

Harvest scheduling of southern highbush blueberries  
(*Vaccinium corymbosum* L. interspecific hybrids) in a climate  
with moderate winter chilling

By

Philippus Swart



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Supervisor: Prof. K.I Theron

Dept. of Horticultural Science

University of Stellenbosch

Co-supervisor: Prof. W.J. Steyn

Dept. of Horticultural Science

University of Stellenbosch

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## **Declaration**

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

March 2015

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In Deo Sapientiae Lux

## Summary

Profitability in the export driven South African blueberry industry is dependent on early spring harvests. The George region in the Western Cape accumulates too few chill-units to release buds of some southern highbush (SHB) blueberry cultivars from endodormancy. This causes problems like delayed budbreak and extended harvests. Growers of other temperate fruit crops are also affected by these problems and chemical rest breaking agents (RBAs) are applied in orchards in an attempt to overcome these. Application of the RBA hydrogen cyanamide (HC) occurs commonly in commercial pome and stone fruit orchards while thidiazuron (TDZ), another RBA is applied on a limited scale in apple orchards. The effect of RBA application on berry ripening, berry size and yield in SHB cultivars Bluecrisp, Emerald and Star was investigated for two seasons in an orchard near George, in order to determine to what extent harvest scheduling with RBAs is possible. Following Dormex® (HC, 520 g L<sup>-1</sup>) application during 2010, when a warm winter was experienced, the berry ripening of 'Bluecrisp' was accelerated. Dormex® application before reproductive bud scales opened, but after some chilling, resulted in acceptable yield and berry size without damage to reproductive buds. A 1% rate gave similar results as a 2% rate, but at a lower risk of reproductive bud damage. Lift® (TDZ, 3 g L<sup>-1</sup>) application reduced the number of days to 75% harvest in 'Star' during 2010. Lower yielding plants produced larger berries than those from higher yielding plants. Lift® application after reproductive buds scales have opened caused malformed and damaged flowers.

Delaying the initiation of reproductive buds could delay spring reproductive budbreak until after new leaves had formed. In turn, this should induce a faster berry ripening rate in some SHB cultivars than would otherwise be the case following unseasonably warm winters. Reproductive bud initiation in SHB blueberries occurs under long (16 hours) nights with the mediation of phytochrome. It is possible in a controlled environment to suppress SHB blueberry reproductive bud initiation by night interruption (NI). The effect of NI on berry ripening, berry size and yield in 'Emerald' and 'Snowchaser' was investigated for two seasons, to determine what degree of harvest scheduling is possible with this technique. NI did not suppress reproductive bud development under these trial conditions, since both cultivars flowered and produced fruit. The effect on berry size and yield was cultivar specific. During 2011 NI decreased the number of berries harvested and total yield per plant in 'Emerald', and this decrease was linear the longer the NI lasted.

## Opsomming

Wingsgewendheid in die uitvoer-gedrewe Suid-Afrikaanse bloubessie-bedryf is van vroeë lante oeste afhanklik. In die George-omgewing in die Wes-Kaap bou te min winterkoue op om die endodormansie van sommige ‘southern highbush’ (SHB) bloubessie kultivars natuurlik op te hef, wat probleme soos vertraagde bot en uitgerekte oestye veroorsaak. Producente van ander gematigde vrugtesoorte, word ook deur hierdie probleme geraak en chemiese rusbreekmiddels (RBs) word in boorde aangewend in ’n poging om dit te oorkom. In kern- en steenvrugboorde vind aanwending van die RB waterstofsianamied (WS) algemeen plaas. Thidiazuron (TDZ), ’n ander RB word op beperkte skaal in appelboorde aangewend. Die uitwerking van RBs op bessierypwording, -grootte en opbrengs van SHB kultivars Bluecrisp, Emerald en Star is oor twee seisoene in ’n boord naby George ondersoek, om vas te stel tot watter mate bloubessie-oesskedulering met behulp van RB aanwending moontlik is. Na Dormex® (WS, 520 g L<sup>-1</sup>) aanwending in 2010, waarin ’n warm winter ondervind is, is die bessierypwording van ‘Bluecrisp’ versnel. Dormex® aanwendingstye voordat blomknopskubblare oopmaak, maar nadat winterkoue opgebou het, het ’n aanvaarbare opbrengs en bessiegrootte met geen blomknopskade tot gevolg gehad nie. ’n 1% Konsentrasie gee soortgelyke reaksies as ’n 2% aanwending maar teen ’n laer risiko vir blomknopskade. Lift® (TDZ, 3 g L<sup>-1</sup>) aanwending het die aantal dae tot 75% oesinsameling van ‘Star’ in 2010 verminder. Plante wat ’n laer opbrengs lewer produseer groter bessies as die wat ’n hoër opbrengs lewer. Lift® aanwending nadat blomknopskubblare oopgemaak het, het misvormde en beskadigde blomme tot gevolg gehad.

Vertraging van blomknopinisiasie kan die oopmaak van blomknoppe uitstel tot na nuwe blare in die lente gevorm het. Dit kan vinniger bessie rypwording meebring as wat die geval vir sommige SHB kultivars na warm winters is. Die aanvang van blomknopontwikkeling in SHB bloubessies vind tydens lang nagte (16 ure) plaas en staan onder beheer van fitochroom. Onder beheerde toestande kan bloubessie blomknopinisiasie deur onderbreking van die lang donker (nag) tydperk (ON) in ’n lig-donker siklus onderdruk word. Die uitwerking van ON op bessierypwording, -grootte en opbrengs van ‘Emerald’ en ‘Snowchaser’ is oor twee seisoene ondersoek, om die mate waartoe oesskedulering met hierdie tegniek in ’n boord moontlik is aan te spreek. ON het nie die blomknopinisiasie onder hierdie eksperimentele toestande onderdruk nie, aangesien beide kultivars in albei seisoene kon blom en opbrengste lewer. Die effek op bessiegrootte en opbrengs was kultivar spesifiek. In 2011 is die totale opbrengs en hoeveelheid bessies per plant geoes van ‘Emerald’, deur ON verminder en dié vermindering was liniêr met toename in aantal ON siklusse.

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

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## General introduction

The blueberry species (*Vaccinium* spp.) are native to North America. Cultivated rabbiteye (*V. virgatum* Ait., syn. *V. ashei* Reade, syn. *V. amoenum* Ait.), northern highbush (*V. corymbosum* L., syn. *V. constablaei* Gray) and southern highbush (SHB) blueberry (interspecific hybrids of *V. corymbosum* L., *V. darrowi* Camp and *V. virgatum* Ait.) largely satisfy the world demand for fresh blueberries. Similar to other horticultural crops, market price for fresh blueberries is highly influenced by time of marketing. Higher prices are offered for fresh berries between the main harvest seasons of the well-established blueberry production regions at high latitudes. This has caused interest in commercial cultivation elsewhere. The development and release of blueberry cultivars with increasingly lower chilling requirements enabled recent industry expansion into new production regions (Darnell and Williamson 1997; Greeff 2003; Greeff and Greeff 2006; Williamson et al. 2012) like the Mediterranean-type climate Western Cape province of South Africa.

Blueberry plants in regions that frequently experience warm winters, like the Western Cape, exhibit delayed foliation during spring. This leads to a delayed and extended harvest season and reduction in berry size (Williamson and Lyrene 2004; Williamson et al. 2012). The most lucrative export market window for fresh blueberries from the Western Cape stretches from mid-September until the end of November. Thus, the only cultivars planted by the majority of growers here are those that ripen early, such as the SHB cultivars Bluecrisp, Emerald, Jewel, Snowchaser and Star (Lyrene 2005; Greeff and Greeff 2006; Müller 2011).

Following warm winters, many temperate-zone deciduous crops cultivated in South Africa and the Western Cape are subject to delayed foliation. Various cultural practices, including the application of chemical rest breaking agents, are employed to manipulate vegetative budbreak in fruit and nut crops. Hydrogen cyanamide (HC) and thidiazuron (TDZ) are two chemical rest breaking agents that have long been used successfully for this purpose. Information in the current literature regarding the use of chemical rest breaking agents on blueberry under South African conditions is limited. In other mild wintered regions, HC has shown potential to accelerate vegetative budbreak in rabbiteye and SHB blueberry (Williamson et al. 2001, 2002; Stringer et al. 2004; Jaldo et al. 2009). Responses were mostly cultivar specific. Williamson and Lyrene (2004) therefore recommended cultivar-specific local evaluation of HC.

Bud dormancy has been overcome by using TDZ on apple (Wang et al. 1986; Steffens and Stutte 1989; Costa et al. 2004) and other fruit crops (Costa et al. 2004). When applied after petal fall, TDZ was shown to improve the yield, as well as the fruit and berry size of kiwifruit and seedless grapes, respectively (Reynolds et al. 1992; Jindal et al. 2003). Recently, improved foliation was observed on fig cultivars in South Africa following a 6% Lift® (TDZ) application during midwinter or at an early stage of bud swell (Theron et al. 2011). Nothing is known about the effects of exogenous TDZ application on blueberry plants.

Darnell and Williamson (1997) proposed investigating the effect of photoperiod on blueberry flower bud initiation to help ensure successful production at lower latitudes. In both rabbiteye and SHB blueberry, vegetative buds on current-season growth are best converted into flower buds (flower bud initiation) under moderate temperatures (21°C) and short days (8 hour photoperiods). During long days (16 hour photoperiods), the rate of flower bud development and vegetative growth is greatest. However, SHB cultivars initiate flower buds under different photoperiod ranges in different growing regions (Spann et al. 2003). A temperature/photoperiod interaction on flower bud initiation was shown (Spann et al. 2004).

Considering the above, a literature review was done, paying particular attention to blueberry flowering, plant phenology at low latitudes, as well as elements like dormancy, chilling requirement and photoperiod that contribute to this. Literature covering chemical rest breaking agents commonly used on blueberry was also reviewed.

In this thesis we report on the efficacy of HC and TDZ applications at different rates and timings on harvest scheduling, average berry weight and yield of SHB cultivars Bluecrisp, Emerald and Star. In addition, we report on the efficacy of night interruption around midnight with low light intensity incandescent light on the same parameters of SHB cultivars Emerald and Snowchaser.

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# Literature review: Harvest scheduling of southern highbush blueberries (*Vaccinium corymbosum* L. interspecific hybrids) in a climate with moderate winter chilling

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## 1. Background information

### 1.1 Aspects of bud dormancy

Bud dormancy is widely regarded as a development phase, during which visible growth is delayed for a time (Gough et al. 1978; Martin 1991; Crabbe 1994; Erez 2000). Some

understanding of what leads to bud dormancy (induction), its maintenance and eventual end (release), might help make cultural practices used for manipulating dormancy more profitable (Erez 1995, 2000; Faust et al. 1997; Faust 2000). During most winters, temperate zone plants are exposed to adverse growing conditions like freezing and dehydration. Woody perennials adapted in order to tolerate and survive these conditions. These plants are able to enter a state of bud dormancy, and also became cold-hardy (i.e. have the ability for cold acclimation during autumn) (Martin 1991; Crabbe 1994; Faust et al. 1997; Erez 2000; Rowland et al. 2005; Kalberer et al. 2006).

Lang et al. (1987) introduced the terminology eco-, endo- and paradormancy to classify different bud dormancy types. Endodormancy (syn. winter dormancy, true dormancy or rest) follows an environmental, or internal stimulus (e.g. photoperiod, ambient temperatures, hormones) that is perceived within (i.e., “endo”) the bud it has an effect on. This is regulated by physiological factors from within the effected plant structure. Paradormancy (syn. summer dormancy, pre-dormancy or correlative inhibition) follows a stimulus that is perceived by a plant structure other than (i.e., “para”) the one it has an effect on (e.g. apical dominance). Ecodormancy (syn. imposed dormancy, summer dormancy or quiescence) is induced by unsuitable environmental (i.e. “eco”) factors (e.g., hot or cold temperatures, dehydration, nutrient deficiencies) that have a general effect on all aspects of plant development and physiology, including those of a bud that becomes dormant (Saure 1985; Fuchigami and Nee, 1987; Lang et al. 1987; Martin 1991; Crabbe 1994; Crabbé and Barnola 1996; Faust et al. 1997; Erez 2000; Arora et al. 2003).

The progression of bud paradormancy into endodormancy is believed to be induced by shorter photoperiods and/or colder temperatures during autumn (Phatak and Austin 1990; Darnell 1991; Couvillon 1995; Horvath et al. 2003; Rhode and Bhalerao 2007), and controlled on a physiological level by plant hormones (Powell 1987; Bernier et al. 1993; Amasino 1996; Faust et al. 1997; Faust 2000; Horvath et al. 2003). The transition into bud dormancy is not necessarily linked to cold-hardiness (Faust et al. 1997; Erez 2000; Arora et al. 2003). Fennel and Hoover (1991) demonstrated that bud endodormancy in grapes (*Vitis labruscana* L.) can be induced by short (8 hour) photoperiods without cold treatment, while in apples and pears, Heide and Prestrud (2005) determined that bud endodormancy can be induced by low temperatures (<12 °C) without exposure to short photoperiods.

For temperate zone woody perennials, the release from endodormancy, and also becoming fully dehardened during spring, depends on 1) the winter fulfillment of chilling requirements of buds that exists from autumn, and 2) spring temperatures that are suitable for growth (Faust 2000; Fuchigami and Wisniewski 1997; Rowland et al. 2005; Kalberer et al. 2006; Rohde and Bhalerao 2007). Low ambient temperatures are not required for the release of paradormant buds, but it is for endodormant buds (Lang et al. 1987). A model developed by Weinberger (1950) quantifies chill-unit accumulation as the time (hours) during which endodormant buds perceive exposure to temperatures between 0 and 7.2 °C (45 °F, often converted into 7 °C). Richardson et al. (1974) developed the Utah-model for peach trees. It adds predetermined values assigned to specific chill-units (Fishman et al. 1987). Each hour of exposure at 6.1 °C contributes a maximum chill-unit value of one. Hourly intervals of exposure between 6 and 8 °C also contribute high values, but intervals outside of this temperature range, down to 1.5 °C and up to 12.4 °C, contribute progressively less. No contribution is made by hours below 1.5 °C. Progressively stronger negative values are contributed by intervals above 15.9 °C. A minimum value of minus one chill-unit is contributed by intervals at temperatures higher than 18 °C (Richardson et al. 1974). Thus, the Utah-model takes into account that cool or high temperatures can respectively contribute to chill-unit accumulation, or cancel prior chilling. Some later dormancy completion models were based on adaption of the Utah-model, e.g. the Florida-model for nectarines by Gilreath and Buchanan (1981), one for blueberries by Norvell and Moore (1982) and one for apples by Shaltout and Unrath (1983). Norvell and Moore (1982) observed that in Northern highbush (NHB) blueberry (*Vaccinium corymbosum* L.) cultivars Bluecrop and Coville, chilling occurs between 1 and 12 °C, with 6 °C being the optimum. They therefore adapted the Utah-model, to allow each hour of exposure at 1 °C to contribute 0.5 chill-units.

The Utah-model and derivatives thereof, gives satisfactory results when applied in temperate climates, but was found to be less reliable in mild wintered growing regions (Erez et al. 1979a; Linsley-Noakes and Allan 1994; Allan 1999). To some extent, this is because the cancellation of prior chilling by high temperatures is dependent on the length of the period of exposure to chilling temperatures (Erez et al. 1979a). Furthermore, mild temperatures could also contribute to chill-unit accumulation (Erez et al. 1979b; Erez and Couvillon 1987). Erez et al. (1979a) observed how vegetative peach buds reacted to different periods (1, 3, 6 or 9 days) of exposure to a combination of cool and high temperatures. Two thirds of each period was kept at 4 to 6 °C, and the remaining third at 24 °C. Periods were repeated, so that all

plants eventually received the same chilling. The result was that increasingly better bud break followed increasingly longer periods of exposure. They concluded that warm temperature only cancel prior chilling if it is experienced during short periods of chill-unit accumulation. Also, after a certain period (6 days), prior chill-unit accumulation become fixated and thus could not be cancelled thereafter (Erez et al. 1979a). During a following trial, Erez et al. (1979b) also examined the reaction of vegetative peach buds during 24 hour cycles of fluctuating temperatures. In two thirds of such a cycle, buds were exposed to cool (6 °C) temperature, and during the remainder of a cycle to either mild (15 or 18 °C) or high (21 or 24 °C) temperatures. Compared with continuous chilling at 4 °C, mild temperatures in a cycle contributed to bud break, by way of reducing the amount of chilling hours required for good budbreak. High temperatures in a cycle cancelled prior chilling, as no bud break occurred following cycles that included 21 or 24 °C (Erez et al. 1979b). Most endodormancy completion models developed hereafter, especially those focused on deciduous fruit crops in mild winter growing regions, factor in these findings by Erez et al. (1979a, 1979b), e.g. the two-step model by Fishman et al. (1987), and the dynamic-model by Erez et al. 1990 (Erez and Couvillon 1987; Seeley 1996; Allan 1999). Linsley-Noakes et al. (1994) focused their attention on South African stone fruit. They proposed a modification to the Utah-model, so that the cancellation effect of high (>15.9 °C) temperatures on chilling is not transferred between successive diurnal cycles (Linsley-Noakes et al. 1994). This modification made the estimation of reproductive bud break in nectarines more reliable (Allan 1999).

Buds differ in chilling requirement (Erez et al. 1979b; Saure 1985; Martin 1991; Horvath et al. 2003). For instance, southern highbush (SHB) blueberry (*V. corymbosum* L. interspecific hybrids) vegetative buds have a higher chilling requirement than reproductive buds on the same shoot (Williamson and Lyrene 2004; Lyrene 2005). Chilling requirement, together with cold acclimation, help to ensure that plant growth only continues during favourable environmental conditions. A high chilling requirement tends to delay spring flowering, reducing the risk of flowers or fruit being damaged by a late spell of very cold weather (Saure 1985; Martin 1991; Bernier et al. 1993; Crabbe 1994). The amount of chilling required is determined genetically, and varies between species and cultivars within the same species (Erez 2000; Ballington 2001; Lyrene and Ballington 2006).

Identifying the point when chilling unit accumulation begins and ends is a point of controversy (Crabbe 1994; Fuchigami and Wisniewski 1997; Rohde and Bhalerao 2007). It appears that buds do not start to record chill units if they have not become endodormant, and

that chilling accumulation may end during spring before the last cold is past (Arora et al. 2003; Rhode and Bhalerao 2007). Buds differ in their sensitivity to chilling temperature at different stages of their dormancy (Austin and Bondari 1987; Erez and Couvillon 1987; Crabbe 1994; Couvillon 1995). Photoperiod and temperature interaction, as well as the influence of the amplitude of diurnal temperature fluctuation also have an influence on chill-unit accumulation (Fishman et al. 1987; Fennel and Hoover 1991; Couvillon 1995; Arora et al. 2003; Spann et al. 2003, 2004; Bañados and Strik 2006).

NHB and SHB blueberries enter endodormancy, and thus require chilling (Norvell and Moore 1982; Lyrene 1990, 2002; Darnell 2006). Blueberry cultivars differ in chilling requirement, a trait that is highly heritable. High-chill parents produce high-chill offspring. Progeny of high and low-chill parents are intermediate and second generation populations segregate as though the chilling requirement is controlled by many genes (Lyrene 1990, 2002, 2005). High-chill cultivars are short lived and not consistently productive in mild wintered regions (Erez 1995; Erez 2000; Darnell and Williamson 1997; Darnell 2006). For this reason, there has been and continues to be much effort focused on developing blueberry cultivars with low chilling requirements (Sharpe and Sherman 1971; Lyrene 1990, 2002, 2005; Hancock et al. 1996). Such cultivars should be adapted for cultivation in growing regions where the mean temperature of the three coldest months is as high as 15 °C (Lyrene and Sherman 2000).

## 1.2 Commercially cultivated blueberries

Commercially cultivated blueberries are classified as belonging to the section *Cyanococcus* Rydb. in the Ericaceae plant family and *Vaccinium* genus (Hancock and Draper 1989; Ballington 2001; Darnell 2006). NHB blueberry is a deciduous species that has its origin in North America, latitude 40 to 45 °N (Hancock and Draper 1989; Hancock et al. 1996). At these latitudes it is the most cultivated species and is largely responsible for the annual supply of fresh blueberries (Hancock and Draper 1989; Eck et al. 1990; Ballington 2001). Rabbiteye (RE) blueberry cultivars were bred from a deciduous species, *V. ashei* Reade (syn. *V. virgatum*), which is indigenous to and well adapted in northern Florida (Eck 1988; Lyrene 1989; Darnell and Davies 1990; Darnell 2006; Lyrene and Ballington 2006). RE cultivars require 300 to 650 hours of exposure at 0 to 7 °C for dormancy completion (Austin and

Bondari 1987; Eck 1988; Darnell and Davies 1990; Tamada 1997). Although this is a relatively low chilling requirement with regards to blueberries species in general, the same RE cultivar usually yields much less at lower than at higher latitudes (Lyrene 1989; Erez 1995; Darnell and Williamson 1997). NHB cultivars have a higher chilling requirement (800 to 1500 hours at 0 to 7 °C) than RE cultivars (Norvell and Moore 1982; Eck 1988; Hancock and Draper 1989; Ballington et al. 1990), and are badly adapted to lower latitudes (Lyrene 1989; Erez 1995; Darnell and Williamson 1997).

