國立臺灣大學生物資源暨農學院園藝暨景觀學系

碩士論文

Department of Horticulture and Landscape Architecture

College of Bioresources an Agriculture

National Taiwan University

Master Thesis

藍莓頂梢開花形態形成與物候研究

Study on the Morphgenesis and Phenology of Apical Flowering in Blueberries

鄭杰騏

Chieh-Chi Cheng

指導教授：李國譚 博士

Advisor: Kuo-Tan Li, Ph. D.

中華民國 109 年 1 月

January 2020

doi:10.6342/NTU202000429

國立臺灣大學碩士學位論文
口試委員會審定書

藍莓頂梢開花形態形成與物候研究

Study on the Morphogenesis and Phenology of Apical Flowering in Blueberries

本論文係鄭杰騏君（R06628124）在國立臺灣大學園藝暨景觀學系完成之碩士學位論文，於民國 109 年 1 月 6 日承下列考試委員審查通過及口試及格，特此證明

口試委員：

______________________（簽名）
（指導教授）

____

____

____

致謝

一本論文的完成，需要諸多因素的配合。在兩年的試驗期間，除了氣候和植物材料為這本論文提供了大部分的題材外，也要感謝許多人的協助。首先要感謝李國譚老師帶領我進入小果類作物的世界，並在試驗與論文撰寫的過程中適時地給予指正、建議和督促，讓我的研究之路避開了很多不必要的錯誤與困擾。謝謝陳香君老師教導植物解剖與顯微技術方面的知識，還有李金龍老師和張哲嘉老師在進度考核與口試時的建議與鼓勵，讓我的論文內容更加完整豐富。謝謝大氣系林博雄老師提供氣象觀測數據、謝謝理學院電顯室的林錦燕技術員和楊雅雲技術員協助操作電子顯微鏡；另外也要感謝生科館的以君學姐、佩穎學姐、還有謦竹學長提供儀器操作和統計分析上的協助。

謝謝這段時間曾經在實驗室一起做實驗及照顧植物的岱耘、佳翰、法平、紹剛、意親、育全、永城、智丞、和享、暐哲、德瑜和真史，為平凡的研究生活增添許多色彩與樂趣，也讓我在實驗遇到瓶頸時能有交換意見的對象。感謝宏林、法平、有堂、博寬、季芃跟立弘，和你們的聚餐總是充滿歡笑，卻又每次都讓我對農業這個領域產生新的見解。謝謝鐵道暨火車研習社和群風棒球隊的朋友們；讓我得以在社課與球場紓解課業與研究上的壓力。謝謝農藝系 B06 雙號班的同學，你們在實驗課堂上成長之餘，也讓身為助教的我反覆提醒自己，要更加努力成為你們的榜樣。謝謝知林分享自己和指導教授互動的經驗、還有晚思提供和日本人學術交流時要注意的細節，讓我在心情極度煩躁的時候，找到平靜下來、繼續完成論文的動力。

最後要感謝我的家人，在我求學的路上持續給予精神與經濟上的支持，讓我能專心一意、無後顧之憂地完成學業。

doi:10.6342/NTU202000429

摘要

藍莓(*Vaccinium* spp.)為原生於北美洲的落葉或常綠性灌木植物。在溫帶地區，藍莓花芽於秋季形成於葉腋後，進入休眠並於翌年春季開花。然而栽培於臺灣平地的藍莓，於秋季仍持續生長的枝梢頂端，常發生直接開花結果的現象，此不時花具一年二收或產期調節之潛力。本論文透過調查田間栽培之藍莓植株、與觀察各品種頂梢於秋季芽體分化之顯微結構，期望找出較易發生頂梢開花現象之品種，並探討頂梢開花可能的產生機制。試驗假說為臺灣平地秋季氣溫有利於藍莓營養生長，同時期之光週期則有助於花芽分化。兩年度的田間調查結果顯示，兔眼藍莓(*V. virgatum* Aiton)秋梢停止生長時間點較南高叢(*V.* hybrid)及北高叢藍莓(*V. corymbosum* L.)晚，在 9 月底至 10 月底之間，尚有高比例的秋梢尚未停梢，而多數易頂梢開花之品種亦屬於兔眼藍莓。2019 年 9 月 22 日至 10 月 22 日間之氣溫較 2018 年同時期高，頂梢花芽分化之數量則較少。芽體觀察試驗結果顯示，若枝梢於適合花芽創始之環境條件下仍持續發育，即產生頂梢開花。比較兩試驗年度秋季之氣溫差異，日均溫介於 20 與 25℃間似可促進頂梢花芽分化，超過 27℃或低於 20℃則有抑制作用。

關鍵字：不時花、花芽創始、光週期、溫度、產期調節

doi:10.6342/NTU202000429

Abstract

Blueberries (*Vaccinium* spp.) are deciduous or evergreen bushes native to North America. In temperate regions, flower buds of blueberries differentiate in leaf axil before winter dormancy and bloom in the next spring. However, in lowland Taiwan, blueberries often produce flowers and bloom directly on growing shoot apices in autumn, such off-season blooming offers potentials for forcing culture. This thesis investigated field grown blueberry plants and observed bud microstructures of apical and axillary buds sampled from various blueberry cultivars to evaluate the tendency and the possible mechanism of apical flowering. The hypothesis was that in lowland Taiwan, mild autumn temperature favors vegetative growth, and the shortening photoperiod favors reproductive growth of blueberries, consequently inducing apical flowering.

The result of two-year observations showed that during late September to late October, a high percentage of growing shoot tips was observed in rabbiteye blueberries (*V. virgatum* Aiton) than in southern (*V.* hybrid) or northern highbush blueberries (*V. corymbosum* L.). Cultivars that produced more apical flowering shoots belonged to rabbiteye blueberries. Between late September and late October, less apical flowering shoots were observed in 2019 than in 2018 during the same period. Weather data indicated that during this period, mean temperature was higher in 2019, which might

doi:10.6342/NTU202000429

have reduced of the tendency of apical flower differentiation.

Microstructure of apical bud showed that in conditions favored flower differentiation, shoots that were still actively growing tend to develop flowers directly in the shoot tips. Daily mean temperature around 20-25℃ seemed to promote apical flower bud differentiation, while temperatures above 27 or below 20℃ posed negative effects.

Key words: off-season flower, flower bud initiation, photoperiod, temperature, and forcing culture

doi:10.6342/NTU202000429

Table of Contents

doi:10.6342/NTU202000429

doi:10.6342/NTU202000429

doi:10.6342/NTU202000429

List of Table

doi:10.6342/NTU202000429

doi:10.6342/NTU202000429

List of Figure

doi:10.6342/NTU202000429

doi:10.6342/NTU202000429

doi:10.6342/NTU202000429

Chapter one

Literature review and hypothesis

1.1. Introduction

Blueberries (*Vaccinium* spp.) are species in the North American native subgenus *Cyanococcus* in Ericaceae (Eck et al., 1990). Commercial cultivars are classified into northern highbush blueberries (*V. corymbosum* L.), southern and half- highbush blueberries (*V.* hybrid), rabbiteye blueberries (*V. virgatum* Aiton), and lowbush blueberries (mainly *V. angustifolium* Aiton) (Retamales and Hancock, 2018).

In the past decades, the world blueberry industry have grown drastically. The annual production increased from 174,143 to 596,813 tons between 1997 and 2017, while the area harvested grew from 48,707 to 109,541 ha (Food and Agricultural Organization, 2019a, b). The production area also expanded from North America to Europe, Australia, New Zealand, Chile, and China (Retamales and Hancock, 2018). In Taiwan, the Fruit Crop Physiology Lab at National Taiwan University (NTU) began blueberry research since 2007. The preliminary field experiment indicated that most evaluated rabbiteye and southern highbush cultivars developed well in northern to central lowland Taiwan, revealing the potential of developing blueberry industry in Taiwan (Li, 2009). Gifted by the various terrain types of Taiwan, northern highbush blueberries are suitable for cultivation in the mountains, while southern highbush and

doi:10.6342/NTU202000429

rabbiteye blueberries are well-adapted to lowland Taiwan (Yang, 2016).

When cultivated in the lowland Taiwan with a warm autumn climate, some blueberry cultivars display a character of flowering on shoot apices (Fig. 1.1). The apical bloom, similar to what Hall and Ludwig (1961) had described in lowbush blueberries, mainly occurs in autumn. The phenomenon may offer potentials for off-season fruit production (Li, 2009). However, the mechanism of apical flowering remains unclear, and the intensity varies between years and cultivars. The purposes of this thesis was to document the timing and cultivar variations of apical flowering in blueberries in lowland Taiwan with a subtropical climate.

1.2. Growth habits of blueberries

1.2.1. Annual growth cycle of blueberries

The annual growth cycle of blueberries begins when new shoots emerge from dormant vegetative buds in the spring (Eck et al., 1990). Depending on species and regional climate patterns, different seasonal growth patterns have been described. In Rhode Island, growth of shoots and roots of highbush blueberries occurs prior bloom but is suppressed by fruit set followed by a second fast growth after harvest (Abbott and Gough, 1987). In northern Florida with a warm climate, the annual growth cycle of rabbiteye blueberries was categorized into five stages: (1) vegetative growth following fruit harvest that occurs between 1 June and 1 October, (2) flower bud initiation that

doi:10.6342/NTU202000429

occurs between 1 October and 1 December, (3) dormancy that starts from 1 December and ends in 15 March, (4) anthesis that begins around 15 March and lasts to 1 April, and (5) fruit development that occurs between 1 April and 1 June (Darnell et al., 1992).

In lowland Taiwan, rabbiteye blueberries are not completely defoliated in winter, thus carrying a semi-evergreen growth habit. Bud break occurs from late February through April. The canopy development maximizes in summer and completes around November. Flower bud differentiation starts in September and flower buds are visible in October. Sporadic flowering occurs from November to the following spring, while the main bloom takes place in March and April. Fruits ripen from mid-May and main harvests occurs from May through July and ends in August (Tsai, 2016).

1.2.2. Shoot growth of blueberries

The shoot growth of blueberries is sympodial and episodic (Eck et al., 1990). New leaf buds are initiated every five days in highbush blueberry (Gough and Shutak, 1978). Every shoot growth cycle, or a growth flush, ends with apical abortion that can be easily observed by the wilted and blackened shoot tip. The cause of apical abortion might be a high concentration of auxin accumulated in the meristem (Barker and Collins, 1963). New shoot growth or flush occurs when the axillary buds next to the apical abortion point break dormancy (Yang, 2016). When blueberry bushes enter dormancy, all shoots undergo growth cessation and apical abortion, therefore, the terminal buds are in fact

doi:10.6342/NTU202000429

the uppermost lateral buds (Bae et al., 2006).

1.2.3. Reproductive growth of blueberries

The reproductive growth of blueberries starts with flower buds formed basipetally on current-year shoots (Eck et al., 1990). In an individual flower bud, the contained florets are developed acropetally, while floral organs are differentiated from outer to inner whorl (Tamada, 1997).

Blueberries differentiate flower buds in response to shortening photoperiod or cool temperatures. Blueberry leaves are the receptors of short day stimulus that trigger flower initiation. Early defoliation reduces flower bud production in southern highbush and rabbiteye blueberries (Lyrene, 1992; Williamson and Miller, 2002). Ye et al. (2005) reported that timing of flower bud differentiation varied among blueberry types and cultivars. Northern highbush blueberries in Rhode Island differentiated flower buds by early October (Gough and Shutak, 1978). In Argentina, ‘O’Neal’ southern highbush blueberry also differentiated flower buds in a 15-h long day condition with an average temperature of 22.5°C (Pescie et al., 2011).

1.2.4. Developmental stages of blueberry flower buds

According to Kovaleski et al. (2015), the differentiation of blueberry flower buds can be divided into five stages: the first internal developmental stage (Stage I-1) with a single vegetative apical meristem; the second internal developmental stage (Stage I-2)

doi:10.6342/NTU202000429

with the appearance of lateral floral meristems. Widening or doming of meristem, which is typical in many plants, is not observed in blueberries at this stage due to their racemic inflorescences (Kovaleski et al., 2015; Tamada, 1997).

The third internal developmental stage (Stage I-3) is associated with the appearance of sepals. The early-developed sepals are initially a ridge around the meristems, and then converted into triangular shapes. In the meantime, the two bracts enclosing the floret are separate. The fourth stage (Stage I-4) is referred to the total development of petals, with the two bracts concealed the floret. In Florida, most southern highbush blueberry buds have developed into this stage by late autumn, then the bud development is suspended until the following spring. The final stage (Stage I-5) is recognized when the peduncle, pedicle, and the flowers are semi expanded. Stage I-4 and Stage I-5 correspond with external bud developmental stages 1 and 2 described by Spiers (1978).

In Stage I-4, anthers and pistils are formed, but in a compacted form (Kovaleski et al., 2015). In Stage I-5, flower buds begin to swell, accompanied with formation of microspore mother cells, macrosporogenesis, and embryo sac differentiation before anthesis (Bieniasz, 2012).

1.3. Environmental factors influencing blueberry growth

1.3.1. Photoperiod

doi:10.6342/NTU202000429

Though some lowbush blueberry clones were reported to develop flower buds in 14-h daylength (Hall and Ludwig, 1961), most blueberries are relative short-day plants and the critical daylength for flower bud initiation is c.a. 12 h (Darnell, 1991). In photoperiod of 16 h light and 8 h dark, northern highbush blueberry produced four to five flushes with no flower bud formed. In contrast, shoot growth ceased after short day treatment with 8 h light and 16 h dark for two to three weeks, and flower bud differentiated after four weeks of the treatment (Bañados and Strik, 2006). 'Misty' southern highbush blueberry produced flower buds at 21°C after four weeks of 8 h photoperiod treatment. Continued photoperiod treatment for another four weeks produced more flower buds and a more concentrated bloom (Spann et al., 2004).

'Tifblue' and 'Bluebelle' rabbiteye blueberries produced flower buds after six weeks of 12 h photoperiod treatment at 27°C-18°C day-night temperature (Phatak and Austin, 1990). They also noticed that insufficient short-day treatment resulted in abnormal inflorescences. Similarly, lowbush blueberries produced abnormal inflorescences when the bushes were treated with short-day photoperiod for only two to four weeks (Hall and Ludwig, 1961). However, in rabbiteye blueberries, the effect of photoperiod on flower bud development seemed to be cultivar dependent. Number of flower buds of 'Beckyblue' rabbiteye blueberry was increased by short-day treatment and the bloom period of the following spring was shortened. However, 'Climax' was

doi:10.6342/NTU202000429

unaffected by the same treatment (Darnell, 1991).

1.3.2. Temperature

High temperatures suppress flower initiation in blueberries. 'Misty' southern highbush blueberry did not produce any flower buds at 28°C after four weeks of short day (8 h) photoperiod. Plants in such condition for another four weeks produced little poorly developed flower buds and did not bloom during the post-experiment observation period (Spann et al., 2004).

'Tifblue' and 'Bluebelle' rabbiteye blueberries produced flower buds at 27°C-18°C day-night temperature after exposure to photoperiod between 10 and 14 h for six weeks (Phatak and Austin, 1990). On the other hand, low temperatures may encourage flower bud differentiation in long-day conditions. Some lowbush blueberry clones differentiated flower bud at 10°C even in long day photoperiod (Hall and Ludwig, 1961). At mild to high temperatures, blueberries may flower after differentiation without entering and breaking dormancy (Darrow, 1942; Phatak and Austin, 1990).

1.3.3. Nutrition

Blueberries have relatively low nutrient requirements than other fruit crops given rise by the acidic soil conditions they prefer (Korcak, 1989). However, adequate fertilization is still needed to improve bush vigor and yield (Krewer and NeSmith, 1999). Yield of 'Star' southern highbush blueberries fertilized with 12N-5.2P-9.9K was

doi:10.6342/NTU202000429

positively correlated with fertilization rates. Nevertheless, continuous over fertilization deceased fruit yield and canopy volume in 'Misty' southern highbush blueberries due to excessive fruit set and thus inducing vegetative growth stress (Wilber and Williamson, 2008).

In Hawaii, monthly fertigation resulted in two major and two minor harvest peaks of southern highbush blueberries in an evergreen cultivation system. The major production peak occurred between mid-August to mid-September, and two weeks in mid-February. The minor peaks occurred between late-May and early-June, and between late-November and early-December (Hummer et al., 2007). However, autumn fertilization did not promote autumn or winter flower amount in Taiwan (Huang, 2014).

1.3.4. Weather differences between Taiwan and northern temperate regions

The weather pattern regarding temperature and daylength of Taipei, Taiwan is different from those of Tifton, Ga. and Raleigh, N.C., where most rabbiteye blueberry cultivars were bred and released. The monthly mean temperature of Taipei declines from 29.6°C in July to 16.1°C in January, while in Tifton and Raleigh, the mean temperature drops from 26.8 to 9.3°C and from 25.8 to 4.2°C, respectively, during same period (Central Weather Bureau, 2011; Climate-data, 2012a, b).

