the majority of the sampling time. The only time leaves turned colour (to reddish brown) and growth stopped, was between growth cessation and reproductive budbreak in the first season when soil and air temperatures were decreasing (Fig. 1 and 2). After vegetative budbreak the plants were photosynthetically active for the remainder of the sampling period, thus providing carbohydrates to sustain vegetative and reproductive development. Excess carbohydrates were likely used for increased reproductive development instead of being stored as reserves. This phenomenon was also observed by Swain and Darnell (2001) in SHB ‘Sharpblue’ and ‘Wannabe’ that were grown in a non-dormant system. In the beginning of the season decreases in starch coincided with increases in sugars, indicating the break-down of starch to sugars, although this trend was not very clear throughout the season.

The present study clearly highlighted the effect of growing conditions on seasonal carbohydrate patterns and although small differences between the two cultivars existed, the overall pattern for ‘Snowchaser’ and ‘Emerald’ were similar and could thus be an indication of the carbohydrate status of SHB in the Paarl district of South Africa. The maintenance of

healthy leaves that photosynthesize is of absolute importance, because SHB grown in this region depends mainly on carbohydrates derived from photosynthesis rather than from reserves. This emphasizes the importance of adequate water and N fertilizer throughout the season as well as pest and disease management to ensure healthy leaves.

### **Literature Cited**

- Abbott, J.D. and R.E. Gough. 1987. Seasonal development of highbush blueberry roots under sawdust mulch. *J. Amer. Soc. Hort. Sci.* 112(1):60-62.
- Bañados, M.P. 2006. Dry Weight and <sup>15</sup>N-Nitrogen and partitioning, growth, and development of young and mature blueberry plants. Oregon State Univ., PhD Diss.
- Birkhold, K.T., K.E. Koch, and R.L. Darnell. 1992. Carbon and nitrogen economy of developing rabbiteye blueberry fruit. *J. Am. Soc. Hort. Sci.* 117(1):139-145.
- Buysse, J. and R. Merckx. 1993. An improved colorimetric method to quantify sugar content of plant tissue. *J. Exp. Bot.* 44(10):1627-1629.
- Darnell, R.L. and K.B. Birkhold. 1996. Carbohydrate contribution to fruit development in two phenologically distinct rabbiteye blueberry cultivars. *J. Amer. Soc. Hort. Sci.* 121(6):1132-1136.
- Dubois, M., K.A. Gilles, J.K. Hamilton, P. Rebers, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28(3):350-356.
- Garcia-Salazar, C. 2002. Crop timeline for blueberries in Michigan and Indiana. Prepared for the US Environmental Protection Agency. 23 April 2013. <<http://www.cipm.info/croptimelines/pdf/RCblueberry.pdf>>

- Gough, R.E. 1994. The highbush blueberry and its management. The Haworth Press, Inc., Binghampton, N.Y.
- Kramer, P.J. and T.T. Kozlowski. 1979. Physiology of woody plants. Academic Press. Inc., N.Y.
- Loescher, W.H., T. McCamant, and J.D. Keller. 1990. Carbohydrate reserves, translocation, and storage in woody plant roots. HortScience 25(3):274-281.
- Maust, B., J. Williamson, and R.L. Darnell. 1999. Flower bud density affects vegetative and fruit development in field-grown southern highbush blueberry. HortScience 34(4):607-610.
- Maust, B., J. Williamson, and R.L. Darnell. 2000. Carbohydrate reserve concentrations and flower bud density effects on vegetative and reproductive development in southern highbush blueberry. J. Am. Soc. Hort. Sci. 125(4):413-419.
- Retamales, J.B. and J.F. Hancock. 2012. Crop production science in horticulture: Blueberries. Cabi, Wallingford, Oxfordshire (UK).
- Spann, T.M., R.H. Beede, and T.M. Dejong. 2008. Seasonal carbohydrate storage and mobilization in bearing and non-bearing pistachio (*Pistacia vera*) trees. Tree Physiol. 28(2):207-213.
- Stassen, P.J.C., D.K. Strydom, and H.W. Stindt. 1981. Seasonal changes in carbohydrate fractions of young Kakamas peach trees. Agroplanta 13:63-72.
- Stephenson, R., E. Gallagher, and T. Rasmussen. 1989. Effects of growth manipulation on carbohydrate reserves of macadamia trees. Scientia Hort. 40(3):227-235.

Swain, P. and R.L. Darnell. 2001. Differences in phenology and reserve carbohydrate concentrations between dormant and non-dormant production systems in southern highbush blueberry. *J. Am. Soc. Hort. Sci.* 126(4):386-393.

Yoshioka, H., K. Nagai, K. Aoba, and M. Fukumoto. 1988. Seasonal changes of carbohydrates metabolism in apple trees. *Scientia Hort.* 36(3):219-227.



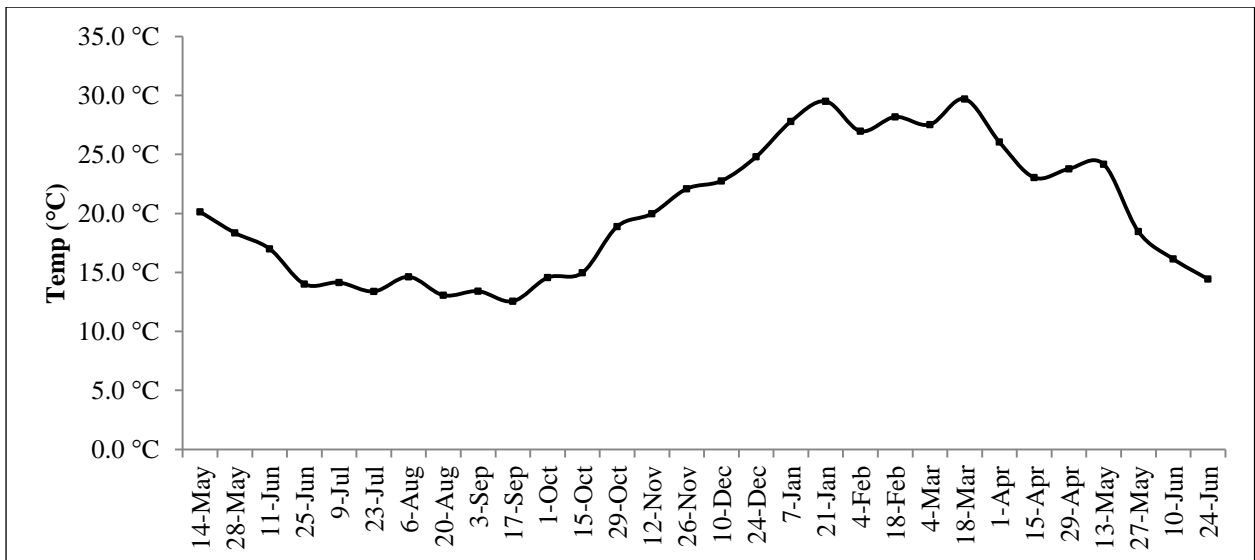


Fig. 1. Average soil temperature in °C in the plant bag at 10 cm depth under a netted tunnel at Backsberg Wine Estate (S 33° 49, 684' E 18° 54, 917') in the Paarl district in the Western Cape of South Africa during the 2013/2014 growing season. Each data point represents the average temperature of the two preceding weeks.

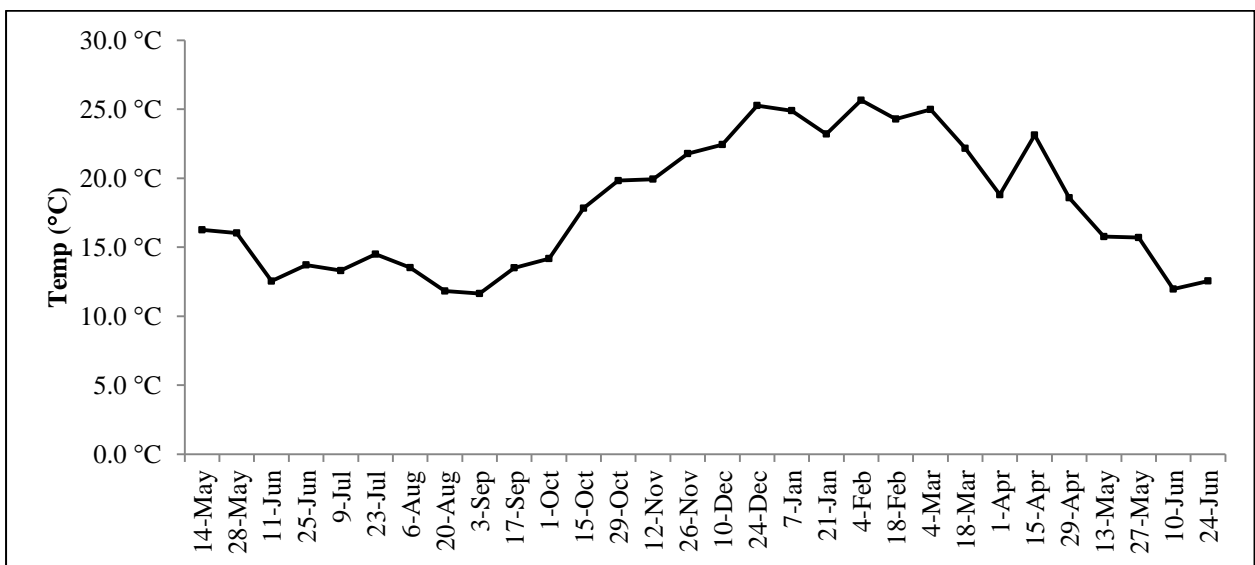


Fig. 2. Average air temperature in °C under a netted tunnel at Backsberg Wine Estate (S 33° 49, 684' E 18° 54, 917') in the Paarl district in the Western Cape of South Africa during the 2013/2014 growing season. Each data point represents the average temperature of the two preceding weeks.

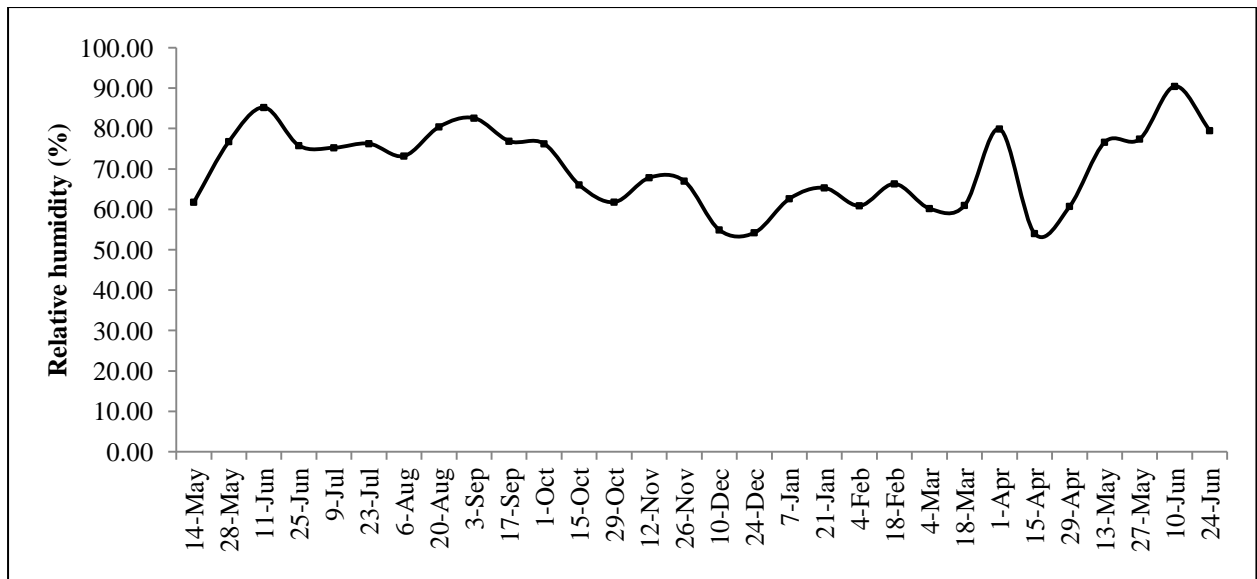


Fig. 3. Average relative humidity under a netted tunnel at Backsberg Wine Estate(S 33° 49, 684' E 18° 54, 917') in the Paarl district in the Western Cape of South Africa during the 2013/2014 growing season. Each data point represents the average relative humidity of the two preceding weeks.

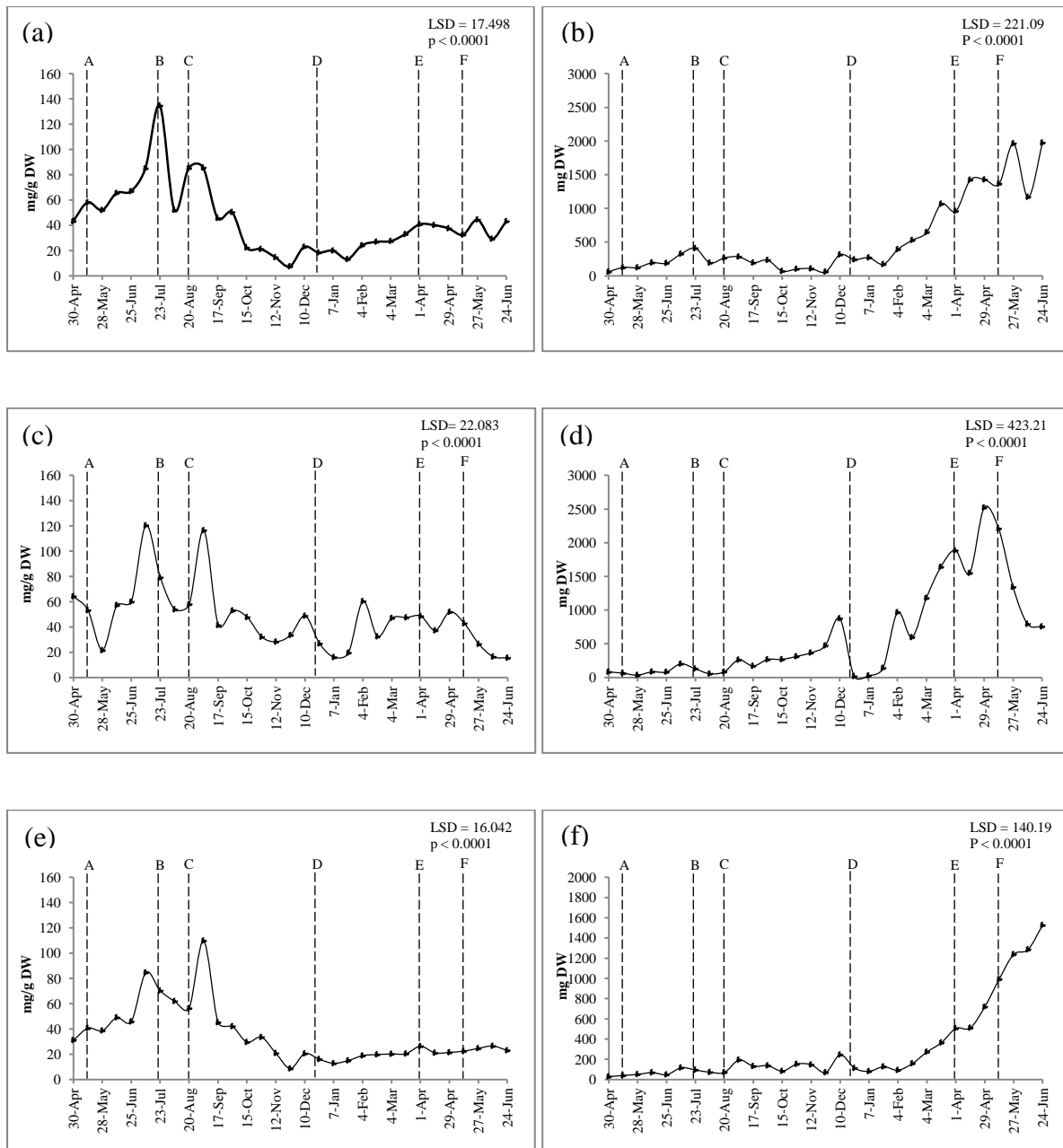


Fig. 4. Starch (as glucose equivalents) concentration and content of 'Snowchaser' southern highbush blueberry roots, leaves and shoots over a 14 month period, from 30 April 2013 to 24 June 2014. The vertical dashed lines represent the following stages: A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom. Graph (a) depicts root starch concentration, (b) root starch content, (c) leaf starch concentration, (d) leaf starch content, (e) shoot starch concentration and (f) shoot starch content.

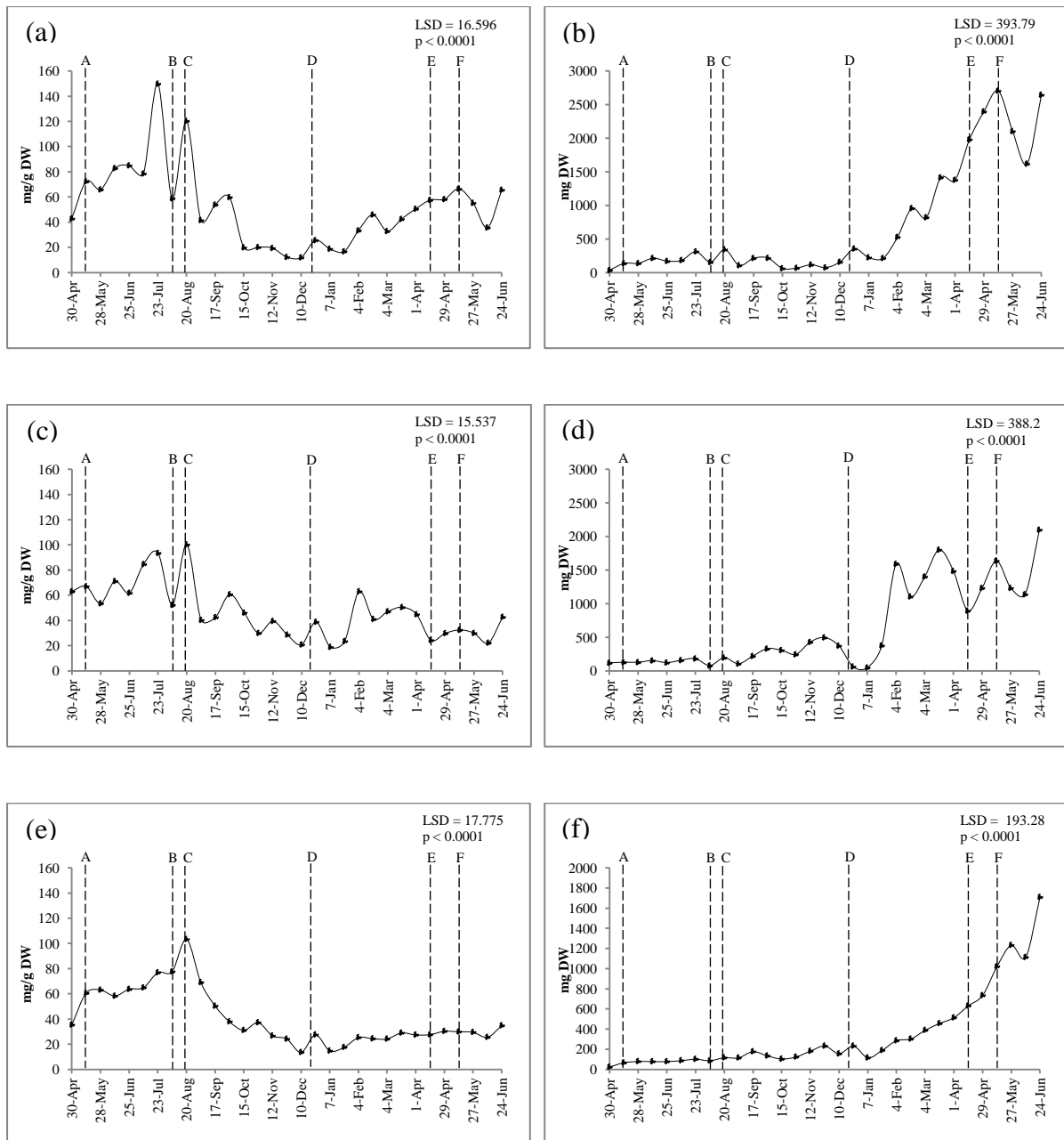


Fig. 5. Starch (as glucose equivalents) concentration and content of 'Emerald' southern highbush blueberry roots, leaves and shoots over a 14 month period, from 30 April 2013 to 24 June 2014. The vertical dashed lines represent the following stages: A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom. Graph (a) depicts root starch concentration, (b) root starch content, (c) leaf starch concentration, (d) leaf starch content, (e) shoot starch concentration and (f) shoot starch content.

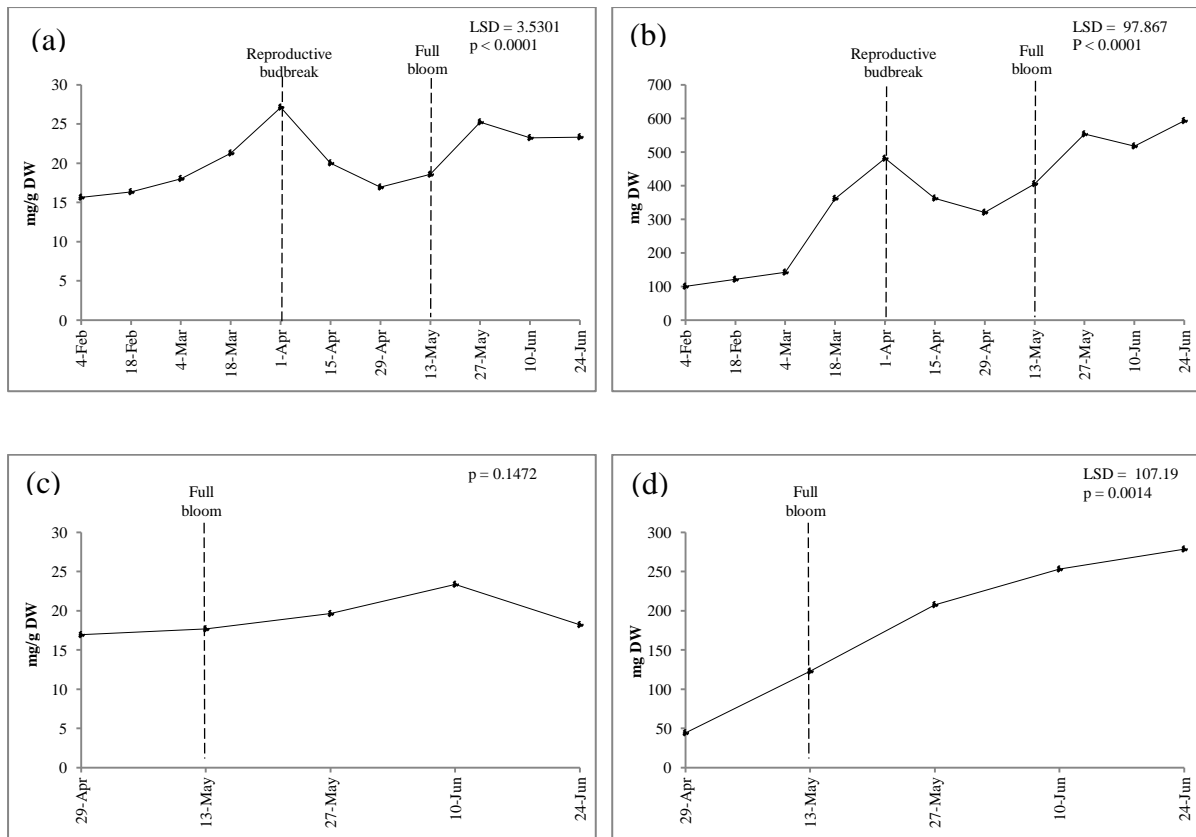


Fig. 6. Starch (as glucose equivalents) concentration and content of ‘Snowchaser’ southern highbush blueberry canes and flowers in the 2014 season. Graph (a) depicts cane starch concentration, (b) cane starch content, (c) flower starch concentration and (d) flower starch content.

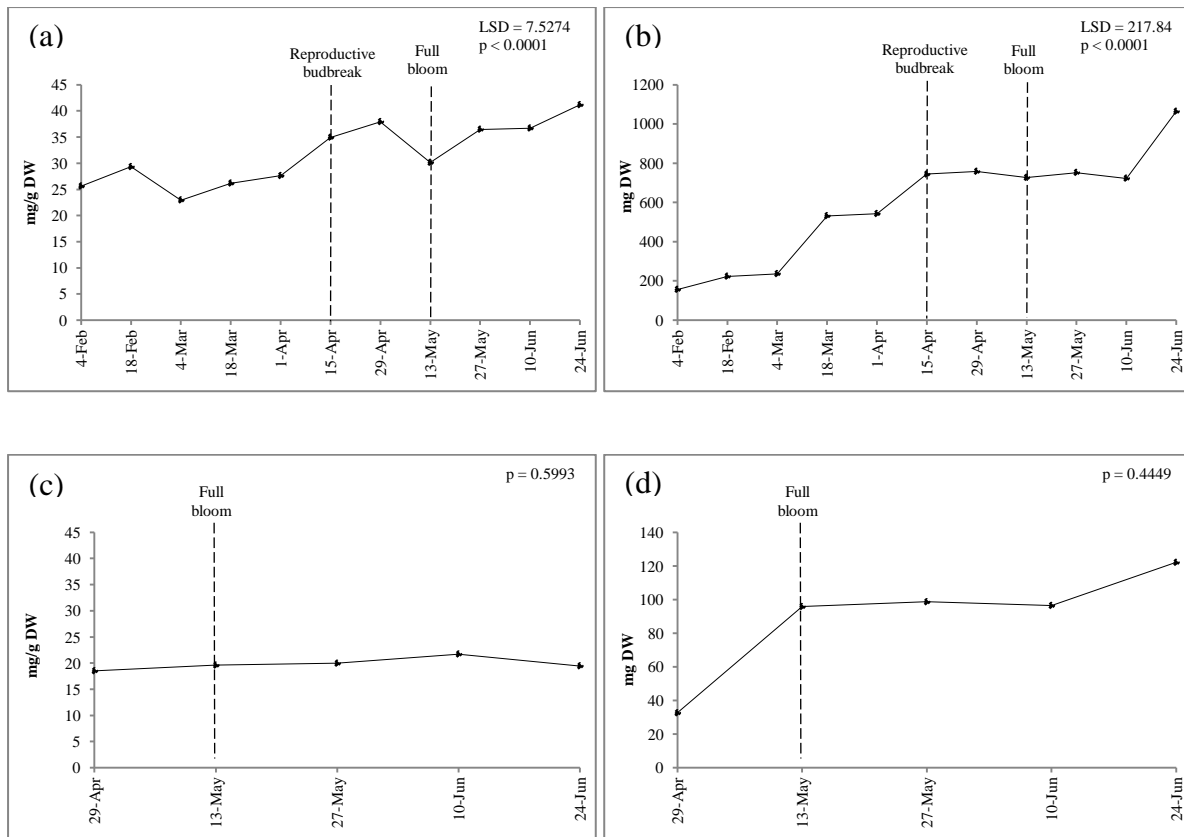


Fig. 7. Starch (as glucose equivalents) concentration and content of 'Emerald' southern highbush blueberry canes and flowers in the 2014 season. Graph (a) depicts cane starch concentration, (b) cane starch content, (c) flower starch concentration and (d) flower starch content.

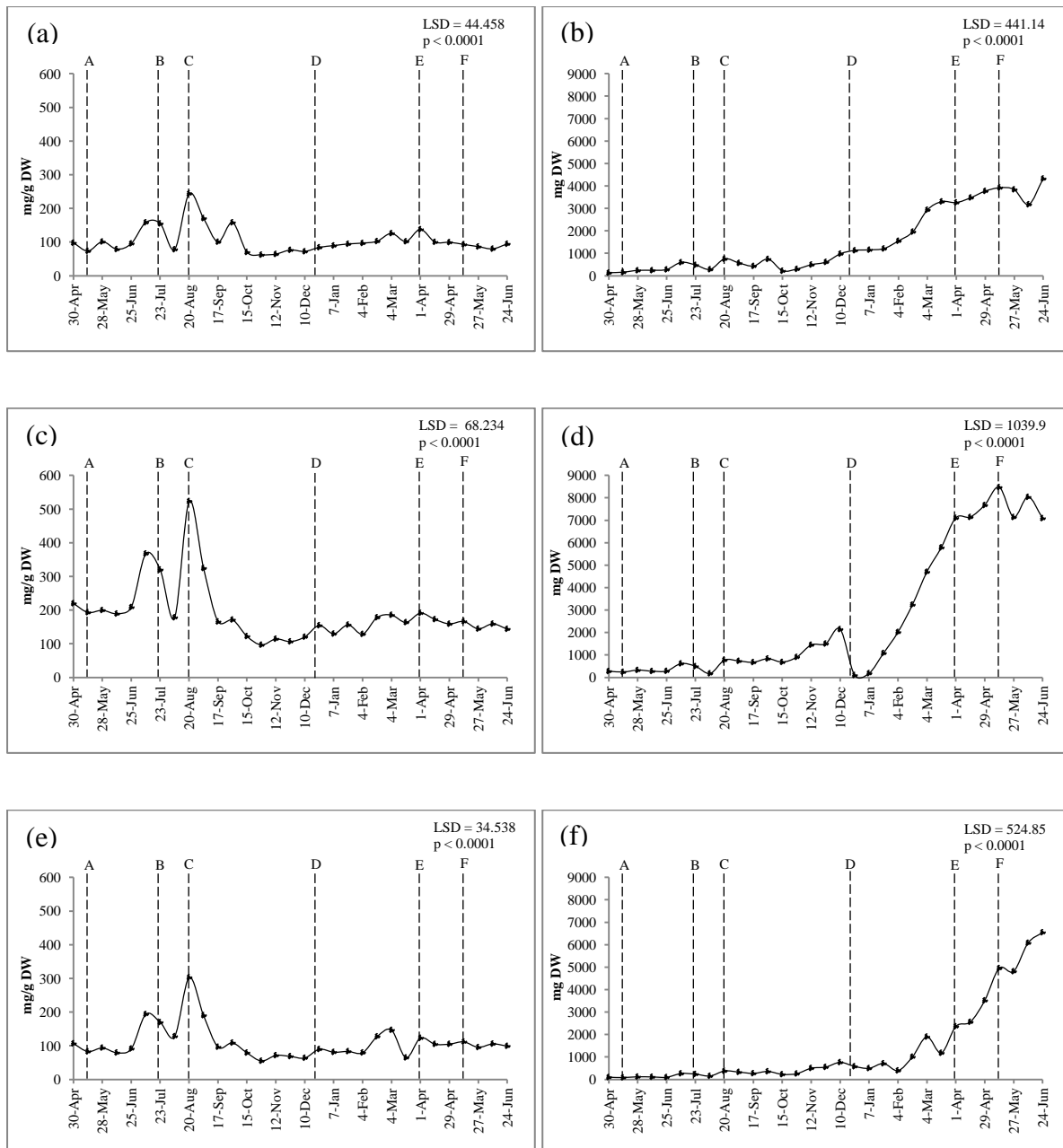


Fig. 8. Total sugar concentration and content of 'Snowchaser' southern highbush blueberry roots, leaves and shoots over a 14 month period, from 30 April 2013 to 24 June 2014. The vertical dashed lines represent the following stages: A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom. Graph (a) depicts root sugar concentration, (b) root sugar content, (c) leaf sugar concentration, (d) leaf sugar content, (e) shoot sugar concentration and (f) shoot sugar content.

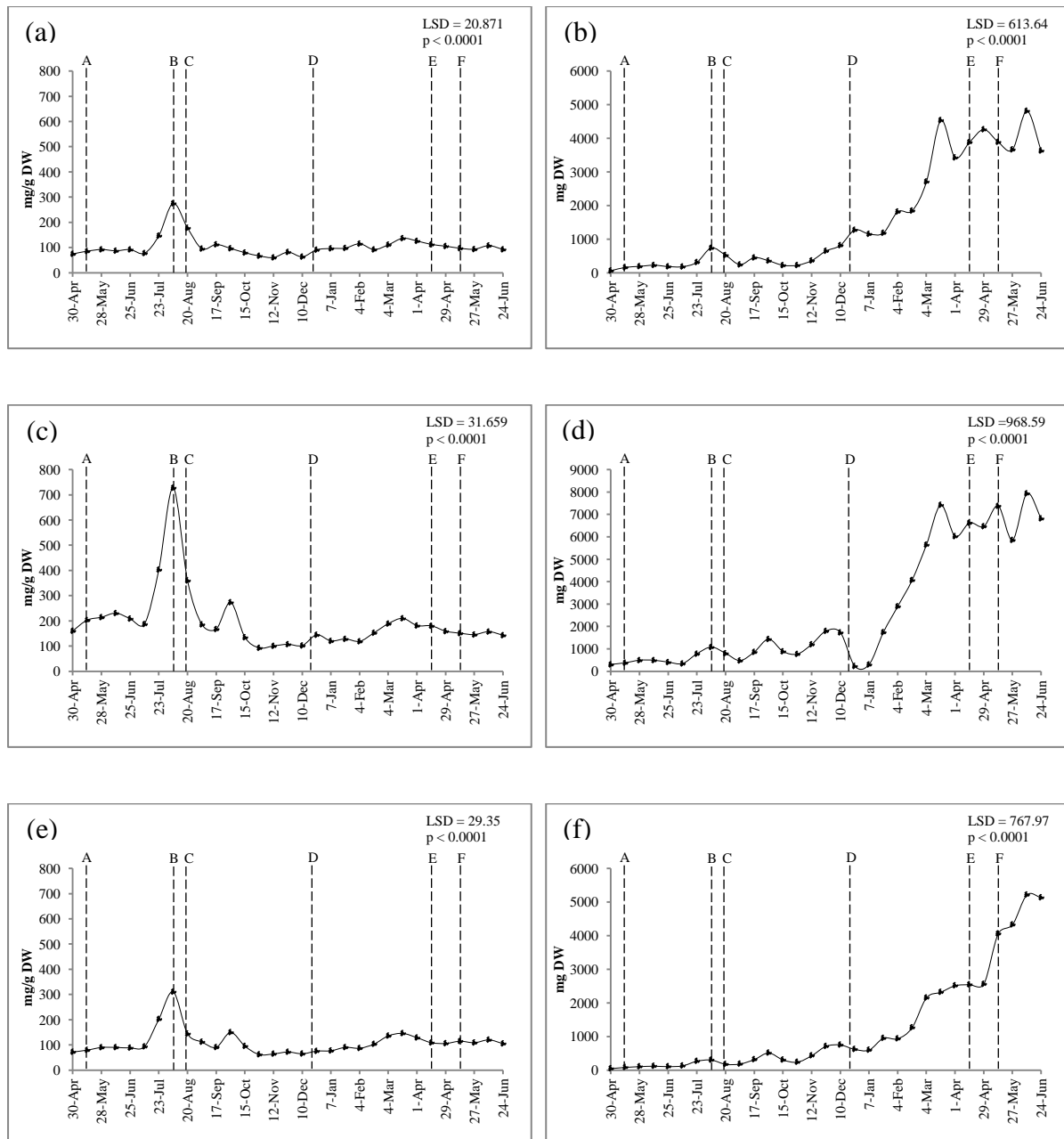


Fig. 9. Total sugar concentration and content of 'Emerald' southern highbush blueberry roots, leaves and shoots over a 14 month period, from 30 April 2013 to 24 June 2014. The vertical dashed lines represent the following stages: A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom. Graph (a) depicts root sugar concentration, (b) root sugar content, (c) leaf sugar concentration, (d) leaf sugar content, (e) shoot sugar concentration and (f) shoot sugar content.



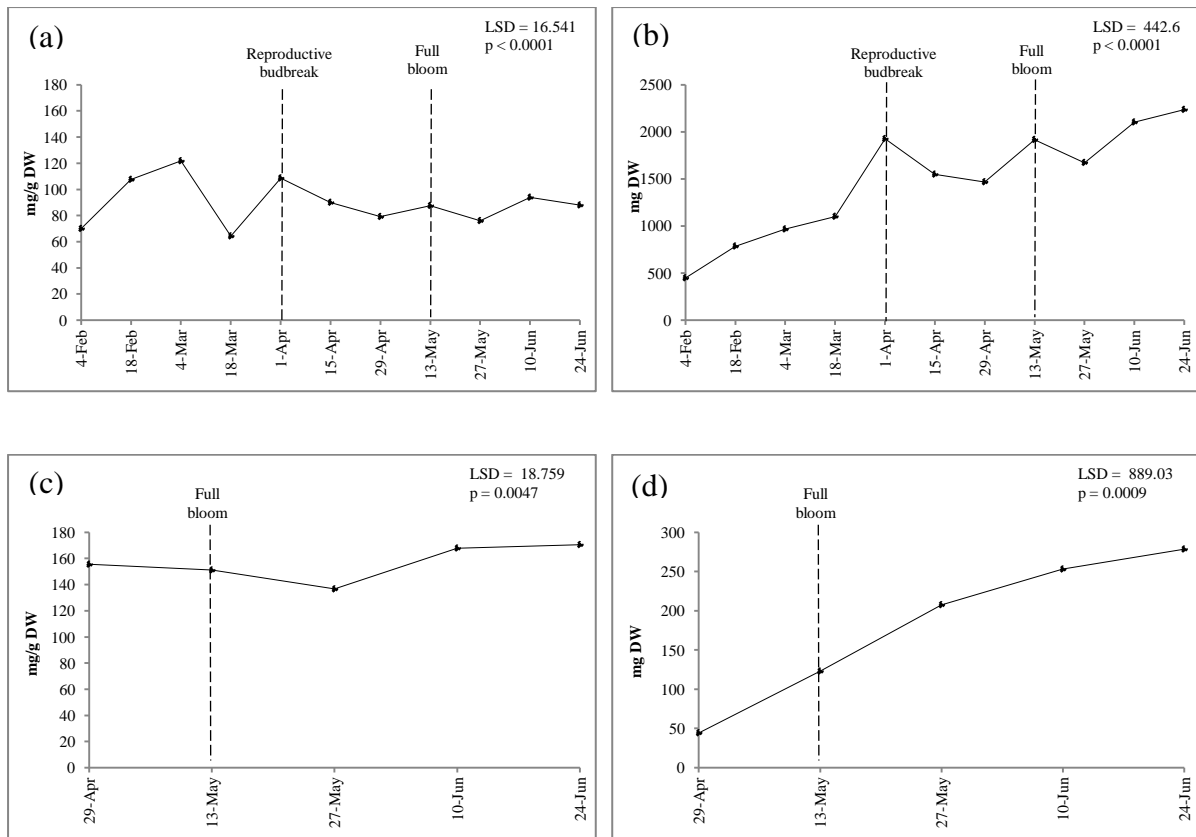


Fig. 10. Total sugar concentration and content of ‘Snowchaser’ southern highbush blueberry canes and flowers in the 2014 season. Graph (a) depicts cane sugar concentration, (b) cane sugar content, (c) flower sugar concentration and (d) flower sugar content.

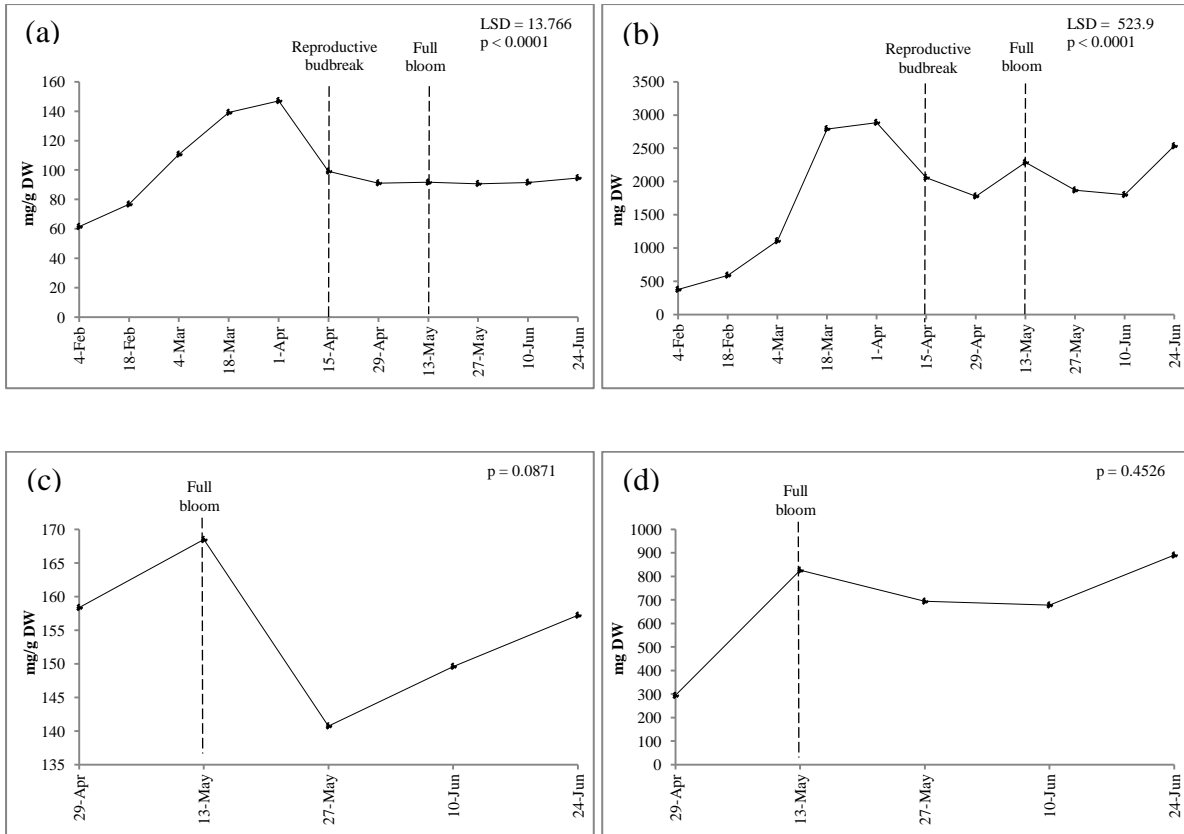


Fig. 11. Total sugar concentration and content of ‘Emerald’ southern highbush blueberry canes and flowers in the 2014 season. Graph (a) depicts cane sugar concentration, (b) cane sugar content, (c) flower sugar concentration and (d) flower sugar content.

## **PAPER 2: Seasonal change in macro nutrients in ‘Emerald’ and ‘Snowchaser’ southern highbush blueberries**

---

*Abstract.* Five-month old tissue culture plants of the southern highbush blueberry cultivars Snowchaser and Emerald were sampled in four-weekly intervals from 14 May 2013 to 10 June 2014 from a netted tunnel. Macro nutrient analysis was conducted on the roots, leaves and shoots in the first season and in the second season the canes and flowers were included and a seasonal pattern of macro nutrients for each plant part was graphically presented. In both cultivars macro nutrient reserves accumulated prior to reproductive budbreak in the second season, but not in the first season. Remobilization patterns differed between the elements and between cultivars. Rapid accumulation of all macro nutrients occurred after summer pruning in both cultivars, when plants were growing vigorously.

---

The South African blueberry industry is expanding. A tenfold increase in blueberry production over the past five years resulted from increased demand, both local and international counter-season demand. The rise in growth of the South African blueberry industry will persist as long as global demand increases (Eurafruit SA Pty Ltd, personal communication).

The continually increasing blueberry cultivation in South Africa requires knowledge on appropriate production practices, including fertilization. The growth in production will potentially lead to blueberries grown on more marginal soils and optimizing fertilization

could make up for the lack of fertile soil and inadequate soil pH (Retamales and Hancock, 2012).

Leaf nutrient analysis is currently used as a guide for fertilizer recommendations. Concentrations of different nutrients in the leaves are determined in autumn when levels are most steady (Spiers, 1982), and then compared to the set optimal leaf concentration range for that particular element (Bryla et al., 2012). According to Weinbaum et al. (2001), the best way to determine nutrient uptake and demand is to sample whole plants in set intervals throughout the season and determine dry mass and nutrient concentration of different tissue types. They also reported that plants take up fertilizer more readily when nutrient availability in the soil equals the nutrient requirement of the plant. A better understanding of the plant's nutrient requirement can improve nutrient management and result in more sustainable fertilizer programs (Rivadeneira, 2012).

Knowledge of the nutrient requirement of the plant as well as the nutrient sources, are important to plan a sustainable fertilizer program. If the pattern of nutrient uptake through the season is established, fertilizer can be applied at intervals that coincide with a specific need of the plant. Setting up a flexible fertilization technique such as fertigation can make control more precise (Tagliavini et al., 2005), resulting in plants with increased physiological status, yield and fruit quality (Yadong et al., 2009).

There is little information on the nutrient status and requirements of southern highbush (SHB) blueberries. Braswell et al. (1997), Clark et al. (1994) and Rivadeneira (2012) studied the foliar nutrient concentrations of deciduous SHB blueberries, but no research has yet been conducted on the nutrient status of other parts of the plant. There is no information regarding the seasonal patterns and partitioning of nutrients in SHB blueberries. The aim of this study was to determine the macro nutrient patterns of 'Emerald' and 'Snowchaser' SHB blueberry

plants grown in an evergreening system over a 13 month period. The seasonal nutrient pattern of evergreen SHB could possibly differ from deciduous SHB, but seeing as no research has yet been conducted on the seasonal patterns of deciduous SHB, this is only a speculation. Nevertheless, deductions from this study only apply to SHB grown in an evergreening system.

### **Material and Methods**

*Plant material.* The trial was conducted on ‘Emerald’ and ‘Snowchaser’ SHB blueberries at Backsberg Wine Estate (S 33° 49, 684' E 18° 54, 917') in the Paarl district in the Western Cape of South Africa during the 2013/2014 growing season. Plants derived from tissue culture were planted into 1 L pots in Dec. 2012. In Apr. 2013 these plants were transplanted into 20 L black plastic plant bags. A mixture of peat moss, coir and perlite in a 7:2:11 ratio was used as growing medium. All plants were grown in an evergreen system under 20% white net.

*Treatments and trial design.* A continuous fertigation system with single dripper lines was installed in the nursery. All plants received the same standard commercial nursery nutrient solution containing nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and all the micro-elements. Air temperature, relative humidity and soil temperature was measured using Tinytag Plus 2 TGP 4500 and 4020 loggers with a thermistor probe PB-5001-1M5 (Gemini Data Loggers, UK).

Flowers were removed during the first season in order to stimulate vegetative growth. Plants from each cultivar were randomly sampled for 13 months at four-weekly intervals from 14 May 2013 to 10 June 2014. Six replications, consisting of two plants per replication (in order to obtain enough dry material for analysis), were sampled for each cultivar for six months,

where after only one plant was sampled per replication. On 20 Dec. 2013 summer pruning was performed and strong, thick shoots were pruned to about 20 cm above the ground, while all thin, weak shoots were removed completely. Between two and four strong shoots were left, depending on how many strong shoots were present. The phenological stage of development was noted at each sampling time. The following stages were noted; root growth, growth cessation, reproductive budbreak, vegetative budbreak and full bloom.

*Preparation for analysis.* Initially plants were separated into shoots, leaves and roots but after 10 months they were separated into canes (thickened shoots older than one year), new shoots, leaves and roots. Flowers were also sampled from 13 May 2014. The fresh weight of each plant part was determined and plant material was stored at -80 °C before being lyophilized. Dried plant material was weighed and ground to pass through a 500 µm sieve. Ground material was stored in 15 mL plastic tubes, vacuum sealed and stored at room temperature until analysis.

*Nutrient analysis.* Material was sent to Bemblab (Pty) Ltd (16 Van der Berg Crescent, Gant's Centrum, Strand 7140, South Africa) agricultural analytical laboratory for macro nutrient analysis. Samples were analyzed via the standard method using the ICP-OES (Inductively Coupled Plasma – Optical Emission Spectrometer) procedure (Hou and Jones, 2000) together with a nitrogen analyzer.

*Statistical analysis.* Comparisons were made within cultivars. Data were subjected to analysis of variance (ANOVA) and least significant difference (LSD) values were determined when the significance level was <0.05 using Fisher's least significant difference test in the SAS enterprise guide 5.1 program (SAS Institute Inc., Cary, NC, USA).