NHB cultivars are harvested from mid-May through to the end of September in the northern hemisphere and from mid-November through to the end of March in the southern hemisphere (Lyrene and Sherman 2000). The remaining gaps in the harvest year can be filled successfully by growing blueberry cultivars adapted to mild wintered regions, where temperatures are also warm during late winter and early spring. For this reason plant breeders have been developing vigorous, high-yielding highbush type cultivars that produce early harvests of good quality berries for cultivation in such regions (Sharp and Sherman 1971; Eck 1988; Hancock et al. 1996; Lyrene and Sherman 2000; Lyrene and Ballington 2006). SHB cultivars are interspecific hybrids, with an upright growth habit and berries large enough to meet fresh market requirements. The gene pool from which SHB cultivars are bred, were developed by crossing NHB cultivars with other blueberry species, e.g. the evergreen *V. myrsinites* Lam. and *V. darrowi* Camp species native to Florida. *V. darrowi* has a low chilling requirement of approximately 200 hours below 7 °C. It has been used extensively in breeding the SHB blueberry at the University of Florida. Initially, *V. darrowi* was crossed with NHB and RE blueberries. Later other species such as *V. tenellum* Ait. and *V. angustifolium* Ait., were included in this breeding program. During 1970, seven low-chill highbush type blueberries were selected. Sharpblue and Flordablue, the first SHB cultivars, were released by the Univ. of Florida in 1975 (Sharpe and Sherman 1971; Eck 1988; Lyrene 1989; Hancock et al. 1996; Ballington 2001). Other low-chill blueberry cultivars have subsequently been released from breeding programs in Florida, Mississippi, North Carolina, and Georgia (Ballington et al. 1990; Lyrene and Sherman 2000; Lyrene 1990, 2002, 2005; Lyrene and Ballington, 2006). Modern SHB cultivars ripen early (commonly before RE cultivars) and range in chilling requirement from 150 to 600 hours at 0 to 7 °C (Sharpe and Sherman 1971; Ballington et al. 1990; Lyrene 1989, 1990, 2002, 2005; Lyrene and Sherman 2000; Lyrene and Ballington 2006). New cultivars are bred with specific growing regions in mind, so that berries ripen early to mid-season (Lyrene and Ballington 2006). The mean temperatures of

the coldest month and the average annual duration of temperatures below 7 °C are usually considered by breeders (Ballington et al. 1990; Lyrene 1990, 2002). SHB cultivars can provide fresh blueberries during April and early May in the northern hemisphere, and during October and early November in the southern hemisphere (Lyrene and Sherman 2000). An earlier time of fruit ripening allows for a longer period of production and the ability to supply fresh markets at an earlier, more lucrative time. Berry firmness and a dry picking scar are also important characteristics, because such berry attributes are more suitable for picking, post harvest handling and storage (Ballington et al. 1990; Lyrene and Sherman 2000). Modern SHB cultivars are grown successfully in the northern hemisphere as far south as 27.5 °N latitude (Darnell and Williamson 1997).

Lowbush blueberries, *V. angustifolium* and *V. myrtilloides* Michx., occur naturally at higher latitudes than NHB blueberry. Lowbush blueberry plants are managed and harvested in the wild for the processing market (Hall and Ludwig 1961; Aalders and Hall 1964; Eck et al. 1990).

### **1.3 Flower bud induction and initiation in highbush blueberry**

The different stages of flower formation are usually referred to as flower bud induction, initiation, differentiation and development (Aalders and Hall 1964; Tamada 1997; Williamson and Lyrene 2004). To best manipulate fruiting season, while maintaining satisfactory yield and fruit quality, it is important to understand the factors that control reproductive development (Bernier et al. 1993; Amasino 1996; Williamson and Lyrene 2004; Wilkie et al. 2008). This begins with flower bud induction and initiation (FBI), usually during the preceding autumn (Bernier et al. 1993; Wilkie et al. 2008). It is then interrupted by endodormancy, before ending with spring anthesis (Gough et al. 1978; Eck et al. 1990; Bernier et al. 1993; Williamson and Lyrene 2004; Wilkie et al. 2008). Blueberry flower bud differentiation and development is influenced by the climate, cultivar, plant age, type of bearing wood and pruning (Aalders and Hall 1964; Austin and Bondari 1987; Tamada 1997; Williamson and Lyrene 2004; Rowland et al. 2005). FBI is well documented in blueberry (Hall and Ludwig 1961; Hall et al. 1963; Gough et al. 1978; Phatak and Austin 1990; Darnell 1991; Spann et al. 2003, 2004; Williamson and Lyrene 2004; Bañados and Strik 2006). It seems to occur under the relatively short days (long nights) of late summer to early autumn,

and more towards the distal section (apical bud) of current season shoots. For NHB blueberry (cultivars Duke, Bluecrop, Elliott), eight weeks of exposure to 8 or 10 hour photoperiods, resulted in significantly more reproductive buds in comparison to 14 or 16 hour photoperiods (Hall et al. 1963). Extended (eight weeks) exposure to short days increased FBI (Bañados and Strik 2006). Following limited (four weeks) exposure to short (8 hour) photoperiods, flower bud differentiation and development appeared incomplete, and bloom was delayed when measured against that of plants experiencing extended exposure (Bañados and Strik 2006). Similarly, at least 5 to 6 weeks of short photoperiods (long nights) were required for normal flower bud initiation in RE blueberry cultivar Beckyblue (Phatak and Austin 1990; Darnell 1991).

Spann et al. (2003) observed that SHB blueberry 'Misty' initiated reproductive buds under short days (8 hour photoperiod), i.e. long dark periods. No FBI occurred during exposure to short dark periods (16 hour photoperiod), and long dark periods that were each interrupted in the middle for an hour, inhibited FBI (Spann et al. 2003). During a subsequent trial, they noted that 'Misty' reproductive buds were visibly smaller, and budbreak delayed and reduced, when plants were subjected to only four weeks of short days in contrast to eight weeks. FBI was found to be significantly reduced at a constant high (28 °C) compared to moderate (21 °C) temperature under short days. They also noted that flower bud development remained incomplete at high temperature (Spann et al. 2004). Above results indicate that both *V. darrowi* and SHB blueberry are short-day plants in which FBI is a phytochrome mediated response (Salisbury 1985; Spann et al. 2003). The same holds true for lowbush, NHB and RE blueberries (Hall and Ludwig 1961; Hall et al. 1963; Darnell 1991).

Short-day plants initiate reproductive buds when the nights are longer than a critical minimum length, but don't necessarily flower under such conditions (Salisbury 1985; Izawa et al. 2000). Mature leaves contain a blue-green plant pigment called phytochrome (Imaizumi and Kay 2006). Phytochrome functions as a photoreceptor (light signaling molecule). This molecule exists in an active and inactive form, which is interconverted by red and far-red light (Amasino 1996). The far-red form is believed to be the biologically active form, but it is unstable and reverts in darkness back to the inactive red absorbing form (King and Bagnall 1996). It appears that for short day plants, the far-red activated form of phytochrome is essential in the production of floral stimuli during inductive darkness (Amasino 1996; Izawa et al. 2000). Phytochrome is also required for the red-light night interrupted inhibition of FBI (Izawa et al. 2000; Imaizumi and Kay 2006). In terms of red or far-red wavelengths, distinct

irradiance conditions exists during the diurnal cycle, and as day length varies with the changing seasons (Imaizumi and Kay 2006). By way of controlling phytochromes, such predictable seasonal variations are believed to help control the transcription of certain genes at certain times of the day and year. These genes could specify enzymes and other proteins with roles in the timing of FBI (Amasino 1996; Hayama and Coupland 2003; Imaizumi and Kay 2006). Phytochrome might also form part of a complex day length measuring mechanism in plants, the circadian system (syn. circadian clock, or circadian rhythms) (Hayama and Coupland 2003; Imaizumi and Kay 2006).

## **2. Blueberry cultivation at low latitudes**

### **2.1 Plant phenology**

To obtain a large, early (e.g. September in the South African context) crop at low latitudes, blueberry cultivars should be grown that flower early (e.g. the beginning of July) and at the same time produce new leaves, while most of their mature leaves are maintained (Lyrene 2004). A very undesirable phenological pattern is when a cultivar maintains its mature leaves until flowering, but then drops them before new leaves emerge (Lyrene 2004). Mild winter temperatures, differences in day length (photoperiod), growing season length and high temperature are all climatic factors that influence blueberry phenology at low latitudes (Darnell and Williamson 1997). As discussed earlier, blueberry cultivars differ in the number of chilling hours required for a high percentage bud break (Sharpe and Sherman 1971; Norvell and Moore 1982; Darnell and Davies 1990; Lyrene 1990, 2002, 2005; Darnell 2006) and vegetative buds have a higher chilling requirement than reproductive buds on the same shoot (Williamson and Lyrene 2004; Lyrene 2005). At low latitudes, annual chilling hours may vary considerably. Following unseasonably warm winters even SHB cultivars, with their relatively low chilling requirements, suffer from unpredictable flowering and pollination, and leaf emergence during or after fruit set (delayed foliation) (Williamson et al. 2001, 2002; Lyrene 2004; Jaldo et al. 2009), resulting in fruit set, berry quality, yield and in most cases berry ripening being adversely affected (Williamson and Lyrene 2004). If high daily maximum temperatures also prevail, the added stress can result in shoots dying back from the tips as in 'Misty' and 'Marimba' (Lyrene 2004). Winter pruning may benefit these cultivars by removing a number of flower buds (Lyrene 2004; Williamson and Lyrene 2004).

However, following sufficient chilling (approximately 600 hours between 0 and 7.2 °C), ‘Misty’ completes flowering in three weeks and produce sufficient leaves in time to support developing berries (Lyrene 2004). When a blueberry plant suffers from delayed foliation, its subsequent canopy development is suppressed (Maust et al. 1999). Fast growing and developing berries are mostly supported with carbohydrates from the leaves, and also from storage reserves (Maust et al. 1999; Lyrene 2004). For this reason, berry development is strongly influenced by berry load, leaf canopy establishment, and the resulting leaf to berry ratio (Maust et al. 1999). Berry development periods range from 50 to 90 days for NHB, 55 to 110 days for SHB, and 60 to 140 days for RE cultivars (Eck et al. 1990; Carlson and Hancock 1991; Darnell 1991; Mainland 2002; Ciordia et al. 2006; Ogden and Van Iersel 2009). Reproductive bud break precedes vegetative budbreak in ‘Misty’, and coincides with it in ‘Sharpblue’ (Maust et al. 1999). Maust et al. (1999) hand thinned flower buds of ‘Misty’ and ‘Sharpblue’ SHB plants during dormancy and observed that by decreasing the initial flower bud density, and thereby the subsequent berry density, the spring vegetative budbreak and canopy development can be increased. The eventual result being larger, faster ripening berries of better quality (Maust et al. 1999).

As mentioned earlier, blueberries are short day plants (Hall and Ludwig 1961; Hall et al. 1963; Darnell 1991; Spann et al. 2003), but there is genotypic variation in blueberry cultivars with regard to the sensitivity to photoperiod for optimal FBI (Darnell 1991). As late summer and early autumn days are longer at lower compared to higher latitudes, blueberry cultivar selection should bear in mind that photoperiod could limit FBI at low latitudes (Darnell and Williamson 1997). Terminal buds on the shoots of SHB, NHB and RE blueberry plants initiate flowers and flower earlier than the auxiliary buds (Tamada 1997; Williamson and Lyrene 2004). Blueberry flower organs (pedicel, sepal, corolla, stamen and pistil) develop sequentially and from the outside inwards ((Tamada 1997; Williamson and Lyrene 2004). As mentioned earlier high (28 °C plus) temperatures reduce FBI, even when SHB cultivars are exposed to inductive short photoperiods (Spann et al. 2004). Exposure to high temperatures also causes incomplete reproductive bud development. Hence, mild (<21 °C) autumn temperatures are required in addition to long nights for acceptable FBI and reproductive bud development at low latitudes (Spann et al. 2004).

The length of the growing season differs between latitudes. At high latitudes, the growing and harvest seasons of NHB and RE blueberries continues until shortly before blueberry plants enter endodormancy. By this time, new reproductive buds have already formed on current

season shoots (Lyrene 2005). At low latitudes, the latest SHB berries are usually harvested months before the onset of cold winter temperatures. In such cases, blueberry leaves are required to maintain photosynthesis and to perceive shortening photoperiods throughout late summer and autumn (Maust et al. 1999; Spann et al. 2003). SHB blueberry plants grown at low latitudes thus have to retain healthy leaves until the end of autumn for adequate FBI (Lyrene 2004). In order to achieve this, proper fertilization, summer pruning, and strict disease and pest management programs need to be implemented (Williamson and Lyrene 2004).

Maximum spring and summer temperatures also have an indirect effect on blueberry phenology (Darnell and Williamson 1997). Moon et al. (1987) reported that photosynthetic rates increased as leaf temperatures increased from 10 °C to 30 °C and 10 °C to 25 °C in RE and NHB cultivars Woodard and Bluecrop, respectively. In contrast to this increase, temperatures outside these ranges limited photosynthetic rates drastically for both cultivars (Moon et al. 1987). High leaf temperatures are believed to affect reproductive development and berry attributes negatively (Darnell and Williamson 1997). *V. darrowi* Camp, a species native to Florida, has a low chilling requirement and high heat tolerance (Hancock et al. 1992; Ballington 2001). Hancock et al. (1992) observed photosynthetic rates in response to temperature changes for the NHB cultivars Bluecrop and Jersey, *V. darrowi* Camp and progeny generated by crosses between them. They concluded that heat tolerance could be improved in NHB cultivars through the incorporation of genes from *V. darrowi* (Hancock et al. 1992). This has been achieved to some extent in certain SHB cultivars (Lyrene 2005). There is however not necessarily a correlation between photosynthetic heat tolerance and yield (Darnell and Williamson 1997).

Leaf and flower buds will resume growth at temperatures as low as 7.2 °C. In spite of this, leaves, flowers and berries develop much faster during higher daytime temperatures, up to a limit of about 32°C (Lyrene 1989). Carlson and Hancock (1991) observed that the rate of berry development in NHB blueberry decreases under exposure to mild to high (>21 °C) temperatures. Williamson et al. (1995) reported that cool (10 °C) night and mild (26 °C) day temperatures are required for optimal berry size in RE blueberry. For some RE cultivars, berries that ripen early during the season, when nights are still cool, are too acidic, whilst early berries from SHB have good sugar to acid ratios (Lyrene 2005). Berries from NHB cultivars that ripen during hot weather may contain too little acids and thus taste bland. SHB cultivars with *V. darrowi* in their ancestry, do not exhibit such problems (Lyrene 2005).

Relative to many other woody perennial fruit crops, blueberries have a shallow root system, which exposes them to the negative effect of high soil temperatures (Darnell and Williamson 1997). For both NHB and SHB cultivars, soil temperatures above 16 to 18 °C reduce root and subsequently shoot growth (Abbot and Gough 1987; Spiers 1995), but this can be negated by mulching (Abbot and Gough 1987). Ogden and Van Iersel (2009) established young ‘Emerald’ and ‘Jewel’ SHB plants in plastic covered greenhouses. Inside these greenhouses, daily maximum winter air temperatures were increased by 3 to 15 °C, and that of the soil (measured at a depth of approximately 10 cm) 2 to 8 °C when compared to open field conditions. Daily minimum winter air temperatures were however not increased. Compared to the control, petal drop and the stage just before berry expansion, were advanced by between 38 and 44 days for ‘Emerald’, and between 22 and 29 days for ‘Jewel’. Plants of both cultivars were consistently larger, as determined by light interception of the canopy. As a result of severe freeze damage inside and outside of the greenhouses, yield data could not be compared. Ciordia et al. (2006) observed that the flowering date of SHB cultivars Flordabue, Misty and Sharpeblue in Northern Spain was advanced with the use of plastic covered greenhouses. In Southwest Portugal, Baptista et al. (2006) conducted a similar trial on five-year-old plants. They obtained similar results with SHB cultivars O’Neal, Georgiagem and Cape Fear and found satisfactory yields, ranging from 0.81 to 1.22 kg per plant per year (Baptista et al. 2006).

Cultivar adaption to climate is of paramount importance as is illustrated by the problems encountered with RE in Florida. During spring bloom, adverse weather disrupts honey bee pollination, resulting in small, late maturing berries. The quality of berries ripening during hot, rainy summers is poor due to cracking and reduced surface waxes. In addition, harvesting is interfered with and blossom blight (*Botrytis cinerea*) promoted (Lyrene 1989).

## **2.2 Evergreen cultivation systems**

The feasibility of commercial blueberry cultivation in warmer growing regions, depends on using low-chill cultivars combined with certain cultural practices, e.g. cross pollination, pruning, fertilization, chemical rest breaking agents, or the advancement of the evergreen (syn. non-dormant) cultivation systems (Wright 1993; Reeder and Darnell 1994; Lyrene 2005; Darnell and Williams 1997; Hummer et al. 2007).

By preventing low-chill blueberry cultivars from entering endodormancy, the main negative effect of inadequate chilling, i.e. delayed foliation, can be alleviated (Lyrene 2005). This can only be achieved economically in growing regions where ambient temperatures do not drop below 0 °C (Darnell and Williamson 1997; Lyrene 2005). In turn, evergreen production poses its own challenges such as maintaining healthy leaves, dealing with protracted flowering and harvesting periods, as well as with fruit potentially ripening outside a desired market window (Darnell and Williamson 1997; Lyrene 2005).

An important part of the production management involved in evergreen systems, is severe summer pruning directly after harvesting. It is crucial to prevent leaf diseases on the resultant new shoots (Lyrene 2005). Mature leaves that are maintained throughout autumn and winter, continues to perceive short-days (long nights) and therefore FBI also continues. Early berry development is therefore supported by mature leaves developed during the previous summer (Lyrene 2005).

Wright (1993) reported on a commercial Sharpblue SHB and eleven RE cultivars evergreen blueberry cultivation system in eastern Australia established during 1984. The planting of 189 ha (at approximately 30 °S latitude; average 110 m altitude; approximately 7 km from the coast) has a mild coastal type climate, with relatively mild day and cool night temperatures during summer, and mild winter temperatures that do not drop below -1 °C. The peak berry production for RE cultivars was during December and January. Of the eleven RE cultivars, only Climax, Premier and Tifblue grew well and produced satisfactory yields. Their estimated average yields were 4 to 5 kg per plant per annum for both ‘Climax’ and ‘Premier’, and 5 to 8 kg for ‘Tifblue’. Yet, adverse weather (hail, heavy rain and strong wind) made their commercial viability marginal as a consequence of frequently disrupted harvesting, damaged plants and berries, and berry cracking (Wright 1993). In general, soils with a shallow topsoil layer, or those that have a subsoil with a high clay content that limits deep drainage, were found to be much less productive than deep, free draining sandy soils. The SHB ‘Sharpblue’ remained evergreen throughout winter and produced almost continuously throughout the year, with a peak yield during November and a low during May. The estimated average yield in good soil was 6.5 kg per plant per annum. Wright (1993) observed a link between the timing of new vegetative growth and harvest date. The quality of ‘Sharpblue’ berries was good, but unfortunately they do not store well, because of a “wet” picking scar and peel that tears easily during harvesting (Wright 1993).

A similar evergreen system was evaluated in Hawaii, and described by Hummer et al. (2007). The planting site (at approximately 20 °N latitude; average 853 m altitude.; approximately 20 km from the coast) has a mild coastal type climate, with an annual average maximum temperature of 23 °C, average minimum temperature of 10 °C, and a temperature range of 28 °C to 5.5 °C. On average, 200 chilling hours (Utah-model) are received throughout the year (Hummer et al. 2007). Low-chill SHB cultivars (Biloxi, Emerald, Jewel, Misty, Sapphire and Sharpblue) were planted at a relatively narrow spacing of 1.2 m by 1.2 m. Only formative pruning and pruning to control insects or localized disease infections were carried out for the first two years after planting. Flowers and berries were not removed from young plants, as is generally recommended to stimulate growth during the establishment phase of the plants. Nitrogen fertilizers, mainly ammonium types, were applied monthly from April through to July. Vegetative growth was not vigorous for the first year after establishment. Despite this, plants flowered and fruited six months after establishment (Hummer et al. 2007). Various known blueberry insect pests, e.g. thrips, aphids and leafhoppers, accumulated rapidly, presumably due to milder winter temperatures not inhibiting their population growth as much as is the case with cold winter temperatures at high latitudes (Hummer et al. 2007). All cultivars produced good quality berries and yields, ranging from 1.70 to 1.87 kg per plant per annum. Yield appeared cyclic, with major peaks for the combined harvests of all cultivars occurring during February, August and September, and minor peaks late during May and November. Cultivars behaved differently with regards to total yield, berry size and plant vigor. ‘Sapphire’ produced the largest total yield, averaging 3.25 kg per plant for both harvest seasons combined, and ‘Jewel’ the lowest (1.5 kg). ‘Emerald’ and ‘Jewel’ produced the largest berries, and ‘Jewel’ plants were the most vigorous of the six cultivars (Hummer et al. 2007).

