Investigating plant growth dynamics of selected southern highbush blueberry (*V. corymbosum* L. interspecific hybrids) cultivars under South African growing conditions

by

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DECLARATION

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SUMMARY

In South Africa, southern highbush blueberry (SHB) cultivars exhibit an evergreen growth habit, due to the generally warmer climatic conditions. Two SHB cultivars, 8-42 and 9-02, were selected based on their observed differences in their aboveground characteristics. Cultivar 9-02 has a more vigorous growth habit than 8-42, but yields are considerably lower in 9-02 than in 8-42, regardless of the larger plant structure. Therefore, the aim of the study was to quantify the above- and belowground phenology of these cultivars, along with non-structural carbohydrate allocation patterns to provide deeper insights into the observed difference in yield between the cultivars.

In the first trial, the timing, duration and intensity of selected flower bud stages in '8-42' and '9-02' were determined at two production sites (Hex River and Wolseley) in the Western Cape, South Africa. Cultivar 8-42 flowered from April to mid-October and reached a peak during early August, while cultivar 9-02 flowered from April to late October but also reached a peak during early August at the Wolseley site. However, at the Hex River site, peak flowering occurred a month later, in September, thus indicating a possible genetic and climate-related interaction response. Fruit harvesting commenced in early August in '8-42', at both sites, and at the Hex River site for '9-02'; however, harvest was advanced to the end of July, at the Wolseley site. Despite these early harvesting times, more than 90% of the crop was concentrated between late-September and mid-November, for both cultivars, at both sites. Furthermore, the study confirmed the challenges encountered when attempting phenological predictions for SHB grown in an evergreen production system, as flowering and harvest stages can occur continuously, across several weeks, within a single plant.

In the second trial, the number and length of new root production were quantified in '8-42' and '9-02' at the Hex River site to describe the seasonal timing of new root production and root distribution patterns. New root production (number of additional roots recorded on consecutive evaluation dates) was evident throughout the study, with increased root production rates occurring at similar times, for both cultivars. A first root production peak occurred at the onset of early winter and coincided with flowering. New root production continued until increased fruit maturation and harvesting that commenced in early spring. A second root production peak coincided with mid-summer, approximately one month after fruit harvest. New root formation

occurred simultaneous with active shoot growth, until shoot activity declined with the termination of shoot elongation during early May.

In the third trial, non-structural carbohydrate allocation patterns were determined in bearing and non-bearing ‘8-42’ and ‘9-02’ plants, at selected phenological stages at the Hex River site, to gain a better understanding of the underlying causes of the reported differences in yield between the cultivars. Leaves were the major source of carbohydrates to support, not only reproductive development between 50% and 90% flowering in both cultivars, but also root growth, which was evident during this period. Thereafter, reproductive growth and new vegetative growth were sustained by all evaluated plant organs in ‘8-42’, during both seasons, whereas distinctly different results between seasons occurred during this period, in ‘9-02’. Leaf carbohydrates decreased between 90% flowering and peak harvest in the 2020 season, whereas it increased sharply in the 2021 season. It was suggested that this could indicate that ‘9-02’ had excess carbohydrates in the 2021 season which were not utilized to enhance reproductive growth and development.

OPSOMMING

In Suid-Afrika, volg “southern highbush” bloubessie (SHB) kultivars ‘n immergroen groeiwyse as gevolg van die algemene warmer klimaatstoestande. Twee SHB kultivars, 8-4’ en 9-02, is geselekteer op grond van hul waarneembare verskille in bogrondse eienskappe. Kultivar 9-02 het ‘n meer groeikragtige voorkoms as 8-4, maar opbrengs is laer in 9-02 as in 8-42, ongeag die groter plantstruktuur. Die doel van die studie was daarom om die bo- en ondergrondse fenologie, asook die nie-strukturele koolhidraat allokasie patrone van hierdie kultivars te kwantifiseer om insig te kry rakende die waarneembare opbrengs verskille tussen die kultivars.

In die eerste proef, is die tyd, tydsduur en intensiteit van selektiewe blomknopfases in ‘8-42’ en ‘9-02’ bepaal in twee produksie streke (Hexrivier en Wolseley) in die Weskaap, Suid-Afrika. Kultivar 8-42 het geblom vanaf April tot mid-Oktober en het ‘n piek bereik gedurende vroeë Augustus, terwyl kultivar 9-02 geblom het vanaf April tot laat Oktober en ‘n piek bereik het gedurende vroeë Augustus. Nietemin het piekblom ‘n maand later plaasgevind, in September, by die Hexrivier produksie area en is dus ‘n moontlike aanduiding van ‘n genetiese en klimaat-verwante interaksies. Oes het in vroeë Augustus vir ‘8-42’ by beide streke begin, asook vir ‘9-02’ by die Hexrivier produksie area, alhoewel die oesdatum vervroeg was, tot laat Julie, in die Wolseley produksie area. Ten spyte van hierdie vroeë oestye, is meer as 90% van die totale oes gekonsentreer tussen einde-September en mid-November, vir beide kultvars, in beide produksie streke. Verder het die studie die uitdagings bevestig soos ondervind met fenologiese voorspellings vir SHB wat verbou word in ‘n immergroen produksiesisteem, waar blom- en oes-stadia deurlopend oor verskeie weke op ‘n enkele plant kan voorkom.

In die tweede proef, is die aantal en lengte van nuwe wortelproduksie gekwantifiseer in ‘8-42’ en ‘9-02’ in die Hexrivier produksie area, om die seisoenale tydsberekening van nuwe wortelproduksie en wortelverspreidingspatrone te beskryf. Nuwe wortelgroei (aantal bykomende wortels wat by opeenvolgende evalueringsdatums opgeneem is) was deurgans duidelik sigbaar tydens die verloop van die studie, met verhoogde wortelproduksie koerse, wat in ooreenkomstige tye plaasgevind het, vir beide kultivars. ‘n Eerste piek in wortelproduksie het tydens die vroeë winter plaasgevind en saamgeval met die blomperiode. Nuwe wortelproduksie het voort geduur tot en met vrug-volwassewording en oes, wat in vroeë lente in

aanvang geneem het. ‘n Tweede piek in wortelproduksie het saamgeval met mid-somer, ongeveer een maand na die finale oes. Nuwe wortelproduksie het gelyktydig met aktiewe lootgroei plaasgevind totdat lootaktiwiteit afgeneem het, met die beëindiging van lootverlenging, gedurende vroeë Mei.

In die derde proef, is nie-strukturele koolhidraatallokasie patrone bepaal in draende en nie-draende ‘8-42’ en ‘9-02’ plante vir gepesifiseerde fenologiese stadia in die Hexrivier produksie area om dieper insig te kry rondom die oorsake vir die gerapporteerde verskille in opbrengs tussen die kultivars. Blare is as hoof bron van koolhidrate geïdentifiseer en het nie net reproduktiewe ontwikkeling tussen 50% en 90% blom ondersteun nie, maar ook die opmerklike wortelgroei tydens hierdie periode onderhou. Daarna is beide reproduktiewe- en vegetatiewe groei ondersteun deur alle plant organe wat ondersoek is in ‘8-42’, gedurende beide seisoene, terwyl kenmerkende verskille tussen seisoene voorgekom het in ‘9-02’ gedurende hierdie periode. Blaar koolhidrate het afgeneem gedurende hierdie tydperk in die 2020 seisoen, terwyl dit drasties toegeneem het in die 2021 seisoen. Daar word voorgestel dat hierdie verskynsel ‘n aanduiding kan wees dat ‘9-02’ ‘n oormaat koolhidrate het wat nie aangewend is om addisionele reproduktiewe groei te ondersteun en uit te brei nie.

NOTE

This thesis is a compilation of chapters, starting with a literature review, followed by three research papers. The first paper was prepared as a scientific paper for submission to *Scientia Horticulturae.* The second and third papers were prepared as a scientific paper for submission to *HortScience*. Repetition or duplication between papers might, therefore, be necessary.

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GENERAL INTRODUCTION

Blueberry (*Vaccinium* spp.) demand, because of its high phenolic concentrations, has increased globally over the last 10 years, growing by 14% per annum from 2010 to 2020 (Fang et al., 2020). This rising demand has resulted in commercial blueberry cultivation to expand into new growing areas i.e., South Africa (Fang et al., 2020). The South African blueberry industry is still relatively small but is one of the fastest growing horticultural industries in South Africa, both in terms of hectares planted and gross value of production (South African Berry Production Association (SABPA), 2019). Production volumes have increased from 2330 t in 2014 to 36000 t in 2021 and are projected to rise above 52000 t in 2024 (www.berriesza.co.za).

Southern highbush blueberry (SHB) is traditionally grown in a dormant production system at high latitudes, but more recently, low-chill cultivars, grown in an evergreen production system, have been introduced that are adapted to lower latitude subtropical and even tropical production areas (Fang et al., 2020), but also Mediterranean-type climatic areas such as the Western Cape, South Africa. Under these climatic conditions, evergreen blueberry production is managed to prevent defoliation during the winter months, while a healthy canopy that can support fruit development during late winter and spring is maintained through selective, continuous nitrogen fertilizer applications (Lyrene, 2005, 2006; Reeder et al., 1998). SHB grown as an evergreen production system allows growers to harvest berries at least one month earlier than possible in deciduous cultivars (Scalzo et al., 2016). This advantage has created an interest to optimize cultivation practices in evergreen production systems, as SHB tends to stay evergreen in South Africa due to the climatic conditions. However, there is a lack of peer reviewed research describing or improving the evergreen SHB production system at this time, as the research is being carried by the industry (Fang et al., 2020). This thesis therefore aims to contribute to the understanding of the phenology and physiology of SHB cultivars, produced within an evergreen system.

A first objective was to conduct a literature review on blueberry phenology in general and to provide insights into production practices, with emphasis on vegetative and reproductive phenological events and the influence of environmental factors and management practices on blueberry plant growth and phenology. Dormant production

systems contributed to the majority of the content, as little information is currently available on evergreen blueberry production systems.

In a second objective, the timing, duration and rate of selected flower bud phenological stages of SHB cultivars ‘8-42’ and ‘9-02’ grown commercially, in two similar climate regions, in the Western Cape, were studied. Unlike deciduous SHB cultivars, evergreen SHB cultivars do not enter endodormancy during autumn, but maintain healthy leaves throughout the growing season. Evergreen SHB cultivars are adapted to flower during the colder winter months, to produce a crop during late winter to late spring (Swain and Darnell, 2001). However, the phenological stages of evergreen SHB cultivars are more complex than that of deciduous cultivars, as these plants tend to have long, protracted reproductive seasons where flowering, fruit set and berry maturation may occur simultaneously on the same plant, complicating harvest predictions (Scalzo et al., 2016). For deciduous SHB cultivars the harvest period is restricted to a more concentrated period of up to six weeks, while cultivars grown in an evergreen production system can have harvest periods of up to three months (Baptista et al., 2006; Swain and Darnell, 2001). Cultural practices such as water management, fertilization, pest and disease management and harvest planning all depend on the recognition of the various phenological stages (Baptista et al., 2006; Kishore, 2019; Larue et al., 2021). Thus, there is a need to determine the timing of phenological stages of newly introduced cultivars to guide optimization of commercial production systems.

Reliable information on root growth and development is essential to optimize irrigation and fertilizer recommendations (Smith et al., 2005). Therefore, the third objective was to determine the timing of new root production and root growth distribution of the evergreen SHB cultivars ‘8-42’ and ‘9-02’ under field conditions, using minirhizotrons. Blueberry plants are known to have shallow, highly branched root systems, rarely exceeding depths of 60 cm (Paltineanu et al., 2017). Current understanding of blueberry root systems is restricted to studies conducted on deciduous northern highbush- and rabbiteye blueberry cultivars in the northern hemisphere (Abbott and Gough, 1987; Bryla et al., 2017).

In the final objective, the carbohydrate allocation patterns within whole plants (bearing and non-bearing) of two cultivars (‘8-42’ and ‘9-02’), differing in aboveground vigor and yield, was investigated to explore their vegetative and reproductive growth during two consecutive seasons.

The information aims to contribute towards implementing precision farming and to inform on decision making for developing management strategies for SHB, based on above- and belowground phenology and resource acquisition between organs within the latter compartments during critical phenological stages.

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LITERATURE REVIEW: Blueberry phenology and production practices

1. Introduction

Blueberries, which are classified as perennial evergreen or deciduous shrubs, is placed within the genus *Vaccinium* and the Ericaceae family (Hancock et al., 2008). Several species of *Vaccinium* are grown commercially, with most production relying on species in the section, Cyanococcus (Hancock et al., 2008). This includes cultivars of northern highbush blueberry (NHB) (*V. corymbosum* L.), southern highbush blueberry (SHB) (*V. corymbosum* L. interspecific hybrids), rabbiteye (RE) blueberry (*V. ashei* Reade) and native stands of lowbush blueberry (*V. angustifolium* Ait.) (Hancock et al., 2008; Lyrene, 2006; Retamales and Hancock, 2018). SHB is the most recent acquisition among these species and originated by inter-crossing *V. corymbosum, V. angustifolium, V. arboretum, V. ashei, V. constable, V. fuscatum, V. tenellum and V. darrowii* (Lang and Parrie, 1992, Retamales and Hancock, 2018). SHB are distinguished from the other blueberry species, specifically regarding its low chilling requirement and cold hardiness, its ability to reach fruit maturity earlier in the season, as well as that they exhibit a second vegetative growth period in summer (Hancock et al., 2008; Pescie et al., 2018).

The blueberry industry is still relatively young in South Africa, with extensive plantings only starting during the 1990's (Retamales and Hancock, 2018). South African berry production is predominantly focussed on SHB (Kritzinger, 2014). Due to the generally warmer climatic conditions in South Africa, SHB can be grown in an evergreen production system, especially near the southern coastal areas (Kritzinger, 2014; Retamales and Hancock, 2018). This production system allows growers to advance harvest about one month earlier than SHB cultivars grown in a dormant production system (Lyrene, 2005). However, reliable information for this production system is limited, as research funding is currently dependant on the industry and insufficient to cover this topic to its full extent (Fang et al., 2020).

To indicate development differences between cultivars and in order to develop standard management recommendations based on phenological stages, accurate information regarding vegetative and reproductive development as relevant under commercial production, is required (NeSmith et al.,1998). For timeous intervention in the commercial production cycle, it is also important to understand the time and length of the respective vegetative and reproductive growth stages of blueberry, and the

underlying factors influencing each stage (Baptista et al., 2006; Kirk and Isaacs, 2012). In this review, the growth and development of blueberry in general will be discussed, focusing specially on reproductive and vegetative phenological stages as well as the influence of environmental and management practices on plant phenology and growth that need to be considered to ensure successful commercial cultivation.

2. Vegetative growth and development

2.1 Vegetative bud development scale

NeSmith et al. (1998) developed a vegetative bud development scale for RE blueberries consisting of six distinct stages (Fig. 1). The scale was based on observations of different RE blueberry cultivars as grown under various climatic conditions. According to NeSmith et al. (1998), the observed development stages for RE blueberries can also be applicable to highbush blueberries. The “dormant bud” (1) stage represents a fully dormant vegetative bud showing no signs of bud growth or swelling. During the “early green tip” (2) stage, the bud starts to swell and elongate, and a green tip becomes visible. The “late green tip” (3) stage occurs prior to leaf unfolding. The bud is elongated with tightly rolled, but considerably visible green leaves. The “unfolding” (4) stage describes a fully elongated leaf bud with leaves starting to unfold. The bud is not completely open but shows the initial signs of opening. The “mouse-ear” (5) stage consists of an open bud with readily visible basal leaves, but not fully expanded. Stage (6) represents a “fully opened bud” with the first three to four completely unfolded and visible leaves. At this stage, the tip is considered to be still actively growing (NeSmith et al., 1998).

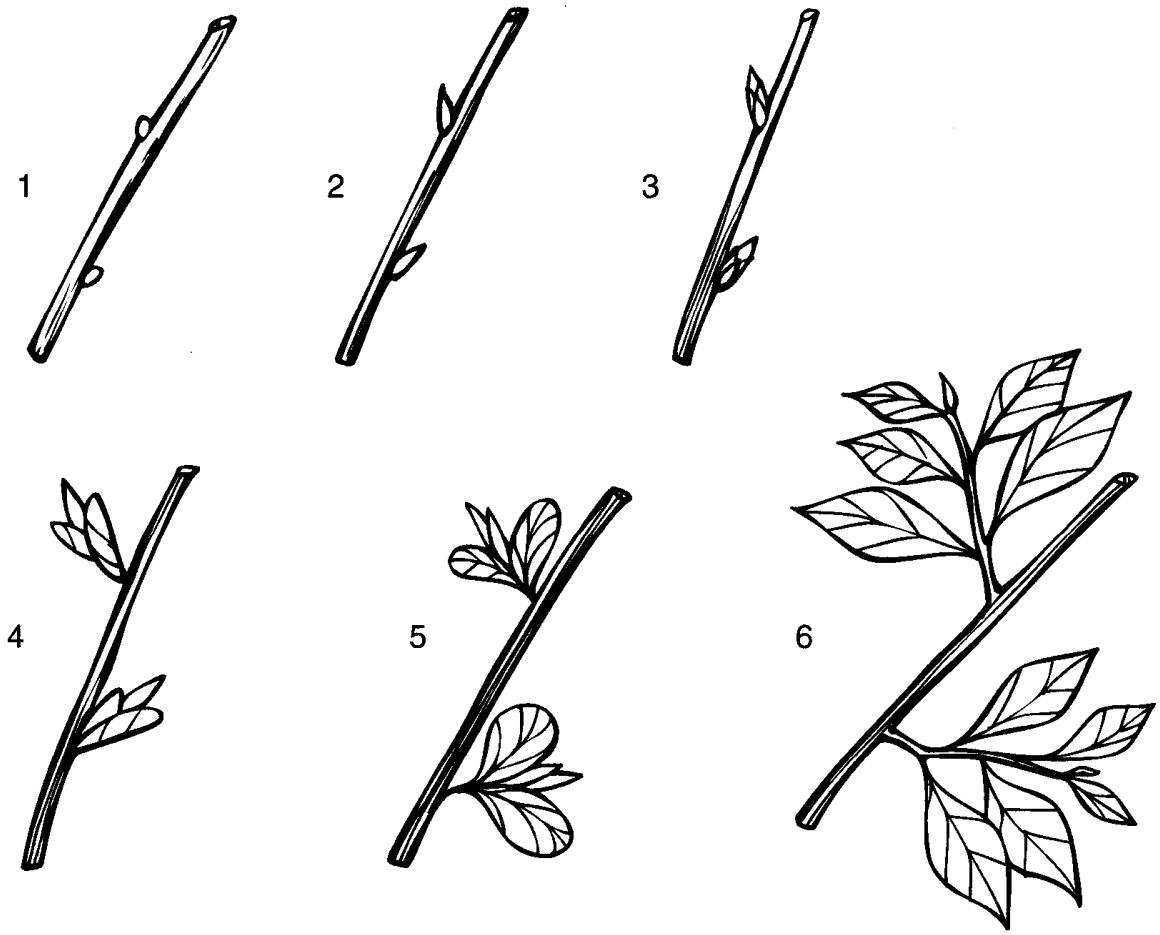

Fig.1. Vegetative bud development scale representing six distinct stages for rabbiteye blueberry: (1) dormant bud; (2) early green tip; (3) late green tip; (4) unfolding stage; (5) mouse-ear stage; (6) fully opened bud [Adapted from NeSmith et al., 1998].

2.2 Vegetative bud growth

Vegetative growth starts in early spring when vegetative buds expand as the leaves develop within bud scales (Pescie et al., 2011; Retamales and Hancock, 2018). The period required to achieve vegetative bud break tends to be longer than recorded for floral bud break, although this relies to some extend on the chilling duration, cultivar, and prevalent spring temperatures. At first, the leaves are closely packed around the stem, but become separated over time as the internodes expand (Retamales and Hancock, 2018). Vegetative buds can contain up to six leaf primordia, but as the shoot elongates the shoot apex initiates additional leaves approximately every five days (Retamales and Hancock, 2018). In NHB, vegetative budbreak occurs prior to flower budbreak, thus allowing flower development to be supported by

carbohydrates, produced by the newly formed leaves. However, in many SHB and RE blueberry cultivars, flower budbreak can occur before or concomitantly with vegetative budbreak, and in some cases, flowering can be advanced by as much as four weeks (Maust et al., 1999). In these cases, there is a period in spring where no leaves are present to support fruit development. Thus, the carbohydrate requirement for flowers and young developing fruit is met through carbohydrate reserves from the previous growing season or by current photosynthesis of the young fruit or flowers themselves (Birkhold et al., 1992).

Blueberry shoot growth is sympodial and episodic (Karimi et al., 2017). Individual shoots grow rapidly initially, before being terminated by a phenomenon known as 'black tip' where the apical meristem is aborted, a process that is completed within a period of one to two weeks (Retamales and Hancock, 2018). Two to five weeks after apical abortion, if conditions are favourable, an underlying axillary bud can be released from dormancy, giving rise to a new growth flush (Kritzinger, 2014; Lindberg, 2013; Retamales and Hancock 2018). New growth continues until the onset of the next 'black tip' which then gives rise to a second growth flush. Only one axillary bud is usually released from dormancy; however, in some cases, two or three axillary buds can break simultaneously (Eck, 1988; Retamales and Hancock, 2018).

Blueberry shoots can have one or even multiple growth flushes, with the majority occurring in mid to late spring and may continue until the end of summer (Karimi et al., 2017; Kovaleski et al., 2015; Lindberg, 2013). The number of shoot growth flushes depends on cultivar, environmental conditions and plant health (Karimi et al., 2017; Lindberg, 2013). SHB can have as many as five growth flushes in a growing season, while NHB typically have two or three growth flushes (Eck, 1988; Retamales and Hancock, 2018). There is also a tendency for early ripening cultivars to have more growth flushes than late-ripening cultivars, although this may not always be the case (Retamales and Hancock, 2018). According to Abbott and Gough (1987), maximum shoot growth coincides with the period of maximum root growth in highbush blueberry. In Argentina, most SHB exhibits two vegetative growth periods in the same year, the first occurring in spring, and the second, in summer, after fruit harvest (Pescie et al., 2011). The first growth period arises from vegetative buds on one-year-old wood, while the second growth period arises from vegetative buds formed on spring growth. In Brazil, 'Jewel' and 'Emerald' SHB cultivars also have two periods of shoot growth, the first in June (southern hemisphere) in winter for both cultivars, followed by

a second growth period in October (southern hemisphere) in spring for ‘Jewel’ and between November and December (southern hemisphere), for ‘Emerald’ (Medina et al., 2018).

2.3 Root growth

Abbott and Gough (1987) reported that ‘Earliblue’, ‘Bluecrop’, and ‘Lateblue’ NHB cultivars exhibit two root growth peaks during a growing season. The first and weaker peak, occurs in spring, near the time of bloom through to fruit set. The second peak occurs after fruit harvest and is completed before plants enter dormancy. Bryla et al. (2017) reported similar results with ‘Bluecrop’ and ‘Duke’ NHB cultivars (Fig. 2) grown in Oregon, USA. As in the case of ‘Earliblue’, ‘Bluecrop’, and ‘Lateblue’, the first root growth peak occurred in May (northern hemisphere) in late spring before harvest and the second peak occurred in September (northern hemisphere), about a month before the onset of dormancy in early autumn. Valenzuela-Estrada et al. (2008) determined root growth dynamics of ‘Bluecrop’ NHB, including that of root lifespan. In their study the first- and second-order root median lifespan ranged from 115 to 120 days, while third-order roots lifespan ranged from 136 to 155 days.

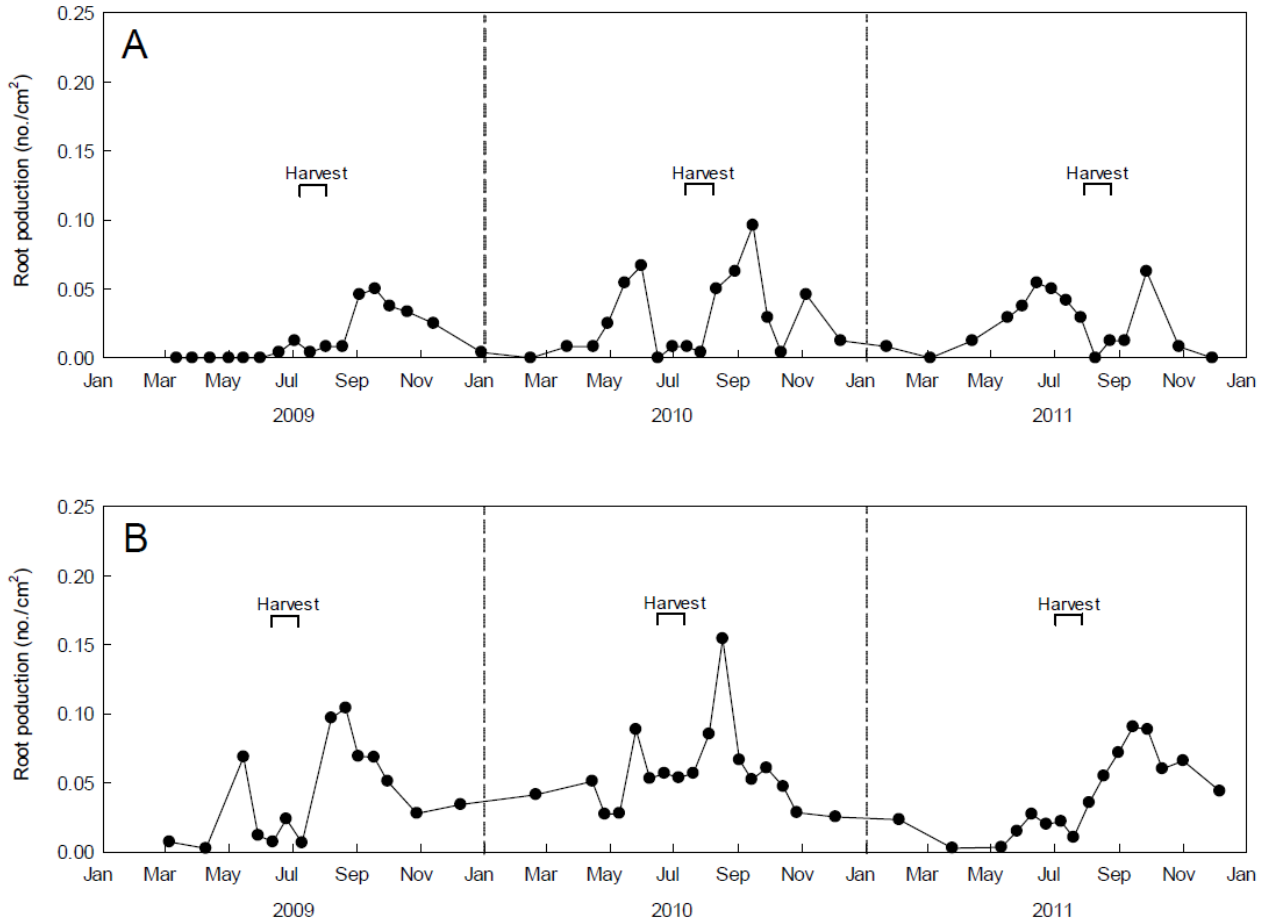

Fig. 2. New root production of 'Bluecrop' (A) and 'Duke' (B) northern highbush blueberry cultivars over three consecutive growing seasons in Oregon, USA (Adapted from Bryla et al., 2017).

3. Reproductive growth and development

3.1 Reproductive bud development scale

Garcia-Salazar (2002) developed a scale representing the flower bud stages of highbush blueberry. These flower bud stages have been widely used for various management practices such as growth regulator applications (NeSmith and Krewer, 1992). During the "dormant" (1) stage, there is no visible swelling of the flower buds and the outer bud scales are tightly closed. "Bud swell" (2) indicates the first sign of growth, where bud sprouting coincides with early spring. At this stage, there is visible swelling of the flower buds and the outer bud scales shows the first signs of separating from one another. During the "bud burst" (3) stage, the flower buds open, and the individual flowers can be seen between the bud scales. The next stage is the "tight

cluster" (4) stage where individual flowers become distinguishable within the flower cluster. "Early pink bud" (5) is when the expanding flowers become readily visible. During this stage, the pink corolla tubes (petals) are still short and closed. At the "late pink bud" (6) stage, most of the flowers on the bush are fully developed. The corollas have expanded, but remain closed, whilst exhibiting a colour change from a pink to white colour. The next stages are "25% bloom" (7) and "full bloom" (8) where 25% of the flowers display completely expanded corollas and where almost all flowers have on a bush have reached anthesis, respectively. During the "petal fall" (9) stage, the corolla tubes senesce and shatter, to reveal a small, green fruit. The initial stage of fruit growth is represented by the "early green fruit" (10) stage where small dark green fruit expands by cell division. The next stage is the "late green fruit" (11) stage where fruit growth occurs by cell expansion. During this stage, the fruit changes from a dark green colour to a pale green colour. "Fruit colouring" (12) implicate the fruit growth stages when colour changes from green to blue is initiated in the fruit along with the development of a softer fruit texture. The next stages comprise of the "first harvest" (13) and "harvesting" (14) stages where the first ripe fruit are harvested, and fruit are picked at various times as the fruit ripens. "Postharvest" (15) is the last phenological stage when the blueberry plant accumulates carbohydrate reserves for the following season's growth.

