



**ALEXANDRE DIAS DA SILVA**

**SILICON ENHANCES QUALITY AND YIELD OF  
RASPBERRY AND CALCIUM UPTAKE IN THE  
DEVELOPMENT OF RASPBERRY AND BLACKBERRY  
FRUITS**

**LAVRAS-MG  
2024**

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agronomia/Fitotecnia, área de concentração em Produção Vegetal, para a obtenção do título de Doutor.

Prof. Dr. Rafael Pio  
Orientador

Prof. Dra. Lisa Wasko Devetter  
Coorientador

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**ALEXANDRE DIAS DA SILVA**

**SILÍCIO MELHORA A QUALIDADE E PRODUTIVIDADE DA FRAMBOESEIRA E  
ACÚMULO DE CÁLCIO NO DESENVOLVIMENTO DE FRUTOS DE FRAMBOESA  
E AMORA PRETA**

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DEFENSE on February 20, 2024.

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**LAVRAS-MG  
2024**

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## RESUMO

O presente trabalho focou na nutrição mineral de framboeseiras e amoreiras, investigando especificamente os efeitos do silício e do cálcio. O objetivo principal do estudo inicial foi avaliar a influência do silício (Si) no crescimento e na qualidade da framboesa (*Rubus idaeus*). O secundário. O objetivo foi determinar o momento específico e a duração do pico de absorção de cálcio (Ca) em frutos de framboesa e amora-preta (*Rubus* subgênero *Rubus*), para orientação para otimizar estratégias de aplicação de fertilizantes. O ensaio inicial consistiu em sete tratamentos, que foram atribuídos aleatoriamente a quatro blocos separados dentro de um local caracterizado por um clima subtropical. Cada tratamento foi constituído com três vasos, com uma planta da cultivar 'Batum' por vaso. Em 2020, o solo foi aplicado com diferentes concentrações de Si (0, 50, 100, 200, 400, 800 ou 1600 mg dm<sup>-3</sup>) 15 dias após o transplante. No segundo experimento realizado em 2022, amostras de frutos foram coletadas em sete estádios diferentes de desenvolvimento de três cultivares de framboesas ('Meeker', 'WakeField' e 'WakeHaven') e três cultivares de amora-preta ('Marion', 'Black Diamond' e 'Columbia Star'). A primeira parte da pesquisa envolveu fazer análises de campo que abrangeram a quantificação de clorofilas a e b, potencial hídrico foliar e produtividade, também foram realizadas as análises pós-colheita em laboratório, e os parâmetros analisados foram cor, firmeza, taxa respiratória, sólidos solúveis e pH. Os resultados indicaram que o uso da adubação com Si resultou em aumento significativo tanto na quantidade de frutos quanto rendimento de framboesa por planta. O Si aumentou o rendimento e a firmeza dos frutos, mas simultaneamente reduziu a água potencial e taxa respiratória. O segundo experimento resultou que a maior absorção de Ca em cultivares de framboesa ocorreram entre o estágio S4 (verde imaturo) e Estágio S6 (fruto branco). Nas cultivares 'Black Diamond', o máximo de absorção ocorreu entre os estágios S4 e S5 (verde maduro), enquanto em 'Columbia Star' e 'Marion', ocorreu entre os estágios S4 e S6. Esses resultados sugerem que aplicação de fertilizante à base de Ca seriam, durante os estágios S4 a S6, mais eficazes, pois se alinha aos períodos de maior absorção pelas plantas.

Palavras-chave: *Rubus idaeus*; *Rubus sp.*; nutriente benéfico; nutrição vegetal; estresses abióticos; marcha de absorção de nutrientes.

## ABSTRACT

The current study focused on mineral nutrition of caneberry, specifically investigating the effects of silicon and calcium. The primary aim of the initial study was to assess the influence of silicon (Si) on the growth and quality of raspberry (*Rubus idaeus*). The secondary objective was to determine the specific timing and duration of peak calcium (Ca) absorption in raspberry and trailing blackberry (*Rubus* subgenus *Rubus*) fruits, in order to provide guidance for optimizing fertilizer application strategies. The initial trial consisted of seven distinct treatments, which were randomly assigned to four separate blocks within a location characterized by a subtropical climate. Each treatment had three pots with a 'Batum' primocane-fruiting raspberry plant. In 2020, the soil was treated with different concentrations of Si (0, 50, 100, 200, 400, 800, or 1600 mg dm<sup>-3</sup>) 15 days after transplanting. In the second experiment conducted in 2022, samples of fruits were collected across seven different phases of development from three florican-fruiting raspberry ('Meeker,' 'WakeField,' and 'WakeHaven') and three blackberry ('Marion,' 'Black Diamond,' and 'Columbia Star') cultivars. The initial research involved doing field analyses that encompassed the quantification of chlorophylls a and b, leaf water potential, and yield. The study of the fruit encompassed parameters such as color, firmness, respiration frequency, soluble solids, and pH. The findings indicated that the use of Si fertilization resulted in a significant augmentation in both the quantity of fruits and raspberry yield per plant. Si enhanced fruit yield and firmness, but concurrently reduced water potential and respiratory rate. The second study revealed that the highest absorption of Ca in raspberry cultivars occurred between the S4 stage (middle developed/"immature green") and S6 stage (immature fruit/"white fruit"). In 'Black Diamond' blackberries, the peak absorption occurred between S4 and S5 stages (mature green), while in 'Columbia Star' and 'Marion' blackberries, it occurred between S4 and S6 stages. These findings suggest that applying Ca fertilizer during the S4 to S6 stages would be most effective, as it aligns with the periods of highest uptake by plants.