On the other hand, the daylength in Tifton shortens from 14 h 12 min in summer solstice to 10 h 6 min in winter solstice. In Raleigh, from summer to winter solstice,

doi:10.6342/NTU202000429

daylength decreases from 14 h 35 min to 9 h 44 min. Due to the lower latitude location of Taipei, daylength is shorter at 13 h 42 min in summer solstice but longer at 10 h 35 min in winter solstice (Central Weather Bureau, 2018; Sunrise Sunset, 2018a, b). The latter is similar to that in November in Tifton and Raleigh. Overall, autumn in the low latitude subtropical regions such as Taipei possesses milder temperatures and less severe photoperiod shortening compared to the mid-latitude temperate regions, such as Tifton.

1.4. Hypothesis

Based on the weather differences between lowland Taiwan and temperate regions, it is expected that the mild autumn temperature in Taiwan favors vegetative growth, while the photoperiod is sufficiently short for flower bud differentiation. In cultivars with the tendency to produce apical flowers, a direct transformation of an active apical meristem from vegetative status to reproductive status occurs.

In chapter two, phenology of different blueberry cultivars was recorded to define their tendency of apical flowering. Detail investigations include the timing of apical flower bud differentiation, apical flowering, and the relationship of apical flowering between vegetative and reproductive growth. The hypothesis was that the apical flowering occurs when the time of vegetative growth overlaps with reproductive growth, which may be affected by both environmental and genetic factors.

In chapter three, microstructures of apical and axillary buds of blueberry cultivars

doi:10.6342/NTU202000429

with different tendency of apical flowering were investigated to determine the developmental difference between easy and rare-apical-flowering cultivars. It was hypothesized that flower organs appear in shoot apices, rather than undergone apical abortion, when apical flowering occurs. The process of apical flower bud differentiation may be able to divide into different stages, as axillary flower buds perform.

1.5. References

Abbott, J.D. and R.E. Gough. 1987. Seasonal development of highbush blueberry roots under sawdust mulch. J. Amer. Soc. Hort. Sci. 112:60-62.

Bae, K., H. Kim, H. Lee, B. Lee, and T. Kim. 2006. Characteristics of flower bud differentiation in highbush blueberry (*Vaccinium corymbosum* L.) cultivars. Kor. J. Hort. Sci. Technol. 24:222-227.

Bañados, M.P. and B. Strik. 2006. Manipulation of the annual growth cycle of blueberry using photoperiod. Acta Hort. 715:65-72.

Barker, W. and W. Collins. 1963. Growth and development of the lowbush blueberry: apical abortion. Can. J. Bot. 41:1319-1324.

Bieniasz, M. 2012. The differentiation of highbush blueberry flower buds. Acta Hort. 932:117-122.

Central Weather Bureau. 2011. Monthly mean temperature of Taipei, Taiwan. <https://www.cwb.gov.tw/V7e/climate/monthlyMean/tx.htm>.

doi:10.6342/NTU202000429

Central Weather Bureau. 2018. Timetable of sunrise, sunset, twilight of Taipei, Taiwan. <https://www.cwb.gov.tw/V8/C/D/astronomy_data.html>.

Climate-data. 2012a. Raleigh climate. <https://en.climate-data.org/north-america/united-states-of-america/north-carolina/raleigh-1714/>.

Climate-data. 2012b. Tifton climate. <https://en.climate-data.org/north-america/united-states-of-america/georgia/tifton-18261/>.

Darnell, R.L. 1991. Photoperiod, carbon partitioning, and reproductive development in rabbiteye blueberry. J. Amer. Soc. Hort. Sci. 116:856-860.

Darnell, R.L., G.W. Stutte, G.C. Martin, G.A. Lang, and J.D. Early. 1992. Developmental physiology of rabbiteye blueberry. Hort. Rev. 13:339-405.

Darrow, G.M. 1942. Rest period requirements for blueberries Proc. Soc. Amer. Hort. Sci. 41:189-194.

Eck, P., R.E. Gough, I.V. Hall, and J.M. Spiers. 1990. Blueberry management, p. 273-333. In: Galetta, G.J. and D.G. Himerick (eds.). Small fruit crop management. Prentice Hall, Englewood Cliffs, N.J.

Food and Agricultural Organization, United Nations. 2019a. Area harvested of blueberries. <http://www.fao.org/faostat/en/#data/QC>.

Food and Agricultural Organization, United Nations. 2019b. Yield of blueberries. <http://www.fao.org/faostat/en/#data/QC>.

doi:10.6342/NTU202000429

Gough, R.E. and V.G. Shutak. 1978. Anatomy and morphology of cultivated highbush blueberry. R.I. Agr. Expt. Sta. Bul. 423.

Hall, I.V. and R.A. Ludwig. 1961. The effects of photoperiod, temperature, and light intensity on the growth of the lowbush blueberry (*Vaccinium angustifolium* Ait.). Can. J. Bot. 39:1733-1739.

Huang, S.H. 2014. The effect of nitrogen and phosphorus on photosynthesis, growth and flowering in rabbiteye blueberry (*Vaccinium ashei* Ait.). MS thesis. Natl. Taiwan Univ. Taipei, Taiwan.

Hummer, K., F. Zee, A. Strauss, L. Keith, and W. Nishijima. 2007. Evergreen production of southern highbush blueberries in Hawai'i. J. Amer. Pomol. Soc. 61:188-195.

Korcak, R. 1989. Variation in nutrient requirements of blueberries and other calcifuges. Hort. Rev. 10:183-227.

Kovaleski, A.P., J.G. Williamson, J.W. Olmstead, and R.L. Darnell. 2015. Inflorescence bud initiation, development, and bloom in two southern highbush blueberry cultivars. J. Amer. Soc. Hort. Sci. 140:38-44.

Krewer, G. and D.S. NeSmith. 1999. Blueberry fertilization in soil. Univ. Ga. Ext. Fruit Publ. 01-1.

Li, K.T. 2009. BLUE FORMOSA - a blueberry initiative program in Taiwan.

doi:10.6342/NTU202000429

HortScience 44:1122 (Abstr.).

Lyrene, P.M. 1992. Early defoliation reduces flower bud counts on rabbiteye blueberry. HortScience 27:783-785.

Pescie, M., M. Lovisolo, A. De Magistris, B. Strik, and C. López. 2011. Flower bud initiation in southern highbush blueberry cv. O’Neal occurs twice per year in temperate to warm-temperate conditions. J. Appl. Hort. 13:8-12.

Phatak, S.C. and M.E. Austin. 1990. The effect of photoperiod on the growth and flowering of two rabbiteye blueberry cultivars. Appl. Agr. Res. 5:350-352.

Retamales, J.B. and J.F. Hancock. 2018. Blueberries 2nd ed. CABI, Oxfordshire, UK.

Spann, T.M., J.G. Williamson, and R.L. Darnell. 2004. Photoperiod and temperature effects on growth and carbohydrate storage in southern highbush blueberry interspecific hybrid. J. Amer. Soc. Hort. Sci. 129:294-298.

Spiers, J.M. 1978. Effect of stage of bud development on cold injury in rabbiteye blueberry. J. Amer. Soc. Hort. Sci. 103:452-455.

Sunrise Sunset. 2018a. Sunrise and sunset times in Raleigh, Wake County, North Carolina, USA. <https://sunrise-sunset.org/search?location=raleigh>.

Sunrise Sunset. 2018b. Sunrise and sunset times in Tifton, Tift County, Georgia, USA. <https://sunrise-sunset.org/search?location=tifton>.

Tamada, T. 1997. Flower-bud differentiation of highbush and rabbiteye blueberries.

doi:10.6342/NTU202000429

Acta Hort. 446:349-355.

Tsai, M.H. 2016. Physiological effects of leaf color and artificial shading on leaf gas exchang and growth in rabbiteye blueberry (Vaccinium ashei). MS thesis. Natl. Taiwan Univ. Taipei, Taiwan

Wilber, W.L. and J.G. Williamson. 2008. Effects of fertilizer rate on growth and fruiting of containerized southern highbush blueberry. HortScience 43:143-145.

Williamson, J.G. and E. Miller. 2002. Early and mid-fall defoliation reduces flower bud number and yield of southern highbush blueberry. HortTechnology 12:214-216.

Yang, S.Y. 2016. Study of mulching effect on growth and weed control in rabbiteye blueberry (*Vaccinium ashei*) cultivation in Taiwan. MS thesis. Natl. Taiwan Univ. Taipei, Taiwan.

Ye, Y.H., N. Aoki, N. Konishi, and N. Patel. 2005. Patterns of flower-bud differentiation, development, and growth habits of several blueberry (*Vaccinium* spp.) cultivar types grown in Japan and New Zealand. J. Japan Soc. Hort. Sci. 74:1-10.

doi:10.6342/NTU202000429

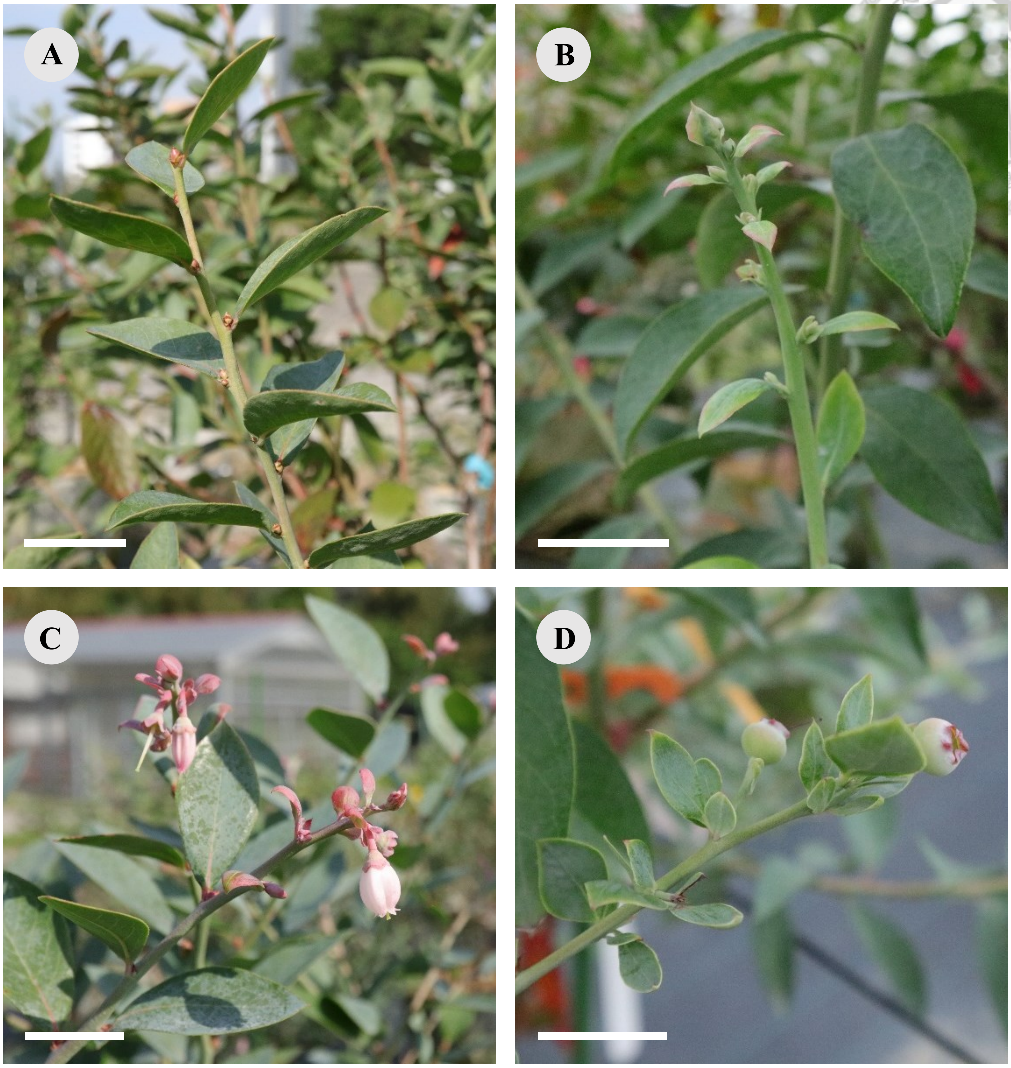


Fig. 1.1. A mature shoot with flower buds in winter (A), a shoot undergoing apical flower bud differentiation, (C) flowering apical inflorescences, and (D) immature fruit produced by apical flowers of ‘Tifblue’ rabbiteye blueberry. Bar = 2 cm.

doi:10.6342/NTU202000429

Chapter two

Autumn apical flowering in blueberries cultivated in subtropical lowland Taiwan

2.1. Abstract

The character of apical flowering in blueberries may be induced by mild autumn temperature and suitable photoperiod. Investigation of mature field grown plants was performed to compare the intensity of apical flowering in different cultivars in natural environment. Further investigations on apical flower bud differentiation and flowering were performed by labeling and observing shoots of easy-apical-flowering blueberry cultivars. The result suggested that most apical flowering cultivars were rabbiteye blueberries (*V. virgatum* Aiton). While still some rabbiteye blueberry cultivars tended to grow vegetatively in autumn. The later timing of shoot growth cessation, leading to the coincidence of vegetative growth and reproductive growth was the main reason why rabbiteye blueberries were more likely to produce apical flowers. The witness of apical abortion and non-flowering shoots after apical flower bud differentiation indicated that apical flowering is affected by environmental factors. Flowering was not guaranteed by the occurrence of apical flower bud differentiation. In 2018, apical flower bud differentiation occurred continuously from 30 September to 23 December, while blooming peaked in mid-November. The apical flower bud differentiation in 2019

doi:10.6342/NTU202000429

mainly occurred between 29 September and 10 November, and only four of the 215 labeled shoots produced apical flowers. The mean temperature between 22 September and 22 October in 2019 was significantly higher than the same period in 2018, probably consequently reduced the intensity of apical flower bud differentiation in 2019.

doi:10.6342/NTU202000429

2.2. 摘要

藍莓的頂梢開花現象可能受溫和的秋季氣溫與適當之光週期促進。本試驗透過田間調查方式比較自然環境下不同品種頂梢開花之發生頻率，並透過標定枝條方式，進一步觀察易發生頂梢開花現象藍莓品種之花芽分化及開花情形。結果顯示，多數易產生此現象的品種屬於兔眼藍莓(*V. virgatum* Aiton)，但亦有部分兔眼藍莓品種傾向營養生長而不易頂梢開花。兔眼藍莓較晚停止枝梢生長可能使營養生長與生殖生長同時發生，因而使之較易產生頂梢開花現象。試驗期間發現部分枝條於枝梢頂端花芽分化後仍發生盲芽現象或並未開花，顯示頂梢開花現象受環境因子影響，且花芽分化不代表頂梢開花現象之必然發生。2018 年頂梢花芽分化於 9 月 30 日至 12 月 23 日間持續發生，於 11 月中達開花高峰；2019 年頂梢花芽分化集中發生於 9 月 29 日至 11 月 10 日，且 215 枝標定枝條中僅有四枝枝條開花。2019 年 9 月 22 日至 10 月 22 日氣溫顯著高於 2018 年同期，可能因此減少 2019 年藍莓頂梢花芽分化之發生頻率。

doi:10.6342/NTU202000429

2.3. Introduction

The nature appearance of apical flowering in blueberries is similar to the abnormal inflorescences described by Hall and Ludwig (1961) and Phatak and Austin (1990), which were induced by artificial forcing with two to four weeks of short day photoperiod at 18.3°C (65°F) in lowbush blueberries and 27°C-18°C day-night temperature in rabbiteye blueberries.

Horiuchi et al. (2013) found that apical flowers were formed on vigorous water sprouts of ‘Emerald’ and ‘Sharpblue’ southern highbush blueberries in October, when the monthly mean air temperature was 17.9°C (Japan Meteorological Agent, 2010). These two cultivars were also well-adapted to tropical highland climates, evidenced by the evergreen production experiment in Hawaii (Hummer et al., 2007), where apical flowers might have also been produced. Regardless of the apical flowers, the successive flowering and fruiting character may be due to the low chilling requirement of these two cultivars that 150-200 h below 7°C is sufficient to break dormancy of lateral flower buds (Krewer and NeSmith, 2000; Lyrene, 2008).

In lowland Taiwan, the mild autumn temperatures between 27.4 and 21.5°C and the shortening daylength from 12 h 9 min to 10 h 35 min may provide an environment that enable blueberry bushes to bloom before dormancy, for warm temperature keeps blueberry plants from entering dormancy, and blueberries are able to flower without

doi:10.6342/NTU202000429

dormancy when kept under growth-favoring environment (Central Weather Bureau, 2011; Horiuchi et al., 2013; Phatak and Austin, 1990). In this chapter, different species and cultivars of blueberries were observed to determine their timing and tendency of apical flowering. The relationship between apical flowering and normal flower bud differentiation was inspected. The hypothesis was that the autumn temperature in lowland Taiwan favors vegetative growth and the photoperiod enables flower bud initiation of blueberries, thus leading to apical flowering in certain cultivars due to the transformation of growing status directly from vegetative to reproductive phase on the growing shoot tip.