3.2 Floral growth

In most climates, vegetative axillary buds located in the leaf axil of the current season's shoot can convert to flower buds during late summer and autumn (Pescie et al., 2011; Strik, 2012; Retamales and Hancock, 2018). This conversion which signals flower bud initiation and development is considered as one of the most important developmental processes in the blueberry productive cycle (Williamson et al., 2020). Flower bud initiation, which is well documented in NHB and RE blueberries, generally occurs in the year preceding flowering and fruiting. Floral initiation commences only after shoot growth cessation occurs (Aalders and Hall, 1964; Banados and Strik 2006; Gough et al., 1978; Pescie et al., 2011). According to Pescie et al. (2011), flower bud initiation occurs twice a year in SHB in temperate to warm-temperate conditions, on spring growth in summer and then again, on summer growth in autumn. Flower bud initiation proceeds basipetally along canes, while flower initiation within individual

racemes occurs acropetally (Kovaleski et al., 2015; Retamoles and Hancock, 2018; Strik, 2012; Tamada, 1997). The period from flower bud initiation to the point of full floral evocation, endures for about two months in NHB grown in New Jersey (USA), while for SHB grown in the southern USA, it spans over a period of two to three months (Gough et al., 1978; Huang et al., 1997; Kovaleski et al., 2015).

In autumn, as temperatures decrease and short days start to prevail, blueberries enter a state of dormancy. Dormancy is released in spring when leaf and floral growth commences, once the winter chilling requirements are satisfied (Retamales and Hancock, 2018). Flower buds start to burst over a period of three to four weeks in spring, depending on cultivar and temperature (Retamales and Hancock, 2018). Following a basipetally sequence, terminal buds generally reach anthesis first, followed by lateral buds (Garcia-Salazar, 2002). Flowers from the terminal buds will typically have the potential to develop into larger fruit than flowers from lower buds, which tends to be smaller (Garcia-Salazar, 2002). The number of flowering peaks observed in blueberry is cultivar dependant (Medina et al., 2018). In Brazil, ‘Emerald’ SHB, exhibits two flowering peaks, the first occurring between mid-June and late July (southern hemisphere) in winter, which is then followed by a second flowering peak, between mid-September and mid-October in spring. ‘Jewel’ SHB however only displays one flowering peak, occurring mainly between mid-September and mid-October (southern hemisphere) in spring (Medina et al., 2018).

According to Eck (1988), the period from flower bud initiation to the onset of flowering is about nine months. Timing of onset of flowering correlates with the timing of flower bud initiation where earlier initiation results in earlier flowering as can be seen for SHB cultivars, ‘Emerald’ and ‘Jewel’, grown in Florida, USA (Kovaleski, 2015). It was noted however that inflorescence bud initiation occurred earlier in ‘Emerald’ compared to ‘Jewel’. The buds of ‘Emerald’ became visible in late summer, in contrast with ‘Jewel’, where the buds only became visible in late autumn. Early initiated inflorescence buds of ‘Emerald’ provided the advantage of larger, more developed buds which led to earlier flowering in ‘Emerald’ in spring compared to ‘Jewel’, where flowering occurred later in spring.

3.3 Fruit growth

Blueberry fruit development is somewhat dispersed and occurs over an extended period (Wang et al., 2018). This is due to blueberry fruit reaching maturity at different rates on a branch, which then requires three to five harvests over a growing season, depending on the cultivar (Wang et al., 2018). Blueberry fruit development is characterized by a double sigmoid growth curve (Fig. 3) (Retamales and Hancock, 2018). During Stage I, rapid cell division and dry weight gain occurs directly after flowering (Birkhold et al., 1992; Tamada, 2002). Stage I ranges between 25 and 35 days, depending on cultivar and environmental conditions (Retamales and Hancock, 2018). Tamada (2002) reported a study in Japan where Stage I lasted for a similar period in NHB and RE blueberries, from mid-May to early June (northern hemisphere) in late spring. During Stage II, also known as the 'slow growth stage', active seed development takes place, whilst little fruit growth is observed (Edwards et al., 1972; Tamada, 2002). Stage II lasts from 30 to 40 days, depending on cultivar and environment, as well as the number of viable seeds (Darnell, 2006). RE blueberries generally have a longer stage II than that of highbush cultivars, but there is considerable overlap (Edwards et al., 1972). Tamada (2002) reported that Stage II lasted for between five and six weeks for RE blueberries and four weeks for NHB. Rapid fruit growth through cell enlargement occurs during Stage III, which lasts for 30 to 60 days until fruit maturation (Tamada, 2002). This stage period also depends on cultivar and environmental conditions. The total length of the fruit development period ranges from 42 to 90 days in NHB, from 55 to 60 days in SHB, and from 60 to 135 days for RE blueberries (Darnell, 2006).

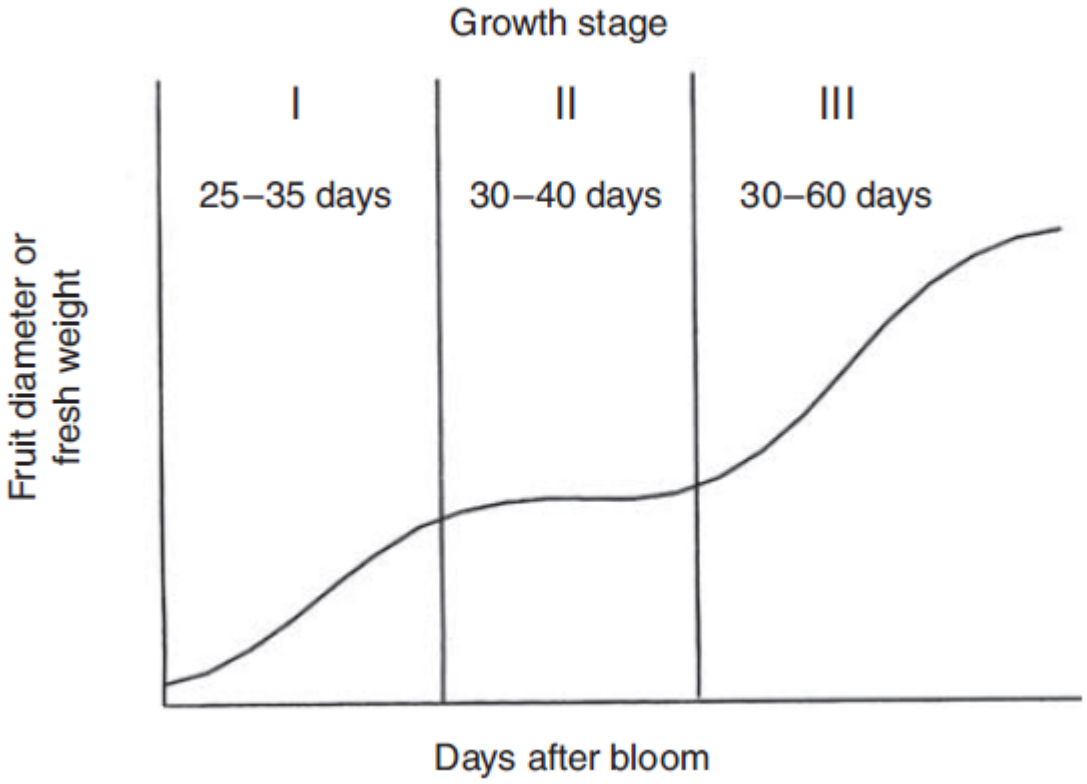

Fig. 3. Double sigmoid growth curve of blueberry fruit development [Adapted from Retamales and Hancock, 2018].

4. Low chill evergreen blueberries

In general, deciduous fruit trees enter endodormancy in autumn, followed by the accumulation of chill hours in winter (Swain and Darnell, 2001). After the chill requirement is satisfied, vegetative and reproductive buds are released from endodormancy and, subsequently, budbreak will proceed, depending on environmental conditions. However, in areas with mild winter temperatures with insufficient chilling hours, budbreak and fruit development will be delayed, prolonged, and/or reduced (Swain and Darnell, 2001). The expansion of deciduous fruit production into warm temperate, subtropical, and tropical areas is therefore limited due to insufficient chilling hours (Darnell and Williamson, 1997; Swain and Darnell, 2001). However, growing low chill blueberry cultivars in an evergreen production system can increase the feasibility of commercial blueberry production in such areas (Darnell and Williamson, 1997).

Evergreen blueberry production systems have been used successfully in subtropical areas such as in subtropical eastern Australia and Southwest Florida, by

avoiding endodormancy and the associated chilling requirement (Swain and Darnell, 2001; Lyrene, 2005). Plants grown in an evergreen production system maintain their leaves longer into winter, allowing these leaves to support fruit development during late winter and spring, without the need to produce strong new leaves in spring (Lyrene, 2005; 2006). Timing of new leafing differs between cultivars, some cultivars leaf mid-way through harvest, whilst in others, leafing may only occur towards the end of harvest (Scalzo et al., 2016). The time of leafing is important as the development of new leaves in evergreen blueberry plants during peak harvest is likely to affect fruit quality. Retaining leaves longer through autumn and winter allows continued perception of short-day photoperiods and therefore, also continued flower bud initiation. This promotes increased flower bud numbers compared to plants grown in dormant production systems (Strik, 2012).

In Florida, USA, SHB cultivars grown in an evergreen production system, allows growers to harvest berries at least one month earlier than possible in low-chill deciduous cultivars (Baptista et al., 2006; Lyrene, 2005; Scalzo et al., 2016; Swain and Darnell, 2001). For evergreen SHB cultivars, flower bud break generally occurs during early winter and provides a crop during late winter to late spring, while for deciduous cultivars, flower bud break occurs during mid-winter to early spring and harvest generally starts during mid-spring (Baptista et al., 2006; Fang et al., 2020; Swain and Darnell, 2001). Cultivars grown in an evergreen production system have long, protracted reproductive seasons (Scalzo et al., 2016). Flowering, fruit set, and berry maturation may happen simultaneous to various degrees on a plant, resulting in a less concentrated harvest season than what is found in a deciduous production system (Baptista et al., 2006; Swain and Darnell, 2001). Harvest is usually concentrated in a time span of two to six weeks for deciduous cultivars, while for cultivars grown in an evergreen system, the harvest season can continue for up to three months (Baptista et al., 2006; Fang et al., 2020; Swain and Darnell, 2001).

5. Environmental factors influencing growth and development

5.1 Temperature and photoperiod

Several studies showed the effect of photoperiod and temperature on flower bud initiation in highbush- and RE blueberries (Darnell, 1991; Hall et al., 1963; Spann et al., 2003; Spann et al., 2004). Flower bud initiation is promoted under short days in

highbush blueberry plants (Darnell, 1991). Flower bud initiation is increased significantly in NHB exposed to 8-, 10- or 12-h photoperiods (short-day, SD) for eight weeks, compared to exposure to 14- or 16-h photoperiods (long-day, LD) (Hall et al., 1963). Darnell (1991) showed that 'Beckyblue' RE blueberry plants exposed to 8-10 h photoperiods in autumn initiated more flower buds and had a more concentrated bloom period than plants exposed to 11-12 h photoperiods. Spann et al. (2003) reported that flower bud initiation only occurred when 'Sharpblue' and 'Misty' SHB were exposed to SD 8-h photoperiods and not in plants exposed to LD 16-h photoperiods. Banados and Strik (2006) also compared early, mid-season as well as a late season highbush blueberry cultivar. Plants were exposed to either SD (8-h of light) or LD (16-h of light) photoperiods, for eight weeks. Similar to previous results, flower bud formation only occurred under SD photoperiods. Vegetative growth was also affected, with plants held under LD growing taller with more growth flushes than plants grown under SD. According to Darnell and Williamson (1997), cultivars vary in their sensitivity to photoperiod, suggesting that careful considerations must be taken in the selection of cultivars, to prevent any flower bud initiation limitations in areas of low latitudes. Although there is limited information on the interaction of photoperiod and temperature on flower bud initiation, Spann et al. (2004) showed that flower bud initiation of 'Misty' SHB was significantly reduced at relative high temperatures of 28 ºC during short day exposure for 4 weeks, compared to 21 ºC.

Abbott and Gough (1987) reported that root growth peaks in highbush blueberries occurred when soil temperatures ranged between 14 °C and 18 °C. However, root growth was reduced at temperatures below 8 °C. Southern highbush- and RE blueberries exposed to different substrate temperatures (16 °C, 27 °C and 38 °C) showed that accumulated root and shoot total dry mass was greatest at 16 °C, with a negative linear response associated with an increase in substrate temperature (Spiers, 1995). Zheng et al. (2017) reported studies on the optimal temperature for the growth of six highbush blueberry cultivars blueberry cultivars ('Bluecrop', 'Duke', 'Brigitta', 'Gulfcoast', 'O'Neal', and 'Blue Ridge'). Plants were grown in four growth chambers for 90 days and subjected to temperatures of 25, 30, 35 and 40 ºC, respectively. For 'Bluecrop', 'Duke', 'Brigitta' and 'Blue Ridge' NHB cultivars the aboveground, belowground, and total biomass was enhanced dramatically with an initial increase in temperature, with an optimum at 32.6, 30.4, 31.8 and 29.0 ºC for the four cultivars respectively. However, a decrease in all characteristics followed, with

any further increase in temperature. However, for 'Gulfcoast' and 'O'Neal' SHB cultivars, the biomass declined linearly with increasing growth temperatures, suggesting that temperatures above 25 ºC had a negative effect on plant growth.

Temperature is known to have a strong influence on the variation in flowering time, ripening intervals and harvest dates of highbush- and RE blueberries (Carlson and Hancock, 1991; Finn et al. 2003; Retamales and Hancock, 2018). Flowering time and petal drop are generally advanced by high spring temperatures (Retamales and Hancock, 2018). Fruit set and size were greater, and harvest period were more extended when highbush blueberries were grown in cooler greenhouse temperatures (8-24 ºC) than in higher temperatures (16-27 ºC) (Knight and Scott, 1964). This trend is similar to that observed for RE blueberries, when grown under warm nights (21 ºC) compared to cool nights (10 ºC) (Williamson et al.,1995).

5.2 Chilling requirement

During autumn, when short days start to prevail and temperatures decline, blueberries enter dormancy, whereafter a period of low temperatures is required for normal growth and development to occur (Retamales and Hancock, 2018). NHB cultivars require chilling hours ranging from 800-1200 h (0-7 ºC), whereas SHB- and RE blueberry cultivars require chilling hours ranging from 100-800 h and 300-700 h, respectively (Darnell and Davies, 1990; Williamson et al., 2002). Insufficient chilling during the dormant period results in delayed and erratic budbreak as well as delayed fruit development (Darnell and Davies, 1990). RE blueberries exposed to temperatures below 7 ºC, ranging from 100-1000 hours, showed that insufficient chilling increased the days to 50% vegetative and flower budbreak, as well as the time to fruit set (Darnell and Davies, 1990). Days to 50% vegetative and flower bud break decreased as chilling increased, for all cultivars.

6. Management practices effect on growth and development

6.1 Irrigation

6.1.1 Water deficit

Blueberry growth and development is strongly influenced by irrigation (Holzapfel and Hepp, 2002). Since blueberry roots are superficial and lack root hairs,

any mild episodes of drought will negatively affect vegetative growth, with a consequence where fruit development, in severe cases, can even be diminished (Holzapfel and Hepp, 2002; Ortega-Farias et al., 2021). The general trend is that vegetative and reproductive growth decrease with a reduction in water levels (Mingeau et al., 2001; Ortega-Farias et al., 2021; Almutairi et al., 2017; Lepaja et al., 2019). Mingeau et al. (2001) reported the vegetative and reproductive growth of 'Bluecrop' highbush blueberries to be affected by water stress. Either a mild (with a 35% reduction in transpiration) or severe water stress (with a 65% reduction in transpiration) was imposed for three weeks during fruit growth (weeks 7 to 4 before peak harvest), fruit ripening (weeks 4 to 1 before peak harvest), harvest (from a week prior to a week after peak harvest) and postharvest (weeks 4 to 7 after peak harvest). Sensitivity to water stress was evident with respect to fruit formation and maturation. Most shoot elongation occurred during the green fruit stage for control plants (no water stress), whereas stressed plants had negligible shoot elongation. Water stressed plants also had no increase in stem diameter throughout the vegetative season. Furthermore, fruit yields were reduced by 31% and 49% when plants were subjected to severe water stress during the initial fruit growth period as well as near harvest, respectively.

Lobos et al. (2016) evaluated the effect of regulated deficit irrigation (RDI) on growth and fruit yield of 'Brigitta' NHB cultivar. Three irrigation treatments were applied to plants: 50, 75, 100% (control) of actual evapotranspiration (ET_a). Severe deficit irrigation (50% ET_a) decreased vegetative growth as well as yield. Almutairi et al. (2017) evaluated new strategies to reduce water use in 'Elliott' NHB cultivar, including RDI, irrigation cut-offs and crop thinning. Treatments consisted of no thinning or 50% crop removal in combination with either full irrigation at 100% of estimated ET_c, deficit irrigation at 50% ET_c (applied for the entire growing season), or full irrigation with irrigation cut-off for 4-6 weeks during early (early- to late green) or late (fruit colouring to harvest) stages of fruit development. Results showed that the harvest season was advanced with either RDI or cut-off during late stages of fruit development, whereas cut-off during early fruit development stages, delayed the harvest season. Yield was reduced when irrigation was cut off during late fruit development, but was unaffected by the other irrigation treatments. Lepaja et al. (2019) showed that total yield and individual fruit weight of 'Bluegold' highbush blueberry was highest when irrigated at 100% of the estimated ET_c, followed by 75% and 50% of the estimated ET_c. Ortega-

Farias et al. (2021) showed similar results when four RDI irrigation treatments [50, 75, 100 and 125% of crop evaporation (ET_c)] were applied to 'Tifblue' RE blueberry cultivar during fruit growth. Yield and individual fruit weight were reduced as cumulated water stress increased during the harvest period.

6.1.2 Excess water

The effects of flooding or supplying excess water to blueberry plants vary with duration, time of season and sometimes the species, with RE blueberries reported to be more flood tolerant than highbush blueberries (Retamales and Hancock, 2018). Highbush blueberry can tolerate extended periods of flooding during the active spring growth period; however, flooding during any other time of the season can have a severe impact on plant growth and development (Darnell, 2006). Abbott and Gough (1987) showed that, after approximately 4 months of flooding, vegetative growth was severely reduced in several highbush blueberry cultivars, with a decrease in shoot and internode length, number of nodes, leaf size and root dry weight. Reproductive growth was also reduced by flooding, with the highest damage occurring when flooding coincided at budbreak. When flooded, plants had fewer inflorescence buds, fewer flowers per inflorescence, exhibited delayed bloom, whereas fruit weight was also decreased significantly. Vegetative growth was similarly restricted when water was applied in excess (125% of ET_c) in 'Bluetta' blueberry plants (Holzapfel and Hepp, 2002).

6.1.3 Irrigation method

Bryla et al. (2011) reported that drip irrigation during the first two years after planting 'Elliot' NHB, produced the largest plants with the highest number of new canes and cane dry weight when compared to micro-sprayers and sprinklers. Root dry weight was also affected by irrigation method, with drip irrigation producing the highest root dry weight at 0.23 $kg.plant^{-1}$, followed by micro sprayers and sprinklers at 0.21 and 0.20 $kg.plant^{-1}$ respectively. It was suggested that 'Elliot' benefitted from drip irrigation because of a higher plant water status due to a higher soil water content in the root locality (Bryla et al., 2011). However, drip irrigation was not beneficial in 'Duke' NHB. Root samples revealed that 'Duke' plants were infected by *Phytophtora cinnamomi*, which causes root rot, with wet soil conditions due to drip irrigation that created more

conducive conditions for this disease. Holzapfel and Hepp (2002) reported that 'Bluetta' blueberry plants obtained a higher yield and fruit size when irrigated with micro-sprayers compared to drip irrigation. According to Patten et al. (1988), micro-sprayers wetted a larger soil volume than drip irrigation and in effect, plants tended to produce larger root systems. This is especially applicable to blueberry plants with its shallow, dense root system. Vargas et al. (2015) evaluated the effect of nitrogen application via drip and alternative micro-irrigation systems (geotextile tape and micro-sprinklers) on growth and early fruit production of several NHB blueberry cultivars. Plant size, in terms of canopy cover, was the highest with the geotextile tape irrigation system, followed by drip irrigation and micro-sprinklers. By the third year, yield was similar for geotextile tape and drip irrigation, but ranked lowest for micro-sprinklers.

6.2 Mulch

Mulching is used to manage weed infestation, but also provides other horticultural benefits. The type of mulch used may affect root growth differently, by altering the soil moisture and temperature (Strik et al., 2020). Sawdust and other organic mulches such as pine, bark and hardwood chips can reduce soil temperature during warm summers (Strik et al., 2020). As the optimum temperature for root growth for highbush blueberry is between 14 and 18 ºC (Abbott and Gough, 1987), reduced soil temperatures in summer will be more favourable to promote root development. This was illustrated in RE blueberry, where sawdust mulch increased both root weight and plant height compared with bare soil (Patten et al., 1988). In recent years, weed mat (synthetic geotextile landscape fabric) is a more preferred option than mulch (Strik et al., 2020). However, weed mat captures more longwave radiation and results in higher soil temperatures than organic mulches (Strik et al., 2017). Although weed mat alone does not show promising results in promoting plant growth characteristics, great promise is showed when adding a layer of organic mulch under the weed mat in NHB. This concept was demonstrated with 'Duke' NHB, where plants grown under weed mat with a sawdust layer underneath resulted in a larger root system in the first year of application, while showing a larger canopy volume in the next season, compared to when just weed mat was used (Strik et al., 2020). Total plant dry weight was also much higher when plants were mulched with weed mat over sawdust. The larger root system in the first season following treatment was ascribed to an increased canopy maximum

temperature together with a deceased soil temperature, with the added sawdust layer (Strik et al., 2020). The sawdust served as an insulator between the weed mat and soil and thus reduced the thermal conduction while increasing convective heat transfer to the canopy (Strik et al., 2020). Studies from Brazil showed that the use of weed mat in RE blueberry ('Climax', 'Delite' and 'Powderblue') and SHB ('Georgiagem', 'Misty' and 'O'Neal') orchards can also produce plants with a higher average cane diameter, cane number and plant height (Pasa et al., 2014).

Mulching encourages the concentration of root systems near the soil surface (Bryla et al., 2017). However, many growers use weed mat as mulch with drip irrigation. Drip irrigation concentrates roots near the drip emitters, while black geotextile fabrics tend to increase soil temperature which in turn results in deeper root development (Bryla et al., 2017). White or reflective mulches tend to reduce soil temperature and could possibly have the opposite effect on root distribution than that of black geotextile fabrics (Bryla et al., 2017).

6.3 Soil pH

Soil pH is an important factor affecting blueberry growth (Jiang et al., 2019). Maximum growth occurs when highbush- and RE blueberries are grown in substrates with a pH in the range of 4.2-5.5 (Retamales and Hancock, 2018). Jiang et al. (2019) studied the effect of different soil pH treatments (4.5, 5.3 and 6.0) on plant growth and fruit yield of 'Climax' RE blueberry- and 'Chaoyue No.1' SHB cultivars. The growth characteristics of plant height, main stem diameter, branch number per plant, total plant dry weight, stem dry weight and leaf dry weight, all decreased with an increase in soil pH. First flowering date, 50% flowering date, first ripening date, 50% ripening date was delayed for both cultivars with an increase in soil pH. Yield also decreased with an increase in soil pH. This result is supported by previous results on 'Tifblue' RE blueberry, where yield decreased with an increase in soil pH from 4.5-7.0, as well as in 'Delite' RE blueberry, where the highest yield occurred in a soil pH lower than 5.0 (Austin and Bondari, 1992).

6.4 Cultivation under high tunnels

In Japan, fruit ripening of many SHB cultivars occurs during the rainy season; however, growers showed an interest in enhancing fruit maturity to harvest before the

onset of the rainy season (Tamada and Ozeki, 2012). Unheated, high tunnels, constructed with metal ribs and covered with polyethylene film, may protect blueberries from frost incidents, and advance fruit ripening (Gaskell, 2004; Ogden et al., 2011; Tamada and Ozeki, 2012; Li and Bi, 2019). Temperature is controlled by passive solar radiation and ventilation, making these tunnels independent of automatic heating or cooling systems (Li and Bi, 2019). High tunnels may raise air temperatures by 10 to 20 ºC and soil temperatures by 4 to 8 ºC above ambient temperatures (Ogden and van Iersel, 2009). Higher air and soil temperatures enable growers to produce fruit out-of-season and access high price windows (Ogden and van Iersel, 2009; Santos and Salame-Donoso, 2012). Positive findings have been reported on NHB- and SHB cultivars grown under unheated, high tunnels (Baptista et al., 2006; Li and Bi, 2019; Ogden and van Iersel, 2009; Renquist, 2005; Retamal-Salgado et al., 2015; Santos and Salame-Donoso, 2012; Tamada and Ozeki, 2012). Most of these studies have similar results, being that blueberry plants grown under high tunnels produced ripe fruit earlier than the same cultivars grown in an open field. Fruit ripening occurred 7 - 40 days earlier for NHB- and SHB cultivars grown under high tunnels in Japan than in an open field (Tamada and Ozeki, 2012). Li and Bi (2019) reported that SHB cultivated under high tunnels in Mississippi advanced fruit ripening by 4 to 5 weeks compared to RE blueberries grown in an open-field system, in the same area. Renquist (2005) demonstrated that plastic tunnels can advance fruit ripening by seven to 22 days in six SHB cultivars and eight NHB cultivars in Oregon, USA.

6.5 Cross-pollination

Various SHB cultivars are commonly inter-planted to enhance cross-pollination (Taber and Olmstead, 2016). The benefits associated with cross-pollination of SHB with compatible pollinators include improved fruit set and fruit size, and advancing harvest by contracting the fruit ripening period (Huang et al., 1997; Lang and Danka, 1991; Lang and Parrie, 1992; Taber and Olmstead, 2016). Lang and Danka (1991) evaluated the effect of self- and cross-pollination on fruit development in ‘Sharpblue’ SHB with other ‘Sharpblue’ or ‘Gulfcoast’ plants, mediated by honeybees. Cross-pollination increased fruit weight, seed number per fruit, as well as shortened the fruit development period. Cross-pollination accelerated the fruit development period and increased the proportions of early-ripening fruit (34.1% vs. 14.2%) while reducing the

proportions of late-ripening fruit (9.5% vs. 30.9%) compared to self-pollination. Similarly, Taber and Olmstead (2016) showed that fruit set, fruit weight, and seed number of several SHB cultivars were increased by cross-pollination, while harvest was advanced by shortening the fruit ripening period.

Müller (2011) showed that the effect of cross-pollination on fruit set, berry weight and diameter and fruit development period is cultivar specific, based on findings in 'Star', 'Emerald', 'Jewel', 'Bluecrisp' and 'Snowchaser' SHB cultivars. Along with increased berry size, diameter, and fruit set, 'Bluecrisp' cross-pollinated with 'Star', 'Jewel' and 'Misty', resulted in a shorter fruit development period than for unpollinated or self-pollinated fruit. Furthermore, 'Star' cross-pollinated with 'Bluecrisp', 'Jewel' cross-pollinated with 'Misty', 'Emerald' cross-pollinated with 'Misty', and 'Snowchaser' cross-pollinated with 'Misty' resulted in a shorter fruit development period, respectively. Therefore, cross-pollination can be a successful approach to shorten the fruit development period in SHB; even though the response to cross-pollination is cultivar specific (Müller, 2011; Taber and Olmstead, 2016).

6.6 Plant growth regulators

Blueberry fruit ripening, as in other fruit, is regulated by multiple plant hormones such as abscisic acid (ABA), auxins, ethylene and jasmonates. Certain plant growth regulators (PGRs) can be used to alter the action of these hormones and potentially accelerate fruit ripening and concentrate the ripening period (Retamales and Hancock, 2018; Wang et al., 2018). Wang et al. (2018) proposed to accelerate fruit ripening with ethephon applications in two RE blueberry cultivars, 'Premier' and 'Powderblue'. Ethephon applied at 250 mg L^{-1} when 30 to 40% of fruit on the plant were ripe, advanced the time required for 50% of fruit to ripen by up to 3 days, compared to when no ethephon was applied. Along with accelerated ripening, the amount of ripe fruit increased by 1.5-1.8- fold within 4 to 7 days after application, in both cultivars. According to Retamales and Hancock (2018), fruit ripening has been accelerated successfully in previous studies, using ethephon to reduce the number of required harvests. However, ethephon application also showed to incur negative effects on postharvest fruit quality parameters such as a decrease in fruit weight, titratable acidity, and fruit firmness, as well as an increase in fruit pH. Although ethephon has the potential to accelerate fruit ripening and concentrate the fruit ripening period,

further studies are needed to determine whether ethephon applications can accelerate fruit ripening of SHB (Wang et al., 2018).

Blueberry cultivars grown in areas with mild winters and insufficient chilling tends to have dispersed and extended budbreak and flowering times in spring (Jaldo et al., 2009). Applications of hydrogen cyanamide or H_2CN_2 (commercially known as Dormex®) during dormancy in various blueberry cultivars reduced the effect of insufficient chilling and enhanced leaf and flower budbreak, as well as concentrated the flowering window and advanced fruit ripening (Williamson et al., 2001, Williamson et al., 2002; Stringer et al., 2002; Jaldo et al., 2009; Arias et al., 2010; Retamales and Hancock, 2018). The effect of H_2CN_2, depending on the timing of applications, varied between cultivars (Arias et al., 2010). Williamson et al. (2002) reported on the use of H_2CN_2 on 'Misty' SHB under field conditions in the southern USA. When H_2CN_2 was applied after 36-40 chilling-hours (hours between 0 and 7.2ºC) had accumulated, earlier and stronger leafing was promoted compared to untreated plants (control). This response led to a secondary effect, advanced fruit ripening by 10 to 14 days, as well as a more concentrated fruit developing period. Vegetative budbreak increased linearly with increased H_2CN_2 concentrations. Swart (2015) showed that fruit ripening in 'Bluecrisp' SHB could be accelerated with H_2CN_2 applications (1 or 2% v/v) at different phenological stages; however, no significant results were obtained for 'Star' and 'Emerald' cultivars. Both H_2CN_2 application rates induced similar results; however, the 1% HCN concentration had a lower risk for flower bud damage than when 2% HCN was applied. Swart (2015) concluded that further studies are required for new cultivars, as the response to H_2CN_2 has been shown to be erratic and differed between cultivars, stage of bud dormancy, H_2CN_2 concentration, and climatic conditions during and after application.