The feasibility of evergreen blueberry production systems has also been trialed by researchers and commercial growers near Immokalee, Southwest Florida (Reeder and Darnell 1994; Williamson and Lyrene 1995; Darnell and Williamson 1997). Such growers install irrigation systems using revolving sprinklers to protect plants against frost. Commercial growers observed that low-chill SHB cultivars were able to continue growing throughout the year. Furthermore, flowering and fruit ripening typically occurred from November until the end of March, and from January until the end of June, respectively, which is earlier than any other Florida location. Harvesting extended over a longer period of time than usual in evergreen production systems (Reeder and Darnell 1994; Williamson and Lyrene 1995).

Evergreen systems together with summer pruning and appropriate nitrogen fertilization can be used for harvest scheduling aimed at high-priced markets (Wright 1993; Reeder and Darnell 1994; Lyrene 2005; Darnell and Williams 1997; Hummer et al. 2007). Some low-chill cultivars are better adapted to these systems than others (Wright 1993; Hummer et al. 2007). Even though evergreen systems show promise, the majority of blueberry growers at low latitudes still utilize low-chill cultivars in a “traditional” (dormant) production system together with chemical rest breaking agents as mechanisms to cope with inadequate winter chilling (Lyrene 2005).

### **3. Chemical regulation of dormancy**

#### **3.1 The use of cyanimides and thidiazuron**

Certain agricultural chemicals and plant growth regulators (gibberellins and cytokinins, or synthesized analogs thereof) are used to induce the release of endodormant buds that experienced insufficient exposure to chilling (Erez 1987; Krisanapook et al. 1990; Erez 1995; Faust et al. 1997). These substances, called rest breaking agents (RBAs), can however only partially compensate for insufficient chilling (Erez 2000). Furthermore, the response of buds to RBAs is complicated by interactions with genotype and cultivar, bud type and structure, stage of bud dormancy, RBA concentration and weather conditions during and after application (Erez 1987; Erez 1995; Faust et al. 1997; Erez 2000). RBAs are nonetheless commonly utilized by deciduous fruit and nut growers around the world, including South Africa (Steffens and Stutte 1989; North 1993; Costa et al. 2004; Rahemi and Asghari 2004; Theron et al. 2011). Calcium cyanamide ( $\text{CaCN}_2$ ) was reported to break bud endodormancy, but owing to the high concentration required for its effectiveness and it not being soluble in water, this substance was found unsuitable for commercial spraying (Shulman et al. 1986; Erez 1987). Hydrogen cyanamide ( $\text{H}_2\text{CN}_2$ ) is an unstable soluble hydrolysis product of  $\text{CaCN}_2$ . A stabilized commercial formulation of  $\text{H}_2\text{CN}_2$  (HC) was originally produced as a herbicide and only later discovered to be a useful RBA (Shulman et al. 1986; Erez 1987). Cyanamide metabolism and mode of action is not clear. Cyanamide and chilling both decrease enzyme activity in plant tissue, specifically that of catalase (Patterson et al. 1984; Nir et al. 1986; Shulman et al. 1986; Bichler 1999). Or et al. (2002) observed that expression of the catalase gene was quickly interrupted by HC. Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) is a normal

but toxic by-product of some oxygen requiring physiological reactions in plant cells.  $H_2O_2$  can reduce the structural integrity of plant cells walls. Catalase plays an important role in reducing hydrogen peroxide  $H_2O_2$  to water and oxygen (Or et al. 2002). For this reason inhibiting catalase activity results in  $H_2O_2$  causing increased oxidative stress (oxidative processes) in grape buds by disruption of cellular respiratory metabolism (Or et al. 2002). Shulman et al. (1986) suggested that bud endodormancy release requires oxidative processes.

HC has been found a successful RBA in apple (Bound and Jones 2004; Sagredo et al. 2005), apricot and plum (Bartolini et al. 1997; Küden and Son 1997; Costa et al. 2004), kiwifruit (McPherson et al. 2001), peaches and nectarines (Dozier et al. 1990; George et al. 1992), pistachio (Rahemi and Asghari 2004), red raspberry (Snir 1988); sweet cherry (Martínez et al. 1999; Costa et al. 2004), wine and table grapes (Lombard 2003; Possingham 2004), fig (Theron et al. 2011), blueberry (Jaldo et al. 2009) and other deciduous crops (Shulman et al. 1986; Klinac et al. 1991; Erez 1995, 2000). Nee and Fuchigami (1992) evaluated the effectiveness of HC as a RBA in red-osier dogwood (*Cornus sericea* L.) at different growth stages and different concentrations (0.5, 1.0 or 2.0 M solutions of a 50%  $H_2CN_2$  formulation). They observed that the most effective HC concentration varied according to dormancy type and stage of endodormancy, i.e. early on, during the middle of, or late during endodormancy. No HC treatment was effective during paradormancy and a high (2.0 M) HC concentration injured buds (was phytotoxic). Early on during endodormancy the percentage bud break increased at increasing HC concentrations. During the middle of endodormancy, first bud break occurred earlier and the percentage bud break was greater with the higher (1.0 and 2.0 M) HC concentrations. Even so, HC treatments had a weaker effect when compared to earlier and later during endodormancy. The HC concentration required for a strong effect decreased from early on until late during endodormancy. During ecodormancy, HC treatments inhibited bud break, retarded first bud break, and phytotoxicity increased at increasing concentrations. The conclusion they came to, was that HC is the most effective as a RBA and least phytotoxic to buds when applied late (approximately the last quarter) during endodormancy (Nee and Fuchigami 1992). This conclusion is in agreement with findings by Shulman et al. (1986). They reported that in most of the deciduous crops included in their trial, HC treatment performed best when applied after a significant degree of chilling exposure, but 1 to 5 weeks before natural bud break (Shulman et al. 1986). Shulman et al. (1986) also observed a general concentration range of 1 to 2% for obtaining desired results with HC treatment in almond, fig, grapes, peach, persimmon and plum. After contemplating past

studies investigating HC as a RBA in stone fruit, Erez (1995) hypothesized that HC treatment after approximately 70% of the required chilling had been received would in most instances show improved foliation. This seems to hold true for stone fruit (Dozier et al. 1990; Bartolini et al. 1997; Küden and Son 1997). Dokoozlian et al. (1995) reported that the efficacy of HC treatments on grapes, and hence the potential profit obtained from its application, decreased as grape buds were exposed to more chilling prior to application. They evaluated the influence of chilling exposure (0, 50, 100, 200 or 800 hours at 3 °C) and HC concentration (1.25 or 2.50%) on the vegetative bud break of ‘Perlette’ grapevine cuttings. ‘Perlette’ has a chilling requirement of 800 hours at 0 to 7 °C. Results in terms of cumulative bud break, number of days required for 50% bud break and the maximum observed bud break, indicated that the desired response of grape buds becomes progressively less after more than 400 hours of chilling exposure (Dokoozlian et al. 1995). Based on results from his trial with five red raspberry (*Rubus idaeus* L.) cultivars over two seasons, Snir (1988) recommended that during an unusually warm winter, i.e. less chill-unit accumulation over a fixed period of time than normal, the HC application date should be postponed for best results. In addition, he proposed a HC concentration of 2% for treating red raspberry cultivars. Differences among cultivars, with regard to effect of HC treatment, were apparent (Snir 1988).

HC is usually phytotoxic during ecodormancy, even at relatively low concentrations (Nee and Fuchigami 1992; Erez 2000). Although HC proved effective in releasing apple vegetative buds from dormancy, results in terms of bloom, fruit set, yield and fruit quality have been conflicting (Petri and Stuker 1995; Bound and Jones 2004; Costa et al. 2004; Sagredo et al. 2005). Erez (2000) propose that such discrepancies are due to varying levels of HC phytotoxicity at different rates and application times, i.e. application during different bud dormancy types and stages thereof. Flower buds seem especially susceptible to HC damage (Nee and Fuchigami 1992; George and Nissen 1993; Williamson et al. 2002). Kiwi and grape flower buds appear to be less sensitive to HC phytotoxicity than those of stone fruit. Powell et al. (2000) observed the highest yield, and no apparent damage to flower buds, in ‘Hayward’ kiwifruit after treatments with 1%, 1.5% or 2% HC concentrations mixed with 0.25% surfactant and applied 3 to 4 weeks before estimated natural bud break. Some deciduous crops, for instance sweet cherries, appear to be less responsive to HC treatments (Erez 1995; Martínez et al. 1999). Reduced sensitivity to HC could be attributed to a bud structure that protects more sensitive inner tissue (Erez 1995). The flower bud within the mixed bud of pome fruit is easily damaged by HC applications, leading to strong vegetative

growth, yet low yields (Erez 1995). Following the application of 1 and 2% HC concentration, Sagredo et al. (2005) found vegetative bud break in 'Golden Delicious' apple increased with increasing concentration, while fruit set and yield were reduced. The higher HC concentration could have been phytotoxic to the more sensitive flower buds (Sagredo et al. 2005). Bound and Jones (2004) examined the effect of HC in red 'Fuji' apple. A 3% Dormex<sup>®</sup> (HC, 520 g L<sup>-1</sup>) treatment was applied at 40, 30, 20, 10 days before and at estimated bud break (dBEB). A month after full bloom, all trees were hand thinned to obtain approximately the same level of blossom clusters. Late applications (20 and 10 dBEB) delayed budbreak and flowering, whereas early applications (40 and 30 dBEB) advance both. Minimal phytotoxicity followed early HC applications (Bound and Jones 2004).

HC is also used in combination with adjuvants or mineral oil (Erez 2000). Under the mild wintered conditions in South Africa, Costa et al. (2004) proposed combining a low (0.5 to 1%) HC concentration with 3 to 4% mineral oil for treating 'Golden Delicious' apples. Petri and Stuker (1995) reported good bud break in 'Gala' apple after also applying HC in combination with mineral oil. For 'Bing' sweet cherry and 'Bon Rouge' pear they proposed a 1.5% or 0.25 to 0.5% HC range, in combination with 4% mineral oil. If applied early (4 to 5 weeks before expected full-bloom), a 0.5% HC in combination with 1.5% mineral oil treatment was effective in promoting earlier vegetative and reproductive bud break in 'Songold' plum (Costa et al. 2004).

It is known that cytokinins (CKs) can induce vegetative bud break in deciduous fruit trees, but the high concentrations required for this proved prohibitively expensive for commercial use (Erez 1987, 1995; Krisanapook et al 1990; Faust et al. 1997). The chemical compound N-phenyl-N'-1,2,3-thiadiazol-5-ylurea (TDZ; thidiazuron) displays CK activity (Mok et al. 1982). Treatment with TDZ has been effective in substituting chilling and inducing bud break (Wang et al. 1986; Steffens and Stutte 1989; Costa et al. 2004; Erez et al. 2008; Theron et al. 2011). The mode of action of TDZ seems to be related to accelerated polyamide synthesis, caused by increases in RNA, DNA, S-adenosylmethionine (SAM) and protein (Wang et al. 1991). TDZ was applied by Steffens and Stutte (1989) to the buds of eleven low-, medium- and high-chill apple cultivars prior to, and at intervals after, the accumulation of predetermined numbers of chill-units at 4 °C. TDZ treatment after chilling only promoted bud break in 'Anna' (low-chill) and 'Redchief' (medium-chill). In all evaluated cultivars bud break was promoted when TDZ was applied before chilling (Steffens and Stutte 1989). This finding is in agreement with a hypothesis by Cook et al. (2001) that the CKs involved with

spring vegetative bud break originate from reserves stored inside affected shoots at a time before endodormancy commences. Cutting et al. (1991) also found that the cytokinin concentration in dormant apple shoots increased rapidly after applying RBAs, and that maximum concentration usually occurred just prior to, or at vegetative bud break. Tromp and Ova (1990) reported similar results.

Wang et al. (1986) reported that TDZ stimulated bud break of 'Gala' and 'Mutsu' apple at concentrations of 3 to 7%. They found 6% to be the optimum concentration for inducing vegetative bud break and canopy development. Furthermore, TDZ was not translocated in apple shoots, as untreated buds remained dormant (Wang et al. 1986). TDZ is a relatively new RBA in South Africa and Costa et al. (2004) reported on the benefits of Lift® (TDZ 3 g L<sup>-1</sup>) applications on various fruit crops. A 5% Lift® application 5 to 6 weeks before expected full bloom (wbfb) resulted in earlier and more rapid budbreak in 'Golden Delicious' apple, compared to a similar application 4 wbfb (Costa et al. 2004). During the following season 3, 4, and 5% Lift® treatments were evaluated. All these concentrations were effective in promoting both vegetative and reproductive budbreak. In 'Bon Rouge' pear good budbreak was obtained with both 4 and 6% Lift® concentrations applied at 4 or 5 wbfb (Costa et al. 2004). Treatment with 4% Lift® 4 to 7 wbfb promoted vegetative bud break in 'Bing' cherries. On 'Songold' plum, a 4% Lift® application 4 or 5 wbfb promoted earlier vegetative and reproductive budbreak (Costa et al. 2004). Theron et al. (2011) evaluated the efficacy of a 6% Lift® concentration applied to dormant fig trees at four predetermined dates, the first being at midwinter (30 June) and the rest equally spread during the last month of winter (3, 15, and 30 August) up until an early stage of bud swell. Advanced foliation occurred in 'Bourjasotte Noire' following treatments on 3 and 15 August. A midwinter treatment retarded vegetative bud break in 'Col de Damme Noire' and 'Noire de Caromb' and retarded breba harvest in the latter cultivar. Furthermore, all treatment dates induced more breba and main crop fruit in 'Noire de Caromb', but the breba fruit were smaller.

Both HC and TDZ are useful to overcome bud dormancy in various deciduous crops. As discussed above, the concentration used and timing of application is critical in terms of obtaining the desired results and preventing phytotoxicity. Research has also been conducted on the effects of HC on foliation, fruit ripening and yield of blueberry and will be discussed in detail in the next section.

### 3.2 Chemical regulation of blueberry dormancy

NeSmith and Krewer (1998) conducted a trial on 'Climax' RE and 'O'Neal' SHB blueberry. After low or moderate pretreatment chilling exposure of endodormant plants, vegetative and reproductive bud break were forced in a greenhouse (exposure to natural daylight, air temperature 24 °C during the day and 18 °C during the night). A 1% HC application was applied once either shortly after forcing, midway during reproductive bud development (bud scales separated) or late (bud scales fully open). HC treatment advanced and increased foliation irrespective of application date. Reproductive buds were however damaged by HC treatment midway during reproductive development, and severely so thereafter.

Williamson et al. (2001) reported that HC treatments can advance and increase foliation, advance and shorten the harvest season, as well as increase berry size in both 'Climax' RE and 'Misty' SHB blueberry. Their first trial involved exposing potted one-year-old, endodormant RE plants to 270 or 600 hours at 5 to 7 °C. Following this, vegetative and reproductive bud break were forced (24 °C/18 °C) and a 1% HC spray subsequently applied once either the day after forcing, 3 days after forcing, early (buds swollen, but scales still closed) or midway during reproductive bud development. A month after exposure to only 270 hours of pretreatment chilling 'Climax' (chilling requirement 400 to 500 hours below 7 °C) still had not developed any leaves. Irrespective of pretreatment chilling exposure, HC treatment midway during reproductive bud development induced the earliest and most prolific foliation in 'Misty'. However, reproductive buds were extensively damaged by HC treatment midway during their development (Williamson et al. 2001). For their second trial potted two-year-old, endodormant 'Misty' plants were exposed to either 150 hours (low-chill) or 300 hours (high-chill) of continuous chilling at 5 to 6 °C, or no chilling at all. A single 1 or 2% HC spray was applied immediately after pretreatment chilling exposure. Bud break was then forced (30 °C/18 °C). As the HC concentration and/or pretreatment chilling exposure increased, the foliation advanced and increased further, and the harvest season advanced and shortened further. Thus, the earliest and most prolific foliation followed after the high-chill pretreatment exposure and 2% HC treatment. This treatment combination also induced the earliest and shortest harvest season. As the HC concentration was increased and/or pretreatment chilling decreased, reproductive bud damage increased. The most reproductive bud damage occurred following the low-chill pretreatment exposure and 2% HC treatment.

Irrespective of pretreatment chilling exposure, yield was highest following the 1% HC treatment, and berries were the largest following 2% HC treatment (Williamson et al. 2001).

Williamson et al. (2002) reported the effects of HC rate and time of application on the vegetative and reproductive growth of various field grown RE and SHB cultivars. In southern Georgia 36 trials were conducted over 8 seasons (1991 to 1998). HC was applied at rates ranging from 0.5 to 2%. At most rates and during most seasons HC advanced and increased foliation in 'Climax' RE and all SHB cultivars evaluated. The lowest HC rate (0.5%) gave the most unpredictable results. Reproductive buds were often damaged when higher HC rates were applied midway or late during their development. In general, the harvest season was advanced and shortened by HC. HC effects were localized on plants, thus thorough coverage is advised (Williamson et al. 2002). Early during the winters of 1996 and 1997 in Florida, before significant pretreatment chilling exposure, endodormant and leafless 'Misty' SHB plants with slightly swollen reproductive buds (bud scales still closed) were sprayed once with a 1% or 2% HC treatment. Both HC treatments advanced and increased spring foliation, advanced and shortened the harvest season, and increased berry size (Williamson et al. 2002). The 2% HC treatment increased foliation the most, induced the shortest harvest season and the largest fruit, however it also damaged reproductive buds (Williamson et al. 2002). During the 1998 season, HC rates were decreased to 0.75 and 1.5%. Both rates induced an earlier harvest and shorter harvest season in 'Climax' and 'Misty'. For both cultivars bud break percentage increased with increasing HC concentration. The 0.75% HC treatment induced the highest total yield in 'Climax'. In 'Misty' both HC treatments reduced the length of the harvest season, while the 0.75% HC treatment induced the highest total yield. The 1.5% HC treatment induced the lowest total yield, but it also tended to induce the largest fruit. The use of HC in combination with a surfactant is recommended (Williamson et al. 2002).

Stringer et al. (2003) evaluated the effects of HC treatments on field grown 'Climax' plants. At three different stages during reproductive bud development (early, midway, or late) a HC treatment were applied at a rate of 1, 1.5 or 2% after significant pretreatment chilling exposure (439 hours below 7 °C). Irrespective of application date, all HC treatments advanced and increased foliation, and increased berry size, but also reduced berry yield. This yield reduction occurred, because berries from the later developing reproductive buds remaining after HC treatments, were more susceptible to late-season frost damage. Only the 2% HC treatment applied late during reproductive bud development damaged reproductive

buds. The 1.5 and 2% HC treatments reduced spring foliation the following season (Stringer et al. 2003).

The efficacy of HC as RBA on blueberry was evaluated at many other low latitude growing regions. Jaldo et al. (2009) reported on a trial conducted at a site (26 °S) in Argentina. Field grown SHB cultivars Bluecrisp, Emerald, Jewel, Misty, O'Neal and Star were treated with different HC concentrations (0.5% to 2.5%) during the winters of 2006 and 2007. During these years plants received 50 and 520 hours pretreatment chilling (0 to 7.2 °C), respectively. HC treatment did not increase the total yield of 'Emerald'. Following the warm winter (2006) an increasingly higher HC concentration tended to increase the total yield of 'Star', and a 2.5% HC treatment increased the yield of 'O'Neal' significantly. A 0.5% HC treatment resulted in an increased yield in 'Jewel' during both seasons. 'Jewel' was the most susceptible to HC phytotoxicity. Following the colder (2007) winter, HC treatment induced an earlier harvest and higher yield in 'Misty'. HC treatments decreased the yield of 'Bluecrisp' following the warm winter.

Arias et al. (2010) conducted trials to determine the effect of HC timing and rate on field grown 'O'Neal' SHB plants at a site (34 °S) in Uruguay. During 2005, a 1% HC treatment was applied to plants early, midway, or both early and midway during endodormancy. A HC treatment midway during endodormancy advanced and increased vegetative and reproductive bud break during both seasons. During 2006, 1, 1.5 or 2% HC concentrations were applied both early and midway during reproductive bud development. The 2% HC treatment damaged reproductive buds when applied midway during development. No other significant results were obtained (Arias et al. 2010).

Above mentioned literature consistently indicate the advantage of HC as a useful RBA to stimulate earlier and stronger spring bud break. When spring bud break was advanced significantly, the harvest season was often earlier and shorter. Larger berries and an increased yield was reported in some instances, usually after reproductive buds had been damaged to a limited extent by higher HC concentrations (2% plus) or HC application during or after reproductive bud scales had separated. However, the timing of HC applications is highlighted as being crucial in order to prevent substantial reproductive bud damage. In general applications were more effective if applied at bud swell before bud scales open, but after significant chilling exposure, but blueberry cultivars responded differently to HC treatments. No literature could be found on the response of blueberry plants to TDZ as a RBA.