## Results and Discussion

Sampling was done on the same plants as used in Paper 1 and therefore the general phenology of the plants was described in Paper 1 (p. 46). In ‘Emerald’ the period between reproductive budbreak and full bloom was two weeks less than in ‘Snowchaser’ and for both cultivars flowering in the second season commenced over an extended period and full bloom was noted as the time that most flowers were open. Root growth was observed throughout the study indicating that young roots were present for nutrient absorption. Where significant increases in the nutrient concentration of a plant tissue did not coincide with a significant decrease in nutrient concentration of another plant tissue, the increase was interpreted as uptake or accumulation. Decreases in nutrient concentration and content in specific plant tissues were ascribed to reallocation.

*Nutrient accumulation.* In ‘Emerald’ N accumulated rapidly three times during the season, as seen by the increase in root, shoot and leaf concentrations (Fig. 1a, c and e). The first occurred during the two months prior to vegetative budbreak during the first season and was most pronounced in the leaf N concentration (Fig. 1c) and the second uptake, indicated by the increase in root, shoot and leaf N concentration, started four weeks before pruning (26 Nov. 2013) and ended four weeks after pruning (21 Jan. 2014) (Fig. 1a, c and e). The last rapid increase started four weeks prior to reproductive budbreak in the second season (18 Mar. 2014) (Fig. 1a, c and e) and was most pronounced in the N concentration of the roots (Fig. 1a), shoots (Fig. 1c), leaves (Fig. 1e) and canes (Fig. 2a and b). The N uptake pattern of ‘Snowchaser’ (Fig. 3a, c and e) was similar to that of ‘Emerald’ and although uptake occurred at exactly the same dates, it differed slightly relative to the phenology of the plant. One would expect that uptake would be correlated to phenology instead of date, but this could have been a result of the irregularities in fertigation as discussed later. Uptake could also have been correlated to soil temperature, air temperature or humidity (Figs. 1 to 3, p. 63-

64 in Paper 1). The last uptake in ‘Snowchaser’ started two weeks prior to reproductive budbreak during the second season (18 Mar. 2014) and was most pronounced in the N concentration in the roots (Fig. 3a), shoots (Fig. 3c), leaves (Fig. 3e) and canes (Fig. 4a and b). In both cultivars there was no significant difference in the N concentration (Fig. 2c and 4c) and content (Fig. 2d and 4d) of the flowers.

The P uptake pattern of ‘Emerald’ (Fig. 5a, c and e) was the same as the N uptake pattern with the first two peaks in P concentration visible in the roots (Fig. 5a) and leaves (Fig. 5e) and the last peak was visible in the roots (Fig. 5a), shoots (Fig. 5c) and leaves (Fig. 5e), but not in the canes (Fig. 6a). The uptake pattern of P in ‘Snowchaser’ was similar to the N uptake pattern, except for the shoots (Fig. 7c). It was clear from the P concentration in the roots (Fig. 7a) and shoots (Fig. 7c) that P was taken up after pruning (24 Dec. 2013) until full bloom (13 May 2014). The P concentration in ‘Snowchaser’ canes also increased slightly before full bloom, possibly due to uptake (Fig. 8a). In both cultivars the P concentration in the flowers decreased over time (Fig. 6c and 8c), but not the P content (Fig. 6d and 8d).

K was taken up rapidly in ‘Emerald’ from two weeks after vegetative budbreak during the first season (3 Sep. 2013) until four weeks thereafter, indicated by the increase in root (Fig. 9a), shoot (Fig. 9c), and leaf (Fig. 9e) K concentration. Another peak in uptake occurred from pruning (26 Nov. 2013) until four weeks thereafter (21 Jan. 2014), as indicated by a rapid increase in shoot K concentration (Fig. 9c). The changes in cane K concentration could be due to reallocation to and from the leaves (Fig. 9e and 10a). Peaks in K concentration in ‘Snowchaser’ occurred three times during the season. K was taken up six weeks before reproductive budbreak during the first season (11 June 2013) until two weeks before reproductive budbreak during this season (9 July 2013), as indicated by the rapid increase in leaf K concentration (Fig. 11e). The second peak in uptake occurred two weeks after vegetative budbreak (3 Sept. 2013) until four weeks thereafter, as indicated by the increase in



root (Fig. 11a), shoot (Fig. 11c) and leaf (Fig. 11e) K concentration. The last peak in uptake occurred during the month after pruning as shown by the increase in shoot K concentration (Fig. 11c). The gradual decrease in the K concentration of the canes was probably due to reallocation (Fig. 12a). In both cultivars there was no significant change in the K concentration (Fig. 12c) or content (Fig. 12d) of the flowers.

In 'Emerald', Ca increased from six weeks before reproductive budbreak during the first season (11 June 2013) until two weeks after reproductive budbreak (6 Aug. 2013), as seen by the increase in root (Fig. 13a), shoot (Fig. 13c) and leaf (Fig. 13e) Ca concentration. Another peak in uptake occurred from two weeks after vegetative budbreak (3 Sep. 2013) until the end of October, also indicated by the increase in root, shoot and leaf Ca concentration (Fig. 13a, c and e). The last peak in uptake occurred from pruning (24 Dec. 2013) until four weeks before reproductive budbreak during the second season (18 Mar. 2014), clearly indicated by the increase in Ca concentration in the shoot (Fig. 13c), leaf (Fig. 13e) and cane tissue (Fig. 14a). In 'Snowchaser' Ca was taken up from growth cessation (14 May 2013) to the beginning of October as seen from the increase in shoot Ca concentration (Fig. 15c) and again from pruning (24 Dec. 2013) to the end of sampling in mid-June, as indicated by the increase in shoot and leaf Ca concentrations (Fig. 15c and e). Small but significant decreases in the Ca concentration of the canes was observed possibly due to reallocation to the leaves, although Ca is known to be relatively immobile in plant tissue (Kramer and Kozlowski, 1979) (Fig. 15e and 16a). There were no changes in the Ca concentrations (Fig. 16c) or content (Fig. 16d) of the flowers in both cultivars.

In 'Emerald' Mg was taken up from six weeks before reproductive budbreak during the first season (11 June 2013) until the beginning of October, as seen from the increase in root (Fig. 17a) and shoot (Fig. 17c) Mg concentration. A sharp decrease in shoot Mg concentration was seen around pruning (Fig. 17c). Uptake occurred again from pruning (24 Dec. 2013) to full

bloom (13 May 2014) as was shown by the increase in shoot (Fig. 17c) and leaf Mg concentration (Fig. 17e). The slight increase in the Mg concentration of the canes, observed from the beginning of sampling (18 Feb. 2014) until four weeks thereafter, could also be ascribed to uptake (Fig. 18a). In 'Snowchaser' Mg was taken up from growth cessation (13 May 2013) until reproductive budbreak during the first season (23 July 2013), as indicated by the increase in shoot (Fig. 19c) and leaf (Fig. 19e) Mg concentration. Another peak in uptake occurred during the eight weeks after pruning, as indicated by the increase in shoot Mg concentration (Fig. 19c). The change in the Mg concentration in the canes was possibly due to reallocation (Fig. 20a). In both cultivars the Mg concentration in the flowers decreased significantly over time (Fig. 18c and 20c), but the Mg content did not (Fig. 18d and 20d). Overall plants started accumulating nutrients rapidly after summer pruning, when plant growth was rapid (data not shown) as seen with the carbohydrates in Paper 1 (p. 41). The relative humidity (Fig. 3 in Paper 1, p. 64) was fairly low from pruning (20 Dec. 2013) to the end of March, while the temperature (Fig. 2 in Paper 1, p. 63) was relatively high, indicating that transpiration rates were high, which could have lead to increased nutrient uptake if water and nutrient supply were sufficient (Kramer and Kozlowski, 1979). The high growth rate of the plants after pruning (data not shown) could also have lead to an increased nutrient demand.

The nutrient concentration patterns in root, shoot and leaf tissues were variable and showed huge fluctuations over the period of sampling in both cultivars (Fig. 1, 3, 5, 7, 9, 11, 13, 15, 17 and 19). The nutrient concentration in leaves changes as leaves age (Faust, 1989) and therefore the fluctuations in leaf tissue could be because leaf samples contained both young and old leaves. Different amounts of old and young leaves on the plant at the same time, is explained by the flushing growth manner of blueberries discussed in Paper 1 (p. 58). The fluctuations generally followed similar patterns for the different nutrients in both cultivars.

For example the low concentration of N and P observed in leaf tissue on 26 Nov. 2013 that was followed by the peak at 21 Jan. 2014, which was also observed in N in the shoot tissue of ‘Snowchaser’ (Fig. 1e, 3c and e, 5e and 7e). Changes in nutrient concentration in plants are dependent on uptake from the growing medium and therefore availability of nutrients and water (Kramer and Kozlowski, 1979). Unfortunately some problems occurred with the fertigation system and irregular fertigation took place and was sometimes replaced by irrigation. Plants therefore did not always receive enough water and/or nutrients, thus restricting nutrient uptake. Unfortunately the times when the fertigation system was faulty is not known and therefore specific peaks and troughs during the season could not definitely be explained by the irregularity in fertigation.

*Nutrient allocation.* Nutrient allocation and distribution patterns were compared to available literature. Unfortunately literature on seasonal nutrient patterns is limited and therefore comparisons were made only with peach, kiwi and grapevine. These crops, however, are deciduous and therefore differences could be expected.

During the first season, there was no clear build up of N, P, K, Ca and Mg reserves, prior to reproductive budbreak, in both cultivars (Figs. 1 to 10). All macro nutrients were accumulated in the plants from pruning until reproductive budbreak during the second season (early Apr. 2014), when plant dry weight (DW) rapidly increased (data not shown), but the remobilization patterns differed between nutrients. The nutrient accumulation pattern is thus opposite to the carbohydrate accumulation pattern discussed in Paper 1 (p. 41), because carbohydrates accumulated in the first season and not in the second season. This was explained by the absence of a crop the previous season that suppressed the build-up of reserves, because carbohydrates were used to increase flowering and was therefore not stored (p. 59). The increased accumulation of nutrients could be due to the higher nutrient demand created by the increased flowering. In ‘Emerald’ no remobilization of N occurred around

reproductive budbreak and therefore N reserves were not important at this stage (Fig. 1 and 2). Uptake of N during this stage could therefore have supplied N demand from flower buds. This is different from peach (Stassen and Stadler, 1988), kiwi (Kotzé and de Villiers, 1989) and grapevine (Conradie, 1980) which remobilized N from the permanent structure at budbreak. In SHB leaves were present during this stage thus enabling transpiration and uptake of nutrients (Kramer and Kozlowski, 1979b), which would not have been possible in the deciduous trees mentioned at this stage. P was remobilized from the roots (Fig. 5a and b) and canes (Fig. 6a and b) at full bloom (13 May 2014) and K was remobilized from the canes (Fig. 10a and b), indicating the importance of P and K reserves for flowering in ‘Emerald’. In peach redistribution of P and K from the permanent structure occurred in the three weeks after budbreak and redistribution thus started earlier than for ‘Emerald’ (Stassen, 1980). In ‘Snowchaser’ N was remobilized from the roots and leaves at full bloom (Fig. 3a and c) and is different from kiwi, which remobilized N from the roots to the leaves (Kotzé and de Villiers, 1989). P (Fig. 7a, b, e and f) and K (Fig. 11a, b, e and f) were remobilized from the roots, leaves and canes (Fig. 8a, b and 12a, b) at full bloom. In kiwi P and K were translocated from the roots to the leaves during this period (Kotzé and de Villiers, 1989). K was also remobilized from the roots (Fig. 11a and b) and canes (Fig. 12a and b) two weeks prior to reproductive budbreak (18 Mar. 2014), but replenishment occurred two weeks after reproductive budbreak (29 Apr. 2014). In grapevines P and K reserves were not remobilized during the period of flowering (Conradie, 1981). After pruning N, P and K in ‘Snowchaser’ were reallocated from the roots to sustain new growth (shoots and leaves) (Fig. 2, 4 and 6). In peach (Stassen and Stadler, 1988), kiwi (Kotzé and de Villiers, 1989) and grapevines (Conradie, 1981), root reserves were also remobilized when new growth started. Ca in the roots of ‘Emerald’ decreased four weeks prior to reproductive budbreak (18 Mar. 2014) (Fig. 13a and b) and two weeks prior to reproductive budbreak for ‘Snowchaser’ (18 Mar. 2014)

(Fig. 15a and b), indicating remobilization even though Ca is very immobile in the plant (Kramer and Kozlowski, 1979a). At full bloom (13 May 2014) Ca was remobilized again from the roots of ‘Snowchaser’ (Fig. 15b), although only slightly. In peach (Stassen and Stadler, 1988) and kiwi (Kotzé and de Villiers, 1989) remobilization of Ca was not apparent. A small amount of Mg was remobilized from the roots of ‘Emerald’ four weeks prior to reproductive budbreak (18 Mar. 2014) (Fig. 17a and b), but the Mg content in the roots recovered again at reproductive budbreak (15 Apr. 2014). This is similar to findings by Stassen and Stadler (1988) on peach. In ‘Snowchaser’ Mg was remobilized from the roots (Fig. 19a and b), leaves (Fig. 19e and f) and canes (Fig. 20a and b) at full bloom (13 May 2014). Kotzé and de Villiers (1989) reported that no remobilization of Mg reserves occurred in kiwi. The difference in reallocation patterns of ‘Emerald’ and ‘Snowchaser’ can be ascribed to the difference in phenology. In ‘Snowchaser’ reproductive budbreak occurred earlier and flower development stretched over a longer period than for ‘Emerald’ and therefore nutrient demands could have been different.

*Nutrient distribution.* The N content in the leaves of ‘Emerald’ (828.18 mg, LSD = 124.56) was the highest at the end of sampling in mid-winter (eight weeks after reproductive budbreak), followed by the roots (663.73 mg, LSD = 99.60) and shoots (559.22 mg, 59.26) that were similar. The leaves of ‘Snowchaser’ (852.34 mg, LSD = 96.69) also contained the most N at the end of the season (nine weeks after reproductive budbreak), but the shoots (672.49 mg, LSD = 46.58) and the roots (620.45 mg, LSD = 42.13) were the same. This is different from peach, which contained the most N in the roots eight weeks after bud movement (Stassen et al., 1983), but is similar to grapevines which also contained the most N in the leaves around this time (Conradie, 1981). In ‘Emerald’ the most P at the end of the season was found in the roots (71.38 mg, LSD = 10.26), but was not significantly higher than the shoots (63.32 mg, LSD = 7.91), while the shoot P content was somewhat higher than the

leaf P content (54.50 mg, LSD = 10.52). The highest P content in 'Snowchaser' was found in the shoots (65.64 mg, LSD = 6.28) at the end of the season, although it did not differ significantly from the roots (61.08 mg, LSD = 6.77) and leaves (61.85 mg, LSD = 8.21). In peach, the most P was present in the roots, but was not much higher than the P content in the wood, while the P content in the leaves was much lower (Stassen et al., 1983), but in grapevines the most P was found in the leaves (Conradie, 1981). The K content of both cultivars, at the end of the season, was the highest in the leaves (465.27 mg, LSD = 68.84 for 'Emerald' and 488.13 mg, LSD = 68.23 for 'Snowchaser'), followed by the shoots (244.52 mg, LSD = 38.14 for 'Emerald' and 276.59 mg, LSD = 28.64 for 'Snowchaser') and the roots (158.14 mg, LSD = 24.94 for 'Emerald' and 124.70 mg, LSD = 16.46 for 'Snowchaser') and is different from peach (Stassen et al., 1983), which contained the most K in the wood, followed by the leaves and roots, and grapevines (Conradie, 1981) which had the most K in the shoots at the end of bloom ( $\pm$  nine weeks after budbreak). The shoots of both cultivars had the most Ca at the end of the season (621.61 mg, LSD = 86.67 for 'Emerald' and 1046.16 mg, LSD = 84.26 for 'Snowchaser'), followed by the leaves (353.56 mg, LSD = 40.27 for 'Emerald' and 308.63 mg, LSD = 32.87 for 'Snowchaser') and then the roots (97.59 mg, LSD = 14.31 for 'Emerald' and 100.49 mg, LSD = 18.68 for 'Snowchaser') and was different from grapevines which had the most Ca in the leaves at the end of bloom (Conradie, 1981). The leaves of both cultivars contained the most Mg at the end of the season (115.00 mg, LSD = 15.00 for 'Emerald' and 114.60 mg, LSD = 15.84 for 'Snowchaser') and is different from peach which had little Ca in the leaves at this stage (Stassen et al., 1983), but is similar to grapevines which also contained the most Ca at this stage (Conradie, 1981). In 'Snowchaser' the shoots (82.897 mg, LSD = 8.28) had a higher Mg content than the roots (66.33 mg, LSD = 7.61), but in 'Emerald' the roots (77.21, LSD = 8.31 mg) and shoots (75.18 mg, LSD = 9.86) had similar amounts of Mg at the end of the season. This is different from peach, which had the highest

Mg content in the wood, followed by the leaves (Stassen et al., 1983) and grapevine which contained the most Mg in the leaves (Conradie, 1981). The macro nutrient content in both cultivars was different from NHB and rabbiteye blueberries in mid-winter (Bañados et al., 2006). As with peach, kiwi and grapevine, these blueberries were deciduous and therefore had no leaves at that stage, explaining the differences observed. The lack of literature on seasonal nutrient patterns of other blueberries makes it difficult to make conclusions on the effect of an ever-greening system on nutrient patterns of blueberries. Therefore comparisons were made with other deciduous crops, in order to establish whether the seasonal nutrient patterns of a naturally deciduous crop, that is grown in an ever-greening system, would be different to the patterns observed in deciduous crops.

### **Conclusion**

Fluctuations in nutrient concentrations could have been a result of irregular fertigation and therefore it is uncertain whether peaks in nutrient uptake was a result of higher nutrient demand by the plant or changes in micro-climate. Rapid accumulation of macro nutrients, however, was evident when plants were rapidly growing after summer pruning. Small amounts of nutrients were reallocated between different plant parts throughout the season as indicated by the changes in the nutrient concentrations during the season. Larger amounts of nutrients were reallocated at full bloom, because a decrease in nutrient content accompanied a decrease in nutrient concentration. Nutrient reserves did not accumulate before reproductive budbreak in the first season, but nutrient accumulation before reproductive budbreak was apparent in the second season. The nutrient patterns thus differed between the vegetative and reproductive seasons. The two cultivars differed in their remobilization patterns and different nutrients were remobilized at full bloom from different plant tissues. Fertilization is especially important during rapid growth and the maintenance of a proper, functioning

fertigation system during the season is important to supply the plant of its nutrient requirements throughout the season.

### Literature Cited

- Bañados, M.P., C. Bonomelli, J. González, and F. Jiullerat. 2006. Dry matter, nitrogen, potassium and phosphorus partitioning in blueberry plants during winter. *Acta Hort.* 715:443-448.
- Braswell, J.H., J.M. Spiers, and F.B. Matta. 1997. Influence of N, P, K, Ca and Mg rates on leaf elemental concentration and plant growth of 'Gulf Coast' blueberry. *Acta Hort.* 446:363-368.
- Bryla, D.R., B.C. Strik, M.P. Bañados, and T.L. Righetti. 2012. Response of highbush blueberry to nitrogen fertilizer during field establishment-II. Plant nutrient requirements in relation to nitrogen fertilizer supply. *HortScience* 47(7):917-926.
- Clark, J.R., D. Creech, M.E. Austin, M.E. Ferree, P. Lyrene, M. Mainland, D. Makus, L. Neuendorff, K. Patten, and J.M. Spiers. 1994. Foliar elemental analysis of southern highbush, rabbiteye, and highbush blueberries in the southern United States. *HortTechnology* 4(4):351-355.
- Conradie, W.J. 1980. Seasonal uptake of nutrients by Chenin blanc in sand culture. I. Nitrogen. *S. Afr. J. Enol. Vitic.* 1.1:59-65.
- Conradie, W.J. 1981. Seasonal uptake of nutrients by Chenin blanc in sand culture. II. Phosphorus, potassium, calcium and magnesium. *S. Afr. J. Enol. Vitic.* 2.1:7-13.
- Faust, M. 1989. *Physiology of temperate fruit trees*. John Wiley & Sons, Inc. NY (USA).



- Hou, X. and B.T. Jones. 2000. Inductively coupled plasma/optical emission spectrometry, p. 9468–9485. In: R.A. Meyers (Ed.). Encyclopedia of analytical chemistry. John Wiley & Sons Ltd Chichester.
- Kotzé, W. and J. De Villiers. 1989. Seasonal uptake and distribution of nutrient elements by kiwifruit vines 1. Macronutrients. S. Afr. J. of Pl. and Soil 6(4):256-264.
- Kramer and Kozlowski, 1979. Physiology of woody plants. Academic Press. Inc., N.Y.
- Retamales, J.B. and J.F. Hancock. 2012. Crop production science in horticulture: Blueberries. Cabi, Wallingford, Oxfordshire (UK).
- Rivadeneira, M. 2012. Nutrient concentration in leaves of different developmental stages in blueberry. 23 April 2013. <<http://ria.inta.gov.ar/english/wp-content/uploads/2013/02/Bu-10013-Rivadeneira-ingles-2.pdf>>
- Spiers, J. 1982. Seasonal variation of leaf nutrient composition in 'Tifblue' rabbiteye blueberry (*Vaccinium ashei*). J. Amer. Soc. Hort. Sci. 107(2):255-257.
- Stassen, P.J.C. 1980. Reserves in deciduous fruit trees and implications to the deciduous fruit grower. Dec. Fruit Grower 30(12):467-472.
- Stassen, P.J.C., M. Du Preez, and J.D. Stadler. 1983. Reserves in full-bearing peach trees. Dec. Fruit Grower 33:200-206.
- Stassen, P. and J. Stadler. 1988. Seasonal uptake of phosphorus, potassium, calcium and magnesium by young peach trees. S. Afr. J. of Pl. and Soil 5(1):19-23.
- Tagliavini, M., E. Baldi, P. Lucchi, M. Antonelli, G. Sorrenti, G. Baruzzi, and W. Faedi. 2005. Dynamics of nutrients uptake by strawberry plants (*Fragaria* × *Ananassa* Dutch.) grown in soil and soilless culture. Eur. J. Agron. 23(1):15-25.

- Weinbaum, S.A., P.H. Brown, R.C. Rosecrance, G.A. Picchioni, F.J.A. Niederholzer, F. Youseffi, and T.T. Muraoka. 2001. Necessity for whole tree excavations in determining patterns and magnitude of macronutrient uptake by mature deciduous fruit trees. *Acta Hort.* 564:41–49.
- Yadong, L., Z. Shuang, D. HanPing, G. XiuWu, K. Hummer, B. Strik, and C. Finn. 2009. Effects of nitrogen, phosphorus and potassium on growth, fruit production and leaf physiology in blueberry. *Acta Hort.* 810:759-764.

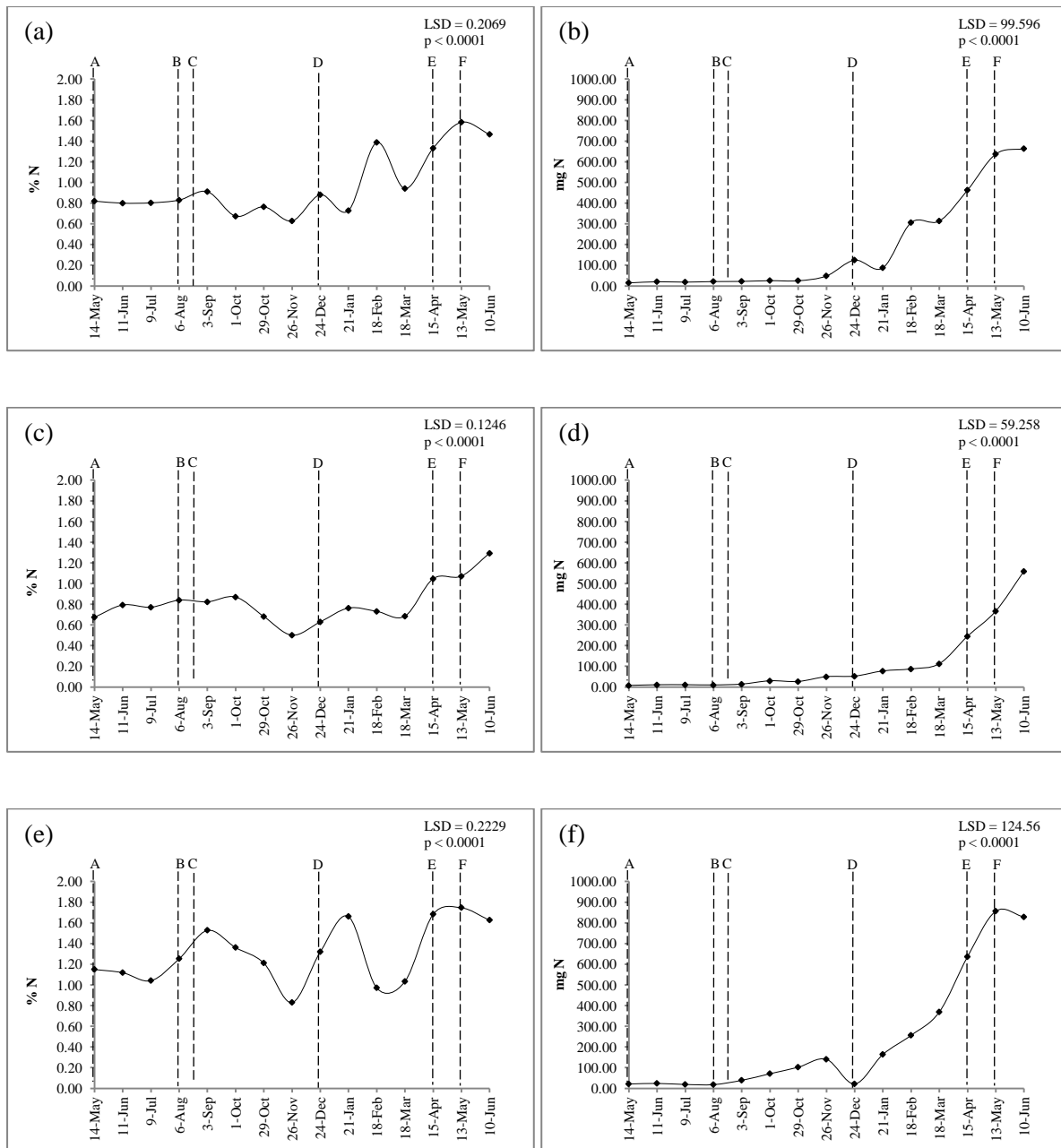


Fig. 1. Nitrogen (N) concentration and content in the roots, shoots and leaves of southern highbush blueberry 'Emerald' from 14 May 2013 to 10 June 2014. (a) is root N concentration, (b) root N content, (c) shoot N concentration, (d) shoot N content, (e) leaf N concentration and (f) leaf N content. A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom.

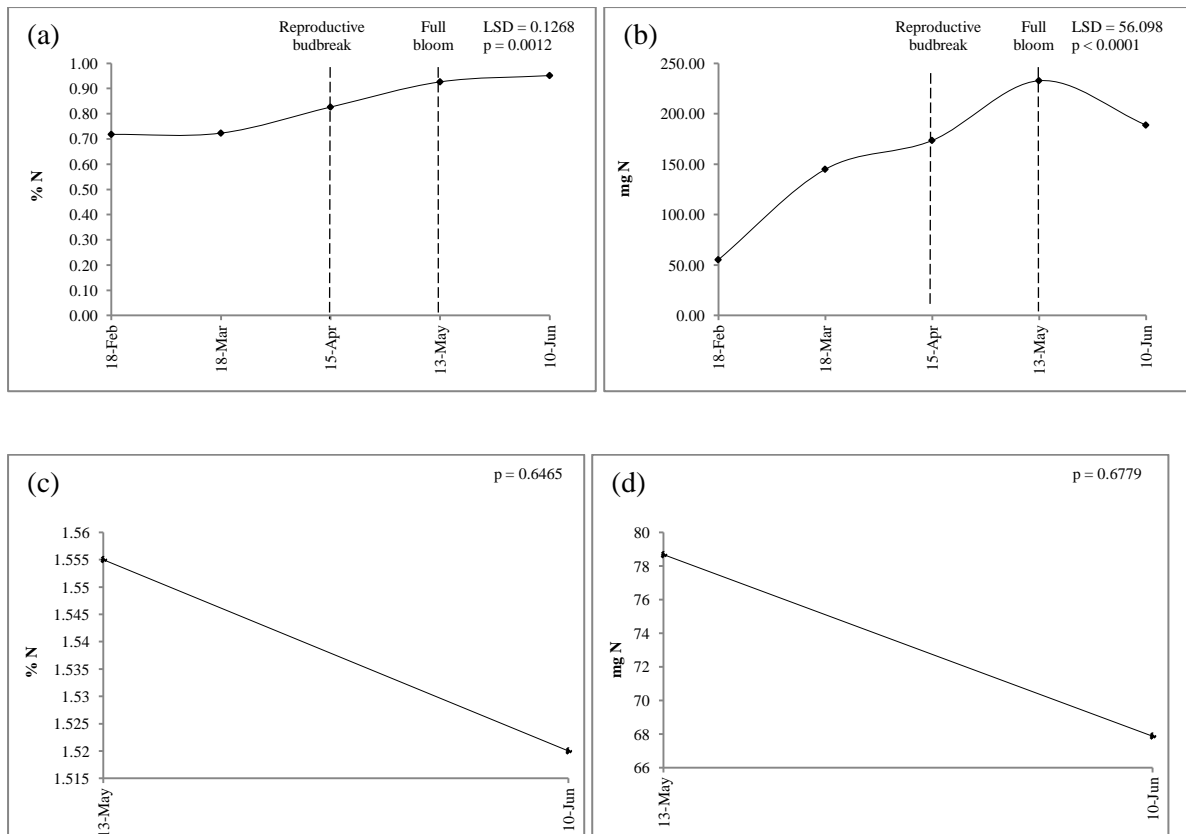


Fig. 2. Nitrogen (N) concentration and content in the canes and flowers of southern highbush blueberry 'Emerald' in the 2014 season. (a) is cane N concentration, (b) cane N content, (c) flower N concentration and (d) flower N content. Reproductive budbreak occurred around 15 Apr. 2014 and full bloom was around 13 May 2014.

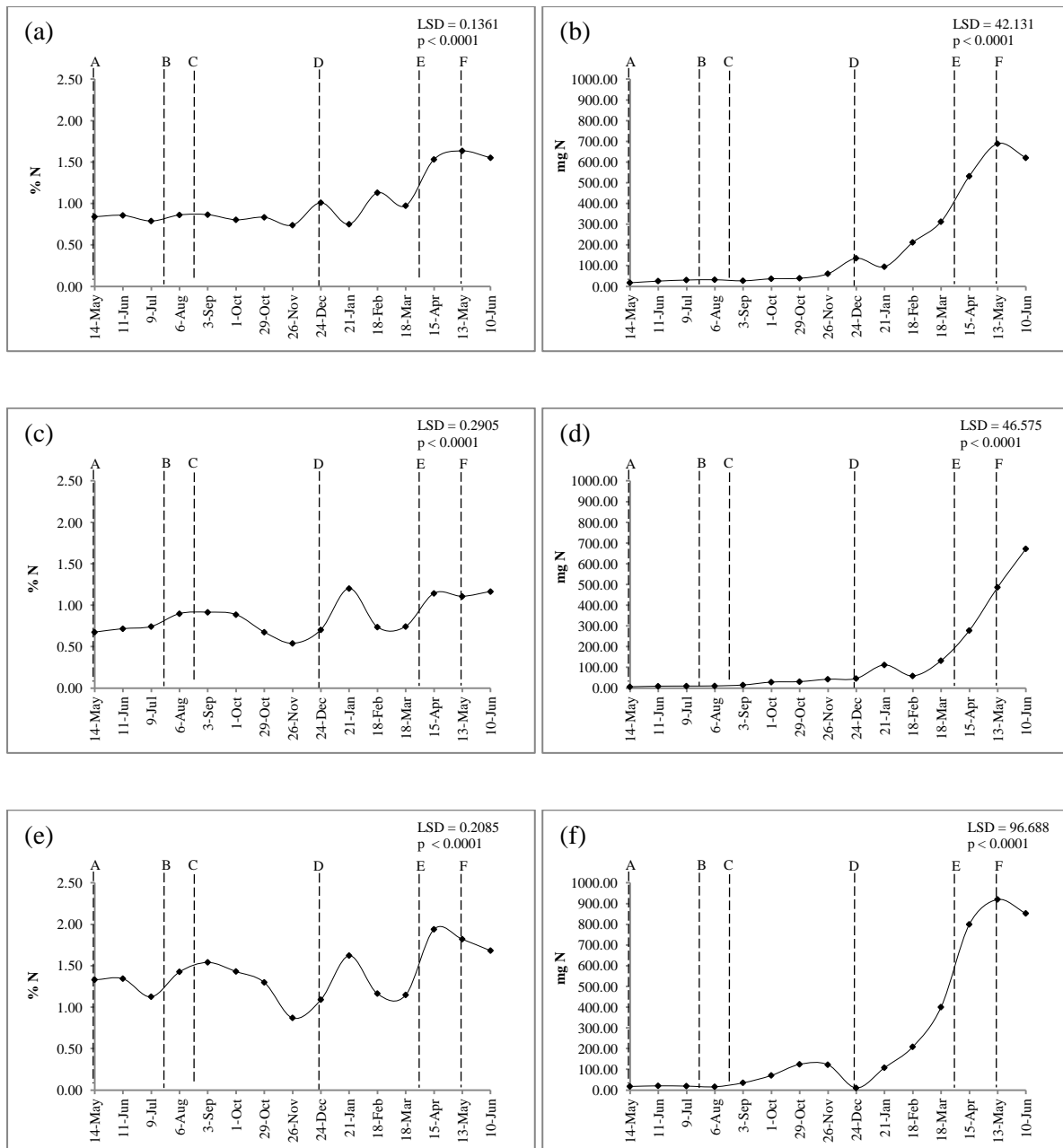


Fig. 3. Nitrogen (N) concentration and content in the roots, shoots and leaves of southern highbush blueberry 'Snowchaser' from 14 May 2013 to 10 June 2014. (a) is root N concentration, (b) root N content, (c) shoot N concentration, (d) shoot N content, (e) leaf N concentration and (f) leaf N content. A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom.

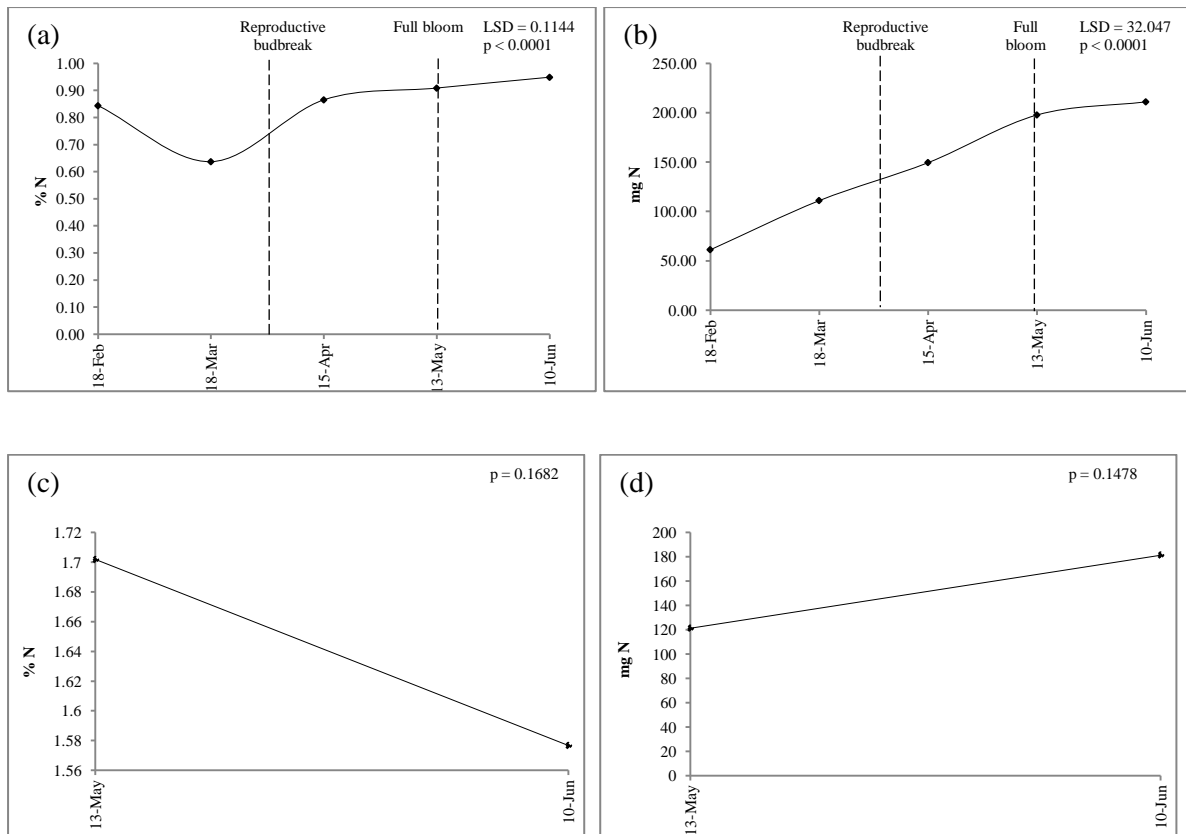


Fig. 4. Nitrogen (N) concentration and content in the canes and flowers of southern highbush blueberry 'Snowchaser' in the 2014 season. (a) is cane N concentration, (b) cane N content, (c) flower N concentration and (d) flower N content. Reproductive budbreak occurred around 1 Apr. 2014 and full bloom was around 13 May 2014.

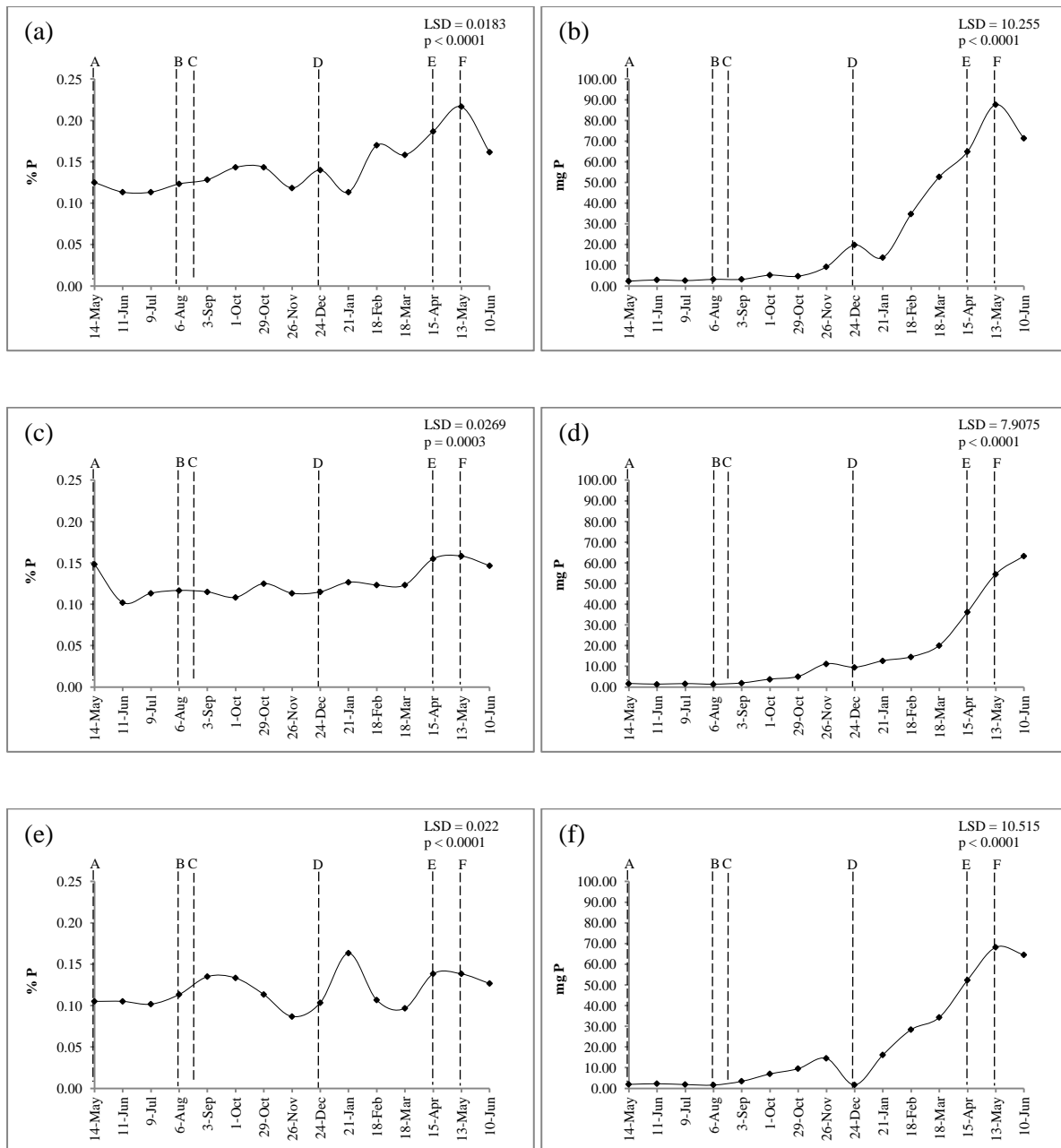


Fig. 5. Phosphorous (P) concentration and content in the roots, shoots and leaves of southern highbush blueberry 'Emerald' from 14 May 2013 to 10 June 2014. (a) is root P concentration, (b) root P content, (c) shoot P concentration, (d) shoot P content, (e) leaf P concentration and (f) leaf P content. A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom.

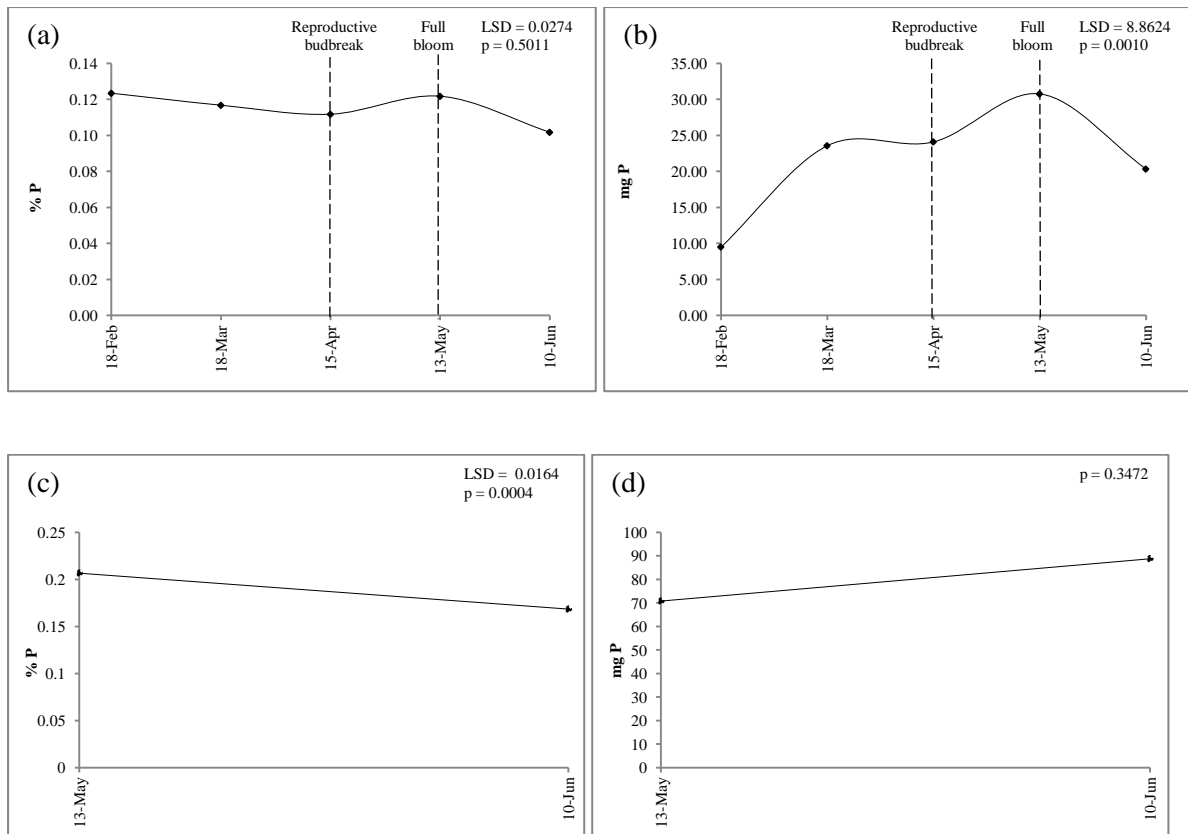


Fig. 6. Phosphorous (P) concentration and content in the canes and flowers of southern highbush blueberry 'Emerald' in the 2014 season. (a) is cane P concentration, (b) cane P content, (c) flower P concentration and (d) flower P content. Reproductive budbreak occurred around 15 Apr. 2014 and full bloom was around 13 May 2014.



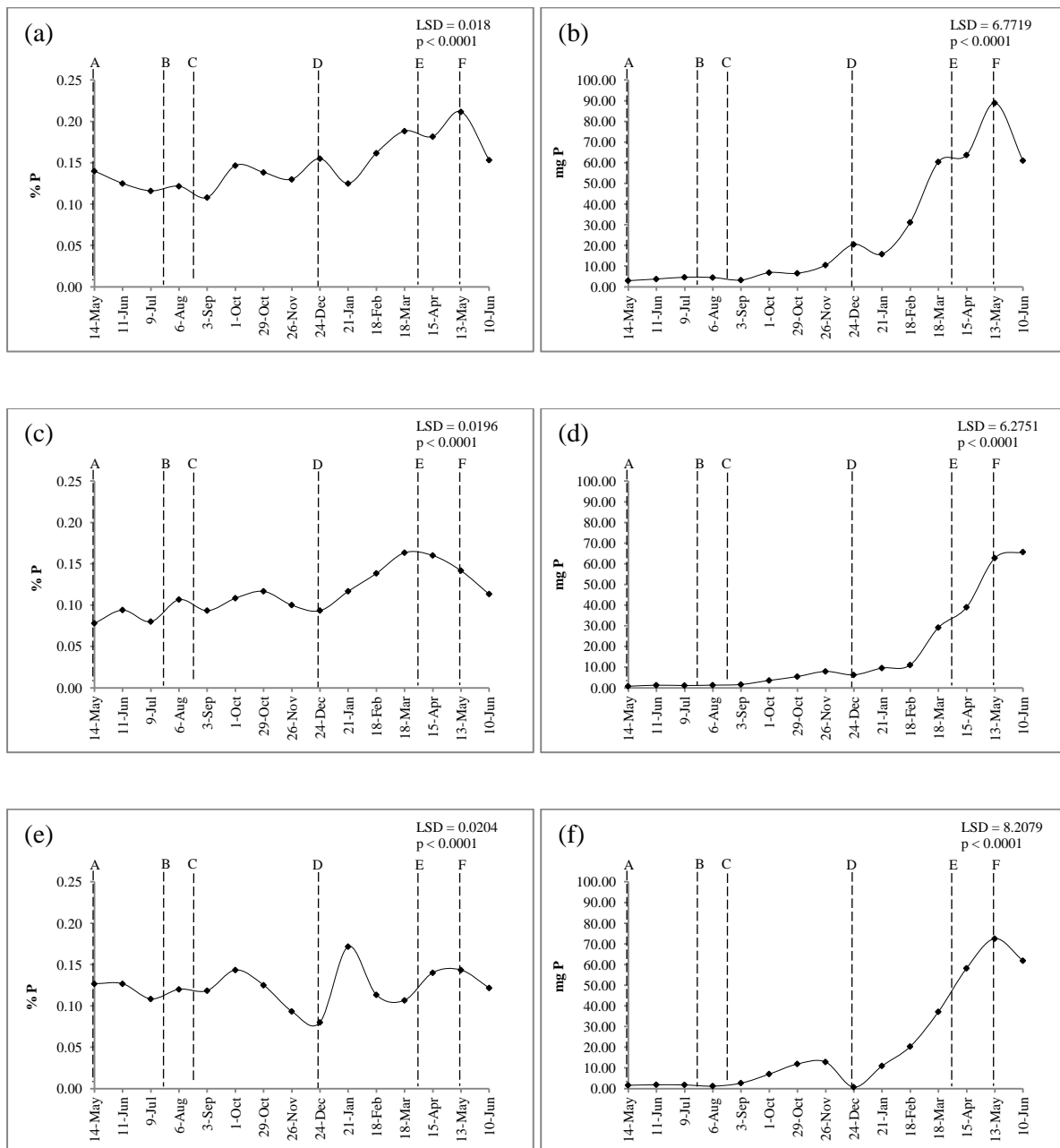


Fig. 7. Phosphorous (P) concentration and content in the roots, shoots and leaves of southern highbush blueberry 'Snowchaser' from 14 May 2013 to 10 June 2014. (a) is root P concentration, (b) root P content, (c) shoot P concentration, (d) shoot P content, (e) leaf P concentration and (f) leaf P content. A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom.