6.7 Timing and severity of pruning

Regular pruning is of utmost importance to sustain productivity in highbush blueberry. NHB is generally pruned in winter being the dormant season, while SHB can be pruned both in summer after harvest, or during the following dormant season (Retamales and Hancock, 2018). However, in warmer production areas, summer pruning is used to complement or even replace winter pruning (Bañados et al., 2009). Summer pruning is performed during the period of active growth, either in spring or

summer, with the objective to stimulate lateral branching by heading back vigorous shoots and to remove excess vigour by shoot thinning (Bañados et al., 2009). The two key factors influencing the plant response are the time and intensity of pruning. Apical dominance is removed when cutting back vigorous shoots which stimulates lateral bud break; however, the degree of the response is influenced by the dormancy stage of the buds (Bañados et al., 2009). Pruning early in summer generally results in more and longer laterals, whereas pruning in late summer will induce growth cessation (Bañados et al., 2009; Müller, 2011).

Flower bud induction can be delayed by pruning in summer, as induction only occurs after growth cessation in blueberry, although this effect is not as evident in regions with warm, long summers where high temperatures stimulate bud formation until late in the season (Bañados et al., 2009). Cutting back of vigorous shoots to 20-30 cm in 'O'Neal', 'Star' and 'Elliott' in Chile in early summer (December, southern hemisphere), resulted in more and longer laterals, compared to pruning in late summer, during February or March (Bañados et al. (2009). Shoots produced no laterals when pruned during late summer (mid-February), for all cultivars. Harvest was also delayed with 14 days in 'O'Neal' and 'Star' with all pruning treatments, and with seven days in 'Elliott', compared to no pruning. Studies by Müller (2011) on 'Star', 'Emerald' and 'Jewel' in South Africa also showed a decrease in total new laterals as time of pruning progressed from mid-December to mid-March, at three-weekly intervals.

The general trend with more severe pruning cuts is the production of longer young shoots that will produce larger berries (Jansen, 1997). The number of flower buds is reduced by pruning and therefore also the number of berries. Thus, pruning results in an increase in berry size, but a decrease in total yield (Jansen, 1997). In Oregon (USA), Strik et al. (2003) reported a 19% increase in berry weight in 'Bluecrop' and 'Berkeley' NHB cultivars when plants were conventionally pruned compared to no pruning, but obtaining a lower yield than that of unpruned plants. Pruning entitled the removal of unproductive canes, thinning of one-year-old shoots at the plant crown, and the removal of unproductive or excessive fruiting shoots near the top of the plant. The harvest season for conventionally pruned plants was also advanced by about a week compared to unpruned plants.

Speed-pruning, where one or two of the most unproductive canes is removed at the base of the plant, in the same trial showed intermediate results for all the

characteristics. This was also confirmed by Müller (2011) on ‘Star’, ‘Emerald’ and ‘Jewel’ SHB cultivars, where five pruning treatments were applied: 1) no pruning; 2) severe pruning where all shoots were headed back to 35 cm above the soil surface or to a lateral thicker than 6 mm, followed by heading the lateral back to 20 cm from the inception; 3) standard pruning which consist of the removal of all unproductive canes, old bearing wood and weak low growing branches and where canes were headed back to 3 or 5 productive laterals or to 35 cm when no productive lateral was present on the cane; 4) standard pruning plus heading which were similar to 3), but all the remaining laterals were headed back by a third; 5) light pruning which consist of the removal of only old bearing wood by heading the laterals to just below the bearing section. It was showed that, for all summer pruning treatments, total vegetative growth and total number of shoots were lower compared to no pruning; however, individual shoot lengths were higher. Berry weight and diameter were increased by summer pruning, but yield decreased. However, plants which received summer pruning produced better quality laterals. Summer pruning delayed termination of growth on more vigorous laterals which resulted in a delay in harvest. The extent to which the plants responded increased with an increase in pruning severity.

6.8 Early cropping

Canopy development during the first years of plant establishment is primarily affected by crop load during this time (Retamales and Hancock, 2018). This is due to the greater demand for assimilates by the fruit compared to the whole-canopy leaf area. NHB cultivars ‘Duke’, ‘Bluecrop’ and ‘Elliott’ illustrated the effects of early cropping on plant growth and fruit yield in the third year since planting, compared to a control where no yield was produced during the first two years following planting (Strik and Buller, 2005). Evaluations in the third year following planting showed that early cropping during the first two years reduced the dry weight of the root system (as much as 57%), crown and one- to three-year-old wood in all cultivars. The percentage fruit buds were also lowered for early cropping compared to control plants. Yield was reduced by 44%, 24% and 19% in the third year of planting for ‘Elliott’, ‘Duke’ and ‘Bluecrop’, respectively, compared to the control. The cumulative yield through the first four years of cropping was similar between the control and early cropping plants in ‘Bluecrop’ and ‘Duke’, while for ‘Elliott’ early cropping reduced the cumulative yield.

Early cropping thus holds a potential long-term risk for fruit yield, depending on cultivar (Strik and Buller, 2005).

7. Conclusion

Numerous advances regarding manipulation of SHB blueberries were achieved during the last decade. Management practices such as drip irrigation and weed mat with an added layer of sawdust as mulch improved plant growth and increased the root system (Bryla et al., 2011; Strik et al., 2020).

Müller (2011) showed that summer pruning decreases yield compared to no pruning, but produces laterals of better quality and larger berries of better quality, which is of utmost importance for the South African industry to compete with other countries for the northern hemisphere market.

Harvest time can be manipulated via regulated deficit irrigation (RDI) (Almutairi et a., 2017) or polyethylene tunnels (Gaskell, 2004; Ogden et al., 2011; Tamada and Ozeki, 2012; Li and Bi, 2019).

Despite these advances, there are still opportunities to obtain premium local prices in South Africa, from the end of summer until early winter (personal communication Laubscher, 2022), when supply in Europe is limited. Thus, more research is required to quantify growth and development of the different SHB blueberry cultivars under evergreen growing conditions to develop suitable management strategies for harvest window manipulation to benefit from premium prices during these periods.

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PAPER 1: The seasonal progression of the reproductive phenology of southern highbush blueberry (*V. corymbosum* L. interspecific hybrids) cultivars 8-42 and 9-02, at two sites, in the Western Cape, South Africa.

Abstract

The objective of this study was to evaluate the timing and duration of selected flower bud phenological stages of two southern highbush blueberry (SHB) cultivars in a Mediterranean climate to optimize the management of commercial production systems. The experiment was conducted in commercial orchards during the 2020 growing season, in the Hex River and Wolseley production areas, Western Cape, South Africa. The timing (date) and duration (days) of selected flower bud stages were recorded on individual plants, from April to November 2020 for bud swell, bud break, tight cluster, closed flower, open flower, petal fall, green fruit, colouring fruit, and fruit harvest respectively. Standard commercial management practices were followed on both sites. Cultivar 8-42 flowered from April to mid-October, with a peak during early August. Cropping occurred from early August to mid-November, and volumes peaked in October, at both sites. Cultivar 9-02 flowered from April to late October, with a peak during early August at the Wolseley site, whereas flowering for this cultivar only peaked during late September, at the Hex River site, indicating a possible climate related response. Subsequently, harvest started two weeks later at the Hex River site for '9-02', while the highest yield volumes were obtained during October to early November, at both sites. This paper provides a first report on the phenological categorization of the reproductive development of commercial SHB blueberry cultivars, as produced in the Western Cape, South Africa. As all major management decisions in blueberry cultivation depend on the phenological phases of the crop, this information will contribute towards decision making regarding production, but also via quantifying the progression of each stage in addition to the peaks, allowing for additional logistical management. The influence of growing conditions on the phenological development of these cultivars was also explored and indicated the need for supplemental research considering the influence of micro-environmental conditions in driving evergreen production blueberry systems.

Key words: bud swell, bud break, tight cluster, flower, petal fall

1. Introduction

Blueberry (*Vaccinium* spp.) is a small fruit-bearing Northern American shrub from the family Ericaceae (*V. ashei* Reade) (Hancock et al., 2008; Lyrene, 2006). Blueberry production increased significantly over the last two decades to be recognized today as one of the foremost health foods valued by consumers worldwide (Lobos, 2015; Wang 2018). The health benefits of blueberry are ascribed to the exceptionally high antioxidant capacity of the fruit, which is mainly attributed to the inclusion of a wide range of phenolic compounds such as flavonoids, phenolic acids, anthocyanins, stilbenes and tannins (Medeiros, 2018; Oliveira, 2017, Leiva-Valenzuela et al., 2013; Wang, 2018).

Blueberries were first introduced into South Africa during the 1970s, but in recent times it has been considered as one of the fastest growing South African agricultural industries (South Africa Berry Producers' Association (SABPA), 2019). Blueberry production is widespread, with most of the blueberries being cultivated in the Western Cape (60%), followed by Limpopo (15%), North-West (10%), Gauteng (8%), Eastern Cape (4%), Free State (2%) and Mpumalanga (1%) (Retamales and Hancock, 2018; SABPA, 2019). The South African blueberry harvesting season generally starts in mid-September and ends in November, with peak exports occurring from October (SABPA, 2019). South African berry production is predominantly focussed on SHB, followed by rabbiteye (RE) blueberry and northern highbush blueberry (NHB) (Kritzinger, 2014). SHB and RE blueberry, with its lower chilling requirements, are cultivated in the southern coastal sites, while NHB is planted more inland where higher chilling requirements can be satisfied (Retamales and Hancock, 2018). Sufficient chilling is paramount for optimal development of blueberries, as it determines the timing and uniformity of bud break (Darnell and Davies, 1990).

Phenology refers to the timing and duration of growth and developmental stage of a crop as linked to the seasons and include events such as bud break, flowering, fruiting and leaf abscission (Larue et al., 2021). Knowledge of phenological stages is essential for optimizing resource utilization with the aim to achieve higher yields and fruit quality, as important cultivation practices such as water management, fertilization, pest and disease management and harvest logistics depend on the recognition of such specific phenological stages (Baptista, 2006; Kishore, 2019; Larue et al., 2021).

NeSmith (2006) observed blueberry flowering and fruit ripening times to vary between years and sites. For example, the flowering period of blueberries can vary up to 24 days, depending on cultivar and accumulated chilling hours of a particular year. Therefore, assessing the growth and development responses of different cultivars is important, as it provides valuable information on the interaction between genotypes and climatic factors (Medeiros et al., 2018).

Conventionally, SHB is grown in a production system where plants enter endodormancy in autumn, followed by the accumulation of a minimum number of chilling hours (Swain and Darnell, 2001). After the chilling requirement is satisfied, reproductive and vegetative buds are released from endodormancy and, subsequently, bud break will proceed from mid-winter to early spring, to produce a crop by mid-spring to late spring, which is usually concentrated over a two- to six-week period (Baptista et al., 2006; Fang et al., 2020; Swain and Darnell, 2001). However, evergreen SHB cultivars do not enter endodormancy and are adapted to flower during the colder winter months, which allows it to produce an earlier crop that is advanced from late winter to late spring (Swain and Darnell, 2001). However, most evergreen SHB cultivars tend to have long, protracted reproductive seasons (Scalzo et al., 2016). Flowering, fruit set, and berry maturation may happen concurrently, to various degrees, on an evergreen plant, making it challenging to predict production factors such as the timing of harvest and total yield (Scalzo et al., 2016).

In South Africa, SHB cultivars exhibit an evergreen growth habit, due to the general warmer climatic conditions (Kritzinger, 2014). However, studies documenting the flowering phenology of SHB cultivars under South African growing conditions are limited. Flower bud break occurred during early April for ‘Snowchaser’ and ‘Emerald’ SHB in the Paarl region (Kritzinger, 2014). Full bloom was evident by mid-May, when most flowers were open, and harvest started in mid-November. In the Porterville region, harvests for ‘Jewel’ and ‘Emerald’ SHB commenced earlier towards the end of October and continued until mid-January (Müller, 2011). For the same cultivars, but produced under different micro-climatic conditions in the Porterville region, harvests were more advanced from mid-October until early December (Müller, 2011). This observation particularly highlights the importance of evaluating plant phenology in different sites, as phenological advancement may vary significantly. Similarly, in the Grabouw region, harvests were advanced by a few weeks compared to the harvests in Porterville for ‘Jewel’ and ‘Star’, from early October until the end of December, again

indicating the influence of climate (Müller, 2011). Swart (2015) also reported studies on 'Jewel' and 'Bluecrisp' SHB in the George region, which produced ripe berries, but only from the beginning of November until the beginning of December.

Blueberry cultivars differ with regard to their above-ground characteristics such as growth vigour and habit, which may result in differences in the timing and duration of various phenological stages. A thorough understanding of the crop phenology forms the basis when developing a management strategy to support a sustainable and profitable production plan for a perennial fruit crop. To date, no information regarding the performance of 8-42 and 9-02 SHB cultivars have been documented under South African conditions. Therefore, the objective of this study was to compare the timing, duration, and intensity of flowering of these two cultivars, as cultivated at two experimental sites in the Western Cape, to guide optimization of commercial evergreen SHB production systems under South African conditions. This study was conducted in conjunction with comparative studies on root growth dynamics and carbohydrate allocation patterns to further aid an understanding of the relationship between crop physiology and phenology and production within evergreen SHB cropping systems.

2. Materials and methods

2.1 Plant material and site description

The study was conducted during the 2020 growing season in the Hex River (33º 37' 01.00" S, 19º 29' 30.71" E, 263 m altitude), as well as Wolseley (33º 29' 06.10" S, 19º 10' 00.20" E, 248 m altitude) production areas, Western Cape, South Africa. The Western Cape typically experiences a Mediterranean climate, which is characterized by warm, dry summers and mild, wet winters. The Wolseley site has an average long-term annual temperature of 16.2 °C, with an average maximum temperature of 22.7 °C for February, and an average minimum temperature of 9.8 °C for July, whilst an average annual rainfall of 706 mm is reported. The Hex River site is comparable, with an average annual temperature of 16.6 °C, an average maximum temperature of 22.3 °C for February, an average of minimum temperature of 11 °C for July, but with a reduced average annual rainfall of 487 mm.

Two Australian bred SHB cultivars (*Vaccinium corymbosum* L. interspecific hybrid '9-02 and '8-42'; US patent 2013/0340 130 P1) were selected for the study.

Cultivar 9-02 has a substantially more vigorous and upright growth habit than 8-42, but obtains significantly lower berry yields. Cultivar 8-42 was propagated from cuttings, while 9-02 was derived from tissue culture. All experimental orchards were established in May 2019, with plants spaced on raised soil beds of 3 x 0.75 m or 2.8 x 0.75 at the Hex River and Wolseley orchards, respectively. Both cultivars were cultivated as an evergreen system, under the protection of 20 % white netting, at a net height of 4.5 m and 3.5 m at the Hex River and Wolseley site, respectively. Cultivars 9-02 and 8-42, at each experimental site, were planted in adjacent commercial blocks, and therefore subjected to the same soil type and management practices, to minimize variability due to environmental conditions and/or cultivation conditions.

2.2.1 Treatments and trial design

Twelve single plants per cultivar were selected according to a completely randomized design as experimental units, at each production site. Beehives were placed in the orchards on 11 May 2020 to enhance fruit set, and again removed on 7 September 2020. Standard maintenance pruning of the plants was carried out during the end of November 2019, according to industry recommendations, whilst commercial fertilization applications were applied based on foliar and soil analyses.

2.3 Data collection

Plant phenology was monitored and quantified at a whole plant level, at weekly intervals, from 28 April 2020 until the end of harvest (17 November 2020). Nine specific growth stages were selected for the quantification of the phenological development, based on the scale developed by Garcia-Salazar (2002) (Fig. 1). The stages included: Stage (1) Bud swell: bud swelling becomes visible and scales start to separate; Stage (2) Bud break: flower buds are open and the bud separation is more advanced, so that flower tips are visible; Stage (3) tight cluster: Individual flowers are distinguishable in the inflorescence cluster; Stage (4) Closed flower: individual flower is distinguishable, with the flower axis clearly visible; (5) Open flower: petals are completely expanded, allowing for pollination to proceed; (6) Petal fall: corolla tubes (petals) drop, revealing the small green fruit; Stage (7): Green fruit - includes all differentiated green fruit, regardless of size; Stage (8) Fruit colouring: fruit colour changes from a green to blue, with fruit softening commencing; Stage (9) Harvest: fruit now a complete blue colour,

ripe and ready for harvesting. The duration (in days) of each phenological stage was calculated, based on the weekly phenological observations, where the duration of each phenological stage was noted as the time between the first and last observation of an organ in each of the respective phenological stages. Due to restrictions associated with the global Covid-19 pandemic which commenced at the end of March 2020, farms could only be accessed from 28 April 2020 onwards. However, phenological stages 1-5 already commenced by then for both cultivars and experimental sites, thus starting dates for these stages could not be quantified for 2020.

Data collection at each phenology stage entailed counting of the total number of plant organs with every assessment date, commencing with the swollen flower bud stage to the fruit harvesting stage. At harvest, all fruit per replicate (single plant) was jointly counted and weighed. Canopy volume of each plant was estimated in autumn (May) after growth cessation, using the following equation for a circular cone (Strik et al., 2020): $V = (1/3)\ \pi r^2 h$; where: r is the plant radius, determined by measuring the width of the canopy at the widest points parallel and perpendicular to the plant row and dividing the average of the measurements by two and h is the plant height, determined by measuring from the top of the raised bed to the highest shoot tip. The intensity of each phenological stage throughout the season was expressed as observations.canopy volume (m^{-3}).

Studies on citrus in the Western Cape revealed that 20% white shade net did not have a significant effect on the microclimate in the tree canopy when compared to open field observations (Prins, 2018). Therefore, ambient hourly temperatures (°C) were recorded by an automatic weather station (iLeaf, Hortec, Somerset West) in the proximity of the trial to allow for comparisons in air temperature and relative humidity between production areas.

2.4 Statistical analysis

For comparisons between cultivars, descriptive statistics were used for analysis in SAS 9.4 (SAS Institute Inc., NC, USA). Data is expressed as mean values and standard errors of the mean.

3. Results

3.1 SHB cultivar 8-42

3.1.1 Flower bud swell

The duration of the flower bud phenological stages were comparable for '8-42' at both the Hex River and Wolseley sites, only differing by one or two days for some phenological stages (Fig. 3; Fig. 4A). The first observations started after bud swell due to logistics associated with COVID 19. However, based on the timing of phenological events during the 2021 growing season (personal observation), flower bud development (bud swell) started most likely at the end of March 2020. Flower bud swell continued until late August 2020, at both sites (Fig. 3; Fig 4A). Two peaks in flower bud swell observations were recorded at both sites (Fig. 4A). At the Hex River site, flower bud swell observations peaked on 26 May at 26.47 observations.m^{-3}, followed by a second peak of 28.05 observations.m^{-3} on 30 June (Fig. 4A). At the Wolseley site, the first larger peak at 68.85 swollen flower bud observations.m^{-3} occurred on 20 May, followed by a smaller peak of 44.55 observations.m^{-3} that was recorded on 1 July (Fig. 4A). Flower bud swell observations were higher through most of the season at the Wolseley site, except during August where flower bud swell observations were comparable between the two sites.

3.1.2 Flower bud break

Flower bud break ended in early September 2020 (Fig. 3; Fig. 4B). Two peaks in flower bud break observations were noted, at both sites (Fig. 4B). At the Hex River site, flower bud break observations first peaked on 26 May at 50.78 observations.m^{-3}, followed by a second, smaller peak of 57.01 flower bud break observations.m-3 on 30 June (Fig. 4B). At the Wolseley site, the first larger peak (107.42.m^{-3}) in observations occurred on 20 May, followed by a smaller peak (64.03 observations.m^{-3}) on 19 June (Fig. 4B). Flower bud break observations were significantly higher at the Wolseley site during the earlier part of the season, followed by a period of four weeks in July when flower bud break observations were marginally higher at the Hex River site. After this, flower bud break observations remained similar between the two sites until the end of the flower bud break stage (Fig. 4B).

3.1.3 Tight cluster

Tight cluster stage continued until mid-September 2020 (Fig. 3; Fig. 4C). Both sites showed variation in observations throughout the tight cluster stage; however, at the Hex River site, these observations peaked at 39.84 observations.m^{-3} on 30 June, while at the Wolseley experimental site, observations peaked at 57.66 observations.m^{-3} on 1 July (Fig. 4C). As with the flower bud swell and bud break, tight cluster observations were significantly higher at the Wolseley site during the earlier part of the season, but were then followed by a few weeks, from late July until early August, where tight cluster observations were noticeably higher at the Hex River site (Fig. 4C). After this, tight cluster observations remained similar between the two sites for the remainder of the tight cluster stage (Fig. 4C).

3.1.4 Closed flower

Closed flower stage continued until early October (Fig. 3; Fig. 4D). For the closed flower stage, primarily only one peak was observed per site, occurring on 30 July at the Hex River site (725.30 closed flower observations.m-^{3}) and on 22 July, at the Wolseley experimental site (726.67 closed flower observations.m^{-3}) (Fig. 4D). Closed flower observations were higher at the Wolseley site during the early part of the season, until the peak of closed flower observations was reached by 22 July (Fig. 4D). From there onwards, from late July until mid-August, closed flower observations were higher at the Hex River site than the Wolseley site. After this period, closed flower observations remained similar between the two sites until the end of closed flower stage (Fig. 4D).

3.1.5 Open flower

Open flower stage continued until mid-October (Fig. 3; Fig. 4E). At the Hex River site, open flower observations peaked at 519.11 observations.m^{-3} on 13 August (Fig. 4E). At the Wolseley site, the open flower stage was inconsistent during the winter months, but open flower observations peaked at 491.44 observations.m^{-3} on 22 July (Fig. 4E). Open flower observations were comparable between the two sites until early June, whereafter open flower observations were higher at the Wolseley site until the

peak occurred at the Hex River site. After this, open flower counts remained similar between the two sites until the end of open flower stage.

3.1.6 Petal fall

Petal fall stage started on 28 April at both sites and continued for a period of 196 and 197 days at the Hex River and Wolseley sites respectively, until the 2nd week of November (Fig. 3; Fig. 4F). Petal fall observations peaked during the end of August for both the Hex River site at 744.97 observations.m^{-3} and for the Wolseley site at 609.50 observations.m^{-3} (Fig. 4F). Petal fall observations were comparable between the two experimental sites for most of the season, except for a few weeks during June and July where petal fall observations were higher at the Wolseley site.

3.1.7 Green fruit

Green fruit stage started on 5 May at both sites and continued for 197 days until 18 November (Fig. 3). One dominant peak in green fruit stage observations occurred on 17 September at the Hex River site at 2787.06 observations.m^{-3}, and almost a week later, on 21 September at the Wolseley site at 3136.29 observations.m^{-3} (Fig. 4G). Green fruit observations were significantly higher at the Wolseley site for most of the season until early October when green fruit observations remained similar between the two sites until the end of green fruit stage.

3.1.8 Fruit colouring

Fruit colouring began at the end of July (Hex River – 30 July; Wolseley – 29 July) at both sites and continued until 18 November (Fig. 3). Fruit colouring observations were comparable between the two sites until early October, whereafter it was higher at the Wolseley site for the remainder of the fruit colouring stage. At the Hex River site, fruit colouring observations showed two prominent peaks, on 6 October at 129.80 observations.m^{-3} and again on 3 November at 133.77 observations.m^{-3}. At the Wolseley site, fruit colouring observations showed much variation from mid-September until the end of fruit colouring stage, with peak observations on 8 October at 165.86 observations.m^{-3}, 23 October at 166.24 observations.m^{-3} and again on 11 November at 168.22 observations.m^{-3} (Fig. 4H).

3.1.9 Harvest

Harvest started a week after fruit colouring, at both sites, in early August and continued for approximately 15 weeks until mid-November (Fig. 3). As with fruit colouring at the Hex River site (Fig. 4H), two visible peaks in observations were observed for the fruit harvest stage, occurring on 21 October (886.27 $g.m^{-3}$) and 10 November (728.24 $g.m^{-3}$) (Fig. 4I). At the Wolseley site, a relatively consistent yield was obtained at each harvest date from 1 October to 11 November, with the peak harvest observations occurring on 15 October (669.24 $g.m^{-3}$) and 28 October (778.46 $g.m^{-3}$) (Fig. 4I). Fruit harvest yields were similar between the two sites for the first few weeks of harvest. After this, fruit harvest yields were significantly higher during both peaks (21 October and 10 November) at the Hex River site, whereas fruit harvest yield was significantly higher during the second peak (28 October) at the Wolseley site.

3.2 SHB cultivar 9-02

3.2.1 Flower bud swell

Based on the timing of phenological events during the 2021 growing season (Personal observation), it could also be extrapolated that flower bud development (bud swell) started during the end of March during the 2020 growing season. In contrast with '8-42' where most phenological stages where comparable between the two experimental sites, cultivar 9-02 displayed differences in the timing and duration of the flower bud and fruiting phenological stages, where most stages were more advanced at the Wolseley site compared to the Hex River site (Fig. 5). Flower bud swell continued until late August at both sites (Fig. 5). One peak in flower bud swell at 27.80 observations.m^{-3} occurred at both sites, occurring on 28 April at the Hex River site (Fig. 6A), whereas a similar single peak was recorded for the Wolseley orchard on 12 May at 49.41 observations.m^{-3}. Flower bud swell observations were noticeably higher at the Wolseley site through most part of the season until mid-July when flower bud swell observations were similar between the two sites, and from there onwards, for the remainder of the bud swell monitoring period (Fig. 6A).

3.2.2 Flower bud break

Flower bud break ended in the 2nd week of September (Fig. 5). At the Hex River site, observations remained comparable throughout the monitoring period, with minor peaks noted on 12 May at 42.41 observations.m^{-3}, 30 June at 38.76 observations.m^{-3} and finally on 22 August at 33.67 observations.m^{-3} (Fig 6B). At the Wolseley site, one prominent peak of 121.18 observations.m^{-3} for flower bud break was noted on 20 May (Fig 6B). Thereafter, the flower bud break pattern remained relatively constant, until a sharp decline was recorded towards late August. Flower bud break observations were significantly higher at the Wolseley site during the earlier part of the season until mid-August when bud break observations were comparable between the two sites for the remainder of the flower bud break stage (Fig. 6B).

3.2.3 Tight cluster

Tight cluster stage continued until late September (Fig. 5). Both sites showed variation in the pattern that emerged for the tight cluster stage (Fig. 6C); however, at the Hex River site, the largest peak at 23.68 observations.m^{-3} occurred on 19 May, while at the Wolseley site, a larger peak of 43.02 observations.m^{-3} were reported on 12 June (Fig. 6C). Tight cluster observations were significantly higher at the Wolseley site during the earlier part of the season until late-July whereafter tight cluster observations were quite similar between the two sites for the remainder of the tight cluster stage monitoring period (Fig. 6C).

3.2.4 Closed flower

The closed flower stage progressed until mid-October (Fig. 5). For the closed flower stage, observations peaked at 365.84 observations.m^{-3} on 30 July at the Hex River site, with higher observations that peaked at 401.17 observations.m^{-3} on 29 July at the Wolseley site (Fig. 6D). As noted in the previous phenological stages, closed flower observations were generally higher at the Wolseley site during the early part of the season. Following the peak in observations that were recorded from mid-July to the end of August, a reverse trend was noticed where closed flower stage observations were generally higher at the Hex River site, until the end of closed flower stage (Fig. 6D).

3.2.5 Open flower

The open flower stage continued until the end of October, for 182 days and 178 days at the Hex River and Wolseley sites, respectively (Fig. 5). At the Hex River site, a first peak in the open flower observations at 174.73 observations.m^{-3} were reported on 6 August, followed by a larger, much later peak at 282.19 observations.m^{-3} on 17 September (Fig. 6E). At the Wolseley site, prominent peaks were not as evident, However, a first peak in open flower observations was obtained on 1 July at 158.80 observations.m^{-3}, followed by a next peak on 5 August at 166.59 observations.m^{-3} (Fig. 6E). Open flower observations were similar between the two sites during the early part of the season until late August, except for a two-week period in late June when open flower observations were significantly higher at the Wolseley site. After this, open flower observations were significantly higher at the Hex River site until the end of open flower stage (Fig. 6E).

3.2.6 Petal fall

Petal fall started on 5 May and 28 April and continued for 197 days until 18 November and 11 November at the Hex River and Wolseley sites, respectively (Fig. 5). At the Hex River site, observations peaked on 22 August at 413.06 observations.m^{-3} and again on 6 October at 481.53 observations.m^{-3}. At the Wolseley site, the larger peak at 380.33 observations.m^{-3} in petal fall occurred on 19 August and was then followed by a smaller peak at 232.50 observations.m^{-3} on 8 October (Fig. 6F). Petal fall observations were comparable between the two sites for most of the season until the end of September, whereafter petal fall observations were significantly higher at the Hex River site for the remainder of the petal fall stage (Fig. 6F).