2.4. Materials and methods

2.4.1. Plant materials

Field grown blueberry plants in the Hort Farm at NTU (121.5°E, 25.0°N, 15 m altitude) were selected as experimental materials. All bushes were at least three years old, planted in PB18 root control bags (diameter = 54 cm; height = 50 cm, Sunny & Wu Enterprises, Changhua, Taiwan) with soilless media mix of peat, perlite, and rice hull (v/v = 1:1:1). All bushes were fully irrigated during the experimental period and were fertigated with a compound fertilizer (N-P-K = 15-15-15-4 (MgO), Taiwan Fertilizer Co., Ltd., Taipei, Taiwan) and an acidifier ($FeSO_2 \cdot 7H_2O$ 0.02M + EDTA-2Na 0.01M + $(NH_4)_2SO_4$ 0.2M + 0.94M concentrated sulfuric acid solution) at a dilution rate of 1,000

doi:10.6342/NTU202000429

on a weekly basis from mid-March to early-October. The field temperature and relative humidity data were obtained from a nearby observation-station operated by Department of Atmospheric Sciences, NTU (121.5°E, 25.0°N, 22 m altitude).

2.4.2. 2018 trial

In autumn of 2018, 22 cultivars and breeding lines, totaling 174 bushes were investigated (Appendix 1). Summer pruning was performed from 18 to 20 July 2018.

From 30 September to 23 December 2018, field investigations were performed on a weekly basis. The information collected included (1) proportions of vegetative growing shoots to total shoots, (2) proportions of axillary flower buds on current year shoots, and (3) number of shoots that showed apical flower bud differentiation on each bush. The proportion of vegetative growing shoots was the approximate percentage of vegetative growing shoots to total shoots on each bush. The proportion of axillary flower buds was the percent flower buds to total lateral buds on five randomly selected shoots on each bush. Number of apical blooming flowers on each bush was also recorded to determine apical flowering peak of each cultivars. The standard for recognition of apical-flower-bud-differentiated shoots was that the shoot growth was not ceased, and the apical bud swelled. However, the identification of whether the flowers were produced by apical flowering or breaking dormancy of axillary flower buds and the exclusion of flowered axillary buds from flowered shoot apices were not successful.

doi:10.6342/NTU202000429

Consequently, number of shoot apices enduring apical-flower-bud-differentiation and apical flowering was recounted on 30 December to determine the true yield of apical-flower-bud-differentiated shoots and flowered apices.

2.4.3. 2019 trial

In 2019, investigations were concentrated to ‘NTU-009’, ‘NTU-031’, ‘NTU-032’, ‘NTU-054’, ‘NTU-103’, ‘NTU-108’, ‘Powderblue’, and ‘Tifblue’ rabbiteye blueberries and ‘Sunshineblue’ southern highbush blueberry. Five bushes of ‘NTU-009’, ‘NTU-032’, ‘NTU-054’, ‘NTU-103’, ‘NTU-108’, ‘Powderblue’, and ‘Tifblue’ each and four bushes of ‘NTU-031’ and ‘Sunshineblue’ each were observed.

On 22 September, five shoots per bush were labeled. All labeled shoots were developed after summer pruning in early August and were still in a vegetative growing phase. Field investigations were performed weekly from 22 September to 22 December. The growth status of each shoot was determined. Numbers of total axillary buds, axillary flower buds, apical flower number and number of flowers produced by axillary flower buds were also recorded. The axillary flower bud percentage of each investigation date were calculated by dividing node number into axillary flower bud number.

2.4.4. Statistical analysis

Data were analyzed by R language (Version 3.4.3., CRAN) using analysis of

doi:10.6342/NTU202000429

variances (ANOVA). Data reached 95% significant standard were further compared by Fisher least significant difference procedure (Fisher LSD). The final production of flower-bud-differentiated apices of different cultivars in 2018 was compared by Tukey honestly significant difference procedure (Tukey HSD). Considering the distribution of investigation data, all percentage data were angular transformed ($y = sin^{-1}\sqrt{X}$) before analysis (Shen, 2014). Figures were plotted by Excel (Version 2016, Microsoft Inc., Redmond, Wa., USA), and modified by Inkscape (Version 0.92.4.).

2.5. Results

2.5.1. 2018 trial

The daily mean temperature from 22 September to 23 December ranged between 29.9 and 15.4°C, but only six days were measured greater than 26°C (Appendix 3). The maximum temperature, 34.1°C, occurred on 23 September, while the minimum temperature was recorded as 11.9°C on 18 December (Appendix 3).

On 30 September, 22.0% of rabbiteye shoots were still growing without shoot tip abortion. At the meantime, non-terminated shoot percentage of northern highbush and southern highbush blueberries were 2.8% and 8.0%, respectively, which was significantly lower than rabbiteye cultivars (Fig. 2.1). The non-terminated shoot percentage of northern and southern highbush blueberries reduced to less than 1% on 21 October. In contrast, rabbiteye blueberries remained non-terminated shoot percentage

doi:10.6342/NTU202000429

around 1% until 11 November, though sharply reduced in October (Fig. 2.1). The non-terminated shoot percentage of rabbiteye blueberries was significantly higher than northern highbush and southern highbush blueberries from 30 September to 11 November (Fig. 2.1).

More axillary flower buds (18.1%) were observed in southern highbush blueberries than rabbiteye blueberries (13.7%) and northern highbush blueberries (8.3%) (Fig. 2.2). Axillary flower buds in southern highbush blueberries were majorly found in cultivar ‘Misty’, ‘O’Neal’, and ‘Sunshineblue’. However, axillary flower bud percentage of rabbiteye cultivars surpassed southern highbush blueberries on 25 November, and became significantly higher than southern highbush blueberries on 23 December (Fig. 2.2). ‘Weymouth’ northern highbush blueberry differentiated flower bud later than all the other species, as the date when axillary flower bud percentage reached 30% was 21 days later than rabbiteye blueberries, or 28 days later than southern highbush blueberries, and the yield of flower buds was the least on all investigation dates (Fig. 2.2).

Apical inflorescences were observed before 30 September and throughout the investigation period mainly in rabbiteye cultivars. However, more apical inflorescences were produced in late-October, while major blooming period lay between mid-November to mid-December, depending on cultivar (Data not shown). There were

doi:10.6342/NTU202000429

cultivars producing more apical blooms in autumn than others by 30 December. In 'NTU-031' rabbiteye blueberry, the number of apical meristems that differentiated flowers was 102 per bush, followed by 'Tifblue', averaging 53.8. 'Sunshineblue' southern highbush blueberry produced 32.8 flower-bud-differentiated apices. On the other hand, some cultivars barely show autumn apical blooming. 'Weymouth' northern highbush blueberry and 'Georgiagem' and 'O'Neal' southern highbush blueberries had less than 2.5 apical blooming shoots per bush (Fig. 2.3).

2.5.2. 2019 trial

The temperature from 22 September to 23 December in 2019 ranged from 34.1 to 10.9°C, which was measured on 2 October and 8 December, respectively (Appendix 4). Daily mean temperature was measured between 28.9 and 14.4°C (Appendix 4). If divide the 93-day investigation period into three equal segments, the mean temperature between 22 September and 22 October in 2019 was significantly higher than in 2018 (Table 2.1).

Overall, during the investigation period of 2019, labeled blueberry shoots decelerated vegetative growth in mid-November, and underwent transformation from vegetative to reproductive growth or growth cessation on the shoot tip thereafter (Table 2.2, Table 2.3), except 'NTU-031' and 'Sunshineblue', whose vegetative shoot percentage had decreased in October (Table 2.2).

doi:10.6342/NTU202000429

Axillary flower buds on labeled shoots were observed after 6 October. Among all cultivars, ‘Sunshineblue’ southern highbush blueberry developed flower buds slightly earlier to other cultivars. By the end of the investigation period, 25.5%-43.5% of axillary buds on the labeled shoots differentiated flower buds (Table 2.4, Table 2.5).

Flower-bud-differentiated apices were visible on labeled shoots in most cultivars on 29 September (Table 2.6), while most cultivars stopped apical flower bud differentiation in early-November, ‘NTU-032’ and ‘NTU-054’ kept differentiating apical inflorescences in December (Table 2.7). 86.9% of apical inflorescences were produced between 29 September and 10 November (Table 2.6, Table 2.7). Among all observed cultivars, ‘NTU-031’ rabbiteye blueberry differentiated 2.05 apical inflorescences per labeled shoot, which is the most throughout the 2019 investigation period. In contrast, ‘NTU-054’ and ‘Sunshineblue’ differentiated least apical inflorescences per shoot, by only approximately 0.5 (Table 2.7). Although apical inflorescences were produced in all investigated cultivars, much less apical flowers were observed in 2019 than previous year between 29 September and 22 December. Only four of 215 labeled shoots total produced apical flowers, which comprised of one ‘Powderblue’ shoot, one ‘NTU-054’ shoot, and two ‘NTU-103’ shoots, producing 14 flowers in total (Data not shown). However, flowers were observed on non-labeled shoots, and heavy flower and fruit load were found on ‘NTU-031’ and ‘Sunshineblue’

doi:10.6342/NTU202000429

bushes (Fig. 2.4).

2.6. Discussions

The result of 2018 trial implied that rabbiteye blueberries ceased shoot growth later than northern and southern highbush blueberries (Fig. 2.1), and less axillary flower buds had differentiated before 30 September, but more flower buds were observed on 23 December, compared with southern highbush blueberries (Fig. 2.2). In fall 2018, apical flowering was mostly observed in rabbiteye blueberries and ‘Sunshineblue’ southern highbush blueberries. While other southern highbush and northern highbush blueberries investigated rarely flowered on the shoot apices (Fig. 2.3). Some rabbiteye cultivars, e.g. ‘Austin’, ‘Gloria’, and ‘Homebell’, also produced very few apices that directly transform into inflorescences in autumn. Shoots of these cultivars mostly terminated with the typical apical abortion before flower bud formation in the lateral buds, as their typical growth pattern in the temperate region.

‘Tifblue’ and ‘Powderblue’ rabbiteye blueberries were witnessed differentiating more apical inflorescences than other cultivars. Given that ‘Tifblue’ is the female parent of ‘Powderblue’, it is possible that apical flowering is more expressive in genes carried by specific cultivars. However, rare-apical-flowering rabbiteye cultivars such as ‘Austin’ and ‘Gloria’ also contained 25% and 50% of ‘Tifblue’ pedigree, respectively (Hall and Draper, 1997). In addition, no apparent linkages between parentage and apical

doi:10.6342/NTU202000429

flowering intensity were found among rabbiteye cultivars (Appendix 2) (Watanabe, 2006). Therefore, the likeliness of apical flowering in different blueberry cultivars might be defined by their growth phenology. The breeding lines grown at NTU displayed higher apical flowering occurrence, possibly indicating that they were more adapted to autumn climate in lowland Taiwan. Nevertheless, they were open pollinated seedlings with unknown male parentages (Appendix 2). The relationship between genetic background and apical flowering intensity might require further analyses.

When a growing blueberry shoot endures apical flower bud differentiation, the shoot tip becomes piercing, the apical bud swells, and the newly formed leaves are smaller than the normal ones. Considering that some axillary flower buds emerged in autumn also develop an elongated peduncle, which also looks like apical-flower-bud-differentiated shoots (Horiuchi et al., 2013), we may regard the portion above these leaves of an apical flowering shoot as an inflorescence, which is greatly elongated and is located on a growing shoot.

Shoot apices that differentiated flowers did not guarantee continuous development to full bloom. During the experiment period, some flower-bud-differentiated shoot apices were aborted later. Some apices stopped further growth after apical flower bud differentiation, but the apical bud was not aborted, instead, a flower bud was observed on the shoot tip. This might correspond to the terminal floret in the axillary flower buds,

doi:10.6342/NTU202000429

as observed by Kovaleski et al. (2015). Browning of florets were also observed during the investigations, showing that the viability of apical flowers was affected by environmental factors. The flowering period of highbush blueberries in spring is affected by temperature (Carlson and Hancock, 1991). Temperature might also be a crucial factor in determining the timing and viability of apical flowers.

According to previous studies, temperature and photoperiod interact and cause various effects on blueberry growth (Hall and Ludwig, 1961); this was evidenced by the formation of flower buds on field-grown lowbush blueberries under 15-h photoperiod (Aalder and Hall, 1964). Some Ericaceae plants such as blueberry cultivars and azaleas were found to have decreasing response to photoperiod with plant age (Darnell, 1991; Pettersen, 1972). However, in 2019, apical flowers and fruits were still observed on non-labeled shoots (Fig. 2.4), indicating that the shoot sampling in 2019 investigation might have obscured the real progress of apical flowering on cultivars observed.

Comparing with air temperature from autumnal equinox to apical flower bud differentiation period of both years, daily mean temperature in 2018 was lower than in 2019, as the recorded temperatures were lower than 26°C throughout October (Appendix 3). In contrast, mean air temperature was above 27°C from 1 to 8 October in 2019 (Appendix 4). Conversely, higher daily mean temperature between 18.1 and 27.0°C was recorded in 2018 November, when apical flowers were witnessed blooming

doi:10.6342/NTU202000429

(Appendix 3), whereas daily mean air temperature in 2019 November was lower, ranged from 17.6 to 24.5°C (Appendix 4).

In 2018, apical flower bud differentiation continuously occurred from 30 September to 23 December, whereas in 2019, 86.9% of apical inflorescences were formed between 29 September and 10 November (Table 2.6, Table 2.7), when the temperature was significantly higher than 2018 (Table 2.1). Although it seemed that the high temperature between 22 September and 22 October did not inhibit the apical flower bud differentiation in 2019, and the temperature pattern between 23 October and 22 November, which was non-significant to those in 2018, did not pose an encouraging effect on differentiation of apical inflorescences, either. It was possible that the high temperature between 22 September and 22 October had limited the apical flower bud differentiation potential, which cause more shoot tips become aborted, rather than differentiating apical inflorescences. In addition, though non-significant, the lower temperature from 23 October to 23 December in 2019 made it more difficult for differentiated apical inflorescences to accumulate heat unit for blooming (Table 2.1), which fitted the observation of terminal-flower-bud-forming apical inflorescences. Consequently, in 2019, both differentiation and flowering of apical inflorescences were not as prosperous as in 2018.

2.7. Conclusions

doi:10.6342/NTU202000429

In natural autumnal environment of lowland Taiwan, blueberries were encouraged to produce apical flowers, for the mild temperature favors vegetative growth, and the photoperiod is suitable for flower differentiation. Among all cultivars, certain rabbiteye cultivars were more likely to develop apical flowers due to the overlapping of shoot vegetative growth and flower bud initiation. In 2018, apical inflorescences differentiated continuously from 30 September to 23 December, and blooming peaked in mid-November. In 2019, apical inflorescences were formed on labeled shoots mainly between 29 September and 10 November, with few flowers observed. The higher temperature between 22 September and 22 October in 2019 might have limited the differentiation of apical inflorescences, and thus leaded to lower intensity of apical flower bud differentiation and flowering.

2.8. References

Aalder, L.E. and I.V. Hall. 1964. A comparison of flower-bud development in the lowbush blueberry, *Vaccinium angustifolium* Ait. under greenhouse and field conditions. Proc. Amer. Soc. Hort. Sci. 85:281-284.

Carlson, J. and J. Hancock. 1991. A methodology for determining suitable heat-unit requirements for harvest of highbush blueberry. J. Amer. Soc. Hort. Sci. 116:774-779.

Central Weather Bureau. 2011. Monthly mean temperature of Taipei, Taiwan.

doi:10.6342/NTU202000429

<https://www.cwb.gov.tw/V7e/climate/monthlyMean/tx.htm>.

Darnell, R.L. 1991. Photoperiod, carbon partitioning, and reproductive development in rabbiteye blueberry. J. Amer. Soc. Hort. Sci. 116:856-860.

Hall, I.V. and R.A. Ludwig. 1961. The effects of photoperiod, temperature, and light intensity on the growth of the lowbush blueberry (*Vaccinium angustifolium* Ait.). Can. J. Botany 39:1733-1739.

Hall, M.R. and A.D. Draper. 1997. 'Austin' rabbiteye blueberry. Hortscience 32:1295-1296.

Horiuchi, N., J. Che, H. Hoshino, and I. Ogiwara. 2013. Influence of high temperature and long-day treatments before endodormancy on morphological and ecological responses of three blueberry species. Hort. Res. (Japan) 12:281-288.

Hummer, K., F. Zee, A. Strauss, L. Keith, and W. Nishijima. 2007. Evergreen production of southern highbush blueberries in Hawai'i. J. Amer. Pomol. Soc. 61:188-195.

Japan Meteorological Agent. 2010. Climate data of Fuchu Station, Tokyo, Japan. <http://www.data.jma.go.jp/gmd/risk/obsdl/index.php#!table>.

Kovaleski, A.P., J.G. Williamson, J.W. Olmstead, and R.L. Darnell. 2015. Inflorescence bud initiation, development, and bloom in two southern highbush blueberry cultivars. J. Amer. Soc. Hort. Sci. 140:38-44.

doi:10.6342/NTU202000429

Krewer, G. and D.S. NeSmith. 2000. Blueberry cultivars for Georgia. Univ. Ga. Fruit Publ. 00-2.

Lyrene, P.M. 2008. ‘Emerald’ southern highbush blueberry. HortScience 43:1606-1607.