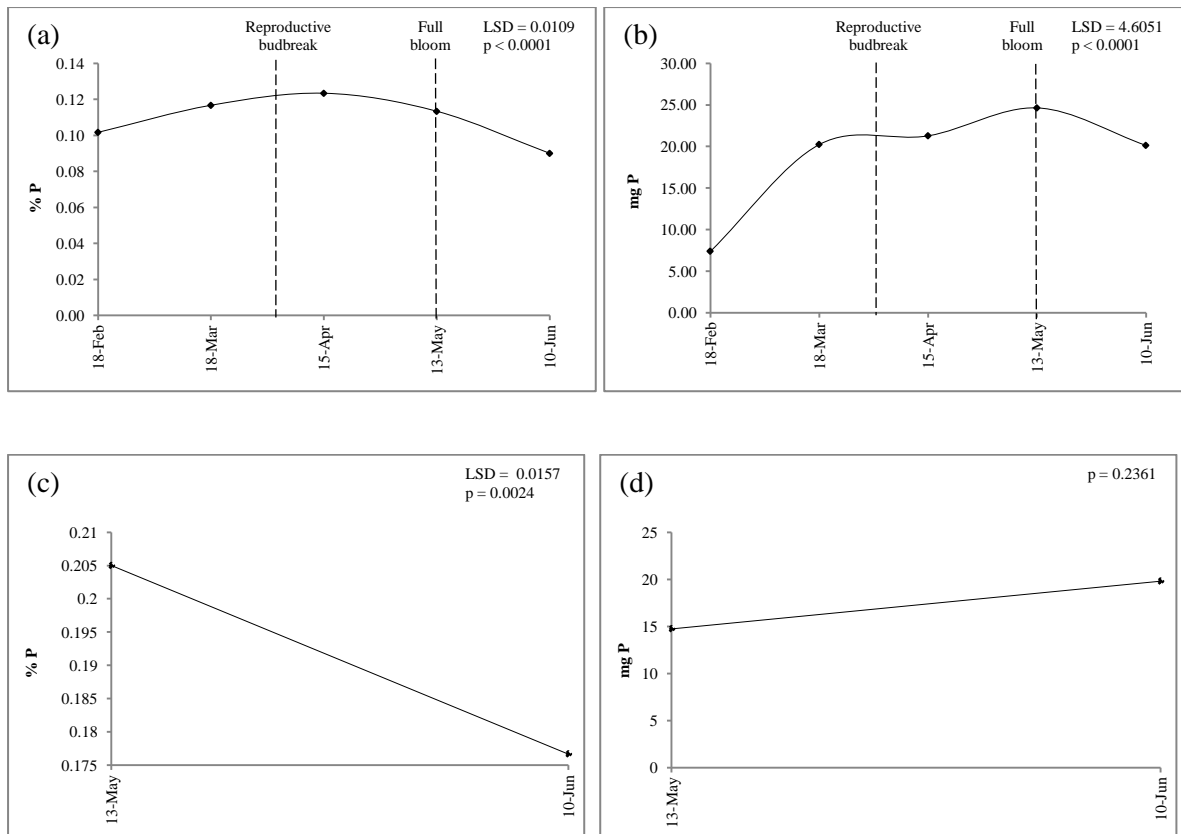


Fig. 8. Phosphorous (P) concentration and content in the canes and flowers of Southern highbush blueberry 'Snowchaser' in the 2014 season. (a) is cane P concentration, (b) cane P content, (c) flower P concentration and (d) flower P content. Reproductive budbreak occurred around 1 Apr. 2014 and full bloom was around 13 May 2014.

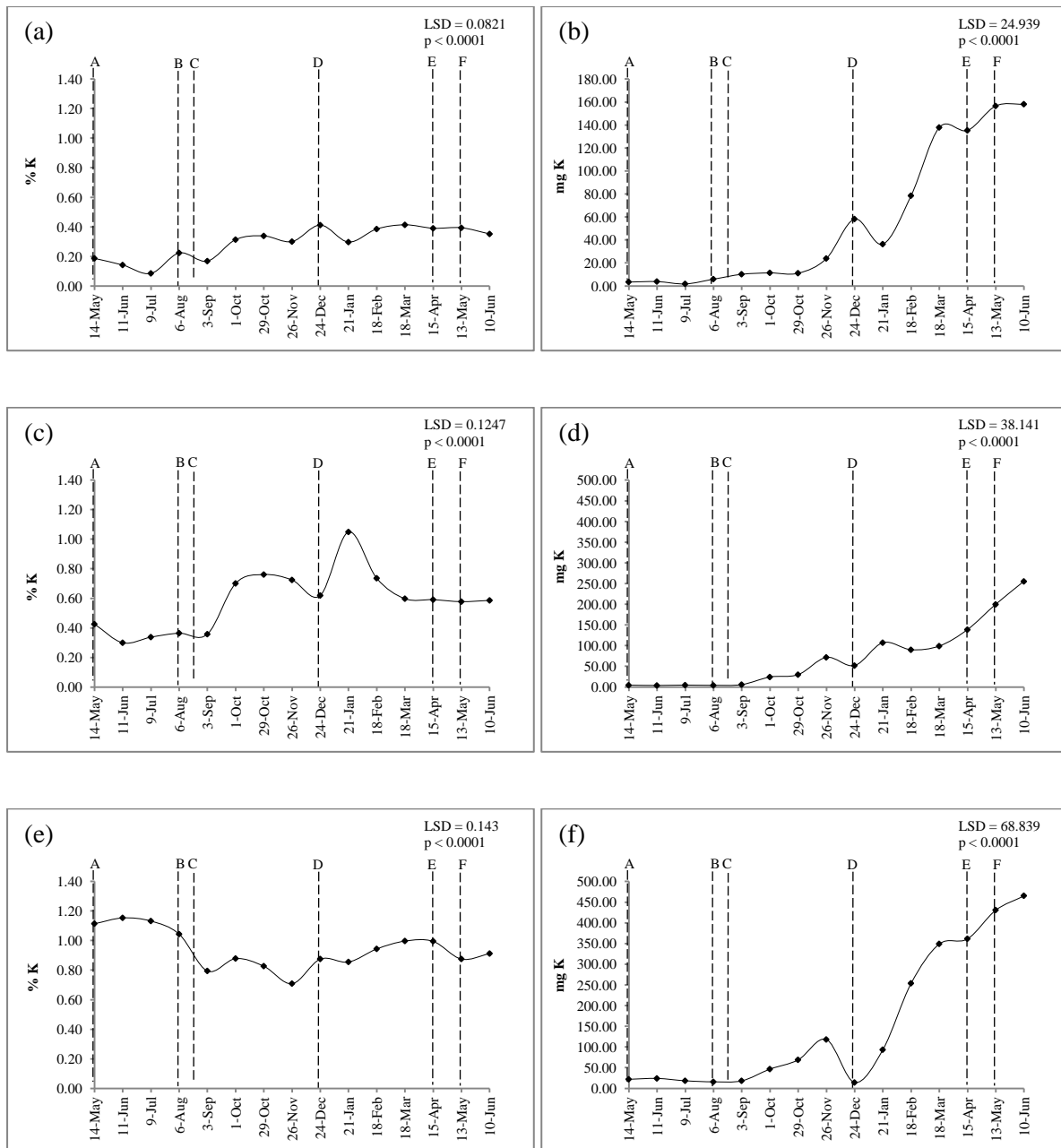


Fig. 9. Potassium (K) concentration and content in the roots, shoots and leaves of southern highbush blueberry 'Emerald' from 14 May 2013 to 10 June 2014. (a) is root K concentration, (b) root K content, (c) shoot K concentration, (d) shoot K content, (e) leaf K concentration and (f) leaf K content. A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom.

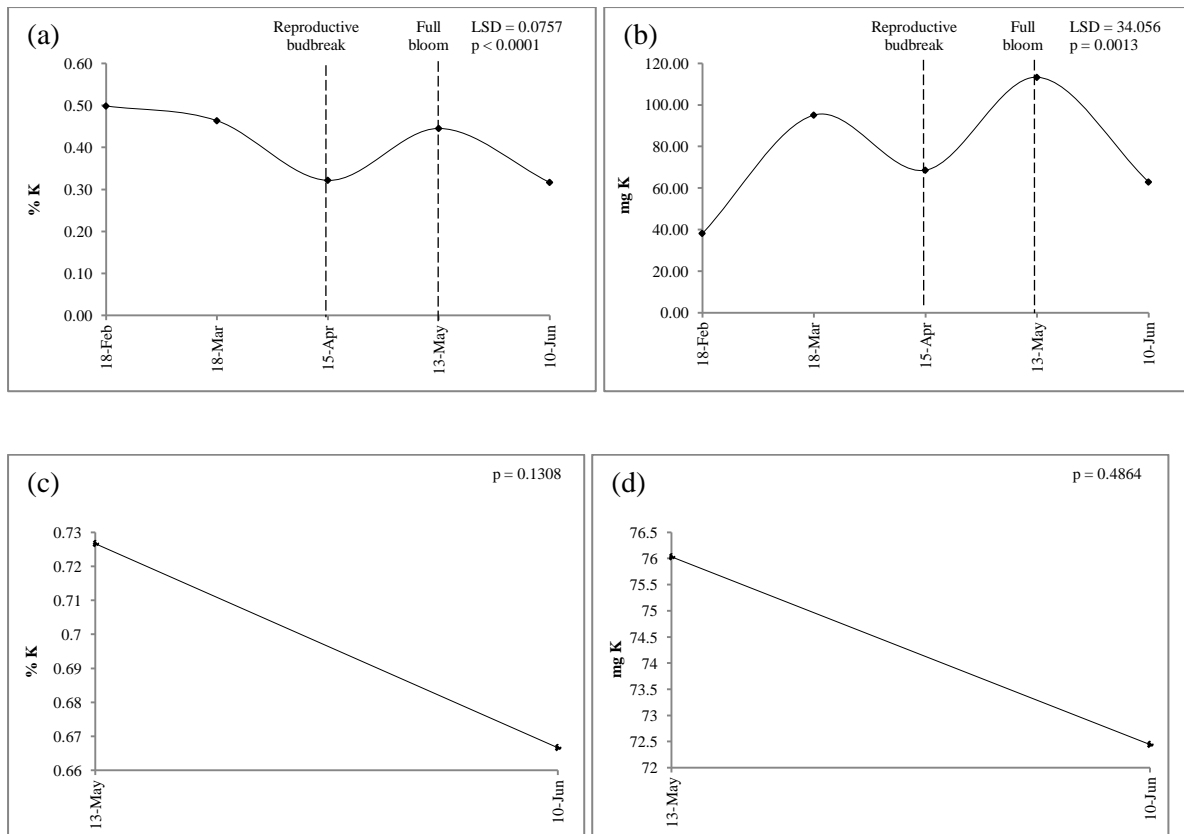


Fig. 10. Potassium (K) concentration and content in the canes and flowers of southern highbush blueberry 'Emerald' in the 2014 season. (a) is cane K concentration, (b) cane K content, (c) flower K concentration and (d) flower K content. Reproductive budbreak occurred around 15 Apr. 2014 and full bloom was around 13 May 2014.

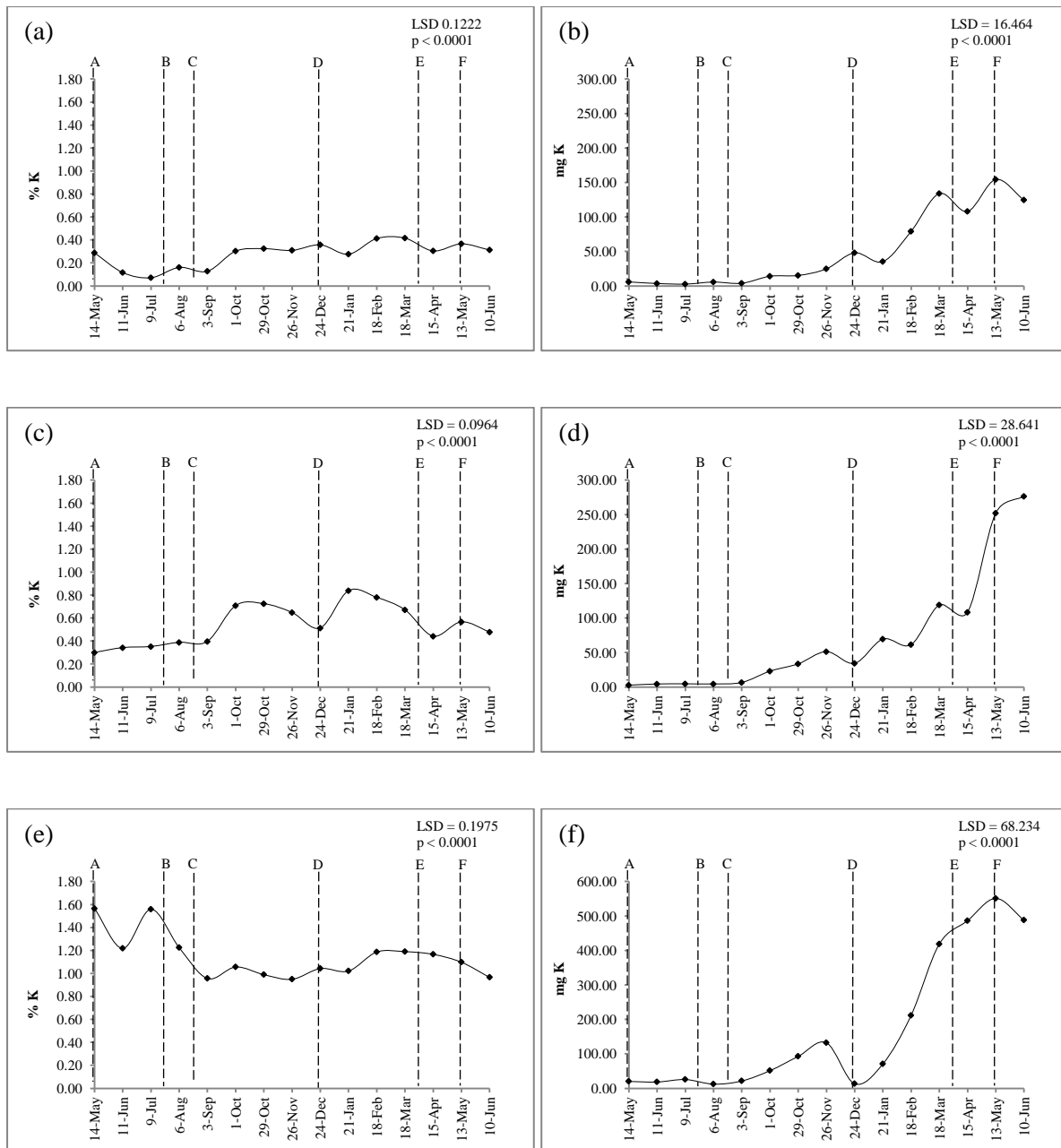


Fig. 11. Potassium (K) concentration and content in the roots, shoots and leaves of southern highbush blueberry 'Snowchaser' from 14 May 2013 to 10 June 2014. (a) is root K concentration, (b) root K content, (c) shoot K concentration, (d) shoot K content, (e) leaf K concentration and (f) leaf K content. A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom.

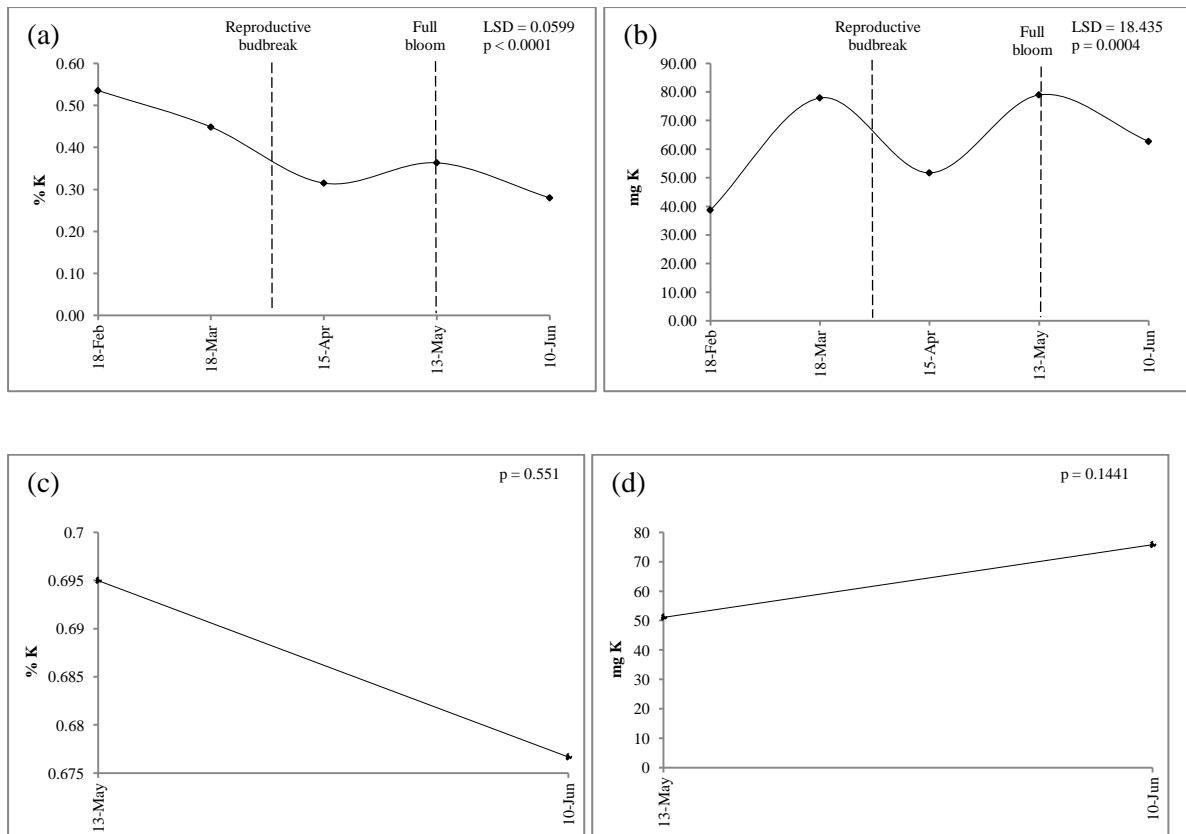


Fig. 12. Potassium (K) concentration and content in the canes and flowers of southern highbush blueberry ‘Snowchaser’ in the 2014 season. (a) is cane K concentration, (b) cane K content, (c) flower K concentration and (d) flower K content. Reproductive budbreak occurred around 1 Apr. 2014 and full bloom was around 13 May 2014.

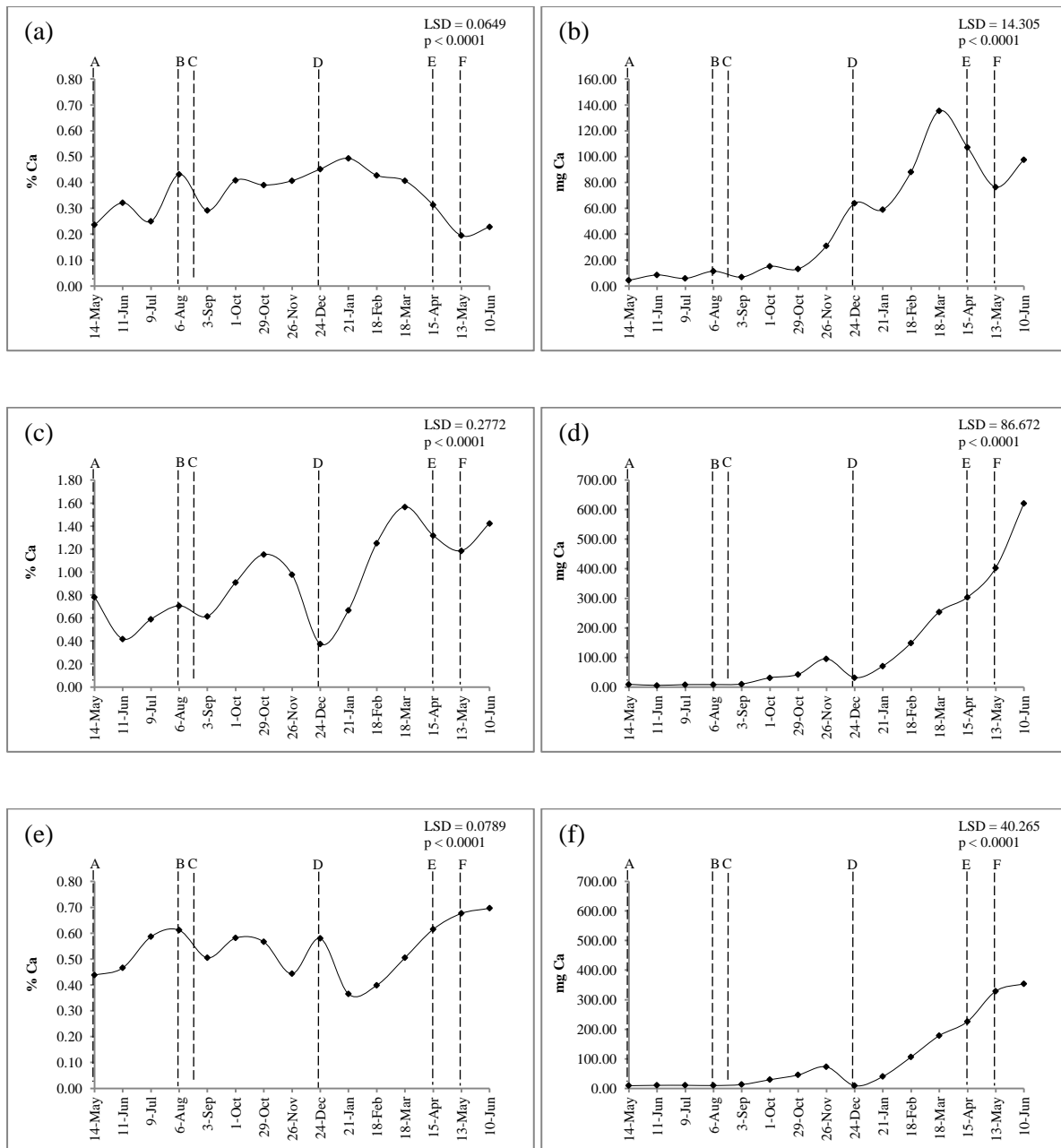


Fig. 13. Calcium (Ca) concentration and content in the roots, shoots and leaves of southern highbush blueberry 'Emerald' from 14 May 2013 to 10 June 2014. (a) is root Ca concentration, (b) root Ca content, (c) shoot Ca concentration, (d) shoot Ca content, (e) leaf Ca concentration and (f) leaf Ca content. A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom.

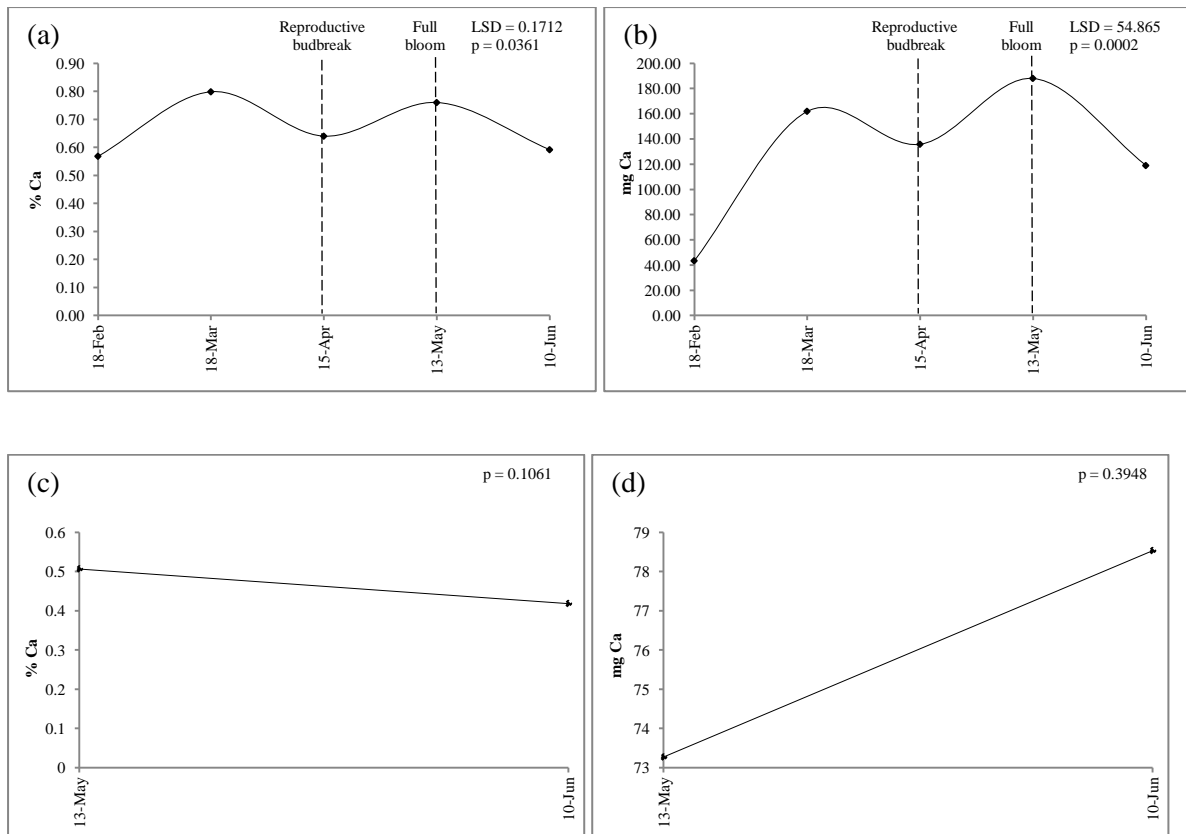


Fig. 14. Calcium (Ca) concentration and content in the canes and flowers of southern highbush blueberry 'Emerald' in the 2014 season. (a) is cane Ca concentration, (b) cane Ca content, (c) flower Ca concentration and (d) flower Ca content. Reproductive budbreak occurred around 15 Apr. 2014 and full bloom was around 13 May 2014.



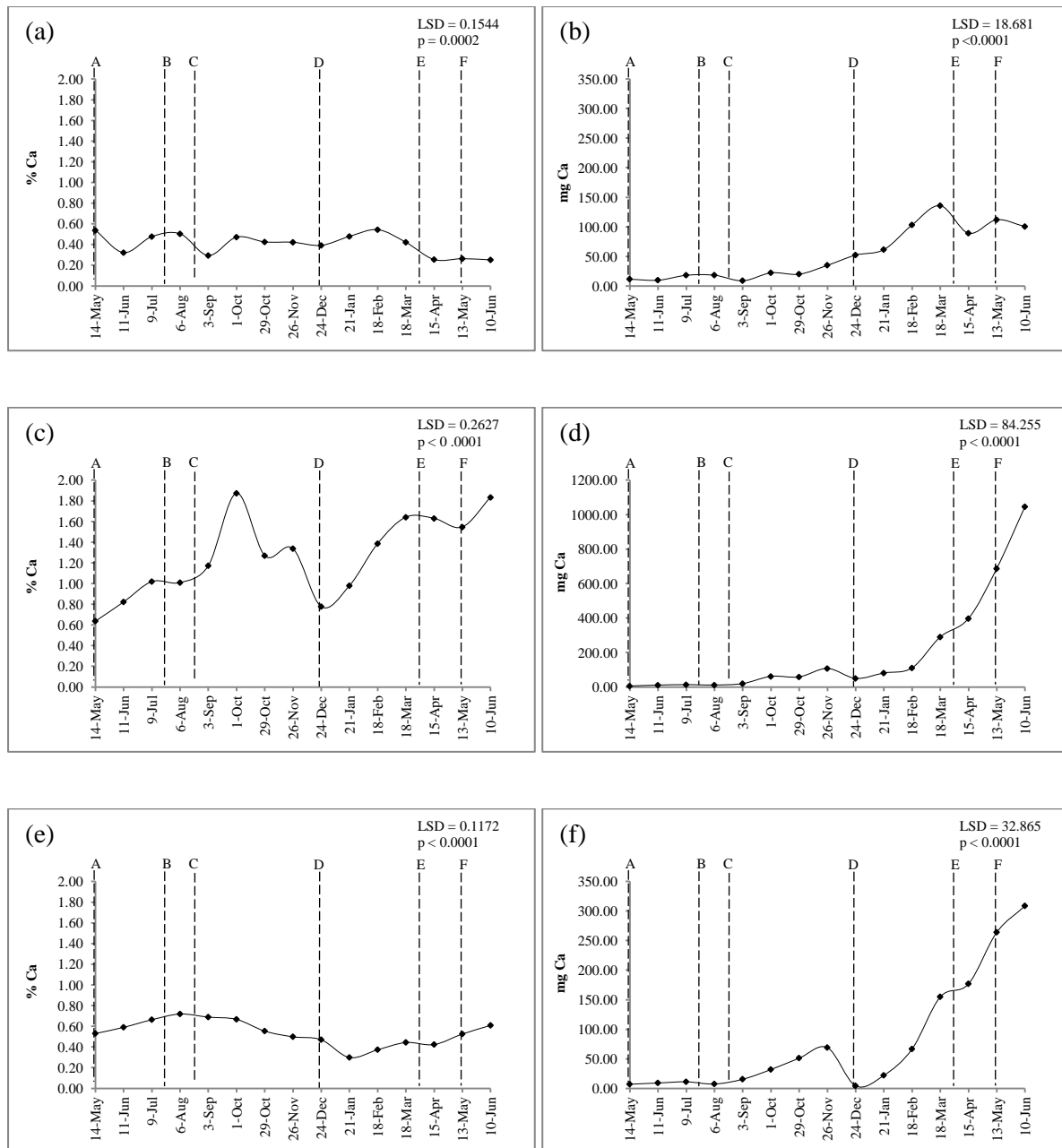


Fig. 15. Calcium (Ca) concentration and content in the roots, shoots and leaves of southern highbush blueberry ‘Snowchaser’ from 14 May 2013 to 10 June 2014. (a) is root Ca concentration, (b) root Ca content, (c) shoot Ca concentration, (d) shoot Ca content, (e) leaf Ca concentration and (f) leaf Ca content. A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom.

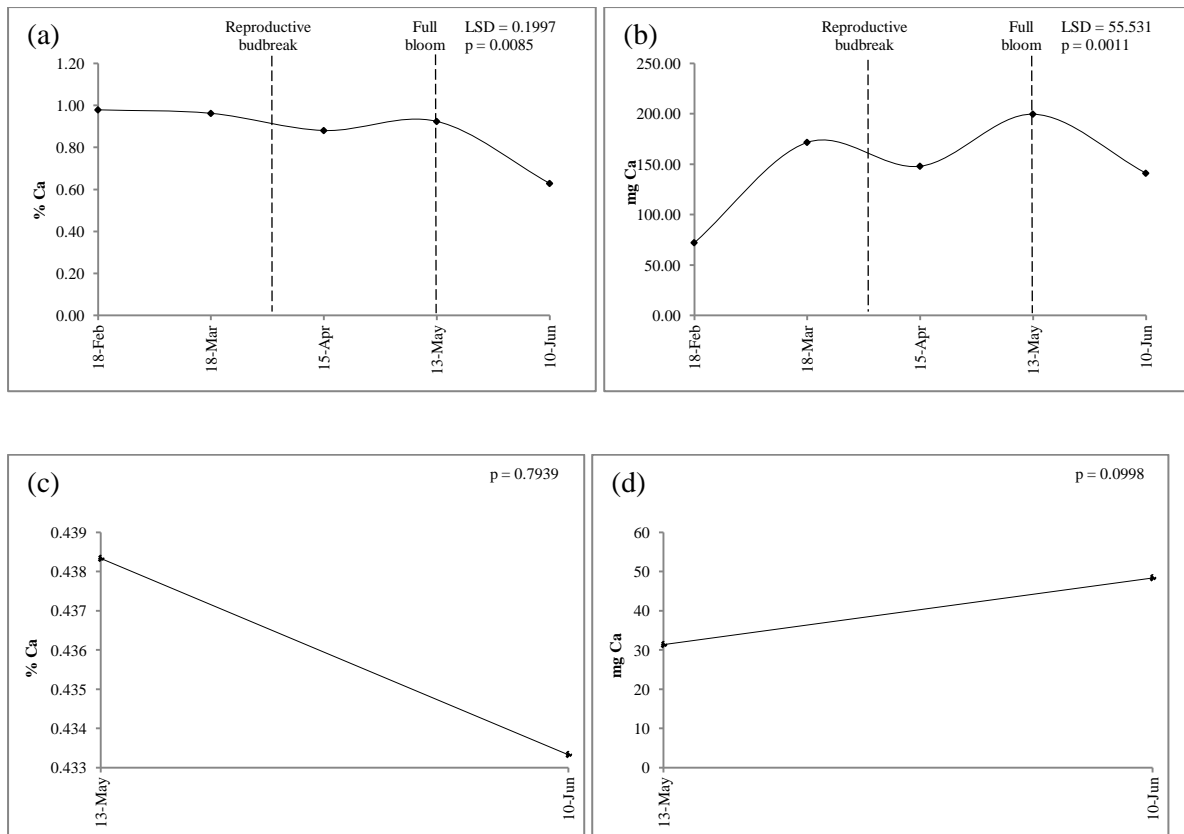


Fig. 16. Calcium (Ca) concentration and content in the canes and flowers of southern highbush blueberry 'Snowchaser' in the 2014 season. (a) is cane Ca concentration, (b) cane Ca content, (c) flower Ca concentration and (d) flower Ca content. Reproductive budbreak occurred around 1 Apr. 2014 and full bloom was around 13 May 2014.

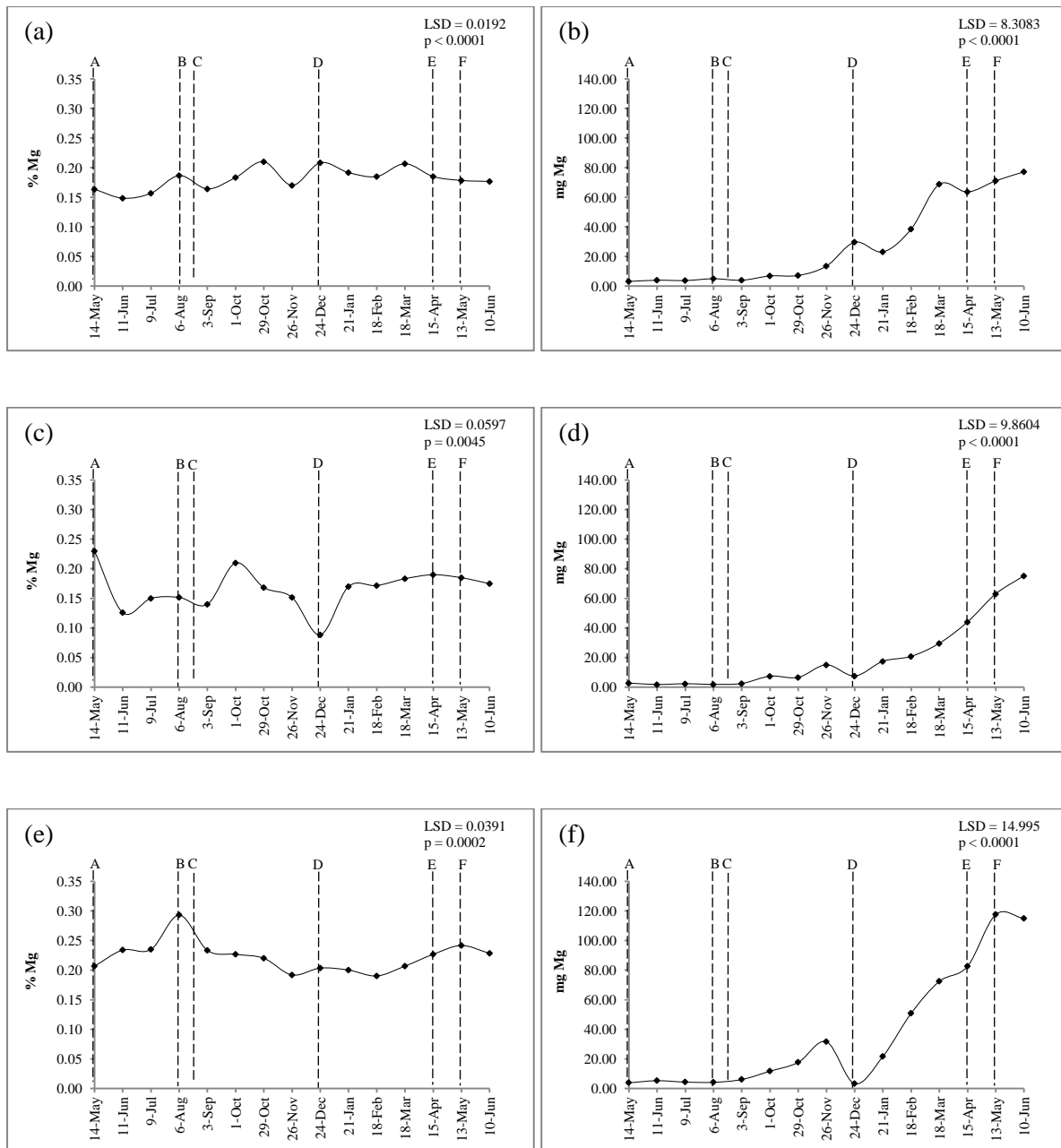


Fig. 17. Magnesium (Mg) concentration and content in the roots, shoots and leaves of southern highbush blueberry 'Emerald' from 14 May 2013 to 10 June 2014. (a) is root Mg concentration, (b) root Mg content, (c) shoot Mg concentration, (d) shoot Mg content, (e) leaf Mg concentration and (f) leaf Mg content. A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom.

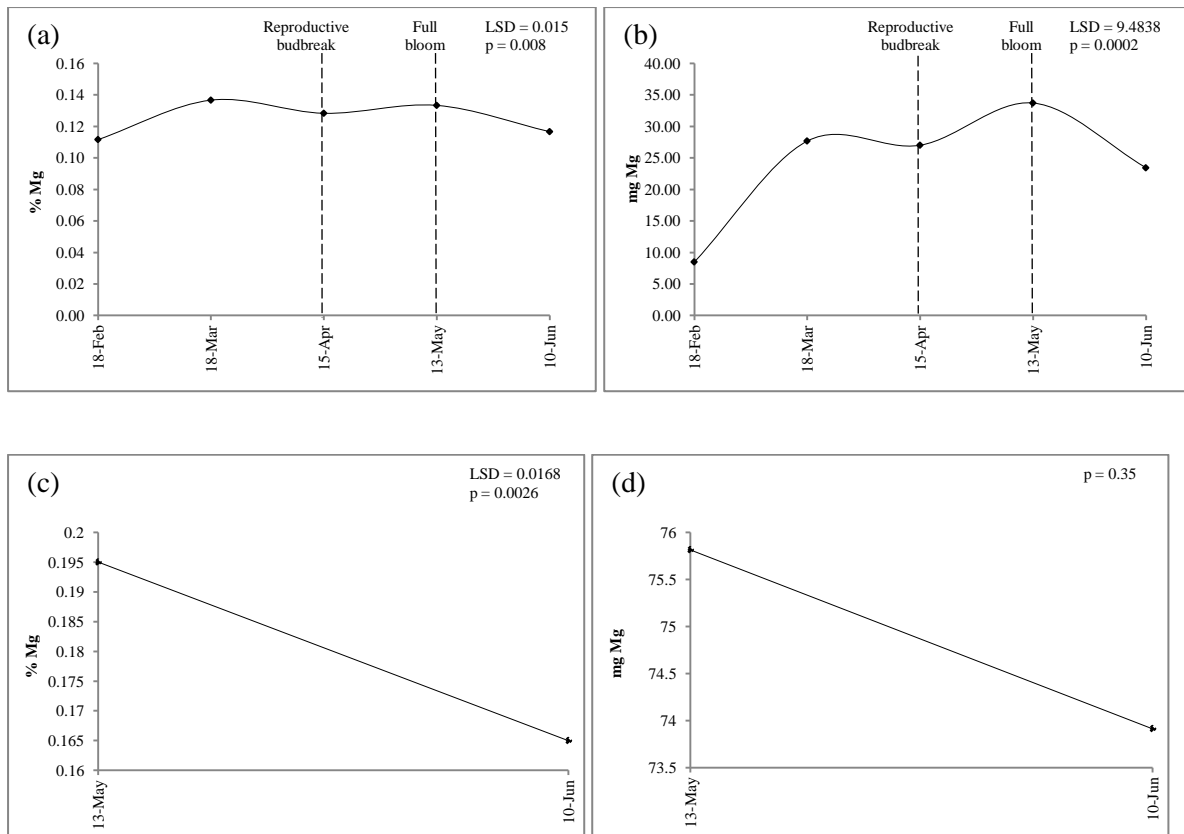


Fig. 18. Magnesium (Mg) concentration and content in the canes and flowers of southern highbush blueberry 'Emerald' in the 2014 season. (a) is cane Mg concentration, (b) cane Mg content, (c) flower Mg concentration and (d) flower Mg content. Reproductive budbreak occurred around 15 Apr. 2014 and full bloom was around 13 May 2014.

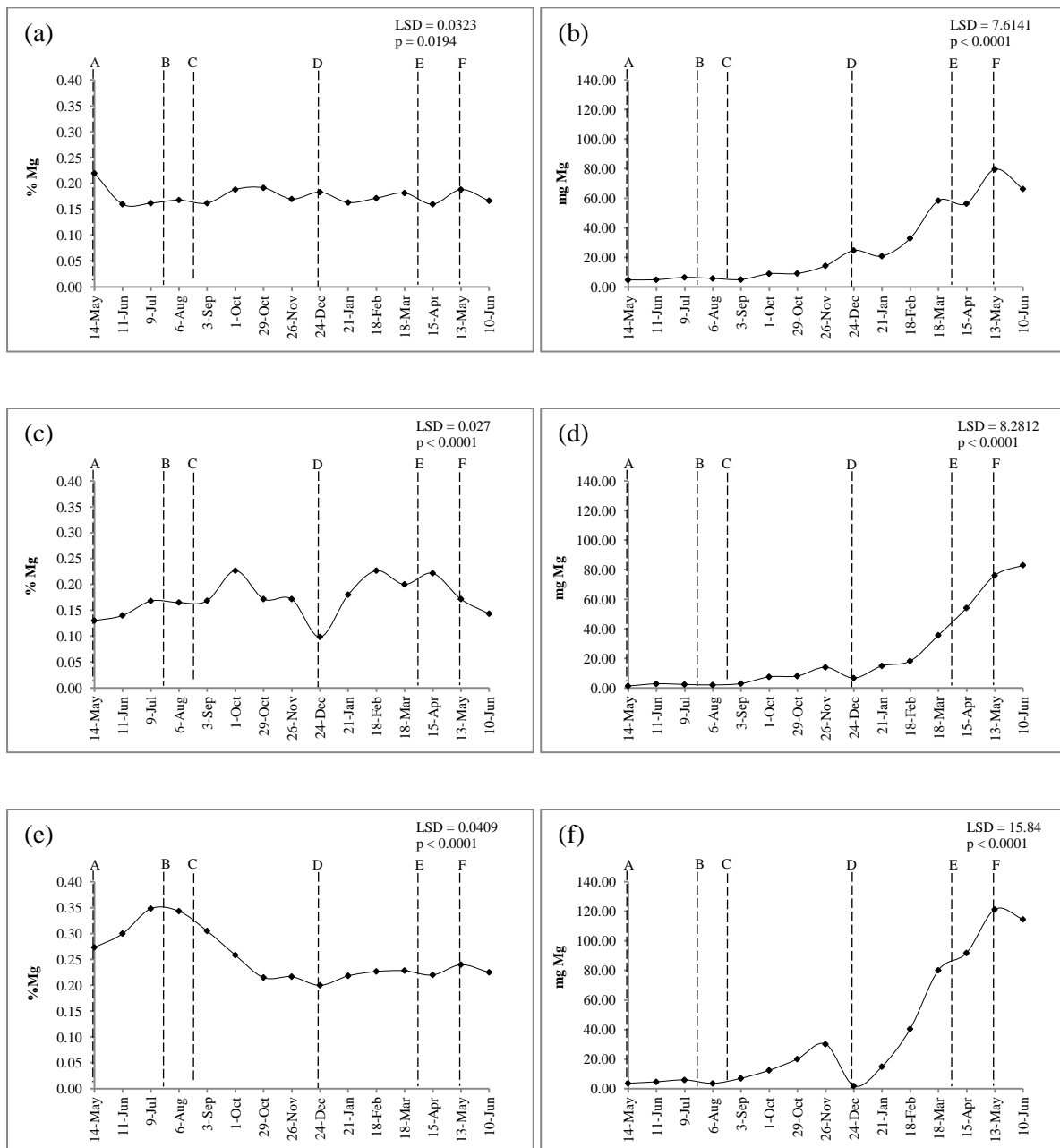


Fig. 19. Magnesium (Mg) concentration and content in the roots, shoots and leaves of southern highbush blueberry 'Snowchaser' from 14 May 2013 to 10 June 2014. (a) is root Mg concentration, (b) root Mg content, (c) shoot Mg concentration, (d) shoot Mg content, (e) leaf Mg concentration and (f) leaf Mg content. A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom.