3.2.7 Green fruit

Green fruit stage started on 12 May at the Hex River site and was noted to occur earlier on 28 April at the Wolseley site, whereafter this stage continued until 18 November, at both sites (Fig. 5). One peak in green fruit stage observations were noted at both sites, occurring on 17 September at the Hex River at 1503.33 observations.m^{-3} and somewhat later, on 21 September at the Wolseley site at 1748.86 observations.m^{-3} (Fig. 6G). Green fruit observations were generally higher at the Wolseley site for most part of the season, until late September when green fruit

observations were similar between the two sites for the remainder of the green fruit stage monitoring period (Fig. 6G).

3.2.8 Fruit colouring

Fruit colouring was initiated on 30 July and 15 July at the Hex River and Wolseley sites, respectively, and continued until 18 November at both sites (Fig. 5). Fruit colouring observations peaked on 17 September at 121.21 observations.m^{-3} and again on 10 November at 120.95 observations.m^{-3} at the Hex River site (Fig. 6H). Three peaks in observations were noted for fruit colouring at the Wolseley site, on 21 September at 100.57 observations.m^{-3}, on 8 October at 111.68 observations.m^{-3} and again on 18 November, at 96.70 observations.m^{-3} (Fig. 6H). Fruit colouring observations were significantly higher during both peaks (17 September and 10 November) at the Hex River site, whereas fruit colouring observations were significantly higher during the second peak (8 October) at the Wolseley site. Other than this, fruit colouring observations were similar between the two sites during the season (Fig. 6H).

3.2.9 Harvest

The harvest period occurred for approximately 15 weeks at the Hex River site from 6 August until 18 November, and for 17 weeks at the Wolseley site, from 22 July until 18 November (Fig. 5). At the Hex River site, fruit harvest yields peaked on 29 September at 384.13 g.m^{-3}, then on 21 October at 520.42 g.m^{-3} and lastly on 10 November at 608.58 g.m^{-3} (Fig. 6I). At the Wolseley site, a relatively consistent yield was obtained at each harvest date from 21 September to 18 November, with the highest yield count obtained on 1 October at 515.69 g.m^{-3}, on 15 October at 533.46 g.m^{-3} and again on 11 November at 517.18 g.m^{-3} (Fig. 6I). Fruit harvest yield was generally higher at the Wolseley site for most of the season until early November when fruit harvest yield was significantly higher at the Hex River site for the last two observation weeks (Fig. 6I).

4. Discussion

4.1 Flower bud swell, bud break and tight cluster

For the first three phenological stages, bud count intensity (observations.m^{-3}) was generally higher through most of the season at the Wolseley site when compared to the Hex River site, for both cultivars. However, from stage 4 onwards, count intensities (observations.m^{-3}) were mostly similar between sites. The reason for this observation is unclear as ambient temperatures between the two areas were similar and could not explain the difference in timing. However, it is possible that soil temperatures and micro-climates under the different netting heights could have contributed towards this observation. However, as these parameters were not recorded in this study, these aspects warrant further investigation.

4.2 Closed and open flower and petal fall

Both cultivars showed one main period of flowering (closed and open flowers), concentrated between the first week of June and the last week of August (‘8-42’) or first week of October (‘9-02’). Flowering began at similar dates but ended approximately two weeks later for ‘9-02’ than for ‘8-42’ (26 weeks vs. 24 weeks). However, at the Hex River site, the major peak in open flower count for ‘9-02’ occurred only during mid-September, a time when beehives were already removed from the orchard. During this time, open flower observations also remained high for ‘9-02’ at the Wolseley site, but did not peak. This resulted in a second peak in petal fall observations for ‘9-02’, at both sites. However, it is suspected that a substantial number of flowers did not complete their development, but aborted due to possible poor fruit set in the absence of bees, in addition to insufficient time for development into mature fruit before pruning of the plants which was scheduled for late November. The flowering progression of ‘8-42’ and ‘9-02’ in the current study differed from that observed in ‘Jewel’ and ‘Emerald’ SHB grown in Brazil, under low-chill conditions, where two flowering peaks occurred during the growing season (Medina et al., 2018). Knowledge of the progression of the open flower stage is of critical value to predict when most flowers are open and viable for pollination by bees, thus allowing growers to optimize beehive management (Kishore, 2019).

4.3 Green fruit, fruit colouring and harvest

For both cultivars, and at both sites, the green fruit stage observations rose steeply from mid-July onwards, to produce the highest count of green fruit during mid-September. Information on the phenological progression of green fruit development as presented in this study is of particular importance to optimise management of irrigation, as water stress during the initial fruit growth stages, as well as close to harvest, can reduce yield significantly (Mingeau et al., 2001). Harvests coincided with early August for '8-42' at both sites, while for '9-02' harvesting started during early August at the Hex River, and was advanced to the end of July, at the Wolseley site. However, despite these early harvesting times, most of the yield (> 90%) was concentrated between late-September and mid-November, for both cultivars, at both sites. Soon after harvest, evergreen blueberry plants are routinely pruned to promote vegetative growth that is to provide support for the next crop (Scalzo et al., 2016). In our study, plants were pruned during the last week of November, approximately a week after the last harvest date. Unharvested fruit present on the plants at the time of pruning were thus removed by pruning and was not reflected in the potential yield recorded per individual plants, similarly, as would be the case in commercial practice.

During August, there is a period of approximately three weeks where all phenological stages could be witnessed on the plants simultaneously, for both cultivars, in agreement with previous studies (Baptista et al., 2006, Phillips et al., 2020, Scalzo et al., 2016, Swain and Darnell, 2001). This phenomenon is also observed in blackberries and is known to complicate the calculation of the exact period fruit requires to complete its development on a single plant (Hussain, 2016). In evergreen blueberries, fruit ripening is supported during winter and early spring by leaves that developed in the main growing season of the previous year. New leafing, for which the timing differs between cultivars, occurs prior to completion of the harvest (Scalzo et al., 2016). In some cultivars new leafing occurs mid-way through harvest, while in others the phenological stage only occurs towards the end of harvest. However, for '8-42' and '9-02', new leafing became evident from late August (personal observation), approximately three weeks after the first harvest date, so that plants continued to produce new leaves throughout the harvest period, including peak harvest. Of future interest, although not the focus for this study, there was a report by Scalzo et al. (2016)

that indicated a decrease in fruit quality when the production of new leaves coincided with the peak fruit harvest window.

The success of growing SHB cultivars in an evergreen production system is largely reliant on the ability to maintain healthy leaves throughout the winter months and avoiding sub-zero temperatures (Darnell and Williams, 1997; Reeder et al., 1998; Swain and Darnell, 2001). In this study, winter temperatures remained above freezing temperatures, whilst the annual temperatures were mild enough to allow continued vegetative growth throughout the year (Fig. 2), thus making the Hex River and Wolseley regions suitable for cultivating '8-42' and '9-02' blueberries in such an evergreen production system.

The phenology of '8-42' and '9-02' were generally comparable with that reported for other SHB cultivars in the Western Cape (Kritzinger, 2014; Müller, 2011; Swart, 2015). From our study, cultivars 8-42 and 9-02 were shown to be earlier cropping cultivars compared to the more established cultivars in the Western Cape. Furthermore, the advanced harvest time in October for '8-42' and '9-02' is considered a favourable trait for the marketing of blueberries in the Northern hemisphere during their off-season (SAPBA, 2019).

5. Conclusion

This study evaluated the reproductive bud and fruiting phenology of the 8-42 and 9-02 SHB cultivars grown under Mediterranean climatic conditions within the Western Cape, South Africa. Cultivar 8-42 presented a flowering period from April to mid-October, with a peak during early August and produced a crop from early August to mid-November, with peak volumes that were delivered during October. Cultivar 9-02 presented a flowering period from April to late October, with a peak during early August at the Wolseley site, whereas flowering for this cultivar only peaked during late September, at the Hex River site. Subsequently, harvest started two weeks later at the Hex River site for '9-02', while the highest yield volumes were obtained during October to early November, at both sites.

The phenological categorization that was developed and explored in this study is a useful management tool in assisting growers and breeders to more accurately predict, i.e. the start of harvest, based on the occurrence of the first flowering observations. This will inform timeous optimization of management practices such as

the application of fertilizers and pesticides, as well as irrigation and beehive management during times when plants are most vulnerable to water stress or ready for pollination to improve fruit set.

This study also highlighted the challenges encountered when attempting phenological predictions for SHB when cultivated in an evergreen production system, as flowering and harvest stages can occur continuously, across several weeks within a single plant. This then results in an extended period of varying fruit maturity stages at the same time, which may compromise fruit quality at harvest. For this reason, future studies should consider the interaction of the various phenological stages with the prevailing microclimate. This will enable quantification of the impact of additional factors such as heat unit accumulation within different production systems and relate this to the varying phenological developmental time frames of evergreen SHB cultivars under South African conditions.

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Fig. 1. The selected phenological stages of flower and fruit development in SHB according to the phenological scale developed by Garcia-Salazar (2002). A) Bud swell; B) Bud break; C) Tight cluster; D) Closed flower; E) Open flower; F) Petal fall; G) Green fruit; H) Fruit colouring; I) Harvest. Photos by J. Steyn.

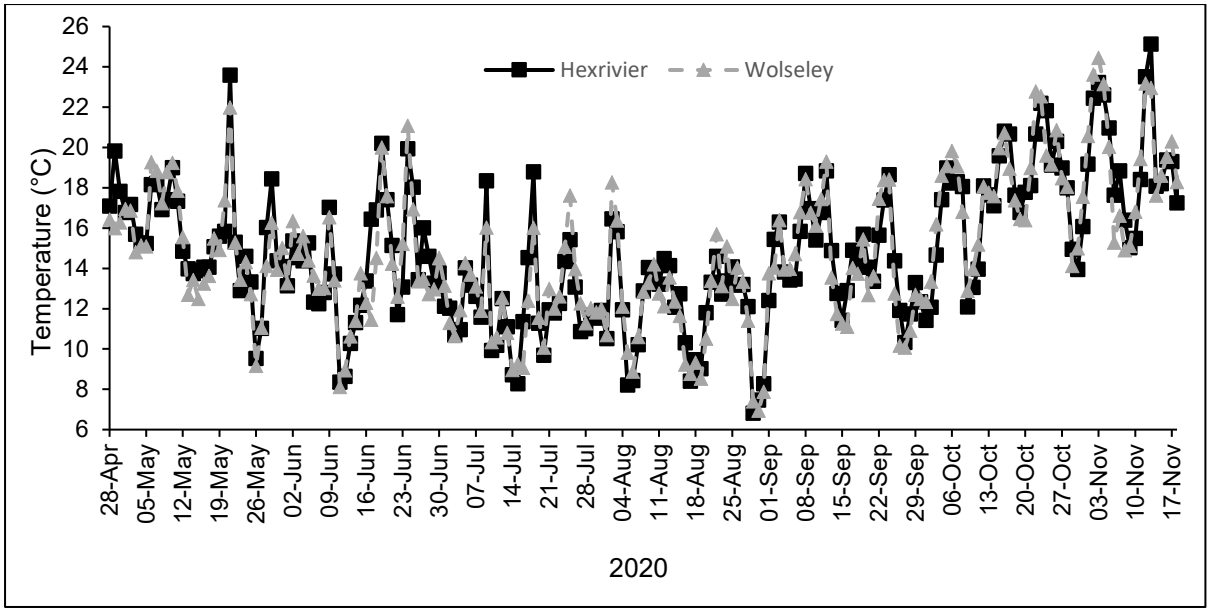

Fig. 2. Average ambient temperature (°C) from 28 April to 17 November for the Hex River and Wolseley experimental sites used to monitor the various floral and fruiting phenological stages of southern highbush blueberry during the 2020 growing ason.

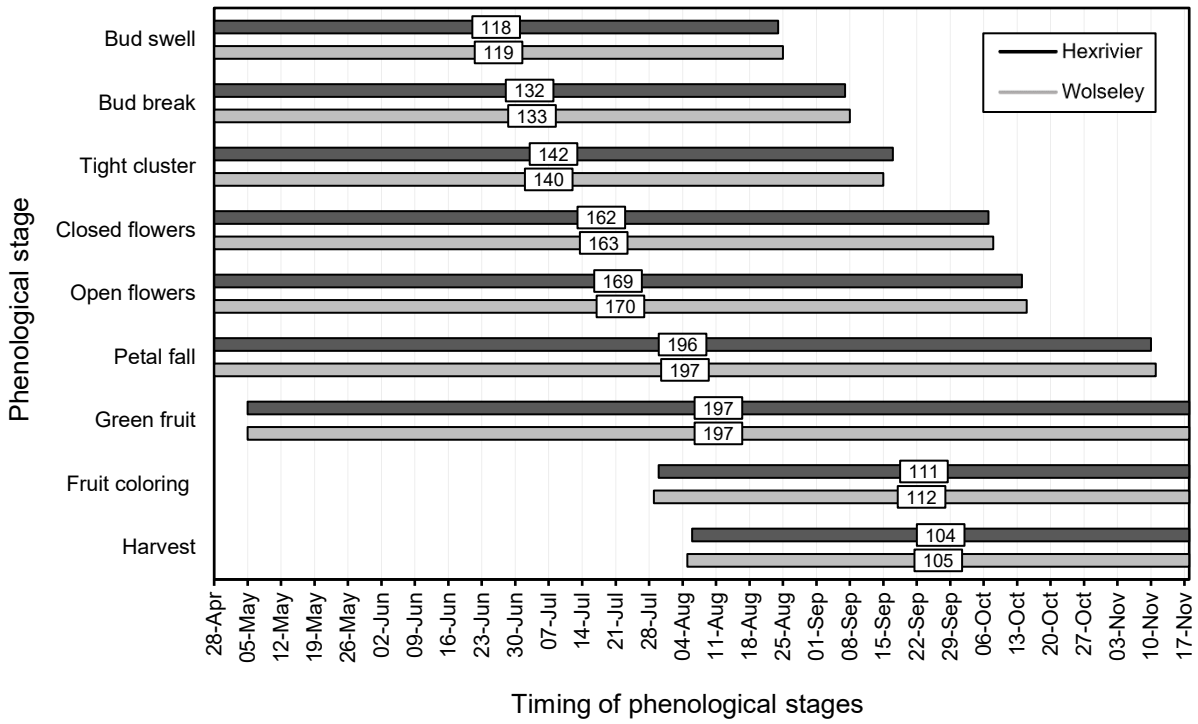

Fig. 3. Timing (blocks) and duration (numbers within blocks indicate the duration in days) of the various flower bud and fruiting phenological growth stages of '8-42' southern highbush blueberry (SHB) for the Hex River and Wolseley experimental sites during the 2020 growing season.

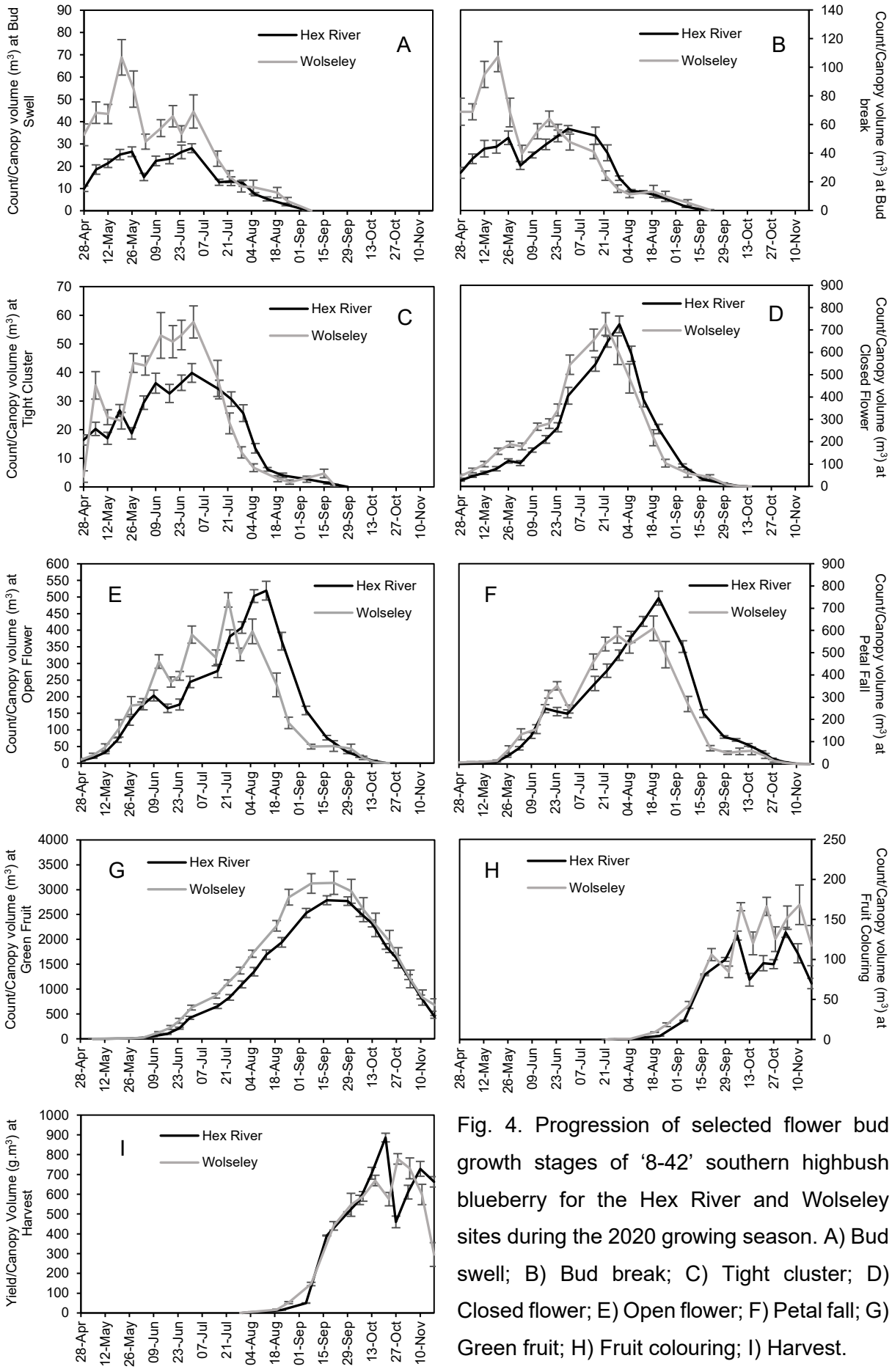

Fig. 4. Progression of selected flower bud growth stages of '8-42' southern highbush blueberry for the Hex River and Wolseley sites during the 2020 growing season. A) Bud swell; B) Bud break; C) Tight cluster; D) Closed flower; E) Open flower; F) Petal fall; G) Green fruit; H) Fruit colouring; I) Harvest.

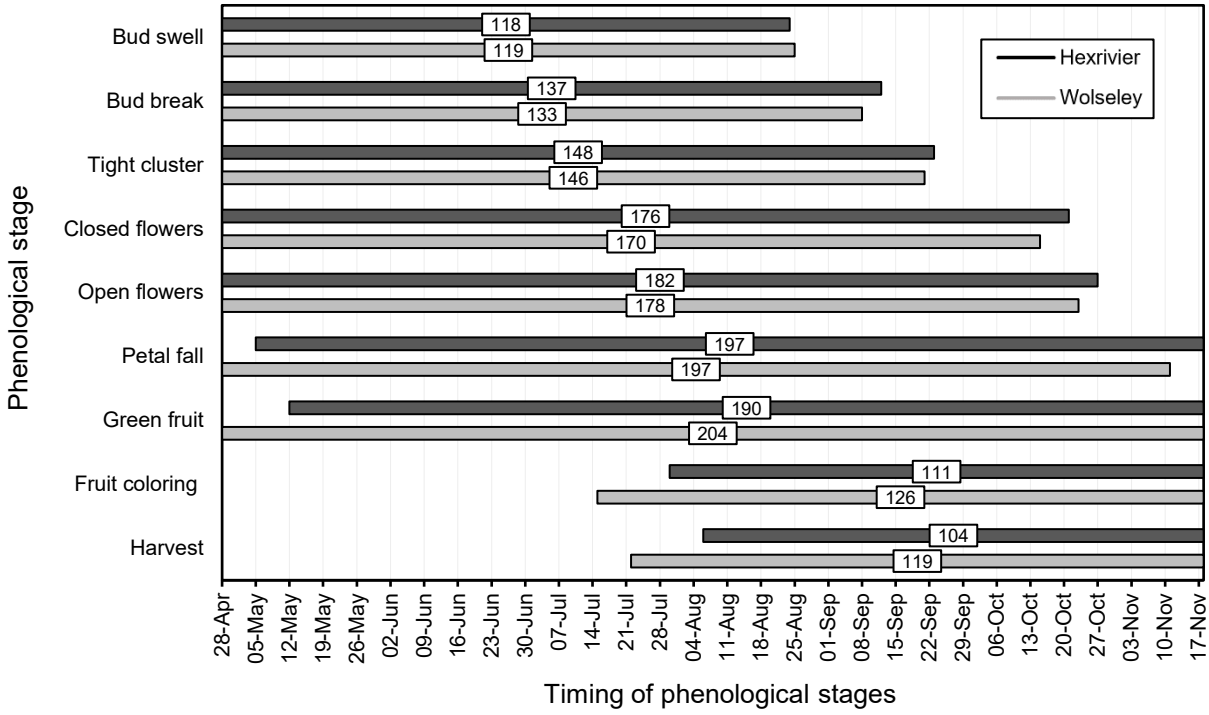

Fig. 5. Timing (bars) and duration (numbers within blocks indicate the duration in days) of the various flower bud and fruiting phenological growth stages of '9-02' southern highbush blueberry (SHB) for the Hex River and Wolseley experimental sites during the 2020 growing season.

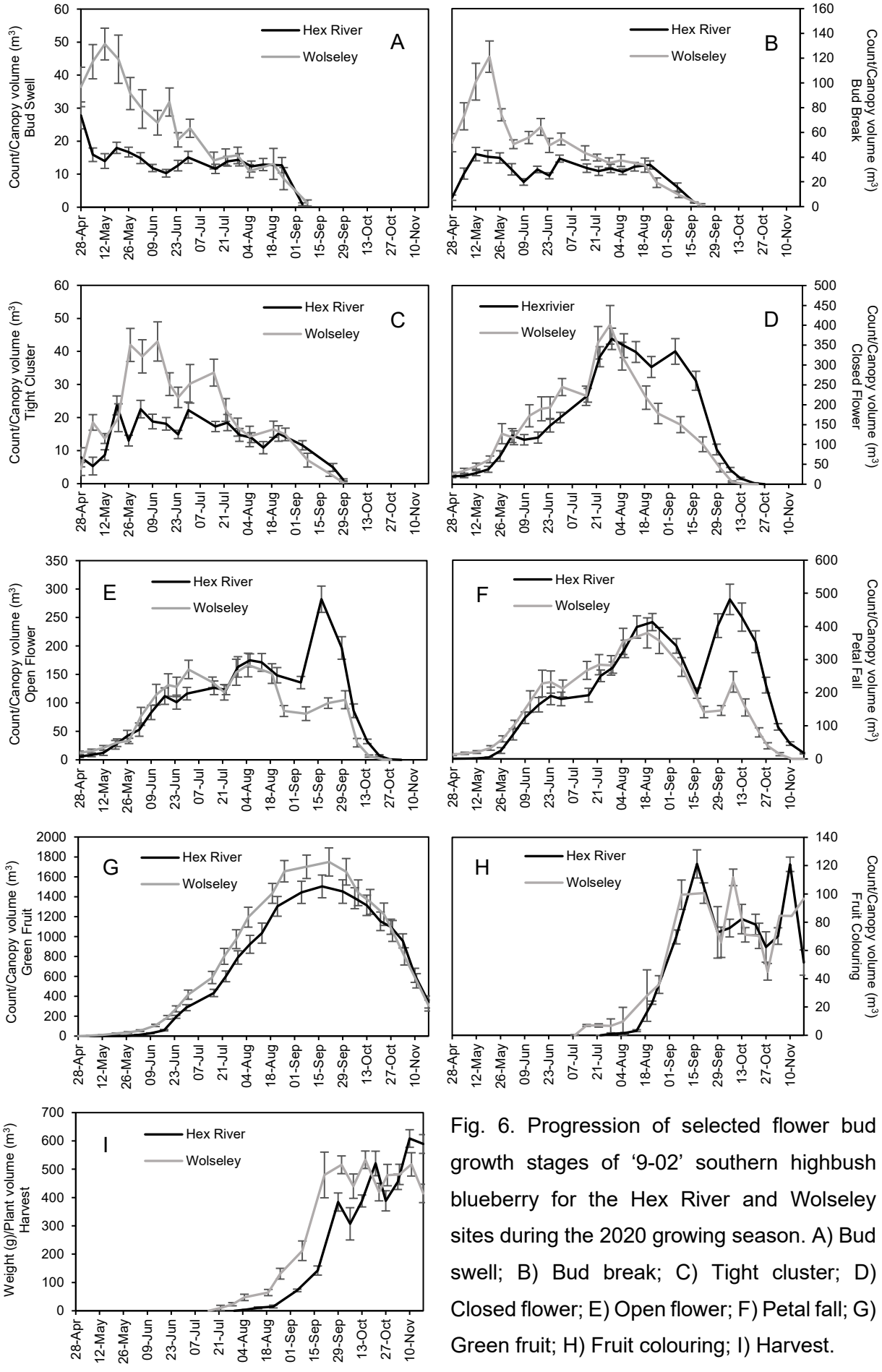

Fig. 6. Progression of selected flower bud growth stages of '9-02' southern highbush blueberry for the Hex River and Wolseley sites during the 2020 growing season. A) Bud swell; B) Bud break; C) Tight cluster; D) Closed flower; E) Open flower; F) Petal fall; G) Green fruit; H) Fruit colouring; I) Harvest.

PAPER 2: Root growth dynamics of two southern highbush blueberry (*V. corymbosum* L. interspecific hybrids) cultivars, 8-42 and 9-02, in the Western Cape, South Africa

Abstract

The objective of this study was to obtain a better understanding of the root growth dynamics of the two southern highbush blueberry (SHB) cultivars '8-42' and '9-02' in a Mediterranean-like climate to optimize the management within such commercial production systems. The experiment was conducted in commercial orchards during the 2020/21 growing season, in the Hex River production area, Western Cape, South Africa. The number and length of new root production of individual plants were quantified, from June 2020 to June 2021, to report on the timing and extent of peaks in root production and root distribution patterns. New root production was evident throughout the study, with similar timing and trends of additional root production, for both cultivars. A first root production peak occurred at the onset of early winter and coincided with flowering. New root production continued until fruit yield increased from late September. This suggests that reproductive growth during the winter months may rely on current photosynthesis and does not depend on storage carbohydrates located in the roots. A second peak in root production coincided with mid-summer, approximately one month after fruit harvest, and overlapped with summer pruning (late November). New root formation occurred simultaneously with active shoot growth, until shoot activity declined with the termination of shoot elongation, during early May. Soil temperatures ranged between 11°C and 20°C during the first root production peak, but were elevated to between 17°C and 31°C during the second root production peak, for both cultivars. These results indicate that root production rates for evergreen SHB grown in the Western Cape, South Africa, are likely to be determined by the stage of plant development and resource acquisition between above- and belowground growth, rather than being primarily driven by soil temperature. This paper provides a first report on the root production trends for evergreen SHB, as produced in the Western Cape, South Africa. This information aims to contribute towards implementing precision irrigation and aid in fertigation decision-making for evergreen SHB cultivars, as well as contribute to possible cost-saving by reducing fertilizer inputs in using a more targeted and scientific guided approach.

Key words: root production peak, minirhizotron, soil temperature

1. Introduction

Root systems, unlike that of shoot systems, are hidden in the soil and are therefore much more complex to observe and gain information on, without disturbing their growth and development (Tajima, 2021; Vanzuela-Estrada et al., 2008). This is especially the case for relatively new crops, such as blueberry, where the understanding of the root phenology is lacking compared to what is known about their above-ground counterpart (Hoppers, 2017). Yet, insights into the root growth and development of a crop, as pertaining to specific production and climatic conditions, are critical for growers to optimize irrigation and fertigation recommendations (Ohashi et al., 2015). Emerging technologies, such as visual quantification of root systems *in situ,* is becoming more common, and is a powerful tool to improve our understanding of root growth dynamics (Tajima, 2021).

Two major root types have been identified in highbush- and rabbiteye (RE) blueberries. The first type is observed to be thick storage roots responsible for anchoring the plant, and have associated storage functions. The second root type resembles fine, thread-like roots, which are adapted for water and nutrient absorption (Bryla, 2017; Retamales and Hancock, 2018; Vargas et al., 2015).

The root system of blueberries is relatively shallow and highly branched, with about 50% of roots located in the top 30 cm of the soil and approximately 80-85%, within the top 60 cm (Paltineau et al., 2017). Overall, more than 80% of the root dry mass is observed to occur in the top 36 cm of the soil (Paltineau et al., 2017). A peculiarity of blueberries is that their fine roots are not equipped with root hairs. Instead, these roots are inhabited by endotrophic mycorrhiza (Bryla et al., 2017; Eck, 1988; Pinto et al., 2017; Retamales and Hancock, 2018). Optimal root growth is obtained in well-drained, porous soils, consisting of a sandy-loam or loamy sand structure with a high organic matter content, with readily available iron, and nitrogen, which is preferred in the ammonium form (Darnell et al. 2015; Pinto, 2017).