Keywords: *Rubus idaeus*; *Rubus* sp.; beneficial nutrient; plant nutrition; abiotic stresses; tissue analysis; foliar feeding; accumulation of nutrients; march of nutrient absorption; dry matter accumulation.

## **IMPACTOS SOCIAIS, TECNOLÓGICOS, ECONÔMICOS E CULTURAIS**

A tese intitulada “Silicon enhances quality and yield of raspberry and calcium uptake in the development of raspberry and blackberry fruits” desenvolvida pelo discente Alexandre Dias da Silva, sob a orientação do Professor Rafael Pio da Universidade Federal de Lavras (UFLA) e com a coorientação da Professora Lisa Wasko DeVetter da Washington State University (WSU), terá impactos positivos para os produtores de framboesas e amora-preta e para toda a cadeia de produção, reduzindo custos e otimizando recursos e processos, uma vez que esses estudos desenvolvidos no presente trabalho foi resultados de uma demanda vinda dos produtores, um dos questionamentos dos produtores respondido foi o efeito do Silício (Si) na cultura da framboesa, para isso a primeira parte do presente estudo concentrou-se na nutrição mineral de framboeseiras, investigando especificamente os efeitos do silício, o objetivo foi avaliar a influência do silício (Si) no crescimento e na qualidade da framboesa (*Rubus idaeus*). A segunda parte foi determinar a marcha de absorção de cálcio (Ca) em frutos de framboesa e amora-preta (*Rubus* subgênero *Rubus*), a fim de fornecer orientação para otimizar estratégias de aplicação de fertilizantes pelos produtores. A primeira parte do trabalho foi desenvolvida na UFLA, no qual regou a publicação do artigo científico “Silicon application for the production and quality of raspberry fruit in a subtropical region” publicado na revista Pesquisa Agropecuária Brasileira – PAB, essa pesquisa também gerou a publicação e apresentação na forma de postes de 3 resumos científicos e 1 resumo de extensão apresentados a comunidade acadêmica nos congressos de iniciação científica e de extensão da universidade. A segunda parte do trabalho foi realizada no Estado de Washington – Estados Unidos, gerando a publicação do artigo “Calcium Accumulation in Developing Fruits of Raspberry and Blackberry” na revista *Acta Horticulturae*, 1 artigo de extensão e uma apresentação oral no WA Small Fruit Conference 2022. Os resultados de ambos os trabalhos continuam sendo apresentados em eventos científicos e de extensão, orientando tanto os produtores brasileiros sobre o uso do Si na cultura da framboesa, quando os produtores de amora e framboesa, sobre o uso eficiente do Ca nos Estados Unidos.

## **SOCIAL, TECHNOLOGICAL, ECONOMIC AND CULTURAL IMPACTS**

The thesis entitled “Silicon improves raspberry quality and yield and calcium absorption in the development of raspberry and blackberry fruits” developed by student Alexandre Dias da Silva, under the guidance of Professor Rafael Pio from the Federal University of Lavras (UFLA) and with the co-supervision by Professor Lisa Wasko DeVetter from Washington State University (WSU), will have positive impacts for raspberry and blackberry growers and the entire production chain, reducing costs and optimizing resources and processes, since these studies developed in the present work were the result of a demand from growers, one of the growers questions answered was the effect of Silicon (Si) on raspberry cultivation, for this reason the first part of the present study focused on the mineral nutrition of raspberry trees, specifically investigating the effects of silicon, the objective was to evaluate the influence of silicon (Si) on the growth and quality of raspberries (*Rubus idaeus*). The second part was to determine the rate of calcium (Ca) absorption in raspberry and blackberry fruits (*Rubus* subgenus *Rubus*), in order to provide guidance for optimizing fertilizer application strategies by growers. The first part of the work was developed at UFLA, which led to the publication of the scientific article “Application of silicon for production and quality of raspberry fruits in the subtropical region” published in the journal *Pesquisa Agropecuária Brasileira – PAB*, this research also generated the publication and in the form of posts with 3 scientific abstracts and 1 extension summary presented to the academic community, presented at the university's scientific initiation and extension conferences. The second part of the work was carried out in the State of Washington – United States, generating the publication of the article “Calcium Accumulation in Development Fruits of Raspberry and Blackberry” in the journal *Acta Horticulturae*, 1 extension article and an oral presentation at the WA Small Fruit Conference 2022 The results of both works continue to be presented at scientific and extension events, guiding both Brazilian producers on the use of Si in raspberry cultivation, and blackberry and raspberry producers on the efficient use of Ca in the United States.

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## **FIRST PART**

## 1 INTRODUÇÃO

Red raspberry (*Rubus idaeus* L.) and blackberries (*Rubus* subspecies *Rubus*) are species in the Rosacea family, this family includes plants such as blackberries and raspberries, which are cultivated for the production of fruit suitable for both direct to the flesh market and processing. In recent years, there has been a significant increase in the global production of blackberries and raspberries. This growth may be attributed to the rising demand for these fruits, which are known to have positive effects on human health, particularly in reducing the risk of degenerative illnesses (Guedes *et al.*, 2013). The plant is extensively farmed in areas characterized by a moderate environment. Through genetic enhancement efforts in Brazil and the USA, several cultivars have been modified to withstand low temperatures (Curi *et al.*, 2015; Oliveira *et al.*, 2020).

The cultivation of raspberry and blackberries has become increasingly popular in Brazil over the past decade. In particular, these caneberry species has garnered attention and piqued the curiosity of small rural producers. It has emerged as a new cultivation option for diversifying small rural holdings. Among the category of small temperate fruits, this crop distinguishes itself due to its cost-effective implementation and rapid capital turnover, owing to the high value contributed by the fruits (Antunes; Hoffmann, 2012).