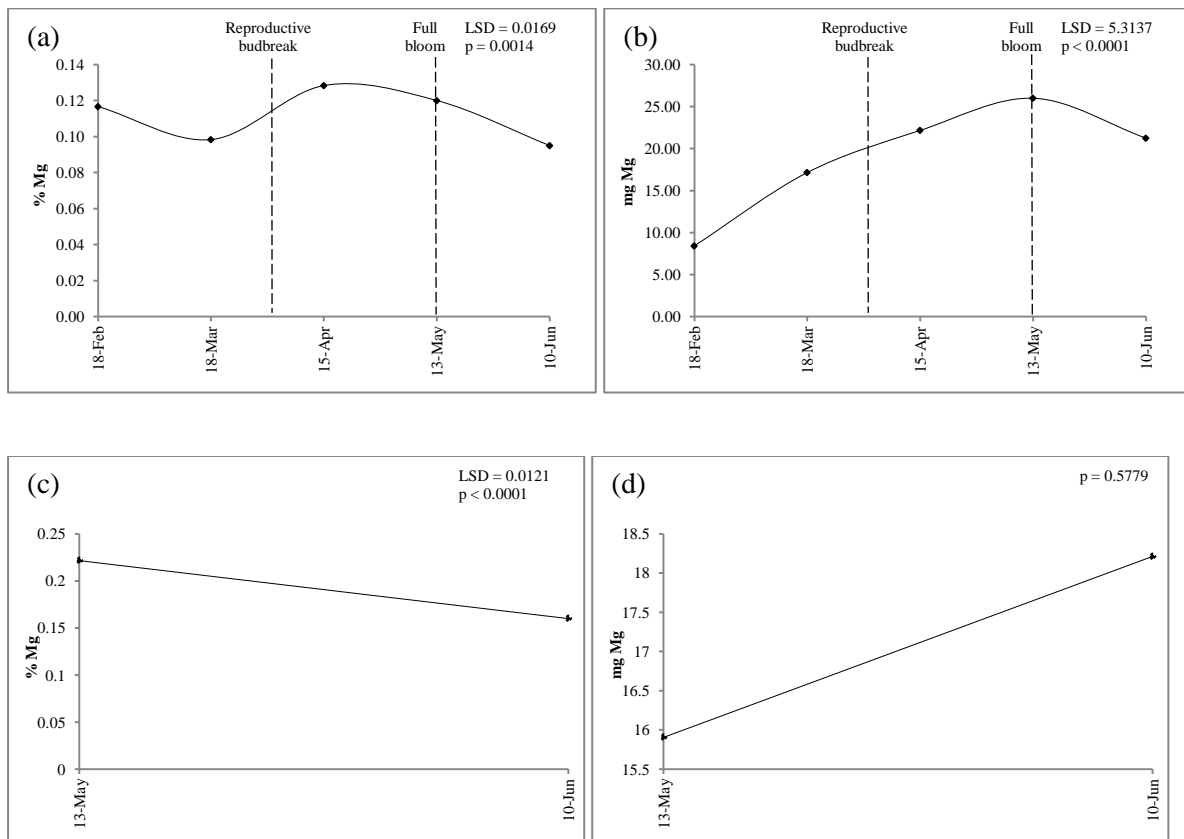


Fig. 20. Magnesium (Mg) concentration and content in the canes and flowers of southern highbush blueberry 'Snowchaser' in the 2014 season. (a) is cane Mg concentration, (b) cane Mg content, (c) flower Mg concentration and (d) flower Mg content. Reproductive budbreak occurred around 1 Apr. 2014 and full bloom was around 13 May 2014.

## **PAPER 3: Seasonal changes and allocation of macro nutrients in ‘Emerald’ and ‘Snowchaser’ southern highbush blueberries**

---

**Abstract.** The macro nutrient content in the different parts of southern highbush cultivars Emerald and Snowchaser, grown under net, were determined at five distinct phenological stages over one season. In ‘Emerald’ N and P were remobilized from the leaves to the roots and in ‘Snowchaser’ only N was remobilized from the leaves to the roots after growth cessation. Reserves were more important for new growth in ‘Emerald’ than for ‘Snowchaser’, as ‘Snowchaser’ depended more on nutrient uptake between growth cessation and early fruit set. ‘Snowchaser’, however, was more dependent on reserves between early fruit set and harvest. Uptake varied through the season as demand fluctuated. Most of the nutrients were taken up after summer pruning in both cultivars. For ‘Emerald’ 73% of the N, 70% of the P, 49% of the K and 64% of the Ca and Mg was taken up after summer pruning. For ‘Snowchaser’ 75% of the N, 64% of the P, 49% of the K and 61% of the Ca and 63% of the Mg was taken up after summer pruning. Fertilization recommendations for ‘Emerald’ and ‘Snowchaser’ spaced at 2.5 x 1 m (4000 plants/ha) were made according to the macro nutrient uptake pattern per plant.

---

Fertilization is the process of adding inorganic or organic fertilizers to a crop in order to optimize growth and fertility (Smagula and Kreider, 2008; Yadong et al., 2009). The improper application of fertilization will result in crops that are under- or over-fertilized. Excess fertilizer could result in poor fruit quality and cause nutrient toxicity or imbalances,

and losses due to leaching, causing pollution. Over-application of fertilizers is also very unprofitable as fertilizers are expensive. A shortage of fertilizer will lead to plants with less vigour, nutrient deficiencies and ultimately a lower yield and quality.

The rate of fertilizer uptake depends on the nutrient demand and growth rate differences of plants during the season (Throop and Hanson, 1997). The time and amount of fertilizer to be applied is greatly dependant on the duration and rate of uptake from the plant as well as nutrient availability of the soil (Stassen et al., 1983). Although uptake of nutrients is affected by various factors, the availability of nutrients in the soil will ultimately determine the amount and extent of nutrient uptake. Phosphorus (P) ions are normally adsorbed to soil particles or organic matter while nitrogen (N) ions are very mobile in the soil (Kramer and Kozlowski, 1979a). The calcium (Ca) and magnesium (Mg) content in the soil are correlated with the soil acidity and the potassium (K) content highly depends on the soil type (Stassen et al., 1983).

The accumulation of biomass is correlated with nutrient uptake and therefore nutrients are taken up most readily when growth rate is high or fruit are developing (Rose, 1999). Much of the nutrients taken up by the plant are lost through the season by processes such as leaf abscission, fruit harvest and pruning and need to be corrected by the application of fertilizer. Bryla et al. (2012) reported that in 'Bluecrop' northern highbush (NHB) blueberries half of the N, P, K, copper (Cu) and zinc (Zn) were lost because of harvest and most of the Ca, Mg, sulphur (S), iron (Fe), manganese (Mn) and boron (B) were lost by leaf abscission.

In deciduous fruit trees nutrients from the leaves are re-allocated to the permanent structural parts of the plant just before winter. Decrease of a nutrient in the permanent structure of a plant in spring is normally attributed to redistribution to growing parts (Stassen et al., 1983). The remobilization of nutrients within the plant is, however, affected by the extent to which



the elements are mobile in the plant. N, P, K and Mg are very mobile in the plant while Ca is fairly immobile in plants (Kramer and Kozlowski, 1979a).

Many studies have been performed on the nutrient status of a variety of crops. Stassen et al. (1983) studied the macro-element content of full bearing 'Kakamas' peach trees at four phenological stages during a season and the seasonal nutrient uptake of young apple trees was determined by Terblanche (1972). Various others studies were conducted on the seasonal nutrient requirement of crops e.g. kiwi (Clark and Smith 1992; Kotze and De Villiers, 1989a and b), apple (Kangueehi et al., 2011), pistachio (Rosecrance et al., 1996), pear (Stassen and North, 2004) and peach (Stassen and Stadler, 1988). A few studies have been conducted on blueberries, e.g. Bañados et al. (2006a) looked at N, P and K partitioning of NHB during the winter, Spiers and Marshall (2012) determined the macro-element distribution of rabbiteye blueberries and the macro and micro nutrient requirements of young NHB blueberry plants were determined by Bryla et al. (2012). No research, up to date, has been conducted regarding seasonal demands and losses of southern highbush (SHB) blueberries. This study aimed to investigate the macro nutrient content of different plant parts of 'Emerald' and 'Snowchaser' SHB blueberries at five distinct phenological stages during the season.

### **Material and Methods**

*Plant material.* The trial was conducted on SHB blueberries, 'Emerald' and 'Snowchaser' at Backsberg Wine Estate (S 33° 49, 684' E 18° 54, 917') in the Paarl district in the Western Cape of South Africa during the 2013/2014 growing season. Two-year old, bearing plants in 20 L bags were replanted into 35 cm pots in Jan. 2014. A mixture of peat moss, coir and perlite in a 7:2:11 ratio was used as growing medium. All plants were grown in an evergreen system under 20% white net.

*Treatments and trial design.* A continuous fertigation system with single dripper lines was installed in the tunnel. All plants received the same standard balanced nutrient solution, containing N, P, K, Ca, Mg and all the micro-elements. Plants were sampled at five distinct phenological stages; growth cessation in the first season, early fruit set (which coincides with vegetative budbreak), harvest, summer pruning, and growth cessation the second season. Six replications were sampled randomly for each cultivar. Summer pruning occurred on 20 Dec. 2013 and old unproductive canes, old bearing wood and low growing branches were removed as well as thin, weak new growth. Laterals were headed back to 20 cm from the inception and if no laterals were present, the cane was pruned to about 30 cm from the ground. This pruning method resulted in almost 100% of the leaves being removed.

*Preparation for analysis.* Plants were divided into canes ( $\geq 1$  year), young shoots, leaves, roots, flowers and berries. Fresh mass of each plant part was determined where after plant material was stored at  $-80^{\circ}\text{C}$  and then lyophilized. Dried material was weighed and ground to pass through a 500  $\mu\text{m}$  sieve. Ground material was stored in 15 mL plastic tubes, vacuum sealed and stored at room temperature until analysis.

*Nutrient analysis.* Material was sent to Bemlab (Pty) Ltd (16 Van der Berg Crescent, Gant's Centrum, Strand 7140, South Africa), an agricultural analytical laboratory for macro and micro nutrient analysis. Samples were analyzed via the standard method using the ICP-OES (Inductively Coupled Plasma – Optical Emission Spectrometer) procedure (Hou and Jones, 2000) together with a nitrogen analyzer.

*Statistical analysis.* Standard errors of the means were calculated per cultivar for each nutrient and plant part.

## Results and Discussion

The changes in macro nutrient levels over the five phenological stages of the two cultivars will be presented and briefly discussed before a more general discussion will follow.

*'Emerald': Nitrogen.* Total N per 'Emerald' plant increased from 912.97 ( $\pm$  89.51) mg at the time of growth cessation (21 May 2013) to 1105.20 ( $\pm$  60.16) mg at early fruit set (21 Aug. 2013) (Fig. 1). Of the total N, 43% was present in the roots at growth cessation and increased from 393.40 ( $\pm$  30.63) mg to 628.58 ( $\pm$  46.52) mg at early fruit set. 25% of the total N content was present in the canes and decreased from 228.83 ( $\pm$  25.09) mg to 113.43 ( $\pm$  16.43) mg at early fruit set, indicating reallocation possibly to the roots and new growth. The shoots only contained 6% of the total N content in the plant and it increased from growth cessation (58.63  $\pm$  8.91 mg) to early fruit set (89.59  $\pm$  15.52) mg. The leaves contained 25% of the total N content and it decreased from 232.12 ( $\pm$  32.75) mg to 185.38 ( $\pm$  7.83) mg at early fruit set. Thus, 115.40 mg and 46.74 mg were redistributed from the canes and leaves, respectively, to the roots and shoots and 192.23 mg was taken up per plant (Table 1) between growth cessation and early fruit set.

From early fruit set (21 Aug. 2013) to harvest (19 Nov. 2013), the total N in the plant increased from 1105.20 ( $\pm$  60.16) mg to 1826.14 ( $\pm$  115.28) mg (Fig. 1). Of the total N at early fruit set, 57% was present in the roots and decreased to 454.75 ( $\pm$  38.43) mg at harvest. 10% of the total N was present in the canes at early fruit set and increased to 194.57 ( $\pm$  14.15) mg at harvest. The shoots contained 8% of the total N at early fruit set and increased to 170.18 ( $\pm$  18.92) mg at harvest. At early fruit set 17% of the total N was present in the leaves and increased to 653.90 ( $\pm$  90.10) mg at harvest. Only 5% of the total N was present in the berries (58.79  $\pm$  7.25 mg) and 3% was present in the flowers (29.43  $\pm$  8.00 mg). 173.83 mg

was redistributed from the roots to the rest of the plant and 720.94 mg was taken up (Table 1) between early fruit set and harvest.

19% of the total N content at harvest ( $352.74 \pm 44.82$  mg) was present in the fruit and was lost by harvesting  $254.08 (\pm 33.64)$  g berries (Fig. 1). After fruit were removed,  $1473.40 (\pm 123.20)$  mg N was present in the plant and increased to  $2023.40 (\pm 75.63)$  mg at pruning (20 Dec. 2013). Of the total N at harvest (including the fruit), 25% was present in the roots ( $454.75 \pm 38.43$  mg) and did not change significantly until pruning ( $456.55 \pm 41.90$  mg). The canes contained 11% of the total N at harvest and increased from harvest to pruning ( $313.87 \pm 18.42$  mg). At harvest 9% of the total N was present in the shoots and increased to  $324.03 (\pm 12.15)$  mg (including the shoot prunings) at pruning. At harvest, 36% of the total N was contained in the leaves and increased to  $928.95 (\pm 33.01)$  mg (including the leaf prunings) at pruning. The increase in N content in the leaves, canes and shoots from harvest to pruning does not coincide with a significant decrease in the roots and therefore cannot be ascribed to reallocation. The increase was due to the uptake of 550.00 mg N during this period (Table 1).

$915.92 (\pm 31.34)$  mg N (45% of total N at pruning) and  $239.26 (\pm 11.87)$  mg N (12% of total N at pruning) for leaves and shoots, respectively, were lost due to pruning (Fig. 1). After plants were pruned,  $868.23 (\pm 42.67)$  mg N was present in the plant and increased to  $4765.33 (\pm 334.35)$  mg at growth cessation (21 May 2014). Of the total N (including the prunings), 23% was present in the roots at pruning and increased to  $1878.51 (\pm 201.34)$  mg at growth cessation. Roots accumulated N, for the first time during the season, after pruning, suggesting that N is first distributed to the other plant parts and then to the roots. The canes contained 16% of the total N at pruning and increased to  $522.97 (\pm 48.63)$  mg at growth cessation. The shoots (excluding the shoot prunings) contained 4% of the total N at pruning and increased from  $84.77 (\pm 10.43)$  mg to  $800.92 (\pm 62.20)$  mg at growth cessation. 1% of the total N content was present in the leaves (excluding the leaf prunings) and increased from pruning

( $13.03 \pm 2.54$  mg) to growth cessation ( $1455.19 \pm 85.45$  mg). 3897.10 mg N was taken up after pruning (20 Dec. 2013) and growth cessation (Table 1). Flowers for the following season were already present at growth cessation in the second season and contained 2% of the total N ( $107.75 \pm 11.48$  mg).

A total of 5360.27 mg N was taken up during the season (Table 1) and 1507.92 mg N was lost through pruning and harvest. 73% of the N fertilizer that was taken up during the season was taken up after summer pruning (Table 1), indicating the importance of N fertilizer during this stage. Only 4% of the N fertilizer that was taken up during the season was taken up between growth cessation in the first season and early fruit set (Table 1) and therefore N fertilizer is not very important during this stage.

*'Emerald': Phosphorus.* Total P per 'Emerald' plant increased from 124.94 ( $\pm 10.87$ ) mg at the time of growth cessation (21 May 2013) to 144.51 ( $\pm 10.45$ ) mg at early fruit set (21 Aug. 2013) (Fig. 2). Of the total P content, 46% was present in the roots at growth cessation ( $58.04 \pm 2.42$  mg) and increased to early fruit set ( $95.37 \pm 7.17$  mg). The canes contained 28% of the total P and decreased from growth cessation ( $34.94 \pm 2.82$  mg) to early fruit set ( $12.86 \pm 2.10$  mg). 11% of the total P was present in the shoots and did not change from growth cessation ( $13.56 \pm 4.81$  mg) to early fruit set ( $10.39 \pm 1.98$  mg). The leaves contained 15% of the total P content and decreased from growth cessation ( $18.40 \pm 1.81$  mg) to early fruit set ( $15.78 \pm 1.56$  mg). 19.57 mg P was taken up during this period (Table 1) while 22.08 mg and 2.62 mg P were redistributed to the roots from the canes and leaves, respectively. The reallocation pattern of P during this stage was similar to that of N.

Between early fruit set (21 Aug. 2013) and harvest (fruit included) (19 Nov. 2013), the total P content increased from 144.51 ( $\pm 10.45$ ) mg to 230.16 ( $\pm 13.32$ ) mg (Fig. 2). Of the total P content, 66% was present in the roots and decreased from early fruit set to harvest to 85.11 ( $\pm$

7.90) mg. The canes contained 9% of the total P content at early fruit set and increased to 32.07 ( $\pm$  3.47) mg at harvest. 7% of the total P content was present in the shoots at early fruit set. The P content in the shoots increased from early fruit set to harvest (28.68  $\pm$  4.16 mg). The leaves contained 11% of the total P content and increased to 45.97 ( $\pm$  5.83) mg at harvest. Fruit P content increased from 7.25 ( $\pm$  1.13) mg at early fruit set to 38.34 ( $\pm$  5.30) mg at harvest. 2% of the total P was present in the flowers (2.86  $\pm$  0.77 mg) at early fruit set. 85.65 mg P was taken up during this period (Table 1) and 10.3 mg was redistributed from the roots to the other plant parts. The reallocation pattern of the P content during this stage was also similar to that of the N content.

17% (38.34  $\pm$  5.30 mg) of the total P at harvest was lost due to the harvest of 254.08 ( $\pm$  33.64) g fruit (Fig. 2). After fruit were removed, 191.82 ( $\pm$  15.84) mg P was present in the plant and increased to 227.88 ( $\pm$  11.69) mg at pruning (20 Dec. 2013). 37% of the total P content (including the fruit) at harvest was present in the roots and decreased slightly to 76.09 ( $\pm$  7.02) mg at pruning. 14% of the total P content was present in the canes at harvest and increased slightly to pruning (37.18  $\pm$  2.61 mg). The shoots contained 12% of the total P content at harvest and increased to 48.81 ( $\pm$  3.56) mg (including the shoot prunings) at pruning. 20% of the total P content was present in the leaves and increased to 65.56 ( $\pm$  2.73) mg (including the leaf prunings) at pruning. Only 9 mg P was redistributed from the roots to the other plant parts, but 36.06 mg P was taken up during this period (Table 1).

64.81 ( $\pm$  2.60) mg (28% of the total P content at pruning) and 36.63 ( $\pm$  2.88) mg P (16% of the total P content at pruning) were lost from the leaves and shoots, respectively, due to pruning (Fig. 2). After plants were pruned, 126.44 ( $\pm$  9.58) mg P was present in the plant and increased to 455.03 ( $\pm$  36.55) mg at growth cessation (21 May 2014) (Fig. 2). Of the total P content, 33% was present in the roots (76.09  $\pm$  7.02 mg) before prunings were removed and increased to 214.57 ( $\pm$  26.14) mg at growth cessation. The canes contained 16% of the total P

content and increased from pruning to growth cessation ( $51.27 \pm 4.98$  mg). 5% of the total P content was present in the shoots (excluding the shoot prunings) and increased from  $12.17 (\pm 1.49)$  mg to  $80.81 (\pm 5.86)$  mg at growth cessation. The leaf DW after pruning was almost 0 g resulting in a P content representing 0% of the total P at pruning. The P content in the leaves then increased to  $97.60 (\pm 7.84)$  mg at growth cessation. A total of 328.60 mg P was taken up after pruning (20 Dec. 2013) until growth cessation (Table 1) and no redistribution occurred. 2% of the total P was present in the new flowers ( $10.78 \pm 1.20$  mg) at growth cessation.

A total of 469.88 mg P was taken up during the season (Table 1) and 139.79 mg P was lost through pruning and harvest. The uptake pattern of P was similar to that of N (Table 1). The most P was also taken up after pruning while only small amounts of P fertilizer was taken up between growth cessation in the first season and early fruit set (Table 1).

*'Emerald': Potassium.* The total K content per 'Emerald' plant decreased from  $488.86 (\pm 118.93)$  mg at the time of growth cessation (21 May 2013) to  $359.43 (\pm 48.28)$  mg at early fruit set (21 Aug. 2013) (Fig. 3). Of the total K content, 39% was present in the roots and decreased, although not significantly, from growth cessation ( $192.76 \pm 79.73$  mg) to early fruit set ( $137.22 \pm 19.02$  mg). 17% of the total K content was present in the canes and decreased from growth cessation ( $82.57 \pm 24.98$  mg) to early fruit set ( $18.40 \pm 4.18$  mg). The shoots contained 6% of the total K content at growth cessation and it did not change significantly between growth cessation ( $28.48 \pm 10.04$  mg) and early fruit set ( $37.86 \pm 11.22$  mg). 38% of the total K was found in the leaves and decreased from  $185.05 (\pm 39.32)$  mg to  $127.43 (\pm 15.35)$  mg at early fruit set. A decrease of 129.43 mg K occurred during this period and can be ascribed to differences in plant DW (data not shown). Due to plant variation, plants sampled at early fruit set were slightly smaller than at growth cessation and therefore had less DW and thus a smaller total nutrient content than plants sampled at growth cessation.

From Fig. 3 it can be seen that total K per plant increased from 359.43 ( $\pm$  48.28) mg at early fruit set (21 Aug. 2013) to 1090.01 ( $\pm$  57.29) mg at harvest (19 Nov. 2013). Of the total K per plant, 38% was present in the roots and did not change significantly between early fruit set (137.22  $\pm$  19.02 mg) and harvest (154.31  $\pm$  13.53 mg). 5% of the total K was present in the canes at early fruit set and increased to 106.27 ( $\pm$  10.19) mg at harvest. The shoots contained 11% of the total K content and it increased significantly from early fruit set to harvest (168.38  $\pm$  21.86 mg). 35% of the total K content was present in the leaves and increased to 305.22 ( $\pm$  45.14) mg at harvest. Fruit K increased from early fruit set (26.85  $\pm$  5.17 mg) to harvest (355.83  $\pm$  45.47 mg). At early fruit set the flowers contained 3% of the total K (11.68  $\pm$  3.36 mg). No redistribution occurred during this stage, but a total of 730.58 mg K was taken up (Table 1).

355.83 ( $\pm$  45.47) mg K (33% of the total K at harvest) was present in the fruit at harvest and lost due to harvest of 254.08 ( $\pm$  33.64) g fruit. Between harvest (19 Nov. 2013) and pruning (20 Dec. 2013), the total K content in the plant which remained after fruit were harvested increased from 734.18 ( $\pm$  76.54) mg to 1184.42 ( $\pm$  76.32) mg (Fig. 3). Of the total K at harvest (before fruit were removed), 14% was present in the roots and increased significantly until pruning (213.41  $\pm$  20.13 mg). 10% of the K was present in the canes and increased from harvest (106.27  $\pm$  10.39 mg) to pruning (132.42  $\pm$  10.19 mg). The shoots contained 15% of the total K at harvest and increased to 320.57 ( $\pm$  15.94) mg (shoot prunings included) at pruning. 28% of the total K content was found in the leaves and increased to 516.81 ( $\pm$  33.26) mg at pruning (leaf prunings included). No redistribution occurred during this stage, but 450.24 mg K was taken up (Table 1).

510.74 ( $\pm$  32.31) mg (43% of the total K content at pruning) and 230.42 ( $\pm$  12.86) mg K (19% of the total K content at pruning) were lost as leaves and shoots, respectively, through pruning (Fig. 3). 443.26 ( $\pm$  31.73) mg K was present in the plant after pruning and increased



to 1555.97 ( $\pm$  102.33) mg at growth cessation (21 May 2014). Of the total K (including the prunings), 18% was present in the roots at pruning and increased to 391.55 ( $\pm$  41.51 mg) at growth cessation. 11% of the total K content was present in the canes and stayed fairly constant from pruning (132.42  $\pm$  10.19 mg) to growth cessation (143.52  $\pm$  20.48 mg). The shoots (excluding the shoot prunings) contained 8% of the total K at pruning and increased to 288.36 ( $\pm$  23.69) mg at growth cessation. 1% of the total K was found in the leaves (leaf pruning excluded) and increased to 698.08 ( $\pm$  61.74) mg from pruning to growth cessation. No redistribution occurred during this stage, but a total of 1112.71 mg K was taken up after pruning (20 Dec. 2013) until growth cessation (Table 1). 2% (34.47  $\pm$  3.73 mg) of the total K was present in the new flowers that had already developed at the time of growth cessation during the second season.

A total of 2293.53 mg K was taken up during the season (Table 1) and 1096.99 mg K was lost through pruning and harvest. 32% of the total K that was taken up during the season was taken up between early fruit set and harvest and 49% was taken up after pruning, while no K was taken up between growth cessation in the first season and early fruit set (Table 1).

*'Emerald': Calcium.* The total Ca content of 'Emerald' decreased from 699.56 ( $\pm$  129.8) mg at the time of growth cessation (21 May 2013) to 502.42 ( $\pm$  51.10) mg at early fruit set (21 Aug. 2013) (Fig. 4). Of the total Ca content per plant, 45% was present in the roots and decreased from growth cessation (318.16  $\pm$  92.88 mg) to early fruit set (219.74  $\pm$  31.55 mg). 27% of the total Ca content was present in the canes at growth cessation and decreased significantly from growth cessation (185.56  $\pm$  83.77 mg) to early fruit set (32.74  $\pm$  4.00 mg). Therefore the decrease in total Ca content was seen in roots and canes and must be due to a lower DW (data not shown) at early fruit set due to plant variation. The shoots contained 11% of the total Ca content and increased from growth cessation (73.70  $\pm$  30.73 mg) to early fruit set (112.36  $\pm$  17.94 mg). 17% of the total Ca was found in the leaves and stayed unchanged

between growth cessation ( $122.14 \pm 16.75$  mg) and early fruit set ( $117.13 \pm 12.89$  mg). The increase in shoot Ca could be due to reallocation from the roots or canes.

Between early fruit set (21 Aug. 2013) and harvest (19 Nov. 2013) the total Ca content in the plant increased from  $502.42 (\pm 51.10)$  mg to  $1076.51 (\pm 93.87)$  mg (Fig. 4). Of the total Ca, 44% was present in the roots and increased from early fruit set ( $219.74 \pm 31.55$  mg) to harvest ( $251.18 \pm 18.73$  mg), although not significantly. The canes contained 7% of the total Ca at early fruit set and increased to  $108.26 (\pm 12.70)$  mg at harvest. 22% of the total Ca was found in the shoots and increased to  $301.36 (\pm 45.25)$  mg at harvest. The leaves contained 23% of the total Ca and increased to  $382.54 (\pm 50.69)$  mg at harvest. Fruit Ca content increased from  $15.49 (\pm 3.05)$  mg at early fruit set to  $33.18 (\pm 6.84)$  mg at harvest. 1% of the total Ca was present in the flowers ( $4.97 \pm 1.35$  mg) at early fruit set. No redistribution occurred between early fruit set and harvest, but 574.09 mg Ca was taken up (Table 1).

$33.18 (\pm 6.84)$  mg (3% of the total Ca at harvest) was lost due to the harvest of  $254.08 (\pm 33.64)$  g fruit (Fig. 4). The total Ca content per plant increased from  $1043.33 (\pm 96.76)$  mg at the time of harvest (after fruit were picked) (19 Nov. 2013) to  $1553.66 (\pm 70.16)$  mg at the time of pruning (20 Dec. 2013). Of the total Ca in the plant at harvest (including the fruit), 23% was present in the roots and decreased to  $176.30 (\pm 16.29)$  mg at the time of pruning. The canes contained 10% of the total Ca at harvest and increased until pruning ( $175.66 \pm 14.38$  mg). 28% of the total Ca was present in the shoots and increased to  $640.65 (\pm 29.49)$  mg at pruning. The leaves contained 36% of the total Ca and increased to  $559.96 (\pm 11.60)$  mg at pruning. 510.33 mg Ca was taken up and 74.88 mg was redistributed from the roots to the rest of the plant during this period.

$554.51 (\pm 11.38)$  mg (36% of the total Ca content at pruning) and  $529.97 (\pm 32.69)$  mg (34% Ca of the total Ca content at pruning) were lost due to the pruning of the leaves and shoots,

respectively (Fig. 4). The total Ca content in the plant that was present after pruning increased from 469.18 ( $\pm$  38.75) mg to 2386.20 ( $\pm$  181.06) mg at growth cessation (21 May 2014). Of the total Ca (including the prunings), 11% was present in the roots at pruning and increased until growth cessation (488.62  $\pm$  63.68 mg). The canes also contained 11% of the total Ca and increased to 433.39 ( $\pm$  83.11) mg at growth cessation. 7% of the total Ca was present in the shoots (prunings excluded) and increased to 903.89 ( $\pm$  82.4) mg at growth cessation. The leaves (prunings excluded) contained 0% of the total Ca at pruning (due to the DW being almost 0 g) and increased to 534.32 ( $\pm$  32.54) mg at growth cessation. A total of 1917.02 mg Ca was taken up after pruning (20 Dec. 2013) until growth cessation and was the highest amount taken up during the season (Table 1). 1% of the total Ca was present in the new flowers (25.99  $\pm$  3.00 mg) at growth cessation at the end of the second season.

A total of 3001.44 mg Ca was taken up during the season and 1117.66 mg Ca was lost through pruning and harvest. 64% of the total Ca taken up during the season was taken up after pruning while no Ca was taken up between growth cessation in the first season and early fruit set (Table 1).

*'Emerald': Magnesium.* The total Mg content of 'Emerald' plants decreased from 201.08 ( $\pm$  26.96) mg at the time of growth cessation (21 May 2013) to 179.02 ( $\pm$  15.40) mg at early fruit set (21 Aug. 2013) (Fig. 5). Of the total Mg, 47% was present in the roots and did not change between growth cessation (94.21  $\pm$  17.83 mg) and early fruit set (108.78  $\pm$  12.35 mg) while 26% of the total Mg was present in the canes and decreased from growth cessation (52.53  $\pm$  19.31 mg) to early fruit set (11.39  $\pm$  1.95 mg). The reason for the decrease in Mg in the canes was the same as seen in Ca. The shoots contained 8% of the total Mg and did not differ between growth cessation (15.94  $\pm$  5.38 mg) and early fruit set (16.88  $\pm$  2.63 mg). The leaves contained 18% of the total Mg content at growth cessation and the content did not change between growth cessation (38.40  $\pm$  4.04 mg) and early fruit set (36.05  $\pm$  2.80 mg). No

uptake occurred during this period and a decrease in the total Mg content can be ascribed to plant variation.

Between early fruit set (21 Aug. 2013) and harvest (19 Nov. 2013) the total Mg content in the plant increased 179.02 ( $\pm$  15.40) mg to 326.12 ( $\pm$  13.34) mg (Fig. 5). Of the total Mg, 61% was present in the roots and did not change from early fruit set to harvest (118.59  $\pm$  7.27 mg). The canes contained 6% of the total Mg content and increased to 31.55 ( $\pm$  2.21) mg at harvest. 9% of the Mg was present in the shoots and increased from early fruit set to harvest (36.78  $\pm$  3.87 mg). The leaves contained 20% of the total Mg and increased significantly until harvest (103.63  $\pm$  9.80 mg). Fruit Mg content increased from early fruit set (3.53  $\pm$  1.03 mg) to harvest (35.58  $\pm$  4.55 mg). The flowers contained 1% of the total Mg content at early fruit set (2.40  $\pm$  0.56 mg). 147.10 mg Mg was taken up during this period (Table 1) and no redistribution occurred.

35.58 ( $\pm$  4.55) mg Mg (11% of the total Mg at harvest) was present in the fruit and lost due to harvest of 254.08 ( $\pm$  33.64) g fruit (Fig. 5). The total Mg content per plant increased from 290.54 ( $\pm$  15.77) mg at the time of harvest (after fruit were picked) (19 Nov. 2013) to 366.62 ( $\pm$  22.07) mg at the time of pruning (20 Dec. 2013). Of the total Mg in the plant (fruit included), 36% was present in the roots and decreased slightly from harvest to pruning (106.17  $\pm$  11.48 mg). The canes contained 10% of the total Mg and increased to 40.99 ( $\pm$  1.67) mg at pruning. 11% of the total Mg was present in the shoots and increased to 63.77 ( $\pm$  3.73) mg (shoot prunings included) at pruning. The leaves contained 32% of the total Mg and increased to 155.40 ( $\pm$  5.17) mg (leaf prunings included) at pruning. 76.08 mg Mg was taken up during this period (Table 1) and 12.42 mg was redistributed from the roots to the other plant parts.

153.93 ( $\pm$  5.03) mg (42% of the total Mg at pruning) and 49.94 ( $\pm$  3.75) mg (14% of the total Mg at pruning) Mg from the leaves and shoots was lost by pruning, respectively (Fig. 5). The total Mg content increased from 162.75 ( $\pm$  13.37) after pruning (20 Dec. 2013) to 558.89 ( $\pm$  48.26) mg at growth cessation (21 May 2014). Of the total Mg content (including the prunings), 29% was present in the roots at pruning and increased until growth cessation (232.8  $\pm$  28.32 mg). 11% of the total Mg was present in the canes and increased to 59.22 ( $\pm$  8.71) mg at growth cessation. The shoots (excluding the shoot prunings) contained 4% of the total Mg and increased to 100.64 ( $\pm$  6.54) mg at growth cessation. 0% of the total Mg was found in the leaves (leaf prunings excluded) and increased until growth cessation (157.53  $\pm$  13.62 mg). A total of 396.15 mg Mg was taken up after pruning (20 Dec. 2013) to growth cessation and was the most Mg taken up during the season (Table 1). The increase of Mg in the canes, shoots and leaves between early fruit set (21 Aug. 2013) and growth cessation (21 May 2014) was mainly due to uptake although some of the Mg was remobilized from the roots between harvest and pruning. At growth cessation, the new flowers contained 2% of the total Mg content (8.62  $\pm$  0.92 mg).

A total of 619.33 mg Mg was taken up during the season and 239.46 mg Mg was lost through pruning and harvest. The Mg uptake pattern was similar to that of Ca (Table 1), with the most taken up after pruning and nothing taken up between growth cessation in the first season and early fruit set (Table 1).

*'Snowchaser': Nitrogen.* Total N per 'Snowchaser' plant increased from 1881.96 ( $\pm$  89.55) mg at the time of growth cessation (21 May 2013) to 2396.22 ( $\pm$  92.15) mg at the time of early fruit set (21 Aug. 2013) (Fig. 6). Of the total N, 44% was present in the roots at growth cessation and increased from 818.97 ( $\pm$  47.82) mg to 1306.08 ( $\pm$  82.01) mg at early fruit set. 19% of the total N content was present in the canes and decreased from 351.02  $\pm$  (23.73) mg to 260.80 ( $\pm$  25.62) mg at early fruit set, indicating reallocation possibly to the roots. The

shoots contained 12% of the total N content in the plant and it increased from growth cessation ( $228.20 \pm 35.64$  mg) to early fruit set ( $271.82 \pm 16.24$  mg). The leaves contained 26% of the total N content and it decreased from  $483.77 (\pm 29.09)$  mg to  $404.76 (\pm 11.28)$  mg at early fruit set.  $514.26$  mg N was taken up during this period (Table 2) and  $169.24$  mg N was redistributed from the canes and leaves.

The total N in the plant did not change significantly between early fruit set ( $2396.22 \pm 92.15$  mg) (21 Aug. 2013) and harvest ( $2384.16 \pm 98.34$  mg) (19 Nov. 2013) (Fig. 6). Of the total N, 55% was present in the roots and decreased greatly to harvest ( $466.63 \pm 112.0$  mg). 11% of the total N was present in the canes and increased to  $366.91 (\pm 37.57)$  mg at harvest. The shoots also contained 11% of the total N at early fruit set and increased to  $410.50 (\pm 15.73)$  mg at harvest. 17% of the total N was present in the leaves and increased to  $768.05 (\pm 49.88)$  mg at harvest. The fruit N content increased from  $113.96 (\pm 15.79)$  mg to  $372.08 (\pm 103.27)$  mg from early fruit set to harvest. 2% of the total N at early fruit set was present in the flowers ( $38.81 \pm 5.43$  mg).  $839.45$  mg was redistributed from the roots to the rest of the plant, but no significant uptake occurred during this period.

$372.08 (\pm 103.27)$  mg (16% of the total N at harvest) N was present in the fruit and lost due to the harvest of  $231.67 (\pm 59.94)$  g fruit (Fig. 6). Total N per plant increased from  $2012.08 (\pm 165.54)$  mg after fruit were removed (19 Nov. 2013) to  $3532.34 (\pm 227.99)$  mg at the time of pruning (20 Dec. 2013). At harvest the roots contained 20% of the total N in the plant (fruit included) and increased to  $908.07 (\pm 55.21)$  mg at pruning. The canes contained 15% of the total N and increased to  $536.91 (\pm 52.24)$  mg at pruning. 17% of the total N was present in the shoots and increased to  $701.42 (\pm 43.11)$  mg (shoot prunings included) at pruning. At harvest, 36% of the total N was contained in the leaves and increased to  $1380.86 (\pm 119.29)$  mg (leaf prunings included) at pruning. No redistribution occurred during this period, but  $1520.26$  mg N was taken up (Table 2).

1355.47 ( $\pm 120.49$ ) mg (38% of the total N at pruning) and 514.16 ( $\pm 38.08$ ) mg (15% of the total N at pruning) N from the leaves and shoots, respectively, were lost by pruning (Fig. 6). After plants were pruned, 1657.64 ( $\pm 83.36$ ) mg N was present in the plant and increased to 7867.68 ( $\pm 308.14$ ) mg at growth cessation (21 May 2014). Of the total N (prunings included), 26% was present in the roots and it increased to 3093.19 ( $\pm 203.73$ ) mg at growth cessation. The canes contained 15% of the total N at pruning and increased to 1086.80 ( $\pm 33.65$ ) mg at growth cessation. The shoots (excluding the shoot prunings) contained 5% of the total N at pruning and increased to 982.42 ( $\pm 100.58$ ) mg at growth cessation. 1% of the total N content was present in the leaves (leaf prunings excluded) and increased to 2023.23 ( $\pm 92.37$ ) mg at growth cessation. 6204.97 mg were taken up after pruning (20 Dec. 2013) to growth cessation (Table 2). At growth cessation new flowers, that have already formed for the next season, contained 9% of the total N content ( $682.05 \pm 60.74$  mg).

A total of 8239.49 mg N was taken up during the season and 2241.71 mg N was lost due to pruning and harvest. 75% of the total N taken up during the season was taken up after pruning, while almost no N was taken up between growth cessation in the first season to harvest (Table 2).

*'Snowchaser': Phosphorus.* Total P per 'Snowchaser' plant increased from 276.37 ( $\pm 14.56$ ) mg at the time of growth cessation (21 May 2013) to 356.99 ( $\pm 17.09$ ) mg at early fruit set (21 Aug. 2013) (Fig. 7). Of the total P content, 54% was present in the roots and increased from growth cessation ( $148.13 \pm 10.23$  mg) to early fruit set ( $218.64 \pm 15.07$  mg). The canes contained 22% of the total P and decreased from growth cessation ( $59.64 \pm 7.26$  mg) to early fruit set ( $31.95 \pm 2.89$  mg). 11% of the total P was present in the shoots and increased slightly from growth cessation ( $29.63 \pm 4.84$  mg) to early fruit set ( $35.52 \pm 2.46$  mg). The leaves contained 14% of the total P content and increased slightly from growth cessation ( $38.96 \pm$

3.66 mg) to early fruit set ( $46.79 \pm 2.64$  mg). 80.63 mg P was taken up during this stage (Table 2) and 27.69 mg was redistributed from the canes.

Between early fruit set (21 Aug. 2013) and harvest (19 Nov. 2013), the total P content decreased from  $356.99 (\pm 17.09)$  mg to  $293.81 (\pm 13.32)$  mg (Fig. 7). Of the total P content, 61% was present in the roots and decreased from early fruit set to harvest ( $77.92 \pm 18.32$  mg). The canes contained 9% of the total P content at early fruit set and increased from early fruit set to harvest ( $46.69 \pm 4.61$  mg). 10% of the total P content was present in the shoots at early fruit set and increased to  $60.43 (\pm 5.41)$  mg at harvest. The leaves contained 13% of the total P content and increased to  $60.87 (\pm 3.84)$  mg at harvest. The P content in the fruit increased from early fruit set ( $18.69 \pm 2.34$  mg) to harvest ( $47.90 \pm 12.69$  mg). The flowers contained 2% of the total P at early fruit set ( $5.41 \pm 0.81$  mg). 82.9 mg P was remobilized from the roots to the canes, shoots, leaves and fruit and 57.8 mg P was lost from the roots, possibly due to loss of DW during sampling.

$47.90 (\pm 12.69)$  mg (16% of the total N at harvest) were present in the fruit and was lost due to the harvest of  $231.67 (\pm 59.94)$  g fruit (Fig. 7). The total P content per plant after fruit were removed (19 Nov. 2013) increased from  $245.90 (\pm 20.31)$  mg to  $422.09 (\pm 23.89)$  mg at the time of pruning (20 Dec. 2013). At harvest the roots contained 27% of the total P content (including the fruit) and increased to  $159.56 (\pm 14.65)$  mg at pruning. 16% of the total P content was present in the canes and increased to  $69.37 (\pm 6.99)$  mg at pruning. The shoots contained 21% of the total P content at harvest and increased to  $92.52 (\pm 5.55)$  mg (shoot prunings included) at pruning. 21% of the total P content was present in the leaves and increased to  $100.19 (\pm 9.82)$  mg at pruning. No redistribution occurred, but 176.18 mg P was taken up during this period (Table 2).



98.33 ( $\pm 9.74$ ) mg (23% of the total N at pruning) and 68.00 ( $\pm 5.31$ ) mg (16% of the total N at pruning) were lost from the leaves and shoots, respectively, due to pruning (Fig. 7). Total P content per plant increased from 255.38 ( $\pm 15.62$ ) mg after pruning (20 Dec. 2013) to 706.77 ( $\pm 32.11$ ) mg at growth cessation (21 May 2014). Of the total P content at pruning (prunings included), 38% was present in the roots and increased to 314.21 ( $\pm 26.62$ ) mg at growth cessation. The canes contained 16% of the total P content and increased to 100.93 ( $\pm 5.84$ ) mg at growth cessation. 6% of the total P content was present in the shoots (excluding the shoot prunings) and increased to 90.40 ( $\pm 10.48$ ) mg at growth cessation. The leaves (excluding the leaf prunings) contained 1% of the total P at pruning and increased to 135.84 ( $\pm 5.30$ ) mg at growth cessation. 451.01 mg P was taken up during this period (Table 2) and no redistribution occurred. At growth cessation the flowers that have already formed for the next season contained 9% of the total P content ( $65.39 \pm 4.67$  mg).

A total of 707.82 mg P was taken up during the season and 214.23 mg P was lost due to pruning and harvest. The most P was taken up after pruning, while only a small amount of P fertilizer was taken up early in the season (Table 2).

*'Snowchaser': Potassium.* The total K content per 'Snowchaser' plant increased from 1015.91 ( $\pm 121.04$ ) mg at the time of growth cessation (21 May 2013) to 1253.20 ( $\pm 27.89$ ) mg at early fruit set (21 Aug. 2013) (Fig. 8). Of the total K content, 30% was present in the roots decreased from growth cessation ( $305.06 \pm 82.42$  mg) to early fruit set ( $243.45 \pm 8.82$  mg) 10% of the total K content was present in the canes and decreased from growth cessation ( $104.10 \pm 27.50$  mg) to early fruit set ( $73.18 \pm 7.54$  mg). The shoots contained 12% of the total K content at growth cessation and increased from growth cessation ( $119.65 \pm 20.24$  mg) to early fruit set ( $158.54 \pm 12.83$  mg). 48% of the total K was found in the leaves and increased from growth cessation ( $487.11 \pm 29.32$  mg) to early fruit set ( $678.67 \pm 26.42$

mg). An uptake of 237.29 mg K occurred during this period (Table 2) and 92.53 mg was redistributed from the roots and canes to the leaves and shoots.

Total K per plant increased from 1253.20 ( $\pm$  27.89) mg at early fruit set (21 Aug. 2013) to 1639.77 ( $\pm$  36.15) mg at harvest (19 Nov. 2013) (Fig. 8). Of the total K per plant, 19% was present in the roots and decreased from early fruit set to harvest (119.91  $\pm$  29.73 mg). 6% of the total K was present in the canes at early fruit set and increased to 179.71 ( $\pm$  18.98) mg at harvest. The shoots contained 13% of the total K content and increased from early fruit set to harvest (388.85  $\pm$  50.77 mg). 54% of the total K content was present in the leaves at early fruit set and decreased to 585.81 ( $\pm$  51.29) mg at harvest. The fruit K content increased from 75.75 ( $\pm$  10.57) mg at early fruit set to 365.50 ( $\pm$  83.71) mg at harvest. 2% of the total K at early fruit set was present in the flowers (23.62  $\pm$  4.05 mg). 386.56 mg was taken up during this period (Table 2) and 216.41 mg was redistributed from the leaves and roots.

365.50 ( $\pm$  83.71) mg K (22% of the total K at harvest) was present in the fruit and lost due to the harvest of 231.67 ( $\pm$  59.94) g fruit (Fig. 8). The total K content in the plant increased from 1274.27 ( $\pm$  82.2) mg after harvest (19 Nov. 2013) to 2542.00 ( $\pm$  146.20) mg at pruning (20 Dec. 2013). Of the total K at harvest (fruit included), 7% was present in the roots and increased to 330.61 ( $\pm$  10.16 mg) at pruning. 11% of the K was present in the canes and increased until pruning (261.64  $\pm$  29.90 mg). The shoots contained 24% of the total K at harvest and increased to 608.96 ( $\pm$  24.77) mg (shoot prunings included) at pruning. 36% of the total K content was found in the leaves at harvest and increased to 1334.85 ( $\pm$  101.36) mg (leaf prunings included) at pruning. No redistribution occurred, but 1267.73 mg K was taken up during this period (Table 2).

1305.05 ( $\pm$  105.12) mg K (51% of the total K at pruning) and 458.94 ( $\pm$  19.07) mg K (18% of the total K at pruning) was lost from leaves and shoots, respectively, as a result from pruning

(Fig. 8). Total K per plant increased from 772.06 ( $\pm$  35.12) mg after pruning (20 Dec. 2013) to 2561.01 ( $\pm$  101.41) mg at growth cessation (21 May 2014). Of the total K in the plant (prunings included), 13% was present in the roots at pruning and increased to 411.38 ( $\pm$  29.15) mg at growth cessation. 10% of the total K content was present in the canes and the K content stayed constant from pruning (261.64  $\pm$  29.90 mg) to growth cessation (275.39  $\pm$  14.37 mg). The shoots (shoot prunings excluded) contained 6% of the total K at pruning and increased to 314.56 ( $\pm$  40.69) mg at growth cessation. 1% of the total K was found in the leaves (leaf prunings excluded) and increased until growth cessation (1335.47  $\pm$  47.68 mg). 1782.99 mg K was taken up after pruning (20 Dec. 2013) until growth cessation. At growth cessation the flowers that have already formed for the next season contained 9% of the total P content (224.21  $\pm$  18.26 mg).

A total of 3674.57 mg K was taken up during the season (Table 2) and 2129.49 mg K was lost due to pruning and harvest. 35% of the total K taken up was taken up between harvest and pruning and 49% was taken up after pruning (Table 2), indicating the importance of K fertilizer after harvest.

*'Snowchaser': Calcium.* The total Ca content per 'Snowchaser' plant increased from 1121.25 ( $\pm$  132.91) mg at the time of growth cessation (21 May 2013) to 1512.59 ( $\pm$  46.98) mg at early fruit set (21 Aug. 2013) (Fig. 9). Of the total Ca content per plant, 43% was present in the roots and increased slightly from growth cessation (477.16  $\pm$  97.93 mg) to early fruit set (531.09  $\pm$  40.33 mg) although not significantly. 15% of the total Ca content was present in the canes at growth cessation and there was no significant difference in the Ca content of the canes between growth cessation (164.61  $\pm$  44.64 mg) and early fruit set (131.32  $\pm$  21.71 mg). The shoots contained 23% of the total Ca content and increased from growth cessation (255.95  $\pm$  38.31 mg) to early fruit set (538.19  $\pm$  39.37 mg). 20% of the total Ca was found in the leaves and did not differ between growth cessation (223.53  $\pm$  47.68 mg) and early fruit set

( $241.55 \pm 14.22$  mg). 391.35 mg was taken up during this period (Table 2) and 33.29 mg was redistributed from the canes, although this decrease in cane Ca content wasn't significant. Ca taken up by the plant during this period was mainly allocated to the shoots, because only the shoots had a significant increase in Ca during this period.

Between early fruit set (21 Aug. 2013) and harvest (19 Nov. 2013) the total Ca content in the plant increased from  $1512.59 (\pm 46.98)$  mg to  $1815.46 (\pm 103.88)$  mg (Fig. 9). Of the total Ca, 35% was present in the roots and decreased from early fruit set to harvest ( $262.41 \pm 62.18$  mg). The canes contained 9% of the total Ca at early fruit set and increased to  $281.59 (\pm 27.24)$  mg at harvest. 36% of the total Ca was found in the shoots and increased to  $733.13 (\pm 50.83)$  mg at harvest. The leaves contained 20% of the total Ca and increased to  $457.87 (\pm 21.68)$  mg at harvest. Fruit Ca content increased from early fruit set ( $55.36 \pm 7.52$ ) to harvest ( $80.47 \pm 19.49$  mg). 1% of the total Ca was present in the flowers at early fruit set ( $15.08 \pm 2.70$  mg). 302.87 mg Ca was taken up (Table 2) and 268.68 mg Ca was redistributed from the roots during this period.