Pettersen, H. 1972. The effect of temperature and daylength on shoot growth and bud formation in azaleas. J. Amer. Soc. Hort. Sci. 97:17-24.

Phatak, S.C. and M.E. Austin. 1990. The effect of photoperiod on the growth and flowering of two rabbiteye blueberry cultivars. Appl. Agr. Res. 5:350-352.

Shen, M.L. 2014. Data transformation, p. 81-104. In: M.L. Shen. Experimental designs. 5th ed. Jeou Chou Book. Taipei, Taiwan.

Watanabe, J. 2006. Blueberry pictorial book. Marumo Publishing. Tokyo, Japan.

doi:10.6342/NTU202000429

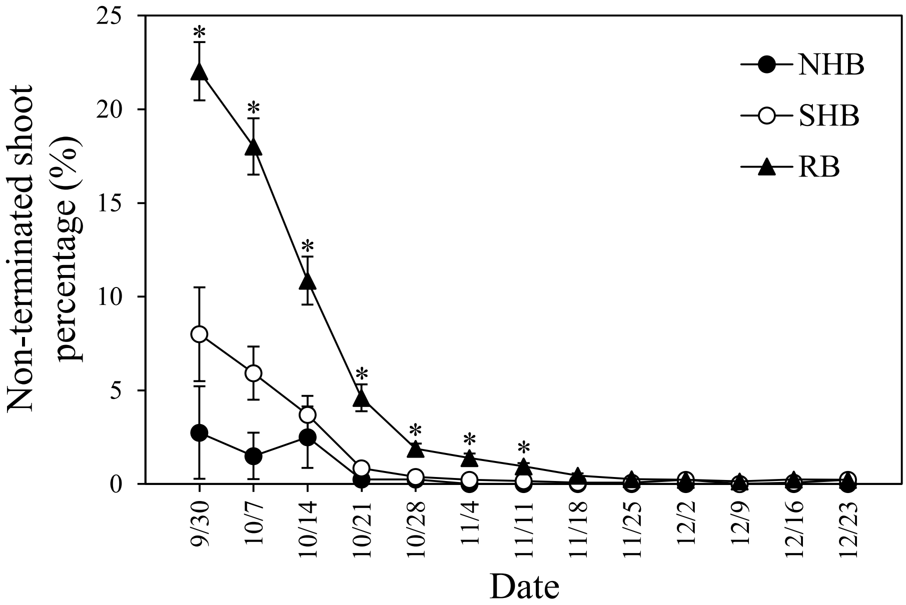


Fig. 2.1. Percentage of non-terminated shoots in northern highbush blueberries (NHB), southern highbush blueberries (SHB), and rabbiteye blueberries (RB) in 2018. Data represented by means ± standard error. Asterisks represent significant differences at $p \leq 0.05$ by Fisher LSD test.

doi:10.6342/NTU202000429

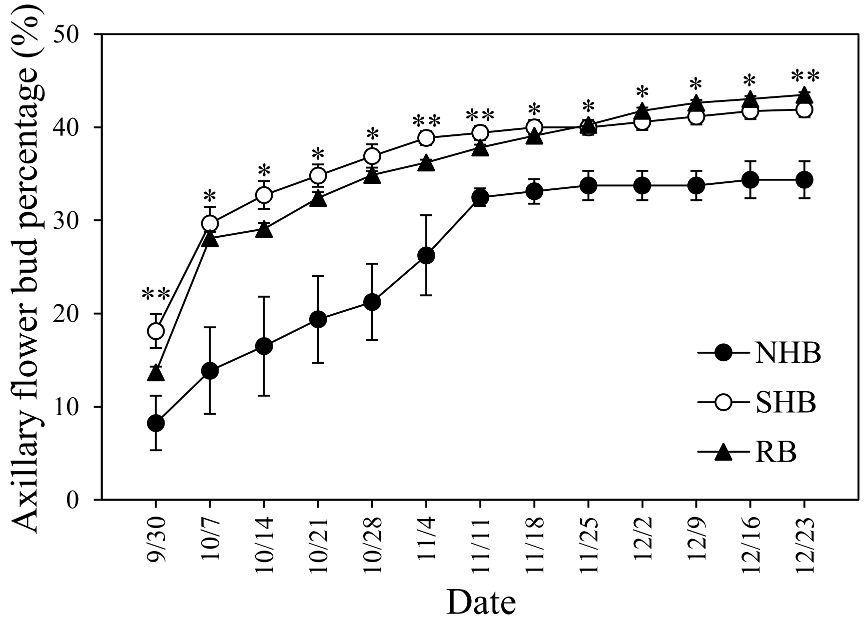


Fig. 2.2. Percentage of visible axillary flower buds to total lateral buds per shoot in northern highbush blueberries (NHB), southern highbush blueberries (SHB), and rabbiteye blueberries (RB) in 2018. Asterisks represent significant differences between NHB and the other two species, with no significant differences between SHB and RB at a given date. Double asterisks represent significant differences between any two species at a given date (Fisher LSD, $p \leq 0.05$).

doi:10.6342/NTU202000429

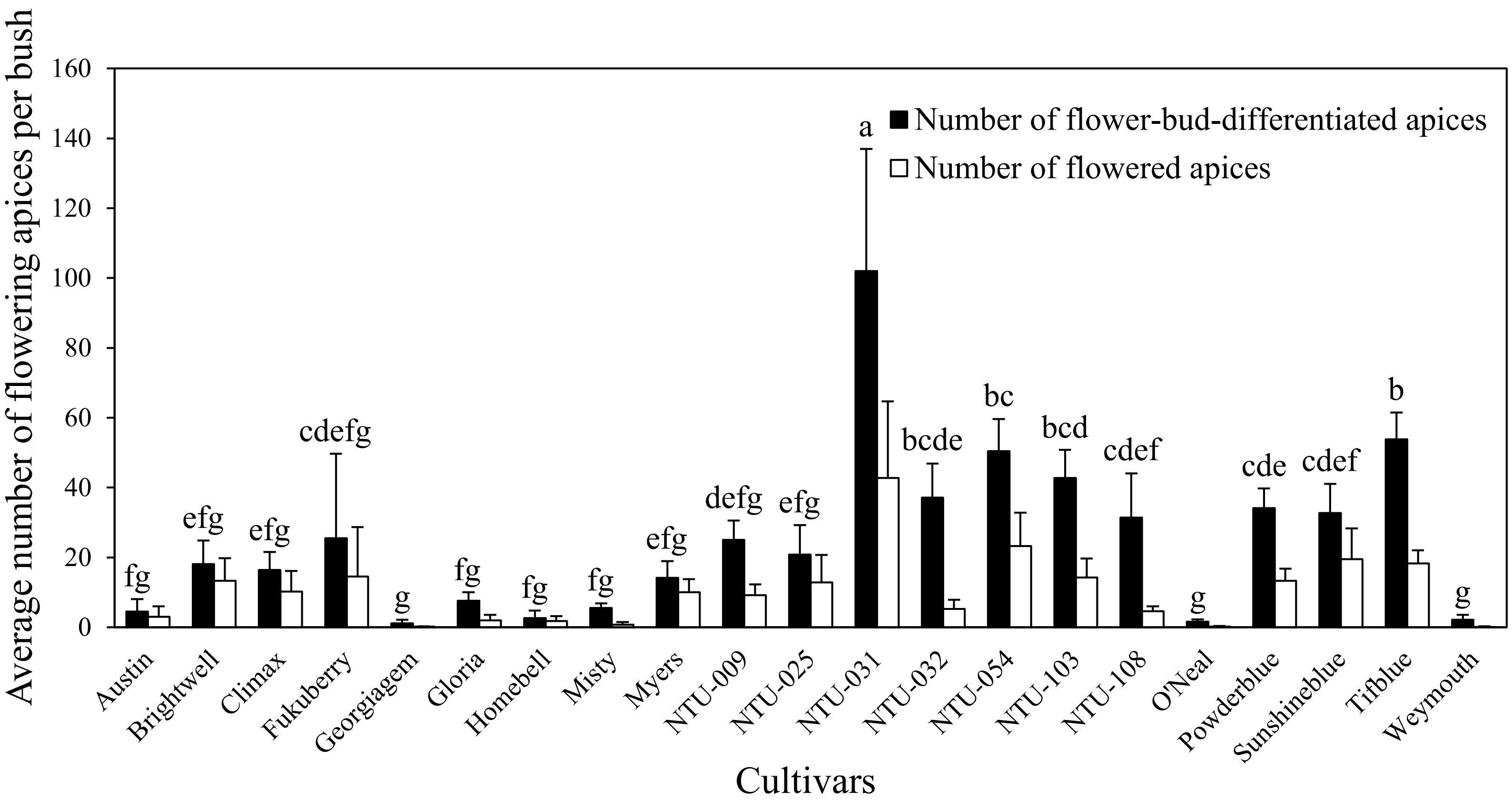


Fig. 2.3. Number of flower-bud-differentiated and flowered apices of blueberry cultivars with a sample size greater than four bushes in year 2018. Bar = standard error. Means followed by same alphabet represent no significant difference between flower-bud-differentiated apices of different cultivars (Tukey HSD, $p \leq 0.05$).

doi:10.6342/NTU202000429

Table 2.1. Comparison of mean temperature data in 2018 and 2019 by paired t-test.

	Mean temperature (°C)		
Date	2018	2019	t-value
22 September - 22 October	24.10 ± 0.42	25.53 ± 0.35	-2.53*
23 October - 22 November	23.10 ± 0.39	22.63 ± 0.33	0.93
23 November - 23 December	20.39 ± 0.46	19.49 ± 0.47	1.24

Means represented with standard error. Asterisks represent significant differences in mean temperature between 2018 and 2019 at $p \leq 0.05$ by paired t-test.

doi:10.6342/NTU202000429

Table 2.2. Non-terminated shoot percentage of all investigated cultivars between 22 September and 3 November 2019.

	Vegetative shoot percentage (%)						
Cultivar	9/22	9/29	10/6	10/13	10/20	10/27	11/3
NTU-009	40.00 ± 6.32 d	50.97 ± 12.81	67.55 ± 2.43 ab	60.54 ± 5.19 a	49.83 ± 8.68 a	16.87 ± 6.02 bc	5.57 ± 2.50 c
NTU-031	62.32 ± 8.82 cd	51.55 ± 8.43	23.91 ± 7.80 cd	17.67 ± 11.83 bc	11.78 ± 7.89 c	0.86 ± 0.86 d	0 c
NTU-032	88.00 ± 4.90 ab	64.00 ± 4.00	24.57 ± 7.80 cde	22.00 ± 6.96 bc	46.89 ± 3.04 a	40.86 ± 9.37 a	29.64 ± 9.59 a
NTU-054	93.78 ± 4.06 a	79.50 ± 7.40	74.85 ± 3.99 a	60.48 ± 7.09 a	46.18 ± 9.45 ab	33.81 ± 10.92 ab	27.10 ± 8.93 ab
NTU-103	50.90 ± 6.09 d	66.93 ± 4.04	70.81 ± 4.37 a	69.27 ± 5.88 a	53.40 ± 5.54 a	45.28 ± 4.73 a	20.71 ± 2.26 ab
NTU-108	58.76 ± 10.36 cd	60.15 ± 5.49	17.81 ± 8.98 de	19.24 ± 14.76 bc	20.58 ± 6.63 c	5.83 ± 3.63 cd	6.28 ± 3.85 c
Powderblue	76.48 ± 2.65 bc	60.78 ± 3.60	19.58 ± 7.01 de	7.37 ± 3.12 c	7.92 ± 4.88 c	4.94 ± 2.62 cd	8.41 ± 5.58 c
Tifblue	92.67 ± 4.52 a	56.29 ± 4.67	42.94 ± 6.92 bc	37.75 ± 7.81 ab	17.62 ± 4.66 c	25.84 ± 8.76 ab	8.36 ± 3.67 bc
Sunshineblue	79.02 ± 8.47 abc	42.22 ± 15.50	7.50 ± 4.79 e	21.47 ± 16.40 bc	23.75 ± 15.54 bc	1.79 ± 1.79 d	0 c

Means represented with standard error. Data followed by same alphabet indicate no significant difference in vegetative shoot percentage between cultivars (Fisher LSD, $p \leq 0.05$).

doi:10.6342/NTU202000429

Table 2.3. Non-terminated shoot percentage of all investigated cultivars between 10 November and 22 December 2019.

	Vegetative shoot percentage (%)						
Cultivar	11/10	11/17	11/24	12/1	12/8	12/15	12/22
NTU-009	6.24 ± 3.00 abc	11.28 ± 6.11	13.59 ± 8.96	14.77 ± 9.35	9.09 ± 5.79	9.09 ± 5.79	6.94 ± 4.52
NTU-031	0.86 ± 0.86 bc	1.47 ± 1.47	0.86 ± 0.86	0.86 ± 0.86	0.86 ± 0.86	0	0
NTU-032	18.91 ± 6.58 a	17.09 ± 5.54	7.45 ± 3.43	11.27 ± 3.36	3.64 ± 2.23	3.82 ± 2.34	4.00 ± 2.45
NTU-054	20.04 ± 8.51 a	12.75 ± 5.31	11.59 ± 5.29	14.05 ± 6.35	11.02 ± 5.34	5.45 ± 3.64	2.00 ± 2.00
NTU-103	15.94 ± 5.08 a	12.77 ± 4.90	22.41 ± 7.36	17.42 ± 7.39	18.74 ± 6.05	17.14 ± 6.37	11.68 ± 6.66
NTU-108	3.61 ± 1.62 abc	3.33 ± 3.33	12.15 ± 6.44	0	0	1.82 ± 1.82	1.82 ± 1.82
Powderblue	0 c	2.50 ± 2.50	4.86 ± 3.64	6.88 ± 3.60	5.77 ± 3.44	4.66 ± 3.63	4.66 ± 3.63
Tifblue	10.66 ± 3.74 ab	5.18 ± 2.37	2.30 ± 1.42	5.84 ± 2.91	4.41 ± 3.09	4.41 ± 3.09	4.58 ± 3.25
Sunshineblue	1.79 ± 1.79 bc	1.79 ± 1.79	0	0	0	0	0

Means represented with standard error. Data followed by same alphabet indicate no significant difference in vegetative shoot percentage between cultivars (Fisher LSD, $p \leq 0.05$).

doi:10.6342/NTU202000429

Table 2.4. Average axillary flower bud percentage of all investigated cultivars between 6 October and 10 November 2019.

	Average axillary flower bud percentage (%)					
Cultivar	10/6	10/13	10/20	10/27	11/3	11/10
NTU-009	0	0	0.81 ± 0.56 cd	2.10 ± 1.29 bc	6.45 ± 1.90 bc	9.22 ± 1.43 cd
NTU-031	0.05 ± 0.05	1.14 ± 0.64	5.27 ± 1.94 a	7.45 ± 2.58 a	11.03 ± 3.17 ab	19.65 ± 3.47 ab
NTU-032	0	0	1.16 ± 0.77 bcd	3.11 ± 0.92 ab	7.12 ± 1.68 bc	10.27 ± 2.44 cd
NTU-054	0	0.07 ± 0.07	0.07 ± 0.07 d	0.13 ± 0.13 c	0.99 ± 0.33 d	3.58 ± 1.09 e
NTU-103	0.19 ± 0.19	0.17 ± 0.17	0.50 ± 0.50 d	0.57 ± 0.57 c	1.38 ± 1.24 d	2.61 ± 1.44 e
NTU-108	0	0	3.37 ± 1.55 abc	4.63 ± 1.37 a	10.46 ± 2.05 ab	11.91 ± 1.74 bc
Powderblue	0.24 ± 0.24	0.35 ± 0.35	2.90 ± 0.72 ab	3.60 ± 0.86 ab	6.29 ± 1.24 bc	13.87 ± 2.44 bc
Tifblue	0	0.16 ± 0.16	0.16 ± 0.16 d	0.64 ± 0.33 c	3.78 ± 1.56 cd	5.52 ± 2.19 de
Sunshineblue	0	0.11 ± 0.11	3.19 ± 0.77 ab	5.95 ± 0.68 a	17.82 ± 5.22 a	25.97 ± 6.26 a

Means represented with standard error. Data followed by same alphabet indicate no significant difference in axillary flower bud percentage between cultivars (Fisher LSD, $p \leq 0.05$).

doi:10.6342/NTU202000429

Table 2.5. Average axillary flower bud percentage of all investigated cultivars between 17 November and 22 December 2019.