In Brazil, there has been a noticeable rise in the cultivation of blackberry and raspberry, particularly in subtropical regions. These regions experience higher temperatures during autumn, winter, and especially summer, compared to areas with a temperate climate where raspberry is traditionally cultivated (Campagnolo; Pio, 2012; Moura *et al.*, 2012). Cultivating caneberry outside of a temperate climate can result in declines in productivity and quality, necessitating the exploration of techniques to improve its cultivation. This work focused on the role of silicon on improving productivity and quality in a subtropical environment as well as uptake periods of calcium in fruits of raspberry and blackberry. Both nutrients can be deficient and are known to have positive effects on fruit quality

## 2 Referencial Teorico

### 2.1 Raspberry

Raspberry (*Rubus idaeus* L.) is a member of the Rosaceae family. It is native to central and northern Europe, particularly in hilly areas of the Mediterranean and Asia. The fruits of this plant are characterised by their red colour and possess a taste that is both sweet and sour. However, certain genotypes may produce yellow berries as a result of recessive genes that cause production of very low concentration of anthocyanin (Oliveira *et al.*, 2017). European red fruit cultivars are the most widely grown and commercially significant, according to Aprea, Biasioli and Gasperi (2015). Marchi *et al.* (2019) stated that raspberry cultivars grown in Brazil originate from North America and Europe. As a result, Brazilian production relies on imported cultivars, which may differ in terms of phenology, fruit production, and quality compared to their original location. Additionally, raspberry cultivation in Brazil is characterized by high production per unit area and a significant need for labour. Therefore, the economic sustainability of this industry depends on the efficiency of the agricultural practices employed in the production system. The fruits of red raspberries are economically significant because of their appealing shape and high content of polyphenols and antioxidants (Gündeşli; Korkmaz; Okatan, 2019). Red raspberries also have a distinctive phytochemical profile, containing abundant ellagitannins and anthocyanins, which sets them apart from other berries and fruits (Rao; Snyder, 2010). The product can be consumed in its raw state, as well as in frozen or processed forms, such as jellies and juices. Raspberry fruits offer the advantage of containing anthocyanins, which are significant natural organic compounds. These compounds serve as edible pigments and play a crucial role in preventing tumours, senile diseases, cardiovascular diseases, and oxidative stress (Teng *et al.*, 2017). Anthocyanins, a kind of flavonoid, has potent antioxidant properties, enabling them to safeguard cells and the body from oxidation by neutralising free radicals (Huynh *et al.*, 2019). Despite the development of cultivars that are more durable with firmer fruit, raspberries remain very perishable (Huynh *et al.*, 2019). This is mostly due to their fragile nature, since the fruit continues to respire at high rates and rapidly becomes soft after being harvested (Zheng; Hrazdina, 2010). As a result, the shelf life of raspberries is often limited. Hence, in order to effectively market fresh fruit, it is imperative to enhance the fruit's quality, prolong its post-harvest lifespan, and provide proper storage conditions.

## 2.2 Blackberry

Blackberries include trailing, erect, and semi-erect types with trailing blackberry considered (*Rubus* subspecies *Rubus*) as having highest fruit quality (Finn *et al.*, 2014). Blackberry is cultivated for the purpose of producing fruits that are consumed fresh or used in processing. The growth of blackberries can be attributed to various factors, including the development of better cultivars, increased marketing initiatives, enhanced fruit availability, and a general rise in berry consumption, particularly in the form of fresh fruit, across many regions worldwide (Clark *et al.*, 2014). The plant is adapted to a moderate climate, but some erect types show adaptation to higher temperatures. Through genetic enhancement efforts in Brazil and the USA, several cultivars have been modified to withstand low temperatures (Curi *et al.*, 2015; Oliveira *et al.*, 2020).

Blackberries belongs to the *Rubus* genus of the Rosaceae family. This genus contains around 750 species, highlighting the subgenus *Rubus*, which include *R. armeniacus*, *R. laciniatus* and *Rubus* hybrids (Daubeney, 1996).

The *Rubus* genus represents the largest group of commercial blackberry cultivars in the world. world due to its rusticity and adverse conditions in the cultivation environment, which cause its location is currently more diverse compared to other genres, with possible to find it on the American, European, African and Asian continents, containing among 400 to 500 species (Poling, 1996).

The blackberry is a perennial species, with a decumbent growth habit, semi-erect and erect, spinescent or inert (Lorenzi *et al.*, 2006). Some species are described as deciduous and others vegetate all year round, the types of reproduction range from sexual the apomictic (Moore, 1993). However, the plant produces fruits on secondary stems, the apomixis a recessive genetic character for blackberries (Raseira, 2004).

The branches of the blackberries are generally erect or creeping shrubs, and may present different colors, from green to brown, depending on the stage of the vegetative development (Attilio, 2009).

The leaves are described as alternate imparifoliolate, green and shiny on the side. upper, white and tomentose on the lower (Attilio, 2009). They are made up of leaflets cartaceous, glabrous above, with hair scattered in various directions on the upper part lower, with a variable size of 4-7 cm in length (Attilio, 2009).