$80.47 (\pm 19.49)$  mg Ca (4% of the total Ca at harvest) was present in the fruit and lost due to the harvest of  $231.67 (\pm 59.94)$  g fruit (Fig. 9). The total Ca content per plant increased from  $1734.99 (\pm 104.56)$  mg after harvest (19 Nov. 2013) to  $3386.90 (\pm 172.66)$  mg at pruning (20 Dec. 2013). Of the total Ca in the plant at harvest (fruit included), 14% was present in the roots and increased to  $444.95 (\pm 54.53)$  mg at pruning. The canes contained 16% of the total Ca at harvest and increased to  $465.04 (\pm 69.19)$  mg at pruning. 40% of the total Ca was present in the shoots and increased until pruning ( $1710.96 \pm 92.20$  mg). The leaves contained 25% of the total Ca and increased to  $762.82 (\pm 49.43)$  mg at pruning. No redistribution occurred during this period, but 1651.90 mg Ca was taken up (Table 2).

747.17 ( $\pm$  50.09) mg (22% of the total Ca at pruning) and 1338.10 ( $\pm$  86.63) mg (40% of the total Ca at pruning) were lost from the leaves and shoots, respectively, due to pruning (Fig. 9). After pruning, 1298.50 ( $\pm$  126.84) mg Ca was present in the plant and increased to 5014.02 ( $\pm$  298.43) mg at growth cessation (21 May 2014). Of the total Ca content (prunings included), 13% was present in the roots at pruning and increased to growth cessation (837.34  $\pm$  84.77 mg). The canes contained 14% of the total Ca and increased from pruning to growth cessation (1541.09  $\pm$  162.93 mg). 11% of the total Ca was present in the shoots (shoot prunings excluded) and increased to 1930.79 ( $\pm$  230.03) mg at growth cessation. The leaves (leaf prunings excluded) contained 1% of the total Ca at pruning and increased until growth cessation (569.76  $\pm$  16.88 mg). 3712.39 mg was taken up after pruning (Table 2) and no redistribution occurred. At growth cessation 3% of the total Ca was present in the flowers (135.04  $\pm$  18.19 mg) that have already formed for the following season.

A total of 6058.51 mg Ca was taken up during the season and 2165.74 mg Ca was lost due to pruning and harvest. The most Ca was taken up at the season (Table 2).

*'Snowchaser': Magnesium.* The total Mg content per 'Snowchaser' plant increased from 333.85 ( $\pm$  42.28) mg at the time of growth cessation (21 May 2013) to 414.87 ( $\pm$  13.72) mg at early fruit set (21 Aug. 2013) (Fig. 10). Of the total Mg, 53% was present in the roots and increased from growth cessation (178.56  $\pm$  29.92 mg) to early fruit set (226.93  $\pm$  11.92 mg). 15% of the total Mg was present in the canes and decreased from 50.80 ( $\pm$  18.69) mg at the time of growth cessation to 28.87 ( $\pm$  3.66) mg at the time of early fruit set. The shoots contained 12% of the total Mg and increased from growth cessation (39.53  $\pm$  6.16 mg) to early fruit set (54.0  $\pm$  4.08 mg). The leaves contained 19% of the total Mg content and increased from 64.96 ( $\pm$  3.03) mg at growth cessation to 81.05 ( $\pm$  4.62) mg at early fruit set. 81.02 mg Mg was taken up during this period (Table 2) and 21.93 mg Mg was redistributed from the canes to the rest of the plant.

Between early fruit set (21 Aug. 2013) and harvest (19 Nov. 2013) the total Mg content in the plant decreased from 414.87 ( $\pm$  13.72) mg to 379.64 ( $\pm$  21.48) mg (Fig. 10). Of the total Mg, 55% was present in the roots and decreased from early fruit set to harvest (104.17  $\pm$  26.11 mg). The canes contained 7% of the total Mg content and increased to 39.56 ( $\pm$  3.14) mg at harvest. 13% of the Mg was present in the shoots and increased from early fruit set to harvest (85.0  $\pm$  11.99 mg). The leaves contained 20% of the total Mg content at early fruit set and increased to 114.07 ( $\pm$  6.61) mg at harvest. Fruit Mg content increased from early fruit set (16.48  $\pm$  1.94 mg) to harvest (36.55  $\pm$  8.37 mg). The flowers contained 2% of the total Mg content at early fruit set (6.95  $\pm$  1.18 mg). Some of the Mg could have been reallocated from the roots or taken up and incorporated in the canes, shoots, leaves and fruit, while the decrease in total Mg content was due to the lower DW of the roots at harvest due to plant variation or loss of root DW during sampling.

36.55 ( $\pm$  8.37) mg Mg (10% of the total Mg at harvest) was present in the fruit at harvest and lost by harvesting 231.67 ( $\pm$  59.94) g fruit (Fig. 10). The total Mg content per plant increased from 343.09 ( $\pm$  28.15) mg after fruit were removed (19 Nov. 2013) to 593.49 ( $\pm$  40.60) mg at pruning (20 Dec. 2013). Of the total Mg in the plant (fruit included), 27% was present in the roots and increased from harvest to pruning (179.78  $\pm$  14.65 mg). The canes contained 10% of the total Mg and increased to 59.71  $\pm$  (6.49) mg at pruning. 11% of the total Mg was present in the shoots and increased to 134.41 ( $\pm$  7.90) mg (shoot prunings included) at pruning. The leaves contained 30% of the total Mg and increased to 218.86 ( $\pm$  17.58) mg (leaf prunings included) at pruning. 250.39 mg Mg was taken up during this period (Table 2) and no redistribution occurred.

215.24 ( $\pm$  17.44) mg Mg (36% of the total Mg at pruning) and 108.09 ( $\pm$  6.39) mg Mg (18% of the total Mg at pruning) were lost from leaves and shoots, respectively, due to pruning (Fig. 10). 269.43 ( $\pm$  16.25) mg Mg was present in the plant after pruning (20 Dec. 2013) and

increased to 831.47 ( $\pm$  30.34) mg at growth cessation (21 May 2014). Of the total Mg in the plant (prunings included), 30% was present in the roots and increased until growth cessation ( $294.42 \pm 24.64$  mg). 10% of the total Mg was present in the canes and increased to 133.11 ( $\pm$  8.17) mg at growth cessation. The shoots (excluding the shoot prunings) contained 4% of the total Mg and increased until growth cessation ( $132.65 \pm 15.82$  mg). 1% of the total Mg was found in the leaves (leaf prunings excluded) and increased until growth cessation ( $216.79 \pm 6.60$  mg). 561.32 mg Mg was taken up after pruning until growth cessation (Table 2) and no significant redistribution occurred. At growth cessation, 7% of the total Mg was present in the flowers ( $54.51 \pm 5.71$  mg) that were already formed for the following season's crop.

A total of 892.73 mg Mg was taken up during the season (Table 2) and 359.89 mg Mg was lost due to pruning and harvest. The uptake pattern of Mg was similar to that of Ca (Table 2). The most Ca was taken up at the end of the season while almost nothing was taken up before harvest (Table 2).

### **General Discussion**

Early fruit set in 'Emerald' occurred a week later than in 'Snowchaser' and harvest started two weeks later in 'Emerald' than in 'Snowchaser', which might explain some of the differences in nutrient patterns between the cultivars. In 'Emerald' the decrease of N and P in the leaves and the increase of N and P in the roots, after growth cessation during the first season (Fig. 1 and 2), indicate remobilization from the leaves to the roots for storage during winter. This is common in deciduous trees that lose their leaves during winter and therefore reallocate nutrients to the permanent parts of the tree (Kanguechi et al., 2011; Kotzé and de Villiers, 1989; Stassen et al., 1983; Stassen and North, 2004). It could be that even though the plants were grown in an evergreening system that the deciduous nature of blueberries influenced the plants to remobilize nutrients from the leaves as would have happened if the

plants lost their leaves. In ‘Snowchaser’ this was evident for N (Fig. 6), but not for P (Fig. 7). Unlike peach (Stassen et al., 1983; Stassen and Stadler, 1988), kiwi (Kotzé and de Villiers, 1989) and pear (Stassen and North, 2004), SHB blueberries in this study did not redistribute nutrients from the leaves to the permanent parts of the tree at the end of the second season, possibly because leaves did not go through the senescing phase that signalled the plant to reallocate its nutrients from the leaves. Remobilization in the first season was observed after growth cessation and sampling did not continue after growth cessation in the second season and therefore it could be that remobilization only started after the last sampling date (Figs. 1 to 10).

Macro nutrients in ‘Emerald’ and ‘Snowchaser’ were generally higher in the roots than the other plant parts at growth cessation (Figs. 1 to 10). Other researchers concluded that the higher nutrient content indicated that roots are the main storage organs during winter and are thus important for new growth in spring (Bañados et al., 2006a; Bryla et al., 2012). Kotzé and de Villiers (1989) determined that in kiwi N and K reserves from the roots played an important role during early fruit set and Stassen et al. (1983) determined that for peach, 80% of N and 40% of K and 43% P used for new growth, came from reserves. According to Stassen et al. (1983) Mg, however, seemed to be taken up instead of being redistributed from reserves in order to sustain new growth. In ‘Emerald’ new spring growth was partly dependent on root N and P reserves (Fig. 1 and 2), but these amounts were much less than the amounts of nutrients taken up and therefore did not play such an important role during early fruit set. In ‘Snowchaser’, a cultivar that starts growing about two weeks earlier than ‘Emerald’, however, new spring growth was mainly dependent on root N and P and partly dependent on K, Ca and Mg root reserves probably as temperatures were still lower (Paper 1) at the time of growth resumption (Figs. 6 to 10). Lower temperatures would limit



transpiration, resulting in limited uptake of nutrients and therefore plants relied more on reserve nutrients (Kramer and Kozlowski, 1979).

In both ‘Emerald’ and ‘Snowchaser’, rate of absorption of nutrients was almost proportional to growth rate. This trend was observed by various researchers (Bañados et al., 2006b; Bryla et al., 2012; Rose, 1999; Throop and Hanson, 1997). According to Throop and Hanson (1997), the highest uptake of N in NHB ‘Bluecrop’ occurred from late bloom to after harvest, when the plant was actively growing. Although N uptake by ‘Emerald’ and ‘Snowchaser’ was high during this period, the highest N uptake only occurred after pruning and before growth cessation (Table 1 and 2). According to Stassen et al. (1983) N will be taken up by a plant as long as there are active leaves on the tree and the root temperature is favorable for root activity. In South Africa, these conditions typically occur during the whole of autumn (Stassen et al., 1983), explaining the rapid uptake of N after harvest.

The biggest uptake of N, P, K, Ca and Mg occurred between pruning and growth cessation (Table 1 and 2). Bañados et al. (2006a) found that NHB blueberry ‘Bluecrop’ also accumulated the most N at the end of the season and Bryla et al. (2012) found that the most K also accumulated late in the season in ‘Bluecrop’. Ca uptake by ‘Bluecrop’, however was higher earlier in the season (Bryla et al., 2012). In ‘Emerald’ little or no N, P, K, Ca and Mg were taken up during the period that the plant was less active (between growth cessation and early fruit set) (Table 1). N and P in ‘Emerald’ were taken up during the whole season and K, Ca and Mg were not taken up between growth cessation and early fruit set (Table 1). In ‘Snowchaser’ N, P and Mg was not taken up between early fruit set and harvest while K and Ca was taken up during the whole season (Table 2). A longer period between two phenological stages of one cultivar vs. the other did not necessarily result in more nutrients being taken up and therefore the time difference in the phenology of the two cultivars could not explain the difference in uptake patterns between the cultivars.

Bryla et al. (2012) determined that more than half of the N, P and K in NHB were lost due to harvest, but this was not the case for ‘Emerald’ or ‘Snowchaser’. 254.08 ( $\pm$  33.64) g fruit were harvested from ‘Emerald’ plants and 231.67 ( $\pm$  59.94) g fruit were harvested from ‘Snowchaser’ plants. Recommendations for correcting nutrient losses by harvest as well as pruning can be seen in Table 3 and 4. These amounts can be adjusted according to the crop load and extent of pruning. The N, P, K, Ca and Mg given during this trial were sufficient to replace losses and increase further uptake. Table 1 and 2 summarizes the total amounts of nutrients that should be applied per plant and the proportion of nutrients that should be given at the different stages during the season if a fertigation system is used. Macro nutrient requirements per ha for plants spaced at 2.5 x 1 m (4000 plants/ha) are presented in Table 5 and 6. If granular fertilizers are applied, these amounts should be adjusted to account for poor placement, leaching and volatilisation. This amount will depend on the soil type and the nutrient content of the soil (Stassen et al., 1983). Increasing the N, P, K, Ca and Mg fertilizer could possibly result in more fertilizer being taken up, but further studies need to be done to confirm this.

### **Conclusion**

Differences in nutrient uptake were observed between the two cultivars and could not be explained fully by difference in phenology or uptake periods. The growth rate of ‘Snowchaser’ was higher than that of ‘Emerald’ and the total DW of ‘Snowchaser’ plants were generally higher, which could explain differences in nutrient uptake. Fertilizer should be applied according to the uptake rates of the plant during the different growth stages. For ‘Emerald’ N and P should be applied through the whole season at different rates depending on the time of the season as indicated in Table 5, while K, Ca and Mg do not need to be applied between growth cessation and early fruit set. For ‘Snowchaser’ K and Ca should be

applied throughout the whole season at different rates depending on the time of the season as indicated in Table 6, while N, P and Mg do not need to be applied between early fruit set and harvest. When the cultivars are planted in the same fertigation block or in alternating rows, the fertilizer program with the highest rates, in this case the recommendations for ‘Snowchaser’, should be used. Both cultivars received the same nutrient solution at the same rates and no toxicity symptoms were observed and therefore the higher rates should not be a problem for the cultivar receiving an excess of fertilizer, in this case ‘Emerald’. Fertilizer rates used during this trial were sufficient to correct losses due to harvest and pruning as well as increase growth. By decreasing the fertilizer rates, plant growth, crop load and/or fruit quality could be affected negatively and further research needs to be conducted in order to know whether increasing fertilizer rates would result in even higher quality plants in terms of vegetative growth, crop load and fruit quality.

### Literature Cited

- Bañados, M.P., C. Bonomelli, J. González, and F. Juillerat. 2006a. Dry matter, nitrogen, potassium and phosphorus partitioning in blueberry plants during winter. *Acta Hort.* 715:443-448.
- Bañados, M.P., B. Strik, and T. Righetti. 2006b. The uptake and use of <sup>15</sup>N-nitrogen in young and mature field-grown highbush blueberries. *Acta Hort.* 715:357-364.
- Bryla, D.R., B.C. Strik, M.P. Bañados, and T.L. Righetti. 2012. Response of highbush blueberry to nitrogen fertilizer during field establishment-II. Plant nutrient requirements in relation to nitrogen fertilizer supply. *HortScience* 47(7):917-926.
- Clark, C. and G. Smith. 1992. Seasonal dynamics of biomass and mineral nutrient partitioning in mature kiwifruit vines. *Ann. Bot.* 70(3):229-237.

- Hou, X. and B.T. Jones. 2000. Inductively coupled plasma/optical emission spectrometry, p. 9468–9485. In: R.A. Meyers (Ed.). Encyclopedia of analytical chemistry. John Wiley & Sons Ltd Chichester.
- Kangueehi, G.N., P. Stassen, K. Theron, and J. Wooldridge. 2011. Macro and micro element requirements of young and bearing apple trees under drip fertigation: short communications. S. Afr. J. of Pl. and Soil 28(2):136-141.
- Kotze, W. and J. De Villiers. 1989a. Seasonal uptake and distribution of nutrient elements by kiwifruit vines 1. Macronutrients. S. Afr. J. of Pl. and Soil 6(4):256-264.
- Kotze, W. and J. de Villiers. 1989b. Seasonal uptake and distribution of nutrient elements by kiwifruit vines 2. Micronutrients. S.Afr. J. of Pl. and Soil 6(4):265-270.
- Kramer and Kozlowski, 1979. Physiology of woody plants. Academic Press. Inc., N.Y.
- Rose, M.A. 1999. Nutrient use patterns in woody perennials: implications for increasing fertilizer efficiency in field-grown and landscape ornamentals. HortTechnology 9(4):613-617.
- Rosecrance, R.C., S.A. Weinbaum, and P.H. Brown. 1996. Assessment of nitrogen, phosphorus, and potassium uptake capacity and root growth in mature alternate-bearing pistachio (*Pistacia vera*) trees. Tree Physiol. 16(11\_12):949-956.
- Smagula, J. and L. Kreider. 2008. Effects of timing of N and phi foliar sprays on lowbush blueberry leaf nutrient concentrations, growth, and yield. Acta Hort. 810:733-740.
- Spiers, J.M. and D.A. Marshall. 2012. Macronutrient distribution in ‘Tifblue’ rabbiteye blueberry. Int. J. Fruit Sci. 12(1-3):48-53.

- Stassen, P.J.C., M. Du Preez, and J.D. Stadler. 1983. Reserves in full-bearing peach trees. *Dec. Fr. Grower* 33:200-206.
- Stassen, P. and J. Stadler. 1988. Seasonal uptake of phosphorus, potassium, calcium and magnesium by young peach trees. *S. Afr. J. of Pl. and Soil* 5(1):19-23.
- Stassen, P. and M. North. 2004. Nutrient distribution and requirement of 'Forelle' pear trees on two rootstocks. *Acta Hort.* 671:493-500.
- Terblanche, J.H. 1972. Seisoensopname en verspreiding van tien voedingselemente by jong appelbome gekweek in sandkultuur. Stellenbosch Univ. PhD. Diss.
- Throop, P.A. and E.J. Hanson. 1997. Effect of application date on absorption of  $^{15}\text{N}$  by highbush blueberry. *J. Am. Soc. Hort. Sci.* 122(3):422-426.
- Yadong, L., Z. Shuang, D. HanPing, G. XiuWu, K. Hummer, B. Strik, and C. Finn. 2009. Effects of nitrogen, phosphorus and potassium on growth, fruit production and leaf physiology in blueberry. *Acta Hort.* 810:759-764.

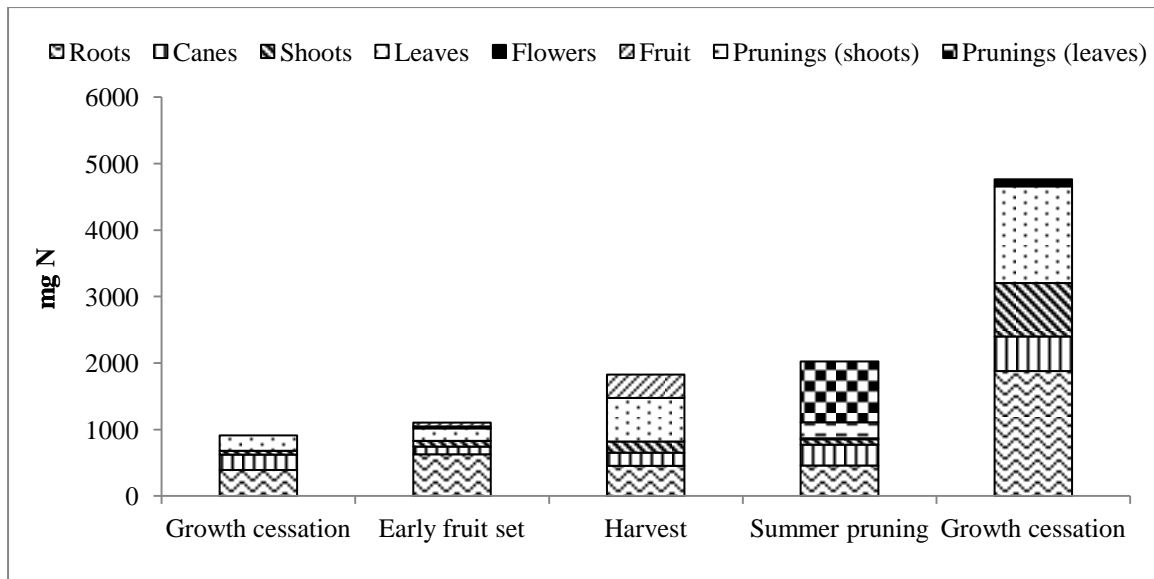


Fig. 1. Nitrogen (N) content of 'Emerald' southern highbush blueberry at five phenological stages during a 12 month period. Growth cessation occurred on 21 May 2013, early fruit set occurred on 21 Aug. 2013, harvest started 19 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

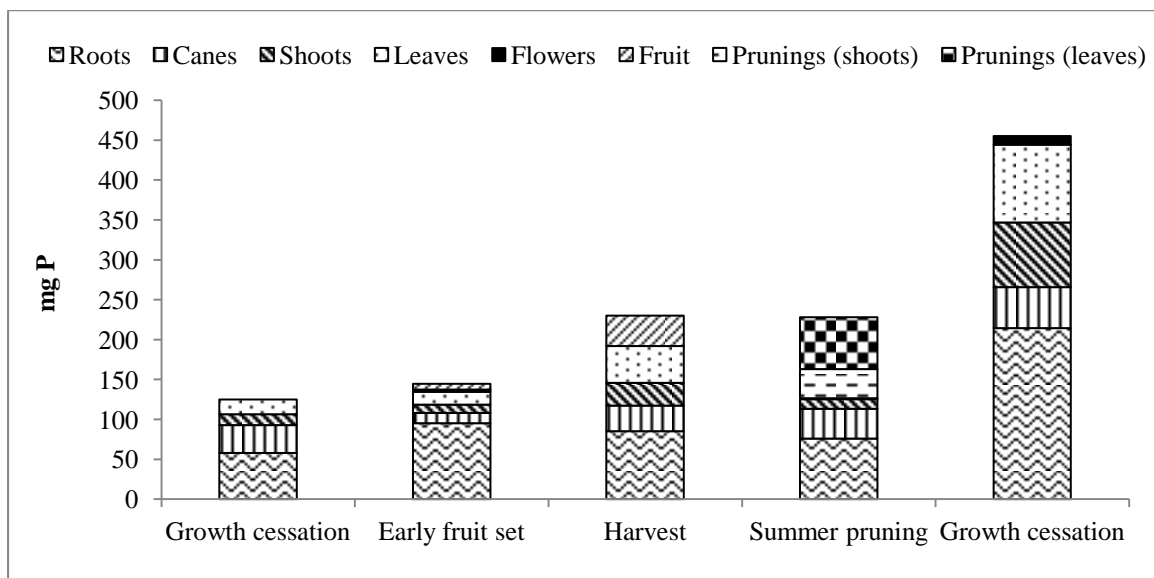


Fig. 2. Phosphorus (P) content of 'Emerald' southern highbush blueberry at five stages during a 12 month period. Growth cessation occurred on 21 May 2013, early fruit set occurred on 21 Aug. 2013, harvest started 19 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

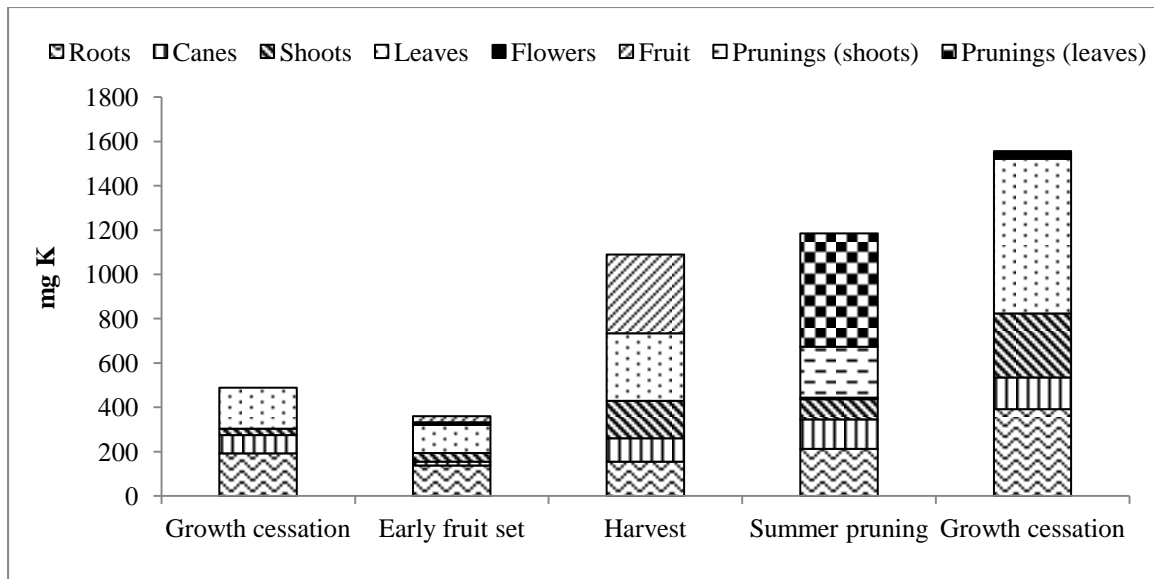


Fig. 3. Potassium (K) content of 'Emerald' southern highbush blueberry at five stages during a 12 month period. Growth cessation occurred on 21 May 2013, early fruit set occurred on 21 Aug. 2013, harvest started 19 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

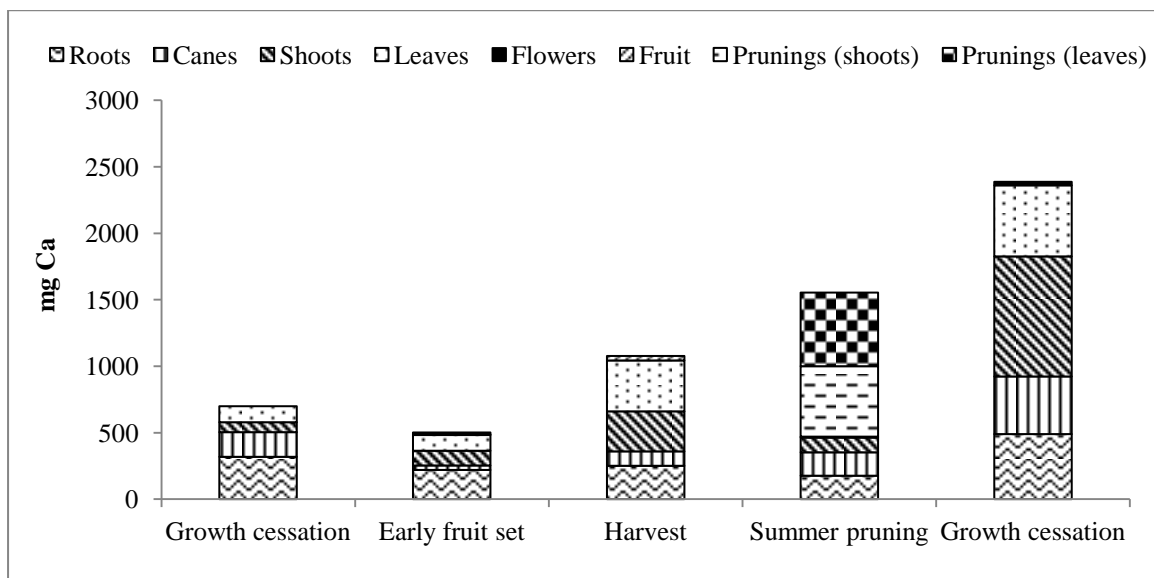


Fig. 4. Calcium (Ca) content of 'Emerald' southern highbush blueberry at five stages during a 12 month period. Growth cessation occurred on 21 May 2013, early fruit set occurred on 21 Aug. 2013, harvest started 19 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

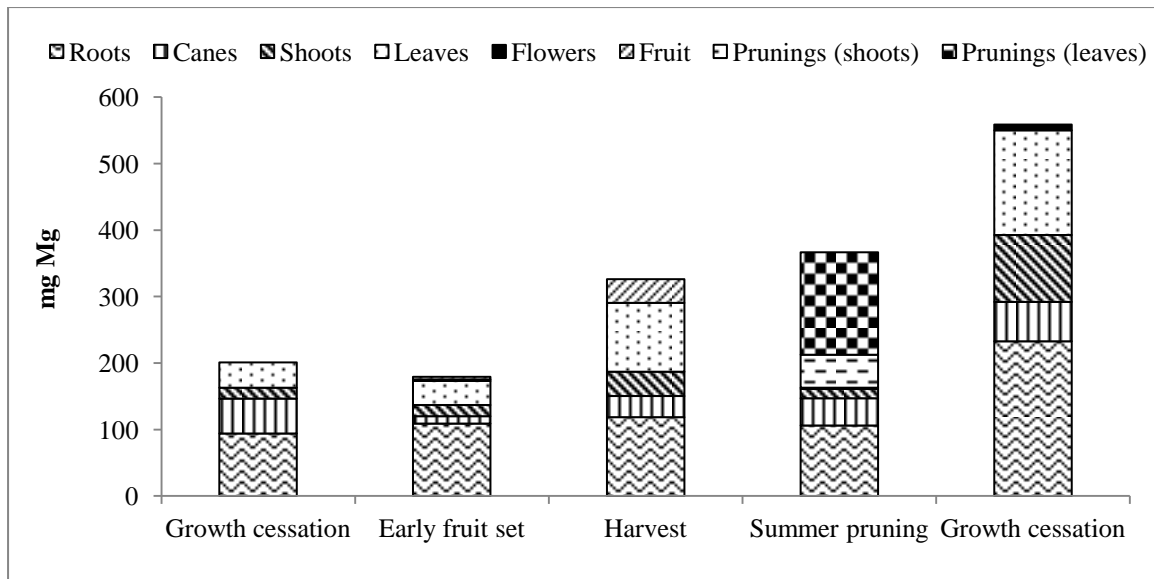


Fig. 5. Magnesium (Mg) content of 'Emerald' southern highbush blueberry at five stages during a 12 month period. Growth cessation occurred on 21 May 2013, early fruit set occurred on 21 Aug. 2013, harvest started 19 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

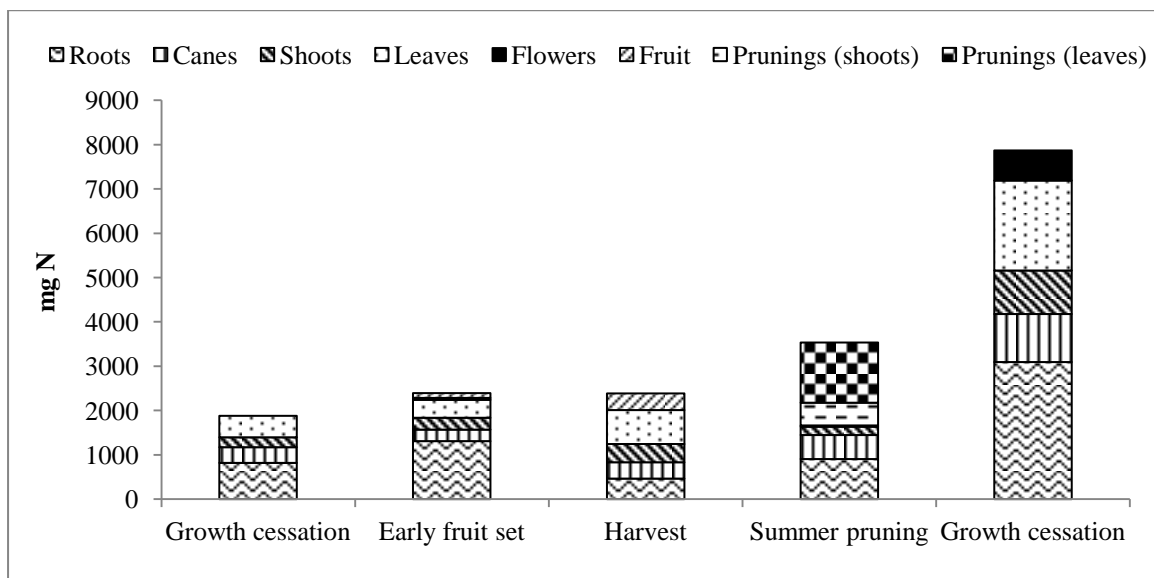


Fig. 6. Nitrogen (N) content of 'Snowchaser' southern highbush blueberry at five stages during a 12 month period. Growth cessation occurred on 21 May 2013, early fruit set occurred on 13 Aug. 2013, harvest started 5 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.



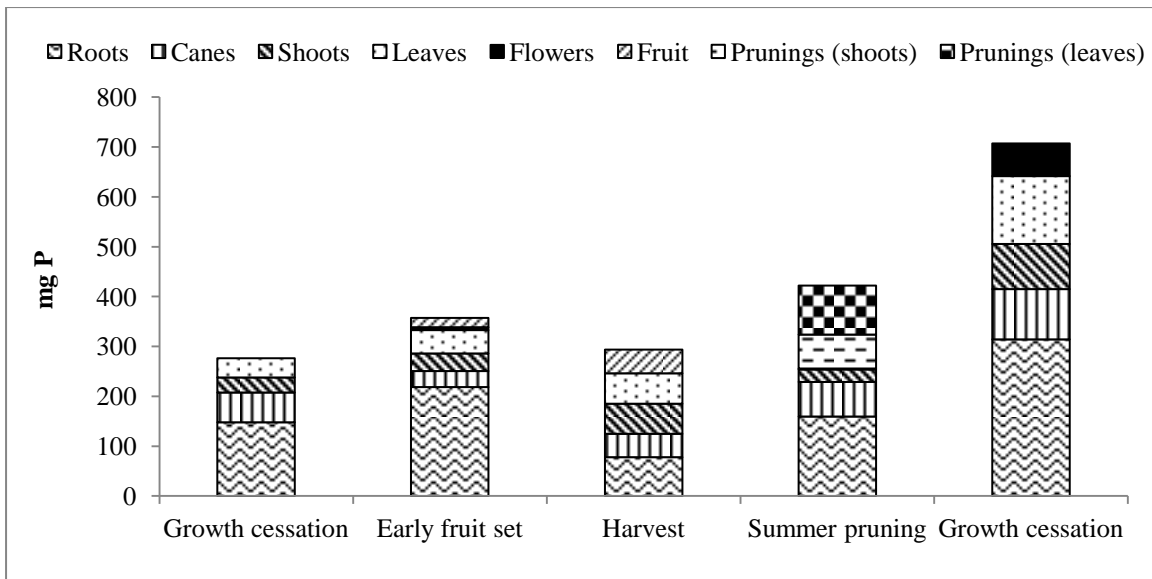


Fig. 7. Phosphorus (P) content of ‘Snowchaser’ southern highbush blueberry at five stages during a 12 month period. Growth cessation occurred on 21 May 2013, early fruit set occurred on 13 Aug. 2013, harvest started 5 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

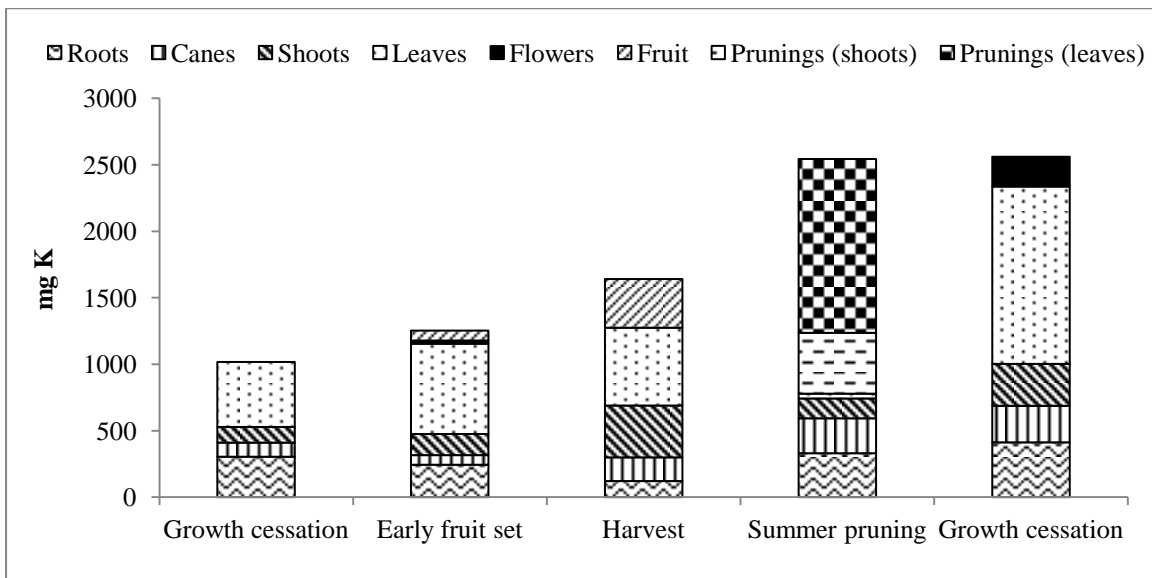


Fig. 8. Potassium (K) content of ‘Snowchaser’ southern highbush blueberry at five stages during a 12 month period. Growth cessation occurred on 21 May 2013, early fruit set occurred on 13 Aug. 2013, harvest started 5 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

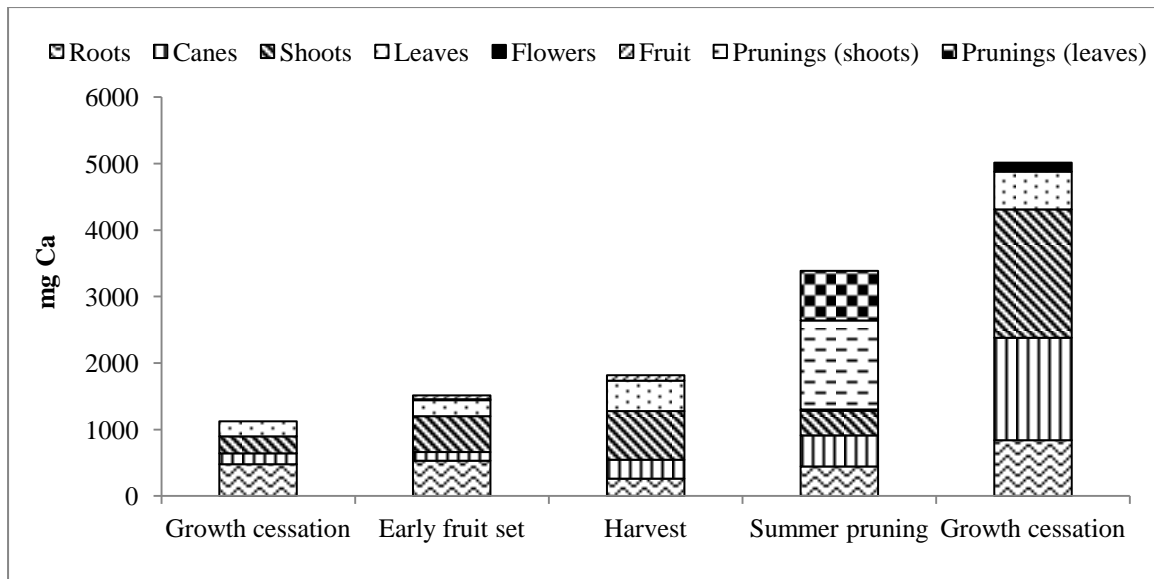


Fig. 9. Calcium (Ca) content of 'Snowchaser' southern highbush blueberry at five stages during a 12 month period. Growth cessation occurred on 21 May 2013, early fruit set occurred on 13 Aug. 2013, harvest started 5 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

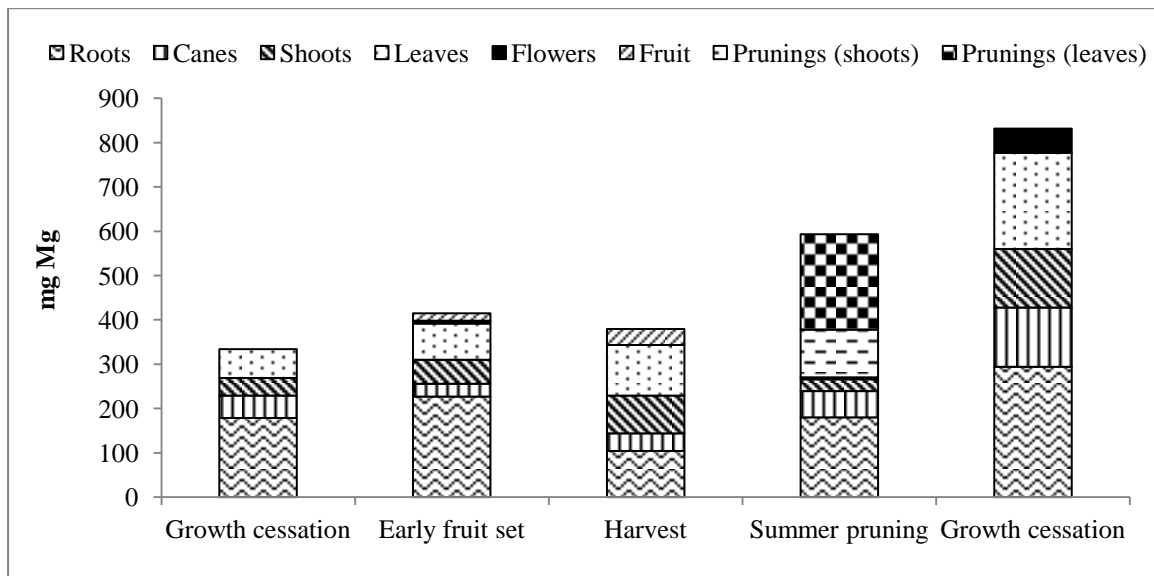


Fig. 10. Magnesium (Mg) content of 'Snowchaser' southern highbush blueberry at five stages during a 12 month period. Growth cessation occurred on 21 May 2013, early fruit occurred on 13 Aug. 2013, harvest started 5 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

Table 1. Macro nutrient uptake in mg/plant and the percentage of total uptake of ‘Emerald’ southern highbush blueberries during the season.

	Growth cessation to early fruit set 21/05-21/08		Early fruit set to harvest 21/08-19/11		Harvest to summer pruning 19/11-20/12		Summer pruning to growth cessation 20/12-21/05		Total
Nitrogen	192.23	4%	720.94	13%	550	10%	3897.1	73%	5360.27
Phosphorus	19.57	4%	85.65	18%	36.06	8%	328.6	70%	469.88
Potassium	0	0%	730.58	32%	450.24	20%	1112.71	49%	2293.53
Calcium	0	0%	574.09	19%	510.33	17%	1917.02	64%	3001.44
Magnesium	0	0%	147.1	24%	76.08	12%	396.15	64%	619.33

Table 2. Macro nutrient uptake in mg/plant and the percentage of total uptake of ‘Snowchaser’ southern highbush blueberries during the season.

	Growth cessation to early fruit set 21/05-13/08		Early fruit set to harvest 13/08-05/11		Harvest to summer pruning 05/11-20/12		Summer pruning to growth cessation 20/12-21/05		Total
Nitrogen	514.26	6%	0	0%	1520.26	18%	6204.97	75%	8239.49
Phosphorus	80.63	11%	0	0%	176.18	25%	451.01	64%	707.82
Potassium	237.29	6%	386.56	11%	1267.73	35%	1782.99	49%	3674.57
Calcium	391.35	6%	302.87	5%	1651.90	27%	3712.39	61%	6058.51
Magnesium	81.02	9%	0	0%	250.39	28%	561.32	63%	892.73

Table 3. Nutrients lost by pruning and harvesting of ‘Emerald’ southern highbush blueberries and subsequent requirements for plants spaced at 2.5 x 1 m (4000 plants/ha).

Nutrient	Nutrient losses		Fertilizer recommendations	
	Harvest (mg/100g fruit)	Prunings (mg/100g FW)	Nutrient requirement after harvest of 1 ton fruit (kg/ha)	Nutrient requirement after pruning 1 ton FW (kg/ha)
Nitrogen	138.83	491.92	1.39	4.92
Phosphorus	15.09	43.2	0.15	0.43
Potassium	140.05	315.61	1.4	3.16
Calcium	13.06	461.81	0.13	4.62
Magnesium	14	86.82	0.14	0.87

FW = fresh weight

Table 4. Nutrients lost by pruning and harvesting of ‘Snowchaser’ southern highbush blueberries and subsequent requirements for plants spaced at 2.5 x 1 m (4000 plants/ha).

Nutrient	Nutrient losses		Fertilizer recommendations	
	Harvest (mg/100g fruit)	Prunings (mg/100g FW)	Nutrient requirement after harvest of 1 ton fruit (kg/ha)	Nutrient requirement after pruning 1 ton FW (kg/ha)
Nitrogen	160.61	485.54	1.61	4.86
Phosphorus	20.68	43.2	0.21	0.43
Potassium	157.77	458.11	1.58	4.58
Calcium	34.73	541.55	0.35	5.42
Magnesium	15.78	83.97	0.16	0.84

FW = fresh weight

Table 5. Macro nutrient requirements of ‘Emerald’ southern highbush blueberries spaced at 2.5 x 1 m (4000 plants/ha) during the season.

	Growth cessation to early fruit set (kg)	Early fruit set to harvest (kg)	Harvest to summer pruning (kg)	Summer pruning to growth cessation (kg)	Total (kg)
Nitrogen	0.77	2.88	2.20	15.59	21.44
Phosphorus	0.08	0.34	0.14	1.31	1.88
Potassium	0.00	2.92	1.80	4.45	9.17
Calcium	0.00	2.30	2.04	7.67	12.01
Magnesium	0.00	0.59	0.30	1.58	2.48

Table 6. Macro nutrient requirements of ‘Snowchaser’ southern highbush blueberries spaced at 2.5 x 1 m (4000 plants/ha) during the season.

	Growth cessation to early fruit set (kg)	Early fruit set to harvest (kg)	Harvest to summer pruning (kg)	Summer pruning to growth cessation (kg)	Total (kg)
Nitrogen	2.06	0.00	6.08	24.82	32.96
Phosphorus	0.32	0.00	0.70	1.80	2.83
Potassium	0.95	1.55	5.07	7.13	14.70
Calcium	1.57	1.21	6.61	14.85	24.23
Magnesium	0.32	0.00	1.00	2.25	3.57

## GENERAL DISCUSSION AND CONCLUSIONS

The climatic conditions under 20% white net in the Paarl district in the Western Cape of South Africa induced the southern highbush (SHB) blueberry cultivars Emerald and Snowchaser to remain evergreen, even though these blueberries are normally deciduous plants when cultivated in Mediterranean-type climates. This was probably mainly due to the higher temperatures experienced in winter under net. The carbohydrate and nutrient patterns of these plants were therefore unlike any other previously reported for blueberry plants.

Both cultivars accumulated reserves for spring growth in the first season. In the second season, however, a build-up of reserves did not occur possibly due to adequate photosynthates produced by current season growth. At the beginning of sampling, plants were very small, with a small leaf area and were possibly still adjusting after being replanted and therefore growth was slow. Plants, therefore, were not able to supply enough carbohydrates from current photosynthesis and depended on reserves for new growth. In the second season plants were significantly bigger and therefore had higher total leaf area and the temperature was favorable for optimal photosynthesis and therefore carbohydrates from current photosynthesis were enough to supply new growth, making reserves less important. It could also be that carbohydrates were used to increase flower bud development in the second season instead of being stored as reserves.

Although small differences between the two cultivars existed, the overall carbohydrate pattern of 'Snowchaser' and 'Emerald' were similar and could thus be an indication of the carbohydrate status of SHB cultivated under net in the Paarl district of South Africa. The maintenance of healthy leaves that photosynthesize is of absolute importance in an evergreening system, as SHB in such a system depend mainly on carbohydrates derived from photosynthesis rather than from reserves. Although we did not determine this, one could

expect to see faster growth when plants utilize both reserves and new photosynthates for growth in spring, than when they are more dependent on reserves. This emphasizes the importance of adequate water and N fertilizer throughout the season as well as pest and disease management to ensure healthy leaves that can photosynthesize.