Abbott and Gough (1987) reported that ‘Earliblue’, ‘Bluecrop’ and ‘Lateblue’ northern highbush blueberry (NHB) cultivars exhibit two root growth peaks during a growing season. The first and lower peak occurred in spring, near the time of flowering and fruit set. The second root peak was noted after fruit harvest and was completed before plant dormancy commences. Both these peaks coincided when soil temperatures ranged between 14°C and 18°C. Root growth continued throughout the

growing season, yet soil temperatures below 8°C greatly reduced root growth. Bryla et al. (2017) reported similar results with 'Bluecrop' and 'Duke' NHB cultivars. As in the case of Abbott and Gough (1987), the first root peak in the study of Bryla et al. (2017) occurred in May in the northern hemisphere, before harvest, whilst the second peak occurred in September, about a month before plants entered dormancy. Valenzuela-Estrada et al. (2008) reported that first- and second-order root median lifespan ranged from 115 to 120 days, while third-order root lifespan ranged from 136 to 155 days, in 'Bluecrop' NHB blueberry.

Knowledge of blueberry root systems is mostly restricted to studies on northern highbush- and rabbiteye blueberry cultivars in the northern hemisphere, and is almost exclusively conducted on plants grown in a dormant system (Abbott and Gough, 1987; Bryla et al., 2017). Therefore, the objective of this study was to obtain a better understanding of the root growth dynamics of southern highbush blueberry (SHB) by quantifying the root growth and development of two early-cropping soil-established SHB cultivars, cultivated commercially in the Western Cape, South Africa. This study was conducted in conjunction with comparative studies on shoot-based phenological observations and carbohydrate allocation patterns of these two cultivars, with the aim to guide optimization of commercial evergreen SHB production systems under South African conditions.

2. Material and methods

2.1. Plant material and site description

The study was conducted during the 2020/21 growing season in the Hex River (33º 37' 01.00" S, 19º 29' 30.71" E, 263 m altitude), Western Cape, South Africa. Two Australian bred early cropping SHB cultivars (*Vaccinium corymbosum* L. interspecific hybrid '9-02' and '8-42'; US patent 2013/0340 130 P1) were selected for the study. Cultivar 9-02 exhibited a substantially more vigorous and upright growth habit than '8-42', but with a significantly lower berry yield. Cultivar 8-42 was propagated from cuttings, while '9-02' was derived from tissue culture. Orchards were established in May 2019, on low ridges (300 mm in height from the bottom of the interrow), with a crown width of 400 mm. The soil type is a loamy sand type and was mulched with peat (200 m^3/hectare) prior to planting and drip irrigation was used, with four drippers (3

$L.h^{-1}$ delivery rate) per plant. All plants were spaced at 3 x 0.75 m and grown as an evergreening system, under 20% white netting.

2.2. Treatments and trial design

The trial layout was a complete randomised design, with single plants as experimental units, and five replications per cultivar as treatment. The two commercial cultivar blocks were planted adjacent to one another, and were thus subjected to similar soil and management practices, which minimized field environmental condition variability. Fertilization applications were applied based on commercial foliar and soil analysis, for both cultivars.

2.3. Data collection

One acrylic minirhizotron tube per plant (100 cm in length) was installed manually on 20 May 2020, using a soil auger. Tube bottoms were sealed and the top, which extended above the soil surface, was closed off with a white cap to reflect sunlight, and prevent water from infiltrating the tube. All tubes were installed at an angle of 30°, in the same direction, perpendicular to the plant row, and followed the same canopy orientation for all plants (Fig. 1). The root environment around minirhizotron tubes was allowed a four-week period to stabilize before root images (Fig. 2A) were collected, at monthly intervals from 8 June 2020, using a root scanner (CI-600 In-Situ Root Imager, CID-BioScience Inc., Camas, WA, USA), capturing three incremental vertical colour images, with a size of 21.6 x 19.6 cm. Root images were analysed using “RootSnap!” analysis software (Fig. 2A) to determine the number and length of new root production, to inform on the time and extending of peaks in root production and to compile reliable root distribution patterns. In this paper, root production values were based on the number of roots counted per sampling date per root image/tube. New root production indicates the number of additional roots recorded on consecutive evaluation dates.

Soil temperature (°C) (Fig. 3) and moisture (%) (Fig. 4) were monitored throughout the study using continuous logging, capacitance AquaCheck probes (AquaCheck Soil Moisture Management, Durbanville, CPT, RSA). The soil moisture data is qualitative data that was primarily used to indicate soil moisture trends.

2.4. Statistical analysis

All analyses were done using SAS software (Version 9.4; SAS Institute Inc, Cary, USA). Analysis of variance (ANOVA) was performed based on the increase in number of roots with observation time per cultivar. Mean separations were carried out using Fisher's least significant difference test, at $P \leq 0.05$. For total cumulative root length, a linear regression through the origin was fitted per tube per plant over time, and slopes were subjected to ANOVA testing at the 5% confidence level, using the GLM procedure, to compare rates of cumulative root growth between cultivars or soil depths for each cultivar.

3. Results and Discussion

3.1. Timing of root production

New root production was evident throughout the study, for both cultivars (Fig. 5). A similar timing and trend of additional root production was noted for '8-42' and '9-02'. A first root peak coincided with flowering (closed and open flowers; Paper 1), with production rates that increased from late June (Fig. 5). High root production rates continued for approximately three months, but declined when fruit maturation was advanced, towards the end of September, and with peak harvest, until the end of November (Fig. 5). In evergreen blueberry cultivars, the photosynthetically active leaves remain intact throughout winter (Swain and Darnell, 2001). It is thus speculated that the high root production rates that occur concomitantly with the peak flowering period in winter may rely on current photosynthates and are not reliant on storage carbohydrates located in the roots. Abbott and Gough (1987) and Bryla et al. (2017) reported similar results in root production phenology for 'Earliblue', 'Bluecrop', 'Lateblue' and 'Duke' NHB cultivars grown in the northern hemisphere. In both studies, the first root production peak coincided with the onset of flowering and continued through to fruit set, during late spring. Root production continued concomitantly with high shoot growth rates for approximately one month, but was then followed by a decline in root production with the onset of fruit maturation and through to harvesting, during mid-summer. This decline in root production rates that was noted for '8-42' and '9-02' during the period of fruit maturation and with harvest, corresponded with declining trends in starch concentration in the roots, similar to that reported in Paper 3, for this phenological phase. Fruit acts as highly competitive sinks for carbohydrates,

which then results in the reverting of carbohydrates away from root production, to prioritize fruit growth and maturation, until the time of harvest (Bryla et al., 2017).

Our results confer with that of Abbott and Gough (1987) and Bryla et al. (2017), where the first root production peak produced a lower number of new roots than was recorded in the second peak, for both cultivars. The second root peak in our study started in mid-summer, after fruit harvest and summer pruning (late November), and occurred simultaneously with active shoot growth, for approximately four months, until it declined with the termination of shoot elongation during early May (Fig. 5). However, vegetative bud break was already evident during the first week of September (personal observation), approximately four months before the second root production peak started. In the studies of Abbott and Gough (1987) and Bryla et al. (2017), the second peak occurred concomitantly with increased shoot growth that coincided with late summer, and continued for almost two months, until early autumn, until the time the plants entered dormancy.

According to Bryla et al. (2017), the second root peak occurred as soon as the fruit sink was removed at the end of harvest; however, in our study, there was a period of approximately a month after the last fruit were harvested, where the root production rate remained very low in both cultivars. This could partly be due to the effect of pruning on resource acquisition during this time. Abbott and Gough (1987) stated that root and shoot growth in blueberries are not antagonistic processes, as they follow the same general trend. However, our study revealed that, for evergreen SHB grown in the Western Cape, South Africa, root and shoot growth displayed each their own distinctive trend. Although shoot growth rates were not quantified in our study, active vegetative growth was only observed from late August onwards and occurred continuously until shoot growth cessation, in early May. In contrast, high root production rates only coincided with active shoot growth, for about four months during this period.

Abbott and Gough (1987) suggested that the production rate of white, non-suberized root growth is interrelated with three major factors: soil temperature, shoot growth and stage of plant development. White root growth occurred primarily under conditions where soil temperatures ranged between 7°C and 20°C. Their respective peaks occurred at temperatures of between 14°C to 18°C, whilst any soil temperatures outside of this range caused a decline in white root growth rates. In the study of Bryla et al. (2017), the soil temperature was 18°C to 20°C when the maximum number of

roots was recorded during the first peak, whereas soil temperatures ranged between 19°C and 22°C, when the maximum number of roots was recorded during the second peak. In the current study, soil temperatures ranged between 11°C and 20°C in the top 30 cm (Fig. 3) during the first mature and white root production peak, while with the second root production peak, soil temperatures ranged between 17°C and 31°C, for both cultivars. Thus, in our study, soil temperature alone could not fully explain the timing of the root production peaks on the early cropping cultivars '8-42' and '9-02'. Root production trends for these cultivars appear to be more than just a simple function of soil temperature under local conditions and is also determined by the stage of plant development and the resource partitioning between above- and belowground growth.

3.2. Root growth accumulation and distribution

The accumulated root length per season and root growth rate per day was significantly higher for '9-02' compared to '8-42' (Fig. 6). The accumulative root length and growth rate per day for '8-42' in the middle 10-20 cm root zone was significantly higher than in the upper 0-10 cm and lower 20-30 cm root zones, which did not differ distinctly from each other (Fig. 7). The lower accumulative root growth in the 20-30 cm root zone was unexpected, as previous studies reported that most of the roots were found in this root zone (Bryla and Strik, 2007). Temperature data could not explain the differences observed between root reactions (Fig. 3A). However, a high variation in soil moisture percentage at 10 and 30 cm soil depth was observed throughout the study (Fig. 4A). Soil moisture was also notably lower at 30 cm compared to the other depths, which could partly explain the unexpected reduction in accumulated root growth, as roots are very sensitive to moisture stress. In '9-02', the accumulative root length and growth rate per day was similar between the lower 10-20 cm and 20-30 cm root zones, which were significantly higher than the upper 0-10 cm root zone (Fig. 8). Soil temperature and -moisture trends did not show the variation between depths as observed in '8-42' (Fig. 3B and 4B). These results are similar to findings by Bryla and Strik (2007), who reported that most of the roots of 'Duke', 'Bluecrop', and 'Elliot' NHB, grown on raised beds, mulched with sawdust and irrigated by sprinklers, were located in the top 20-30 cm of the soil profile. In our study, plants were also grown on raised beds, but used drip irrigation and black geotextile fabrics ("weed mat") as mulch, which could have influenced root growth differently from that reported for NHB. Firstly, drip

irrigation is known to concentrate roots near the drip emitter, especially when fertigated with nitrogen and other nutrients through the drip system (Bryla et al., 2011). However, in our study, minirhizotron tubes were installed adjacent to drippers and not directly under drippers. Dripper positions should thus not be considered a determining factor in the outcome of our results. Secondly, black geotextile fabric has a tendency to increase soil temperature and generally result in a deeper root system (Larco, 2010), and a decline in fine root growth in the shallow soil depths (Kotze, 2012; Nicholson, 2012). Our study confirmed the lowest root numbers in the 0-10 cm soil profile, validating previous findings that black geotextile fabric influences root distribution and should be taken into consideration when discussing root growth dynamics.

4. Conclusion

This study investigated the root growth dynamics of SHB cultivars 8-42 and 9-02 grown under Mediterranean-like climatic conditions, in an evergreen system. Results obtained for both cultivars, concurred with previous studies on NHB blueberries grown in the northern hemisphere, indicating two root production peaks during a growing season. Although root peaks occurred before and after peak fruit harvest, confirming observations in NHB in the northern hemisphere, root production peaks for these cultivars occurred at different phenological stages and continued for longer periods than reported in NHB cultivars. The first peak occurred from early winter, concomitantly with the peak flowering period, until fruit maturation and harvest volumes increased in early spring. The second peak occurred from mid-summer, after fruit harvest, and continued until termination of shoot elongation, during early May.

Peak root production rates in ‘8-42’ and ‘9-02’ occurred at different soil temperatures than the suggested optimal ranges in NHB. Our study therefore suggests that root production rates for SHB, cultivated in an evergreen production system, are likely to be determined more by the stage of plant development and resource allocation dynamics between above- and belowground growth, than soil temperature alone.

This study highlights the distinct differences between the timing and duration of peak root production rates of SHB under evergreen production systems compared to those described for NHB. Not only could this information lay a foundation for more target specific fertigation in SHB under evergreen production systems, but it also

initiates further interest on the relation of these root growth peaks to specific nutrient uptake efficiency. Quantification of seasonal carbohydrate allocation patterns in below- and above-ground plant organs for the various phenological stages should also be investigated to determine the role of carbohydrates in driving and supporting the observed root production peaks.

Acknowledgments

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Fig. 1. Minirhizotron tube placed at an angle of 30°, perpendicular to the plant row, for the individual plant root studies in ‘8-42’ and ‘9-02’ southern highbush blueberry orchards, cultivated in the Hex River, Western Cape, South Africa.

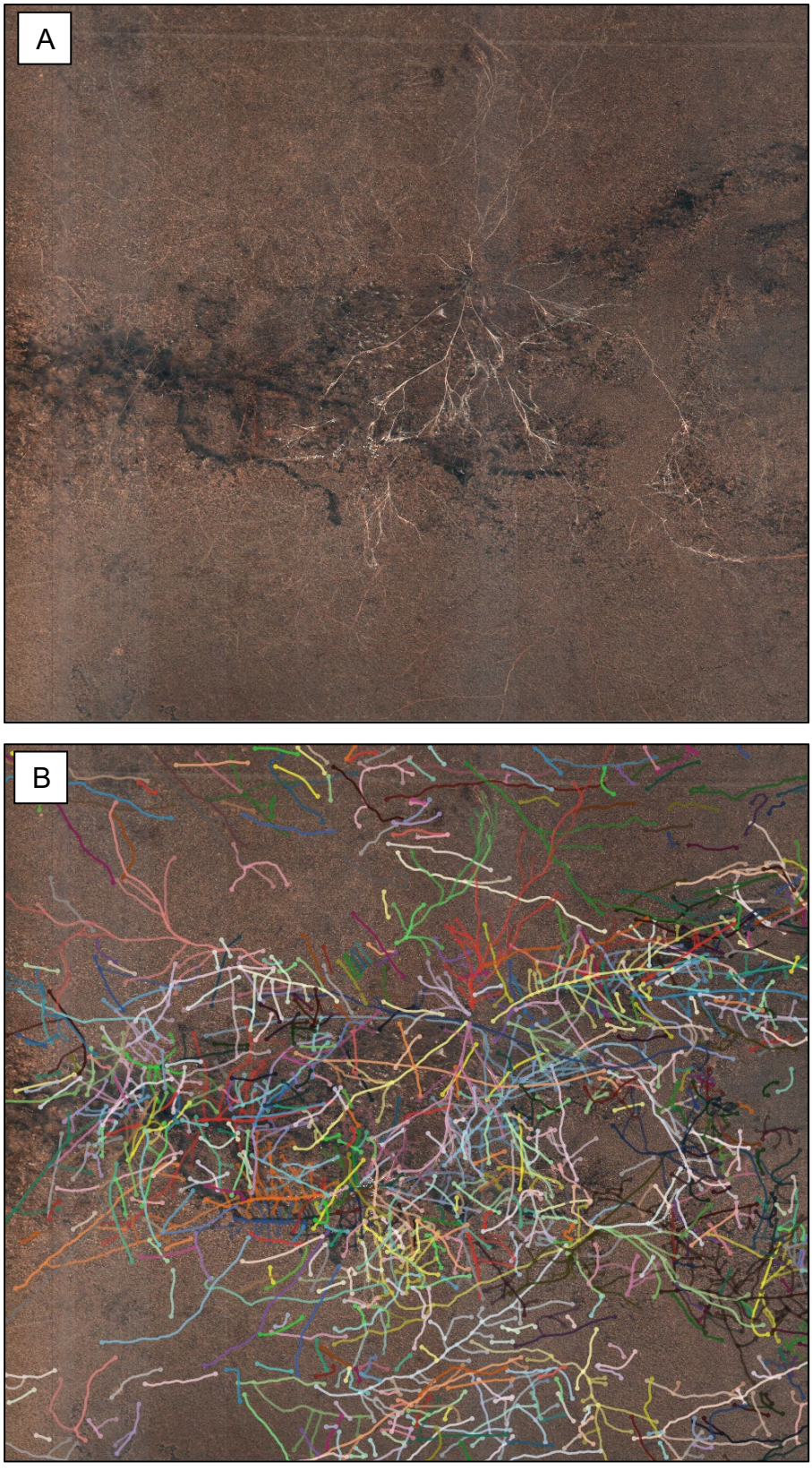

Fig. 2. (A) A root image collected at one depth, using a root scanner (CI-600 In-Situ Root Imager, CID-BioScience Inc., Camas, WA, USA), that represents an area of 21.6 x 19.6 cm. (B) Root length manual quantification of the same image, analysed with Rootsnap! Software to determine number and length of new root production.

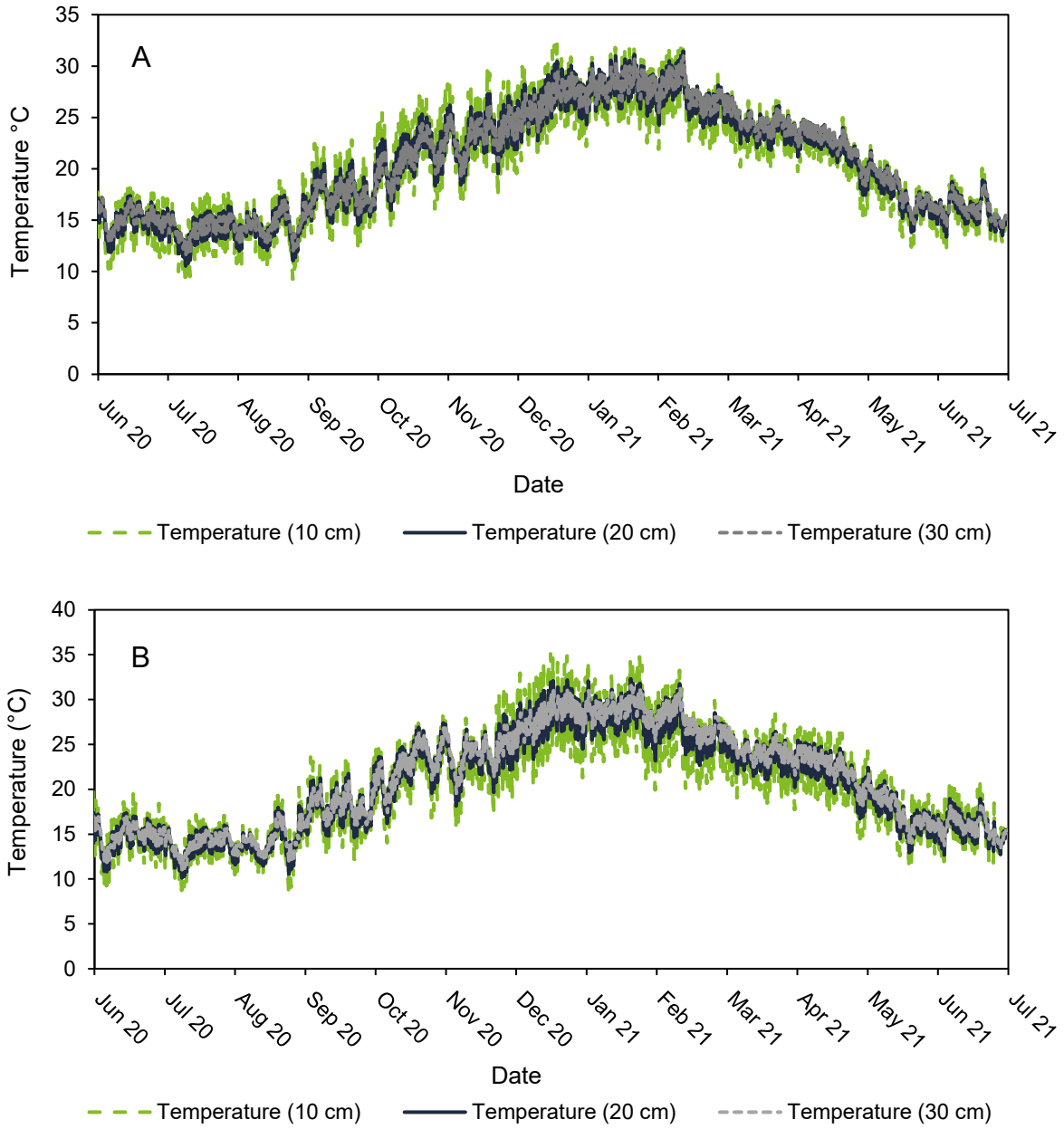

Fig. 3. Average hourly soil temperature (°C) at 10, 20 and 30 cm depths in the soil profile of '8-42' (A) and '9-02' (B) southern highbush blueberry orchards, cultivated in the Hex River, Western Cape, South Africa.

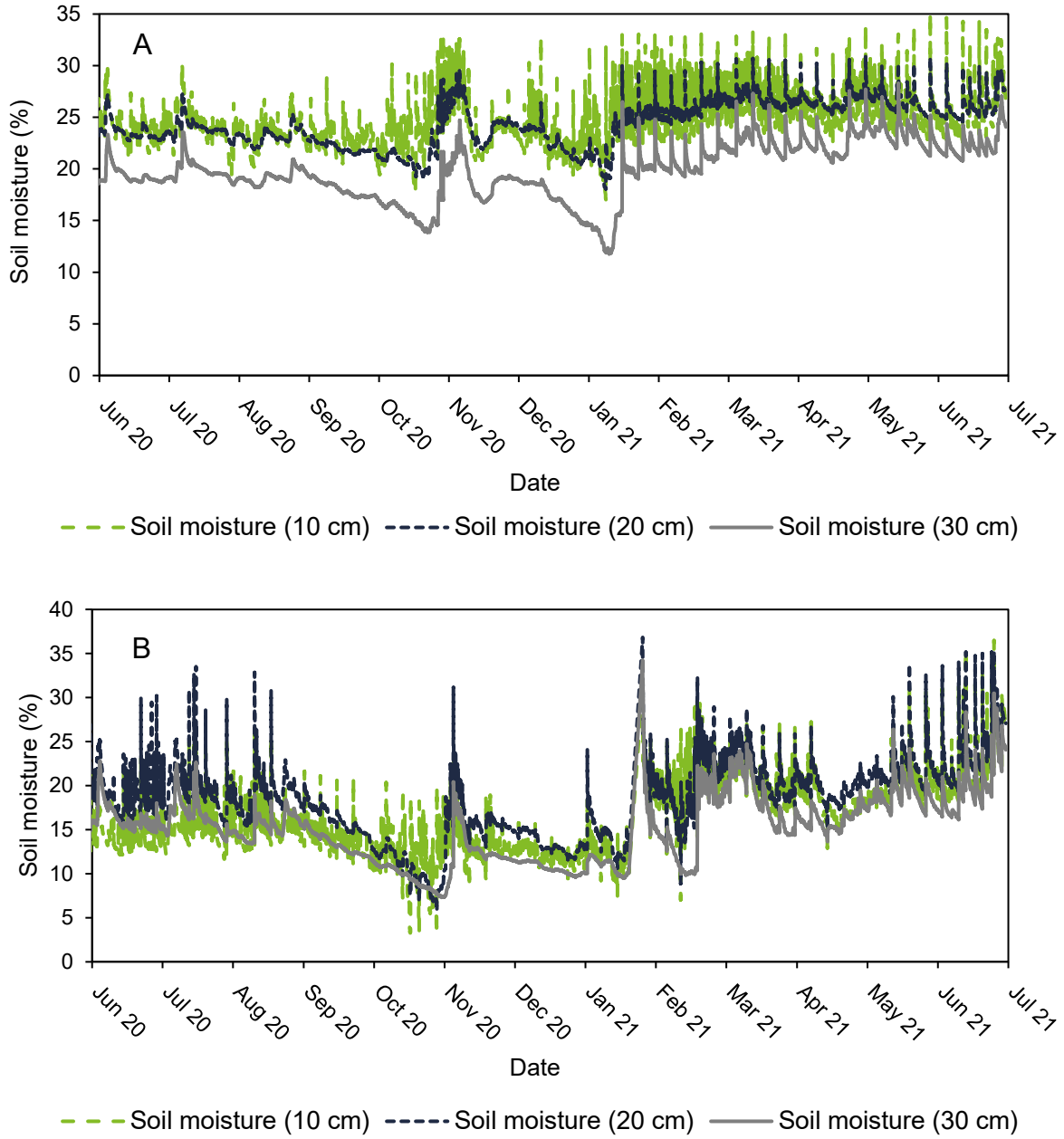

Fig. 4. Average hourly soil moisture (%) at 10, 20 and 30 cm depth in the soil profile of '8-42' (A) and '9-02' (B) southern highbush blueberry orchards, cultivated in the Hex River, Western Cape, South Africa.

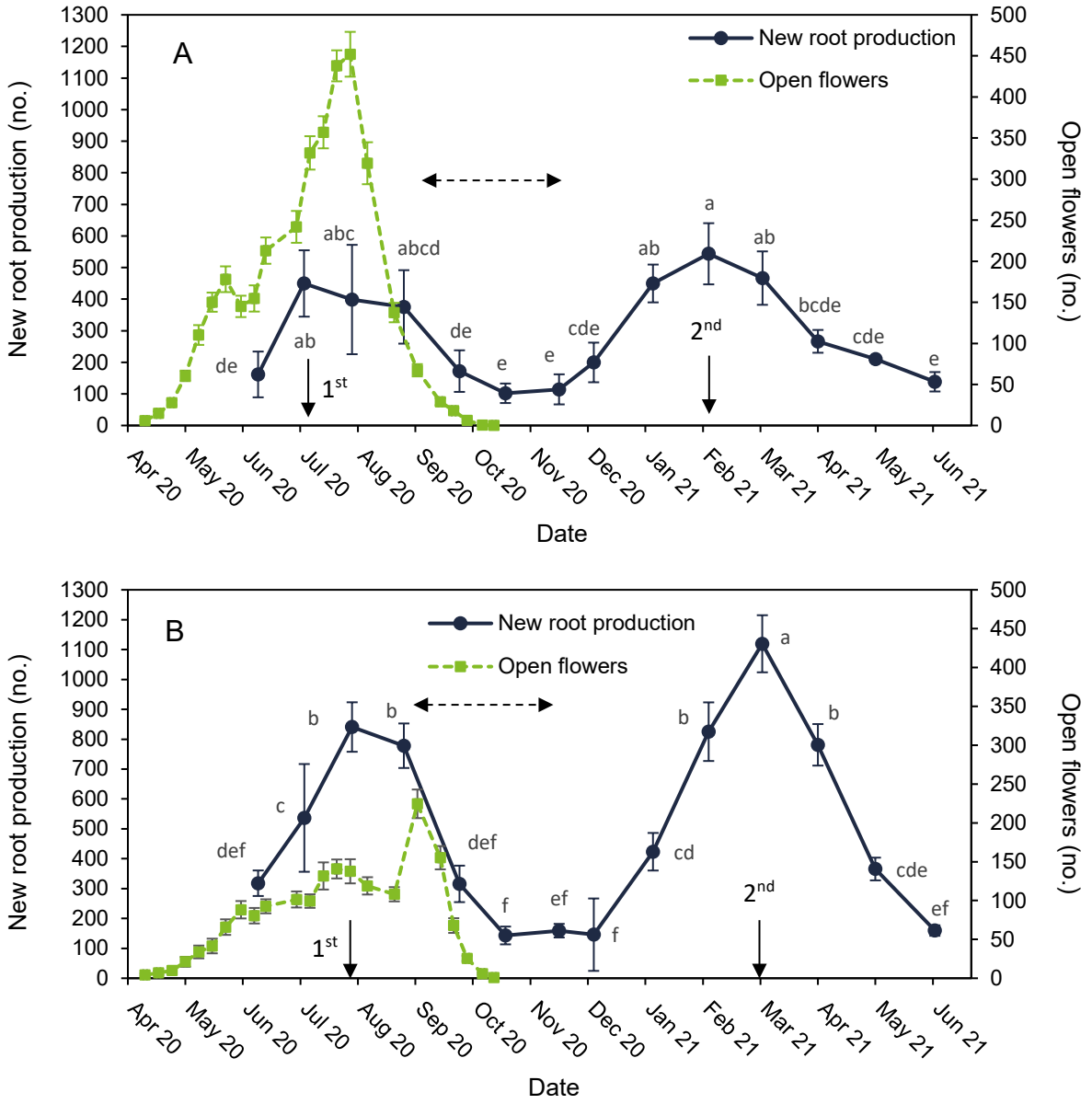

Fig. 5. Seasonal new root production and open flower progression by '8-42' (A) and '9-02' (B) southern highbush blueberry cultivars in the Hex River, Western Cape, South Africa. Data is expressed as the mean (n=5) number of additional roots per cultivar. Bars denote standard errors of the means, and different letters, significant differences between values (P<0.05; Fisher's LSD test; n=5). Dashed line with arrows indicates peak harvest period (>90% of total yield) between 21 September and 17 November for both cultivars. Solid arrows indicate 1st and 2nd root production peaks.