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# **Paper 1: Effects of hydrogen cyanamide on yield of southern highbush blueberry (*Vaccinium corymbosum* L. interspecific hybrids) cultivars Bluecrisp, Emerald and Star**

## **Abstract**

Production areas in the Western Cape often receive inadequate winter chilling for some of the southern highbush (SHB) blueberry cultivars grown here, resulting in a delayed, prolonged harvest period and smaller berries of poor quality. Hydrogen cyanamide (HC) is often used as a rest breaking agent in deciduous fruit and nut orchards. The effect of HC application at different rates and phenological stages on berry ripening, berry size and yield of SHB ‘Bluecrisp’, ‘Emerald’ and ‘Star’ were evaluated, to ascertain if there is interaction between HC application date and rate, and to help develop recommendations for best practices in this regard. A trial was conducted during 2010 and 2011 in a commercial orchard near the town of George (34 °S, 194 m altitude). It included two concentrations (1 or 2% v/v) of Dormex® (HC, 520 g L<sup>-1</sup>) applied on six dates, plus an untreated control. Dormex® application accelerated berry ripening in ‘Bluecrisp’ during the 2010 harvest period following an unseasonably warm winter. Application was best done before reproductive bud scales opened, but after at least fair chilling exposure if possible. The 1% concentration induced similar results as the 2% concentration, but with less risk of reproductive bud damage.

*Keywords:* southern highbush blueberries, hydrogen cyanamide, rest breaking, harvest timing

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## **Introduction**

Buds of southern highbush (SHB) blueberries (*Vaccinium corymbosum* L. interspecific hybrids) enter endodormancy during autumn and require 100 to 600 hours of chilling (exposure at 0 to 7 °C) for full release from endodormancy (Ballington et al. 1990; Lyrene 1999, 2001, 2008; Lyrene and Sherman 2000; Darnell 2006; Lyrene and Ballington 2006).

Vegetative buds of SHB blueberries have a higher chilling requirement than reproductive buds on the same shoot (Williamson and Lyrene 2004; Lyrene 2005). Therefore, following insufficient chilling, reproductive budbreak precedes vegetative budbreak in some SHB cultivars (Williamson et al. 2001, 2002).

In blueberry, fast growing and developing berries are mainly supported with carbohydrates from current photosynthates from mature leaves and also from storage reserves (Maust et al. 1999; Lyrene 2004). Thus, the rate of berry ripening is strongly influenced by berry load, foliage development, and the resultant leaf to berry ratio (Maust et al. 1999). Too many developing berries in relation to mature leaves on a plant result in a delayed, prolonged harvest period and smaller berries of poor quality (Maust et al. 1999; Williamson et al. 2002). Currently the most lucrative market window for exporting fresh blueberries from South Africa is early to mid-spring. Growers producing for this market are interested in early ripening cultivars, and cultural practices that could accelerate berry ripening.

In mild wintered growing regions, applying a rest breaking agent (RBA) to partially compensate for the insufficient chilling of deciduous fruit and nuts trees is common practice (Shulman et al. 1986; Erez 1987, 1995; Faust et al. 1997). Commercial formulations of hydrogen cyanamide (HC;  $H_2CN_2$ ) are often recommended as RBAs in the South African deciduous fruit industry (North 1993; Costa et al. 2004; Sagredo et al. 2005; Theron et al. 2011). On certain SHB cultivars, e.g. Misty, O'Neal and Jewel, HC has been shown to induce earlier and more condensed spring vegetative budbreak than would otherwise occur after mild winters (NeSmith and Krewer 1998; Williamson et al. 2001, 2002; Jaldo et al. 2009; Arias et al. 2010). A concentration range of 0.5 to 2.5% HC is commonly recommended. Treatment with HC concentrations at the lower end of this range (0.5 to 0.75%) gives the most unpredictable results, while those at the higher end (2.0 to 2.5%) could damage reproductive buds. Reproductive buds were damaged more when little chilling exposure occurred and/or when treatments were applied from midway during their development (bud scales separated) and severely so if applied even later (NeSmith and Krewer 1998; Williamson et al. 2001, 2002; Jaldo et al. 2009; Arias et al. 2010). In some trials HC treatment also resulted in larger berries and a slightly higher yield, usually after reproductive buds had been damaged to a limited extent resulting in some blossom thinning (Williamson et al. 2001, 2002; Jaldo 2009). In general, HC application is more effective if applied early during reproductive bud development (buds swollen and bud scales closed), but after significant chilling exposure. Similar results were obtained on 'Climax' rabbiteye blueberry (*V. ashei* Reade) after HC

treatment (Williamson et al. 2002; Stringer et al. 2003). Responses to HC treatment were mostly cultivar specific in SHB blueberries (Williamson et al. 2001, 2002; Jaldo 2009). Since the effects of HC treatments are localized on plants, thorough coverage is advised (Williamson et al. 2002).

For deciduous fruit in general, the response of buds to RBA is complicated by interactions with genotype and cultivar, bud type and structure, stage of bud dormancy, RBA concentration and weather conditions during and after application (Erez 1995, 2000; Faust et al. 1997, Faust 2000). This seems to hold true for blueberry as well.

In this paper we report on the effect of exogenous HC application at different rates and phenological stages on berry ripening, berry size and yield of the SHB blueberries 'Bluecrisp', 'Emerald' and 'Star' grown in the Western Cape.

## **Materials and Methods**

### *Plant material and site*

The trial was conducted on 'Bluecrisp', 'Emerald' and 'Star' SHB blueberry plants during 2010 and 2011 in a commercial orchard near George, Western Cape province, South Africa (34°00'19.60"S, 22°17'37.83"E, 194 m altitude). These cultivars have a reported chilling requirement of 400-600, 100-400 and 400 hours below 7 °C, respectively (Lyrene 1999, 2001, 2008; Lyrene and Sherman 2000; Lyrene and Ballington 2006). The area accumulated 325 chill-units (Utah model) from 01 June 2010 until 30 September 2010, and 502 chill-units from 01 June 2011 until 30 September 2011. Orchards were established during 2008 under 20% net using two-year-old plants from a commercial quarantine nursery. Plants were spaced 1.0 x 2.5 m apart on low ridges and the soil surface was mulched. Two rows of 'Bluecrisp' alternate with two rows of 'Star' in the first production block, and in the next two rows of 'Emerald' alternate with two rows of 'Jewel'. For 'Bluecrisp', 'Emerald' and 'Star' the 2009 average yield was 6.1, 21.0 and 12.5 ton ha<sup>-1</sup>, respectively.

### *Treatments and trial design*

The experimental design was a randomized complete block with 13 treatments (2 concentrations x 6 application times plus an untreated control) and ten single plant replicates. The two concentrations of HC (1 or 2% v/v Dormex®; 520 g L<sup>-1</sup> HC; Degussa Ag, Trostberg,

Germany) were applied with a pressurized backpack sprayer until runoff (i.e.  $\pm 0.30$  L plant<sup>-1</sup>) to tagged 'Bluecrisp' and 'Star' plants on six dates during both seasons (Table 1). On these same dates only the lower HC concentration (i.e. lower risk for phytotoxicity) was applied to Emerald as this cultivar has a low chilling requirement. Application dates were aimed at including a broad range of reproductive bud development stages and different levels of pretreatment chilling exposure (Table 1). Buffer plants and border rows occurred between plots. Besides the treatments, only standard commercial practices for irrigation, fertigation, pruning, and pest management were followed in the orchard during both years.

#### *Data recorded*

The developmental stages of reproductive buds as well as chill unit accumulation at the time of application were recorded (Table 1). A week after treatment reproductive bud damage was recorded (Table 1). At each commercial harvest date berries were harvested and weighed and total mass of berries per plant recorded and average berry mass determined. Average berry mass was used as an indication of berry size.

#### *Data analysis*

Analysis of variance (ANOVA) were performed on the data using SAS software (version 9.2; SAS Institute Inc., Cary, USA). Single degree of freedom, orthogonal contrasts were fitted to the factorial component of the data to test for interaction between concentration and date as well as to determine which polynomial function best described application date effect. When interaction was present data is presented as such, but also when some parameters did not show interaction, the data is presented in this way except where all parameters did not show interaction. A probability level of 5% was considered significant. Additionally regression analysis was performed using the NLIN Procedure of SAS. The Gompertz function with harvest day as independent variable was fitted for each experimental unit (treatment x replication combination) to describe trends in cumulative percentage harvest mass over time. Regression parameters obtained were used to calculate the estimated number of days from the first harvest until 25%, 50% and 75% respectively of total harvest mass. This could lead to the calculation of negative values.

## Results

### *2010 Season*

#### ‘Star’

Generally Dormex® application delayed days to 25% harvest ( $p=0.0277$ ) (Table 2). The quadratic interaction between Dormex® concentration and date of application showed that with the 1% concentration the different application dates resulted in a similar response in days to 25% harvest, but at the higher concentration of 2% Dormex® a sharp quadratic response was seen in delaying harvest with later applications (Table 2). This same interaction was observed in days to 50% harvest. The number of days to 75% harvest showed a significant quadratic delay with the last application date. Both the 1% and 2% Dormex® applied on 26 July increased the number of days to 25%, 50% and 75% harvest (Table 2). The total yield, as well as the berry size (as measured by mean berry mass) showed a quadratic effect with application date with the first and last application dates resulting in the lowest yield and smallest berries (Tables 2 and 3). On the first harvest date berry size decreased following Dormex® application ( $p=0.0286$ ) (Table 3). The linear interaction between Dormex® concentration and application date on the third harvest date, showed that with the 1% application berry size increased in response to delayed applications, but at the higher concentration of 2% Dormex® berry size decreased in response to delayed applications (Table 3). On all other harvest dates berry size showed a quadratic trend with June application dates resulting in the largest berries (Table 3).

#### ‘Bluecrisp’

Dormex® application generally accelerated days to 25% harvest ( $p=0.0140$ ) (Table 4). No interaction was observed between concentration and date of application, but a linear trend was found with date of Dormex® application in days to 25% harvest. This was because the response did not differ with delaying application date (Table 4). Days to 50% and 75% harvest were in general not affected by Dormex® application. The linear interaction between Dormex® concentration and date of application showed that with the 1% application the different application dates resulted in a constant response for days to 50% harvest, but at the higher concentration of 2% Dormex® a linear response was found with a slight delay in harvest with later applications (Table 4). The number of days to 75% harvest showed a

significant quadratic effect with the 26 July application delaying harvest (Table 4). An interaction between concentration and date of application was found for total yield (Table 4). This was due to the quadratic trend with application date as yield increased with later application date but decreased again with the last application date (26 July). This trend was stronger for the 2% Dormex® application. Berry size increased with later applications but decreased following the last application date (Table 5). On the first harvest date the 2% Dormex® concentration reduced berry size compared to the 1% concentration. On the second and third harvest dates Dormex® applications also generally decreased berry size ( $p=0.0072$  and  $p=0.0052$ ) (Table 5). Berries harvested on 18 Nov. were smaller with later applications, especially following application of the 2% Dormex® concentration. This same trend was observed on the fourth harvest date, while on the last harvest date no significant differences were observed (Table 5).

#### ‘Emerald’

Dormex® application displayed a quadratic effect in days to 25% harvest with the earliest and last applications delaying harvest more than mid applications ( $p<0.0001$ ) (Table 6). A similar effect was observed for days to 50% and 75% harvest. Generally Dormex® application increased total yield ( $p=0.0365$ ), but the quadratic trend clearly illustrates that this was only due to the 17 May application (Table 6). On most harvest dates later applied Dormex® resulted in smaller berries except on the last harvest date where the trend was reversed, but in general Dormex® did not reduce berry size (Table 7). Berry size displayed a quadratic effect with only the last application date resulting in smaller berries (Table 7).

#### *2011 Season*

##### ‘Star’

Generally Dormex® application accelerated days to 50% and 75% harvest ( $p=0.0277$  and  $p=0.0022$ ) (Table 8). Number of days to 25%, 50% and 75% harvest showed a quadratic delay with application date (Table 8). The 1% Dormex® concentration increased the total yield compared to the 2% concentration ( $p<0.0001$ ) and the control (Table 8). With both Dormex® concentrations the total yield decreased linearly in response to delayed application

(Table 8). With the 1% Dormex® concentration application date had little effect on berry size, but with the 2% concentration a linear decrease in berry size was observed (Table 9). Berries harvest on the first date also displayed this interaction while berries harvested on 30 Nov. and 6 Dec. showed a clear linear effect with application date as Dormex® concentration had little effect in berry size (Table 9).

#### ‘Bluecrisp’

With both Dormex® concentrations days to 25%, 50% and 75% harvest increased linearly from the first to the last application date ( $p < 0.0001$ ) (Table 10). Overall, applying Dormex® decreased total yield ( $p = 0.0148$ ), but the 1% concentration did so less than the 2% concentration ( $p < 0.0001$ ) (Table 10). The 1% Dormex® concentration increased berry size compared to the 2% concentration ( $p = 0.0078$ ), but overall Dormex® did not affect berry size compared to the control ( $p = 0.9683$ ) (Table 11). On the first harvest date Dormex® application decreased berry size ( $p = 0.0070$ ), and berry size showed a quadratic effect with application on 28 June resulting in the smallest berries (Table 11). This quadratic effect was reversed on the second harvest date, with the 28 June application date resulting in the largest berries. On the third harvest date berry size decreased linearly with delayed Dormex® application while no differences were found on the last harvest date (Table 11).

#### ‘Emerald’

Dormex® application tended to accelerate days to 50% and 75% harvest in ‘Emerald’ ( $p = 0.0030$  and  $p < 0.0001$ ) (Table 12) and days to 25%, 50% and 75% harvest increased linearly in response to delayed application of the 1% Dormex® concentration (Table 12). Generally, Dormex® application decreased total yield ( $p < 0.0001$ ), and this decrease followed a linear trend towards the last application date (Table 12). Generally Dormex® application increased berry size ( $p < 0.0001$ ) (Table 13). This overall increase in berry mass was also seen on the first, second and third harvest dates ( $p = 0.0001$ ,  $p = 0.0206$  and  $p = 0.0173$ ) (Table 13). On the first harvest date berry size decreased linearly with delayed Dormex® application (Table 13). This linear effect was reversed on the final harvest date. On the second harvest date berry size showed a quadratic effect with the first and last application dates resulting in the largest berries (Table 13).

## Discussion

Unfortunately no detailed data could be recorded on budbreak patterns following the RBA application. The accelerated berry ripening early during the harvest period of 'Bluecrisp' as a result of Dormex® application (Table 4) was not unexpected and is in agreement with observations by NeSmith and Krewer (1998), Williamson et al. (2001, 2002), Jaldo et al. (2009) and Arias et al. (2010), who reported that HC application can advance and shorten the harvest season of SHB cultivars following insufficient prior chilling exposure. Such a result was not observed during the 2011 season with its much higher chill-unit accumulation (Table 1 and 10). 'Bluecrisp' has the highest chilling requirement of the three evaluated cultivars. Following an unseasonably warm winter, as was the case during 2010, 'Bluecrisp' reproductive budbreak precede vegetative budbreak (personal observation). As with other fruit and nut trees (Shulman et al. 1986; Erez 1987, 1995; Faust et al. 1997), Dormex® application could have partially compensated for the insufficient chilling exposure of 'Bluecrisp' plants, thereby inducing earlier and more condensed spring vegetative budbreak than was the case for the untreated control plants. Improved early foliar development can result in accelerated berry ripening (Maust et al. 1999).

Compared to other application dates, the last application date tended to induce a delay in berry ripening in 'Bluecrisp' and 'Star' during both seasons and for both Dormex® concentrations (Tables 2, 4, 8 and 10). Similarly, compared to other application dates the total yield (Tables 2, 4, 6, 8, 10 and 12) and mean berry mass (Tables 3, 5, 7, 9, 11 and 13) were lower in 'Bluecrisp' and 'Star' following Dormex® application on the last date. On the last application date during both seasons reproductive bud scales of all cultivars were open, or plants already had some flowers and bud damage was observed one week after Dormex® application (Table 1). This was not unexpected and is in agreement with observations by NeSmith and Krewer (1998), Williamson et al. (2001, 2002) and Jaldo et al. (2009).

During both seasons and for both concentrations, the first application date seldom increased total yield or berry size in 'Bluecrisp' and 'Star' following Dormex® application (Tables 2, 3, 4, 5, 9, 10 and 11). The George region normally does not start to accumulate chill-units until the beginning of June (Table 1). Shulman et al. (1986) reported that in most of the deciduous crops included in their trial, HC treatment performed best when applied after a significant degree of chilling exposure. This seems to be the case for SHB and rabbiteye blueberries as well (Williamson and Lyrene 2004). The delayed berry ripening observed after

the 26 July Dormex® application during both seasons (Tables 2 and 8) was unexpected since ‘Star’ and ‘Bluecrisp’ have a relatively high chilling requirement compared to ‘Emerald’. However, during 2010 and 2011 the total yield in ‘Star’ was reduced following Dormex® application at the higher 2% concentration on the last date (Tables 2 and 8), and during 2011 the mean berry mass of ‘Star’ for each application date was reduced after application of Dormex® at both rates on the last date (Table 3). It is possible, that the reproductive buds of ‘Star’ were damaged more severely during the last application date than those of ‘Bluecrisp’. Variable sensitivity to HC has been reported and is attributed to slight changes in bud structure (Erez 1995).

‘Emerald’ responded differently to Dormex® application than ‘Bluecrisp’ and ‘Star’ at the first application date, as during both seasons this date resulted in a good total yield (Tables 6 and 12) and mean berry mass (Tables 7 and 13) compared to other application dates. Furthermore significantly higher berry mass was observed in ‘Emerald’ during the 2011 season (Table 13), and Dormex® application did not induce a significant effect with regards to the berry ripening rate in ‘Emerald’ during 2010 (Table 6). Jaldo et al. (2009) also found that Dormex® application did not induce a significant effect on the berry ripening rate of ‘Emerald’ even following an unseasonably warm winter. The general increase in the total yield of ‘Emerald’ during 2010 was also unexpected. This can however be attributed to an exceptionally high yield that followed the first Dormex® application date (Table 6) and a general decrease in total yield followed Dormex® application in 2011 (Table 12). ‘Emerald’ has the lowest reported chilling requirement of the cultivars evaluated, and these plants could have received their full chilling requirement even during the unseasonably warm 2010 winter. Increased yield and berry mass is not often reported following Dormex® application (NeSmith and Krewer 1998; Williamson et al. 2001, 2002; Jaldo et al. 2009; Arias et al. 2010), and further trials on this matter are required before recommendations can be made.

## **Conclusion**

HC application is not recommended for the low-chill cultivar Emerald. Using either 1 or 2% HC, concentration was of less significance than application timing with regards to promoting early berry ripening and increased yield in field grown ‘Bluecrisp’ SHB blueberry plants that had experienced insufficient prior chilling. However a 1% Dormex® concentration can induce similar results as a 2% concentration in ‘Bluecrisp’ but with less risk of reproductive

bud damage. During unseasonably warm winters the timing of HC application could be based on the visual appearance of reproductive buds and should best be carried out before bud scales open but after at least fair chilling exposure if possible. Further studies are required to identify the best HC application time for ‘Star’ as the results were inconclusive. SHB cultivars with a relatively low chilling requirement, like ‘Emerald’, which seem to be better suited to production in the mild, coastal climate of the George area, should be continuously identified and evaluated in field trials.

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Table 1: Dormex® application dates for both seasons, with the reproductive bud development of different cultivars and chill-units (Utah model) accumulated on each date.

Application date	2010				2011			
	Bluecrisp	‘Emerald	Star	Chill-units	Bluecrisp	Emerald	Star	Chill-units
17 May	Not swollen	Not swollen	Not swollen	-154	Not swollen	Not swollen	Not swollen	-65
31 May	Not swollen	Not swollen	Not swollen	-77	Not swollen	Swollen, scales closed	Not swollen	-33
14 June	Not swollen	Swollen, scales closed	Not swollen	45	Swollen, scales closed	Scales separated	Swollen, scales closed	62
28 June	Swollen, scales closed	Scales separated	Swollen, scales closed	89	Scales separated	Scales open <sup>Z</sup>	Scales separated	123
12 July	Scales separated	Scales open <sup>Z</sup>	Scales separated	98	Scales open <sup>Z</sup>	First flowers <sup>Y</sup>	Scales open <sup>Z</sup>	205
26 July	Scales open <sup>Z</sup>	First flowers <sup>Y</sup>	Scales open <sup>Z</sup>	197	First flowers <sup>Y</sup>	5% Bloom <sup>Y</sup>	First flowers <sup>Y</sup>	287

<sup>Y</sup> phytotoxicity observed one week after applying the 1% or 2% concentration

<sup>Z</sup> phytotoxicity observed one week after applying the 2% concentration only

Table 2: Effect of hydrogen cyanamide (Dormex®) on the berry ripening, harvest distribution and total yield of ‘Star’ during the 2010 harvest season. Means were separated by LSD (5%).