The nutrient concentration patterns in the root, shoot and leaf tissues were variable and showed huge fluctuations over the period of sampling in both cultivars. This could have been a result of irregular fertigation and therefore it is uncertain whether flushes in nutrient uptake was a result of higher nutrient demand by the plant. Rapid accumulation of macro nutrients, however, was evident when plants were rapidly growing after summer pruning. Small amounts of nutrients were reallocated between different plant parts throughout the season and larger amounts of nutrients were reallocated at full bloom, indicating a large nutrient demand by flowers.

Nutrient reserves did not accumulate before reproductive budbreak in the first season, but nutrient accumulation before reproductive budbreak was apparent in the second season. Nutrient accumulation in the second season accompanied dry weight (DW) accumulation, indicating that nutrient uptake increases as dry weight increases. Cultivars differed in their remobilization patterns and different nutrients were remobilized at full bloom from different plant tissues. Fertilization is especially important during rapid growth and the maintenance of a proper, functioning fertigation system during the season is important to supply the plant of its nutrient requirements throughout the season.

Nutrient losses due to harvest and pruning should be corrected by applying the right amounts of fertilizer. In Paper 3 fertilizer recommendations for the whole season were made and guidelines for correcting nutrient losses due to harvest and pruning were given. Differences in nutrient uptake were observed between cultivars and could not be explained by the difference

in phenology or uptake periods. ‘Emerald’ plants were overall smaller than ‘Snowchaser’ and the growth rate of ‘Snowchaser’ was higher than ‘Emerald’, possibly explaining why ‘Snowchaser’ accumulated more nutrients during the season.

Fertilizers should be applied according to the uptake rates of the different cultivars during the different growth stages, but this is not practical where different cultivars are planted in the same fertigation block. Fertilizer recommendations per ha for ‘Emerald’ and ‘Snowchaser’ were not very different and therefore the higher rate, which was for ‘Snowchaser’, can be applied. This would lead to ‘Snowchaser’ plants receiving optimum fertilizer, while ‘Emerald’ will receive somewhat more than necessary, but this is not enough to cause toxicity, because otherwise symptoms would have been visible as all plants received the same amount of fertilizer during these trials. It is also possible to fertilize according to the lower demand and then applying foliar or granular fertilizers to the plants with the higher nutrient demand, thus overcoming overfertilization of the one cultivar. Fertilizer rates used during this trial were sufficient to correct losses by harvest and pruning as well as increase growth, but it is uncertain whether increasing fertilizer rates would result in even higher quality plants in terms of vegetative growth, crop load and fruit quality.

This study only looked at one growing system, an ever-greening system under net and therefore recommendations cannot be made for any other system as plant responses would be different. Research on carbohydrate and nutrient patterns of blueberries grown under open field conditions need to be studied and different climatic areas could be included as blueberries might be deciduous under different circumstances.

In addition to this work the uptake and partitioning of a certain element such as nitrogen, could also be determined by applying  $^{15}\text{N}$  fertilizer and then determining the amount of  $^{15}\text{N}$  uptake and the allocation to the different plant tissues. Because the uptake of nutrients is



highly dependent on the pH of the soil, it would greatly benefit nutrient uptake studies to regularly take pH measurements of the soil.

## **APPENDIX A**

The micro nutrient concentration and content was determined for the same plants used in Paper 1 and 2 and the materials and methods described in Paper 2 (p. 75) also applies to all samples used to determine the results in this appendix.

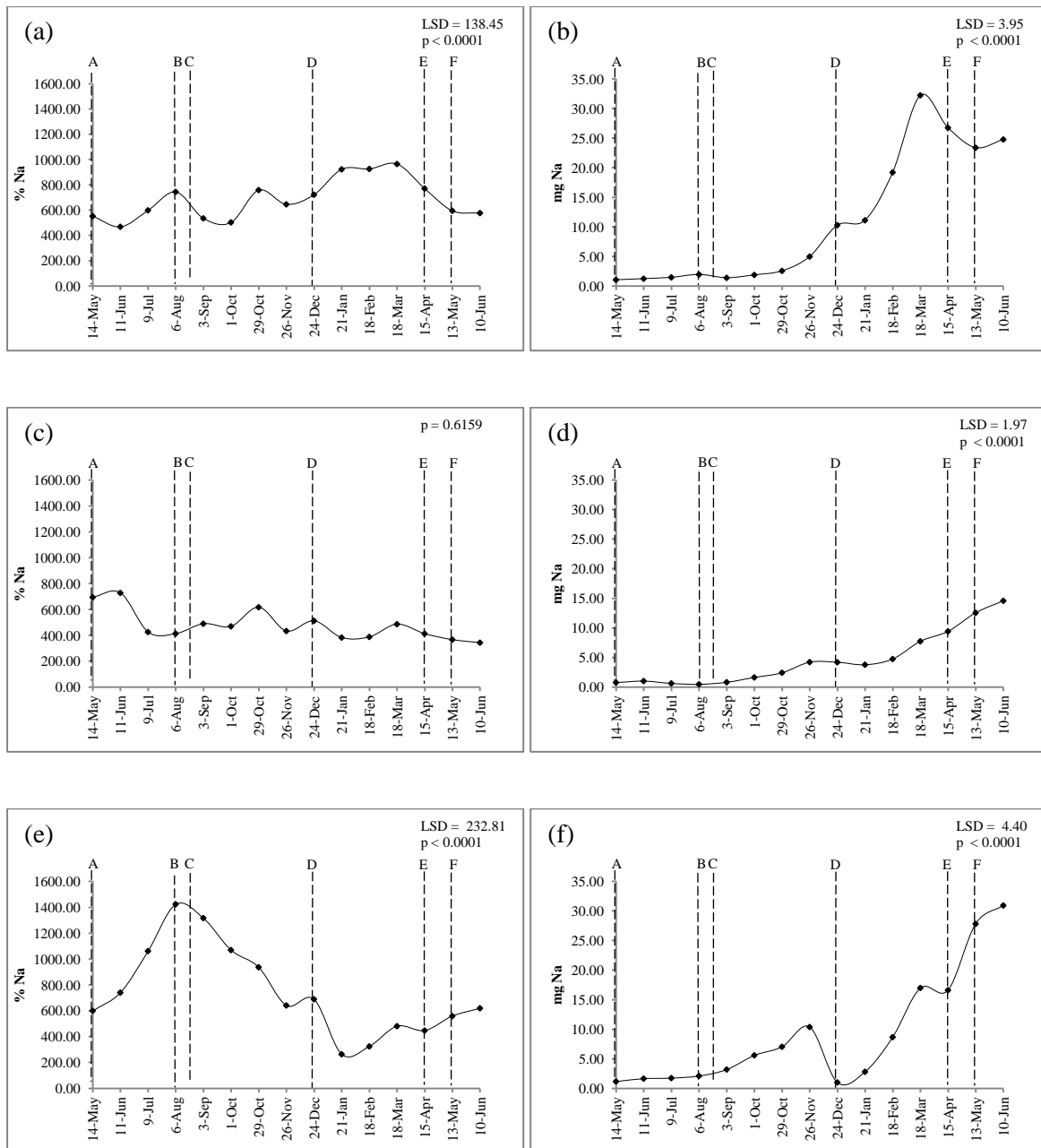


Fig. 1. Sodium (Na) concentration and content in the roots, shoots and leaves of southern highbush blueberry 'Emerald' from 14 May 2013 to 10 June 2014. (a) is root Mg concentration, (b) root Na content, (c) shoot Na concentration, (d) shoot Na content, (e) leaf Na concentration and (f) leaf Na content. A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom.

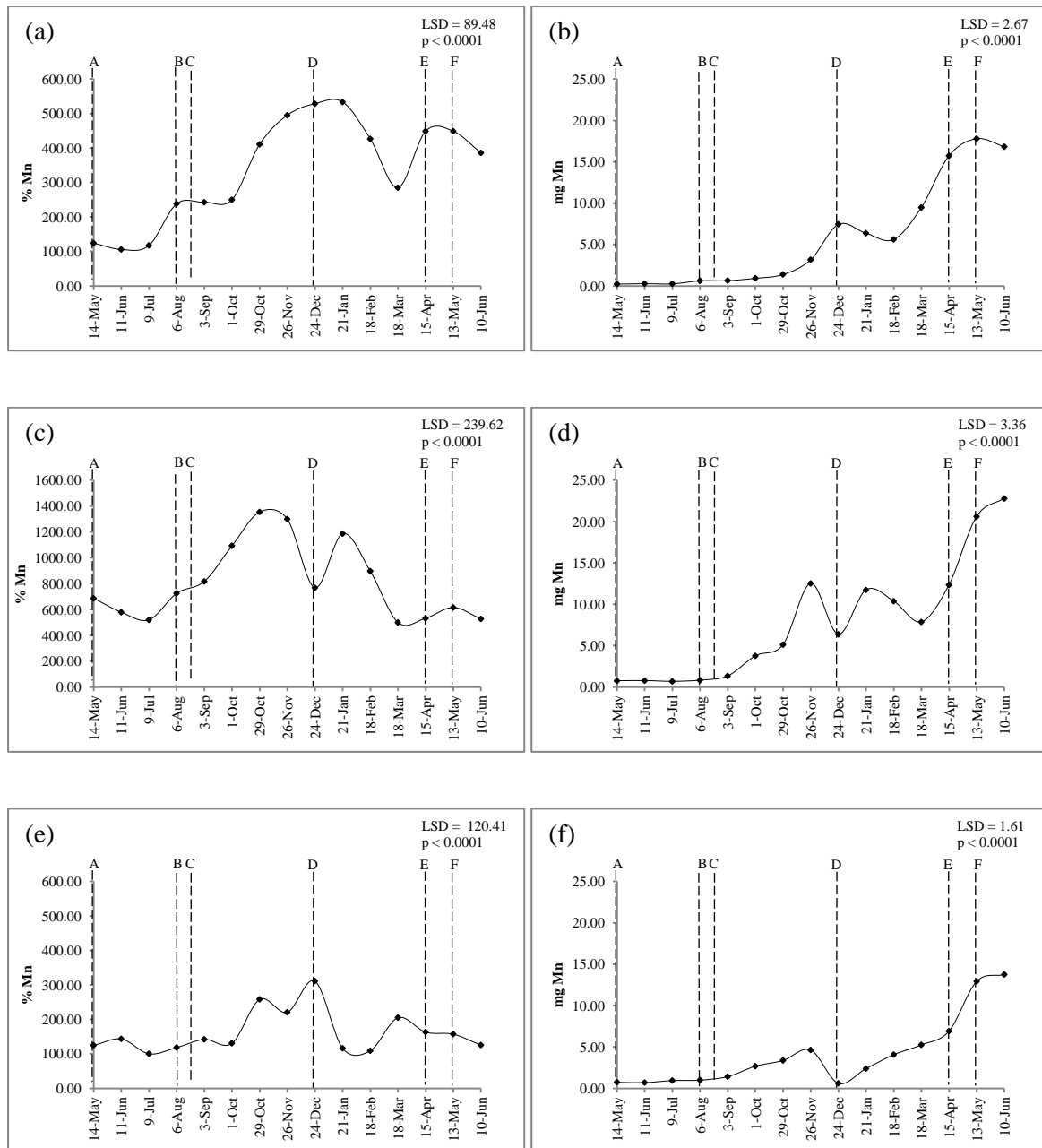


Fig. 2. Manganese (Mn) concentration and content in the roots, shoots and leaves of southern highbush blueberry 'Emerald' from 14 May 2013 to 10 June 2014. (a) is root Mn concentration, (b) root Mn content, (c) shoot Mn concentration, (d) shoot Mn content, (e) leaf Mn concentration and (f) leaf Mn content. A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom.

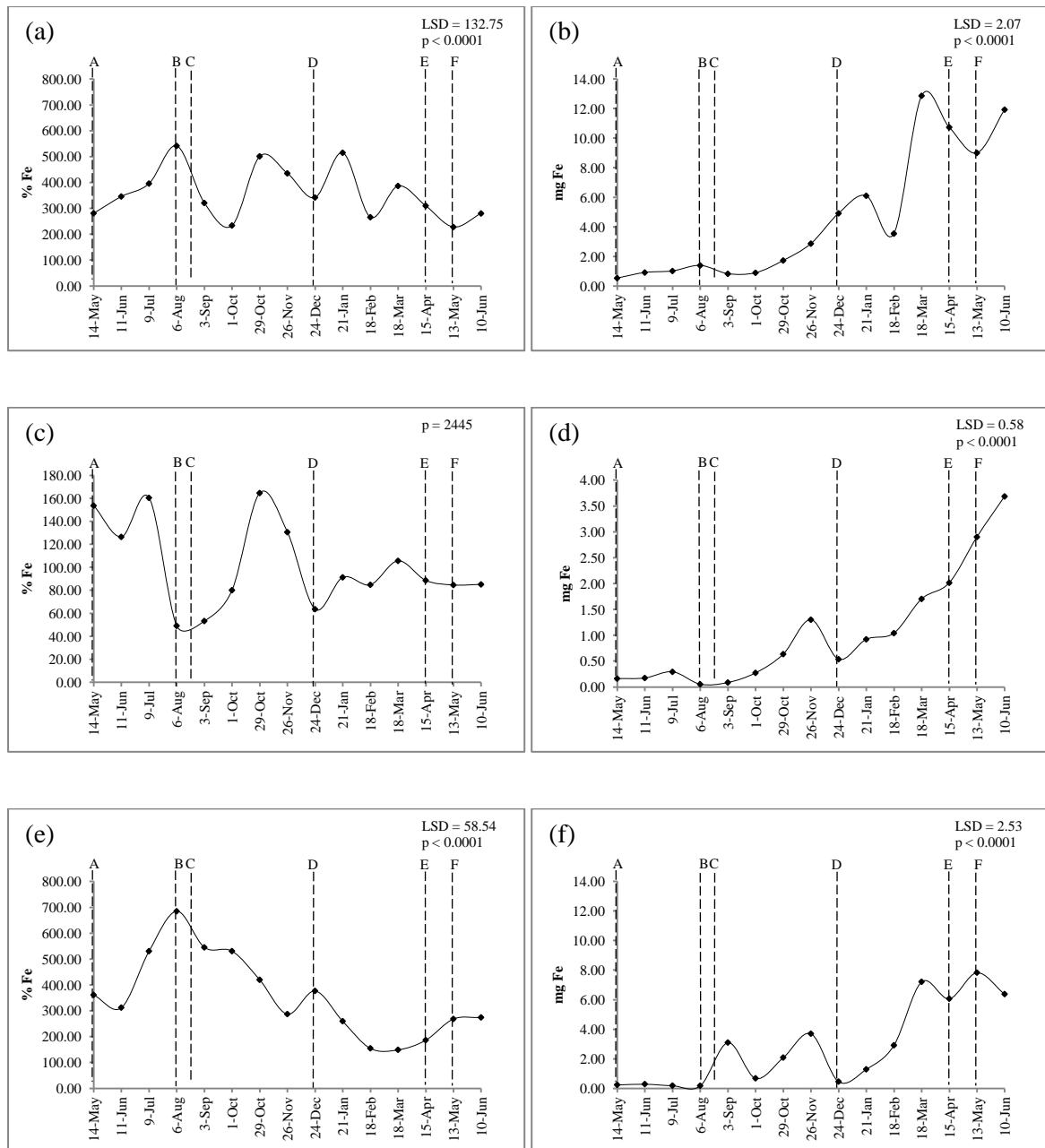


Fig. 3. Iron (Fe) concentration and content in the roots, shoots and leaves of southern highbush blueberry 'Emerald' from 14 May 2013 to 10 June 2014. (a) is root Fe concentration, (b) root Fe content, (c) shoot Fe concentration, (d) shoot Fe content, (e) leaf Fe concentration and (f) leaf Fe content. A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom.

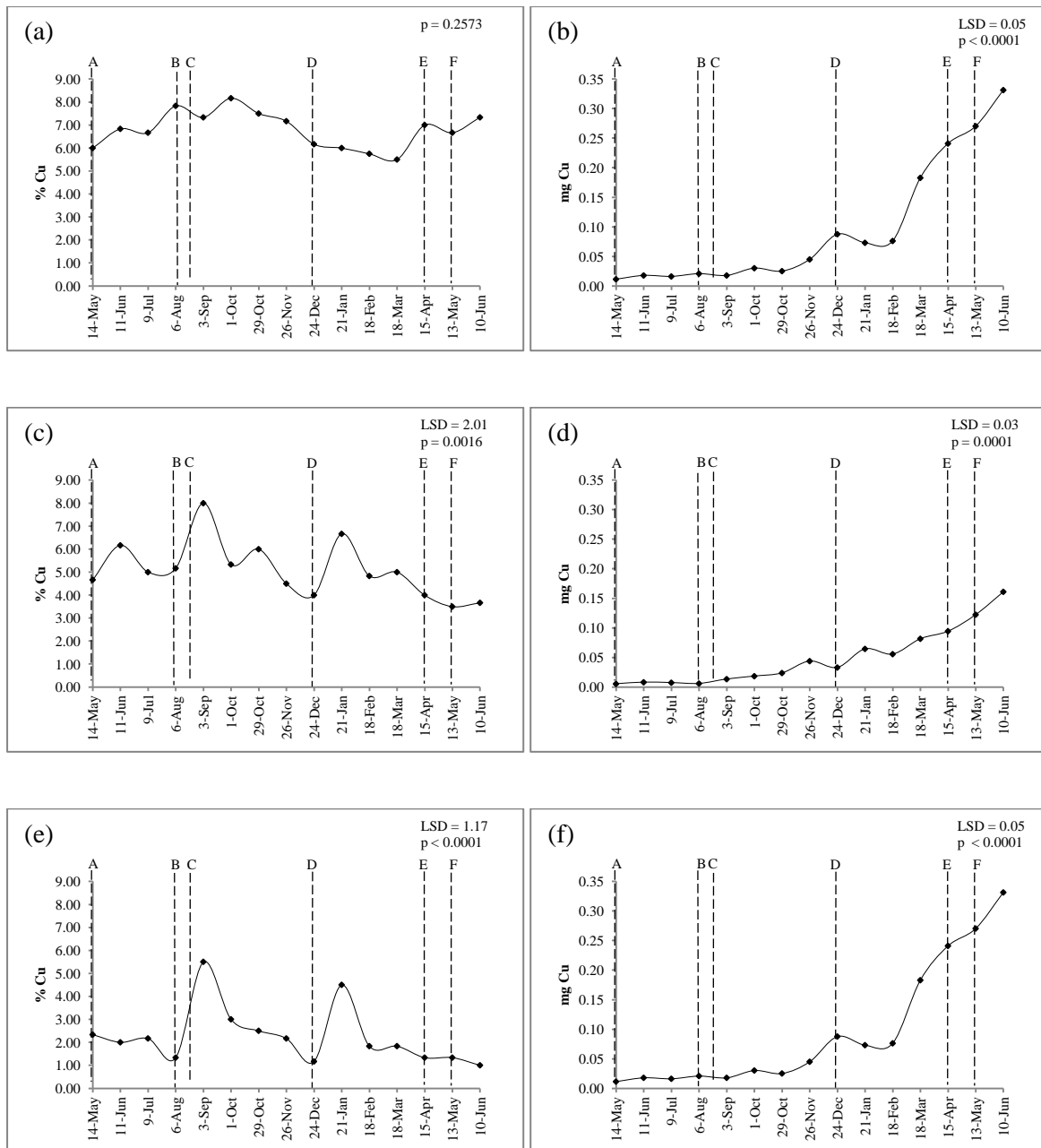


Fig. 4. Copper (Cu) concentration and content in the roots, shoots and leaves of southern highbush blueberry 'Emerald' from 14 May 2013 to 10 June 2014. (a) is root Mg concentration, (b) root Cu content, (c) shoot Cu concentration, (d) shoot Cu content, (e) leaf Cu concentration and (f) leaf Cu content. A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom.

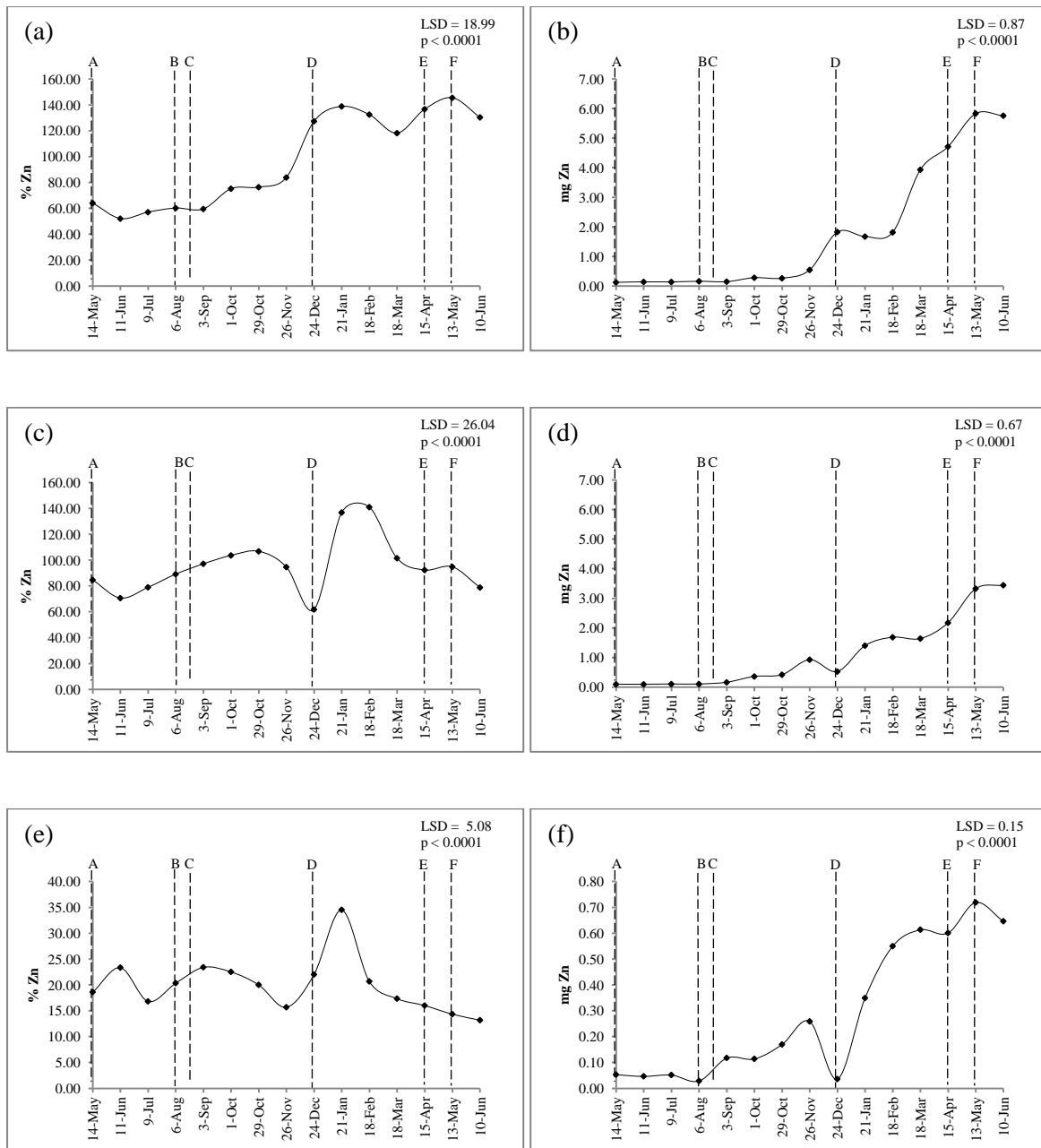


Fig. 5. Zinc (Zn) concentration and content in the roots, shoots and leaves of southern highbush blueberry 'Emerald' from 14 May 2013 to 10 June 2014. (a) is root Zn concentration, (b) root Zn content, (c) shoot Zn concentration, (d) shoot Zn content, (e) leaf Zn concentration and (f) leaf Zn content. A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom.

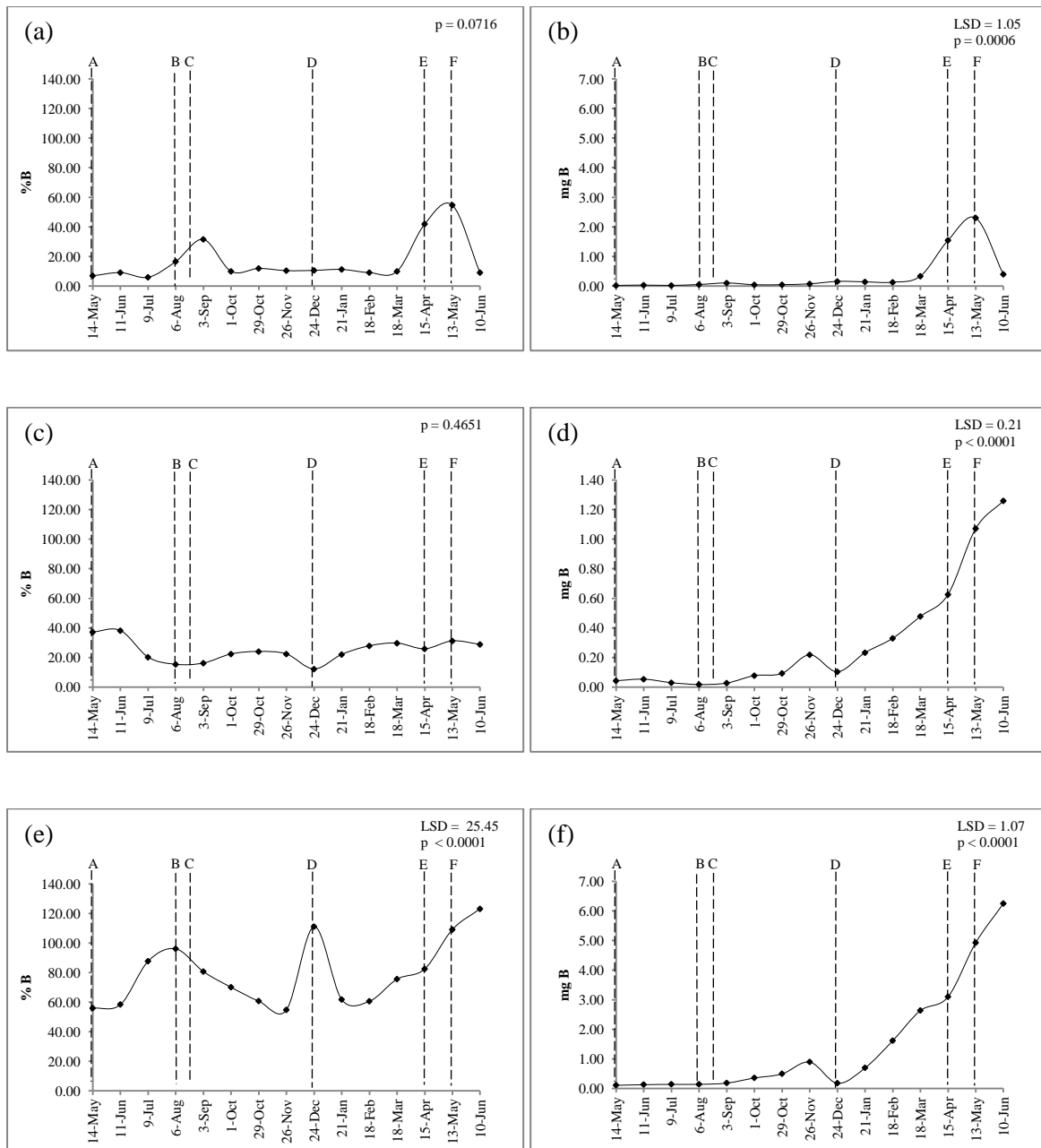


Fig. 6. Boron (B) concentration and content in the roots, shoots and leaves of southern highbush blueberry 'Emerald' from 14 May 2013 to 10 June 2014. (a) is root B concentration, (b) root B content, (c) shoot B concentration, (d) shoot B content, (e) leaf B concentration and (f) leaf B content. A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom.



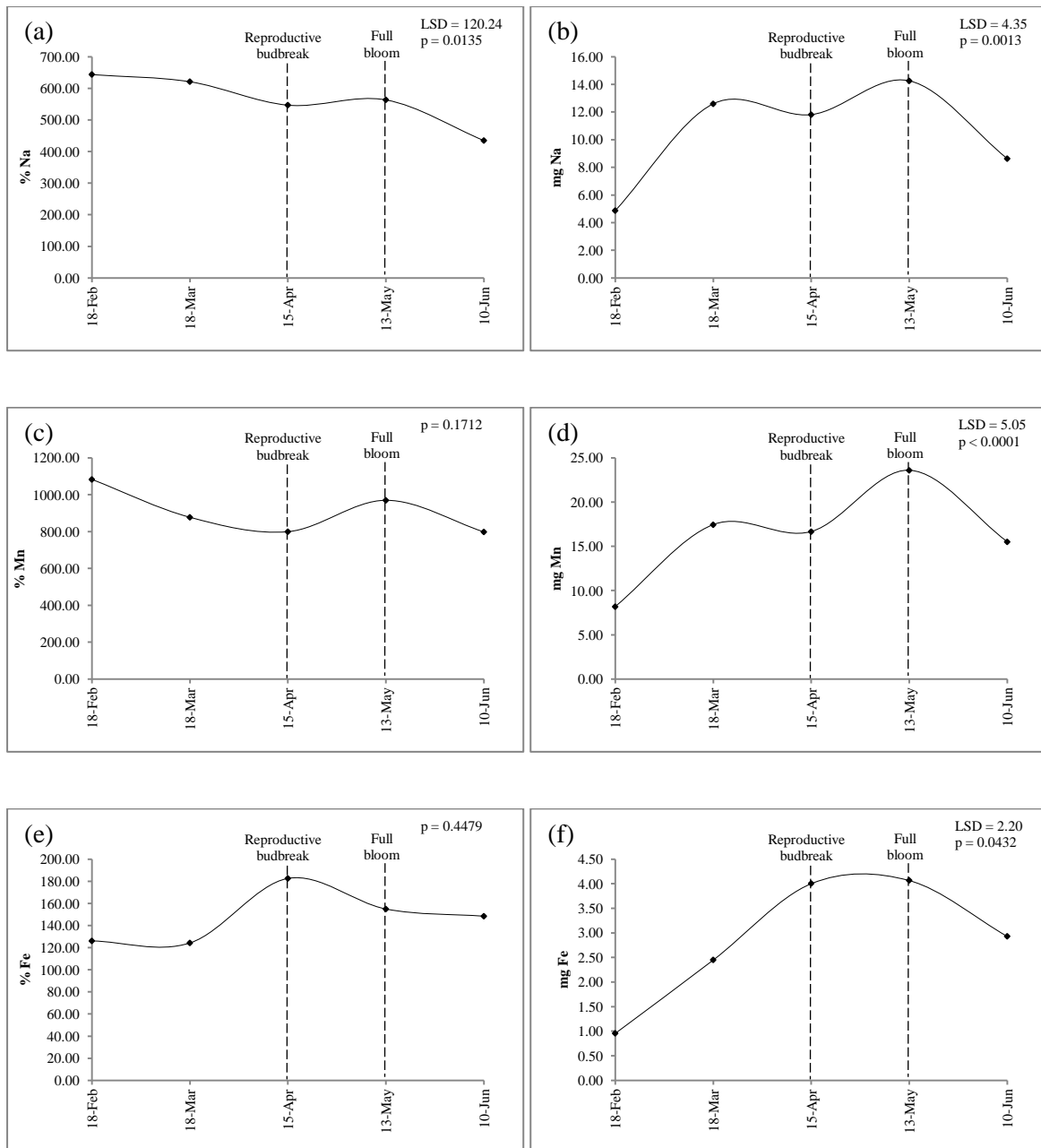


Fig. 7. Micro-element concentration and content in the canes of 'Emerald' southern highbush blueberry in the 2014 season. (a) sodium (Na) concentration, (b) sodium (Na) content, (c) manganese (Mn) concentration, (d) manganese (Mn) content, (e) iron (Fe) concentration, (f) iron (Fe) content.

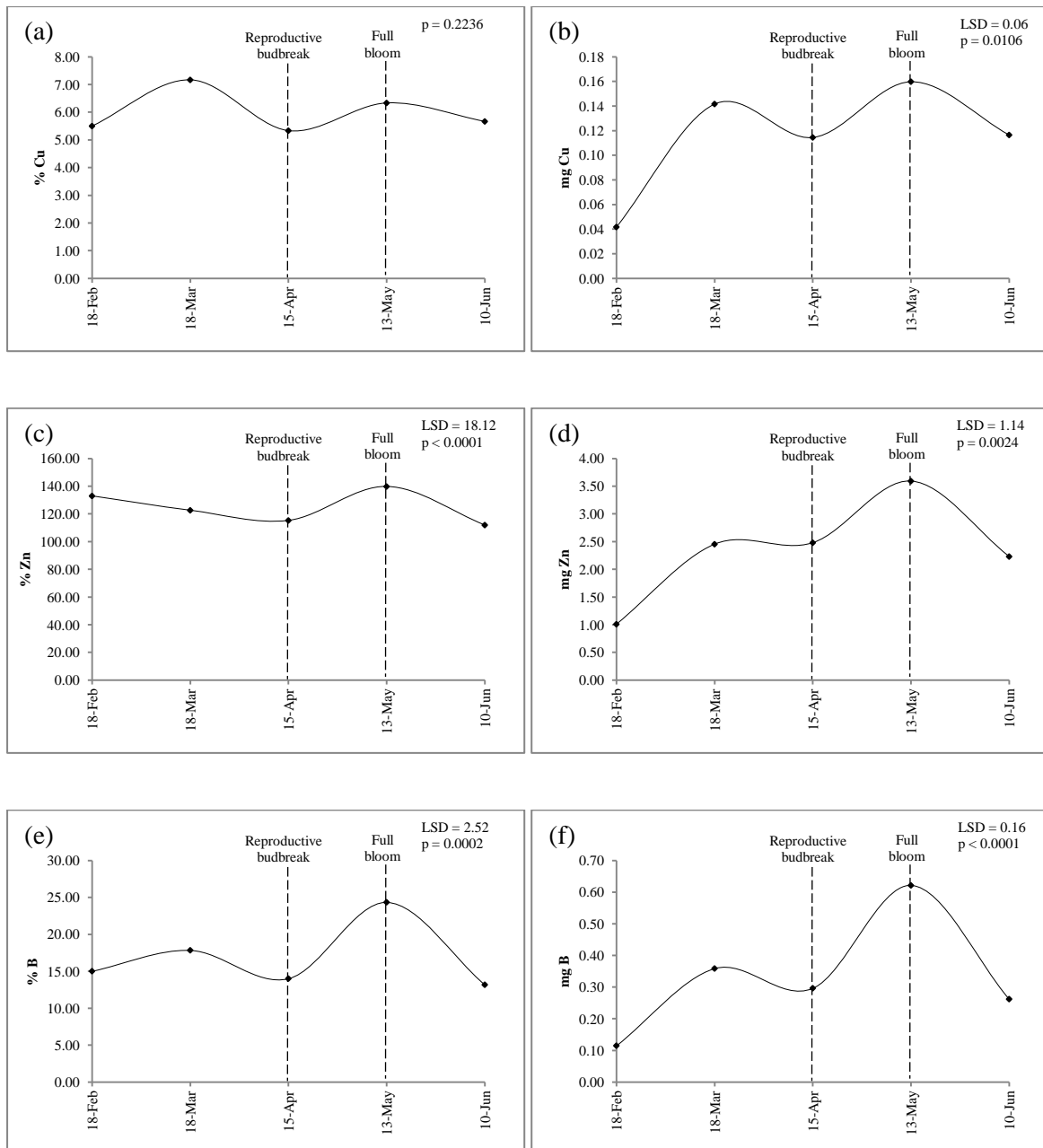


Fig. 8. Micro-element concentration and content in the canes of 'Emerald' southern highbush blueberry in the 2014 season. (a) copper (Cu) concentration, (b) copper (Cu) content, (c) zinc (Zn) concentration, (d) zinc (Zn) content, (e) boron (B) concentration, (f) boron (B) content.

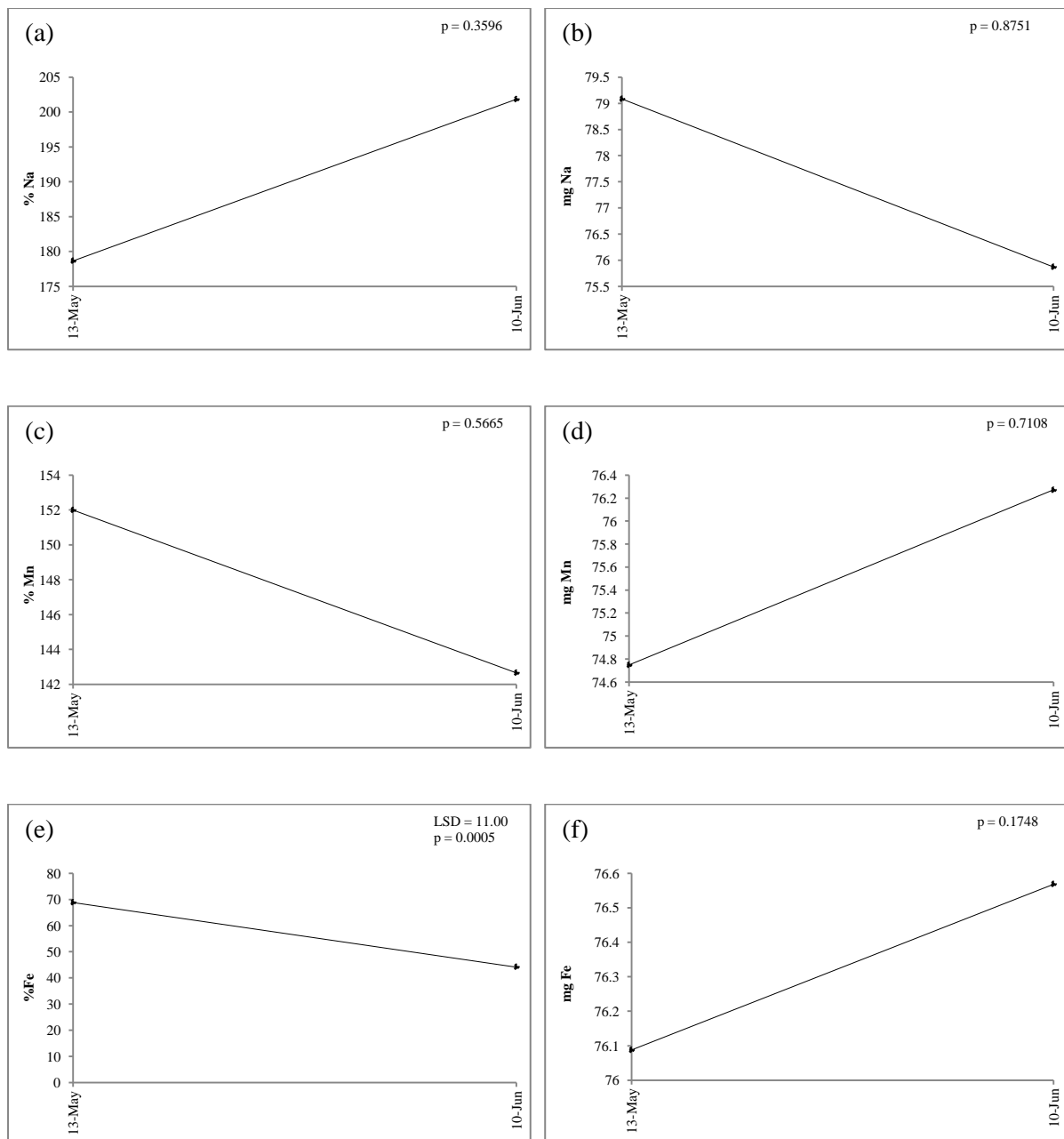


Fig. 9. Micro-element concentration and content in the flowers of 'Emerald' southern highbush blueberry in the 2014 season. (a) sodium (Na) concentration, (b) sodium (Na) content, (c) manganese (Mn) concentration, (d) manganese (Mn) content, (e) iron (Fe) concentration, (f) iron (Fe) content. Full bloom occurred on 13 May.

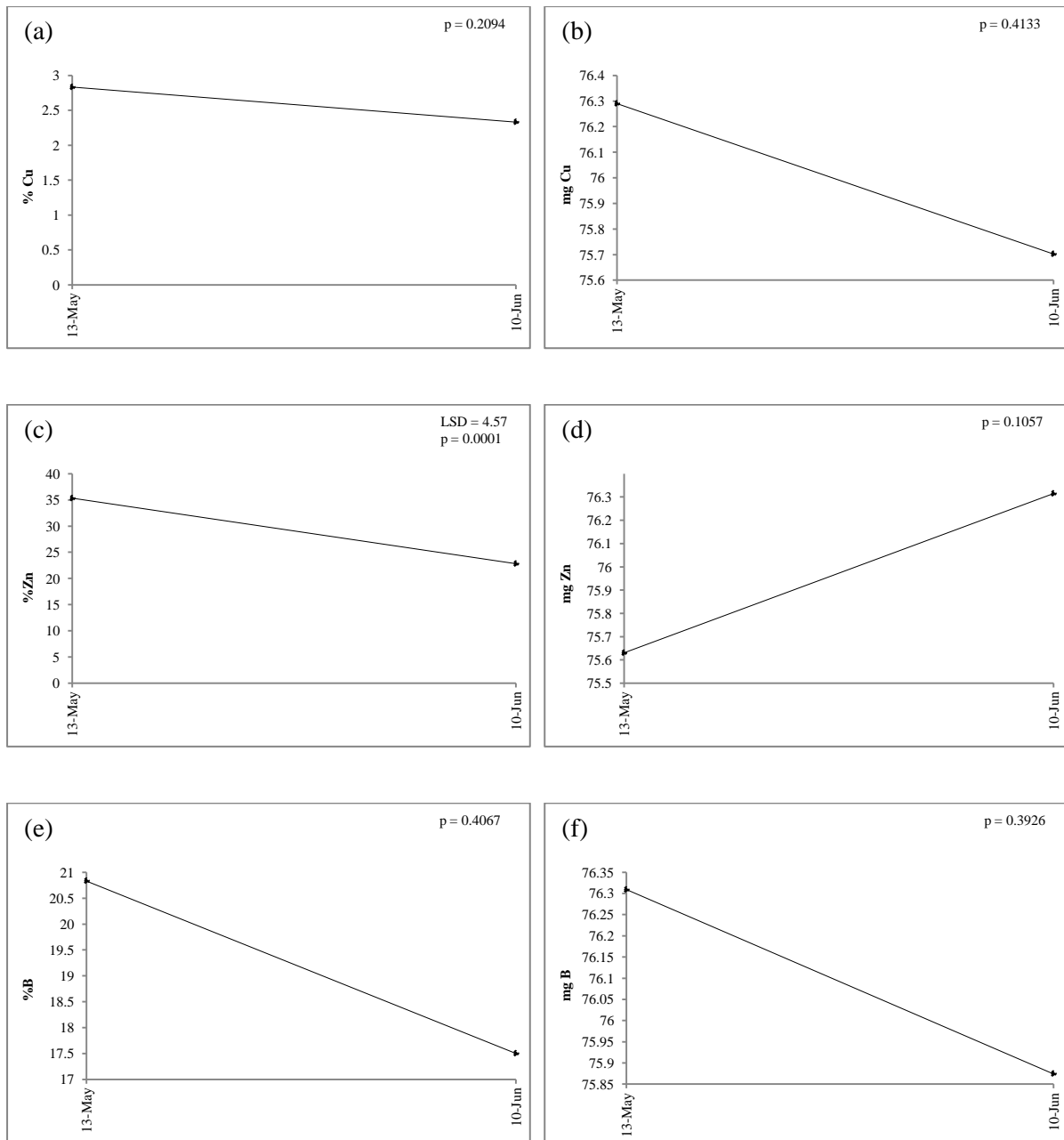


Fig. 10. Micro-element concentration and content in the flowers of 'Emerald' southern highbush blueberry in the 2014 season. (a) copper (Cu) concentration, (b) copper (Cu) content, (c) zinc (Zn) concentration, (d) zinc (Zn) content, (e) boron (B) concentration, (f) boron (B) content. Full bloom occurred on 13 May.

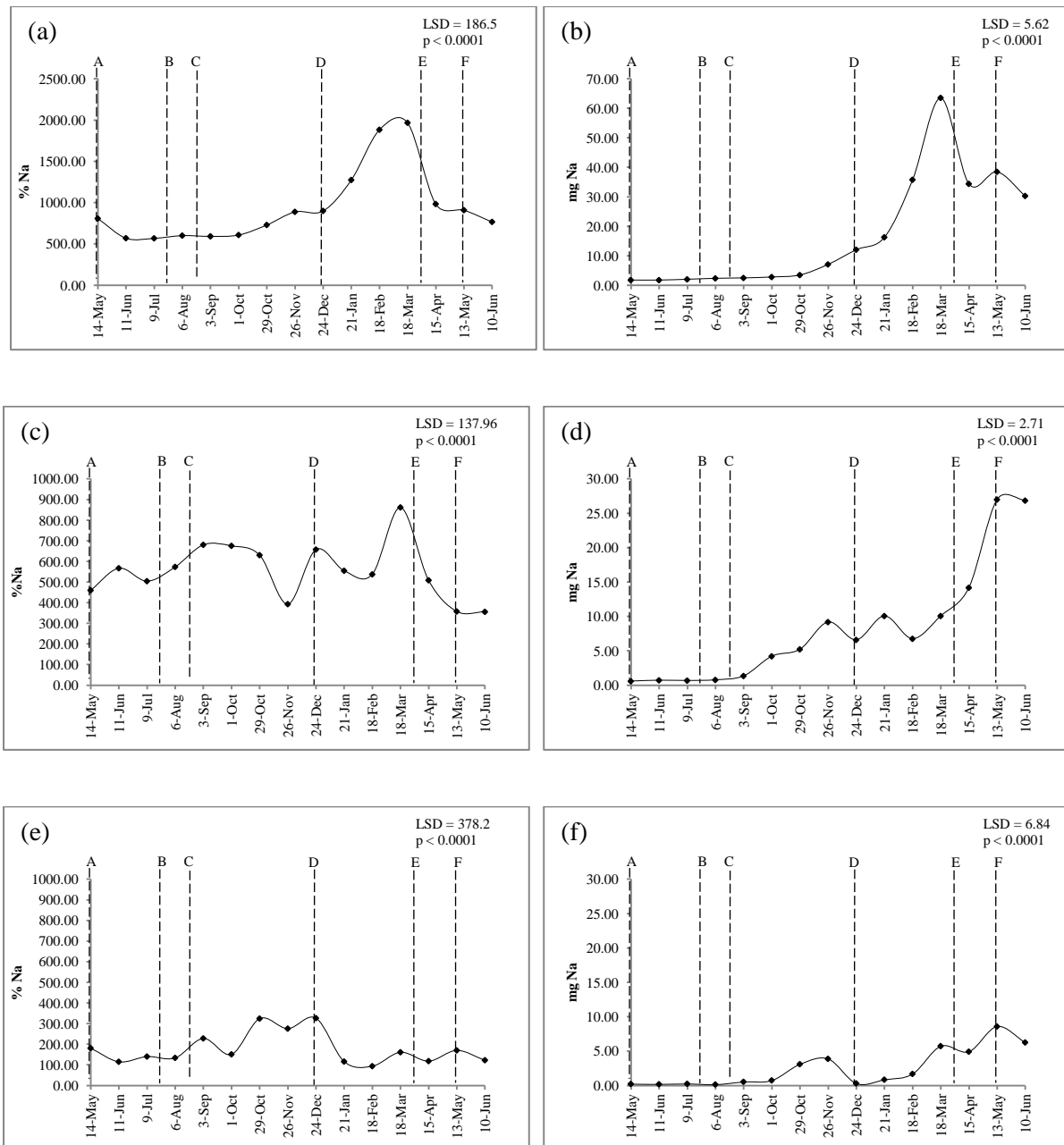


Fig. 11. Sodium (Na) concentration and content in the roots, shoots and leaves of southern highbush blueberry 'Snowchaser' from 14 May 2013 to 10 June 2014. (a) is root Na concentration, (b) root Na content, (c) shoot Na concentration, (d) shoot Na content, (e) leaf Na concentration and (f) leaf Na content. A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom.