	Average axillary flower bud percentage (%)					
Cultivar	11/17	11/24	12/1	12/8	12/15	12/22
NTU-009	19.14 ± 1.53 b	27.95 ± 1.40 ab	30.03 ± 1.51 ab	30.68 ± 1.45 bc	32.47 ± 2.16 bc	33.17 ± 2.19 bcd
NTU-031	25.00 ± 1.90 ab	28.58 ± 1.30 ab	31.00 ± 2.08 ab	31.51 ± 1.91 abc	32.35 ± 2.19 bcd	33.16 ± 2.53 bcd
NTU-032	18.33 ± 3.44 b	23.48 ± 3.97 bc	28.69 ± 2.97 b	29.73 ± 3.35 bcd	33.30 ± 3.21 abc	35.69 ± 2.48 abc
NTU-054	9.76 ± 1.55 d	17.60 ± 2.58 cd	20.08 ± 2.07 c	23.07 ± 2.20 cd	26.54 ± 1.94 cd	27.63 ± 1.91 cd
NTU-103	9.06 ± 1.76 d	14.47 ± 1.27 d	20.76 ± 1.92 c	25.47 ± 2.78 bcd	29.06 ± 2.32 bcd	32.55 ± 1.82 bcd
NTU-108	16.88 ± 1.29 bc	23.58 ± 2.02 bc	29.33 ± 2.07 b	33.76 ± 2.11 ab	36.29 ± 2.45 ab	38.39 ± 2.12 ab
Powderblue	21.83 ± 2.52 b	27.72 ± 2.84 b	28.79 ± 2.49 b	30.58 ± 2.43 bc	31.59 ± 2.43 bcd	32.34 ± 2.24 bcd
Tifblue	11.37 ± 3.45 cd	17.60 ± 3.26 cd	19.47 ± 3.17 c	21.85 ± 3.21 d	24.06 ± 3.09 d	25.51 ± 2.79 d
Sunshineblue	32.49 ± 5.37 a	37.83 ± 6.41 a	38.92 ± 6.61 a	41.21 ± 7.60 a	42.57 ± 7.25 a	43.52 ± 7.85 a

Means represented with standard error. Data followed by same alphabet indicate no significant difference in axillary flower bud percentage between cultivars (Fisher LSD, $p \leq 0.05$).

doi:10.6342/NTU202000429

Table 2.6. Accumulated number of apical inflorescences produced per labeled shoot by each cultivar between 22 September and 3 November 2019.

Cultivar	Accumulated number of apical inflorescences per labeled shoot						
	9/22	9/29	10/6	10/13	10/20	10/27	11/3
NTU-009	0.12 ± 0.07 b	0.24 ± 0.09 bc	0.32 ± 0.11 bc	0.36 ± 0.14 bc	0.40 ± 0.15 cd	0.64 ± 0.22 cde	0.88 ± 0.25 cde
NTU-031	0.65 ± 0.30 a	0.80 ± 0.30 a	1.45 ± 0.45 a	1.65 ± 0.44 a	1.85 ± 0.43 a	1.95 ± 0.46 a	2.05 ± 0.44 a
NTU-032	0 b	0.08 ± 0.06 c	0.48 ± 0.10 bc	0.72 ± 0.21 b	0.72 ± 0.21 bc	0.76 ± 0.21 bcd	0.80 ± 0.21 cde
NTU-054	0 b	0.04 ± 0.04 c	0.04 ± 0.04 c	0.12 ± 0.07 c	0.12 ± 0.07 d	0.20 ± 0.08 de	0.28 ± 0.11 e
NTU-103	0.04 ± 0.04 b	0.08 ± 0.06 c	0.08 ± 0.06 c	0.08 ± 0.06 c	0.08 ± 0.06 d	0.08 ± 0.06 e	0.60 ± 0.19 de
NTU-108	0 b	0.04 ± 0.04 c	0.68 ± 0.22 b	0.84 ± 0.22 b	1.24 ± 0.25 ab	1.28 ± 0.26 ab	1.28 ± 0.26 bc
Powderblue	0.20 ± 0.08 b	0.48 ± 0.14 b	1.24 ± 0.26 a	1.72 ± 0.25 a	1.72 ± 0.25 a	1.84 ± 0.26 a	1.84 ± 0.26 ab
Tifblue	0 b	0.24 ± 0.09 bc	0.32 ± 0.10 bc	0.48 ± 0.10 bc	0.80 ± 0.19 bc	0.84 ± 0.20 bc	0.96 ± 0.21 cd
Sunshineblue	0 b	0 c	0.30 ± 0.25 bc	0.35 ± 0.25 bc	0.40 ± 0.26 cd	0.40 ± 0.26 cde	0.45 ± 0.26 de

Means represented with standard error. Data followed by same alphabet indicate no significant difference in accumulated apical inflorescence number between cultivars (Fisher LSD, $p \leq 0.05$).

doi:10.6342/NTU202000429

Table 2.7. Accumulated number of apical inflorescences produced per labeled shoot by each cultivar between 10 November and 22 December 2019.

	Accumulated number of apical inflorescences per labeled shoot						
Cultivar	11/10	11/17	11/24	12/1	12/8	12/15	12/22
NTU-009	1.12 ± 0.25 cd	1.12 ± 0.25 cd	1.12 ± 0.25 cd	1.12 ± 0.25 cd	1.12 ± 0.25 cd	1.12 ± 0.25 cd	1.12 ± 0.25 cd
NTU-031	2.05 ± 0.44 a	2.05 ± 0.44 a	2.05 ± 0.44 a	2.05 ± 0.44 a	2.05 ± 0.44 a	2.05 ± 0.44 a	2.05 ± 0.44 a
NTU-032	0.80 ± 0.21 cde	0.88 ± 0.21 cde	0.88 ± 0.21 cde	0.88 ± 0.21 cde	0.92 ± 0.22 cd	0.92 ± 0.22 cd	0.92 ± 0.22 cd
NTU-054	0.36 ± 0.17 e	0.36 ± 0.17 e	0.40 ± 0.17 e	0.40 ± 0.17 e	0.48 ± 0.18 d	0.48 ± 0.18 d	0.56 ± 0.20 d
NTU-103	0.68 ± 0.21 cde	0.72 ± 0.20 cde	0.72 ± 0.20 cde	0.72 ± 0.20 cde	0.72 ± 0.20 cd	0.72 ± 0.20 cd	0.72 ± 0.20 cd
NTU-108	1.28 ± 0.26 bc	1.28 ± 0.26 bc	1.28 ± 0.26 bc	1.28 ± 0.26 bc	1.28 ± 0.26 bc	1.28 ± 0.26 bc	1.28 ± 0.26 bc
Powderblue	1.88 ± 0.25 ab	1.88 ± 0.25 ab	1.88 ± 0.25 ab	1.88 ± 0.25 ab	1.88 ± 0.25 ab	1.88 ± 0.25 ab	1.88 ± 0.25 ab
Tifblue	0.96 ± 0.21 cde	0.96 ± 0.21 cde	0.96 ± 0.21 cde	0.96 ± 0.21 cde	0.96 ± 0.21 cd	0.96 ± 0.21 cd	0.96 ± 0.21 cd
Sunshineblue	0.45 ± 0.26 de	0.45 ± 0.26 de	0.45 ± 0.26 de	0.45 ± 0.26 de	0.45 ± 0.26 d	0.45 ± 0.26 d	0.45 ± 0.26 d

Means represented with standard error. Data followed by same alphabet indicate no significant difference in accumulated apical inflorescence number between cultivars (Fisher LSD, $p \leq 0.05$).

doi:10.6342/NTU202000429

Fig. 2.4. Apical flowers and fruits produced by unlabeled shoots of ‘Sunshineblue’ southern highbush blueberry (A) and ‘NTU-031’ rabbiteye blueberry (B-D) photographed on 10 December 2019. Bar = 2 cm.

doi:10.6342/NTU202000429

Chapter three

Microstructure of apical flowering blueberry buds

3.1. Abstract

To determine the microscopic structure development of apical flowering, easy and rare-apical-flowering cultivars were compared in the developmental stages of buds, and the bud developmental stages of apical bud and underneath three or five axillary buds were recorded from 26 September to 23 October. The floret differentiation in blueberry inflorescences follows the principle of acropetal formation and the flower organs form from the outer to inner whorls. The bud anatomy result indicated that all bud internal developmental stages in axillary buds are observable in apical buds. In 2019, Total shoot growth cessation was found in 'Brightwell' in mid to late-October. However, shoots of 'Powderblue' and 'NTU-031' without growth cessation produced flower organs on 23 October. More apical inflorescences were produced between 29 September and 8 Octover 2018 when daily mean temperature lay between 20 and 25°C, rather than over 27°C in 2019 during the same dates. Reduced proportion of developing apical inflorescences to total sampled shoots was observed when daily mean temperature decreased below 20°C. The result suggests that under daylength between 11 and 12 h, the optimum daily mean temperature for apical flower bud differentiation was 20 to 25°C, while daily mean temperature below 20 or above 27°C reduced formation of

doi:10.6342/NTU202000429

apical inflorescences. Occurrence of apical flowering is irrelevant to timing of flower bud initiation, but determined by whether the shoot has ceased growth when flower bud initiation takes place. If the shoot does not cease vegetative growth, apical flower bud differentiation will occur in both easy and rare-apical-flowering cultivars at a sequence of fruit maturity time in different cultivars. However, growth cessation may occur after apical flower bud initiation.

doi:10.6342/NTU202000429

3.2. 摘要

本試驗自 9 月 26 日至 10 月 23 日間採集易產生頂梢開花與不易頂梢開花藍莓品種之頂芽及下方三或五個腋芽，比較其生長點型態，並記錄其芽體發育階段，以了解頂梢開花過程中的芽體顯微構造變化。試驗結果顯示，藍莓花芽分化遵守小花由基部向頂分化與花器由外圈向內圈分化之原則，所有藍莓腋芽之芽體內部發育階段皆可在頂梢開花現象發生之頂芽內部發現。2019 年'Brightwell'兔眼藍莓枝梢在十月中下旬完全停止生長，而未停梢之'Powderblue'及'NTU-031'枝梢於 10 月 23 日時頂芽皆有花器形成。2018 年 9 月 29 日至 10 月 8 日間，日均溫介於 20 與 25℃時，花芽分化頂梢產量多於 2019 年日均溫超過 27℃之期間。而日均溫降至 20℃以下時，花芽分化頂梢數量亦隨之減少。顯示在日長 11 至 12 小時下，藍莓頂梢開花行為之最適日均溫為 20 至 25℃，但頂梢花芽分化比例在日均溫低於 20 或高於 27℃時減少。頂梢開花現象之發生與否取決於在適合花芽創始的條件下，枝梢是否仍持續生長。若枝梢未停止營養生長，頂梢花芽分化即會依各品種果實成熟期之順序發生，惟頂梢花芽分化枝梢仍有可能停止生長。

doi:10.6342/NTU202000429

3.3. Introduction

Microstructural transformation from vegetative to reproductive status in a typical leaf axillary bud have been well documented in blueberries (Bieniasz, 2012; Kovaleski et al., 2015; Tamada, 1997). When flower initiation occurs, the vegetative meristems in an axillary or lateral bud convert to floral meristems with distinguishable bracts (Kovaleski et al., 2015). Follows the formation of floral meristems are the sequential developments of sepals, petals, anthers and pistil before the bud became dormant (Kovaleski et al., 2015; Tamada, 1997). After the flower bud break dormancy in the following spring, microspore and macrospore cells develop and mature, and the peduncle, pedicle, and flowers elongate, which referred to the beginning of flowering (Bieniasz, 2012; Kovaleski et al., 2015).

In apical flowers, the florets differentiate while the stem apical meristem are still growing, thus provides a development progress different from axillary buds. However, no studies have been made to understand the microstructural changes during apical flower bud differentiation in blueberries.

In this chapter, the microstructures of blueberry apical and axillary buds from cultivars with different autumn blooming tendency were observed in two years to determine the timing and the seasonal variations in apical blooming. The hypothesis of the experiment was that easy-flowering cultivars develop floral organs directly in the

doi:10.6342/NTU202000429

apical bud, while the apical bud of rare-flowering cultivars undergo typical apical abortion and the shoot tip enter dormancy.

3.4. Materials and methods

3.4.1. Plant materials

‘Brightwell’, ‘Powderblue’, and ‘NTU-031’ rabbiteye blueberries (*Vaccinium virgatum* Aiton) were used in this experiment. Previous investigations indicated that ‘Brightwell’ rarely develops apical flowers, while the other two cultivars frequently produce flowers on shoot apices in autumn in Northern Taiwan. All plant materials were at least three years old and were cultivated as described in Chapter two.

3.4.2. Sampling and observation

On 22 September 2018, 30 shoots on ‘Brightwell’ and ‘Powderblue’ rabiteye blueberries each were labeled. The labeled shoots were emerged after summer pruning, containing at least five fully expanded mature leaves and three developing young leaves, and were still extending as checked by the lack of apical abortion.

From 26 September to 23 October, three shoots on each cultivar were randomly sampled on a three-day interval. The apical bud and the following five axillary buds on each shoot sample were abscised and dissected under a stereomicroscope (Nikon SMZ-10, Tokyo, Japan). All dissected buds were photographed using a digital eyepiece (Observer version 3.1.1.2., KData Co., Ltd., Hsinchu, Taiwan). The internal

doi:10.6342/NTU202000429

developmental stages of each bud were recorded according to Kovaleski et al. (2015). Half values were used when bud internal developmental stages of sampled buds were considered being in the middle between two stages. The internal developmental stage of a browning or aborted apical meristems were considered as Stage zero (SI-0).

The observation was repeated in 2019 with the addition of 'NTU-031' rabbiteye blueberry. Thirty shoots of each cultivar were labeled on 24 and 25 September. Shoots were periodically sampled and observed from 26 September to 23 October.

The representative samples of each bud internal developmental stages of apical buds in 'NTU-031' and 'Powderblue' were treated with standard protocol of specimen preparation, and were photographed under a scanning electron microscope (Hitachi S4800, Tokyo, Japan).

3.4.3. Statistical analysis

The experiment was randomized complete block design (RCBD); buds at different node positions were designated into different blocks. Experiment data were analyzed by R language (Version 3.4.3., CRAN). Bud internal developmental stages of same sampling date at a given node were compared by f-test and two-sample t-test in 2018. In case of missing data, the Welch t-test was used instead of two-sample t-test. The data of 2019 were analyzed by analysis of variance (ANOVA), data reached 95% significant standard were compared by Tukey honestly significant difference procedure (Tukey

doi:10.6342/NTU202000429

HSD).

3.5. Results

3.5.1. Bud anatomy 2018

In investigation period of 2018, daily mean temperature ranged between 19.5 and 26.4°C, flower bud differentiation in the shoot apex was observed in both cultivars since 29 September, and 11 ‘Brightwell’ and 18 ‘Powderblue’ of 30 sampled shoots for each cultivar were apical flower bud differentiated (Fig. 3.1). Five bud internal developmental stages from Stage I-1 to I-5 in the axillary buds were also observable in the apical buds (Fig. 3.2). ‘Brightwell’ apical buds developed flower earlier than ‘Powderblue’. On 2 October, developed sepals were distinguishable in all three sampled apical buds of ‘Brightwell’ (Table. 3.1). However, after 20 October, the apical meristems of all ‘Brightwell’ bud samples were aborted. In contrast, seven sampled ‘Powderblue’ shoots were apical aborted between 26 September and 14 October, which was more than six aborted shoots observed in ‘Brightwell’ during the same period, but only one of the nine sampled ‘Powderblue’ shoots ceased growth from 17 October to 23 October, and seven of the eight non-terminated shoots were found apical-flower-bud-differentiated (Table 3.1). On 23 October, a sampled shoot of ‘Brightwell’ was found producing two lateral shoots, while the apical bud became browning and aborted (Fig. 3.3).

doi:10.6342/NTU202000429

On 26 September, all sampled axillary buds in both ‘Brightwell’ and ‘Powderblue’ were categorized as Stage I-1, when the investigation ended on 23 October, the bud internal developmental stages of ‘Brightwell’ and ‘Powderblue’ from basal to apical node position were averaged between 1 and 3 and 2.33 to 4.17, respectively (Table 3.1). Nevertheless, the axillary bud developmental stage did not strictly follow the basipetal order (Table 3.1). The third axillary bud on 20 October and the second on 23 October were the only two data with significant difference between the two cultivars (Table 3.1).

3.5.2. Bud anatomy 2019

The daily mean temperature during 2019 investigation period was measured from 22.5 to 29.0°C, flower bud differentiating shoot apices were observed from 29 September to 23 October, the final sampling date (Fig. 3.4). For the 30 sampled shoots in each cultivar, four ‘Brightwell’ shoots, 13 ‘Powderblue’ shoots, and 21 ‘NTU-031’ shoots had begun apical flower bud differentiation when observed (Fig. 3.4).

The apexes of all shoots of ‘Brightwell’ sampled on and after 14 October were aborted (Fig. 3.5). In ‘Powderblue’, all shoots sampled on 11 and 17 October were also apical aborted, but flower bud differentiation was observed in two of the three sampled on 20 and 23 October each (Fig. 3.6). In ‘NTU-031’, all but one shoot samples continued growing without apical abortion from 26 September to 23 October (Table 3.7). Significant differences in apical bud internal developmental stages was observed

doi:10.6342/NTU202000429

among all cultivars from 11 October to 23 October except 17 October. Sepals were first observed in apical buds of ‘Powderblue’ and ‘NTU-031’ shoot samples on 2 October, and all non-terminated sampled ‘Powderblue’ and ‘NTU-031’ apical meristems developed petals by 23 October (Fig. 3.6, Fig. 3.7). Only two of the 30 apical buds of ‘Brightwell’ developed sepals, which was found on 2 and 11 October, respectively (Table 3.2). Bud internal developmental stages from Stage I-1 to I-4 were found among sampled apical buds (Fig. 3.2).