Treatment	Days to 25% harvest <sup>Y</sup>	Days to 50% harvest <sup>Y</sup>	Days to 75% harvest <sup>Y</sup>	Average total yield per plant (g)
Untreated control	0 d	8 c	18 c	243 abcd
1% Dormex – 17 May	0 d	8 c	18 c	203 d
1% Dormex – 31 May	3 bd	10 bc	18 c	334 a
1% Dormex – 14 June	3 bd	9 c	17 c	284 abcd
1% Dormex – 28 June	4 bd	11 bc	20 bc	267 abcd
1% Dormex – 12 July	2 bd	9 c	18 c	325 ab
1% Dormex – 26 July	6 b	14 b	23 ab	303 abc
2% Dormex – 17 May	1 d	7 c	16 c	226 bcd
2% Dormex – 31 May	2 bd	9 c	17 c	307 abc
2% Dormex – 14 June	3 bd	9 c	18 c	271 abcd
2% Dormex – 28 June	1 d	8 c	17 c	278 abcd
2% Dormex – 12 July	5 b	11 bc	19 bc	304 abc
2% Dormex – 26 July	13 a	19 a	25 a	218 cd
<b>Pr &gt; F</b>				
<i>Treatment</i>	<i>&lt;0.0001</i>	<i>&lt;0.0001</i>	<i>0.0021</i>	<i>0.1449</i>
<i>Control vs Treatment</i>	<i>0.0277</i>	<i>0.0773</i>	<i>0.4713</i>	<i>0.3219</i>
<i>Conc. 1% vs 2%</i>	<i>0.3290</i>	<i>0.6524</i>	<i>0.7178</i>	<i>0.5321</i>
<i>Date linear</i>	<i>&lt;0.0001</i>	<i>&lt;0.0001</i>	<i>&lt;0.0001</i>	<i>0.2479</i>
<i>Date quadratic</i>	<i>0.0273</i>	<i>0.0097</i>	<i>0.0157</i>	<i>0.0315</i>
<i>Conc.*Date linear</i>	<i>0.0548</i>	<i>0.0936</i>	<i>0.3617</i>	<i>0.3123</i>
<i>Conc.*Date quadratic</i>	<i>0.0099</i>	<i>0.0464</i>	<i>0.4978</i>	<i>0.4647</i>

<sup>Y</sup> counted from first harvest date

Table 3: Effect of hydrogen cyanamide (Dormex®) on the berry mass of ‘Star’ during the 2010 harvest season. Means were separated by LSD (5%).

Treatment	Mean berry mass (g) on different harvest dates					Mean berry mass per plant (g)
	03 Nov.	11 Nov.	18 Nov.	25 Nov.	07 Des.	
Untreated control	2.30 a	2.11 abc	1.95 ab	1.67 ab	1.58 abcd	1.99 a
1% Dormex – 17 May	1.84 d	1.76 d	1.22 d	0.99 d	1.17 e	1.57 b
1% Dormex – 31 May	2.28 ab	2.08 abc	1.95 ab	1.66 ab	1.40 bcde	1.95 a
1% Dormex – 14 June	2.23 ab	2.32 ab	1.84 ab	1.64 ab	1.60 abcd	1.97 a
1% Dormex – 28 June	2.29 ab	2.25 ab	1.88 ab	1.67 ab	1.71 abc	1.95 a
1% Dormex – 12 July	2.03 bcd	2.38 a	1.92 ab	1.71 a	1.59 abcd	2.02 a
1% Dormex – 26 July	2.12 abc	2.03 bcd	1.63 bc	1.68 ab	1.15 e	1.57 b
2% Dormex – 17 May	1.87 cd	1.84 cd	1.49 cd	1.19 cd	1.40 bcde	1.58 b
2% Dormex – 31 May	2.19 ab	2.11 abc	2.02 a	1.58 ab	1.75 ab	1.98 a
2% Dormex – 14 June	2.02 bcd	2.20 ab	1.90 ab	1.58 ab	1.37 cde	1.96 a
2% Dormex – 28 June	2.28 ab	2.30 ab	1.84 ab	1.76 a	1.84 a	2.01 a
2% Dormex – 12 July	2.03 bcd	2.14 abc	1.87 ab	1.65 ab	1.48 abcde	1.91 a
2% Dormex – 26 July	1.78 d	1.75 d	1.21 d	1.41 bc	1.26 de	1.44 b
<b>Pr &gt; F</b>						
<i>Treatment</i>	<i>0.0001</i>	<i>0.0001</i>	<i>&lt;0.0001</i>	<i>&lt;0.0001</i>	<i>0.0026</i>	<i>&lt;0.0001</i>
<i>Control vs Treatment</i>	<i>0.0286</i>	<i>0.9523</i>	<i>0.0839</i>	<i>0.2085</i>	<i>0.4474</i>	<i>0.0155</i>
<i>Concentration 1% vs 2%</i>	<i>0.0614</i>	<i>0.276</i>	<i>0.8304</i>	<i>0.6139</i>	<i>0.3238</i>	<i>0.5341</i>
<i>Date linear</i>	<i>0.9342</i>	<i>0.0965</i>	<i>0.8358</i>	<i>&lt;0.0001</i>	<i>0.7883</i>	<i>0.4255</i>
<i>Date quadratic</i>	<i>&lt;0.0001</i>	<i>&lt;0.0001</i>	<i>&lt;0.0001</i>	<i>&lt;0.0001</i>	<i>&lt;0.0001</i>	<i>&lt;0.0001</i>
<i>Conc.*Date linear</i>	<i>0.2055</i>	<i>0.0846</i>	<i>0.0056</i>	<i>0.0865</i>	<i>0.2895</i>	<i>0.1491</i>
<i>Conc.*Date quadratic</i>	<i>0.655</i>	<i>0.7482</i>	<i>0.6656</i>	<i>0.8458</i>	<i>0.2641</i>	<i>0.4883</i>

Table 4: Effect of hydrogen cyanamide (Dormex®) on the berry ripening, harvest distribution and total yield of ‘Bluecrisp’ during the 2010 harvest season. Means were separated by LSD (5%).

Treatment	Days to 25% harvest <sup>Y</sup>	Days to 50% harvest <sup>Y</sup>	Days to 75% harvest <sup>Y</sup>	Average total yield per plant (g)
Untreated control	11.2 a	14.7 ab	19.1 abcd	38.56 cde
1% Dormex – 17 May	8.7 abcd	13.9 abc	20.5 abc	23.89 ef
1% Dormex – 31 May	8.3 abcd	13.0 bcd	19.0 abcd	55.6 abc
1% Dormex – 14 June	7.7 cd	12.2 cd	17.9 bcd	63.5 ab
1% Dormex – 28 June	10.0 abc	13.9 abc	18.9 abcd	47.78 bcd
1% Dormex – 12 July	8.2 bcd	12.6 bcd	18.1 bcd	52.67 abc
1% Dormex – 26 July	9.2 abcd	14.3 abc	20.9 ab	53.3 abc
2% Dormex – 17 May	7.1 cd	11.4 d	16.8 d	31.1 def
2% Dormex – 31 May	8.9 abcd	12.6 bcd	17.4 cd	61.56 ab
2% Dormex – 14 June	9.4 abcd	13.3 bcd	18.3 bcd	48.8 bcd
2% Dormex – 28 June	7.0 d	11.3 d	16.7 d	59.38 ab
2% Dormex – 12 July	9.3 abcd	12.7 bcd	16.9 d	71.1 a
2% Dormex – 26 July	10.9 ab	15.9 a	22.2 a	14.4 f
<b>Pr &gt; F</b>				
<i>Treatment</i>	<i>0.0625</i>	<i>0.0165</i>	<i>0.0530</i>	<i>&lt;0.0001</i>
<i>Control vs Treatment</i>	<i>0.0140</i>	<i>0.0582</i>	<i>0.7212</i>	<i>0.2340</i>
<i>Con. 1% vs 2%</i>	<i>0.7300</i>	<i>0.4386</i>	<i>0.0906</i>	<i>0.7174</i>
<i>Date linear</i>	<i>0.0283</i>	<i>0.0135</i>	<i>0.0952</i>	<i>0.3170</i>
<i>Date quadratic</i>	<i>0.8466</i>	<i>0.0805</i>	<i>0.0070</i>	<i>&lt;0.0001</i>
<i>Conc.*Date linear</i>	<i>0.1107</i>	<i>0.0430</i>	<i>0.1107</i>	<i>0.0458</i>
<i>Conc.*Date quadratic</i>	<i>0.4595</i>	<i>0.7282</i>	<i>0.7913</i>	<i>0.0819</i>

<sup>Y</sup> counted from first harvest date

Table 5: Effect of hydrogen cyanamide (Dormex®) on the berry mass of ‘Bluecrisp’ during the 2010 harvest season. Means were separated by LSD (5%).

Treatment	Mean berry mass (g) on different harvest dates					Mean berry mass per plant (g)
	03 Nov.	11 Nov.	18 Nov.	25 Nov.	07 Des.	
Untreated control	2.04 a	1.41 a	1.40 a	1.17 a	1.17 a	1.28 ab
1% Dormex – 17 May	1.59 ab	1.36 a	1.34 ab	1.14 a	1.14 ab	1.03 c
1% Dormex – 31 May	1.53 abc	1.33 a	1.28 abc	1.13 a	1.07 ab	1.23 ab
1% Dormex – 14 June	1.48 abc	1.32 a	1.25 abc	1.10 a	0.94 ab	1.24 ab
1% Dormex – 28 June	1.43 abc	1.31 a	1.23 abc	1.09 a	0.94 ab	1.22 ab
1% Dormex – 12 July	1.35 abc	1.25 a	1.19 abc	1.08 a	0.94 ab	1.24 ab
1% Dormex – 26 July	1.22 bc	1.25 a	1.17 abc	1.07 a	0.87 ab	1.19 ab
2% Dormex – 17 May	1.14 bc	1.21 ab	1.17 abc	1.06 a	0.86 ab	1.21 ab
2% Dormex – 31 May	1.06 bc	1.18 ab	1.11 bcd	1.06 a	0.85 ab	1.16 bc
2% Dormex – 14 June	1.04 bc	1.10 ab	1.09 bcd	1.04 a	0.83 ab	1.28 ab
2% Dormex – 28 June	1.00 bc	0.96 ab	1.03 cde	0.96 ab	0.82 ab	1.23 ab
2% Dormex – 12 July	0.86 bc	0.92 ab	0.86 de	0.93 ab	0.74 b	1.30 a
2% Dormex – 26 July	0.79 c	0.66 b	0.81 e	0.69 b	0.72 b	1.15 bc
Pr > F						
<i>Treatment</i>	<i>0.0954</i>	<i>0.4208</i>	<i>0.0046</i>	<i>0.1065</i>	<i>0.5282</i>	<i>0.0327</i>
<i>Control vs Treatment</i>	<i>0.8213</i>	<i>0.0072</i>	<i>0.0052</i>	<i>0.5533</i>	<i>0.2406</i>	<i>0.3321</i>
<i>Concentration 1% vs 2%</i>	<i>0.0450</i>	<i>0.5222</i>	<i>0.0901</i>	<i>0.2038</i>	<i>0.7112</i>	<i>0.2596</i>
<i>Date linear</i>	<i>0.2648</i>	<i>0.9181</i>	<i>0.2800</i>	<i>0.4091</i>	<i>0.7546</i>	<i>0.1499</i>
<i>Date quadratic</i>	<i>0.8247</i>	<i>0.2833</i>	<i>0.0070</i>	<i>0.0196</i>	<i>0.6669</i>	<i>0.0029</i>
<i>Conc.*Date linear</i>	<i>0.3083</i>	<i>0.2681</i>	<i>0.0103</i>	<i>0.0303</i>	<i>0.0797</i>	<i>0.1982</i>
<i>Conc.*Date quadratic</i>	<i>0.1408</i>	<i>0.9363</i>	<i>0.4952</i>	<i>0.3097</i>	<i>0.3823</i>	<i>0.3707</i>

Table 6: Effect of hydrogen cyanamide (Dormex®) on the berry ripening, harvest distribution and total yield of ‘Emerald’ during the 2010 harvest season. Means were separated by LSD (5%).

Treatment	Days to 25% harvest <sup>Y</sup>	Days to 50% harvest <sup>Y</sup>	Days to 75% harvest <sup>Y</sup>	Average total yield per plant (g)
Untreated control	13.9 abc	21.8 ab	21.8 ab	595 bc
1% Dormex – 17 May	16.2 a	24.4 a	24.4 a	1654 a
1% Dormex – 31 May	12.1 c	19.6 bc	19.6 bc	767 b
1% Dormex – 14 June	12.0 c	19.0 c	19.0 c	703 b
1% Dormex – 28 June	12.8 bc	21.0 bc	21.0 bc	644 b
1% Dormex – 12 July	12.0 c	19.3 bc	19.3 bc	756 b
1% Dormex – 26 July	14.9 ab	21.5 bc	21.5 bc	341 c
<b>Pr &gt; F</b>				
<i>Treatment</i>	<i>0.0037</i>	<i>0.0007</i>	<i>0.0017</i>	<i>&lt;0.0001</i>
<i>Control vs Treatment</i>	<i>0.4346</i>	<i>0.2776</i>	<i>0.2590</i>	<i>0.0365</i>
<i>Date linear</i>	<i>0.6961</i>	<i>0.2095</i>	<i>0.0740</i>	<i>&lt;0.0001</i>
<i>Date quadratic</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0027</i>	<i>0.0018</i>

<sup>Y</sup> counted from first harvest date

Table 7: Effect of hydrogen cyanamide (Dormex®) on the berry mass of ‘Emerald’ during the 2010 harvest season. Means were separated by LSD (5%).

Treatment	Mean berry mass (g) on different harvest dates										Mean berry mass per plant (g)
	28 Sep.	05 Oct.	09 Oct.	19 Oct.	26 Oct.	11 Nov.	18 Nov.	25 Nov.	7 Des.		
Untreated control	1.54 a	1.95 ab	1.56 b	1.93 b	1.83 ab	1.59 a	1.49 a	1.15 a	1.08 ab	1.70 a	
1% Dormex – 17 May	0.55 bc	2.08 a	1.81 a	2.04 ab	1.94 a	1.72 a	1.42 a	1.04 a	0.88 b	1.78 a	
1% Dormex – 31 May	1.29 ab	1.77 b	1.70 ab	1.86 b	1.71 b	1.54 a	1.39 a	1.11 a	0.85 b	1.71 a	
1% Dormex – 14 June	1.53 a	2.01 a	1.64 ab	2.14 a	1.82 ab	1.56 a	1.36 a	0.99 a	0.93 b	1.77 a	
1% Dormex – 28 June	1.84 a	1.85 ab	1.62 b	1.95 b	1.91 ab	1.54 a	1.31 a	0.98 a	1.11 ab	1.77 a	
1% Dormex – 12 July	1.56 a	1.77 b	1.55 b	1.89 b	1.77 ab	1.55 a	1.32 a	0.98 a	1.11 ab	1.72 a	
1% Dormex – 26 July	0.23 c	1.51 c	1.23 c	1.38 c	1.41 c	1.30 b	1.44 a	1.13 a	1.31 a	1.40 b	
Pr > F											
<i>Treatment</i>	<i>0.0006</i>	<i>0.0006</i>	<i>&lt;0.0001</i>	<i>&lt;0.0001</i>	<i>0.0001</i>	<i>0.0223</i>	<i>0.5652</i>	<i>0.8795</i>	<i>0.1684</i>	<i>&lt;0.0001</i>	
<i>Control vs Treatment</i>	<i>0.2087</i>	<i>0.0585</i>	<i>0.6831</i>	<i>0.3855</i>	<i>0.3876</i>	<i>0.4966</i>	<i>0.1477</i>	<i>0.3795</i>	<i>0.7346</i>	<i>0.8746</i>	
<i>Date linear</i>	<i>0.8321</i>	<i>0.0002</i>	<i>&lt;0.0001</i>	<i>&lt;0.0001</i>	<i>0.0004</i>	<i>0.0019</i>	<i>0.8462</i>	<i>0.9625</i>	<i>0.0057</i>	<i>&lt;0.0001</i>	
<i>Date quadratic</i>	<i>&lt;0.0001</i>	<i>0.2521</i>	<i>0.0696</i>	<i>&lt;0.0001</i>	<i>0.0189</i>	<i>0.5580</i>	<i>0.1640</i>	<i>0.4072</i>	<i>0.4763</i>	<i>&lt;0.0001</i>	

Table 8: Effect of hydrogen cyanamide (Dormex®) on berry ripening, harvest distribution and total yield in ‘Star’ during the 2011 harvest season.

Treatment	Days to 25% harvest <sup>Y</sup>	Days to 50% harvest <sup>Y</sup>	Days to 75% harvest <sup>Y</sup>	Average total yield per plant (g)
Untreated control	-1.7 <sup>Z</sup>	4.5	12.3	447
<u>Concentration:</u>				
1% Dormex	-5.3	0.4	7.6	671
2% Dormex	-4.9	0.1	6.4	485
<u>Date:</u>				
17 May	-13.85	-8.05	-0.65	834
31 May	-11.65	-6.4	0.3	559
14 June	-8.55	-3.6	2.7	557
28 June	-4.1	1.45	8.4	585
12 July	-0.55	5.35	12.8	613
26 July	8.3	12.85	18.55	321
<b>Pr &gt; F</b>				
<i>Treatment</i>	<0.0001	<0.0001	<0.0001	<0.0001
<i>Control vs Treatment</i>	0.1234	0.0277	0.0022	0.1081
<i>Conc. 1% vs 2%</i>	0.7064	0.8665	0.2792	<0.0001
<i>Date linear</i>	<0.0001	<0.0001	<0.0001	<0.0001
<i>Date quadratic</i>	0.0197	0.0069	0.0025	0.9493
<i>Conc.*Date linear</i>	0.3079	0.3820	0.6035	0.1743
<i>Conc.*Date quadratic</i>	0.5004	0.5335	0.6454	0.8109

<sup>Y</sup> counted from first harvest date

<sup>Z</sup> negative values indicate specified harvest percentage would have been achieved prior to the first harvest date

Table 9: Effect of hydrogen cyanamide (Dormex®) on berry mass in ‘Star’ during the 2011 harvest season. Means were separated by LSD (5%).

Treatment	Mean berry mass (g) on different harvest dates								Mean berry mass per plant (g)	
	07 Nov.		30 Nov.		6 Dec.		14 Dec.			
Untreated control	1.96	de	2.14	abcd	1.87	abcd	0.89	abc	2.08	def
1% Dormex – 17 May	2.11	bcde	2.12	abcd	1.49	cdef	0.91	abc	2.10	cdef
1% Dormex – 31 May	2.43	abc	1.91	d	1.29	f	0.77	abc	2.29	abcd
1% Dormex – 14 June	2.35	abcd	2.17	abcd	1.44	ef	0.41	c	2.32	ab
1% Dormex – 28 June	1.82	ef	2.15	abcd	1.63	cdef	1.07	ab	2.00	ef
1% Dormex – 12 July	2.34	abcd	2.33	a	1.90	abc	1.23	a	2.26	abcd
1% Dormex – 26 July	2.06	cde	2.26	ab	2.21	a	1.25	a	2.22	abcd
2% Dormex – 17 May	2.53	a	2.03	bcd	1.23	f	0.46	bc	2.40	a
2% Dormex – 31 May	2.50	ab	1.94	cd	1.53	cdef	0.45	bc	2.31	abc
2% Dormex – 14 June	2.25	abcd	1.98	cd	1.46	def	0.49	bc	2.27	abcd
2% Dormex – 28 June	2.37	abc	2.19	abc	1.79	bcde	0.37	c	2.26	abcd
2% Dormex – 12 July	1.96	de	2.01	bcd	1.83	abcde	1.15	a	1.93	f
2% Dormex – 26 July	1.44	f	2.20	abc	2.05	ab	1.19	a	2.16	bcde
<b>Pr &gt; F</b>										
<i>Treatment</i>	<0.0001		0.0827		<0.0001		0.0094		0.0006	
<i>Control vs Treatment</i>	0.1360		0.7309		0.1737		0.7443		0.1088	
<i>Concentration 1% vs 2%</i>	0.9388		0.0819		0.9153		0.0500		0.5778	
<i>Date linear</i>	<0.0001		0.0071		<0.0001		0.0006		0.0176	
<i>Date quadratic</i>	0.0541		0.4928		0.1564		0.0387		0.8072	
<i>Conc.*Date linear</i>	0.0008		0.5653		0.8973		0.4910		0.0055	
<i>Conc.*Date quadratic</i>	0.1903		0.9368		0.1191		0.9110		0.5101	

Table 10: Effect of hydrogen cyanamide (Dormex®) on berry ripening, harvest distribution and total yield in 'Bluecrisp' during the 2011 harvest season.

Treatment	Days to 25% harvest <sup>Y</sup>	Days to 50% harvest <sup>Y</sup>	Days to 75% harvest <sup>Y</sup>	Average total yield per plant (g)
Untreated control	20.0	22.2	24.9	269
<u>Concentration:</u>				
1% Dormex	20.47	22.55	25.27	236
2% Dormex	19.78	22.2	25.27	146
<u>Date:</u>				
17 May	16.05	19.25	23.3	223
31 May	16.85	19.4	22.7	153
14 June	19.85	21.75	24.25	206
28 June	21.85	23.9	26.55	146
12 July	22.35	24.55	27.3	335
26 July	23.8	25.4	27.45	82
<b>Pr &gt; F</b>				
<i>Treatment</i>	0.0002	<0.0001	<0.0001	<0.0001
<i>Control vs Treatment</i>	0.9536	0.9718	0.8046	0.0148
<i>Conc. 1% vs 2%</i>	0.6055	0.7338	0.8641	<0.0001
<i>Date linear</i>	<0.0001	<0.0001	<0.0001	0.2563
<i>Date quadratic</i>	0.6194	0.7447	0.8660	0.0727
<i>Conc.*Date linear</i>	0.9588	0.9597	0.7804	0.1711
<i>Conc.*Date quadratic</i>	0.4848	0.5825	0.9633	0.2536

<sup>Y</sup> counted from first harvest date

Table 11: Effect of hydrogen cyanamide (Dormex®) on the berry mass in ‘Bluecrisp’ during the 2011 harvest season.