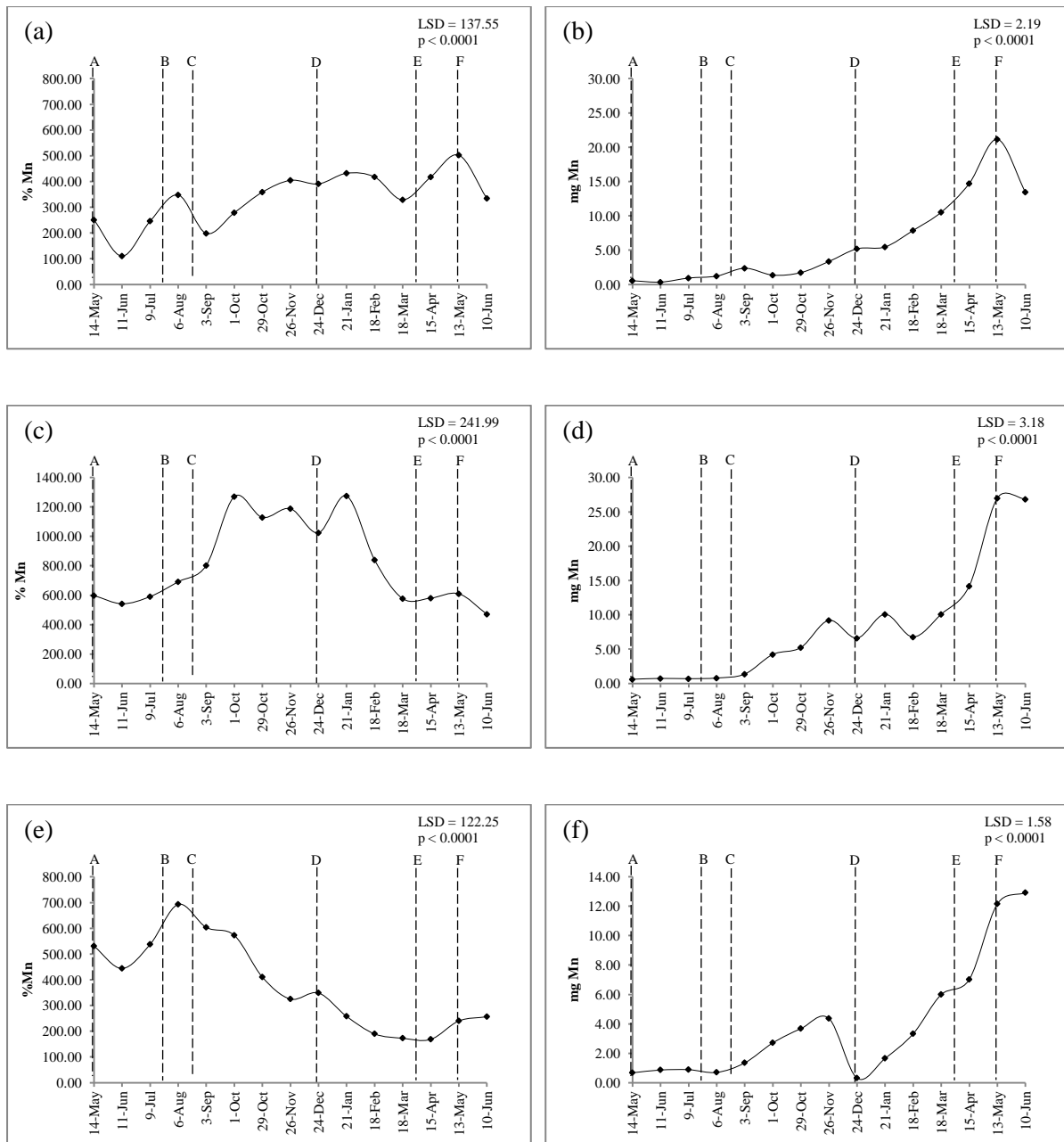


Fig. 12. Manganese (Mn) concentration and content in the roots, shoots and leaves of southern highbush blueberry 'Snowchaser' from 14 May 2013 to 10 June 2014. (a) is root Mn concentration, (b) root Mn content, (c) shoot Mn concentration, (d) shoot Mn content, (e) leaf Mn concentration and (f) leaf Mn content. A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom.

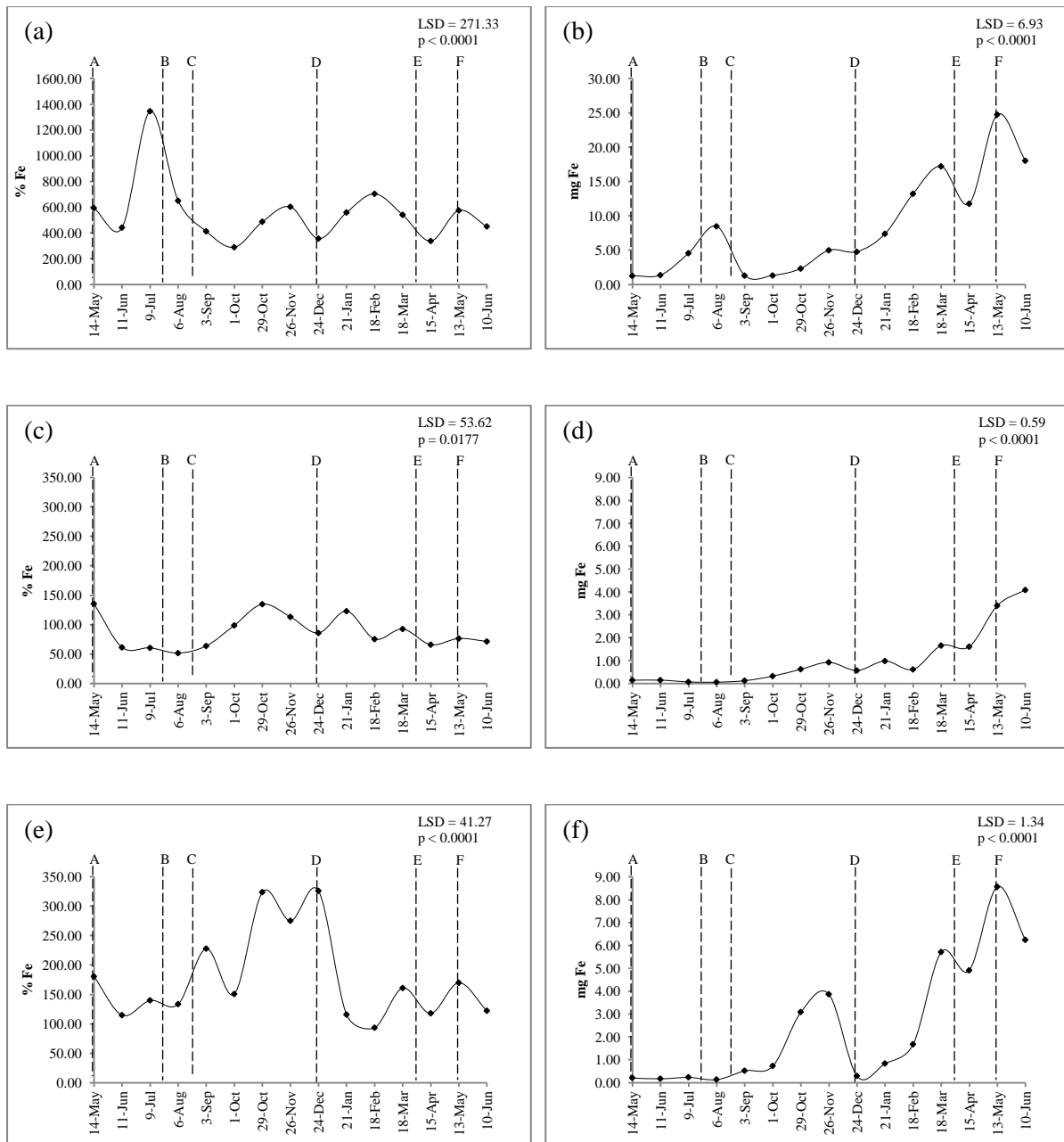


Fig. 13. Iron (Fe) concentration and content in the roots, shoots and leaves of southern highbush blueberry 'Snowchaser' from 14 May 2013 to 10 June 2014. (a) is root Fe concentration, (b) root Fe content, (c) shoot Fe concentration, (d) shoot Fe content, (e) leaf Fe concentration and (f) leaf Fe content. A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom.

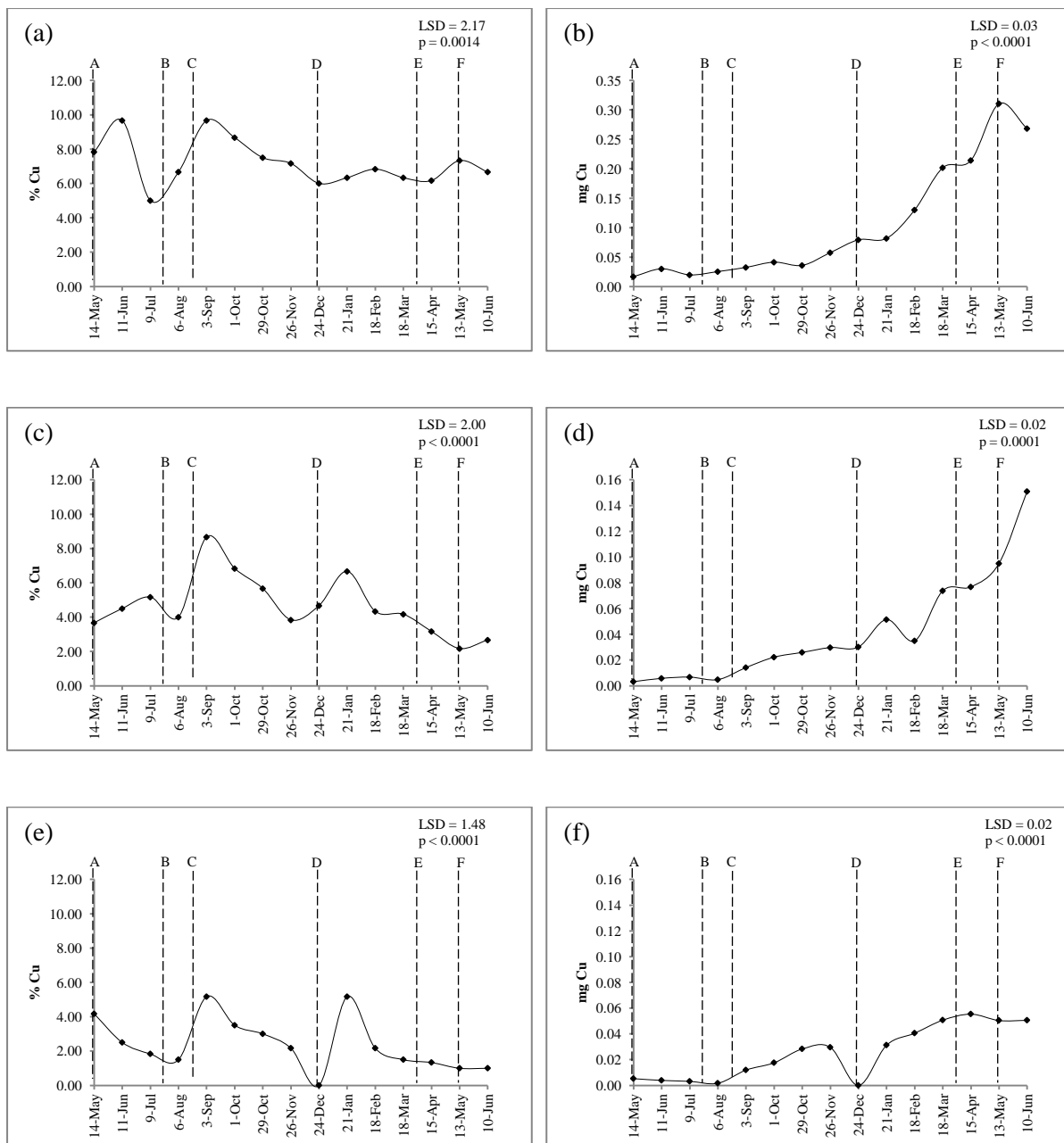


Fig. 14. Copper (Cu) concentration and content in the roots, shoots and leaves of southern highbush blueberry 'Snowchaser' from 14 May 2013 to 10 June 2014. (a) is root Cu concentration, (b) root Cu content, (c) shoot Cu concentration, (d) shoot Cu content, (e) leaf Cu concentration and (f) leaf Cu content. A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom.



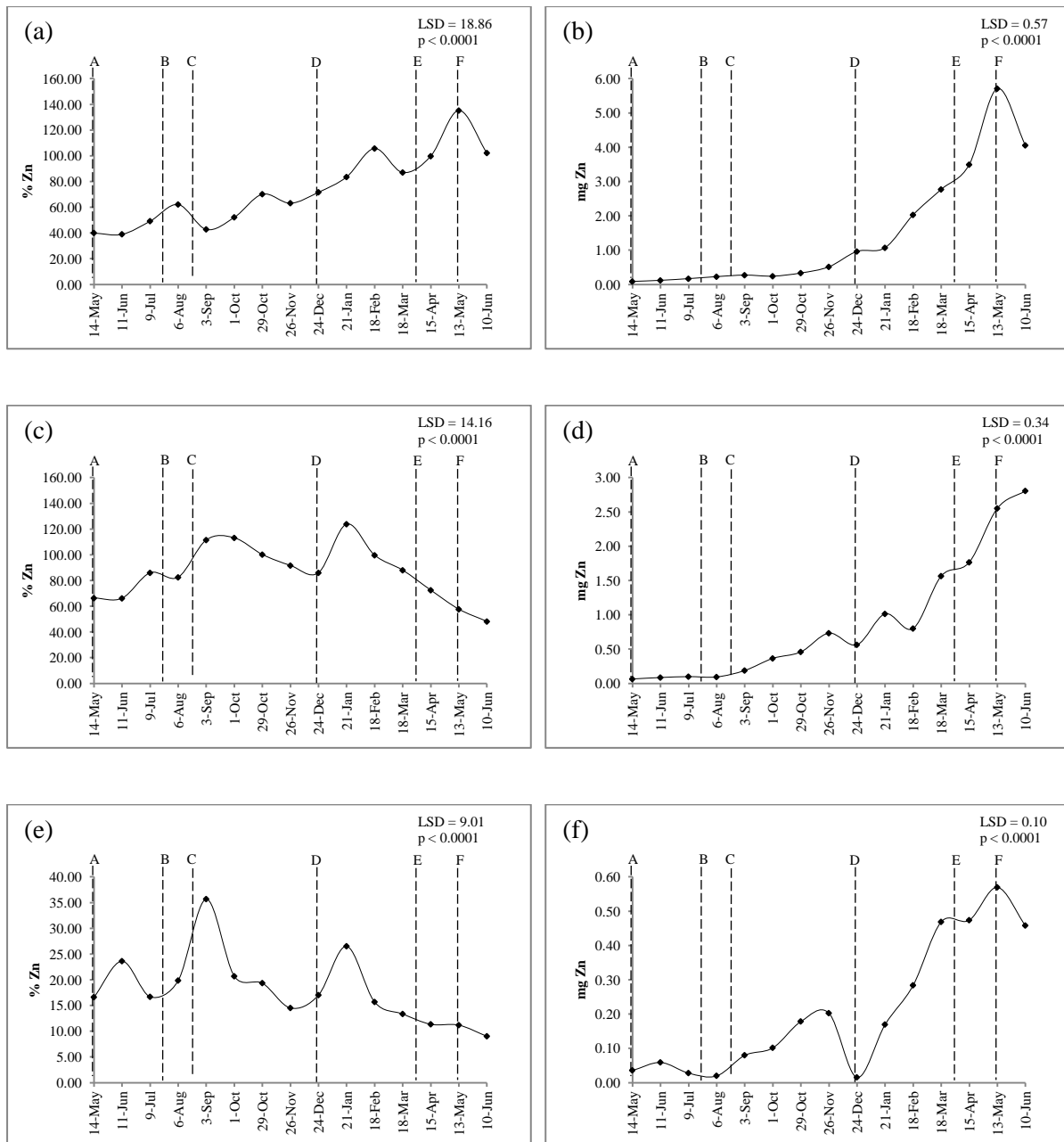


Fig. 15. Zinc (Zn) concentration and content in the roots, shoots and leaves of southern highbush blueberry 'Snowchaser' from 14 May 2013 to 10 June 2014. (a) is root Zn concentration, (b) root Zn content, (c) shoot Zn concentration, (d) shoot Zn content, (e) leaf Zn concentration and (f) leaf Zn content. A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom.

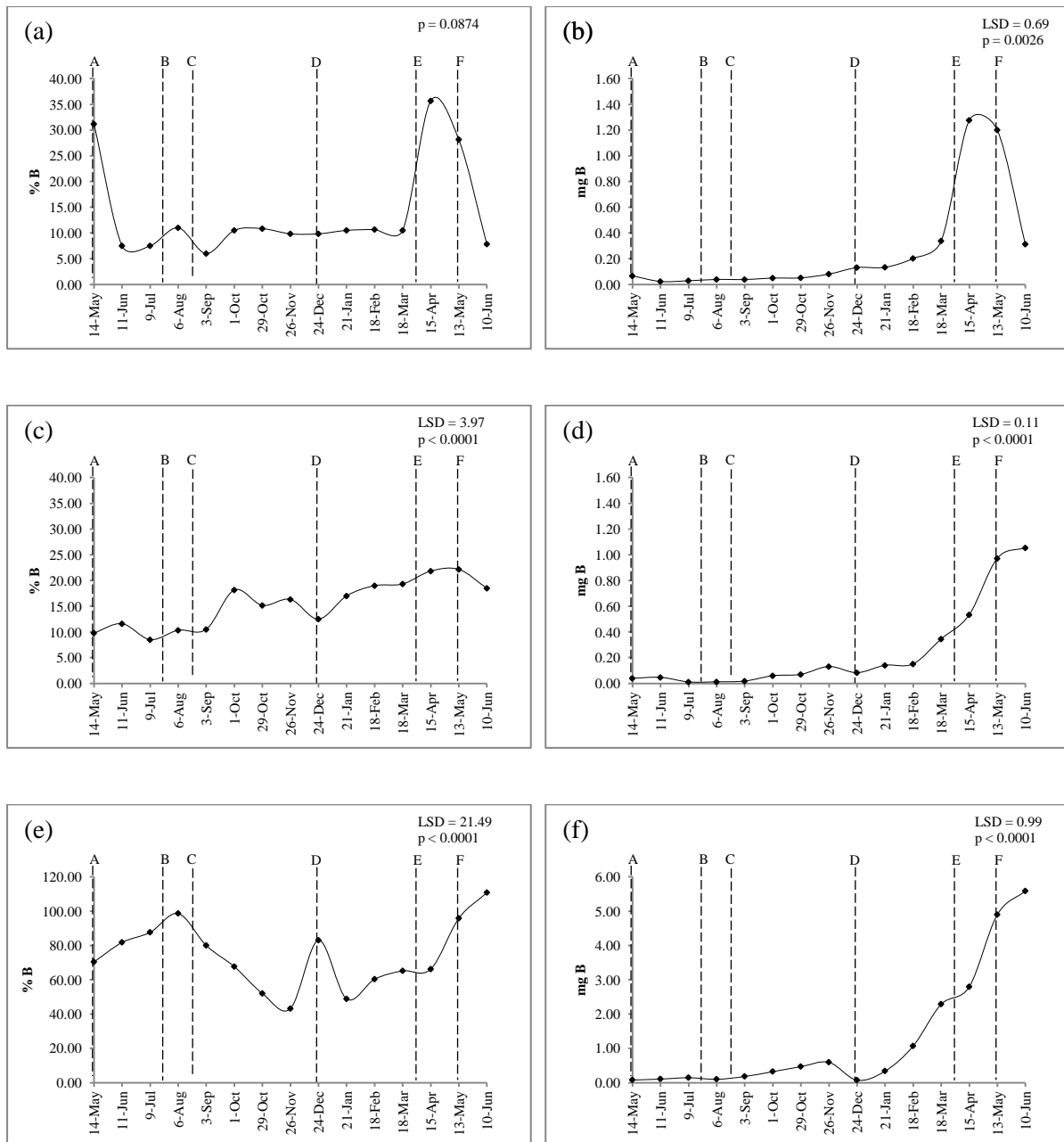


Fig. 16. Boron (B) concentration and content in the roots, shoots and leaves of southern highbush blueberry 'Snowchaser' from 14 May 2013 to 10 June 2014. (a) is root B concentration, (b) root B content, (c) shoot B concentration, (d) shoot B content, (e) leaf B concentration and (f) leaf B content. A = growth cessation, B = reproductive budbreak, C = vegetative budbreak, D = pruning, E = reproductive budbreak and F = full bloom.

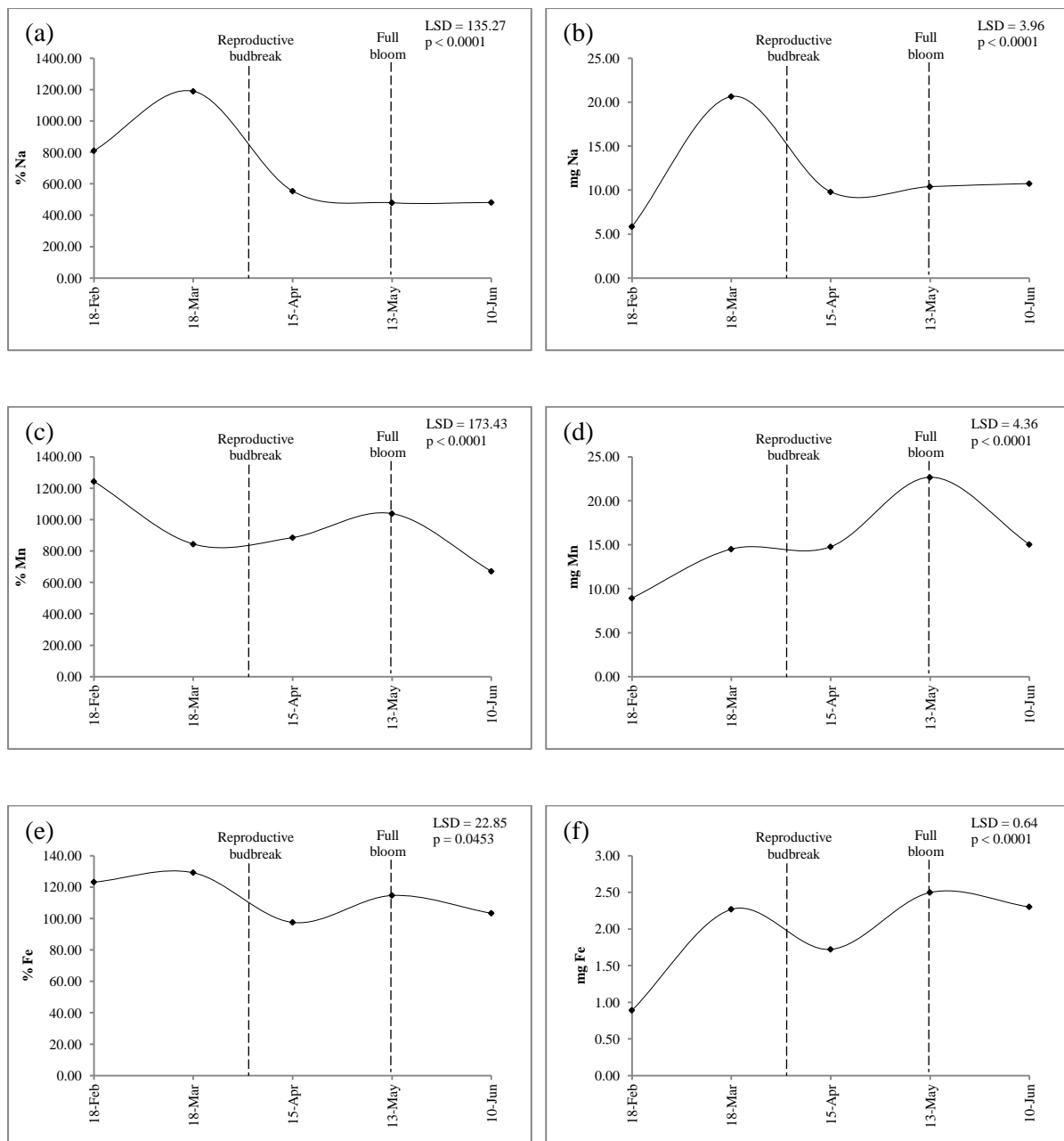


Fig. 17. Micro-element concentration and content in the canes of 'Snowchaser' southern highbush blueberry in the 2014 season. (a) sodium (Na) concentration, (b) sodium (Na) content, (c) manganese (Mn) concentration, (d) manganese (Mn) content, (e) iron (Fe) concentration, (f) iron (Fe) content.

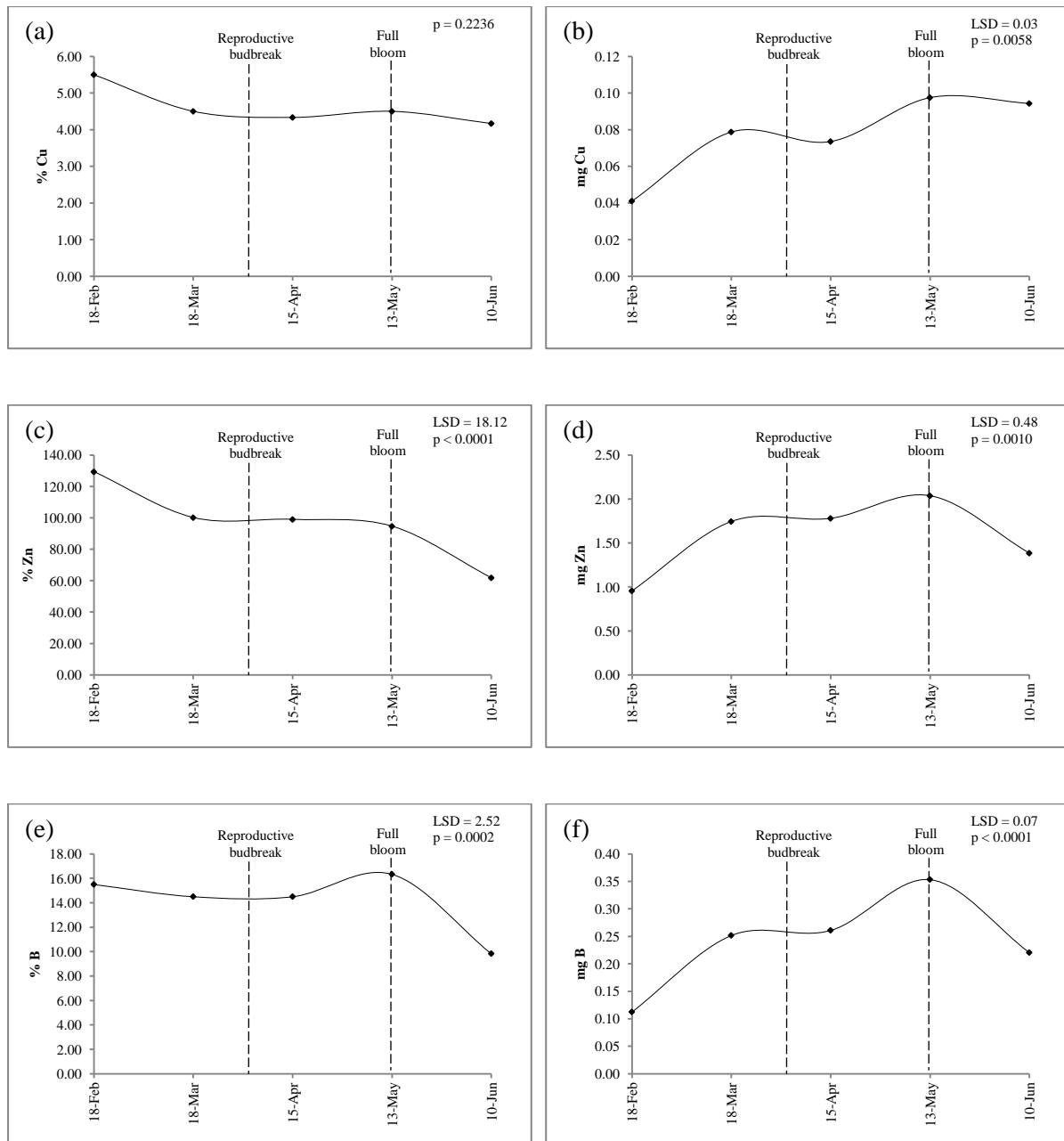


Fig. 18. Micro-element concentration and content in the canes of 'Snowchaser' southern highbush blueberry in the 2014 season. (a) copper (Cu) concentration, (b) copper (Cu) content, (c) zinc (Zn) concentration, (d) zinc (Zn) content, (e) boron (B) concentration, (f) boron (B) content.

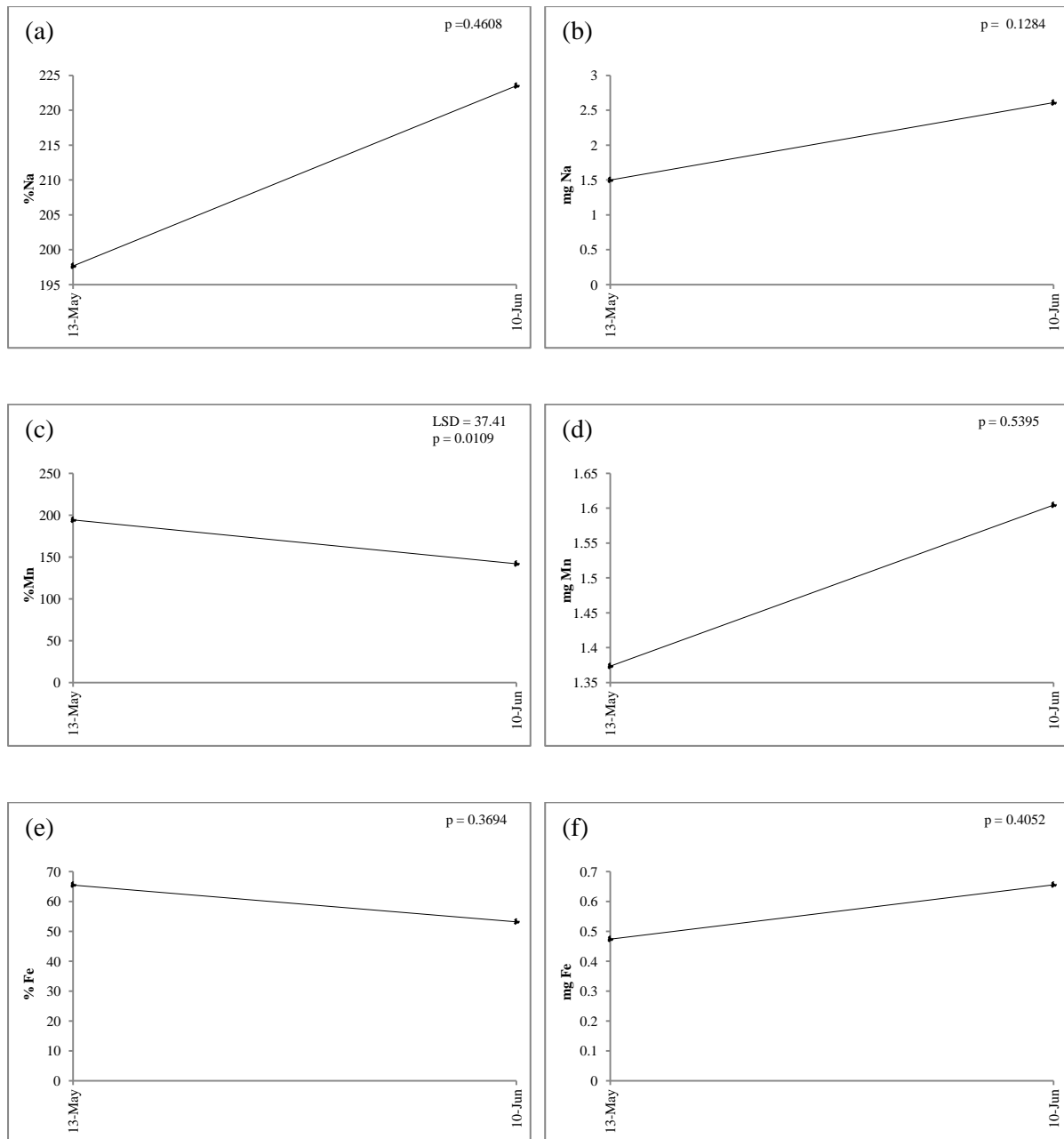


Fig. 19. Micro-element concentration and content in the flowers of 'Snowchaser' southern highbush blueberry in the 2014 season. (a) sodium (Na) concentration, (b) sodium (Na) content, (c) manganese (Mn) concentration, (d) manganese (Mn) content, (e) iron (Fe) concentration, (f) iron (Fe) content. Full bloom occurred on 13 May.

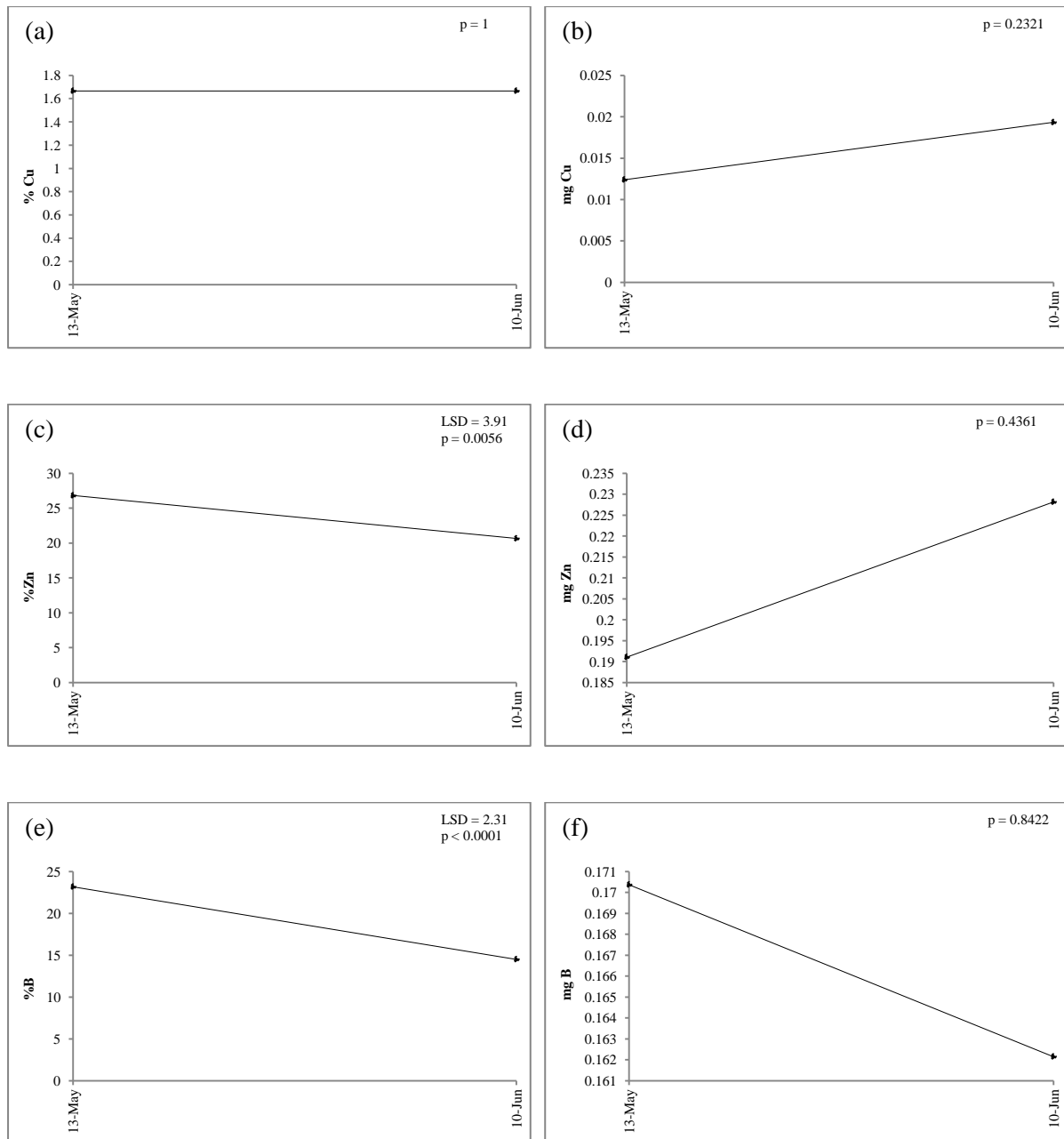


Fig. 20. Micro-element concentration and content in the flowers of 'Snowchaser' southern highbush blueberry in the 2014 season. (a) copper (Cu) concentration, (b) copper (Cu) content, (c) zinc (Zn) concentration, (d) zinc (Zn) content, (e) boron (B) concentration, (f) boron (B) content. Full bloom occurred on 13 May.

## **APPENDIX B**

The micro nutrient content and the starch and total sugar content was determined for the plants sampled in Paper 3 and is presented in this appendix. The same materials and method described in Paper 3 (p. 111) was used to obtain the micro nutrient results while the same carbohydrate procedure as described in Paper 1 (p. 44-46) was used to obtain the starch and sugar results.

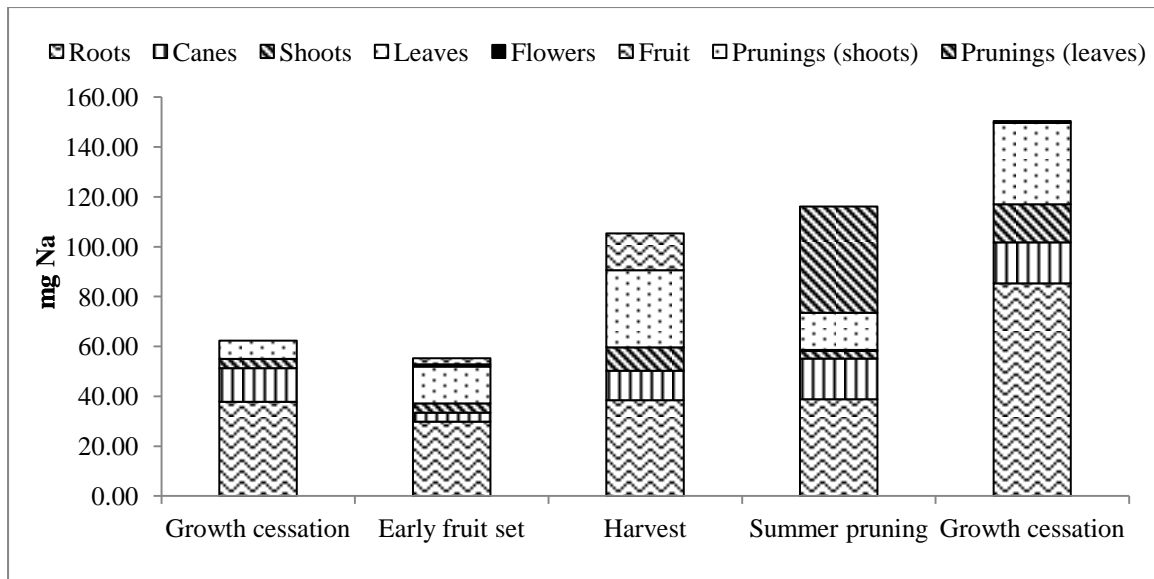


Fig. 1. Sodium (Na) content of 'Emerald' southern highbush blueberry at five stages during phenology. Growth cessation occurred on 21 May 2013, early fruit set occurred on 21 Aug. 2013, harvest started 19 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

Table 1. Sodium (Na) content in mg in different plant tissues of 'Emerald' southern highbush blueberry at five stages during a 12 month period.

	Growth cessation 21/05/2013	Early fruit set 21/08/2013	Harvest 19/11/2013	Summer pruning 20/12/2013	Growth cessation 21/05/2014
Roots	37.78 ± 8.52	29.81 ± 3.46	38.39 ± 2.75	38.79 ± 3.83	85.34 ± 10.11
Leaves	7.34 ± 0.41	14.76 ± 1.90	30.88 ± 3.38	0.48 ± 0.10	32.70 ± 2.86
Shoots	3.65 ± 1.47	3.77 ± 1.01	9.43 ± 1.07	3.07 ± 0.31	15.28 ± 0.73
Canes	13.54 ± 1.63	3.57 ± 0.62	11.86 ± 1.13	16.30 ± 1.41	16.35 ± 1.85
Fruit	.	2.42 ± 0.38	14.72 ± 2.00	.	.
Flowers	.	0.95 ± 0.25	.	.	0.75 ± 0.11
Prunings (leaves)	.	.	.	42.63 ± 1.31	.
Prunings (shoots)	.	.	.	14.80 ± 1.39	.
Total	62.30 ± 10.57	55.29 ± 4.34	90.56 ± 6.50	58.64 ± 3.55	150.42 ± 12.43



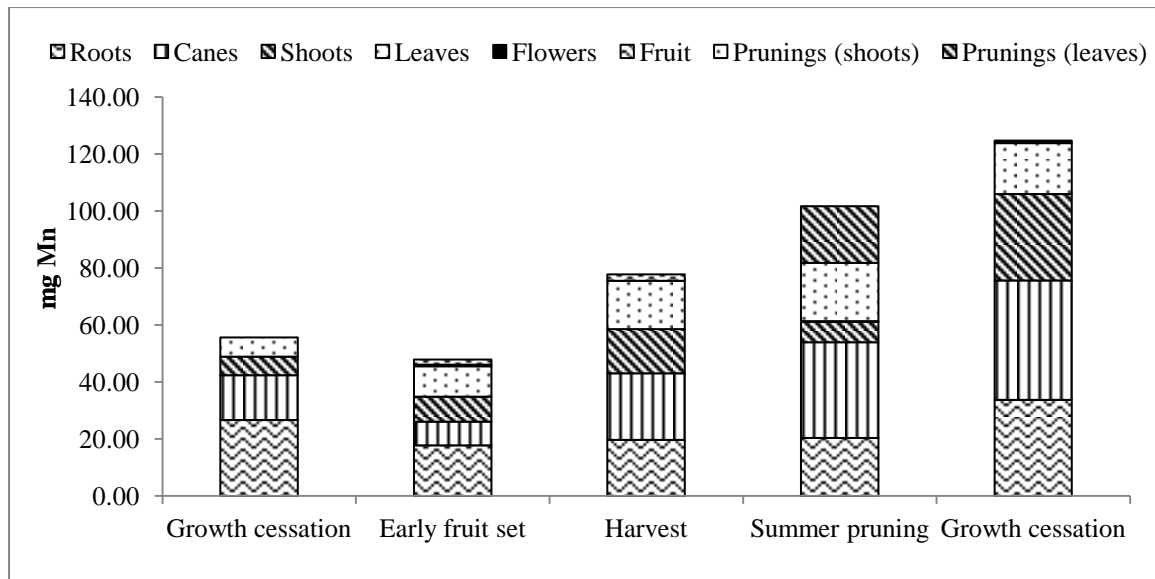


Fig. 2. Manganese (Mn) content of 'Emerald' southern highbush blueberry at five stages during phenology. Growth cessation occurred on 21 May 2013, early fruit set occurred on 21 Aug. 2013, harvest started 19 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

Table 2. Manganese (Mn) content in mg in different plant tissues of 'Emerald' southern highbush blueberry at five stages during a 12 month period.

	Growth cessation 21/05/2013	Early fruit set 21/08/2013	Harvest 19/11/2013	Summer pruning 20/12/2013	Growth cessation 21/05/2014
Roots	26.66 ± 7.95	17.79 ± 1.73	19.71 ± 2.13	20.35 ± 1.98	33.75 ± 3.34
Leaves	6.73 ± 1.11	10.74 ± 0.89	16.99 ± 2.31	0.17 ± 0.04	17.86 ± 1.17
Shoots	6.52 ± 2.99	8.72 ± 1.53	15.53 ± 1.97	7.17 ± 1.19	30.26 ± 3.66
Canes	15.77 ± 3.52	8.33 ± 0.74	23.33 ± 3.00	33.63 ± 1.71	41.94 ± 4.6
Fruit	.	1.75 ± 0.34	2.26 ± 0.26	.	.
Flowers	.	0.56 ± 0.13	.	.	0.94 ± 0.14
Prunings (leaves)	.	.	.	19.96 ± 0.55	.
Prunings (shoots)	.	.	.	20.46 ± 0.99	.
Total	55.69 ± 11.69	47.89 ± 2.43	75.55 ± 5.04	61.33 ± 4.02	124.75 ± 6.55

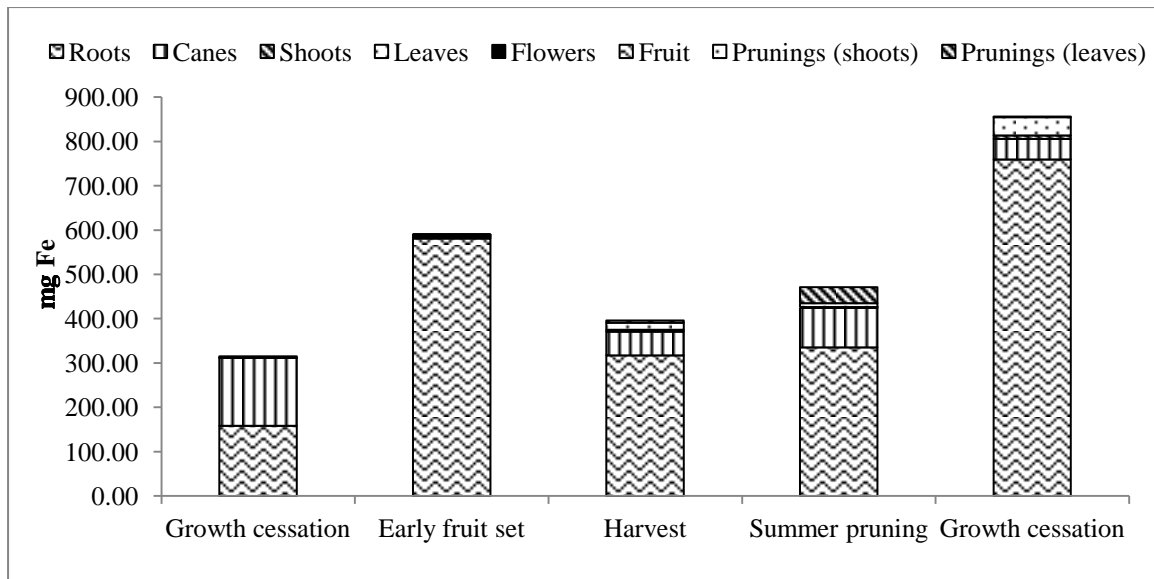


Fig. 3. Iron (Fe) content of 'Emerald' southern highbush blueberry at five stages during phenology. Growth cessation occurred on 21 May 2013, early fruit set occurred on 21 Aug. 2013, harvest started 19 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

Table 3. Iron (Fe) content in mg in different plant tissues of 'Emerald' southern highbush blueberry at five stages during a 12 month period.