Scanning electron microscopy showed that the meristems within an apical bud of blueberries were arranged counter-clockwise; the uppermost floret of a flower-bud-differentiated apex had a lower bud internal developmental stage than more basal florets (Fig. 3.8).

The axillary bud internal developmental stages did not strictly follow the basipetal order, as observed in 2018 (Table 3.2). All bud development stages of axillary buds at the same position sampled on the same date were non-significant between cultivars (Table 3.2).

3.6. Discussions

Bud internal developmental stages ranging from vegetative meristem to elongated florets were observed in sampled apical buds (Fig. 3.2). The first stage was referred to vegetative bud (Fig. 3.2 a), followed by the development of bracts and subtly widening

doi:10.6342/NTU202000429

of the meristems (Fig. 3.2 b). The third stage was featured by the early development of sepals (Fig. 3.2 c), while in the fourth stage; the petals were clearly visible (Fig. 3.2 d). The final stage of apical inflorescence development was corresponded with the elongation of peduncle and pedicels (Fig. 3.2 e). Browning florets were found in some apical buds (Fig. 3.3), implying that apical abortion may occur even after apical flower bud differentiation in case of encountering unfavorable environmental factors.

Scanning electron microscopy showed that the meristems within an apical bud of blueberries were arranged counter-clockwise; the uppermost floret of a flower-bud-differentiated apex had a lower bud internal developmental stage than more basal florets (Fig. 3.5), indicating that the florets on the apical-flower-bud-differentiated apices developed acropetally from the first differentiated meristem. This also revealed the fact that the apical flowering apex could be considered as a single inflorescence. A foliage leaf was observable on the top of the apical inflorescence (Fig. 3.2) and was found increased in size during the experiment (Fig. 3.2 a, b, d), showing that during the development of apical inflorescences, vegetative growth continued even after flower bud initiation. The determinant of whether vegetative growth would continue after initiation of apical inflorescence might be the result of interaction between environmental factors and assimilation and allocation of nutrition. Take southern highbush blueberries for example, coexistence of apical flowers, fruits, and vegetative

doi:10.6342/NTU202000429

shoots were achieved in ‘Emerald’ in March when grown under forcing culture by treating artificial light supplement and nocturnal heating, to maintain 15-16 h photoperiod and temperature above 10°C from November in the previous year (Horiuchi et al., 2013). This implied that vegetative and reproductive growth of blueberries might be able to occur simultaneously under specific environment. On the perspectives of nutrition, flowering required over 60% of applied nitrogen for two-year-old ‘Emerald’ plants, while approximately the same percentage of fertilized nitrogen was allocated to the final growth flush (Fang et al., 2017). The development of apical inflorescences might pose strong competition between flower bud differentiation and vegetative growth. Consequently, the coexistence of apical inflorescence development and vegetative growth in the shoot tip might be the result of negotiation between vegetative and reproductive growth with attached strings of offering favorable environment and sufficient nutrients.

The average bud internal developmental stage changes in axillary buds did not strictly follow the basipetal order of flower bud formation, possibly due to low maturity of new formed bud on growing shoots. In 2018, some axillary buds on the labeled shoots broke dormancy and formed a new growth flush. Causing browning of buds above the site of branching, revealing that vigorous new growth flushes were more competitive nutrition sink, compared to apical inflorescences. This could be again

doi:10.6342/NTU202000429

explained by the approximately 60%-80% allocation of fertilized nitrogen to new stems and leaves of highbush blueberry plants in the postharvest vegetative growth stage (Fang et al., 2017), which caused competition between vegetative and reproductive growth, and even competition of vegetative growth between apical and axillary buds. In contrast, only one axillary flower bud of 'Powderblue' swelled in 2019. The different intensity of axillary bud break might be given rise by the inconsistent maturity of labeled shoots in 2018. Most average bud internal developmental stages in axillary buds between cultivars were not significant different, implying the fact that the occur of apical flowering is irrelevant to the progress of axillary flower bud differentiation, but determined by whether the shoot tip is still vegetative growing when flower bud initiation occurs.

The expected result of the experiment is that the apical bud of 'Brightwell' would undergo apical abortion, while 'Powderblue' would encounter apical flower bud differentiation. Overall, the result of 2019 was closer to the hypothesis, and more sampled shoots were found to differentiate flower organs inside the apical bud in 2018 (Table 3.1, Table 3.2). However, in both years, 'Brightwell' had flower bud differentiation in some observation date, and the development of sepals was found simultaneous or prior to the date when flower organs were visible in sampled shoot apices of 'Powderblue'. On the other hand, sampled shoot apices of 'Powderblue' were

doi:10.6342/NTU202000429

all apical aborted on certain dates in the middle of investigation period, which might be caused by the light interception or microclimate difference due to the different position of the sampled shoots (Yáñez et al. 2009).

The maximum and minimum field temperature during investigation period in 2018 were 33.4 and 17.6°C, respectively, while the field temperature in 2019 ranged from 34.5 to 18°C (Appendix 3, Appendix 4), which was slightly higher than in 2018. Daily mean temperature during the experiment was also higher in 2019, ranging between 21.5 and 29.2°C comparing to 18.7 and 26.7°C in 2018 (Fig. 3.1, Fig. 3.4). The daylength shortened from 12 h 3 min to 11 h 24 min (Central Weather Bureau, 2018), which was considered capable for flower bud initiation, and did not inhibit shoot growth of blueberries (Darnell, 1991; Bañados and Strik, 2006).

Considering that cool temperature encourages flower bud differentiation of 'Misty' southern highbush blueberry (Spann et al., 2004), it is possible that flower bud differentiation of 'Brightwell' and 'Powderblue' was enhanced in 2018 by cooler daily mean temperature, approximately between 20 and 25°C, from late-September to early-October (Fig. 3.1), rather than over 27°C from 2 to 9 October in 2019 (Fig. 3.4). For most blueberry cultivars, the timing of axillary flower bud differentiation and flowering seemed to be correspondent with the timing of fruit maturity (Eck et al., 1990). 'Brightwell' were considered as mid to late ripening rabbiteye cultivar, but earlier than

doi:10.6342/NTU202000429

‘Powderblue’ (Williamson and Lyrene, 2004). This might be the main reason why ‘Brightwell’ developed floral organs prior to ‘Powderblue’. On the other hand, when apical flowering of ‘Brightwell’ and ‘Powderblue’ was less encouraged due to higher temperature in 2019, ‘NTU-031’ displayed higher intensity of apical flower bud differentiation, probably due to better adaptability to climate in lowland Taiwan based on the fact that they were bred in Taiwan (Appendix 2). In ‘NTU-031’, floral organs were observed inside one apical bud on 29 September, and all sampled apical buds underwent apical-flower-bud-differentiation on 11, 14, 20, and 23 October. On 23 October, all sampled apical buds were observed the formation of petals (Fig. 3.7, Table 3.2).

On 14 October, 2018, only one of three sampled shoots produced apical inflorescence in both ‘Brightwell’ and ‘Powderblue’, which occurred two days after daily mean temperature dropped from 24.4 to 18.7°C, from 9 to 12 October (Fig. 3.1). Similar scenario was found in 2019, as only two sampled ‘NTU-031’ shoots differentiated apical inflorescences, and the other sampled shoot was apical aborted on 17 October, which was the only apical aborted shoot for the breeding line throughout the experiment, when daily mean temperature decreased from 28.2°C on 13 October to 21.5°C on 16 October (Fig. 3.4). Combine the results above, it seemed that apical flowering was promoted when daily mean temperature was 20-25°C, but less

doi:10.6342/NTU202000429

encouraged below 20 or above 27°C.

3.7. Conclusions

Apical flower bud differentiation was found in both years. When apical flower bud differentiation occurs, floral organs form directly on the uppermost meristem inside the apical bud, and new floral meristems form acropetally above the first differentiated floral meristem. Axillary flower buds form basipetally on the shoot after apical flower bud differentiation occurs. The occurrence of apical flowering is irrelevant to the progress of axillary flower bud differentiation but determined by the timing of shoot growth cessation: apical flowering occurs when flower bud initiates on shoots without growth cessation. However, growth cessation may occur even after apical flower bud differentiation.

Comparing the results of bud anatomy in 2018 and 2019, it is agreeable that under autumnal photoperiod between 11 and 12 h, apical flower bud differentiation was encouraged under mean air temperature between 20 and 25°C, but less promoted when mean temperature exceeded 27°C. Regardless of apical abortion, apical flower bud differentiation of different blueberry cultivars occurred in sequence of flower bud initiation as in axillary buds.

3.8. References

Bañados, M.P. and B. Strik. 2006. Manipulation of the annual growth cycle of blueberry

doi:10.6342/NTU202000429

using photoperiod. Acta Hort. 715:65-72.

Bieniasz, M. 2012. The differentiation of highbush blueberry flower buds. Acta Hort. 932:117-122.

Central Weather Bureau. 2018. Timetable of sunrise, sunset, twilight of Taipei, Taiwan. <https://www.cwb.gov.tw/V8/C/D/astronomy_data.html>.

Darnell, R.L. 1991. Photoperiod, carbon partitioning, and reproductive development in rabbiteye blueberry. J. Amer. Soc. Hort. Sci. 116:856-860.

Eck, P., R.E. Gough, I.V. Hall, and J.M. Spiers. 1990. Blueberry management, p. 273-333. In: Galetta, G.J. and D.G. Himerick (eds.), Small fruit crop management. Prentice Hall, Englewood Cliffs, N.J.

Fang, Y., J. Williamson, R. Darnell, Y. Li, and G. Liu. 2017. Nitrogen uptake and allocation at different growth stages of young southern highbush blueberry plants. HortScience 52:905-909.

Horiuchi, N., J. Che, H. Hoshino, and I. Ogiwara. 2013. Influence of high temperature and long-day treatments before endodormancy on morphological and ecological responses of three blueberry species. Hort. Res. (Japan) 12:281-288.

Kovaleski, A.P., J.G. Williamson, J.W. Olmstead, and R.L. Darnell. 2015. Inflorescence bud initiation, development, and bloom in two southern highbush blueberry cultivars. J. Amer. Soc. Hort. Sci. 140:38-44.

doi:10.6342/NTU202000429

Spann, T.M., J.G. Williamson, and R.L. Darnell. 2004. Photoperiod and temperature effects on growth and carbohydrate storage in southern highbush blueberry interspecific hybrid. J. Amer. Soc. Hort. Sci. 129:294-298.

Tamada, T. 1997. Flower-bud differentiation of highbush and rabbiteye blueberries. Acta Hort. 446:349-355.

Williamson, J.G. and P.M. Lyrene. 2004. Blueberry varieties for Florida. Univ. Fl. IFAS Ext. Bul. HS215.

Yáñez, P., J.B. Retamales, G.A. Lobos, and A. del Pozo. 2009. Light environment within mature rabbiteye blueberry canopies influences flower bud formation. Acta Hort. 810:471-474.

doi:10.6342/NTU202000429

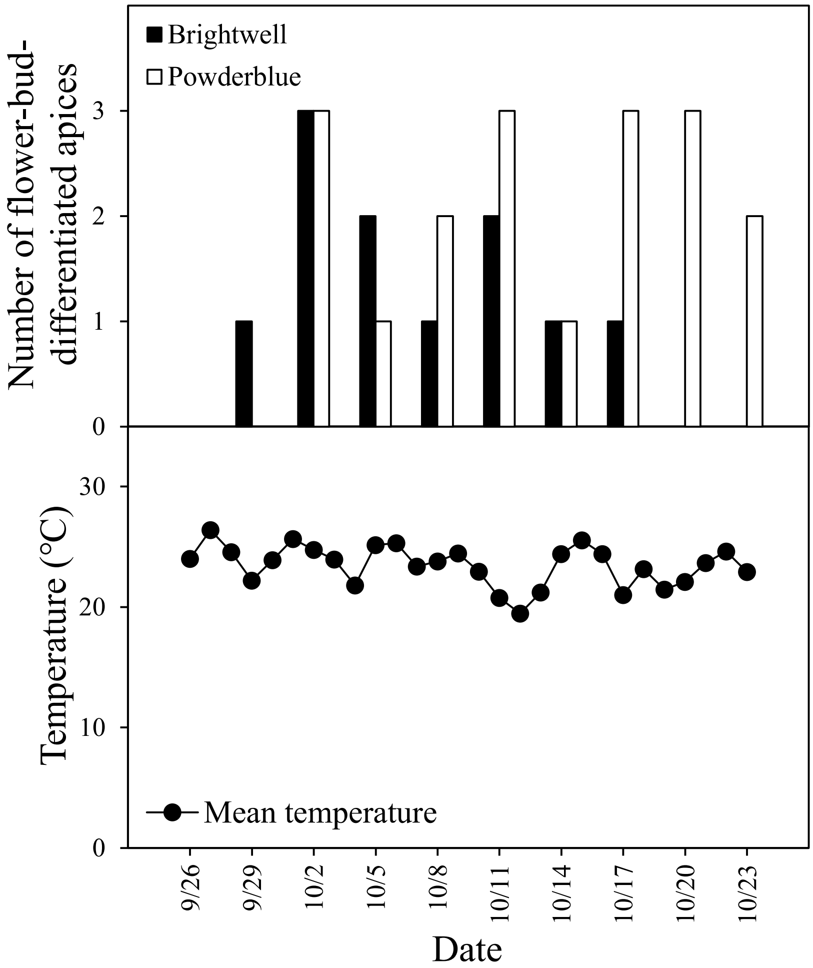


Fig. 3.1. Relationship between numbers of flower-bud-differentiated shoot apices of sampled cultivars and daily mean temperature on each sampling date in 2018.

doi:10.6342/NTU202000429

Table 3.1. Average bud internal developmental stage and shoot growth status of shoot apices of ‘Brightwell’ and ‘Powderblue’ rabbiteye blueberries in year 2018.

Bud position	Bud internal developmental stage									
	9/26	9/29	10/2	10/5	10/8	10/11	10/14	10/17	10/20	10/23
Brightwell										
Apical bud	1	1.33	4.33*	2.83	1.17	2.33	1.5	1.5	0*	0
Axil 1	1	1	3.17	1.17	1.33	3.5	1.83	2.5	2.17	3
Axil 2	1	1	1.83	1.17	1.17	3.5	1.83	0.83	2	2.5*
Axil 3	1	1	1.67	1.5	1	2.33	1.83	1.5	1.67*	1
Axil 4	1	1	1.5	1	1.17	2.17	1.83	1.17	1.5	1
Axil 5	1	1	1	1. 67	1.17	1.83	1.25	1.17	1.33	1

doi:10.6342/NTU202000429

Powderblue										
Apical bud	1.17	0.5	2.33	0.83	1.83	3.83	1.67	4.5	4.17	2.83
Axil 1	1	1.17	1.17	1	1.83	1.67	3.33	3.67	2.5	4.17
Axil 2	1	1	1.17	1.33	1	2	2.67	3.5	2.33	3.67
Axil 3	1	1	1.17	1.17	1.17	1.83	2.17	2	2.67	3
Axil 4	1	1	1	1	1	1.5	1.33	1.33	2.67	2.83
Axil 5	1	1	1	1	1	1.33	1.5	1.67	2	2.33
					Number of non-growth-ceased shoots					
Brightwell	3	1	3	3	1	2	2	1	0	0
Powderblue	3	1	3	1	2	3	1	3	3	2
					Number of flower bud differentiated shoots					
Brightwell	0	1	3	2	1	2	1	1	0	0
Powderblue	0	0	3	1	2	3	1	3	3	2

Means followed by asterisks indicate significant differences between cultivars in the bud internal developmental stage at a given node (two-sample t-test, $P \leq 0.05$).