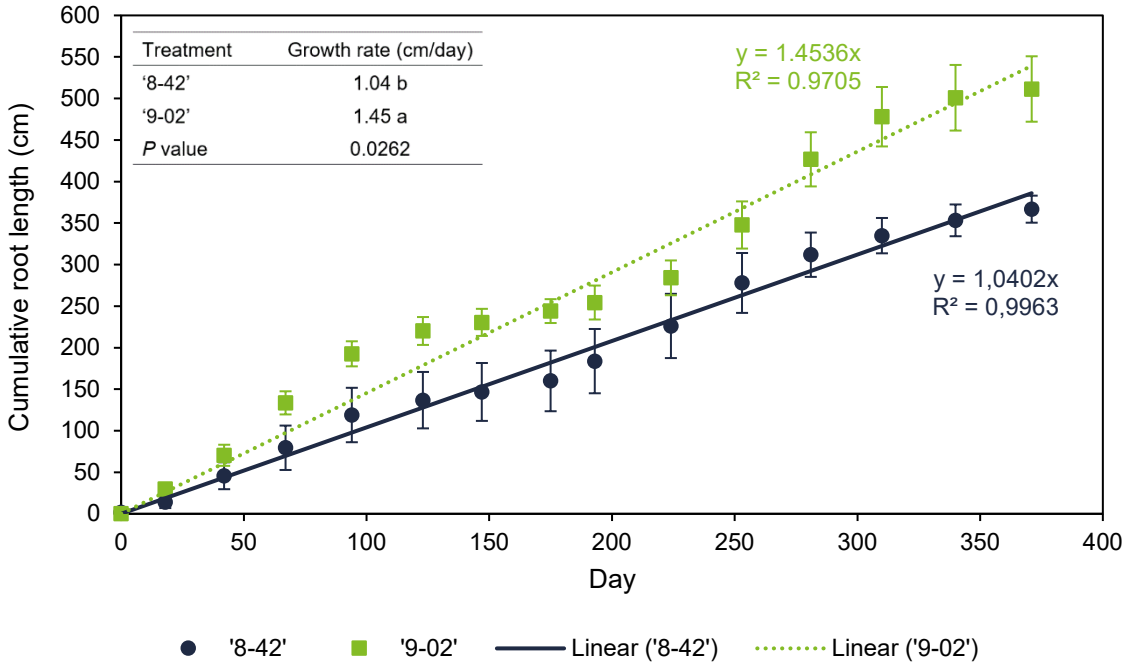

Fig. 6. Seasonal root length accumulation and rate of cumulative root growth of 8-42 and 9-02 southern highbush blueberry cultivars grown in the Hex River, Western Cape, South Africa. Day 0 = 8 June 2020; Day 371 = 14 June 2021. Data is expressed as the mean (n=5) cumulative number of roots per cultivar. Different letters within the same column denote significant differences between values ($P<0.05$; Fisher's LSD test; n=5).

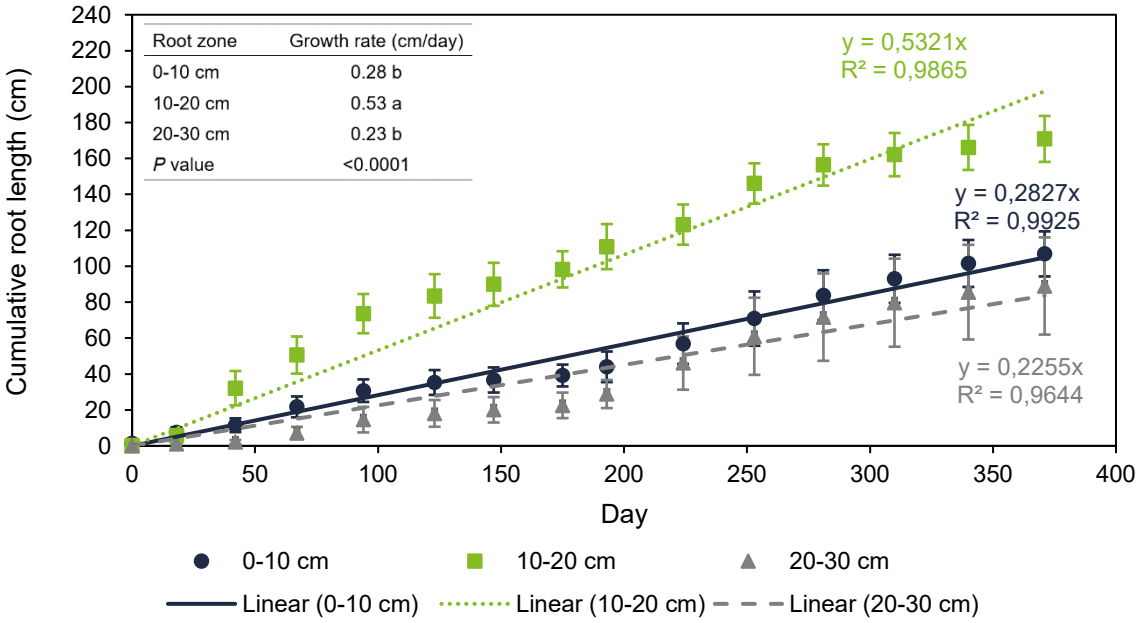

Fig. 7. Seasonal root length accumulation and rate of cumulative root growth in the respective soil depths (0-10 cm; 10-20 cm; 20-30 cm) for '8-42' southern highbush blueberry grown in the Hex River, Western Cape, South Africa. Day 0 = 8 June 2020; Day 371 = 14 June 2021. Data is expressed as the mean (n=5) cumulative number of roots. Different letters within the same column denote significant differences between values (P<0.05; Fisher's LSD test; n=5).

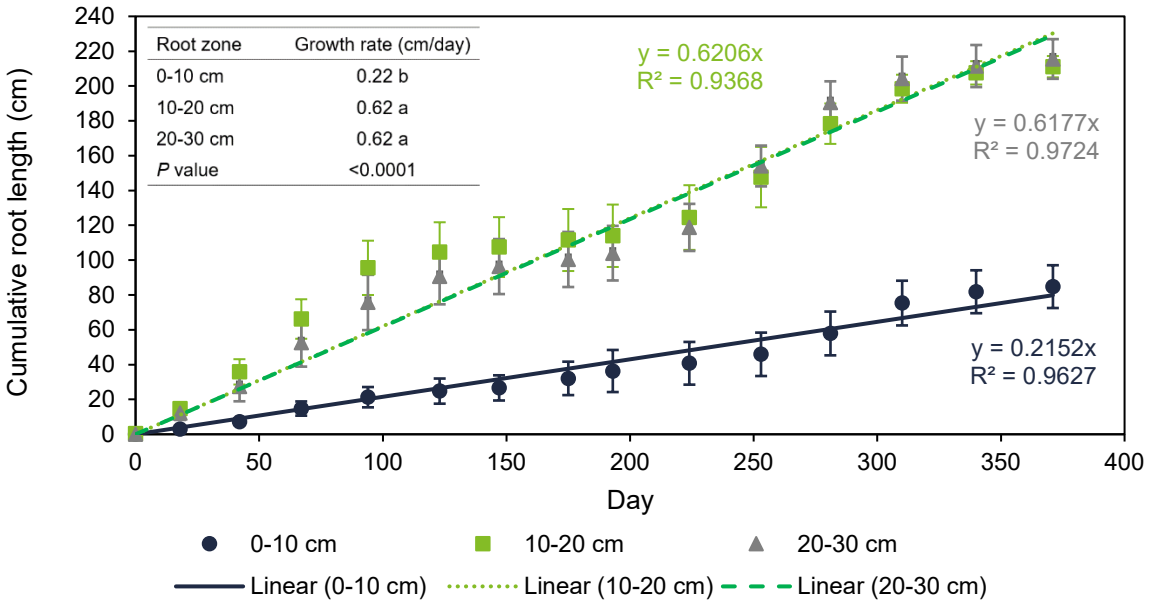

Fig. 8. Seasonal root length accumulation and rate of cumulative root growth in the respective soil depths (0-10 cm; 10-20 cm; 20-30 cm) for '9-02' southern highbush blueberry grown in the Hex River, Western Cape, South Africa. Day 0 = 8 June 2020; Day 371 = 14 June 2021. Data is expressed as the mean (n=5) cumulative number of roots. Different letters within the same column denote significant differences between values (P<0.05; Fisher's LSD test; n=5).

PAPER 3: Seasonal variation in non-structural carbohydrate composition and allocation in two southern highbush blueberry (*V. corymbosum* L. interspecific hybrids) cultivars, 8-42 and 9-02, as cultivated in the Western Cape, South Africa

Abstract

The objective of this study was to quantify the non–structural carbohydrate allocation trends between above- and belowground plant organs in bearing and non-bearing (flowers removed manually) plants, throughout the phenological stages of flowering and fruit development, to investigate the observed difference in yield between two southern highbush blueberry (SHB) cultivars, 8-42 and 9-02, in a Mediterranean-like climate. The experiment was conducted in commercial orchards during the 2019/20 and 2020/21 growing seasons, in the Hex River production area, Western Cape, South Africa. Destructive carbohydrate analyses were performed at 50% flowering, 90% flowering and at peak harvest and total soluble sugars and starch were determined using the phenol-sulphuric acid assay. Throughout the study, sugar was the major carbohydrate component in all plant organs, of both cultivars. Starch concentrations were the highest in either roots or shoots (primary and secondary), while sugar concentrations were highest in leaves. Leaves were the main source of carbohydrates to sustain new root production and early fruit development in both cultivars between 50% and 90% flowering, during both seasons. Between 90% flowering and peak harvest, reproductive development and new vegetative growth was sustained by carbohydrate reserves in '8-42', during both seasons. In contrast, '9-02' showed distinct differences between seasons, especially with regard to roots and leaves. Root carbohydrates were only utilised for reproductive or vegetative development between 90% flowering and peak harvest during 2019/20. Leaf carbohydrates were utilised in all phenological stages in 2020, but increased dramatically between 90% flowering and peak harvest in 2021. Carbohydrate concentrations were generally higher in all plant organs at peak harvest, for non-bearing and bearing plants, in both cultivars. This is explained by a higher carbohydrate availability due to continuous flower removal throughout the season. However, the carbohydrate results alone could not explain the yield differences between the cultivars.

Keywords: flowers, leaves, shoots, sugar, starch, roots

1. Introduction

In deciduous perennial crops, carbohydrates that accumulated after harvest in the previous growing season, are remobilized during spring to support the current season's reproductive and vegetative growth (Darnell, 1996). Developing sink tissues rely heavily on reserve carbohydrates until the photo-assimilates of the current season's photosynthetic tissues becomes available. With the onset of carbohydrate exports from the newly formed vegetative tissue, reserve carbohydrates play a less significant role (Darnell, 1996). In northern highbush blueberry (NHB), flower bud break follows after vegetative bud break (Maust et al., 1999a, 1999b). Floral growth and development are therefore supported by carbohydrates produced by newly formed leaves, and do not rely solely on reserve carbohydrates (Darnell, 1996). However, the respective contribution from carbohydrate reserves and the current season assimilates may vary, depending on the rate at which the sinks deplete the plant reserves, but also according to the developmental sequence in which vegetative and reproductive growth occurs (Maust et al., 1999a, 1999b). The timing of vegetative and reproductive growth also differs between rabbiteye blueberry (RE) and southern highbush blueberry (SHB). In SHB, flower bud break can precede vegetative bud break by as much as four weeks, when no leaves were present in spring to support fruit development (Maust et al., 1999a, 1999b). Under these conditions, both flowers and young fruit are therefore reliant for their carbohydrate requirement on current flower or fruit photosynthesis and/or by remobilization of stored carbohydrates (Birkhold et al., 1992).

In warmer climates, such as the Western Cape, SHB cultivars can be grown in an evergreen production system, where plants are able to maintain their leaves longer into autumn and winter compared to blueberries in dormant production systems (Swain and Darnell, 2001). Results from studies on 'Sharpblue' and 'Wannabe' SHB cultivars showed that plants that were managed as an evergreen production system had a higher carbohydrate availability compared to plants within dormant production systems. As the cane and root carbohydrate reserve pools were shown to be generally similar for both dormant and evergreen production systems, the higher carbohydrate availability in plants within an evergreen system was presumably allocated towards flower bud initiation and development (Swain and Darnell, 2001).

In South Africa, the seasonal variation of carbohydrates in ‘Emerald’ and ‘Snowchaser’ SHB, in the evergreen production system, was evaluated extensively over two growing seasons. Both cultivars accumulated total sugars and starch in the roots, shoots and leaves before reproductive bud break in the first season. However, during the second season, this physiological trend was not evident. In both cultivars, starch concentrated in the roots, whereas sugars accumulated in the leaves. In general, non-structural carbohydrate content for both starch and sugars in the roots, leaves and shoots, in both cultivars, increased from growth cessation until reproductive bud break, followed by a decrease, assumedly due to a reallocation to support reproductive growth (Kritzinger, 2014).

In this study, the two cultivars under investigation showed differences in aboveground characteristics (Paper 1 and 2), with 9-02 being substantially more vigorous than 8-42, but with a lower berry yield in 9-02 than 8-42. As no information is available on the non-structural carbohydrate allocation trends of these two cultivars, the objective of this study was to quantify the carbohydrate concentrations per plant organs (roots, shoots and leaves), throughout the phenological stages of flowering and fruit development, to provide insights into the observed difference in yield between the cultivars. This study was conducted in conjunction with comparative studies on root growth dynamics and phenological observations.

2. Materials and methods

2.1. Plant material and site description

The study was conducted during the 2020 and 2021 growing seasons in the Hex River (33º 37' 01.00" S, 19º 29' 30.71" E, 263 m altitude) production area, Western Cape, South Africa. Two Australian bred SHB cultivars (*Vaccinium corymbosum* L. interspecific hybrid 9-02 and 8-42; US patent 2013/0340 130 P1) were selected for the study. Cultivar 8-42 was propagated from cuttings, while 9-02 was derived from tissue culture. The Western Cape typically experiences a Mediterranean-like climate, which is characterised by warm, dry summers and mild, wet winters. The Hex River site has an average annual temperature of 16.6 °C, an average maximum temperature of 22.3 °C for February, an average of minimum temperature of 11 °C for July, with an annual rainfall of 487 mm (www.Climate-Data.org). Orchards were established in May 2019, with plants spaced on raised soil beds of 3 x 0.75 m. Soil was mulched with peat prior

to planting and drip irrigation was used, with four drippers (3 $L.h^{-1}$ delivery rate) per plant. Both cultivars were cultivated in an evergreen system under 20% white net which was erected at 4.5 m above soil surface.

2.2. Treatments and trial design

The study consisted of two cultivars, planted in adjacent commercial blocks, in a loamy sand soil type, with the same environmental conditions and management practices. A randomised block split plot design was used, with treatment (bearing or non-bearing plants) as main plot factor and phenological stage (50% flowering, 90% flowering and peak harvest) as subplot factor, replicated in five blocks. Single plants were studied as experimental units, over two consecutive seasons (2020 and 2021). To create the non-bearing treatment, all flowers were removed manually on a weekly basis, from the beginning of flowering (28 April 2020/ 30 April 2021) until the end of flowering (7 October 2020/21), while plants in the bearing treatment were left to flower and bear fruit. Standard maintenance pruning of the plants was carried out during the end of November 2020, according to industry recommendations, whilst commercial fertilisation applications were applied based on foliar and soil analyses, for both cultivars.

2.3. Data collection and analysis

2.3.1. Sample preparation

Destructive analyses of the different plant organs were performed at three distinctive phenological events: at 50% flowering (half of all flowers reached anthesis), at the end of flowering (90% of flowers reached anthesis) and at peak harvest. The phenological event dates for the two seasons are presented in Table 1.

Table 1. Sampling dates for whole plant harvests of southern highbush blueberry cultivars 8-42 and 9-02 during the 2020 and 2021 seasons.

Phenological stage	'8-42'		'9-02'	
	2020	2021	2020	2021
50% flowering	7 July	9 July	21 July	6 July
90% of flowering	1 September	31 August	14 September	2 September
Peak harvest	26 October	25 October	9 November	27 October

At each sampling date, one bearing and non-bearing plant were harvested per block. Each plant was separated into roots, primary shoots (> 1 year), secondary shoots (< 1 year), and leaves and fresh weight was determined. Fruit was weighed at every harvest date for the bearing plants. The various plant parts were washed with distilled water and dried in paper bags in an oven [Merck, Merck Chemicals (Pty) Ltd., Germiston, GPT, SA] at 70 °C for 5 days to determine final dry weight (DW). Dried material per plant part was mixed thoroughly, whereafter a sub-sample of approximately 20 g was ground to a fine powder with an analytical grinder (IKA M 20 Universal mill, IKA®-Werke GmbH & Co. KG, Staufen, Germany) and stored in 50 ml plastic tubes at room temperature until analysed. All analyses were performed in the laboratory of the Department of Horticultural Science, Stellenbosch University.

2.3.2. Total soluble sugar extraction.

Ethanol-soluble sugars (mono-, di- and oligosaccharides) were extracted, using an adaptation of the method described by Röhwer (2015). For the extraction of the ethanol soluble sugars, 4 ml 80% ethanol was pipetted to and vortexed with a 100 mg of dried sample, before being placed on a heating block (Grant QBD4, Grant Instruments Cambridge Ltd, Shepreth, England) at 80 °C for 30 min. The samples were centrifuged at 4000 rpm for 4 min at 20 °C, where after the supernatant was decanted and the pellet was re-extracted twice following the first extraction. The respective supernatants were pooled to yield a volume of 12 ml of 80% ethanol extract.

Total water-soluble polysaccharides were determined by extracting the remaining pellet three times with 4 ml deionised water at 80 °C for 22 h and the respective supernatants were pooled to yield a volume of 12 ml of deionized water extract.

To determine total starch content, 3 ml acetate buffer (5 mM, pH 4.8) was added to the remaining pellet following the polysaccharide extractions, vortexed and placed in a heating block at 100 °C for 60 min to extract and gelatinize the starch. The sample was then removed from the heating block and left to cool down to at least 60 °C before 3 ml amyloglucosidase enzyme (AMG) [Sigma Aldrich (Pty) Ltd, Aston Manor, SA] was added, vortexed, and placed in a heating block at 60 °C for 18h. After 18 h in the heating block, the temperature of the heating block was increased to 100 °C for 15 min to deactivate the AGM enzyme. The incubated samples were then centrifuged at 4000 rpm for 4 min and the supernatant decanted for starch determination.

2.3.3. Phenol-sulphuric acid assay

Ethanol-soluble sugars, water-soluble polysaccharides and starch were determined using the phenol-sulphuric acid assay according to the method of Rohwer (2015). The respective 80% ethanol, water and AMG enzyme extracts were diluted according to a final volume of 2 ml to obtain absorbance values ranging between 0.1 and 1. Thereafter, 200 µL phenol was added to 200 µL of the diluted sample and vortexed before 1 ml concentrated sulphuric acid was added. The solution was again vortexed where after it was left to cool at room temperature for 30 min. Each sample extract was assayed in triplicate, and quantified against a glucose standard curve, which was prepared by diluting 0, 50, 100, 150 and 200 µL glucose stock (0.10 $mg.ml^{-1}$) with deionized water to a final volume of 200 µL. The samples were analysed using a spectrophotometer (Genesys 50, Thermo Fisher Scientific, Madison, WI, USA) at 480 nm. The ethanol-soluble sugar, water-soluble polysaccharide and starch concentrations were expressed as $mg.g^{-1}$ sample dry weight (% DW) and referred to as sugars, polysaccharides and starch. The total carbohydrate concentration of each sample was calculated as the sum of the three respective components.

2.3.4. Statistical analysis

Analysis of variance (Anova) was conducted according to the experimental design using the GLM procedure of SAS statistical software (Version 9.4, SAS Institute Inc., Cary, NC, USA). Shapiro-Wilk test was performed on the standardised residuals from the model to test for deviation from normality. Fisher's least significant difference was calculated at the 5% level to compare treatment means for significant effects. A probability level of 5% was considered significant for all significance tests.'

3. Results

3.1. SHB cultivar 8-42

3.1.1. Roots

In both seasons, root total carbohydrate concentrations were similar for 50% and 90% flowering in bearing plants (Table 1). Thereafter, it decreased between 90% flowering and peak harvest. In non-bearing plants, root total carbohydrate concentrations peaked at 90% flowering in the 2020 season, while the total carbohydrate concentrations for this phenological stage were comparable with that of 50% flowering in the 2021 season. Non-bearing plants had significantly higher total root carbohydrate concentrations compared to bearing plants at 90% flowering and peak harvest in the 2020 season, and at 50% flowering and peak harvest in the 2021 season, with no significant differences at any other phenological stages (Table 1).

In bearing plants, a trend was noticed where the starch fraction decreased in relation with the total sugar (ethanol-soluble sugars and water-soluble polysaccharides) fractions between the 50% flowering and peak harvest period in the 2020 season (Fig. 1). However, in the 2021 season, the starch fraction for bearing plants increased between 50% and 90% flowering, only to decrease again between 90% flowering and peak harvest (Fig. 2). In the 2020 season for non-bearing plants, the starch fraction decreased between 50% and 90% flowering, but then remained stable between 90% flowering and peak harvest (Fig. 1). In the 2021 season, an opposite trend to that of the 2020 season was noted where the starch fractions increased between 50% and 90% flowering, before remaining relatively stable between 90% flowering and peak harvest (Fig. 2).

In the 2020 season, root sugar concentrations increased significantly between 50% and 90% flowering, only to decrease again between 90% flowering and peak harvest (Table 1). During the 2021 season, sugar concentrations decreased significantly between 50% and 90% flowering, before remaining stable between 90% flowering and peak harvest.

Root polysaccharide concentrations decreased significantly between 50% and 90% flowering in the 2020 season, for both bearing and non-bearing plants, where after it remained stable between 90% flowering and peak harvest (Table 1). However, for the 2021 season, trends seen in the polysaccharide concentration were different from that noted during 2020 (Table 1). In bearing plants, polysaccharide concentrations were similar for 50% and 90% flowering, before decreasing significantly between 90% flowering and peak harvest, whereas polysaccharide levels in non-bearing plants did not differ significantly between the various phenological stages (Table 1).

Root starch concentration trends in bearing plants decreased significantly between 50% and 90% flowering, with a further significant decrease between 90% flowering and peak harvest in the 2020 season (Table 1). Starch concentrations in non-bearing plants also decreased significantly between 50% and 90% flowering, but then remained stable between 90% flowering and peak harvest (Table 1). In the 2021 season, starch concentrations peaked at 90% flowering in bearing plants, whereas in non-bearing plants, it increased significantly between 50% and 90% flowering, but then remained stable between 90% flowering and peak harvest (Table 1).

3.1.2. Primary shoots

In the 2020 season, primary shoot total carbohydrate concentrations decreased significantly between 50% and 90% flowering in bearing plants, with a further decrease between 90% flowering and peak harvest (Table 1). In the 2021 season, total carbohydrate concentrations were similar for 50% and 90% flowering in bearing plants, only to decrease significantly between 90% flowering and peak harvest (Table 1).

In non-bearing plants, similar total carbohydrate concentration trends were found between seasons (Table 1). Total carbohydrate concentrations were noted to be similar for 50% and 90% flowering, but then decreased significantly between 90% flowering and peak harvest. Non-bearing plants had significantly higher total

carbohydrate concentrations compared to bearing plants at peak harvest, during both seasons (Table 1). Otherwise, no significant differences in primary shoots total carbohydrate concentrations were found between bearing and non-bearing plants at any other phenological stages.

In the 2020 season, for both bearing and non-bearing plants, the starch fraction remained stable in relation to the total sugar fractions between 50% and 90% flowering, but then decreased between 90% flowering and peak harvest (Fig. 1). In the 2021 season, the starch fraction increased between 50% and 90% flowering, before decreasing between 90% flowering and peak harvest, in both bearing and non-bearing plants (Fig. 2).

Sugar concentration trends in primary shoots were comparable between seasons for the respective treatments (Table 1). In bearing plants, sugar concentrations decreased significantly between 50% flowering and 90% flowering, then continued to decrease significantly between 90% flowering and peak harvest, in both seasons. In contrast, sugar concentrations decreased significantly between 50% and 90% flowering for non-bearing plants, but then remained stable between 90% flowering and peak harvest.

Similarly, polysaccharide trends in primary shoots were comparable between seasons for bearing plants (Table 1). Polysaccharide concentrations were similar for 50% and 90% flowering, before decreasing significantly between 90% flowering and peak harvest. In non-bearing plants, polysaccharide concentrations were comparable for all phenological stages in the 2020 season, whereas polysaccharide concentrations were similar for 50% and 90% flowering in the 2021 season, and remained stable between 90% flowering and peak harvest (Table 1).

Starch concentrations were similar for 50% and 90% flowering in the 2020 season in bearing plants, before decreasing significantly between 90% flowering and peak harvest (Table 1). In the 2021 season, starch concentrations increased significantly between 50% and 90% flowering in bearing plants, only to decrease significantly between 90% flowering and peak harvest (Table 1). Starch concentrations peaked at 90% flowering for non-bearing plants during both seasons (Table 1).

3.1.3. Secondary shoots

In both seasons, secondary shoot total carbohydrate concentrations were similar for 50% and 90% flowering in bearing plants, before a significant decrease occurred between 90% flowering and peak harvest (Table 1). Non-bearing plants showed contrasting trends between seasons (Table 1). Total carbohydrate concentrations peaked at 90% flowering in the 2020 season in contrast where the lowest total carbohydrate concentrations were recorded at 90% flowering in the 2021 season. Non-bearing plants had significantly higher secondary shoot total carbohydrate concentrations compared to bearing plants at 90% flowering in the 2020 season, 50% flowering in the 2021 season, and at peak harvest, in both seasons (Table 1). Otherwise, no significant differences in secondary shoots total carbohydrate concentrations were found between bearing and non-bearing plants at any other phenological stages.

Comparable starch fraction trends in relation to the total sugar fraction were shown in secondary shoots as mentioned for primary shoots for the respective treatments across both seasons (Fig. 1 and 2). In the 2020 season, the starch fraction remained stable in relation to the total sugar fractions between 50% and 90% flowering for both treatments, but then decreased between 90% flowering and peak harvest (Fig. 1). In the 2021 season, the starch fraction increased between 50% and 90% flowering for both treatments, before decreasing between 90% flowering and peak harvest (Fig. 2).

Sugar concentrations of secondary shoots did not differ significantly between phenological stages in bearing plants during the 2020 season, whereas it decreased significantly between 50% and 90% flowering and increased significantly between 90% flowering and peak harvest in the 2021 season (Table 1). In non-bearing plants, there was a significant increase between 50% and 90% flowering, before sugar concentrations remained stable between 90% flowering and peak harvest in the 2020 season (Table 1). In the 2021 season, similar sugar concentration trends were observed in non-bearing plants as in bearing plants during the same season (Table 1). Sugar concentrations decreased significantly between 50% and 90% flowering, before increasing significantly between 90% flowering and peak harvest.

Secondary shoot polysaccharide concentrations were similar for 50% and 90% flowering in bearing plants, before it decreased between 90% flowering and peak

harvest during the 2020 season, whereas polysaccharide concentrations were similar for all phenological stages in non-bearing plants (Table 1). In the 2021 season, polysaccharide concentrations decreased significantly between 50% and 90% flowering, but then remained stable between 90% flowering and peak harvest (Table 1). In non-bearing plants, polysaccharide concentrations were lowest at 90% flowering, differing significantly from the other phenological stages (Table 1).

Starch concentrations in secondary shoots were similar for 50% and 90% flowering in bearing plants during the 2020 season, but then decreased significantly between 90% flowering and peak harvest (Table 1). Starch concentrations increased significantly between 50% and 90% flowering, only to decrease significantly between 90% flowering and peak harvest in non-bearing plants, in the 2020 season (Table 1). A similar starch concentration trend as mentioned for non-bearing plants in the 2020 season was followed for both treatments during the 2021 season (Table 1).

3.1.4. Leaves

In the 2020 season, leaf total carbohydrate concentrations for both bearing and non-bearing plants decreased significantly between 50% and 90% flowering, and continued to decrease between 90% flowering and peak harvest (Table 1). In the 2021 season, total carbohydrate concentrations peaked at 90% flowering for both treatments (Table 1). Total carbohydrate concentrations were significantly higher for non-bearing plants than in bearing plants per respective phenological stage during the 2020 season. In the 2021 season, total carbohydrate concentrations were comparable between treatments at 90% flowering, while carbohydrate concentrations were significantly higher in non-bearing plants at 50% flowering and peak harvest than in bearing plants.

In both treatments, the starch fraction decreased in relation with the total sugar fractions between the 50% flowering and peak harvest period in the 2020 season (Fig. 1). However, in the 2021 season, the starch fraction increased between 50% and 90% flowering, only to decrease between 90% flowering and peak harvest (Fig. 2).

Bearing plants exhibited similar sugar concentration trends during both seasons. Sugar concentrations were similar for 50% and 90% flowering, but then decreased significantly between 90% flowering and peak harvest (Table 1). In non-bearing plants, sugar concentrations decreased significantly between phenological

stages from 50% flowering to peak harvest in the 2020 season (Table 1). In the 2021 season, sugar concentrations were similar for 50% and 90% flowering in non-bearing plants, before decreasing significantly between 90% flowering and peak harvest (Table 1).

In the 2020 season, polysaccharide concentrations decreased significantly between 50% and 90% flowering in bearing plants, before remaining stable between 90% flowering and peak harvest (Table 1). In the 2021 season, polysaccharide concentrations were similar for 50% and 90% flowering in bearing plants, before increasing significantly between 90% flowering and peak harvest (Table 1). In non-bearing plants, polysaccharide concentrations were similar for 50% and 90% flowering, before decreasing significantly between 90% flowering and peak harvest during the 2020 season (Table 1). However, during the 2021 season, leaf polysaccharide concentrations were lowest at 90% flowering, differing significantly from the other phenological stages (Table 1).

Starch concentration trends were comparable between treatments within seasons, but differed between seasons for the respective treatments (Table 1). In the 2020 season, starch concentrations decreased significantly between 50% and 90% flowering, and continued to decrease significantly between 90% flowering and peak harvest, whereas starch concentrations peaked at 90% flowering during the 2021 season (Table 1).