	Growth cessation 21/05/2013	Early fruit set 21/08/2013	Harvest 19/11/2013	Summer pruning 20/12/2013	Growth cessation 21/05/2014
Roots	158.08 ± 70.02	581.05 ± 134.21	317.55 ± 72.88	335.32 ± 84.17	759.31 ± 120.41
Leaves	2.87 ± 0.56	3.36 ± 0.64	15.59 ± 1.86	0.32 ± 0.07	41.06 ± 12.46
Shoots	0.94 ± 0.24	2.00 ± 0.77	4.62 ± 0.54	1.80 ± 0.36	7.75 ± 1.28
Canes	153.42 ± 85.39	2.75 ± 0.55	52.99 ± 10.02	89.00 ± 13.64	46.56 ± 12.17
Fruit	.	0.37 ± 0.03	5.49 ± 0.75	.	.
Flowers	.	0.34 ± 0.13	.	.	1.21 ± 0.32
Prunings (leaves)	.	.	.	35.93 ± 5.16	.
Prunings (shoots)	.	.	.	8.59 ± 1.45	.
Total	315.31 ± 80.47	589.88 ± 134.37	390.76 ± 76.80	426.45 ±	855.89 ± 122.99

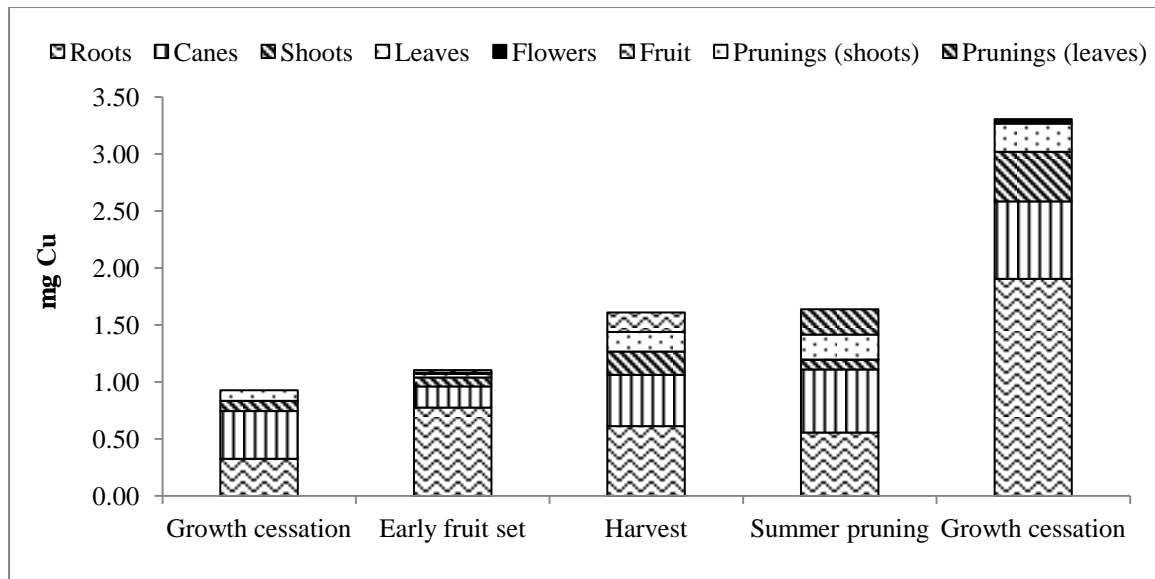


Fig. 4. Copper (Cu) content of 'Emerald' southern highbush blueberry at five stages during phenology. Growth cessation occurred on 21 May 2013, early fruit set occurred on 21 Aug. 2013, harvest started 19 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

Table 4. Copper (Cu) content in mg in different plant tissues of 'Emerald' southern highbush blueberry at five stages during a 12 month period.

	Growth cessation 21/05/2013	Early fruit set 21/08/2013	Harvest 19/11/2013	Summer pruning 20/12/2013	Growth cessation 21/05/2014
Roots	0.33 ± 0.10	0.78 ± 0.10	0.61 ± 0.06	0.56 ± 0.04	1.91 ± 0.18
Leaves	0.09 ± 0.01	0.03 ± 0.01	0.17 ± 0.03	0.00 ± 0.00	0.25 ± 0.02
Shoots	0.09 ± 0.02	0.08 ± 0.02	0.20 ± 0.02	0.08 ± 0.01	0.43 ± 0.03
Canes	0.42 ± 0.08	0.19 ± 0.02	0.45 ± 0.04	0.55 ± 0.04	0.68 ± 0.05
Fruit	.	0.02 ± 0.00	0.17 ± 0.01	.	.
Flowers	.	0.01 ± 0.0	.	.	0.04 ± 0.00
Prunings (leaves)	.	.	.	0.22 ± 0.01	.
Prunings (shoots)	.	.	.	0.22 ± 0.01	.
Total	0.93 ± 0.16	1.10 ± 0.12	1.44 ± 0.10	1.20 ± 0.05	3.31 ± 0.23

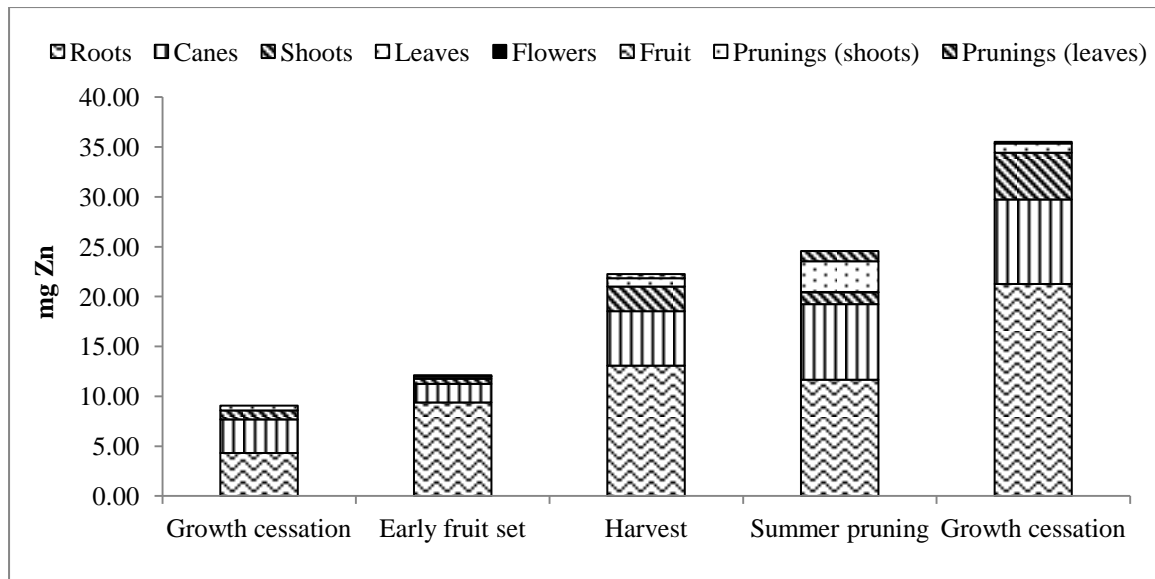


Fig. 5. Zinc (Zn) content of 'Emerald' southern highbush blueberry at five stages during phenology. Growth cessation occurred on 21 May 2013, early fruit set occurred on 21 Aug. 2013, harvest started 19 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

Table 5. Zinc (Zn) content in mg in different plant tissues of 'Emerald' southern highbush blueberry at five stages during a 12 month period.

	Growth cessation 21/05/2013	Early fruit set 21/08/2013	Harvest 19/11/2013	Summer pruning 20/12/2013	Growth cessation 21/05/2014
Roots	4.32 ± 0.75	9.37 ± 0.86	13.08 ± 1.51	11.66 ± 0.04	21.25 ± 2.34
Leaves	0.49 ± 0.23	0.23 ± 0.02	0.84 ± 0.07	0.02 ± 0.00	0.93 ± 0.05
Shoots	0.88 ± 0.51	0.50 ± 0.14	2.48 ± 0.40	1.19 ± 0.19	4.70 ± 0.34
Canes	3.37 ± 0.66	1.88 ± 0.15	5.44 ± 0.54	7.59 ± 0.28	8.48 ± 0.70
Fruit	.	0.09 ± 0.02	0.41 ± 0.06	.	.
Flowers	.	0.03 ± 0.01	.	.	0.14 ± 0.01
Prunings (leaves)	.	.	.	1.04 ± 0.01	.
Prunings (shoots)	.	.	.	3.09 ± 0.01	.
Total	9.05 ± 0.84	12.10 ± 0.95	21.84 ± 2.16	20.45 ± 1.49	35.50 ± 2.76

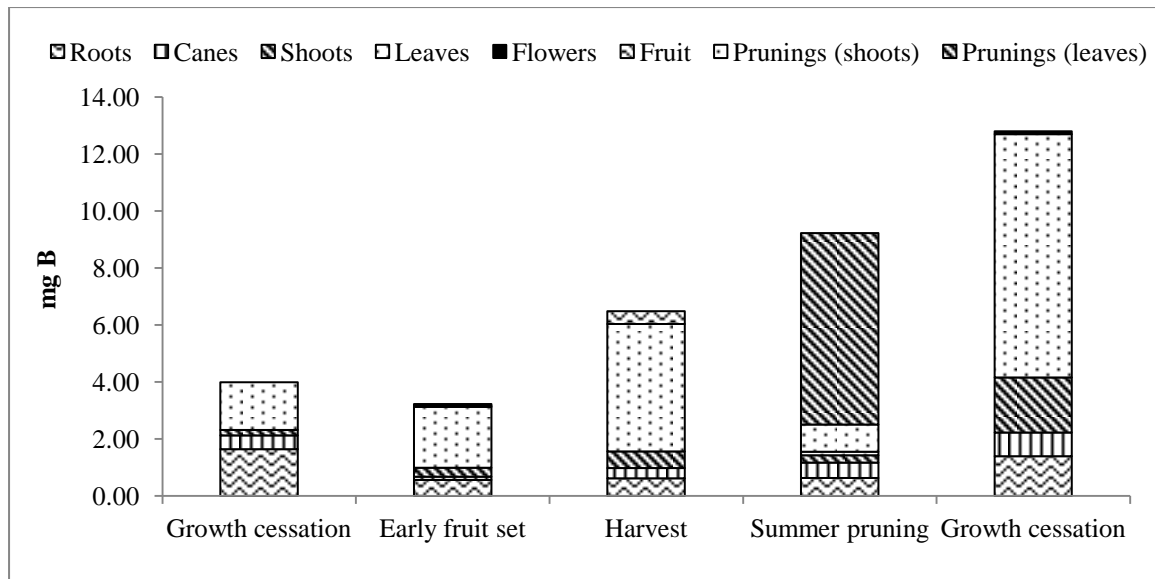


Fig. 6. Boron (B) content of 'Emerald' southern highbush blueberry at five stages during phenology. Growth cessation occurred on 21 May 2013, early fruit set occurred on 21 Aug. 2013, harvest started 19 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

Table 6. Boron (B) content mg in different plant tissues of 'Emerald' southern highbush blueberry at five stages during a 12 month period.

	Growth cessation 21/05/2013	Early fruit set 21/08/2013	Harvest 19/11/2013	Summer pruning 20/12/2013	Growth cessation 21/05/2014
Roots	1.64 ± 0.95	0.56 ± 0.08	0.62 ± 0.03	0.63 ± 0.07	1.40 ± 0.16
Leaves	1.68 ± 0.39	2.14 ± 0.18	4.47 ± 0.57	0.13 ± 0.03	8.54 ± 0.47
Shoots	0.19 ± 0.05	0.31 ± 0.07	0.58 ± 0.09	0.26 ± 0.04	1.94 ± 0.12
Canes	0.49 ± 0.12	0.11 ± 0.02	0.37 ± 0.02	0.54 ± 0.03	0.83 ± 0.13
Fruit	.	0.07 ± 0.01	0.45 ± 0.03	.	.
Flowers	.	0.03 ± 0.01	.	.	0.10 ± 0.01
Prunings (leaves)	.	.	.	6.72 ± 0.29	.
Prunings (shoots)	.	.	.	0.95 ± 0.08	.
Total	3.99 ± 1.31	3.23 ± 0.25	6.04 ± 0.66	1.56 ± 0.11	12.81 ± 0.45

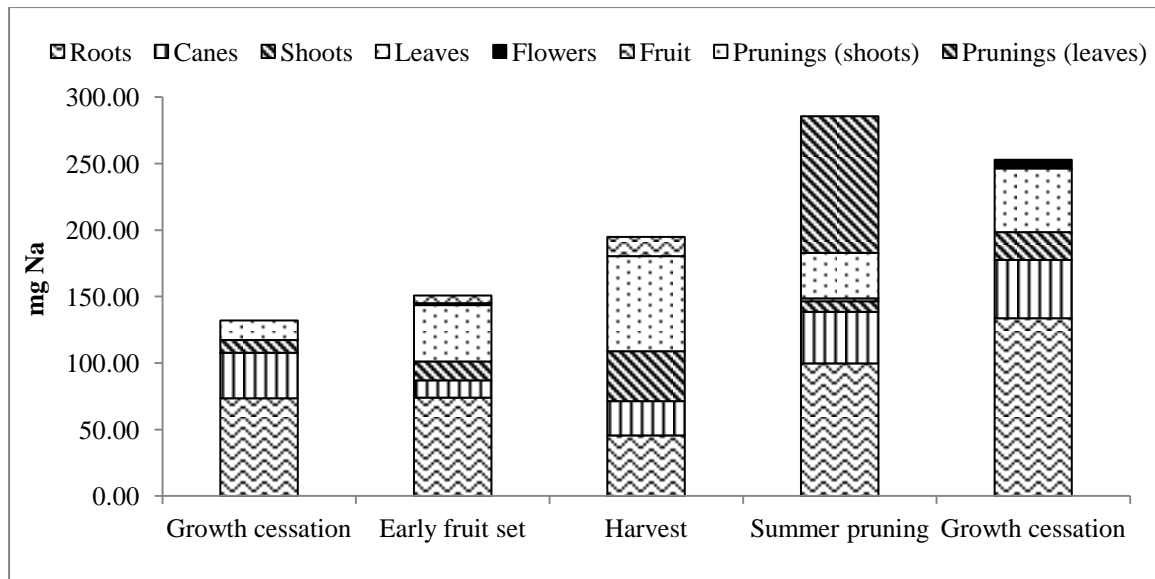


Fig. 7. Sodium (Na) content of 'Snowchaser' southern highbush blueberry at five stages during phenology. Growth cessation occurred on 21 May 2013, early fruit set occurred on 13 Aug. 2013, harvest started 5 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

Table 7. Sodium (Na) content and standard errors in mg in different plant tissues of 'Snowchaser' southern highbush blueberry at five stages during a 12 month period.

	Growth cessation 21/05/2013	Early fruit set 13/08/2013	Harvest 2/11/2013	Summer pruning 20/12/2013	Growth cessation 21/05/2014
Roots	73.38 ± 14.49	74.01 ± 3.10	45.62 ± 12.67	99.70 ± 5.83	133.61 ± 11.33
Leaves	14.79 ± 1.08	42.34 ± 4.07	71.56 ± 4.14	2.14 ± 0.41	47.98 ± 3.89
Shoots	9.44 ± 1.41	14.29 ± 0.85	37.39 ± 13.75	7.95 ± 1.23	20.86 ± 3.29
Canes	34.39 ± 7.13	12.88 ± 1.10	25.77 ± 0.84	38.73 ± 8.08	43.92 ± 5.23
Fruit	.	5.75 ± 1.00	14.60 ± 3.21	.	.
Flowers	.	1.51 ± 0.34	.	.	6.62 ± 0.78
Prunings (leaves)	.	.	.	102.85 ± 11.21	.
Prunings (shoots)	.	.	.	34.18 ± 4.08	.
Total	132.00 ± 15.83	150.78 ± 4.96	180.34 ± 14.04	148.52 ± 12.39	252.99 ± 14.94

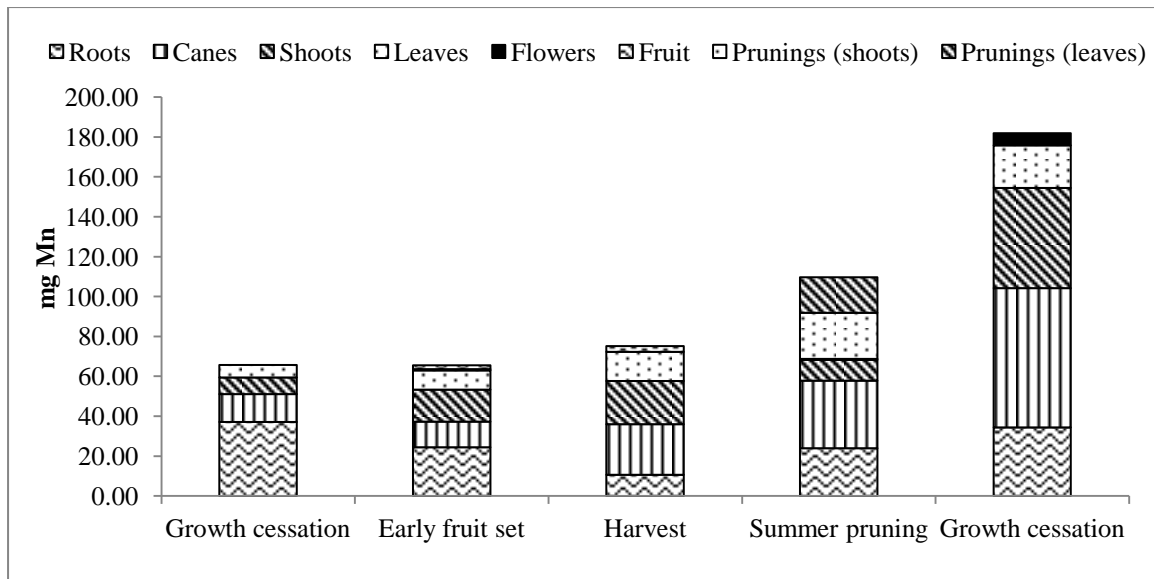


Fig. 8. Manganese (Mn) content of 'Snowchaser' southern highbush blueberry at five stages during phenology. Growth cessation occurred on 21 May 2013, early fruit set occurred on 13 Aug. 2013, harvest started 5 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

Table 8. Manganese (Mn) content and standard errors in mg in different plant tissues of 'Snowchaser' southern highbush blueberry at five stages during a 12 month period.

	Growth cessation 21/05/2013	Early fruit set 13/08/2013	Harvest 2/11/2013	Summer pruning 20/12/2013	Growth cessation 21/05/2014
Roots	37.08 ± 13.22	24.36 ± 1.80	10.61 ± 2.87	24.00 ± 2.00	34.44 ± 3.33
Leaves	6.38 ± 0.99	9.67 ± 0.52	14.63 ± 1.17	0.49 ± 0.10	21.37 ± 0.89
Shoots	8.27 ± 1.32	16.07 ± 1.06	21.71 ± 1.75	10.53 ± 0.87	50.13 ± 6.50
Canes	14.00 ± 2.71	12.91 ± 2.00	25.35 ± 1.98	33.76 ± 3.46	69.82 ± 3.54
Fruit	.	1.92 ± 0.23	2.81 ± 0.89	.	.
Flowers	.	0.54 ± 0.09	.	.	6.22 ± 0.60
Prunings (leaves)	.	.	.	17.74 ± 1.36	.
Prunings (shoots)	.	.	.	23.13 ± 1.37	.
Total	65.74 ± 14.25	65.48 ± 2.54	72.31 ± 3.79	68.78 ± 6.27	181.98 ± 10.97

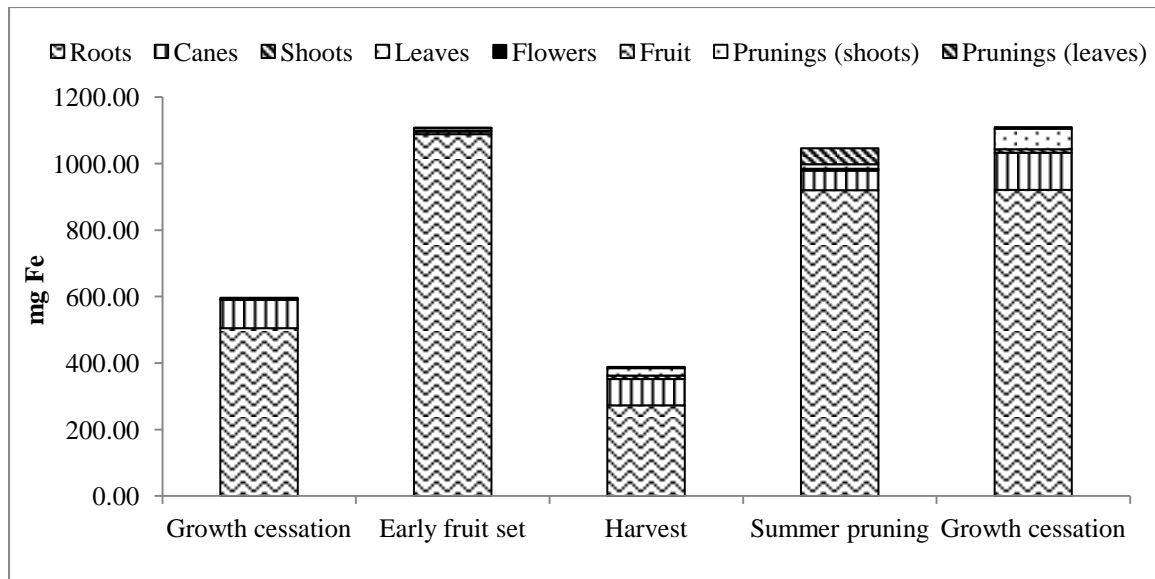


Fig. 9. Iron (Fe) content of 'Snowchaser' southern highbush blueberry at five stages during phenology. Growth cessation occurred on 21 May 2013, early fruit set occurred on 13 Aug. 2013, harvest started 5 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

Table 9. Iron (Fe) content and standard errors in mg in different plant tissues of 'Snowchaser' southern highbush blueberry at five stages during a 12 month period.

	Growth cessation 21/05/2013	Early fruit set 13/08/2013	Harvest 2/11/2013	Summer pruning 20/12/2013	Growth cessation 21/05/2014
Roots	504.84 ± 220.98	1088.33 ± 115.39	272.70 ± 73.12	919.56 ± 436.28	921.17 ± 207.83
Leaves	5.15 ± 0.56	7.58 ± 1.02	22.36 ± 0.92	2.26 ± 0.72	61.29 ± 11.02
Shoots	1.95 ± 0.45	2.99 ± 0.26	10.99 ± 3.89	3.99 ± 0.45	10.87 ± 1.73
Canes	84.91 ± 42.26	7.96 ± 1.57	78.63 ± 21.30	58.99 ± 10.60	111.70 ± 30.80
Fruit	.	0.55 ± 0.09	3.65 ± 1.15	.	.
Flowers	.	0.26 ± 0.07	.	.	4.37 ± 0.79
Prunings (leaves)	.	.	.	47.85 ± 4.73	.
Prunings (shoots)	.	.	.	13.28 ± 1.00	.
Total	596.85 ± 243.34	1107.67 ± 117.08	384.67 ± 59.41	984.80 ± 430.32	1109.40 ± 198.61



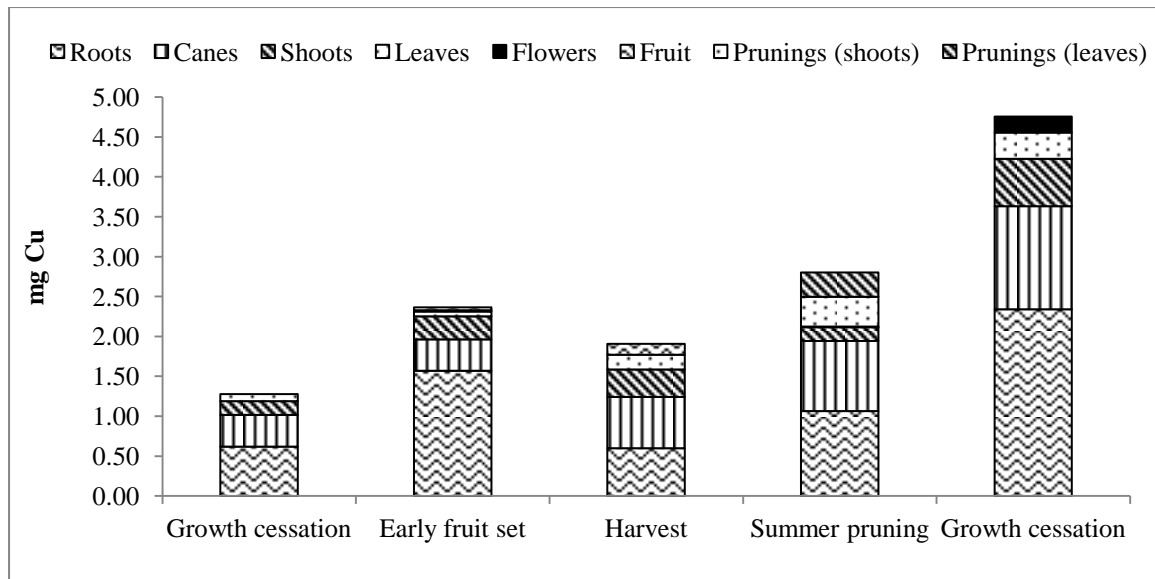


Fig. 10. Copper (Cu) content of ‘Snowchaser’ southern highbush blueberry at five stages during phenology. Growth cessation occurred on 21 May 2013, early fruit set occurred on 13 Aug. 2013, harvest started 5 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

Table 10. Copper (Cu) content and standard errors in mg in different plant tissues of ‘Snowchaser’ southern highbush blueberry at five stages during a 12 month period.

	Growth cessation 21/05/2013	Early fruit set 13/08/2013	Harvest 2/11/2013	Summer pruning 20/12/2013	Growth cessation 21/05/2014
Roots	0.62 ± 0.08	1.57 ± 0.08	0.60 ± 0.14	1.06 ± 0.08	2.34 ± 0.24
Leaves	0.09 ± 0.02	0.06 ± 0.02	0.19 ± 0.02	0.01 ± 0.00	0.33 ± 0.03
Shoots	0.17 ± 0.04	0.29 ± 0.03	0.34 ± 0.04	0.17 ± 0.01	0.59 ± 0.07
Canes	0.39 ± 0.02	0.39 ± 0.05	0.64 ± 0.07	0.88 ± 0.09	1.29 ± 0.10
Fruit	.	0.04 ± 0.00	0.14 ± 0.04	.	.
Flowers	.	0.02 ± 0.00	.	.	0.20 ± 0.01
Prunings (leaves)	.	.	.	0.30 ± 0.03	.
Prunings (shoots)	.	.	.	0.37 ± 0.02	.
Total	1.28 ± 0.08	2.36 ± 0.11	1.77 ± 0.17	2.12 ± 0.22	4.75 ± 0.33

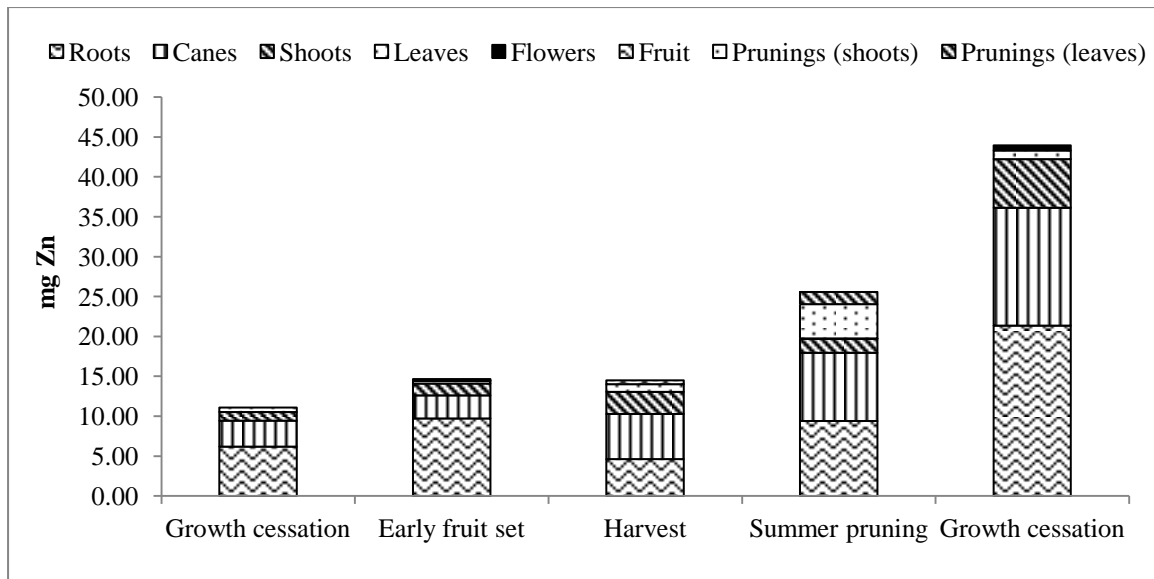


Fig. 11. Zinc (Zn) content of ‘Snowchaser’ southern highbush blueberry at five stages during phenology. Growth cessation occurred on 21 May 2013, early fruit set occurred on 13 Aug. 2013, harvest started 5 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

Table 11. Zinc (Zn) content and standard errors in mg in different plant tissues of ‘Snowchaser’ southern highbush blueberry at five stages during a 12 month period.

	Growth cessation 21/05/2013	Early fruit set 13/08/2013	Harvest 2/11/2013	Summer pruning 20/12/2013	Growth cessation 21/05/2014
Roots	6.20 ± 1.02	9.72 ± 0.68	4.63 ± 1.03	9.42 ± 1.86	21.35 ± 2.27
Leaves	0.53 ± 0.14	0.31 ± 0.04	0.96 ± 0.04	0.04 ± 0.01	1.06 ± 0.04
Shoots	1.11 ± 0.17	1.49 ± 0.10	2.76 ± 0.42	1.77 ± 0.10	6.11 ± 0.91
Canes	3.23 ± 0.32	2.91 ± 0.34	5.65 ± 0.54	8.53 ± 0.75	14.78 ± 0.77
Fruit	.	0.17 ± 0.02	0.48 ± 0.15	.	.
Flowers	.	0.06 ± 0.01	.	.	0.64 ± 0.07
Prunings (leaves)	.	.	.	1.51 ± 0.12	.
Prunings (shoots)	.	.	.	4.28 ± 0.26	.
Total	11.08 ± 1.15	14.65 ± 0.75	14.00 ± 1.01	19.77 ± 2.04	43.94 ± 2.75

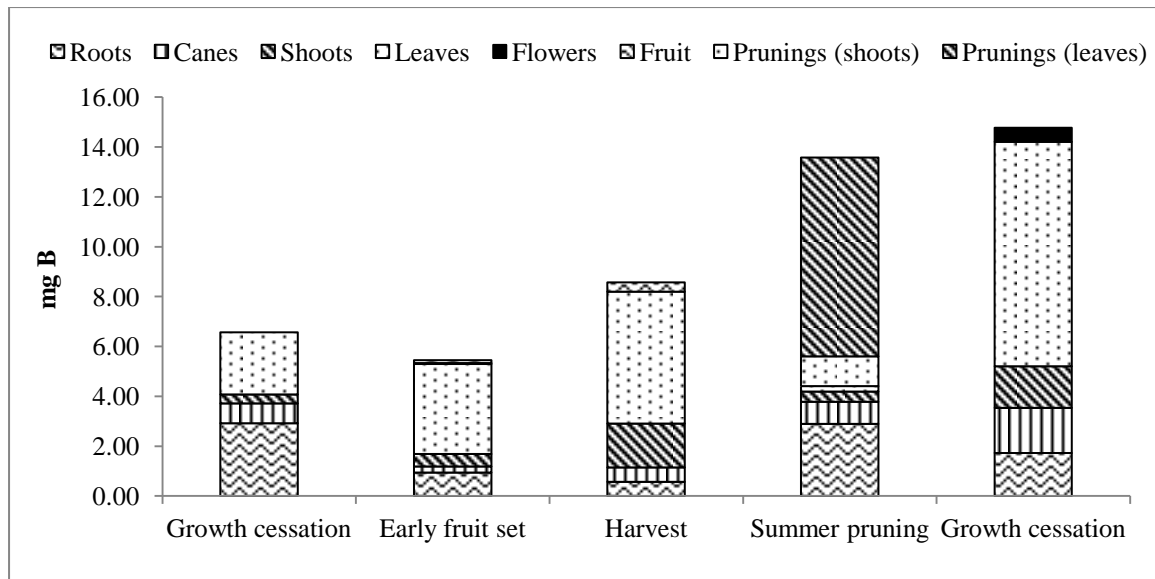


Fig. 12. Boron (B) content of 'Snowchaser' southern highbush blueberry at five stages during phenology. Growth cessation occurred on 21 May 2013, early fruit set occurred on 13 Aug. 2013, harvest started 5 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

Table 12. Boron (B) content and standard errors in mg in different plant tissues of 'Snowchaser' southern highbush blueberry at five stages during a 12 month period.

	Growth cessation 21/05/2013	Early fruit set 13/08/2013	Harvest 2/11/2013	Summer pruning 20/12/2013	Growth cessation 21/05/2014
Roots	2.92 ± 1.33	0.94 ± 0.06	0.57 ± 0.15	2.89 ± 1.53	1.72 ± 0.13
Leaves	2.49 ± 0.10	3.61 ± 0.19	5.29 ± 0.27	0.22 ± 0.04	9.00 ± 0.48
Shoots	0.37 ± 0.07	0.50 ± 0.07	1.76 ± 1.05	0.42 ± 0.04	1.67 ± 0.23
Canes	0.79 ± 0.36	0.24 ± 0.03	0.57 ± 0.05	0.88 ± 0.08	1.81 ± 0.24
Fruit	.	0.13 ± 0.02	0.37 ± 0.09	.	.
Flowers	.	0.04 ± 0.01	.	.	0.56 ± 0.08
Prunings (leaves)	.	.	.	7.96 ± 0.65	.
Prunings (shoots)	.	.	.	1.20 ± 0.06	.
Total	6.57 ± 1.54	5.45 ± 0.21	8.20 ± 1.02	4.40 ± 1.58	14.77 ± 0.76

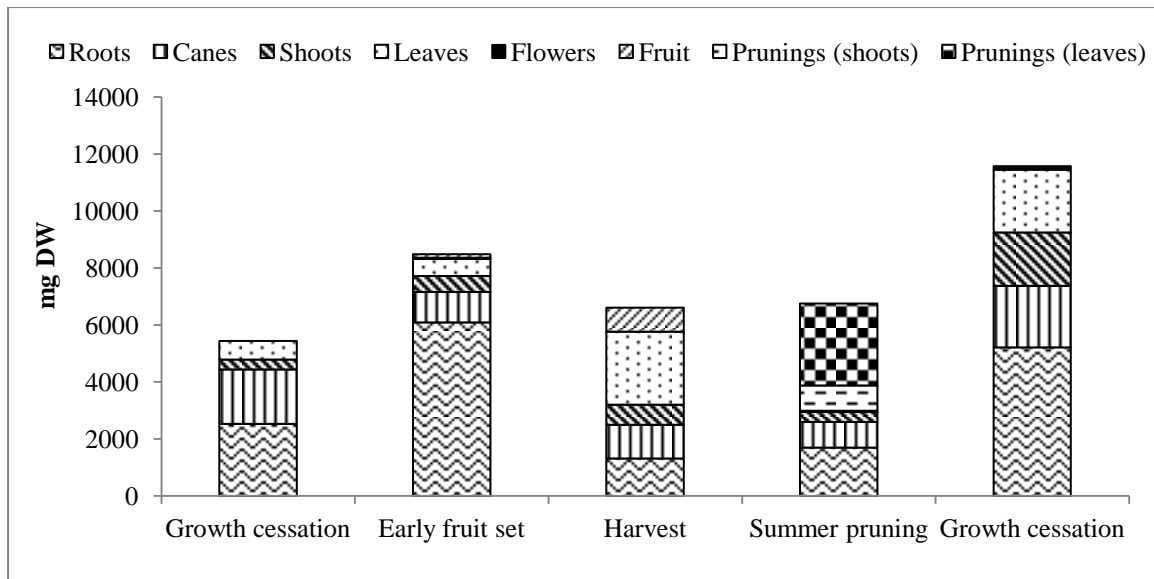


Fig. 13. Starch (as glucose equivalents) content of 'Emerald' southern highbush blueberry at five stages during phenology. Growth cessation occurred on 21 May 2013, early fruit set occurred on 21 Aug. 2013, harvest started 19 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

Table 13. Starch (as glucose equivalents) content in mg in different plant tissues of 'Emerald' southern highbush blueberry at five stages during a 12 month period.

	Growth cessation 21/05/2013	Early fruit set 21/08/2013	Harvest 19/11/2013	Summer pruning 20/12/2013	Growth cessation 21/05/2014
Roots	2531.36 ± 370.69	6095.51 ± 828.02	1315.24 ± 230.40	1697.30 ± 140.33	5214.92 ± 1065.87
Leaves	654.38 ± 133.79	602.72 ± 138.73	2572.17 ± 634.59	45.69 ± 15.53	2204.86 ± 462.87
Shoots	347.23 ± 50.95	558.04 ± 119.84	701.49 ± 92.02	338.18 ± 35.77	1880.05 ± 152.85
Canes	1910.03 ± 123.92	1063.73 ± 178.52	1183.52 ± 124.06	906.29 ± 104.24	2156.18 ± 278.13
Fruit	.	123.13 ± 13.88	835.59 ± 49.08	.	.
Flowers	.	45.04 ± 13.37	.	.	120.08 ± 19.67
Prunings (leaves)	.	.	.	2875.66 ± 503.81	.
Prunings (shoots)	.	.	.	891.69 ± 66.60	.
Total	5442.99 ± 505.91	8395.15 ± 782.55	5772.42 ± 952.30	2874.73 ± 200.73	11576.08 ± 1683.70

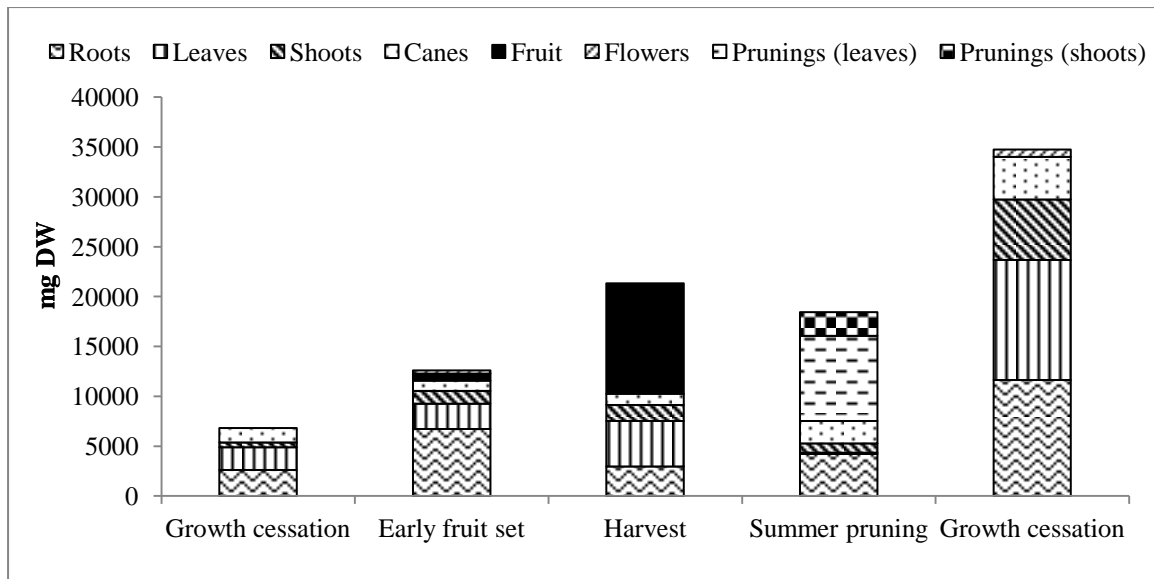


Fig. 14. Total sugar content of 'Emerald' southern highbush blueberry at five stages during phenology. Growth cessation occurred on 21 May 2013, early fruit set occurred on 21 Aug. 2013, harvest started 19 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

Table 14. Total sugar content in mg in different plant tissues of 'Emerald' southern highbush blueberry at five stages during a 12 month period.

	Growth cessation 21/05/2013	Early fruit set 21/08/2013	Harvest 19/11/2013	Summer pruning 20/12/2013	Growth cessation 21/05/2014
Roots	2601.77 ± 211.19	6718.08 ± 715.79	2975.08 ± 173.53	4204.03 ± 375.88	11630.92 ± 1557.61
Leaves	2273.03 ± 270.31	2537.07 ± 184.54	4541.58 ± 474.20	124.61 ± 22.11	12020.96 ± 809.59
Shoots	488.41 ± 78.75	1275.18 ± 256.24	1597.07 ± 142.56	939.42 ± 147.40	6093.33 ± 301.72
Canes	1450.41 ± 78.89	1023.36 ± 175.60	1130.78 ± 251.27	2266.85 ± 114.53	4270.96 ± 565.40
Fruit	.	718.16 ± 128.40	11074.96 ± 1709.62	.	.
Flowers	.	333.42 ± 974.91	.	.	722.23 ± 111.15
Prunings (leaves)	.	.	.	8514.24 ± 687.64	.
Prunings (shoots)	.	.	.	2391.00 ± 157.29	.
Total	6813.62 ± 493.34	12392.73 ± 867.11	10244.50 ± 686.35	7201.01 ± 549.78	34738.40 ± 2589.32

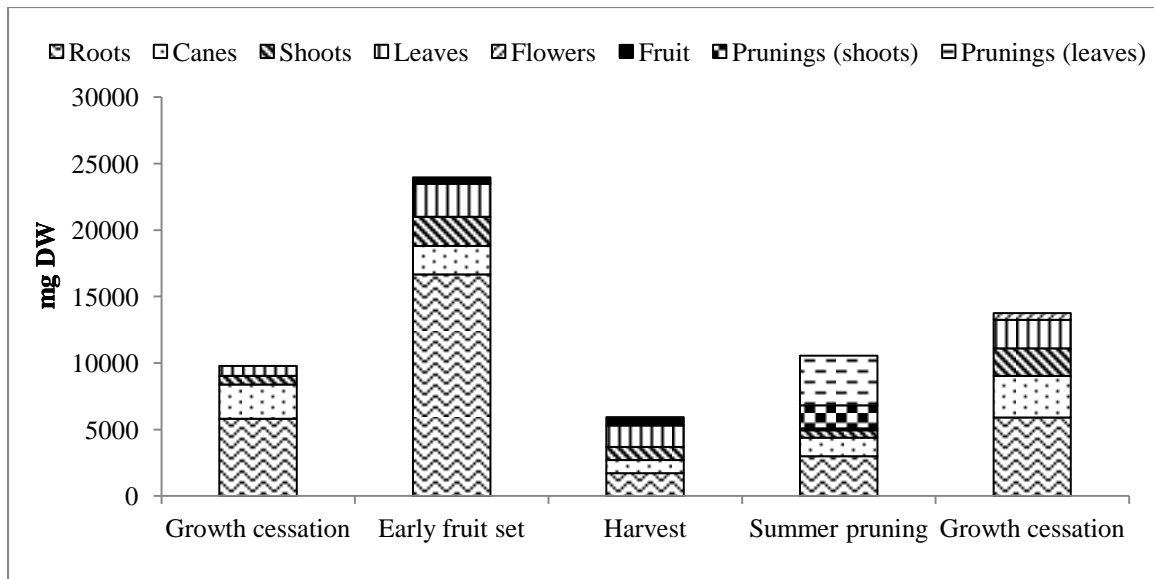


Fig. 15. Starch (as glucose equivalents) content of ‘Snowchaser’ southern highbush blueberry at five stages during phenology. Growth cessation occurred on 21 May 2013, early fruit set occurred on 13 Aug. 2013, harvest started 5 Nov. 2013, summer pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

Table 15. Starch (as glucose equivalents) content in mg in different plant tissues of ‘Snowchaser’ southern highbush blueberry at five stages during a 12 month period.

	Growth cessation 21/05/2013	Early fruit set 13/08/2013	Harvest 2/11/2013	Summer pruning 20/12/2013	Growth cessation 21/05/2014
Roots	5812.71 ± 606.83	16672.45 ± 1729.24	1712.37 ± 442.25	2986.00 ± 471.54	5915.05 ± 952.28
Leaves	756.17 ± 49.49	2490.12 ± 220.12	1619.11 ± 225.77	157.91 ± 44.80	2157.59 ± 161.04
Shoots	659.00 ± 118.72	2187.67 ± 129.78	966.94 ± 116.11	533.76 ± 31.43	2055.07 ± 265.38
Canes	2562.99 ± 246.16	2134.60 ± 173.73	1001.39 ± 186.61	1413.43 ± 105.80	3129.67 ± 314.85
Fruit	.	371.76 ± 55.14	617.39 ± 142.89	.	.
Flowers	.	95.74 ± 14.79	.	.	494.57 ± 51.11
Prunings (leaves)	.	.	.	3714.82 ± 492.22	.
Prunings (shoots)	.	.	.	1738.23 ± 173.14	.
Total	9790.86 ± 762.83	23952.34 ± 1649.47	5132.90 ± 676.28	5064.78 ± 478.40	13779.32 ± 1370.72

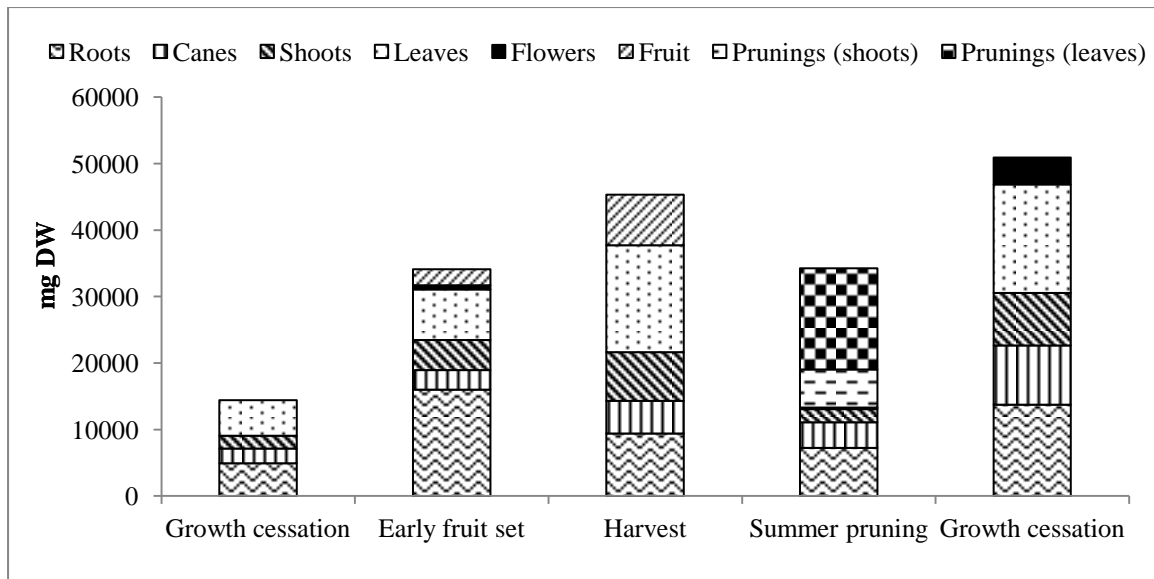


Fig. 16. Total sugar content of ‘Snowchaser’ southern highbush blueberry at five stages during phenology. Growth cessation occurred on 21 May 2013, early fruit set occurred on 13 Aug. 2013, harvest started 5 Nov. 2013, pruning was performed on 20 Dec. 2013 and growth cessation in the following season occurred on 21 May 2014.

Table 16. Total sugar content in mg in different plant tissues of ‘Snowchaser’ southern highbush blueberry at five stages during a 12 month period.

	Growth cessation 21/05/2013	Early fruit set 13/08/2013	Harvest 2/11/2013	Summer pruning 20/12/2013	Growth cessation 21/05/2014
Roots	4912.30 ± 244.19	16018.22 ± 918.06	9401.93 ± 2229.20	7219.71 ± 1035.22	13757.40 ± 1499.15
Leaves	5323.11 ± 392.25	7580.93 ± 316.02	16082.36 ± 951.79	268.02 ± 74.25	16321.83 ± 694.80
Shoots	1951.79 ± 355.48	4548.29 ± 221.40	7307.75 ± 1502.20	1945.58 ± 156.13	7881.55 ± 972.65
Canes	2211.55 ± 113.85	2910.17 ± 430.47	4901.04 ± 1169.43	3884.62 ± 427.22	8884.52 ± 344.05
Fruit	.	2376.14 ± 375.65	7614.50 ± 1514.18	.	.
Flowers	.	652.60 ± 106.16	.	.	4085.24 ± 230.5
Prunings (leaves)	.	.	.	15212.64 ± 1421.53	.
Prunings (shoots)	.	.	.	5724.84 ± 362.15	.
Total	14398.76 ± 683.44	34086.35 ± 1631.64	37693.09 ± 3805.28	12670.50 ± 1424.73	50930.54 ± 2612.49