doi:10.6342/NTU202000429

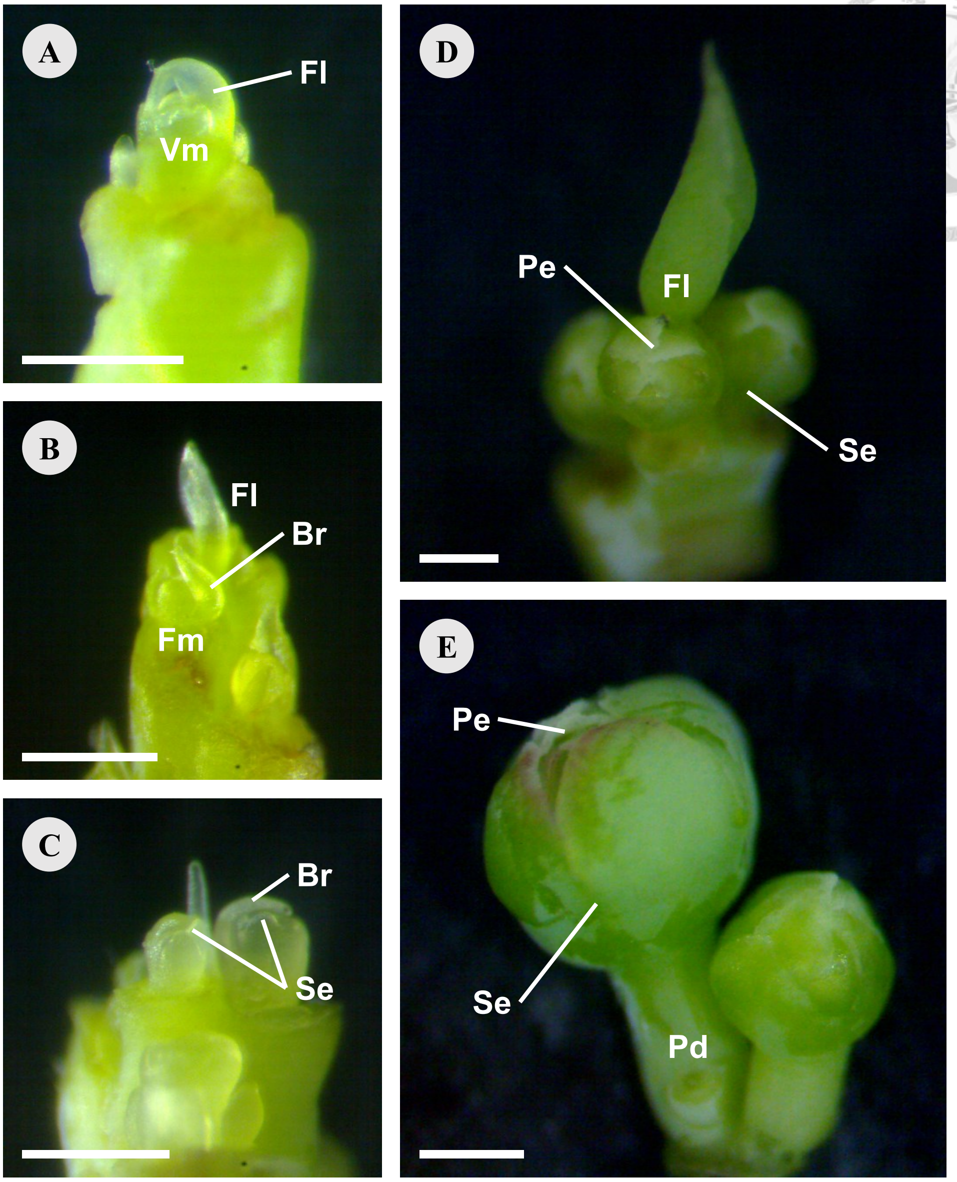


Fig. 3.2. Dissected apical buds of 'Powderblue' rabbiteye blueberry viewed with stereomicroscope; correspond to stages of internal development 1-5 described by Kovaleski et al. (2015). (A) Stage I-1: apical bud with vegetative meristem. (B) Stage I-2: apical bud with floral meristem visible. (C) Stage I-3: preliminary development of sepals. (D) Stage I-4: developed apical inflorescence. (E) Stage I-5: pedicel has expanded; Vm: vegetative meristem, Fl: foliage leaf, Fm: floral meristem, Br: bract, Se: sepal, Pe: petal, Pd: pedicel; bar = 500 μm.

doi:10.6342/NTU202000429

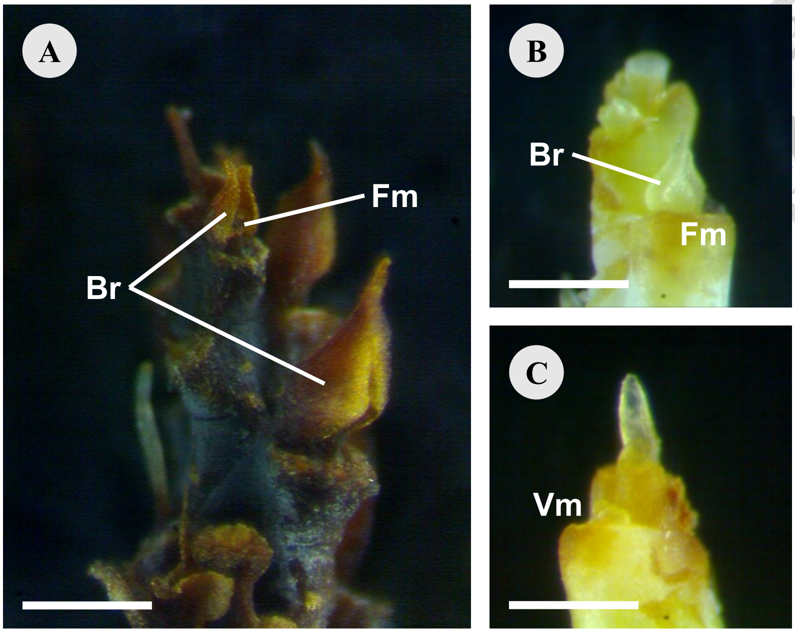


Fig. 3.3. Dissected totally or partially browning apical buds of 'Brightwell' (A, 23 October 2018), 'Powderblue' (B, 2 October 2019), and 'NTU-031' (C, 26 September 2019) viewed with stereomicroscope. Br: bract, Fm: floral meristem, Vm: vegetative meristem. Bar = 500 µm.

doi:10.6342/NTU202000429

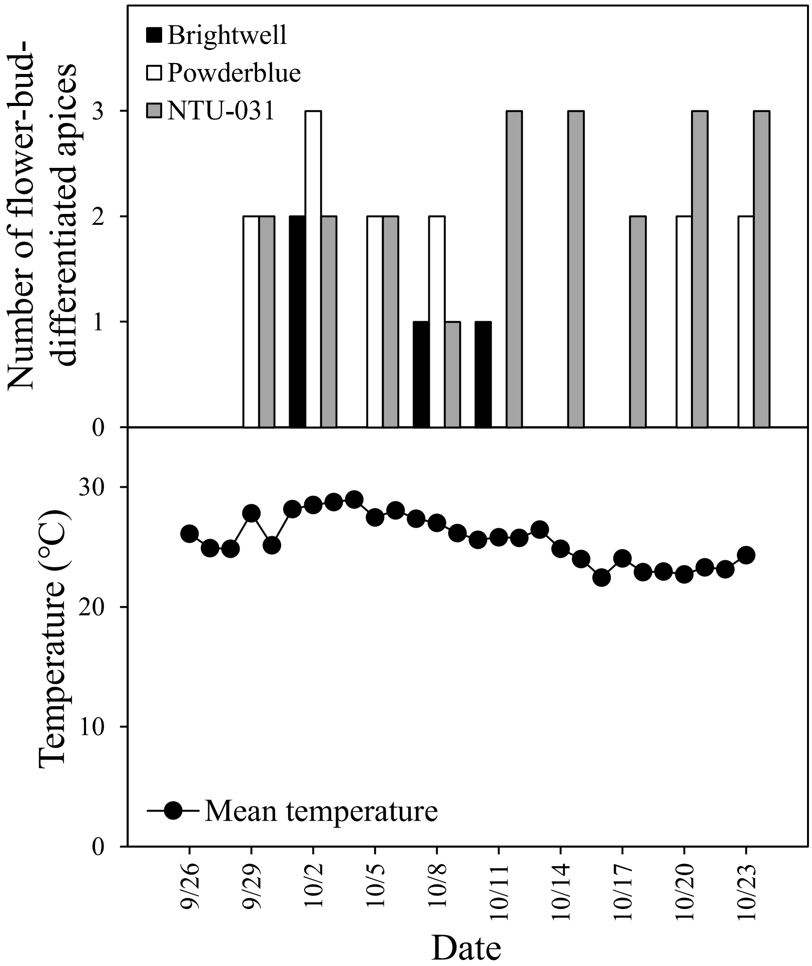


Fig. 3.4. Relationship between numbers of flower-bud-differentiated shoot apices of sampled cultivars and daily mean temperature on each sampling date in 2019.

doi:10.6342/NTU202000429

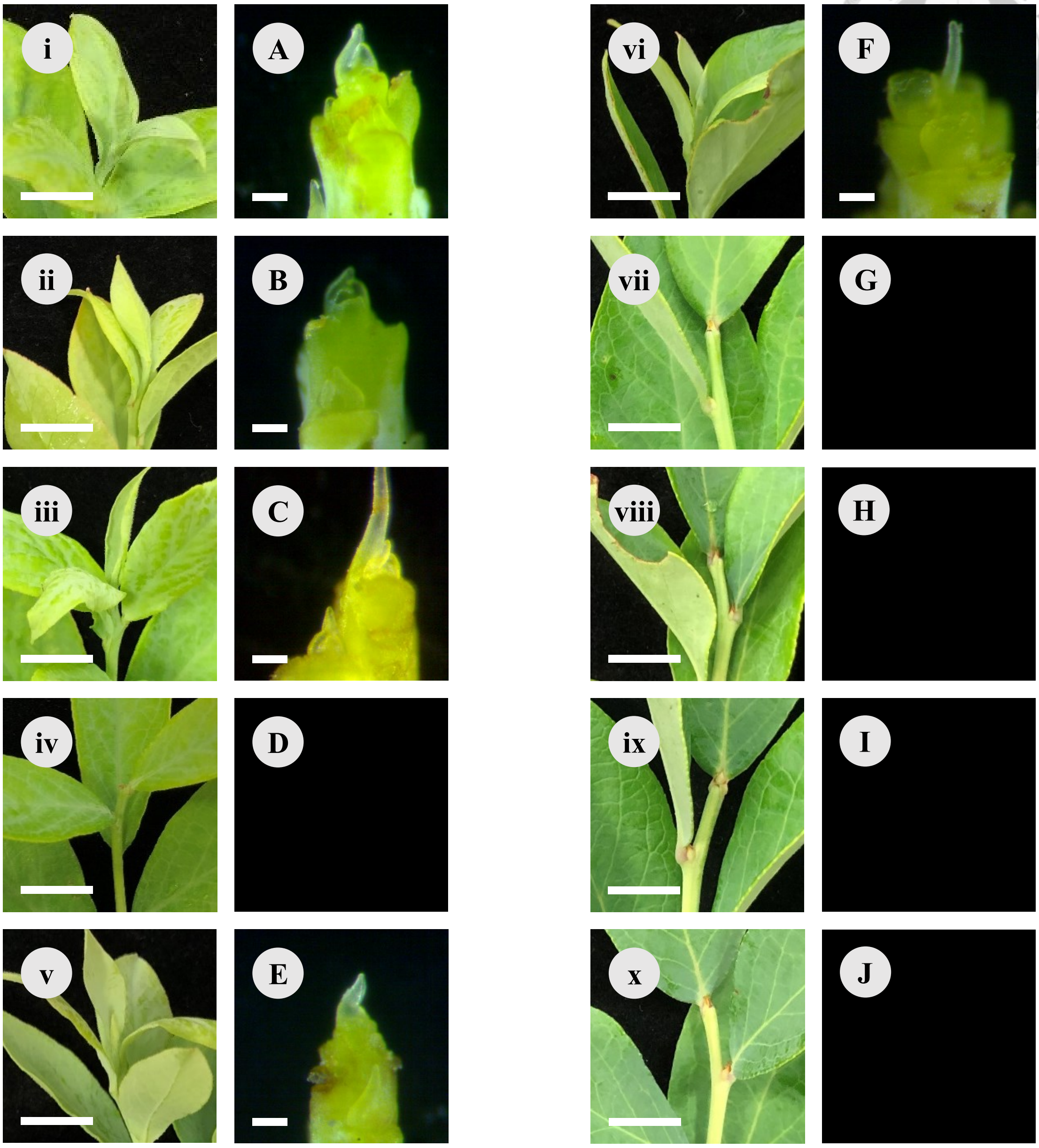


Fig. 3.5. Appearance of apical bud meristems (A to J) and shoot tip (i to x) of 'Brightwell' rabbiteye blueberry on each sample date in 2019. Bar in picture A to J: 200 µm; in picture i to x: 1 cm.

doi:10.6342/NTU202000429

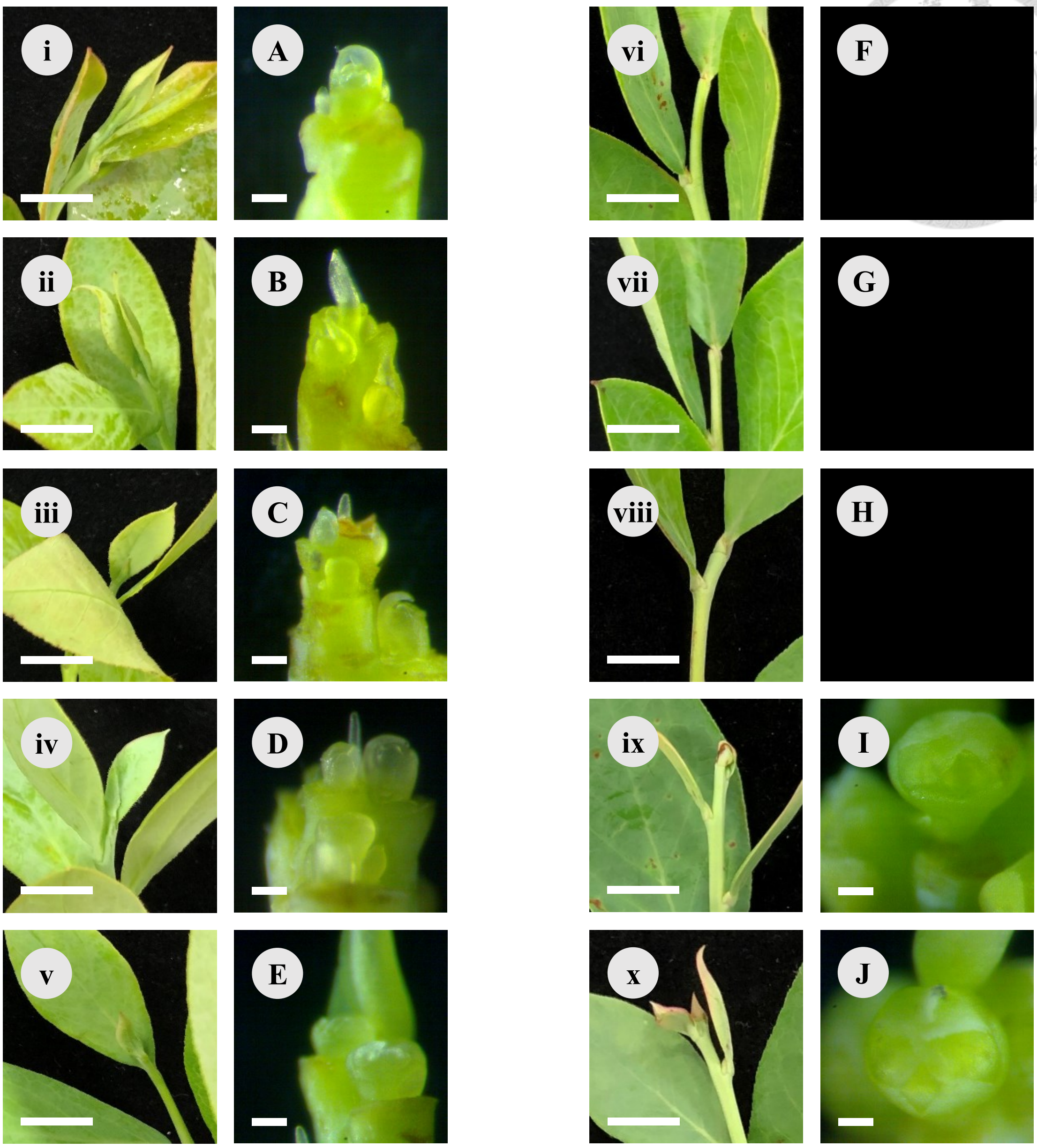


Fig. 3.6. Appearance of apical bud meristems (A to J) and shoot tip (i to x) of ‘Powderblue’ rabbiteye blueberry on each sample date in 2019. Bar in picture A to J: 200 μm; in picture i to x: 1 cm.