The curls they form in the axil of the leaves, regularly from branches of the previous year, and the floral receptacle conical in shape, well developed, elevated in relation to the flower with androgenic characteristics solitary or grouped, axillary, have five sepals and five petals and several stamens and carpels arranged around (Antunes; Raseira, 2004; Poling, 1996). The fruits of the blackberries are generally aggregated, called mini drupes, in that each contains only one hard seed. Since each drupe is a true fruit, the structure that they generally call fruit, is, in fact, an aggregate of fruits, thus, the blackberry is an aggregate of drupeoles that are united to a common receptacle or drupe, containing smaller seeds (Oliveira, 2006). These are normally composed of formed by 75 to 85 drupes (Pagot *et al.*, 2007), of the globose type, presenting varied in green, red and black according to the stage of maturity, and when fully ripe, flavors can range from sweet to sour-sweet or bittersweet, depending on cultivars and management methods and conditions. Blackberry maturation can be determined by the surface color of the fruit, completely black berries, firmness, soluble solids, titratable acidity and characteristic aroma (Coutinho; Machado; Cantillano, 2004).

### **2.3 Economic importance**

The world population's is increasing, leading to a rising need for food, both in terms of quantity and quality (Gupta *et al.*, 2020). Fruits, which are abundant in vitamins, lipids, proteins, fibre, carbohydrates, and mineral nutrients that are crucial for the body's maintenance, regulation, and growth, have become the preferred choice among consumers (Saath; Fachinello, 2018).

Blackberries and raspberries are primarily grown in temperate regions, particularly in Europe (Pio; Gonçalves, 2018). However, their production is global and covers an estimated area of 25 to 30 thousand hectares. The typical yields obtained from traditional farming methods range from 8,000 to 20,000 kg per hectare, contingent upon factors such as soil quality, climatic conditions, crop variety, and the chosen production strategy (Strik *et al.*, 2007; Clark; Finn, 2014).

## 2.4 Groups and cultivars

Raspberry plants are divided into two categories: 1) primocane-fruiting (or ever-bearing), which produces fruit on current-season primocanes, and 2) floricanes-fruiting, which produce fruit on second-year floricanes (Carew *et al.*, 2000).

According to Oliveira (2006) a cultivar is considered remontant means it can flower more than once in a season, also knowing with primocane-fruiting raspberries are considered remontant and therefore produce flowers and fruit twice in a growing season (depending on management) whereas floricanes-fruiting raspberries only flower and fruit once in a growing season also knowing with no Non-remontant. - It should also be noted that vegetative growth differs during periods of flowering and fruiting.

### 2.4.1 Raspberry cultivars

**Batum** is a cultivar that exhibits a low chilling need and has successfully acclimated to the southern region of Minas Gerais, Brazil. This plant has a growth pattern resembling that of primocane-fruiting 'Autumn Bliss'. 'Batum' bears oval-shaped, red fruits. It is particularly suitable for places with moderate cold temperatures (Pagot *et al.*, 2007). Pagot *et al.* (2007) states that 'Batum' is the most widely grown cultivar in the southern region of Minas Gerais. Moura *et al.* (2012) found that in the southern region of Minas Gerais, this particular cultivar yields approximately 5 tonnes per hectare in its second year of production. This is considered a high value compared to other widely grown cultivars in Brazil, such as 'Heritage' and 'Autumn Bliss', which are better suited for the southern region of the country. The raspberry plants of this particular cultivar have a reflowering tendency (Raseira *et al.*, 2004). This cultivar is extensively distributed in Serra da Mantiqueira, although there is no documented evidence of its presence outside this region. There is a possibility that 'Batum' is actually the cultivar 'Autumn Britten', which was named 'Britten' by growers in Brazil and then renamed 'Batum' (CAPRONI *et al.*, 2016).

**Meeker** is a floricanes-fruiting red raspberry hybrid developed by Washington State University in 1967 and it remains a favorite for commercial production as well as for home gardeners. Sturdy canes produce sweet, juicy red berries in late summer. The berries have a high sugar content and are excellent for eating fresh or preserving. 'Meeker' has replaced its parent, 'Willamette' as one of the most widely planted red raspberry cultivars in the Pacific Northwest which is the major production region for the crop in North America. 'Meeker' often

occupies more than 60% of the plantings in the region which includes western parts of Oregon and Washington and southwestern British Columbia.

**WakeField** is a floricanne-fruiting red raspberry from The New Zealand Institute for Plant and Food Research Limited [Plant and Food Research (PFR)] and Northwest Plant Company joint raspberry breeding program (Stephens; Enfield; Hall, 2012). WakeField produces high yields of very firm, medium sized fruit mid-to-late in the season and is well suited to machine harvesting and process markets. Machine-harvested fruit are frequently tunnel frozen to produce individual quick frozen (IQF) products. WakeField plants appear to have some tolerance to Raspberry Bushy Dwarf Virus (RBDV) and to root rot caused by *Phytophthora rubi* (Stephens; Enfield; Hall, 2012). The cultivar was created in 1990 at PFR (formerly HortResearch), Motueka, New Zealand. The seed parent used to make the cross was the unpatented selection ORUS 576-47, since named ‘Lewis’ (Finn *et al.*, 2001) from the Oregon State University, U.S. Department of Agriculture, Agriculture Research Service Rubus breeding program. The selection 86105M57 from the PFR New Zealand breeding program was the pollen parent (Stephens; Enfield; Hall, 2012).

**WakeHaven** is a floricanne-fruiting red raspberry cultivar also from The New Zealand Institute for Plant & Food Research Limited (PFR) and Northwest Plant Company (NWPCo.) (Stephens *et al.*, 2017). Characterized as floricanne fruiting cultivar that produces high yields of medium-large, firm fruit in the midseason, it is similarly well suited to machine harvest and processing applications. WakeHaven’ appears to have resistance to Raspberry bushy dwarf virus (RBDV) and tolerance root rot caused by *Phytophthora rubi* (Stephens *et al.*, 2017). The cultivar was created as the result of a planned cross carried out by The New Zealand Institute for PFR

#### 2.4.2 Blackberry cultivars

**Marion** or “marionberry” is a trailing blackberry cultivar released by the U.S. Department of Agriculture Agricultural Research Service (USDA-ARS) breeding program in Corvallis, Oregon and remains very popular in the worldwide. The fruit quality of ‘Marion’ is more superior than other cultivars such as ‘Thornless Evergreen’. ‘Marion’ precedes ‘Thornless Evergreen’ and produces a higher yield than traditional blackberry cultivars with the added benefit of improved fruit quality (Waldo *et al.*, 1957).