Treatment	Mean berry mass (g) on different harvest dates				Mean berry mass per plant (g)
	07 Nov.	30 Nov.	06 Des.	14 Des.	
Untreated control	1.64	2.10	1.75	1.36	1.88
<u>Concentration:</u>					
1% Dormex	1.10	2.06	1.86	1.35	1.94
2% Dormex	0.73	1.97	1.69	1.30	1.84
<u>Date:</u>					
17 May	1.89	2.03	1.68	1.30	1.90
31 May	1.41	2.07	1.62	1.22	1.93
14 June	0.56	1.98	1.71	1.28	1.87
28 June	0.14	2.18	1.84	1.23	1.92
12 July	0.97	1.96	1.85	1.66	1.85
26 July	0.53	1.87	1.99	1.26	1.87
Pr > F					
<i>Treatment</i>	<i>&lt;0.0001</i>	<i>0.0057</i>	<i>0.0001</i>	<i>0.1648</i>	<i>0.4465</i>
<i>Control vs Treatment</i>	<i>0.0070</i>	<i>0.2460</i>	<i>0.6938</i>	<i>0.8426</i>	<i>0.9683</i>
<i>Conc. 1% vs 2%</i>	<i>0.0148</i>	<i>0.0393</i>	<i>0.0015</i>	<i>0.6318</i>	<i>0.0078</i>
<i>Date linear</i>	<i>&lt;0.0001</i>	<i>0.0444</i>	<i>&lt;0.0001</i>	<i>0.3474</i>	<i>0.3205</i>
<i>Date quadratic</i>	<i>&lt;0.0001</i>	<i>0.0220</i>	<i>0.3204</i>	<i>0.9040</i>	<i>0.6910</i>
<i>Conc.*Date linear</i>	<i>0.2285</i>	<i>0.2382</i>	<i>0.5870</i>	<i>0.4542</i>	<i>0.7985</i>
<i>Conc.*Date quadratic</i>	<i>0.5876</i>	<i>0.5779</i>	<i>0.8268</i>	<i>0.4939</i>	<i>0.9739</i>

Table 12: Effect of hydrogen cyanamide (Dormex®) on berry ripening, harvest distribution and total yield in ‘Emerald’ during the 2011 harvest season. Means were separated by LSD (5%).

Treatment	Days to 25% harvest <sup>Y</sup>	Days to 50% harvest <sup>Y</sup>	Days to 75% harvest <sup>Y</sup>	Average total yield per plant (g)
Untreated control	1.3 bc	11.4 ab	24.2 a	1851 a
1% Dormex – 17 May	-14.3 <sup>Z</sup> d	-6.1 d	4.2 c	681 bc
1% Dormex – 31 May	-14.0 d	-4.3 d	8.1 c	876 b
1% Dormex – 14 June	-5.7 c	3.2 c	14.4 b	682 bc
1% Dormex – 28 June	2.3 ab	8.6 bc	16.7 b	309 d
1% Dormex – 12 July	-2.6 bc	6.1 bc	17.1 b	434 cd
1% Dormex – 26 July	9.7 a	16.5 a	25.1 a	316 d
<b>Pr &gt; F</b>				
<i>Treatment</i>	<0.0001	<0.0001	<0.0001	<0.0001
<i>Control vs Treatment</i>	0.0595	0.0030	<0.0001	<0.0001
<i>Date linear</i>	<0.0001	<0.0001	<0.0001	0.0002
<i>Date quadratic</i>	0.6600	0.7755	0.9835	0.7555

<sup>Y</sup> counted from first harvest date

<sup>Z</sup> negative values indicate specified harvest percentage would have been achieved prior to the first harvest date

Table 13: Effect of hydrogen cyanamide (Dormex®) on berry mass in ‘Emerald’ during the 2011 harvest season. Means were separated by LSD (5%).

Treatment	Mean berry mass (g) on different harvest dates				Mean berry mass per plant (g)
	07 Nov.	30 Nov.	6 Dec.	14 Dec.	
Untreated control	1.99 c	2.21 c	1.71 b	1.70 bc	1.96 c
1% Dormex – 17 May	2.46 a	2.48 ab	2.15 a	1.08 d	2.49 a
1% Dormex – 31 May	2.45 ab	2.26 bc	1.91 ab	1.78 bc	2.38 ab
1% Dormex – 14 June	2.52 a	2.45 ab	2.15 a	1.75 bc	2.38 ab
1% Dormex – 28 June	2.23 b	2.33 bc	1.90 ab	1.31 cd	2.23 b
1% Dormex – 12 July	2.33 ab	2.41 abc	2.24 a	2.01 ab	2.32 ab
1% Dormex – 26 July	2.00 c	2.60 a	2.08 ab	2.46 a	2.34 ab
Pr > F					
<i>Treatment</i>	<i>&lt;0.0001</i>	<i>0.0236</i>	<i>0.1009</i>	<i>0.0003</i>	<i>&lt;0.0001</i>
<i>Control vs Treatment</i>	<i>0.0001</i>	<i>0.0206</i>	<i>0.0173</i>	<i>0.898</i>	<i>&lt;0.0001</i>
<i>Date linear</i>	<i>&lt;0.0001</i>	<i>0.1837</i>	<i>0.7399</i>	<i>&lt;0.0001</i>	<i>0.0594</i>
<i>Date quadratic</i>	<i>0.0659</i>	<i>0.0355</i>	<i>0.5059</i>	<i>0.381</i>	<i>0.1059</i>

## **Paper 2: Effects of thidiazuron on yield of southern highbush blueberry (*Vaccinium corymbosum* L. interspecific hybrids) cultivars Bluecrisp and Star**

### **Abstract**

Some southern highbush (SHB) blueberry cultivars grown in the Mediterranean-type climate of the Western Cape province experience inadequate winter chilling and produce smaller, slower ripening berries of poor quality. The chemical rest breaking agent Lift® (thidiazuron; TDZ; 3 g L<sup>-1</sup> in mineral oil) is recommended in the South African apple industry. Two concentrations (4 or 6% v/v) of Lift® applied at different phenological stages of ‘Bluecrisp’ and ‘Star’ were compared to an untreated control over two seasons in a commercial orchard in the George region (34 °S, 194 m altitude). The effect on berry ripening, berry size and yield were evaluated. Lift® application did not result in the desired harvest scheduling effect, although days to 75% harvest were accelerated in ‘Star’ following the unseasonably warm winter in 2010. The effect of Lift® on total yield appeared to be dependent on application date, as lower total yields in both cultivars followed application on the first or last dates. Berry size was increased by a reduced total yield. To help prevent phytotoxicity and malformed flowers, applications should not be made after reproductive bud scales have opened.

*Keywords:* southern highbush blueberries, thidiazuron, rest breaking.

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### **Introduction**

In Paper 1 we discussed how buds of southern highbush (SHB) blueberries (*Vaccinium corymbosum* L. interspecific hybrids) enter endodormancy during autumn and require chilling to be released. The dependence of rapidly developing berries on photosynthates from leaves and reserves as well as the interest of South African growers in cultural practices that could

accelerate berry ripening were also discussed and these topics will not be further elaborated on in this paper. In order to optimise budbreak, foliage development and harvest scheduling in mild wintered growing regions, applying a rest breaking agent (RBA) to partially compensate for the insufficient chilling of deciduous fruit and nuts is common practice (Erez 1987, 1995; Faust et al. 1997). It is known that cytokinins (CKs) can induce vegetative bud break in deciduous fruit trees (Faust et al. 1997). The chemical compound N-phenyl-N'-1,2,3-thiadiazol-5-ylurea (TDZ; thidiazuron) displays CK-like activity (Mok et al. 1982). Treatments with commercial formulations of TDZ are effective as a RBA in apple (Wang et al. 1986; Steffens and Stutte 1989). Faust et al. (1997) recommend that RBAs are applied after two thirds of the chilling requirement of endodormant buds is satisfied for optimal promotion of early spring bud break. TDZ is a RBA registered in the South African apple industry (Costa et al. 2004). Costa et al. (2004) evaluated TDZ application on apple, pear, cherry and Japanese plum, and advised a concentration range of 3 to 7% when applying Lift® (TDZ; 3 g L<sup>-1</sup> in mineral oil; Almond Agro Chemicals, South Africa). On these crops, Lift® application 4 to 7 weeks before expected full bloom resulted in the best bud break pattern (Costa et al. 2004). Erez et al. (2008) also reported that TDZ has a strong rest breaking effect in stone fruit species. Improved vegetative bud break was observed on fig cultivars following a 6% Lift® application during midwinter or at an early stage of bud swell (Theron et al. 2011). TDZ is not translocated in shoots and must therefore be applied to and cover endodormant buds to be effective (Wang et al. 1986).

The response of buds to RBAs is complicated by the interactions of various endo- and exogenous factors (Erez 1995, 2000; Faust et al. 1997, Faust 2000; Paper 1), and information in the current literature regarding the use of RBAs on SHB blueberry under South African conditions is limited. Paper 1 reported on the effect of exogenous hydrogen cyanamide (HC) application on yield of SHB cultivars 'Bluecrisp', 'Emerald' and 'Star'. It was found that HC accelerated berry ripening in 'Bluecrisp' following an unseasonably warm winter, and recommendations regarding HC application rate and timing were made. In this paper we report on a similar trial the effects of exogenous TDZ application at different rates and dates on the SHB 'Star' and 'Bluecrisp'.

## **Materials and Methods**

### *Plant material and site*

This trial was conducted on ‘Bluecrisp’ and ‘Star’ SHB blueberry plants (Lyrene 1999; Lyrene and Sherman 2000). The same trial site and plant material were used as described in Paper 1.

### *Treatments and trail design*

Two concentrations of TDZ (4 or 6% v/v Lift®) were applied with a pressurized backpack sprayer until runoff (i.e.  $\pm 0.30 \text{ L plant}^{-1}$ ) to tagged plants on six dates during both seasons (Table 1). See Paper 1 for further details on the treatments and trial design.

### *Data recorded*

See Paper 1 for details.

### *Data analysis*

See Paper 1 for details.

## **Results**

Defective, malformed flowers appeared frequently following Lift® treatments of plants with open reproductive bud scales, and severe tissue damage (phytotoxicity) was observed after treating flowering plants (Table 1).

*2010 Season*

## ‘Star’

Days to 25% and 50% harvest were not affected by Lift® application, but it did accelerated days to 75% harvest ( $p=0.0103$ ) (Table 2). No interaction was observed between concentration and date of application in berry ripening or total yield. Application date displayed a strong quadratic effect in total yield with the earliest and the last application dates reducing total yield while the middle dates increased total yield (Table 2). No significant treatment effects on berry size (as measured by mean berry mass) were observed on the first and second harvest dates (Table 3). On the third harvest date berry size were improved with later Lift® application, until the last application date which resulted in much smaller berries. A similar trend was observed on the final harvest date, but here the 4% Lift® concentration also improved berry size compared to the higher 6% concentration (Table 3). Average berry size, as measured by mean berry mass over all harvest dates, was not affected by Lift® application, and no interaction was detected between concentration and application date in berry size (Table 3).

## ‘Bluecrisp’

No significant treatment effects were observed in days to 25% harvest. Lift® application date displayed a quadratic effect in days to 50 and 75% harvest. In the case of days to 50% harvest little differences occurred over application time except for a steep decrease in days at the last application date while for the days to 75% harvest the first and last application dates accelerated the time until this harvest period more than middle application dates (Table 4). No interaction was observed between concentration and date of application in berry ripening and total yield following Lift® application (Table 4). Total yield was lower following the first and last application dates compared to middle application dates. On the first and third harvest dates no significant trends were seen in berry size (Table 5). On the second harvest date a linear response was observed with Lift® application date (Table 5). On the last harvest date a quadratic interaction was detected between Lift® concentration and date of application in berry size. With the 4% concentration berries were significantly smaller following the last application date, but with the higher 6% concentration berries were significantly smaller following the first

application date (Table 5). Average overall berry size following a 4% Lift® application decreased the later the application was made but increased at the last application date (Table 5). In the case of the 6% Lift® application the berry size decreased the later the application.

### *2011 Season*

#### *‘Star’*

A significant increase in number of days to 25% and 50% harvest was observed the later the application date of Lift® (Table 6). Lift® concentration showed a linear interaction with application date for days to 75% harvest as the delay in harvest with delayed application was less severe with the 4% concentration than with the higher 6% concentration (Table 6). A quadratic interaction was displayed between Lift® concentration and date of application in total yield. An increase in yield followed Lift® application up to the third date with the 6% concentration, while no significant differences were found between application dates following the 4% application (Table 6). At the first harvest date Lift® application generally improved berry size ( $p=0.0118$ ), and a quadratic effect was found with application date as the middle application dates resulted in smaller berries than earlier or later application dates (Table 7). At the second harvest date delayed Lift® application improved berry size linearly the later the application. On the third and fourth harvest dates Lift® concentration showed a linear interaction with application date in berry size, with the 4% application resulting in bigger berries with early and late application while the 6% application resulted in an increase in berry weight with later application (Table 7). The overall berry size was very similar for all applications and the control except at the very late application of both 4 and 6% Lift® which significantly improved berry size (Table 7).

#### *‘Bluecrisp’*

A quadratic interaction was found between Lift® concentration and date of application in berry ripening to 25 and 50% harvest. With the 6% concentration a quadratic response was seen in delayed berry ripening between the initial and mid application dates, but with the lower 4% concentration the response was an increase in delayed harvest with later application. With Lift®

treatment days to 75% harvest showed a similar linear delay in harvest from the first to the last application date for 4 and 6% Lift® applications. Lift® application generally decreased total yield ( $p=0.0014$ ) (Table 8). The quadratic interaction between Lift® concentration and date of application in total yield, showed total yield being the highest at mid application dates, however with the higher 6% concentration the total yield was lower than with the 4% concentration. At the first harvest date berry mass showed a quadratic effect in berry size, with the 31 May application date resulting in the largest berries with both concentrations (Table 9). On the second harvest date the quadratic interaction between Lift® concentration and date of application in berry size, showed that with the 6% concentration delayed application resulted in a similar response in berry size, but with the lower concentration of 4% a quadratic response was seen in reduced berry size following the last application date. On the third harvest date berry size improved linearly with delayed application of both 4 and 6% concentration (Table 9). Generally Lift® application resulted in a decrease in berry size on the last harvest date ( $p=0.0003$ ) and the 4% treatments resulted in slightly larger berries than the 6% treatments. Overall average berry size was not affected by Lift® application (Table 9).

## **Discussion**

Although Lift® accelerated days to 75% harvest in ‘Star’ following the unseasonably warm wintered 2010 season (Table 2), it did not result in the desired advancement of earlier harvest percentages (Tables 2, 4, 6 and 8). TDZ releases apple vegetative buds from dormancy (Wang et al. 1986; Steffens and Stutte 1989). The mode of action of TDZ seems to be related to accelerated polyamide synthesis, caused by increases in RNA, DNA, S-adenosylmethionine (SAM) and protein (Wang et al. 1991). Steffens and Stutte (1989) observed that TDZ has the capacity to induce vegetative bud growth in ‘Northern Spy’ (high-chill) apple when applied prior to chilling. In the George area chill-unit accumulation normally starts in June. It was therefore expected that Lift® treatments during May would promote vegetative budbreak in ‘Bluecrisp’ and ‘Star’, which have relatively high chilling requirements compared to other locally grown SHB cultivars. ‘Bluecrisp’ suffers from delayed foliation following unseasonably warm winter (personal observation), as was the case during 2010. Improved early foliar development favours faster berry ripening (Maust et al. 1999). Results in terms of harvest scheduling varied

considerably amongst cultivars and over seasons (Tables 2, 4, 6 and 8). The reason for this variability is unknown, although some of it may be accounted for by differences in phenological stages and prior chilling exposure at treatment application dates (Erez 1995, 2000; Faust et al. 1997, Faust 2000).

When comparing the application dates with each other, Lift® treatment at the late application date tended to decrease the number of days to 25%, 50% and 75% harvest in both cultivars during 2010 (Tables 4 and 6). Yet, during 2011 the opposite seemed to occur as Lift® treatment tended to increase the number of days to the respective harvest percentages (Tables 6 and 8). These opposite results may be partially due to flower tissue damage caused by Lift® treatments on the final application dates during the 2011 season (Table 1) as elimination of more advanced flowers can result in a delay in berry ripening (Stringer et al. 2003; NeSmith 2005). Applications were made on the same dates during both years, but the plants were phenologically more advanced after the colder winter in the 2011 season (Table 1).

The effect of Lift® on total yield appeared to be dependent on application date, because total yield tended to be lower in both cultivars and during both seasons following application on the first or last dates (Tables 2, 4, 6 and 8). Reduced total yield following the 17 May Lift® applications suggests that reproductive buds could have been damaged, for chemical rest breaking agents are known to be phytotoxic to paradormant buds (Erez 2000). Excessive flower injury caused with Lift® to flowering plants (Table 1) may have resulted in the observed reduction in total yield following 26 July applications, and unintentionally in increased fruit size (Tables 5 and 7). Defective, malformed flowers following Lift® treatment was not unexpected as Erez et al. (2008) found flower malformation in apricot, peach and nectarine cultivars following TDZ treatments.

## **Conclusion**

TDZ application is not currently recommended for 'Bluecrisp' and 'Star' as results were inconclusive. The TDZ concentrations we used caused malformation and excessive injury to flowers especially at later application dates, and as a precaution TDZ should not be applied after

reproductive bud scales have opened. Further studies are suggested to clarify possible benefits of TDZ application in SHB cultivars.

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Table 1: Lift® application dates for both seasons, with the reproductive bud development of different cultivars and chill-units (Utah model) accumulated on each date.

Application date	2010			2011		
	Bluecrisp	Star	Chill-units	Bluecrisp	Star	Chill-units
17 May	Not swollen	Not swollen	-154	Not swollen	Not swollen	-65
31 May	Not swollen	Not swollen	-77	Not swollen	Not swollen	-33
14 June	Not swollen	Not swollen	45	Swollen, scales closed	Swollen, scales closed	62
28 June	Swollen, scales closed	Swollen, scales closed	89	Scales separated	Scales separated	123
12 July	Scales separated	Scales separated	98	Scales open <sup>Z</sup>	Scales open <sup>Z</sup>	205
26 July	Scales open <sup>Z</sup>	Scales open <sup>Z</sup>	197	First flowers <sup>Y</sup>	First flowers <sup>Y</sup>	287

<sup>Y</sup> phytotoxicity observed one week after applying the 4% or 6% concentration

<sup>Z</sup> flower malformation observed two weeks after applying the 4% or 6% concentration

Table 2: Effect of thidiazuron (Lift®) on berry ripening, harvest distribution and total yield in ‘Star’ during the 2010 harvest season.

Treatment	Days to 25% harvest <sup>Y</sup>	Days to 50% harvest <sup>Y</sup>	Days to 75% harvest <sup>Y</sup>	Average total yield per plant (g)
Untreated control	0.5	7.5	16.3	150
<u>Concentration:</u>				
4% Lift	-0.6 <sup>Z</sup>	5.1	12.4	151
6% Lift	-0.6	4.9	11.9	161
<u>Date:</u>				
17 May	-1.5	3.7	10.1	41
31 May	-0.1	5.1	11.7	176
14 June	-3.7	3.1	11.6	213
28 June	0.2	6.2	13.7	183
12 July	1.0	7.0	14.7	232
26 July	0.5	5.2	11.1	90
<u>Pr &gt; F</u>				
<i>Treatment</i>	0.0184	0.0065	0.0048	<0.0001
<i>Control vs Treatment</i>	0.6686	0.1308	0.0103	0.7779
<i>Conc. 4% vs 6%</i>	0.2970	0.4490	0.8672	0.3564
<i>Date linear</i>	0.6079	0.4387	0.3534	0.0032
<i>Date quadratic</i>	0.0986	0.6949	0.1508	<0.0001
<i>Conc.*Date linear</i>	0.2948	0.2536	0.3309	0.6263
<i>Conc.*Date quadratic</i>	0.0596	0.0799	0.2651	0.4993

<sup>Y</sup> counted from first harvest date<sup>Z</sup> negative values indicate specified harvest percentage would have been achieved prior to the first harvest date

Table 3: Effect of thidiazuron (Lift®) on mean berry mass in ‘Star’ during the 2010 harvest season.