doi:10.6342/NTU202000429

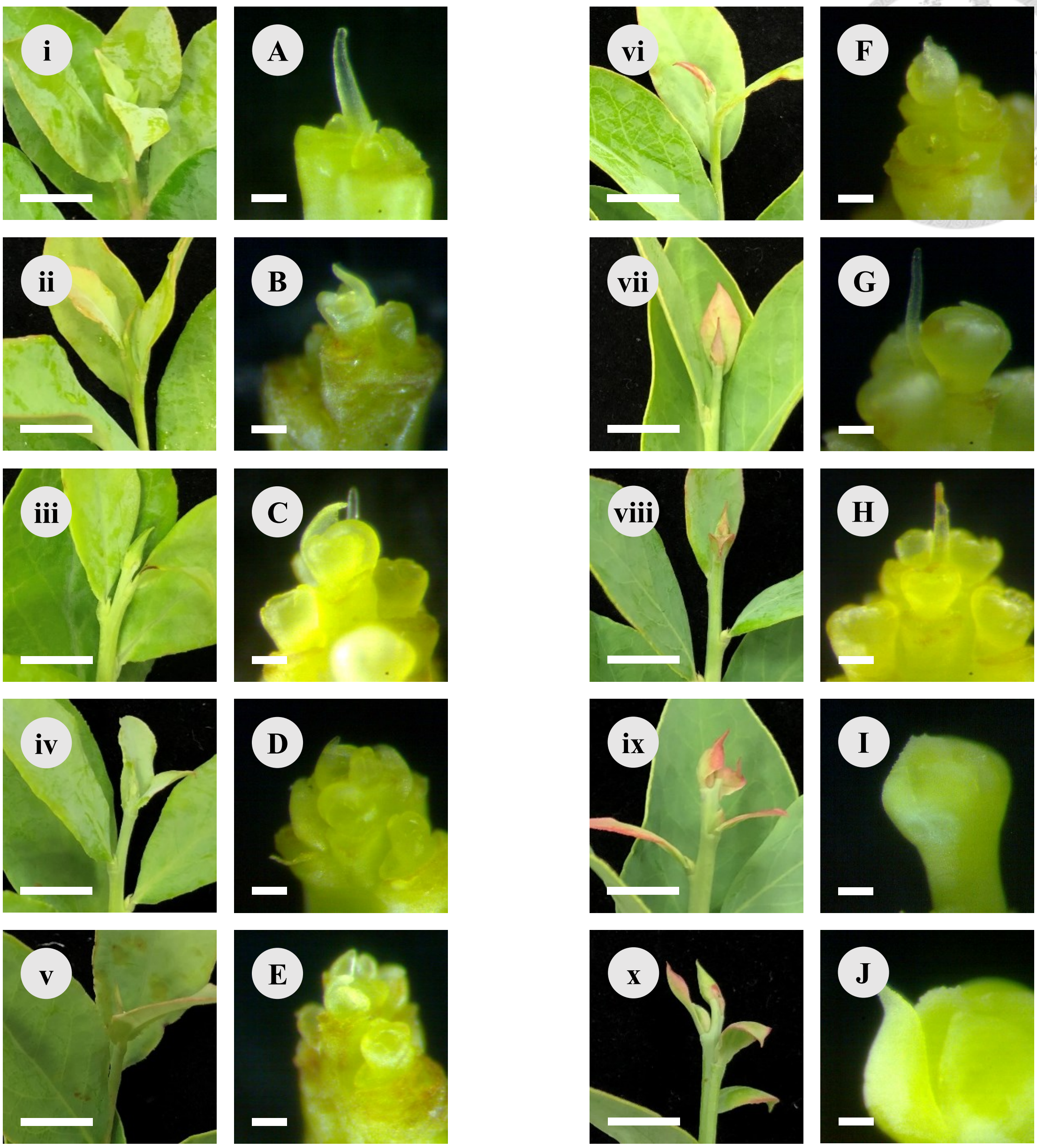


Fig. 3.7. Appearance of apical bud meristems (A to J) and shoot tip (i to x) of 'NTU-031' rabbiteye blueberry on each sample date in 2019. Bar in picture A to J: 200 µm; in picture i to x: 1 cm.

doi:10.6342/NTU202000429

Table 3.2. Average bud internal developmental stage and shoot growth status of shoot apices of ‘Brightwell’, ‘Powderblue’, and ‘NTU-031’ rabbiteye blueberries in year 2019.

Bud position	Bud internal developmental stage									
	9/26	9/29	10/2	10/5	10/8	10/11	10/14	10/17	10/20	10/23
Brightwell										
Apical bud	1.5	1.33	1. 67	0	0.5	1 ab	0 b	0	0 b	0 b
Axil 1	1	1	1	1	1.17	1.17	1.33	1.33	2	1.67
Axil 2	1	1	1	1	1.17	1	1.17	1.5	1.67	1.5
Axil 3	1	1	1	1	1	1.33	1.17	1.5	1.33	1.33
Powderblue										
Apical bud	1.17	1.67	2.5	1.83	2.17	0 b	0 b	0	2.67 ab	2.67 ab
Axil 1	1	1.17	1	1	1.17	1.83	1.5	2	4.17	2.5
Axil 2	1	1.17	1	1	1.33	1.5	1.33	1.5	1.67	1.67
Axil 3	1	1.17	1.17	1.17	1.17	1.33	1.5	1.33	1.83	1.5

doi:10.6342/NTU202000429

NTU-031										
Apical bud	1.33	1.83	2.5	2.17	2	3.17 a	3.83 a	1.83	3.83 a	4.17 a
Axil 1	1	1	1	1	1	1	1.33	1	2.67	3.33
Axil 2	1	1	1	1	1	1	1.17	1.5	2.5	3.33
Axil 3	1	1	1.33	1	1	1.33	1.67	1.67	2	3.17
Number of non-growth-ceased shoots										
Brightwell	3	3	2	0	1	1	0	0	0	0
Powderblue	3	3	3	2	2	0	0	0	2	2
NTU-031	3	3	3	3	3	3	3	2	3	3
Number of flower bud differentiated shoots										
Brightwell	0	0	2	0	1	1	0	0	0	0
Powderblue	0	2	3	2	2	0	0	0	2	2
NTU-031	0	2	2	2	1	3	3	2	3	3

Means followed by same alphabet indicate no significant difference between cultivars in the bud internal developmental stage at a given nod (Tukey HSD, $p \leq 0.05$).

doi:10.6342/NTU202000429

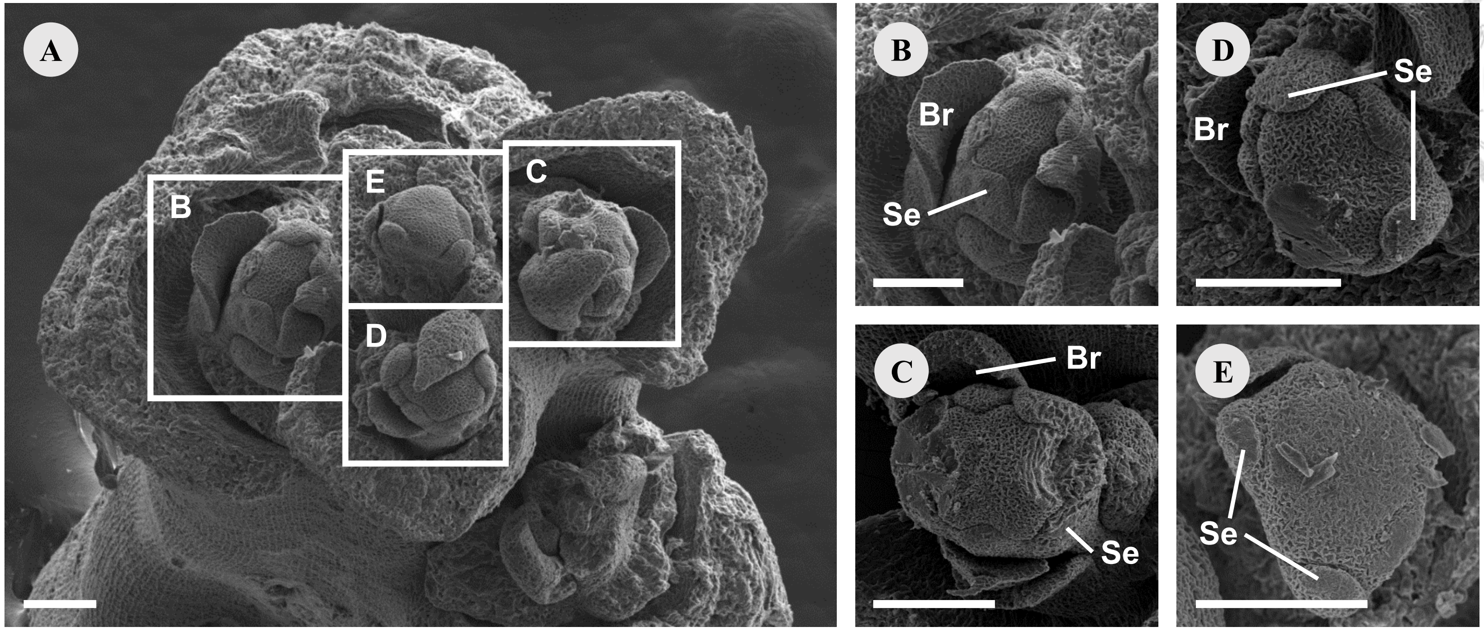


Fig. 3.8. Scanning electron microscopy of apical inflorescence (A) and florets of 'Powderblue' rabbiteye blueberry. Individual florets from picture B to E were arranged from base to top of the shoot apex. Br: bract; Se: sepal. Bar = 100 µm.

doi:10.6342/NTU202000429

Chapter four

General conclusions and future studies

4.1. General conclusions

Blueberries cultivated in lowland Taiwan often display apical flowering in autumn, which may offer potentials for off-season fruit production (Li, 2009). Previous studies indicated that blueberries grow vegetatively under long day photoperiod but cease shoot growth after two weeks of short day treatment (Bañados and Strik, 2006). In contrast, the reproductive growth begins when photoperiod is shorter than 12 h (Darnell, 1991). The phenomenon of apical flowering may be caused by mild temperature and shortening photoperiod of lowland Taiwan in autumn, which favors vegetative growth and reproductive growth simultaneously. In this thesis, field grown blueberry bushes were investigated in both overall and microscopic scale to determine the easy-apical-flowering cultivars under natural environmental conditions of subtropical lowland Taiwan.

The result from chapter two showed that most apical flowering cultivars were rabbiteye blueberries, probably due to the later occurrence of growth cessation coincided with flower bud initiation. The different intensity of apical flower bud differentiation and flowering between 2018 and 2019 might be given rise by high temperature between 22 September and 22 October in 2019. In chapter three, florets

doi:10.6342/NTU202000429

were found developing acropetally in apical buds when apical flowering occurs. The result also revealed that the tendency of apical flowering might be determined by whether the shoot tip is still growing when environmental conditions favor flower bud initiation. The optimum daily mean temperature for apical flower bud differentiation was found to be approximately 20-25°C. In contrast, daily mean temperature above 27 or below 20°C would inhibit apical flowering.

Though some cultivars rarely produce apical flowers in natural environment, flower-bud-initiated apices were still observed. Indicating that apical flowering can be promoted by preventing shoot cessation. Nevertheless, fruit quality was not investigated in this thesis. Consequently, the induction of autumnal shoot growth and apical fruit yield and quality are considerable subjects for future studies. Developing growing degree days model for apical flowering is also suggested to predict intensity and yield more precisely.

4.2. References

Bañados, M.P. and B. Strik. 2006. Manipulation of the annual growth cycle of blueberry using photoperiod. Acta Hort. 715:65-72.

Darnell, R.L. 1991. Photoperiod, carbon partitioning, and reproductive development in rabbiteye blueberry. J. Amer. Soc. Hort. Sci. 116:856-860.

Li, K.T. 2009. BLUE FORMOSA - a blueberry initiative program in Taiwan.

doi:10.6342/NTU202000429

HortScience 44:1122 (Abstr.).

doi:10.6342/NTU202000429

Appendix 1. Plant materials investigated in 2018 trial.

Species	Cultivars	Number of bushes
NHB	Weymouth	8
SHB	Georgiagem	8
	Misty	4
	O'Neal	12
	Sunshineblue	4
RB	Austin	4
	Beckyblue	10
	Brightwell	8
	Climax	6
	Fukuberry	4
	Gloria	4
	Homebell	5
	Myers	6
	NTU-009	7
	NTU-025	10
	NTU-031	5
	NTU-032	8
	NTU-054	10
	NTU-103	13
	NTU-108	5
	Powderblue	12

doi:10.6342/NTU202000429

	Tifblue	21
Total		174

NHB: northern highbush blueberry, SHB: southern highbush blueberry, RB: rabbiteye blueberry.

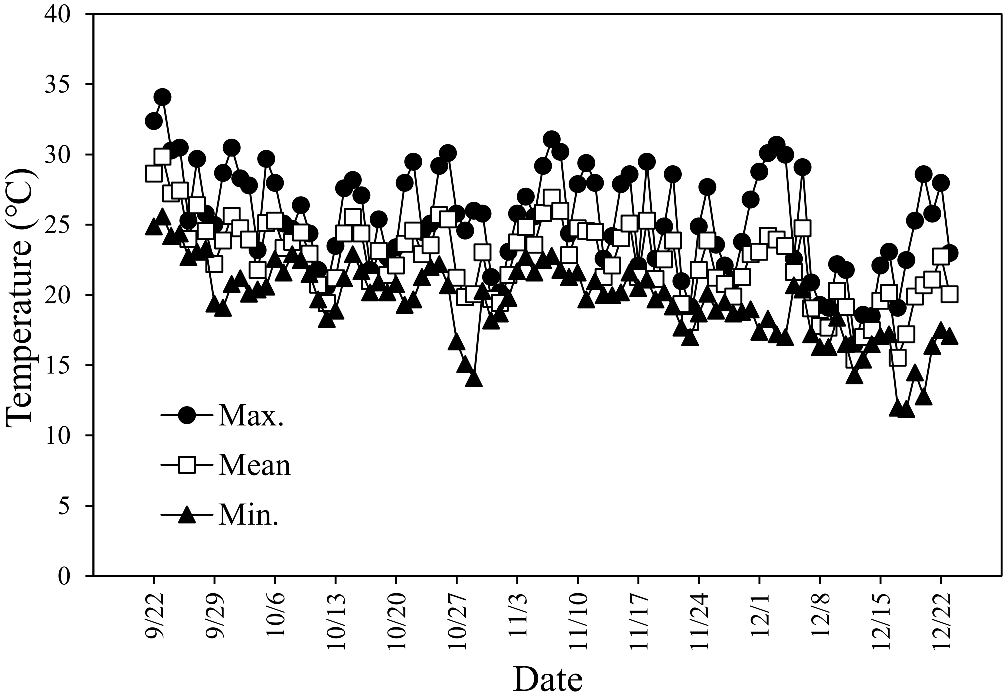
doi:10.6342/NTU202000429

Appendix 2. Parentage and origin of investigated rabbiteye blueberries.

Cultivar	Parentage (F)	Parentage (M)	Origin
Austin	T-110	Brightwell	Ga.
Brightwell	Tifblue	Menditoo	Ga.
Climax	Callaway	Ethel	Ga.
Fukuberry	Woodard	OP	Japan
Gloria	Tifblue	Woodard	Ga.
Homebell	Myers	Black Giant	Ga.
Myers	Wild selection		Fl.
NTU-009	Tifblue	OP	Taiwan
NTU-025	Blueshower	OP	Taiwan
NTU-031	Woodard	OP	Taiwan
NTU-032	Woodard	OP	Taiwan
NTU-054	Tifblue	OP	Taiwan
NTU-103	Blueshower	OP	Taiwan
NTU-108	Woodard	OP	Taiwan
Powderblue	Tifblue	Menditoo	N.C.
Tifblue	Ethel	Clara	Ga.

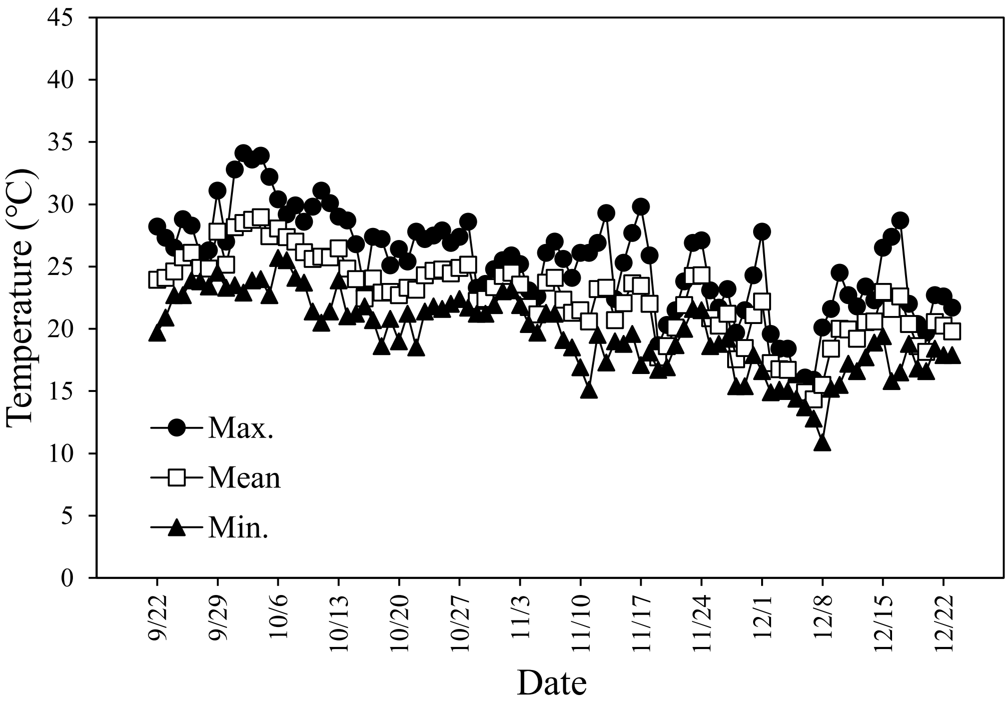
OP: open pollinated.

doi:10.6342/NTU202000429



Appendix 3. Daily maximum, mean, and minimum air temperature from 22 September to 23 December 2018 (source: Department of Atmospheric Sciences, NTU).

doi:10.6342/NTU202000429



Appendix 4. Daily maximum, mean, and minimum air temperature from 22 September to 23 December 2019 (source: Department of Atmospheric Sciences, NTU).

doi:10.6342/NTU202000429