3.2. SHB cultivar 9-02

3.2.1. Roots

In the 2020 season, root total carbohydrate concentrations increased significantly between 50% and 90% flowering for both bearing and non-bearing plants (Table 2). Thereafter, it remained stable between 90% flowering and peak harvest for both treatments. In the 2021 season, total carbohydrate concentrations were similar for 50% and 90% flowering in bearing plants, before decreasing significantly between 90% flowering and peak harvest (Table 2). In non-bearing plants, total carbohydrate concentrations decreased significantly between 50% and 90% flowering in the 2021 season, before remaining stable between 90% flowering and peak harvest (Table 2). Non-bearing plants had significantly higher total carbohydrate concentrations compared to bearing plants at 90% flowering and peak harvest in the 2020 season,

and at 50% flowering and peak harvest during the 2021 season, with no further significant differences at any other phenological stage between treatments (Table 2).

In the 2020 season, the starch fraction remained stable in relation to the total sugar fractions between 50% and 90% flowering, in both treatments (Fig. 3). Thereafter a decrease occurred between 90% flowering and harvest. In the 2021 season, the starch fraction increased slightly in relation to the total sugar fraction between 50% and 90% flowering in both treatments (Fig. 4). Thereafter, the starch fraction decreased between 90% flowering and peak harvest in bearing plants, while it increased slightly in non-bearing plants.

Root sugar concentration trends were comparable for bearing and non-bearing plants in the 2020 season (Table 2). Sugar concentrations increased significantly between 50% and 90% flowering, where after a further significant increase occurred between 90% flowering and peak harvest. In the 2021 season, sugar concentrations decreased significantly between phenological stages in bearing plants from 50% to peak harvest, whereas in non-bearing plants, it decreased significantly between 50% and 90% flowering, before remaining stable between 90% flowering and peak harvest (Table 2).

Root polysaccharide concentrations were similar for all phenological stages within each treatment across both seasons (Table 2). Root starch concentration trends were comparable for bearing and non-bearing plants in the 2020 season (Table 2). Starch concentrations increased significantly between 50% and 90% flowering, only to decrease significantly between 90% flowering and peak harvest. In the 2021 season, starch concentration trends in bearing plants were similar to starch trends in the 2020 season (Table 2). Starch concentrations increased significantly between 50% and 90% flowering, before decreasing significantly between 90% flowering and peak harvest. In non-bearing plants, starch concentrations were similar for 50% and 90% flowering in the 2021 season, before increasing significantly between 90% flowering and peak harvest (Table 2).

3.2.2. Primary shoots

In the 2020 season, primary shoot total carbohydrate concentrations in bearing plants decreased significantly between phenological stages from 50% flowering to peak harvest (Table 2). In non-bearing plants, total carbohydrate concentrations

remained stable between all phenological stages (Table 2). In the 2021 season, total primary shoot carbohydrate concentrations in bearing and non-bearing plants decreased significantly between phenological stages from 50% flowering to peak harvest (Table 2). Non-bearing plants had significantly higher total carbohydrate concentrations than in bearing plants at 90% flowering and at peak harvest in the 2020 season, but only at peak harvest in the 2021 season (Table 2). Otherwise, carbohydrate concentrations between treatments at the various phenological stages were comparable.

The starch fraction in the 2020 season decreased in relation with the total sugar fractions from 50% and 90% flowering to peak harvest period for both treatments (Fig. 3). In the 2021 season, the starch fraction increased in relation with the total sugar fractions between 50% and 90% flowering, for both bearing and non-bearing plants (Fig. 4). Thereafter, the starch fraction decreased between 90% flowering and peak harvest in bearing plants, while it remained stable in non-bearing plants (Fig. 4).

Sugar concentrations in primary shoots of bearing plants were similar for all phenological stages in the 2020 season, whereas in non-bearing plants, sugar concentrations were also similar for 50% and 90% flowering, but then increased significantly from 90% flowering to peak harvest (Table 2). In the 2021 season, sugar concentration trends were comparable between bearing and non-bearing plants (Table 2). Sugar concentrations decreased significantly between phenological stages from 50% flowering to peak harvest.

Polysaccharide concentration trends were comparable for bearing and non-bearing plants and also between seasons (Table 2). Polysaccharide concentrations were similar for 50% and 90% flowering, before decreasing significantly from 90% flowering to peak harvest.

Starch concentration trends in primary shoots were similar for treatments during the respective seasons, but differed between seasons (Table 2). In the 2020 season, starch concentrations decreased significantly between phenological stages from 50% flowering to peak harvest, whereas in the 2021 season, starch concentrations increased significantly between 50% and 90% flowering only to decrease significantly between 90% flowering and peak harvest (Table 2).

3.2.3. Secondary shoots

In the 2020 season, secondary shoot total carbohydrate concentrations decreased significantly between 50% and 90% flowering in bearing plants, before remaining stable between 90% flowering and peak harvest (Table 2). In non-bearing plants, total carbohydrate concentrations were similar for 50% and 90% flowering, before a significant increase occurred between 90% flowering and peak harvest (Table 2). In the 2021 season, total carbohydrate concentrations decreased significantly between phenological stages from 50% flowering to peak harvest for both treatments (Table 2). Non-bearing plants had significantly higher total carbohydrate concentrations compared to bearing plants at 90% flowering and peak harvest in the 2020 season, and at all three phenological stages in the 2021 season (Table 2).

In the 2020 season, the starch fraction remained reasonably similar in relation to the total sugar fractions between 50% and 90% flowering for both treatments, before decreasing between 90% flowering and harvest (Fig. 3). During the 2021 season, the starch fraction increased in relation to the total sugars between 50% and 90% flowering for both treatments, before decreasing between 90% flowering and peak harvest (Fig. 4).

In the 2020 season, sugar concentrations in secondary shoots of bearing plants were lowest at 90% flowering, differing significantly from the sugar concentrations at the other phenological stages (Table 2). In non-bearing plants, sugar concentrations were similar for 50% and 90% flowering, before increasing significantly between 90% flowering and peak harvest (Table 2). During the 2021 season, sugar concentrations decreased significantly between 50% and 90% flowering for both treatments (Table 2). After this, sugar concentrations were similar for 90% flowering and peak harvest in bearing plants, whereas in non-bearing plants, sugar concentrations increased significantly during this period.

Polysaccharide concentrations were similar for 50% and 90% flowering in both bearing and non-bearing plants in the 2020 season (Table 2). Thereafter, polysaccharide concentrations in bearing plants remained stable between 90% flowering and peak harvest, whereas polysaccharide concentrations increased significantly in non-bearing plants. In the 2021 season, polysaccharide concentrations peaked at 90% flowering in bearing plants, whereas in non-bearing plants, polysaccharide concentrations were the lowest at 90% flowering, differing significantly

compared to the polysaccharide concentrations at the other phenological stages (Table 2).

Starch concentrations decreased significantly in bearing plants between phenological stages from 50% flowering to peak harvest in the 2020 season (Table 2). In non-bearing plants, starch concentrations were similar for 50% and 90% flowering, before decreasing significantly between 90% flowering and peak harvest (Table 2). In the 2021 season, starch concentrations peaked at 90% flowering for both treatments (Table 2).

3.2.4. Leaves

Leaf total carbohydrate concentrations decreased significantly between phenological stages in bearing plants from 50% flowering to peak harvest during the 2020 season (Table 2). In non-bearing plants, total carbohydrate concentrations decreased significantly between 50% and 90% flowering, but then remained stable between 90% flowering and peak harvest (Table 2). In the 2021 season, total carbohydrate concentrations decreased significantly between 50% and 90% flowering in bearing plants, whereas in non-bearing plants, carbohydrate concentrations were similar for 50% and 90% flowering (Table 2). Thereafter, total carbohydrate concentrations increased significantly between 90% flowering and peak harvest for both treatments. Except for no significant differences in total carbohydrate concentrations between bearing and non-bearing plants at 50% flowering in the 2020 season, carbohydrate concentrations were significantly higher in non-bearing plants at all other phenological stages across both seasons (Table 2).

In the 2020 season, the starch fraction decreased in relation with the total sugar fractions between 50% and 90% flowering for both treatments (Fig. 3). Thereafter, it increased between 90% flowering and harvest, although the increase was more pronounced in non-bearing plants. During the 2021 season, the starch fraction remained stable between 50% and 90% flowering in bearing plants, whereas the starch fraction increased during this period in non-bearing plants (Fig. 4). Thereafter, the starch fraction decreased between 90% flowering and peak harvest in both treatments.

Sugar concentrations decreased significantly between phenological stages in bearing plants from 50% flowering to peak harvest during the 2020 season, whereas

in non-bearing plants, sugar concentrations peaked at 90% flowering (Table 2). Sugar concentration trends were comparable for treatments in the 2021 season (Table 2). Sugar concentrations decreased significantly between 50% and 90% flowering, before increasing significantly between 90% flowering and peak harvest.

In the 2020 season, polysaccharide concentrations decreased significantly between 50% and 90% flowering in bearing plants, but then remained stable between 90% flowering and peak harvest (Table 2). In non-bearing plants, polysaccharide concentrations were similar for 50% and 90% flowering, before increasing significantly between 90% flowering and peak harvest (Table 2). Polysaccharide concentration trends were comparable for treatments in the 2021 season (Table 2). Polysaccharide concentrations were similar for 50% and 90% flowering, before increasing significantly between 90% flowering and peak harvest.

Starch concentration trends were comparable for treatments in the 2020 season (Table 2). Starch concentrations decreased significantly between 50% and 90% flowering, before increasing significantly between 90% flowering and peak harvest. During the 2021 season, starch concentrations were similar for 50% and 90% flowering in bearing plants, only to decrease significantly between 90% flowering and peak harvest, whereas in non-bearing plants, starch concentrations peaked at 90% flowering (Table 2).

4. Discussion

4.1. SHB cultivar 8-42

In ‘8-42’, leaf total carbohydrate concentrations in bearing plants decreased throughout the 50% flowering to peak harvest period in the 2020 season, whereas in the roots and shoots (primary and secondary), it remained similar for 50% and 90% flowering, before decreasing from 90% flowering towards peak harvest. These results differ from Swain and Darnell (2001), who reported that cane and root carbohydrate levels in evergreen ‘Sharpblue’ and ‘Wannabe’ SHB plants decreased dramatically from 50% flowering throughout the fruit development period until end of harvest. However, our studies showed high new root production rates (Paper 2) during the period between 50% and 90% flowering, whereas a decline in root production rates was associated with the period between 90% flowering and peak harvest. This is reflected in the decrease in root starch and polysaccharide concentrations between

50% and 90% flowering, which was accompanied with an increase in sugar concentrations during the same period, indicating conversion of starch to sugars to facilitate root growth and activity (Hinko-Najera et al., 2015). The decrease in primary shoot sugar concentration and the accompanying increase in root sugar concentrations in bearing plants, suggests that sugars are remobilised from the shoots towards the roots. Furthermore, the decrease in leaf starch, but stable leaf sugar concentrations during the 50% to 90% flowering period, indicates starch breakdown with a reallocation of leaf sugars, probably to sustain early fruit development. Thus, between 50% and 90% flowering, leaves are the main source of carbohydrates to support fruit development, while root growth is a carbohydrate sink, being sustained by remobilisation of carbohydrates from aboveground organs. From 90% flowering onwards, root sugar and starch concentrations decreased, indicating reallocation of resources in support of reproductive development in addition to new vegetative growth which emerged in late August (personal observation), close to 90% flowering. Although the contribution of root carbohydrate reserves to fruit development was not directly assessed in our study, the sharp depletion of starch in roots during the fruit development period suggests mobilization of these reserves from the roots in support of developing fruit (Darnell and Birkhold, 1996). Similarly, between 90% flowering and peak harvest, carbohydrates were remobilized towards reproductive development and new vegetative growth which is supported by the simultaneous decrease in carbohydrate concentrations.

Non-bearing plants showed comparable carbohydrate trends in leaves to that of bearing plants in ‘8-42’, with a decrease between 50% flowering and peak harvest in 2020. Furthermore, carbohydrates accumulated in roots and secondary shoots between 50% and 90% flowering, whereas in the primary shoots, it remained similar for both the 50% and 90% flowering phenological stages. Thereafter, carbohydrates decreased in shoots, as well as in roots, until peak harvest. Although different trends occurred in the roots and shoots compared to bearing plants, comparable patterns were observed in the different carbohydrate fractions. Similar conversions between root starch and sugar occurred in non-bearing and bearing plants between 50% and 90% flowering, but sugar concentrations at 90% flowering were consistently significantly higher in non-bearing than bearing plants. Although root growth trends were not evaluated in non-bearing plants, this phenomenon may have been due to increased root growth in non-bearing plants during this period. However, this was not

reflected in the root dry weight (DW) (Appendix 1, Table 1) of the treatments at 90% flowering. The increase in secondary shoot sugars and decrease in leaf sugars between 50% and 90% flowering suggests remobilization from leaves towards secondary shoots to support new vegetative growth in late August. Furthermore, the substantially higher carbohydrates and higher starch concentrations in all plant organs at peak harvest in non-bearing plants suggests that non-bearing plants probably had a higher carbohydrate availability and that the sugar and polysaccharide components were sufficient to sustain new vegetative growth. Starch serves as the main carbohydrate reserve in blueberry (Darnell and Birkhold, 1996) and Kritzinger (2014) showed that continuous removal of flowers throughout the season increased starch availability for vegetative growth. Roots required allocation of sugars between 50% and 90% flowering to sustain root growth in non-bearing and bearing plants with the highest demand for carbohydrates during 90% flowering to peak harvest.

Carbohydrate concentration trends in the roots and shoots (primary and secondary) of bearing plants were similar between seasons in '8-42', while it differed between seasons in the leaves. Leaf carbohydrate concentrations increased between 50% and 90% flowering, and only decreased towards peak harvest in the 2021 season. Between 50% and 90% flowering, sugar concentrations decreased dramatically in roots and shoots, while starch concentrations increased, suggesting a conversion of sugars to starch, allowing for a build-up of reserves. This is an unexpected result, as starch decreased after reproductive bud break and continued to decrease until the end of harvest in previous studies (Swain and Darnell, 2001). With plant DW (roots, shoots, and leaves) (Appendix 1; Table 1 and 2) that was markedly higher in the second season at 50% flowering than first season, it is speculated that the larger plant structure (biomass) allowed for an adequate carbohydrate reserve status at 50% flowering to sustain reproductive development and allow accumulation of starch reserves between 50% and 90% flowering. In combination with stable carbohydrate levels in primary shoots between 50% and 90% flowering, a lower root demand for carbohydrates was observed in 2021 than 2020. Alternatively, previous studies have shown that carbohydrates accumulate in the roots and shoots prior to reproductive bud break of SHB in an evergreen production system (Kritzinger, 2014; Swain and Darnell, 2001), but differed in the degree of accumulation between seasons (Kritzinger, 2014). Although carbohydrate accumulation prior to reproductive bud break was not evaluated in our study, a similar starch accumulation was noted in

bearing plants of ‘8-42’ between 50% and 90% flowering. After 90% flowering, starch concentrations decreased sharply in all plant organs, indicating mobilization of reserves to sustain reproductive development and new vegetative growth, in late August (personal observation), similar to 2020. However, leaves, which were the major source of carbohydrates in the 2020 season, showed a less dramatic decrease in carbohydrate concentrations between 90% flowering and peak harvest in 2021. Furthermore, leaf DW (Appendix 1, Table 1 and 2) decreased towards peak harvest in the 2021 season, whereas it increased towards peak harvest in the 2020 season. The reason for the observed seasonal difference in carbohydrate levels and allocation patterns is unclear.

Non-bearing plants showed distinct differences between seasons in carbohydrate allocation trends in ‘8-42’, specifically in the roots and secondary shoots, similar to recorded in bearing plants. Root starch concentrations increased between 50% and 90% flowering in the 2021 season, while sugar concentrations decreased, indicating active starch accumulation. However, in contrast with a decrease in root starch in bearing plants between 90% flowering and peak harvest, starch remained constant in roots of non-bearing plants during this period. This suggests an adequate carbohydrate status in non-bearing plants to sustain new vegetative growth during this period, while allowing for additional allocation of reserves in the form of starch to roots. Secondary shoots showed contradicting results in 2021 compared to 2020, revealing a decrease in carbohydrate concentrations between 50% and 90% flowering in 2020, followed by an increase to peak harvest. The starch fraction increased in relation to the other carbohydrate fractions during 50% to 90% flowering, indicating starch accumulation in the secondary shoots. The major carbohydrate component that contributed to the increase in the starch fraction was ethanol soluble sugars, which decreased notably during this period. Kritzinger (2014) showed that carbohydrates in shoots of non-bearing plants decreased when new growth occurred, but increased as new growth could sustain itself by the middle of November (southern hemisphere). This is explained by the growth habit of SHB, with several growth flushes, following two to five weeks apart (Gough, 1994). During a growth flush, shoots grow rapidly, resulting in a sharp decline of carbohydrates, which declines with cessation of growth, when the plant accumulates carbohydrates again. Carbohydrate concentrations were significantly higher in all plant organs of non-bearing than bearing plants at peak

harvest, indicating a higher availability of carbohydrates for new vegetative growth in non-bearing than bearing plants.

4.2. SHB cultivar '9-02'

In '9-02', results from the 2020 season showed that carbohydrate concentrations in bearing plants increased in roots between 50% and 90% flowering, in contrast to '8-42', while it decreased in shoots (primary and secondary) and leaves, as in '8-42'. Similar as in '8-42', high root production rates (Paper 2) were associated with the 50% to 90% flowering period in '9-02', which is supported by the increase in root sugar concentrations between 50% and 90% flowering. From 90% flowering, root starch decreased sharply, whereas soluble sugars increased, indicating a conversion of starch to sugars to support the continuous, although reduced root production rates during this period (Paper 2). Root growth was sustained during 2020 via remobilisation of carbohydrates from aboveground organs towards the roots, as observed for '8-42'. Although direct and quantitative comparisons cannot be made between cultivars, results indicated that leaf sugar concentrations and DW (Appendix 1, Table 1 and 3) were considerably higher in '9-02' than in '8-42' at peak harvest, suggesting a higher photosynthetic capacity for '9-02' than '8-42'. It is proposed that this allowed for a higher availability of free sugars, which reduced the need to mobilize reserves between 90% flowering and peak harvest. Similarly, the increase in secondary shoot sugar concentrations between 90% flowering and peak harvest is suggested to be a result of high photosynthetic activity from the new vegetative growth during this period (Kritzinger, 2014).

Similar carbohydrate concentration trends were found in all plant organs in non-bearing and bearing plants in 2020. However, the degree of carbohydrate accumulation and the level of depletion differed between the treatments. More carbohydrates accumulated in roots of non-bearing plants between 50% and 90% flowering, suggesting this higher sugar concentration could have promoted root growth in non-bearing plants also noted in '8-42'. As in '8-42', leaves in '9-02' were the main source of carbohydrates to sustain root growth between 50% and 90% flowering, indicated by the sharp decline in sugar concentrations during this period. Furthermore, the higher carbohydrates in all plant organs at peak harvest in non-bearing plants, confirm the higher carbohydrate capacity in non-bearing plants also observed in '8-42'. Between 90% flowering and peak harvest, new vegetative growth was the largest

sink, and the carbohydrate demand was sustained by carbohydrates from all plant organs indicated by the dramatic decrease in root and shoot starch, as well as leaf sugar.

As in ‘8-42’, carbohydrate concentration trends differed between seasons in both bearing and non-bearing plants of ‘9-02’. However, when each carbohydrate component trend is interpreted individually, the 2021 season trends between cultivars were comparable and similar conclusions can be drawn for the respective treatments. The primary difference between the cultivars occurred between 90% flowering and peak harvest. Leaf sugar concentrations increased dramatically during this period in both treatments in ‘9-02’, which could be ascribed to higher photosynthetic activity. Alternatively, it could indicate that ‘9-02’ did not utilise the carbohydrates to increase reproductive development, which could possibly explain why lower yields (Table 3) are obtained in ‘9-02’ than in ‘8-42’.

Throughout the study, sugar was the major carbohydrate component in all plant organs of both cultivars, followed by polysaccharides and starch, although starch was present in higher concentrations than polysaccharides at some phenological stages, in certain plant organs. This agrees with results by Kritzinger (2014) in ‘Snowchaser’ and ‘Emerald’ SHB cultivars. However, Kritzinger (2014) did not evaluate the polysaccharide component separately, and thus the starch concentration includes polysaccharides and therefore overestimates the starch concentrations. At most phenological stages, irrespective of treatment or cultivar, starch concentrations were the highest in either roots or shoots (primary and secondary), followed by leaves. This is common in many plant species as roots and shoots are the main storage organs (Stassen et al., 1981). Total sugar concentrations (mono-, oligo- and polysaccharides) were highest in leaves, followed by roots, secondary shoots and primary shoots. It is not uncommon that leaves contain the highest sugar concentrations as photosynthetically active leaves are the sites of carbohydrate synthesis (Retamales and Hancock, 2018).

5. Conclusion

This study investigated carbohydrate allocation trends in 8-42 and 9-02 SHB cultivars grown under Mediterranean-like climatic conditions, in an evergreen production system. Sugar was the major carbohydrate component in all plant organs of both cultivars during both seasons. Starch concentrations were the highest in either

roots or shoots, while sugar concentrations were highest in leaves, irrespective of treatment.

In bearing plants of '8-42', active new root production and the concomitant early fruit development were sustained by carbohydrates from leaves between 50% and 90% flowering in both seasons. From 90% flowering, reproductive development and new vegetative growth, which became evident in late August, were sustained by all plant organs in both seasons. However, in contrast with the 2020 season, starch build-up occurred in all plant organs between 50% and 90% flowering in the 2021 season, suggesting that adequate carbohydrates were available in the form of sugars at 50% flowering to sustain new root production and early fruit development.

Major differences were observed between seasons in the carbohydrate allocation trends of bearing '9-02' plants, especially in the roots and leaves. In the 2020 season, root carbohydrates were not utilised for reproductive development between 90% flowering and peak harvest, whereas it was utilised in the 2021 season. Leaf carbohydrates were utilised throughout all phenological stages in the 2020 season, whereas in the 2021 season, carbohydrate build-up in the form of sugars occurred between 90% flowering and peak harvest. This is possibly an indication that '9-02' had excess carbohydrates in the 2021 season at the time of 90% flowering, which were not utilised for higher reproductive growth.

Carbohydrate concentrations were generally higher at peak harvest in non-bearing plants of both cultivars, due to the continuous removal of a reproductive sink (flowers).

Although this study gave insight into the differences observed between cultivars in carbohydrate allocation trends during the flowering and fruit development stages, the major limitation of the study lies in the period between carbohydrate analyses, which prevented the quantification of precise timing of shifts in carbohydrate allocation trends between plant organs. Previous studies showed that carbohydrate concentrations accumulate to different degrees prior to reproductive bud break between seasons, as well as fluctuate over short periods, especially when new vegetative growth is evident. For this reason, future studies should consider quantifying carbohydrate concentrations at shorter intervals, as well as quantify the carbohydrate status prior to reproductive bud break and post-harvest, to determine the role of excess carbohydrate levels on the following season's crop.

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Table 1. The concentration of extracted sugars (monosaccharides, oligosaccharides, and polysaccharides) and starch within selected plant organs of '8-42' southern highbush blueberry cultivar at three distinct phenological stages during the 2019/20 and 2020/21 seasons.

Carbohydrate	Treatment	Phenological stage	Roots		Primary shoots		Secondary shoots		Leaves	
			2020	2021	2020	2021	2020	2021	2020	2021
			mg.g^{-1} DW							
Monosaccharides + oligosaccharides	Bearing	50% flowering	43.0 e	74.0 b	51.0 a	45.2 a	56.0 bc	49.6 c	119.5 b	125.8 ab
		90% flowering	66.2 b	55.3 c	42.9 b	35.5 b	57.8 abc	35.0 d	120.6 b	132.5 a
		Peak harvest	53.6 c	58.3 c	31.4 c	23.2 c	52.5 c	47.8 c	94.2 d	115.8 c
	Non-bearing	50% flowering	48.0 d	82.8 a	49.6 a	49.4 a	53.5 c	57.3 b	131.3 a	127.6 a
		90% flowering	84.0 a	56.7 c	44.4 b	34.5 b	61.7 ab	33.6 d	117.8 b	128.4 a
		Peak harvest	66.0 b	59.4 c	47.0 ab	37.8 b	62.8 a	75.0 a	103.1 c	117.5 bc
		P-Value	**0.0027**	**0.1288****	**0.0003**	**0.0036**	**0.0147**	**<.0001**	**0.0047**	**0.497***
Polysaccharides	Bearing	50% flowering	25.4 a	29.5 bc	26.7 a	27.4 ab	32.9 a	39.8 b	35.7 a	31.6 d
		90% flowering	17.3 bc	33.9 ab	25.9 ab	27.8 a	33.8 a	35.0 c	28.8 c	30.8 d
		Peak harvest	15.3 c	26.7 c	19.7 c	16.0 c	30.2 b	31.9 cd	28.3 c	41.3 b
	Non-bearing	50% flowering	24.1 a	29.2 bc	23.6 b	28.7 a	32.6 a	44.6 a	37.1 a	35.2 c
		90% flowering	19.1 b	33.4 ab	24.9 ab	27.2 ab	32.9 a	30.0 d	35.5 a	29.9 d
		Peak harvest	18.9 b	37.0 a	24.3 ab	25.1 b	34.6 a	40.0 b	32.1 b	46.9 a
		P-Value	**0.0171**	**0.0142**	**0.0034**	**<.0001**	**0.0081**	**0.0001**	**0.0618****	**0.0249**
Starch	Bearing	50% flowering	27.3 a	25.0 c	17.2 b	24.8 b	20.6 b	15.2 bc	27.4 b	7.7 d
		90% flowering	12.8 b	35.9 b	16.6 b	34.2 a	20.2 b	31.2 a	11.9 e	14.8 b
		Peak harvest	3.3 c	21.2 c	4.3 d	4.4 d	5.6 d	7.3 d	4.5 f	7.3 d
	Non-bearing	50% flowering	26.3 a	25.2 c	16.6 b	24.6 b	20.1 b	17.5 b	31.5 a	12.2 bc
		90% flowering	10.2 b	40.0 ab	20.3 a	35.7 a	24.8 a	34.0 a	22.8 c	25.3 a
		Peak harvest	10.2 b	45.2 a	10.4 c	16.0 c	11.1 c	12.3 c	15.3 d	9.7 cd
		P-Value	**0.0001**	**<.0001**	**0.0011**	**0.0009**	**0.0008**	**0.3708**	**0.0008**	**0.0094**
Total carbohydrates	Bearing	50% flowering	95.72 b	128.5 c	94.9 a	97.3 a	109.5 b	104.6 c	182.6 b	165.1 c
		90% flowering	96.30 b	125.1 c	85.3 bc	97.5 a	111.8 b	101.1 c	161.3c	178.1 ab
		Peak harvest	72.06 c	106.2 d	55.3 d	43.6 c	88.3 c	87.0 d	127.0 e	164.4 c
	Non-bearing	50% flowering	98.26 b	137.3 ab	89.8 ab	102.7 a	106.2 b	119.5 b	199.9 a	175.0 b
		90% flowering	113.28 a	130.1 bc	89.5 ab	97.3 a	119.4 a	97.6 c	176.1 b	183.6 a
		Peak harvest	95.15 b	141.6 a	81.7 c	79.0 b	108.4 b	127.3 a	150.5 d	174.0 b
		P-Value	**0.0001**	**<.0001**	**<.0001**	**<.0001**	**0.0003**	**<.0001**	**0.1739****	**0.6904****

Per carbohydrate fraction, means with the same letter within a column (Phenological stage x Treatment) were not significantly different per organ per year, LSD (P = 0.05).
* Significant difference between phenological stages
** Treatment (bearing or non-bearing) and phenological stage significant differences

Table 2. The concentration of extracted sugars (monosaccharides, oligosaccharides, and polysaccharides) and starch within selected plant organs of '9-02' southern highbush blueberry cultivar at three distinct phenological stages during the 2019/20 and 2020/21 seasons.