Marion blackberry has a striking resemblance to 'Himalaya' blackberry (*Rubus armeniacus*) in terms of its overall look and growth pattern. However, the leaves exhibit a somewhat brighter shade of green and possess a bigger size compared to 'Himalaya'. Typically, there are only a limited number of lengthy canes, usually measuring 4,8 to 6,1 meters in length. These few elongated canes are simple to train.

The vigorous development of Marion and other blackberry cultivars makes it difficult to use various training systems, if more canes are required to adhere to a certain training scheme, a segment of the new cane can be extracted. This will cause the expulsion of several branches. Marion blackberry has high productivity despite its limited number of canes. This is due to the fruiting buds being in very close proximity to one another, while the internodes are short in length. Moreover, the fruiting branches have elongated morphology with many flowers and fruits per lateral (Waldo *et al.*, 1957).

**Black Diamond** is another cultivar of trailing blackberry developed by the USDA-ARS breeding programme in Corvallis, Oregon. It was released in collaboration with the Oregon State University Agricultural Experiment Station and the Washington State University Agricultural Research Centre (Finn *et al.*, 2005). 'Black Diamond' has great productivity, robust growth, is well-suited for mechanical harvesting, and yields an excellent processed product. Furthermore, the fruit possesses sufficient firmness and the outer layer of the drupelets is sufficiently durable, making it suitable for certain fresh market uses (Finn *et al.*, 2005). 'Black Diamond' was developed in 1997 by crossing 'Kotata' with NZ 8610L-163 (E90 × N- 71), a hybrid developed by Harvey Hall of New Zealand HortResearch Inc. 'Kotata' is another trailing blackberry cultivar that is commercially grown. It is heptaploid, meaning it has seven sets of chromosomes ( $2n = 7x = 49$ ). 'Black Diamond' is a result of crossbreeding between eastern and western blackberry varieties as well as well as 'Logan' and 'Boysen' hybrids. The spinelessness trait was obtained from 'Austin Thornless' (Finn *et al.*, 2005).

**Columbia Star** is a thornless, trailing blackberry cultivar also released from the USDA-ARS breeding program in Corvallis, OR, released in cooperation with the Oregon State University's Agricultural Experiment Station. 'Columbia Star' is the first thornless cultivar to be introduced that has inherited its thornlessness from the 'Lincoln Logan' source. This is distinct from the original 'Lincoln Logan' and 'Waimate' cultivars, which have fruit similar to the 'Logan' type, as well as 'Marahau' and 'Boysen'. 'Columbia Star' is a superior cultivar known for its exceptional fruit quality and great output. It can be easily harvested by machines and does not have thorns. The fruit of this blackberry is firm and delicious, and when processed, it is comparable to or even better than the fruit from the well-established varieties 'Marion' and

'Black Diamond'. The cultivation of 'Columbia Star' should be limited to regions where other trailing blackberry varieties can thrive. The name acknowledges the significance of the Columbia River in the geography and history of the Pacific Northwest (Finn *et al.*, 2014).

## 2.5 Mineral nutrition

The supply and uptake of specific chemical elements and their compounds required for normal growth and metabolism of plants is defined as plant nutrition. Elements and chemical compounds that function as raw materials for the synthesis of structural and functional plant substances are called nutrients. Mineral elements are available for absorption by plant roots, as ions present in soil water. In the soil, nutrients can be: in the soil solution, adsorbed on organic or inorganic particles, in the form of an insoluble inorganic compound, and/or as a component of organic compounds.

Most of the elements that are necessary for plant development are present in minerals found in rocks. When rocks weather and break down, their minerals are gradually converted into ions and inorganic compounds that will be made available to plant roots in the soil. Because these ions and inorganic compounds initially come from minerals in rocks, the study of their role in plant nutrition is called mineral nutrition (Mauseth, 2009).

Of all the mineral elements absorbed, not all are considered essential. Essential nutrients or elements are those required for the healthy development of plants. Initially determination of essential elements was done by trial and error, i.e., adding various chemical compounds to the nutrient solution. It was then verified that there were some aspects to take into consideration in these studies (Mauseth, 2009).

Nutrients that are considered essential for plants are divided into the following groups: 1) organic (C, O and H), and 2) minerals. Minerals are further divided into two groups: 1) macronutrients take up in large quantities (N, P, K, Ca, Mg and S) and 2) micronutrients taken up in comparatively smaller quantities (B, Cl, Cu, Fe, Mn, Mo, Ni and Zn). Some elements can affect the growth and development of plants, although conditions have not been determined to characterize them as essential.

Macronutrients generally have their levels expressed as a percentage (%) and micronutrients in parts per million (ppm), all in elemental form. The only distinction in the classification between macro- and micronutrients is the concentration required by plants.

Macronutrients occur in concentrations 10 to 5,000 times higher than micronutrients (Epstein, 1975).

Nutrients may also be classified into three categories:

- a) Essential – these are the plant's mineral nutrients without which the plant cannot live. Calcium is considered essential and is the main theme of the second article in this study.
- b) Useful – these are not essential as the plant can live without them; however, their presence is capable of contributing positively to the growth and production of the plant. Silicon is often considered a useful nutrient and is the main theme for the first article in this study.
- c) Toxic – essential or not, when they are harmful to the plant. An essential element is potentially toxic, depending on its concentration in the medium.