Treatment	Mean berry mass (g) on different harvest dates				Mean berry mass per plant (g)
	04 Nov.	18 Nov.	25 Nov.	07 Des.	
Untreated control	2.02	2.16	1.45	1.40	1.93
<u>Concentration:</u>					
4% Lift	2.10	2.18	1.37	1.29	2.09
6% Lift	2.02	2.08	1.41	1.05	2.01
<u>Date:</u>					
17 May	2.12	2.15	0.95	1.18	2.22
31 May	2.14	2.08	1.36	1.12	1.98
14 June	2.16	2.14	1.45	1.42	2.08
28 June	2.02	2.14	1.59	1.14	1.97
12 July	2.27	2.14	1.61	1.37	2.04
26 July	1.66	2.12	1.37	0.82	2.01
<b>Pr &gt; F</b>					
<i>Treatment</i>	0.5717	0.5743	0.2315	0.0003	0.0976
<i>Control vs Treatment</i>	0.8839	0.8257	0.7584	0.1336	0.1662
<i>Conc. 4% vs 6%</i>	0.5581	0.1784	0.7179	0.0098	0.0764
<i>Date linear</i>	0.1761	0.8381	0.0159	0.2565	0.0458
<i>Date quadratic</i>	0.1019	0.8825	0.0098	0.0065	0.1151
<i>Conc.*Date linear</i>	0.6576	0.7112	0.5862	0.2286	0.4045
<i>Conc.*Date quadratic</i>	0.6984	0.8783	0.3958	0.0629	0.6008

Table 4: Effect of thidiazuron (Lift®) on berry ripening, harvest distribution and total yield in 'Bluecrisp' during the 2010 harvest season.

Treatment	Days to 25% harvest <sup>Y</sup>	Days to 50% harvest <sup>Y</sup>	Days to 75% harvest <sup>Y</sup>	Average total yield per plant (g)
Untreated control	0.7	7.8	16.8	99
<u>Concentration:</u>				
4% Lift	2.22	8.24	15.87	78
6% Lift	0.31	7.19	15.91	68
<u>Date:</u>				
17 May	2.00	8.08	15.78	42
31 May	2.49	8.40	15.90	105
14 June	1.83	8.87	17.80	100
28 June	2.53	9.28	17.84	82
12 July	2.43	8.11	15.31	90
26 July	-3.67 <sup>Z</sup>	3.55	12.70	20
<b>Pr &gt; F</b>				
<i>Treatment</i>	<i>0.3947</i>	<i>0.2915</i>	<i>0.0467</i>	<i>&lt;0.0001</i>
<i>Control vs Treatment</i>	<i>0.3778</i>	<i>0.4293</i>	<i>0.8006</i>	<i>0.1109</i>
<i>Conc. 4% vs 6%</i>	<i>0.2441</i>	<i>0.4589</i>	<i>0.6111</i>	<i>0.0664</i>
<i>Date linear</i>	<i>0.1758</i>	<i>0.0984</i>	<i>0.1074</i>	<i>0.0337</i>
<i>Date quadratic</i>	<i>0.1267</i>	<i>0.0314</i>	<i>0.0091</i>	<i>&lt;0.0001</i>
<i>Conc.*Date linear</i>	<i>0.1579</i>	<i>0.2154</i>	<i>0.7420</i>	<i>0.0839</i>
<i>Conc.*Date quadratic</i>	<i>0.2918</i>	<i>0.8617</i>	<i>0.0697</i>	<i>0.3798</i>

<sup>Y</sup> counted from first harvest date<sup>Z</sup> negative values indicate specified harvest percentage would have been achieved prior to the first harvest date

Table 5: Effect of thidiazuron (Lift®) on mean berry mass in 'Bluecrisp' during the 2010 harvest season. Means were separated by LSD (5%).

Treatment	Mean berry mass (g) on different harvest dates								Mean berry mass per plant (g)	
	04 Nov.		18 Nov.		25 Nov.		07 Des.			
Untreated control	2.03	ab	1.25	bcd	1.31	ab	0.75	bcd	1.47	bcd
4% Lift – 17 May	1.94	ab	1.63	ab	1.29	abc	0.62	cd	1.56	abc
4% Lift – 31 May	1.81	abc	1.50	abcd	1.24	abc	0.84	bc	1.39	cde
4% Lift – 14 June	1.68	bc	1.51	abc	1.25	abc	0.88	bc	1.43	cde
4% Lift – 28 June	1.95	ab	1.47	bcd	1.22	bc	1.12	ab	1.40	cde
4% Lift – 12 July	1.38	c	1.34	bcd	1.24	abc	0.91	bc	1.37	de
4% Lift – 26 July	2.15	a	1.28	bcd	1.44	a	0.38	d	1.64	ab
6% Lift – 17 May	1.93	ab	1.88	a	1.10	c	1.42	a	1.73	a
6% Lift – 31 May	1.60	bc	1.12	d	1.18	bc	0.74	bcd	1.28	e
6% Lift – 14 June	1.94	ab	1.34	bcd	1.20	bc	0.86	bc	1.37	de
6% Lift – 28 June	1.62	bc	1.54	abc	1.19	bc	0.96	bc	1.35	de
6% Lift – 12 July	1.84	ab	1.44	bcd	1.18	bc	0.74	bcd	1.40	cde
6% Lift – 26 July	1.97	ab	1.21	d	1.35	ab	0.94	bc	1.40	cde
Pr > F										
<i>Treatment</i>	0.0813		0.0423		0.5211		0.0320		0.0004	
<i>Control vs Treatment</i>	0.1692		0.1920		0.2715		0.5368		0.6493	
<i>Conc. 4% vs 6%</i>	0.9998		0.4524		0.0622		0.0998		0.2833	
<i>Date linear</i>	0.7677		0.0075		0.0726		0.0788		0.2250	
<i>Date quadratic</i>	0.0612		0.8996		0.2755		0.1695		<0.0001	
<i>Conc.*Date linear</i>	0.5743		0.9146		0.4380		0.6474		0.0636	
<i>Conc.*Date quadratic</i>	0.7087		0.6450		0.3645		0.0006		0.7543	

Table 6: Effect of thidiazuron (Lift®) on berry ripening, harvest distribution and total yield in ‘Star’ during the 2011 harvest season. Means were separated by LSD (5%).

Treatment	Days to 25% harvest <sup>Y</sup>	Days to 50% harvest <sup>Y</sup>	Days to 75% harvest <sup>Y</sup>	Average total yield per plant (g)
Untreated control	-7.1 <sup>Z</sup> cde	0.8 cdef	10.8 cde	431 bcd
4% Lift – 17 May	-11.8 def	-3.7 efg	6.7 efg	424 bcd
4% Lift – 31 May	-11.0 def	-2.4 defg	8.5 def	445 bcd
4% Lift – 14 June	-0.9 bc	6.3 bc	15.4 bc	384 bcd
4% Lift – 28 June	-3.8 bcd	4.1 bcd	14.2 bcd	544 bc
4% Lift – 12 July	0.2 bc	6.7 bc	14.8 bcd	365 cd
4% Lift – 26 July	18.4 a	22.0 a	26.7 a	304 d
6% Lift – 17 May	-18.6 f	-9.8 g	1.4 g	276 d
6% Lift – 31 May	-14.1 ef	-6.1 fg	3.9 fg	554 b
6% Lift – 14 June	-4.8 cd	3.5 cde	14.1 bcd	761 a
6% Lift – 28 June	-7.5 cde	0.1 cdef	9.8 cdef	398 bcd
6% Lift – 12 July	4.9 b	11.3 b	19.4 b	296 d
6% Lift – 26 July	13.7 a	19.7 a	27.3 a	265 d
<b>Pr &gt; F</b>				
<i>Treatment</i>	<0.0001	<0.0001	<0.0001	<0.0001
<i>Control vs Treatment</i>	0.1997	0.1927	0.2362	0.8462
<i>Conc. 4% vs 6%</i>	0.1537	0.1540	0.2092	0.7187
<i>Date linear</i>	<0.0001	<0.0001	<0.0001	0.0187
<i>Date quadratic</i>	0.0191	0.0298	0.1059	<0.0001
<i>Conc.*Date linear</i>	0.2967	0.1406	0.0407	0.5142
<i>Conc.*Date quadratic</i>	0.4794	0.6518	0.9585	0.0291

<sup>Y</sup> counted from first harvest date<sup>Z</sup> negative values indicate specified harvest percentage would have been achieved prior to the first harvest date

Table 7: Effect of thidiazuron (Lift®) on mean berry mass in ‘Star’ during the 2011 harvest season. Means were separated by LSD (5%).

Treatment	Mean berry mass (g) on different harvest dates				Mean berry mass (g)
	07 Nov.	30 Nov.	06 Des.	14 Des.	
Untreated control	2.02 c	2.07 cd	1.81 c	1.14 cd	2.13 c
4% Lift – 17 May	2.32 abc	2.07 cd	1.80 c	1.46 bcd	2.17 cb
4% Lift – 31 May	2.17 bc	2.13 cd	1.66 cd	1.25 bcd	2.06 c
4% Lift – 14 June	2.37 abc	2.27 bc	1.72 cd	1.31 bcd	2.26 cb
4% Lift – 28 June	2.07 c	2.11 cd	1.79 c	1.20 bcd	2.06 c
4% Lift – 12 July	2.70 a	2.14 cd	1.70 cd	1.38 bcd	2.20 cb
4% Lift – 26 July	2.61 a	2.93 a	2.56 a	2.28 a	2.41 b
6% Lift – 17 May	2.34 abc	1.81 d	1.47 d	0.50 e	2.12 c
6% Lift – 31 May	2.33 abc	2.04 cd	1.47 d	1.01 de	2.14 c
6% Lift – 14 June	2.11 bc	2.15 cd	2.17 b	1.73 b	2.12 c
6% Lift – 28 June	2.37 abc	2.23 bc	1.69 cd	1.32 bcd	2.19 cb
6% Lift – 12 July	2.48 ab	2.34 bc	1.93 bc	1.55 bc	2.17 cb
6% Lift – 26 July	2.67 a	2.64 ab	2.77 a	2.73 a	2.72 a
<b>Pr &gt; F</b>					
<i>Treatment</i>	0.0048	0.0003	<0.0001	<0.0001	<0.0001
<i>Control vs Treatment</i>	0.0118	0.3640	0.4828	0.0857	0.2916
<i>Conc. 4% vs 6%</i>	0.8980	0.3882	0.5289	0.9651	0.3167
<i>Date linear</i>	0.0019	<0.0001	<0.0001	<0.0001	<0.0001
<i>Date quadratic</i>	0.0136	0.0842	<0.0001	0.0027	0.0005
<i>Conc.*Date linear</i>	0.7358	0.5679	0.0206	0.0007	0.0911
<i>Conc.*Date quadratic</i>	0.9156	0.1365	0.1287	0.0698	0.2410

Table 8: Effect of thidiazuron (Lift®) on berry ripening, harvest distribution and total yield in 'Bluecrisp' during the 2011 harvest season. Means were separated by LSD (5%).

Treatment	Days to 25% harvest <sup>Y</sup>	Days to 50% harvest <sup>Y</sup>	Days to 75% harvest <sup>Y</sup>	Average total yield per plant (g)
Untreated control	20.5 ab	23.2 ab	26.6 bcd	358 a
4% Lift – 17 May	10.9 c	17.2 cd	25.1 bcd	271 bcde
4% Lift – 31 May	10.6 c	16.6 d	24.1 d	346 ab
4% Lift – 14 June	17.2 b	20.8 bc	25.5 bcd	236 cdef
4% Lift – 28 June	21.0 ab	23.6 ab	26.8 bc	339 ab
4% Lift – 12 July	20.3 ab	23.2 ab	27.0 abc	314 abc
4% Lift – 26 July	24.7 a	26.8 a	29.5 a	103 g
6% Lift – 17 May	7.2 c	15.0 d	25.1 bcd	233 cdef
6% Lift – 31 May	9.9 c	16.3 d	24.4 cd	284 abcd
6% Lift – 14 June	20.5 ab	23.2 ab	26.7 bcd	297 abc
6% Lift – 28 June	19.7 ab	23.2 ab	27.6 ab	202 edf
6% Lift – 12 July	21.0 ab	23.8 ab	27.3 ab	154 fg
6% Lift – 26 July	16.5 b	21.0 b	26.8 bcd	199 ef
<b>Pr &gt; F</b>				
<i>Treatment</i>	<0.0001	<0.0001	0.0053	<0.0001
<i>Control vs Treatment</i>	0.0562	0.1185	0.8238	0.0014
<i>Conc. 4% vs 6%</i>	0.1532	0.2655	0.9926	0.0222
<i>Date linear</i>	<0.0001	<0.0001	<0.0001	<0.0001
<i>Date quadratic</i>	0.0018	0.0179	0.7644	0.0007
<i>Conc.*Date linear</i>	0.3719	0.2798	0.2052	0.6085
<i>Conc.*Date quadratic</i>	0.0069	0.0073	0.0552	0.0373

<sup>Y</sup> counted from first harvest date

Table 9: Effect of thidiazuron (Lift®) on mean berry mass in 'Bluecrisp' during the 2011 harvest season. Means were separated by LSD (5%).

Treatment	Mean berry mass (g) on different harvest dates				Mean berry mass (g)	
	07 Nov.	30 Nov.	06 Des.	14 Des.		
Untreated control	1.64 bcd	2.02 b	1.87 abc	1.99 a	1.99 ab	
4% Lift – 17 May	2.20 ab	2.15 ab	1.87 abc	1.66 bc	2.00 ab	
4% Lift – 31 May	2.32 a	2.07 ab	1.92 abc	1.48 cd	1.99 ab	
4% Lift – 14 June	2.20 ab	2.11 ab	1.81 bc	1.63 bc	1.97 ab	
4% Lift – 28 June	1.36 cde	2.20 ab	2.02 ab	1.81 ab	2.07 a	
4% Lift – 12 July	1.13 def	2.33 a	1.98 ab	1.66 bc	2.00 ab	
4% Lift – 26 July	0.71 efg	0.92 c	2.05 ab	1.71 bc	1.89 ab	
6% Lift – 17 May	1.85 abc	2.13 ab	1.92 abc	1.69 bc	1.95 ab	
6% Lift – 31 May	2.03 ab	1.99 b	1.71 c	1.37 d	1.92 ab	
6% Lift – 14 June	1.65 bcd	2.00 b	1.84 abc	1.53 cd	1.96 ab	
6% Lift – 28 June	1.33 cdef	2.21 ab	1.86 abc	1.59 bcd	1.97 ab	
6% Lift – 12 July	0.70 fg	2.25 ab	2.04 ab	1.63 bc	2.05 a	
6% Lift – 26 July	0.20 g	2.02 b	2.10 a	1.54 cd	1.84 b	
Pr > F						
<i>Treatment</i>	<0.0001	<0.0001	0.2303	0.0031	0.6079	
<i>Control vs Treatment</i>	0.4950	0.8702	0.5860	0.0003	0.7710	
<i>Conc. 4% vs 6%</i>	0.0078	0.0150	0.6216	0.0419	0.3468	
<i>Date linear</i>	<0.0001	<0.0001	0.0065	0.2406	0.5122	
<i>Date quadratic</i>	0.0128	<0.0001	0.1086	0.7008	0.1018	
<i>Conc.*Date linear</i>	0.8077	<0.0001	0.6308	0.5266	0.6901	
<i>Conc.*Date quadratic</i>	0.6681	<0.0001	0.3767	0.5536	0.9824	

## **Paper 3: Effects of night interruption on yield of southern highbush blueberry (*Vaccinium corymbosum* L. interspecific hybrids) cultivars Emerald and Snowchaser**

### **Abstract**

Since commercial blueberry growers in the Western Cape aim to supply export markets at an earlier, more lucrative time, they are interested in cultivation practices that could influence flowering and the rate of early berry ripening. The initiation of southern highbush (SHB) blueberry reproductive buds is controlled by day length and temperature. SHB initiate reproductive buds under short days, i.e. long dark periods, and this process is mediated by phytochrome, therefore night interruption (NI) during the middle of the dark period inhibits reproductive bud development in SHB blueberry. Delaying reproductive bud initiation and therefore bud burst until after vegetative bud burst would favour faster berry ripening than would otherwise be the case in some SHB cultivars following insufficient chilling exposure. The effect of different NI periods on yield, berry size and yield distribution of ‘Emerald’ and ‘Snowchaser’ were evaluated. A trial was conducted during 2010 and 2011 in a commercial orchard near George (34 °S, 194 m altitude). It included three NI durations, plus an untreated control. NI treatments did not suppress reproductive bud initiation. Although cultivars responded differently to NI, the yield, number of berries harvested and berry size were generally reduced by an extended duration of NI.

*Keywords:* southern highbush blueberries, night interruption, reproductive bud initiation.

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### **Introduction**

Most commercial blueberry growers in the Western Cape province of South Africa produce berries for the early to mid spring export market and the effect of cultivation practices on flowering and early berry maturation are therefore important considerations. Blueberry

auxiliary buds are vegetative when they first develop and some of them are initiated to form reproductive buds (Darnell 2006). This conversion is controlled by day length and temperature (Hall and Ludwig 1961; Hall et al. 1963; Phatak and Austin 1990; Darnell 1991; Spann et al. 2003, 2004; Williamson and Lyrene 2004; Bañados and Strik 2006). Spann et al. (2003) observed that SHB blueberry 'Misty' initiated reproductive buds under short days (8 hour photoperiod), i.e. long dark periods. No reproductive bud initiation occurred during exposure to short dark periods (16 hour photoperiod), and long dark periods that were each interrupted in the middle for a hour by incandescent light suppressed reproductive bud initiation (Spann et al. 2003). Mature leaves contain a blue-green plant pigment called phytochrome. Phytochrome functions as a photoreceptor (light signalling molecule) and is believed to form part of a complex day length measuring mechanism in plants (Imaizumi and Kay 2006). Mature blueberry leaves are therefore important for reproductive bud initiation (Williamson and Lyrene 2004). For northern highbush blueberry (*V. corymbosum* L.) it was shown that an extended period (more than four weeks) of exposure to short (8 hour) photoperiods is required for satisfactory reproductive bud differentiation and development (Bañados and Strik 2006). Similarly, at least 5 to 6 weeks of short photoperiods were required for normal reproductive bud initiation in the rabbiteye (RE) blueberry (*V. ashei* Reade) cultivar Beckyblue (Phatak and Austin 1990; Darnell 1991). Genotypic variation exists between blueberry cultivars with regard to the sensitivity to photoperiod for optimal reproductive bud initiation (Darnell 1991).

As discussed in Paper 1 a good balance between foliage development and developing berries is required during spring for fast ripening berries of acceptable quality (Maust et al. 1999). If too little foliage is present the requirement for carbohydrates by developing berries may exceed what can be supported by mature leaves (Maust et al. 1999; Lyrene 2004). When reproductive bud initiation is inhibited while mature leaves are maintained, vegetative bud break could then precede or at least coincide with reproductive bud break. Thereby, the leaf to berry ratio could be improved in favour of faster berry development and ripening than would be the case following insufficient chilling exposure and delayed vegetative bud break. We therefore investigated the efficacy of night interruption with low light intensity incandescent light on berry ripening, berry size and yield for two important SHB cultivars grown in the Western Cape.

## Materials and Methods

### *Plant material and site*

The trial was conducted on ‘Emerald’ and ‘Snowchaser’ SHB blueberry plants (Lyrene 2001, 2008a, 2008b) during 2010 and 2011. After two years in quarantine the plants were planted under 20% white shade net in open soil especially for the trial. Plants were spaced 8 m apart in staggered rows 3.2 m apart to avoid contamination effects from NI treatments. Two plants per plot were arranged in a square pattern 0.6 m apart. The same trial site was used as described in Paper 1.

### *Treatments and trail design*

During both years an untreated control, and night interruption around midnight (NI) with low light intensity incandescent light were applied to plants for three distinct periods from autumn to spring (Table 1). The longest treatment period (184 days) stretched between the autumn and spring equinoxes, when night and day are about the same length. Besides the treatments and plants not being pruned, standard farm cultivation practices were applied during both years. For NI treatments, 100 W incandescent light bulbs were located 1.5 m above the plants in each plot. Light bulbs were connected to an automatic timer that switched them on 30 min before and off 30 min after midnight, i.e. effecting a one hour night interruption around midnight. The experimental design was a randomized complete block with the four treatments replicated 10 times.

### *Data recorded*

The most distal reproductive buds were monitored and dates for first flowering in plants were noted (Table 1). See Paper 1 for further details on data recorded.

### *Data analysis*

Analysis of variance (ANOVA) were performed on the data using SAS software (version 9.2; SAS Institute Inc., Cary, USA). Single degree of freedom, orthogonal contrasts were fitted to

the factorial component of the data to determine which polynomial function best described NI period effect. A probability level of 5% was considered significant.

## Results

Flowering occurred approximately 10 days later during 2011 than 2010. Dates for first flowering in both cultivars and during both years, did not differ by more than two days between NI treatments and between NI treatments and the control, with the exception of 'Emerald' during 2010. Compared to 2011, the date for first flowering in 'Emerald' was not synchronized during 2010, with differences of between three and seven days between treatments and the control (Table 1).

In 'Emerald', NI treatments had no significant effect on total yield, average yield on the two harvest dates, the number of berries harvested per harvest date or in total or average berry size (as measured by mean berry mass) (Tables 2, 3 and 4). At the first harvest date the shortest NI period reduced the yield compared to the NI treatment that continued from March until July but no differences were seen at the second harvest date or in total yield (Tables 5). The number of berries harvested from 'Snowchaser' plants was reduced by the shortest NI treatment compared to the control and the March to July treatment, but no differences in berry number occurred at the second harvest date or in the total number of berries harvested (Table 6). The average berry size did not differ overall or at either harvest date (Table 7).