Carbohydrate	Treatment	Phenological stage	Roots		Primary shoots		Secondary shoots		Leaves	
			2020	2021	2020	2021	2020	2021	2020	2021
			mg.g^{-1} DW							
Monosaccharides + oligosaccharides	Bearing	50% flowering	44.6 d	79.2 b	39.0 bc	64.5 a	51.0 bc	79.9 a	156.9 b	149.8 d
		90% flowering	58.4 c	67.4 c	37.5 c	33.2 c	40.8 d	29.0 cd	135.1 c	133.8 e
		Peak harvest	76.6 b	59.1 d	41.8 bc	20.9 d	54.8 b	25.7 d	127.1 d	186.4 b
	Non-bearing	50% flowering	47.4 d	93.6 a	42.5 b	69.0 a	52.7 bc	80.4 a	155.3 b	167.9 c
		90% flowering	80.5 b	64.1 cd	41.3 bc	39.2 b	48.8 c	32.1 c	164.9 a	147.6 d
		Peak harvest	91.5 a	63.9 cd	53.8 a	23.0 d	67.2 a	45.9 b	126.9 d	215.0 a
		P-Value	**0.0002**	**0.0037**	**0.0171**	**0.452****	**0.0120**	**0.0002**	**<.0001**	**0.0900****
Polysaccharides	Bearing	50% flowering	21.0 b	31.0 ab	23.8 ab	29.5 ab	31.8 b	34.0 c	38.6 b	28.0 c
		90% flowering	22.2 ab	33.0 b	24.0 ab	28.4 b	30.1 b	37.2 b	32.4 cd	27.9 c
		Peak harvest	21.7 ab	29.1 b	22.0 c	15.2 d	31.2 b	30.7 d	28.8 d	34.7 b
	Non-bearing	50% flowering	22.8 ab	32.0 ab	23.5 abc	32.2 a	31.2 b	40.2 a	36.5 b	29.5 c
		90% flowering	24.2 a	32.4 ab	25.0 a	31.0 ab	31.1 b	37.8 b	35.6 bc	31.3 bc
		Peak harvest	22.7 ab	35.1 a	23.1 bc	20.0 c	39.7 a	40.5 a	46.1 a	38.5 a
		P-Value	**0.8841**	**0.1988**	**0.4197***	**0.4277****	**<.0001**	**<.0001**	**<.0001**	**0.6411****
Starch	Bearing	50% flowering	14.7 b	33.9 c	29.6 b	23.3 c	23.8 b	11.6 d	35.8 c	17.1 b
		90% flowering	21.1 a	40.2 ab	22.9 c	32.3 b	17.5 d	29.2 b	6.4 f	13.9 b
		Peak harvest	3.2 c	14.1 d	9.3 e	5.8 d	6.9 e	6.0 e	10.8 e	6.7 c
	Non-bearing	50% flowering	15.6 b	34.8 bc	33.4 a	23.1 c	26.0 a	12.4 d	43.3 a	17.1 b
		90% flowering	18.8 a	36.0 abc	29.2 b	35.2 a	25.6 ab	42.7 a	13.4 d	42.5 a
		Peak harvest	4.0 c	42.0 a	14.9 d	21.6 c	19.6 c	16.4 c	39.9 b	14.2 b
		P-Value	**0.1849***	**<.0001**	**0.4464****	**<.0001**	**<.0001**	**0.0007**	**<.0001**	**<.0001**
Total carbohydrates	Bearing	50% flowering	80.2 c	144.1 b	92.3 b	117.3 a	106.6 b	125.5 b	231.3 a	194.9 d
		90% flowering	101.7 b	140.7 bc	84.4 c	93.9 c	88.4 c	95.4 e	173.8 c	175.5 e
		Peak harvest	101.5 b	102.2 d	73.0 d	41.9 e	93.0 c	62.4 f	166.7 d	227.8 b
	Non-bearing	50% flowering	85.8 c	160.3 a	99.4 a	124.3 a	109.9 b	133.0 a	235.0 a	214.5 c
		90% flowering	123.5 a	132.6 c	95.4 ab	105.4 b	105.5 b	112.6 c	213.9 b	221.4 bc
		Peak harvest	118.2 a	141.0 bc	91.8 b	64.5 d	126.5 a	102.8 d	212.9 b	267.7 a
		P-Value	**0.0077**	**<.0001**	**0.0203**	**0.0118**	**<.0001**	**<.0001**	**<.0001**	**0.0108**

Per carbohydrate fraction, means with the same letter within a column (Phenological stage x Treatment) were not significantly different per organ per year, LSD (P = 0.05).
* Significant difference between phenological stages
** Treatment (bearing or non-bearing) and phenological stage significant differences

Table 3. Mean yield (g/plant) (n = 5) obtained up until peak harvest during the 2020 and 2021 seasons in '8-42' and '9-02' southern highbush blueberry at the Hex River site, Western Cape, South Africa.

2020			2021		
Harvest date	'8-42'	'9-02'	Harvest date	'8-42'	'9-02'
	g/plant			g/plant	
20 Aug. 2020	21.9 ± 2.9	11.1 ± 0.4	16 Aug. 2021	15.0 ± 2.2	23.4 ± 2.9
24 Aug. 2020	4.7 ± 0.4	10.3 ± 1.1	25 Aug. 2021	33.6 ± 5.5	77.2 ± 8.4
7 Sep. 2020	54.0 ± 7.6	87.7 ± 15.4	2 Sep. 2021	16.2 ± 1.1	67.5 ± 13.5
16 Sep. 2020	297.9 ± 11.8	76.9 ± 16.5	8 Sep. 2021	13.6 ± 2.3	83.3 ± 14.9
23 Sep. 2020	265.4 ± 22.1	178.5 ± 22.3	16 Sep. 2021	62.0 ± 10.7	23.9 ± 4.4
29 Sep. 2020	393.7 ± 16.6	267.7 ± 38.4	22 Sep. 2021	20.5 ± 5.6	167.4 ± 13.3
7 Oct. 2020	371.6 ± 25.2	214.4 ± 13.3	30 Sep. 2021	123.2 ± 16.7	204.6 ± 33.3
13 Oct. 2020	399.4 ± 20.7	339.8 ± 22.8	7 Oct. 2021	287.2 ± 18.4	138.8 ± 17.8
21 Oct. 2020	827.6 ± 37.7	566.2 ± 36.4	14 Oct. 2021	390.3 ± 20.9	223.2 ± 20.1
27 Oct. 2020	-	473.6 ± 56.9	19 Oct. 2021	564.0 ± 34.4	223.7 ± 11.0
6 Nov. 2020	-	663.0 ± 49.3	25 Oct. 2021	-	409.2 ± 50.9

Hyphens indicate where peak has already been reached

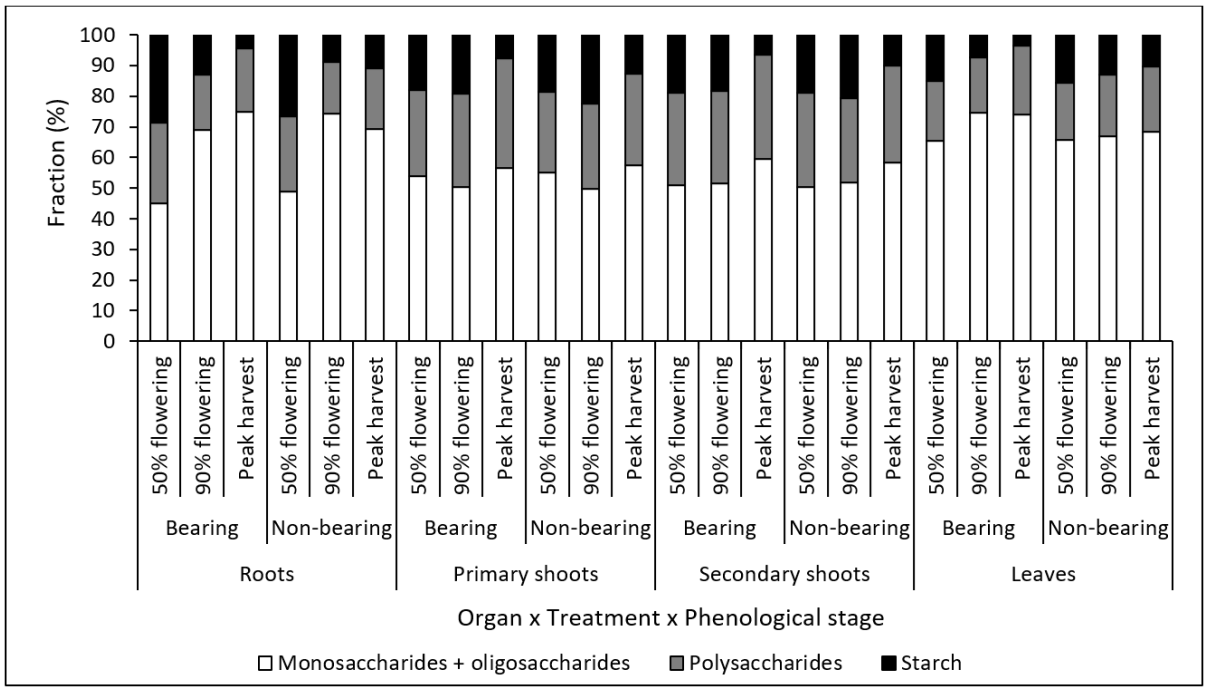

Figure 1. The fractions (%) of extracted sugars (monosaccharides, oligosaccharides, and polysaccharides) and starch, representing the available non-structural carbohydrates of selected plant organs as collected from bearing and non-bearing '8-42' southern highbush blueberry plants at 50% flowering, 90% flowering and peak harvest during the 2020 season.

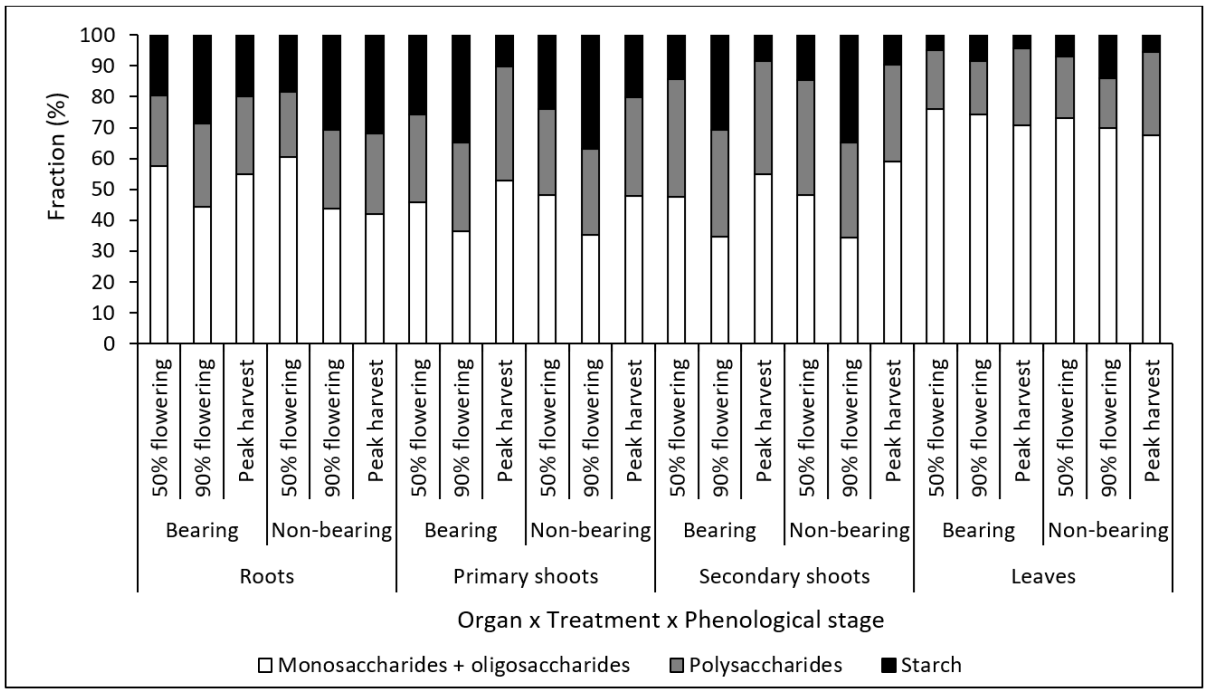

Figure 2. The fractions (%) of extracted sugars (monosaccharides, oligosaccharides, and polysaccharides) and starch, representing the available non-structural carbohydrates of selected plant organs as collected from bearing and non-bearing '8-42' southern highbush blueberry plants at 50% flowering, 90% flowering and peak harvest during the 2021 season.

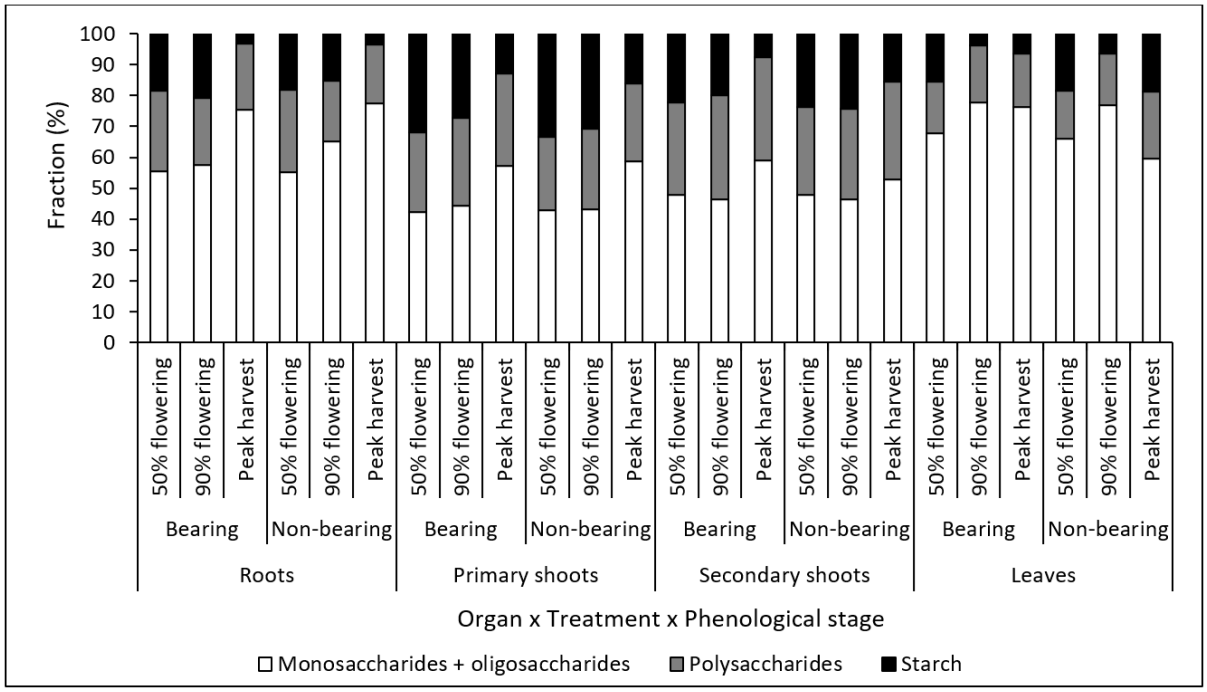

Figure 3. The fractions (%) of extracted sugars (monosaccharides, oligosaccharides, and polysaccharides) and starch, representing the available non-structural carbohydrates of selected plant organs as collected from bearing and non-bearing '9-2' southern highbush blueberry plants at 50% flowering, 90% flowering and peak harvest during the 2020 season.

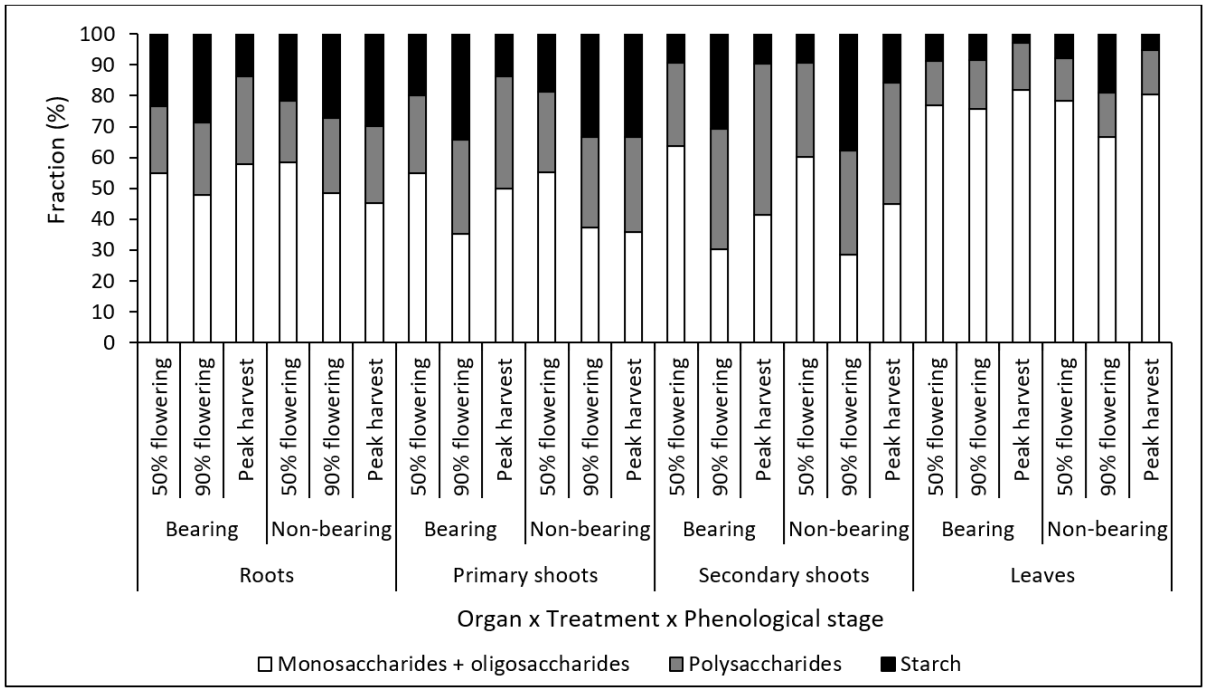

Figure 4. The fractions (%) of extracted sugars (monosaccharides, oligosaccharides, and polysaccharides) and starch, representing the available non-structural carbohydrates of selected plant organs as collected from bearing and non-bearing '9-2' southern highbush blueberry plants at 50% flowering, 90% flowering and peak harvest during the 2021 season.

GENERAL DISCUSSION AND CONCLUSION

The aim of this study was to quantify the above- and belowground phenology, along with non-structural carbohydrate allocation patterns, to provide insights into the observed difference in yield between two southern highbush blueberry (SHB) cultivars, 8-42 and 9-02, cultivated in an evergreen production system in the Hex River and Wolseley production areas, Western Cape, South Africa.

Knowledge of phenological stages is essential for optimizing resource utilization with the aim to achieve higher yields and top fruit quality, as important cultivation practices such as water management, fertilization, pest and disease management and harvest logistics rely on the recognition and prediction of such specific phenological stages (Baptista, 2006; Kishore, 2019; Larue et al., 2021). Both cultivars exhibited one main flowering (open and closed) period, at both sites, concentrated between the first week of June and last week of August (‘8-42’) or first week of October (‘9-02’). Surprisingly, the major peak noted for open flower counts for ‘9-02’, only occurred during mid-September at the Hex River site, a time when beehives were already removed from the orchard. It is suspected that a substantial number of these flowers did not complete their development, but aborted due to possible poor fruit set in the absence of bees, in addition to insufficient time for development into mature fruit before pruning was carried out in late November. Fruit harvests commenced in early August at the Hex River site, and advanced to the end of July, at the Wolseley site. However, despite these early harvesting times, more than 90% of the crop was concentrated between late-September and mid-November, for both cultivars, at both sites.

Our results concurred with previous studies, in that a period during August occurred where all phenological stages were witnessed simultaneously, for both cultivars (Baptista et al., 2006, Phillips et al., 2020). This is explained by the long, protracted reproductive seasons observed in evergreen SHB cultivars (Scalzo et al., 2016). Flowering, fruit set, and berry maturation may happen concurrently to various degrees on an evergreen plant (Scalzo et al., 2016). This phenomenon also occurs in blackberries and is known to complicate the prediction of production factors such as the timing of harvest and total yield (Hussain, 2016).

Root studies into root growth dynamics of blueberry are restricted to studies done on northern highbush blueberry and rabbiteye blueberry cultivars, grown in a

dormant production system in the northern hemisphere (Abbot and Gough, 1987). In our study on SHB within an evergreen cultivation system, comparable timing of increased root production rates was noted for '8-42' and '9-02'. Although root production at some level was evident throughout the study, a first peak was noted to coincide with flowering (closed and open flowers), with production rates that accelerated from late June onwards. High root production rates continued for approximately three months until an advanced state of fruit maturation in late September. The decrease in root production rates could be explained by fruit that are highly competitive sinks for carbohydrates, which results in the reverting of carbohydrates away from root growth, towards fruit growth and maturation, until the end of harvest (Bryla et al., 2017).

A second root production peak occurred towards mid-summer, after fruit harvest and summer pruning (late November), and occurred simultaneously with active shoot growth for approximately four months, until it declined with the termination of shoot elongation, during early May. Our results conferred with that of Abbot and Gough (1987) and Bryla et al. (2017), where also two root production peaks were noted in northern highbush blueberry cultivars, when cultivated in the northern hemisphere. In their studies, the first root peak occurred with the onset of flowering and continued until fruit set, during late spring. The second peak occurred soon after the completion of fruit harvest and continued until plants entered dormancy.

Abbot and Gough (1987) suggested that the production rate of white non-suberized root growth is interrelated with three major factors: soil temperature, shoot growth and stage of plant development. In their study, the respective peaks occurred at temperatures between 14°C to 18°C, whilst temperatures outside of this range caused a decline in root growth rates. In the study of Bryla et al. (2017), the respective peaks occurred when soil temperatures were between 18°C to 20°C and 19°C and 22°C. In our study, soil temperatures ranged between 11°C and 20°C during the first root production peak, while it varied between 17°C and 31°C during the second peak for both cultivars. Thus, our study revealed that soil temperature alone could not fully explain the timing of root production peaks in evergreen '8-42' and '9-02' SHB, but is rather determined by the stage of plant development and the resource acquisition between above- and below-ground growth.

The carbohydrate allocation trends evaluated in '8-42' and '9-02' revealed that sugar was the major carbohydrate component in all plant organs, of both cultivars and

during both studied seasons. Irrespective of bearing or non-bearing habits, starch concentrations were the highest in either roots or shoots, while sugar concentrations were highest in leaves as the sites of carbohydrate synthesis (Retamales and Hancock, 2018).

In bearing plants of '8-42', root growth and reproductive development were sustained by carbohydrates from leaves during the 50% to 90% flowering period, during both seasons. Thereafter, reproductive development and new vegetative growth, which became evident in late August, were supported by all plant organs, during both seasons, as suggested by a decrease in carbohydrates, in all plant organs, between 90% flowering and peak harvest. However, during the 2021 season, starch build-up occurred between 50% and 90% flowering in all plant organs, suggesting that the plants had sufficient sugars and polysaccharides to sustain root growth and reproductive development, and could utilize these reserves after 90% flowering to sustain further reproductive growth and new vegetative growth.

Bearing plants of '9-02' showed major differences in the carbohydrate allocation trends between the two consecutive seasons, especially for the roots and leaves. In the 2020 season, similar root carbohydrate concentrations for 90% flowering and peak harvest, suggested that root carbohydrates were not utilised between 90% flowering and peak harvest, whereas in the 2021 season, the sharp decline between 90% flowering and peak harvest is seen as being indicative of carbohydrate mobilisation from roots to support fruit growth and development. Leaf carbohydrates were extensively utilised between all phenological stages in the 2020 season, as indicated by a significant decrease between phenological stages, whereas in the 2021 season, leaf carbohydrates increased significantly between 90% flowering and peak harvest. It was suggested that this accumulation of carbohydrates imply an excess of carbohydrates in the 2021 season which were not allocated to promote additional reproductive growth and development.

In both cultivars, carbohydrate concentrations were generally higher at peak harvest in all plant organs of non-bearing than in bearing plants. This is explained by a higher carbohydrate availability in non-bearing plants, without the need to serve the demands from sinks such as developing flowers and/or maturing fruit (Kritzinger, 2014).

The outcomes of the studies conducted for this thesis raised a number of important limitations for consideration in future studies. Firstly, shifts in carbohydrate

allocation trends could have occurred during the extended period between evaluation dates, therefore it is recommended that in future studies sampling to quantify carbohydrate concentrations should be done at shorter intervals between target phenological stages, to accurately capture shifts in carbohydrate allocation that may occur between plant organs. Secondly, future studies should include an assessment of carbohydrates concentration levels prior to reproductive bud break to include the degree of carbohydrate accumulation that was reported in previous studies. Sampling to determine carbohydrate content should also be extended to the post-harvest period to determine the possible role of excess carbohydrates within specific plant organs on the following years' cropping behaviour as relating to time of harvest and yield. Lastly, it is acknowledged that the continuous growth habit of evergreen production systems makes predictions and quantification of phenological stages such as 50% flowering, 90% flowering and peak harvest exceedingly difficult and warrants further investigation to increase the accuracy of predictions, regarding phenological stages and crop estimates.

The aim of the thesis was to provide insight into the observed differences in fruit yield between the two evaluated cultivars. Although no major differences were shown in the above- and below-ground phenological traits between the evaluated cultivars, the carbohydrate allocation trends suggested that carbohydrates are produced in '9-02' plants in excess of what is required for the number of fruit produced. This study however could not explain why more energy is dedicated towards a more vigorous plant structure in '9-02'. Further research is thus needed to include the influence of other factors, such as hormonal status at the time of flower bud initiation and fruit development as well as manipulations strategies to increase resource allocation towards reproductive development in '9-02' or other cultivar also exhibiting a similar vigorous growth habit.

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Appendix 1: Supplementary tables (Paper 3)

Table 1. Individual plant organ dry weight (g) (± SE) of bearing and non-bearing '8-42' southern highbush blueberry plants at the Hex River site at 50% flowering, 90% flowering and peak harvest in the 2020 season.

Plant organ	Bearing			Non-bearing		
	50% flowering	90% flowering	Peak harvest	50% flowering	90% flowering	Peak harvest
	Dry weight (g)					
Roots	152.5 ± 3.8	190.7 ± 22.4	180.3 ± 15.7	168.2 ± 2.5	189.5 ± 7.5	172.3 ± 3.8
Primary shoots	208.8 ± 9.2	169.4 ± 27.0	142.3 ± 5.1	222.6 ± 10.3	176.9 ± 13.4	160.6 ± 6.7
Secondary shoots	137.7 ± 13.1	137.4 ± 15.1	171.5 ± 7.8	134.3 ± 9.0	130.3 ± 14.6	212.7 ± 18.9
Leaves	112.2 ± 6.9	188.1 ± 15.2	201.4 ± 9.8	147.7 ± 9.7	175.1 ± 14.5	320.5 ± 10.0
Total	611.2 ± 27.6	685.5 ± 29.0	695.5 ± 22.9	672.8 ± 23.0	671.8 ± 27.9	866.1 ± 31.1

Table 2. Individual plant organ dry weight (g) (± SE) of bearing and non-bearing '8-42' southern highbush blueberry plants at the Hex River site at 50% flowering, 90% flowering and peak harvest in the 2021 season.

Plant organ	Bearing			Non-bearing		
	50% flowering	90% flowering	Peak harvest	50% flowering	90% flowering	Peak harvest
	Dry weight (g)					
Roots	190.8 ± 14.6	209.8 ± 7.6	208.4 ± 12.5	216.8 ± 12.4	216.3 ± 6.9	227.5 ± 11.6
Primary shoots	264.3 ± 21.2	236.3 ± 27.8	292.9 ± 24.2	311.4 ± 43.6	281.7 ± 18.7	244.8 ± 21.0
Secondary shoots	197.5 ± 9.8	159.0 ± 3.6	177.4 ± 12.2	213.3 ± 18.2	198.1 ± 15.4	171.9 ± 19.2
Leaves	204.6 ± 19.3	177.3 ± 9.8	164.3 ± 24.0	228.6 ± 13.1	184.9 ± 14.5	188.9 ± 19.6
Total	857.2 ± 33.4	782.4 ± 36.1	843 ± 52.9	970.1 ± 74.9	881.0 ± 42.6	833.1 ± 55.1

Table 3. Individual plant organ dry weight (g) (± SE) of bearing and non-bearing '9-02' southern highbush blueberry plants at the Hex River site at 50% flowering, 90% flowering and peak harvest in the 2020 season.

Plant organ	Bearing			Non-bearing		
	50% flowering	90% flowering	Peak harvest	50% flowering	90% flowering	Peak harvest
	Dry weight (g)					
Roots	166.5 ± 5.3	213.4 ± 3.9	226.7 ± 15.4	187.3 ± 7.8	226.7 ± 5.7	227.3 ± 2.6
Primary shoots	156.3 ± 6.8	127.5 ± 11.3	136.8 ± 2.0	158.3 ± 7.4	142.4 ± 8.3	171.6 ± 11.8
Secondary shoots	118.5 ± 5.1	172.5 ± 22.0	213.1 ± 11.7	141.4 ± 5.7	167.9 ± 15.2	288.0 ± 14.8
Leaves	185.4 ± 9.7	186.0 ± 18.2	242.3 ± 6.3	206.4 ± 11.7	200.8 ± 11.7	364.4 ± 10.9
Total	626.7 ± 19.8	699.4 ± 46.9	818.9 ± 24.9	693.4 ± 21.0	737.8 ± 35.9	1050.0 ± 24.7

Table 4. Individual plant organ dry weight (g) (± SE) of bearing and non-bearing '9-02' southern highbush blueberry plants at the Hex River site at 50% flowering, 90% flowering and peak harvest in the 2021 season.

Plant organ	Bearing			Non-bearing		
	50% flowering	90% flowering	Peak harvest	50% flowering	90% flowering	Peak harvest
	Dry weight (g)					
Roots	229.9 ± 9.8	226.2 ± 17.7	209.8 ± 15.2	243.1 ± 11.0	229.9 ± 14.8	254.9 ± 21.3
Primary shoots	371.2 ± 19.8	364.7 ± 13.6	259.5 ± 9.2	308.5 ± 36.5	359.4 ± 28.2	313.0 ± 28.0
Secondary shoots	290.5 ± 24.0	291.6 ± 9.3	283.2 ± 31.5	329.3 ± 20.3	338.8 ± 14.1	363.0 ± 32.5
Leaves	260.0 ± 25.4	224.2 ± 21.0	217.5 ± 13.2	238.8 ± 25.1	256.5 ± 13.1	331.9 ± 32.7
Total	1151.6 ± 64.6	1106.7 ± 33.6	970.0 ± 45.2	1119.7 ± 76.9	1184.6 ± 49.1	1262.8 ± 71.5