### **2.5.1 Silicon**

Silicon (Si) is the second most abundant element in the lithosphere and together with oxygen it represents 50-70% of the Earth's crust (Ma; Yamaji, 2008). However, due to intense cultivation, soils can quickly have reduced availability of this element to plants (Korndörfer, 2006).

Silicon has garnered attention from researchers in recent years, owing to the advantageous effects it exerts on some agricultural crops. One notable advantage of this element is its ability to modify the plant's physiology by depositing compounds in the tissues, forming a silicon structure. This structure has been found to reduce water consumption and minimise losses caused by pests and diseases as it becomes difficult for them to penetrate plant tissues (Reis *et al.*, 2007).

Silicon can decrease transpiration and enhance the photosynthetic rate of plants by improving leaf structure (Ref?). Additionally, Si can diminish the respiratory activity of fruits (Reis *et al.*, 2007). In addition, Si can enhance the ability of plants to withstand bending and increase the strength of their tissues. It also offers protection against non-living factors, such as reducing the harmful effects of excess manganese, iron, and sodium (Epstein, 2001), and improving the plant's ability to resist excess aluminium (Wiese, 2007). Furthermore, it may play a role in the metabolic or physiological functions of plants when they are exposed to high salinity or water stress (Gunes *et al.*, 2008).

Silicon induces leaf blade tissue thickening and the accumulation of epicuticular wax (BRAGA *et al.*, 2009). This component enhances the plant-environment system by equipping plants with the ability to tolerate and overcome challenges related to climate, soil conditions, and biological factors. Silicon functions as a suppressor of inherent pressures, alleviating, for instance, the consequences brought about by severe temperatures (Epstein, 2001; Braga *et al.*, 2009). Si may also enhance the post-harvest quality of fruits by affecting the levels of anthocyanins, soluble solids, and titratable acidity, hence influencing their economic value (Silva *et al.*, 2013).

In their study on strawberry (*Fragaria × ananassa*) plants, Figueiredo *et al.* (2010) observed that varying dosages of Si resulted in enhanced production, higher sugar content, and alterations in both internal and exterior colour of the strawberries. In line with Silva *et al.* (2013), who conducted a study on the impact of Si on strawberry fruit production and quality, it was found that Si played a significant role in increasing fruit yield. Regardless of whether it was applied through soil or foliar methods, silicon consistently enhanced the overall cultivation of strawberries. Based on these favorable findings, the primary objective of this study was to assess the impact of varying dosages of Si on the yield, quality, and physiological attributes of raspberries cultivated in a subtropical environment.

### 2.5.2 Calcium

Calcium (Ca) is an essential macronutrient needed for plant growth and development under both non-stressed and stressed conditions through its structural, signaling, and enzymatic functions. One important structural function of Ca is cell wall strengthening and promoting tissue and organ integrity by serving as a key constituent of the middle lamella between cells through pectin binding (Zamil; Geitmann, 2017; Demarty; Morvan; Thellier, 1984).

Calcium is a divalent cation that readily enters the apoplast and is bound to the cell wall and exterior surface of the plasma membrane in an exchangeable form. Calcium maintains the cell wall structure of the fruit by interacting with the pectic acid in the cell walls to form Capectate. Divalent forms of Ca cross-links between pairs of negatively charged homogalacturonans, thus tightening the cell wall (Turmanidze *et al.*, 2017).

The structural function of Ca is frequently associated with fruit firmness and overall quality of multiple horticultural crops, as insufficient Ca levels can cause declines in fruit

quality (De Freitas; Mitcham, 2012). Multiple studies have documented the consequences of insufficient Ca on fruit quality, such as bitter pit in apple (*Malus domestica*), blossom end rot in tomato (*Solanum lycopersicum*), internal flesh breakdown in mango (*Mangifera indica*), and internal tissue browning and premature fruit drop in ‘Draper’ northern highbush blueberry (*Vaccinium corymbosum*) (Ferguson; Watkins, 1989; Gerbrandt; Mouritzen; Sweeney, 2019; MA *et al.*, 2023; Ho; White, 2005).

Accumulation and distribution of Ca in fruits is also highly dependent on water availability, cell wall interactions in the apoplast, and transpiration which in turn influences the concentration of Ca in the xylem (Hocking *et al.*, 2016; Yang *et al.*, 2020). Prior research with other crops including highbush blueberry, grape (*Vitis vinifera*), kiwifruit (*Actinidia deliciosa*), tomato, and apple indicate key periods of Ca accumulation in fruits occurs early in their development (Cline *et al.*, 1991; Ho; White, 2005; Gerbrandt *et al.*, 2019; Montanaro *et al.*, 2015; Rogiers *et al.*, 2006). Xylem vessels have been shown to restrict during later stages of fruit development and impede flow of Ca dissolved in xylem sap to developing fruits (Ho; White, 2005). Accumulation of epicuticular wax at later stages of fruit development can further reduce transpiration and overall xylem flow to developing fruits (ROGIERS *et al.*, 2004). Epicuticular wax development in highbush blueberry was negatively correlated with stomatal conductance (Yang; Bryla; Strik, 2019) and provides further evidence that Ca accumulation may be restricted by micro-surface features of developing fruits.