During 2011 the two longer NI treatments reduced the yield at the first harvest date of 'Emerald' and this trend was also clear in the total yield per plant while no significant effect was noticed at the second harvest date (Table 8). The number of berries harvested from 'Emerald' plants during the first harvest decreased linearly the longer the NI lasted (Table 9). This trend was also seen at the second harvest date and was significant ( $p=0.0207$ ) in the total number of berries harvested (Table 9). At the first harvest date as well as the total harvest, the 'Emerald' berries were significantly smaller following the longest NI period (Table 10). No significant effect was found with NI on yield or total number of berries harvested off 'Snowchaser' in 2011 (Tables 11 and 12). The average berry weight of berries during harvest one decreased the longer the NI and this was also seen in the average berry weight over all harvest (Table 13). No differences were found in average berry size at the second 'Snowchaser' harvest in 2011.

## Discussion

Unsynchronized flowering is one of the known symptoms of a lack of chilling in blueberry (Lyrene 2005). Some of the unsynchronized flowering of ‘Emerald’ during 2010 may have been due to its higher chilling requirement than that of ‘Snowchaser’, the latter requiring only 100-200 hours below 7 °C while ‘Emerald’ requires 100-400 hours below 7 °C (Lyrene 2001, 2008a, 2008b).

Inferred from flowering observations (Table 1) and from yield data during both seasons, the NI treatments used seemed not to have suppressed reproductive bud initiation in ‘Emerald’ and ‘Snowchaser’, yet differences in response between cultivars were observed. ‘Emerald’ responded the strongest during 2011, when the total yield and number of berries harvested per plant were significantly reduced in general, as well as linearly with increased NI duration (Tables 8 and 9). Although general photoperiod requirements for reproductive bud initiation in different blueberry species have been identified, significant cultivar differences in response have been observed (Hall and Ludwig 1961; Hall et al. 1963; Phatak and Austin 1990; Darnell 1991; Spann et al. 2003; Bañados and Strik 2006). For instance, after a two-year trial with ‘Beckyblue’ and ‘Climax’ RE plants, Darnell (1991) reported that the photoperiod used had no effect on reproductive bud initiation, number of berries harvested or berry size in ‘Climax’, while ‘Beckyblue’ plants exposed to naturally long autumn daylengths (gradually decreasing from 12 to 11 hours) initiated significantly fewer reproductive buds than plants exposed to short days (8 hour photoperiods). This decreased reproductive bud initiation under extended photoperiod was similar to that of lowbush (Hall and Ludwig 1961), highbush (Hall et al. 1963; Bañados and Strik 2006) and SHB (Spann et al. 2003) blueberry.

Prior research on the use of NI involving SHB (Spann et al. 2003, 2004) and other blueberry cultivars (Hall and Ludwig 1961; Hall et al. 1963; Phatak and Austin 1990; Darnell 1991; Bañados and Strik 2006) did not report the effect on yield, but from data presented by Darnell (1991) it can be inferred that the effect was insignificant in that trial. Such a yield reduction as observed in this trial may have partially been due to a decrease in number of flowers per bud under NI (Darnell 1991). Unfortunately no detailed data could be recorded on flowering per plant following the NI treatments, so it remains unclear whether reduced yield was due to reduced flower numbers or a decrease in fruit set. Alternatively, or additionally, low carbohydrate content in stem node tissue could have limited reproductive bud initiation. Partitioning of translocated assimilates to stem node tissue has been suggested by Spann et al.

(2004) as a possible prerequisite for floral initiation in SHB blueberries. Darnell (1991) observed an increase in CO<sub>2</sub> assimilation in blueberry leaves under short days and suggested it to be in response to the onset of endodormancy and the initiation of carbohydrate reserves required for its release and subsequent spring budbreak. Spann et al. (2003) found that netto CO<sub>2</sub> assimilation was minimal following one hour NI treatments in their trial.

Low temperatures may also play a role in the reproductive bud initiation response of blueberries (Spann et al. 2003), for initiation in ‘Sharpblue’ occurs year round on the Corindi Plateau (Wright 1993), but not in North central Florida with its similar photoperiod (latitude) yet lower ( $\pm 4$  °C) average temperatures (Spann et al. 2003). In strawberry the short day requirement for initiation can be nullified by low (<9 °C) temperatures (Sønsteby and Heide 2010).

Another possible explanation for the apparent lack of suppression of reproductive bud initiation by the NI treatments is that such buds may have been initiated prior to treatment. In SHB reproductive buds develop on shoots that grew during spring, as well as on young, upright shoots that only emerge after the harvest or summer pruning (Williamson and Lyrene 2004). It could be that the initiation of new reproductive buds on young shoots were suppressed by NI treatments, but that reproductive buds were already initiated on older current season shoots during spring. The plants used in this trial were not summer pruned and therefore most shoots were older. Shoots were unfortunately not monitored separately.

During 2011, berry size tended to decrease with increased NI duration in both cultivars (Tables 10 and 13). This could be interpreted as supportive of the theory that carbohydrate reserves became progressively less with increased NI duration. Otherwise it could be interpreted as a result of poor pollination brought about by unknown factors during 2011 but not 2010. Poor pollination is known to affect berry size in blueberries (Williamson and Lyrene 2004). Furthermore, when flowers were hand-pollinated by Darnell (1991) there was no effect of photoperiod treatment on number of berries harvested or berry size in ‘Beckyblue’ and ‘Climax’.

## **Conclusion**

The possible benefits for blueberry growers of NI during the middle of the dark period requires further investigation. Results from this trial were discouraging, as reproductive bud

initiation was not suppressed by NI, and the yield, number of berries harvested and berry size of 'Emerald' and 'Snowchaser' were reduced by an extended duration of NI.

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Table 1: Dates for first flowering<sup>Y</sup> in trial plants according to season, treatment and SHB cultivar.

Treatment	2010		2011	
	'Emerald	Snowchaser	Emerald	Snowchaser
Untreated control	28 Jul.	21 Jul.	12 Aug.	16 Aug.
NI: 21 Mar. – 21 May (61 days)	26 Jul.	19 Jul.	10 Aug.	15 Aug.
NI: 21 Mar. – 21 Jul. (122 days)	30 Jul.	20 Jul.	12 Aug.	17 Aug.
NI: 21 Mar. – 21 Sept. (184 days)	23 Jul.	21 Jul.	11 Aug.	16 Aug.

<sup>Y</sup> Individual flowers distinctly separated, corollas completely expanded and open.

Table 2: Effect of night interruption on harvest distribution and total yield in 'Emerald' during the 2010 harvest season. Means were separated by LSD (5%).

Treatment	Average yield (g) on different harvest dates		Average total yield per plant (g)
	08 Oct.	11 Nov.	
Untreated control	32.70 a	44.40 a	77.10 a
NI: 21 Mar. – 21 May (61 days)	22.30 a	50.00 a	72.30 a
NI: 21 Mar. – 21 Jul. (122 days)	32.40 a	45.40 a	77.80 a
NI: 21 Mar. – 21 Sept. (184 days)	36.40 a	42.30 a	78.70 a
Pr > F			
<i>Treatment</i>	0.5277	0.9563	0.9772
<i>NI period linear</i>	0.5006	0.8089	0.8364
<i>NI period quadratic</i>	0.3083	0.6663	0.7983

Table 3: Effect of night interruption on number of berries harvested in 'Emerald' during the 2010 harvest season. Means were separated by LSD (5%).

Treatment	Average number of berries harvested on different dates		Average total number of berries per plant
	08 Oct.	11 Nov.	
Untreated control	18 a	17 a	35 a
NI: 21 Mar. – 21 May (61 days)	12 a	20 a	32 a
NI: 21 Mar. – 21 Jul. (122 days)	18 a	18 a	36 a
NI: 21 Mar. – 21 Sept. (184 days)	19 a	19 a	38 a
Pr > F			
<i>Treatment</i>	0.5328	0.9635	0.8737
<i>NI period linear</i>	0.6050	0.8800	0.6085
<i>NI period quadratic</i>	0.3568	0.7724	0.6412

Table 4: Effect of night interruption on mean berry mass in ‘Emerald’ during the 2010 harvest season. Means were separated by LSD (5%).

Treatment	Mean berry mass (g) on different harvest dates		Mean berry mass per plant (g)
	08 Oct.	11 Nov.	
Untreated control	1.47 a	2.49 a	2.25 a
NI: 21 Mar. – 21 May (61 days)	1.88 a	2.37 a	2.26 a
NI: 21 Mar. – 21 Jul. (122 days)	1.85 a	2.51 a	2.11 a
NI: 21 Mar. – 21 Sept. (184 days)	1.63 a	2.19 a	2.01 a
Pr > F			
<i>Treatment</i>	0.3685	0.5510	0.5271
<i>NI period linear</i>	0.5907	0.3306	0.1653
<i>NI period quadratic</i>	0.0987	0.5801	0.6888

Table 5: Effect of night interruption on harvest distribution and total yield in ‘Snowchaser’ during the 2010 harvest season. Means were separated by LSD (5%).

Treatment	Average yield (g) on different harvest dates		Average total yield per plant (g)
	08 Oct.	11 Nov.	
Untreated control	24.50 ab	69.40 a	93.90 a
NI: 21 Mar. – 21 May (61 days)	12.70 b	62.50 a	75.20 a
NI: 21 Mar. – 21 Jul. (122 days)	28.30 a	61.50 a	89.80 a
NI: 21 Mar. – 21 Sept. (184 days)	16.90 ab	52.70 a	69.60 a
Pr > F			
<i>Treatment</i>	0.0473	0.8510	0.5498
<i>NI period linear</i>	0.7003	0.3974	0.3477
<i>NI period quadratic</i>	0.9618	0.9436	0.9567

Table 6: Effect of night interruption on number of berries harvested in ‘Snowchaser’ during the 2010 harvest season. Means were separated by LSD (5%).

Treatment	Average number of berries harvested on different dates		Average total number of berries per plant
	08 Oct.	11 Nov.	
Untreated control	11 a	42 a	53 a
NI: 21 Mar. – 21 May (61 days)	5 b	38 a	43 a
NI: 21 Mar. – 21 Jul. (122 days)	13 a	37 a	50 a
NI: 21 Mar. – 21 Sept. (184 days)	9 ab	34 a	43 a
Pr > F			
<i>Treatment</i>	0.0350	0.9127	0.7300
<i>NI period linear</i>	0.9066	0.4819	0.4670
<i>NI period quadratic</i>	0.7137	0.9338	0.8639

Table 7: Effect of night interruption on mean berry mass in ‘Snowchaser’ during the 2010 harvest season. Means were separated by LSD (5%).

Treatment	Mean berry mass (g) on different harvest dates		Mean berry mass per plant (g)
	08 Oct.	11 Nov.	
Untreated control	1.90 a	1.70 a	1.81 a
NI: 21 Mar. – 21 May (61 days)	2.01 a	1.57 a	1.69 a
NI: 21 Mar. – 21 Jul. (122 days)	2.04 a	1.60 a	1.78 a
NI: 21 Mar. – 21 Sept. (184 days)	2.01 a	1.45 a	1.60 a
Pr > F			
<i>Treatment</i>	0.9623	0.5615	0.4564
<i>NI period linear</i>	0.6850	0.1973	0.2281
<i>NI period quadratic</i>	0.7345	0.9447	0.7965

Table 8: Effect of night interruption on harvest distribution and total yield in ‘Emerald’ during the 2011 harvest season. Means were separated by LSD (5%).

Treatment	Average yield (g) on different harvest dates		Average total yield per plant (g)
	07 Nov.	06 Des.	
Untreated control	359.2 a	10.2 a	369.4 a
NI: 21 Mar. – 21 May (61 days)	299.0 ab	8.9 a	307.9 ab
NI: 21 Mar. – 21 Jul. (122 days)	221.2 bc	7.7 a	228.9 bc
NI: 21 Mar. – 21 Sept. (184 days)	161.7 c	3.3 a	165.0 c
Pr > F			
<i>Treatment</i>	<i>0.0100</i>	<i>0.3571</i>	<i>0.0076</i>
<i>NI period linear</i>	<i>0.0086</i>	<i>0.1336</i>	<i>0.0067</i>
<i>NI period quadratic</i>	<i>0.9933</i>	<i>0.5888</i>	<i>0.9771</i>

Table 9: Effect of night interruption on number of berries harvested in ‘Emerald’ during the 2011 harvest season. Means were separated by LSD (5%).

Treatment	Average number of berries harvested on different dates		Average total number of berries per plant
	07 Nov.	06 Des.	
Untreated control	168 a	6 a	174 a
NI: 21 Mar. – 21 May (61 days)	139 ab	5 ab	144 ab
NI: 21 Mar. – 21 Jul. (122 days)	114 ab	4 ab	118 b
NI: 21 Mar. – 21 Sept. (184 days)	93 b	2 b	95 b
Pr > F			
<i>Treatment</i>	<i>0.0558</i>	<i>0.2163</i>	<i>0.0418</i>
<i>NI period linear</i>	<i>0.0278</i>	<i>0.0660</i>	<i>0.0207</i>
<i>NI period quadratic</i>	<i>0.8276</i>	<i>0.6741</i>	<i>0.8524</i>

Table 10: Effect of night interruption on mean berry mass in ‘Emerald’ during the 2011 harvest season. Means were separated by LSD (5%).

Treatment	Mean berry mass (g) on different harvest dates		Mean berry mass per plant (g)
	07 Nov.	06 Des.	
Untreated control	2.11 a	1.60 a	2.10 a
NI: 21 Mar. – 21 May (61 days)	2.10 a	1.11 a	2.08 a
NI: 21 Mar. – 21 Jul. (122 days)	1.94 ab	1.71 a	1.94 ab
NI: 21 Mar. – 21 Sept. (184 days)	1.73 b	1.48 a	1.75 b
Pr > F			
<i>Treatment</i>	<i>0.0697</i>	<i>0.3601</i>	<i>0.1002</i>
<i>NI period linear</i>	<i>0.0605</i>	<i>0.3869</i>	<i>0.0674</i>
<i>NI period quadratic</i>	<i>0.3746</i>	<i>0.6121</i>	<i>0.4470</i>

Table 11: Effect of night interruption on harvest distribution and total yield in ‘Snowchaser’ during the 2011 harvest season. Means were separated by LSD (5%).

Treatment	Average yield (g) on different harvest dates		Average total yield per plant (g)
	07 Nov.	06 Des.	
Untreated control	80.1 a	0.8 a	80.9 a
NI: 21 Mar. – 21 May (61 days)	76.2 a	2.8 a	79.0 a
NI: 21 Mar. – 21 Jul. (122 days)	112.2 a	2.4 a	114.6 a
NI: 21 Mar. – 21 Sept. (184 days)	46.9 a	0.5 a	47.4 a
Pr > F			
<i>Treatment</i>	<i>0.2874</i>	<i>0.3796</i>	<i>0.2709</i>
<i>NI period linear</i>	<i>0.5468</i>	<i>0.7955</i>	<i>0.5416</i>
<i>NI period quadratic</i>	<i>0.1974</i>	<i>0.0883</i>	<i>0.1741</i>

Table 12: Effect of night interruption on number of berries harvested in ‘Snowchaser’ during the 2011 harvest season. Means were separated by LSD (5%).

Treatment	Average number of berries harvested on different dates		Average total number of berries per plant
	07 Nov.	06 Des.	
Untreated control	61 a	1 a	62 a
NI: 21 Mar. – 21 May (61 days)	65 a	2 a	67 a
NI: 21 Mar. – 21 Jul. (122 days)	88 a	2 a	90 a
NI: 21 Mar. – 21 Sept. (184 days)	44 a	0 a	44 a
Pr > F			
<i>Treatment</i>	0.4305	0.5139	0.4095
<i>NI period linear</i>	0.7300	0.5934	0.7119
<i>NI period quadratic</i>	0.2118	0.1659	0.1911

Table 13: Effect of night interruption on mean berry mass in ‘Snowchaser’ during the 2011 harvest season. Means were separated by LSD (5%).

Treatment	Mean berry mass (g) on different harvest dates		Mean berry mass per plant (g)
	07 Nov.	06 Des.	
Untreated control	1.35 a	0.44 a	1.34 a
NI: 21 Mar. – 21 May (61 days)	1.24 ab	0.55 a	1.25 ab
NI: 21 Mar. – 21 Jul. (122 days)	1.23 ab	0.60 a	1.23 ab
NI: 21 Mar. – 21 Sept. (184 days)	1.04 b	0.25 a	1.04 b
Pr > F			
<i>Treatment</i>	0.1731	0.6153	0.1626
<i>NI period linear</i>	0.0362	0.5660	0.0362
<i>NI period quadratic</i>	0.6895	0.2593	0.5670

## General discussion and overall conclusions

The low chilling requirements of some SHB cultivars make it possible for South African growers to supply world markets with fresh blueberries from mid-September until the end of November. These cultivars have complex ancestry, giving each its unique environmental requirement and hence phenology at low latitudes. During seasons with relatively high winter temperatures some SHB cultivars grown in the Western Cape exhibit late, prolonged spring berry ripening. This inability to fruit reliably makes the application of RBAs to such cultivars an attractive option for growers since RBAs can partially compensate for inadequate chilling.

In this study it was shown that hydrogen cyanamide (HC) is an effective rest breaking agent for 'Bluecrisp', because HC treatment during 2010, when low chill-unit accumulation occurred, accelerated berry ripening early during the harvest period. Such a result was not observed during the 2011 season when much higher chill-unit accumulation occurred. 'Bluecrisp' has the highest chilling requirement of the three evaluated cultivars and shows weak reproductive bud break before it reaches full bloom following an warm winter. HC rate appeared of less significance for harvest scheduling in 'Bluecrisp' than application timing. The 1 and 2% Dormex® rates caused phytotoxicity when applied after reproductive buds scales were open, and this not only delayed berry ripening but also reduced berry size and total yield in 'Bluecrisp' and 'Star'. HC application before chill-unit accumulation started, seldom increased berry size and total yield in 'Bluecrisp' and 'Star'. The practical implication of these two findings is that HC application is best carried out before bud scales open but after as much chilling exposure as possible. It was also concluded that the reproductive buds of 'Star' could possibly be more sensitive to HC damage than those of 'Bluecrisp' due to a slight difference in bud structure. Further studies on 'Star' are required to identify the best HC application time as results were inconclusive. 'Emerald' has the lowest chilling requirement of the cultivars evaluated. These plants received their full chilling requirement during both seasons and responded very differently to HC treatment than 'Bluecrisp' and 'Star' plants. Application two weeks before any chilling improved berry size and total yield compared to later applications, but not compared to the untreated control. In addition, HC treatment induced an increased total yield in 'Emerald' during 2010, but not during 2011. In 2011 however berries were larger compared to the untreated control. HC application is not recommended for 'Emerald', as this cultivar appeared to be well adapted to the local climate.

Thidiazuron (TDZ) application is not currently recommended for ‘Bluecrisp’ or ‘Star’, since results in terms of the harvest scheduling effect, berry size and total yield varied considerably between the cultivars and seasons. During 2010 TDZ treatment accelerated days to 75% harvest in ‘Star’. TDZ applications two weeks before the start of chilling may have damaged paradormant reproductive buds, as the total yield was significantly reduced by such treatments. Defective, malformed flowers were observed after TDZ application to plants with open reproductive bud scales, and TDZ should not be applied from this stage onwards, for later applications also caused excessive flower tissue damage. Further studies are suggested to clarify possible benefits of TDZ application in SHB cultivars. Studies with RBAs like HC and TDZ should include the recording of detailed data on bud break patterns following application.

Attempts to schedule SHB blueberry harvests with NI treatment in an orchard environment did not yield positive results. Low temperatures during reproductive bud initiation could have nullified the short day requirement of the plants, or reproductive buds may have been initiated prior to treatment. The latter seems to be the principle factor causing this lack of response, since plants used in this trial were not summer pruned and therefore most shoots were older. ‘Emerald’ showed a general reduction in total yield and number of berries harvested per plant during 2011, and this reduction occurred linearly with increased NI duration. Such yield reductions may have been due to a decrease in the average number of flowers per bud, or lower carbohydrate content in stem node tissue which is known to limit reproductive bud initiation. The latter theory is supported by the tendency observed during 2010 for berries from both cultivars to be smaller with increased NI duration, yet increasingly poorer pollination conditions could also result in smaller berries. Older and younger current season’s shoots should be monitored separately during further investigations regarding the possible benefits of NI treatment for blueberry growers. Detailed data on flowering per plant following the NI treatments should also be recorded, in order to help determine if yield responses could perhaps occur due to differences in flower bud numbers or fruit set.

It is evident that the cultivation practices we evaluated provide rather little, and unpredictable aid in improving the undesirable phenology of some SHB cultivars. These practices should therefore be seen as short term ameliorations only. For the Western Cape blueberry industry to remain profitable over the long term, efforts should also be directed at breeding and/or identifying SHB cultivars that are potentially well suited to the local climate, and to evaluate their phenology in field trails before promoting the better adapted cultivars to growers.