To mitigate deficiencies and imbalances, growers often apply Ca fertilizers to soil or plant canopies (i.e., “foliar feeding”). However, the overall efficacy of these applications is mixed. Vance, Jones e Strik (2017) found foliar applications of Ca had no effect on fruit quality or shelf life in raspberry, highbush blueberry, strawberry, and blackberry. Arrington and De Vetter (2017) also found similar results for commercially available foliar and soil-applied Ca in highbush blueberry. In contrast, Gerbrandt *et al.* (2019) found foliar Ca was able to correct deficiencies in blueberry when applied frequently and at high concentration from mid-bloom onward. Pre-harvest foliar Ca application also increased fruit Ca content and improved fruit quality in kiwifruit (Morton *et al.*, 2018) and reduced incidence of fruit cracking in pomegranate (*Punica granatum*) (Davarpanah *et al.*, 2018). Applications of calcium on sweet cherries (*Prunus avium*) during bud dormancy can effectively improve sweet cherry fruit characteristics, in terms of calcium content, cracking incidence, and fruit set and a mix of Ca plus copper at post-bloom in cherries and apples improved fruit resistance to cracking and firmness (Michailidis *et al.*, 2021; Brown *et al.*, 1996). The purpose of the second study was to explore timing of Ca uptake in raspberry and blackberry to inform timing of Ca fertilizer.

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**SECOND PART – ARTICLES\***

**ARTICLE 1 - SILICON APPLICATION FOR THE PRODUCTION AND QUALITY OF RASPBERRY FRUIT IN A SUBTROPICAL REGION**

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Pomology/ Original Article

# Silicon application for the production and quality of raspberry fruit in a subtropical region

**Abstract** – The objective of this work was to evaluate the effect of silicon (Si) on the cultivation and quality of raspberries (*Rubus idaeus*). The experiment consisted of seven treatments and four blocks located in a subtropical region. Each plot consisted of three pots with one seedling of 'Batum' raspberry. In each pot, the treatment consisted of Si doses at 0, 50, 100, 200, 400, 800, or 1600 mg dm<sup>-3</sup>, which were applied to the soil 15 days after the transplanting of the seedlings. Field analyses were performed by measuring chlorophyll a and b, water potential, and production. Fruit were analyzed for color, firmness, respiratory rate, soluble solids, and pH. Fertilization with Si stimulates the increase of fruit number and of the raspberry production per plant. The Si application increases the fruit production and fruit firmness; however, it reduces the water potential and respiration rate.

**Index terms:** *Rubus idaeus*, abiotic stresses, plant nutrition.

## Aplicação de silício para a produção e qualidade de frutos de framboesa em região subtropical

**Resumo** – O objetivo deste trabalho foi avaliar o efeito do silício (Si) sobre o cultivo e a qualidade de framboesa (*Rubus idaeus*). O experimento consistiu de sete tratamentos e quatro blocos em região de clima subtropical. Cada parcela foi composta de três vasos com uma muda da framboeseira 'Batum'. Em cada vaso, o tratamento consistiu de doses de Si a 0, 50, 100, 200, 400, 800 ou 1600 mg dm<sup>-3</sup>, que foram aplicadas ao solo 15 dias após o transplante das mudas. As análises de campo foram realizadas pela medição de clorofilas a e b, potencial hídrico e produção. Os frutos foram analisados quanto à cor, firmeza, taxa respiratória, sólidos solúveis e pH. A adubação com Si estimula o aumento do número de frutos e da produção de framboesa por planta. O silício promove o aumento da produção e a firmeza dos frutos, no entanto, reduz o potencial hídrico e a taxa de respiração.

**Termos para indexação:** *Rubus idaeus*, estresse abiótico, nutrição vegetal.

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## Introduction

Fruit trees of the genus *Rubus* are highly appreciated for their organoleptic characteristics (Guedes et al., 2014; Raseira et al., 2020). More recently, they have also been valued for their health benefits that result from their high levels of antioxidants, vitamins, minerals, fibers, and folic acid, among others (Guedes et al., 2013; Maro et al., 2013, 2014).



Raspberry (*Rubus idaeus* L.) stands out among the fruit species of the genus *Rubus* for its good marketing ability and use in the agroindustry. Raspberry is a species traditionally exploited in temperate regions, mainly in North America, Europe, and Asian countries (Pio et al., 2019).

In the last 10 years, the exploitation of raspberry tree began to advance to subtropical regions. Several studies have been done on the selection of cultivars adapted to the subtropical climate and on the adaptation of their cultural management (Moura et al., 2012; Curi et al., 2014). In the subtropical regions where raspberry trees have been grown, the temperatures are higher in the spring and summer than in their natural habitat (Souza et al., 2014; Rizzi et al., 2020). Their productive performance in subtropical areas is encouraging because the production of raspberry concentrates between November and June, with some cultivars producing large amounts of fruit, as is the case of the Batum cultivar (Campagnolo & Pio, 2012b; Moura et al., 2012). Since temperatures in the subtropical regions are high during the harvest of raspberry trees, it is necessary to develop technologies to increase the production and decrease the respiratory rate of raspberries, aiming to increase their durability after harvest.

In recent years, Si has attracted interest from researchers, due to the benefits that this element brings to some agricultural crops. Among the advantages of Si are the ways that this element modifies the physiology of the plant, in the form of compounds that are deposited in the tissues and form a structure of silicon, which reduces the water consumption by the plant and the losses related to the incidence of pests and diseases, due to the difficulty of pests to penetrate the tissues (Silva et al., 2013).

Silicon can act to reduce transpiration, favor the photosynthetic rate of plants by improving the leaf architecture, and reduce the respiratory activity of fruits (Silva et al., 2013). It can provide a greater tolerance to lodging and greater structural rigidity of tissues, as well as protection against abiotic stresses, such as by reducing the toxicity of manganese, iron, and sodium, and greater resistance to excess aluminum. In addition, silicon increases the thickness of the leaf blade tissues and the deposition of epicuticular wax (Braga et al., 2009). This element optimizes the plant-environment system, since it can help plants withstand climatic,

edaphic, and biological adversities. Silicon acts as an inhibitor of natural stresses by mitigating, for instance, the impacts caused by extreme temperatures (Braga et al., 2009). Silicon can also lead to increased postharvest fruit quality by promoting changes in the concentrations of anthocyanins, soluble solids, and titratable acidity, which can translate into higher market quality (Silva et al., 2013).

Kowal et al. (2020) studied strawberry plants and reported higher productivity, increased sugar content, and changes in internal and external color of strawberries exposed to different Si doses. Silva et al. (2013) evaluated the effect of Si on the production and quality of strawberry fruit and reported that Si increased fruit production and promoted improvements in strawberry fruit quality, regardless of the application method (soil or leaf).

The objective of this work was to evaluate the effect of Si on the cultivation and quality of raspberry.

## Materials and Methods

The experiment was conducted in the state of Minas Gerais, Brazil, at 21°14'S, 45°00'W at 918 m above sea level. According to the Köppen-Geiger's classification, the climate of the region is of the Cwb type, that is, tropical high altitude (mesothermal), with dry winters and concentrated rains from October to March (Alvares et al., 2013). The climate data for the experimental period are presented (Figure 1).

The experiment was carried out in 11 L plastic pots, which were filled with 10 L of clay soil sieved through a 4 mm mesh sieve. The chemical and physical attributes (Table 1) were determined from soil analyses. Soil liming was performed to increase base saturation to 80%, by applying 2,465 kg of lime to 1 m<sup>3</sup> soil, with 36% CaO and 12% MgO contents. The limestone was mixed with the soil until a homogeneous mixture was attained. After homogenization, the soil was distributed in the pots and incubated for 90 days with moisture at field capacity. Based on the soil analysis, the planting fertilization was performed on the day the seedlings were planted, using 8 g of N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O of the formula 04-14-08 per pot. For the correction and fertilization of raspberry growing, recommendations were followed according to Pio (2018).

Each pot received one raspberry seedling of 'Batum' raspberry produced by using root cuttings

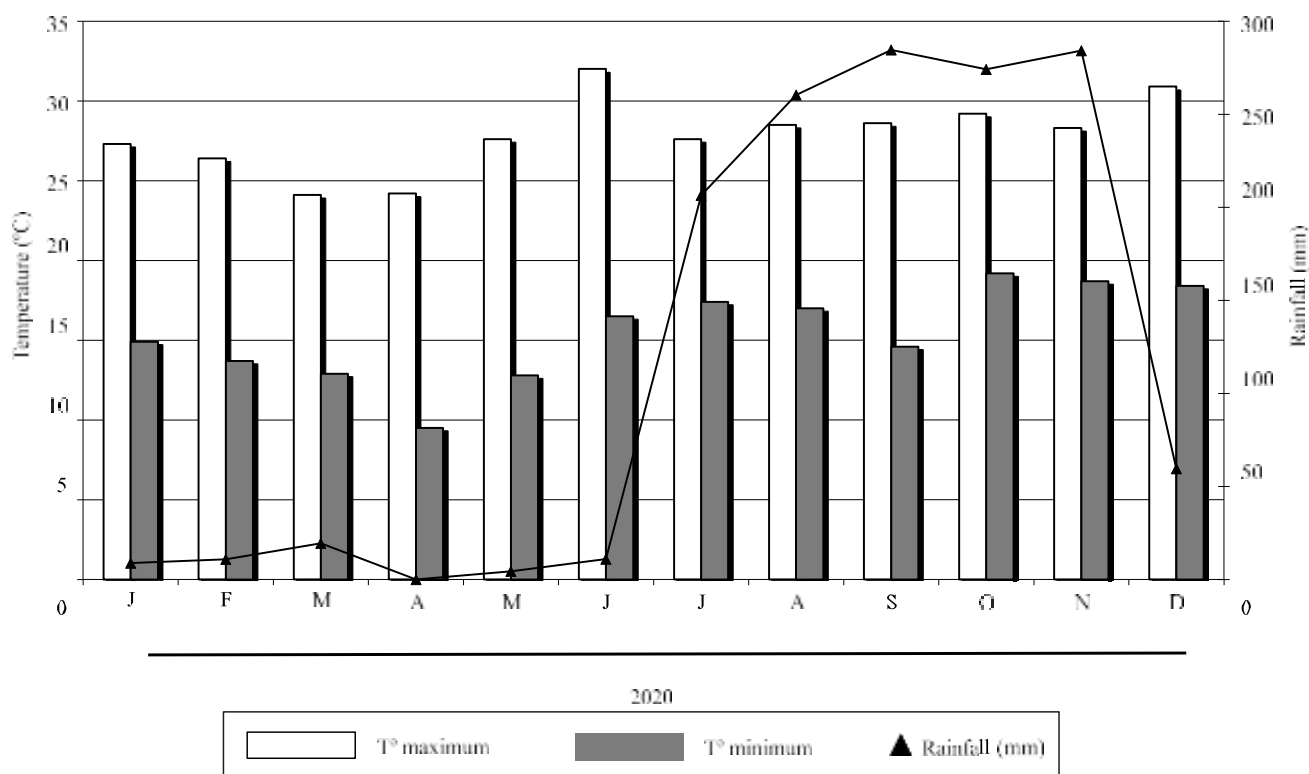
(Campagnolo & Pio, 2012a) in a greenhouse. Before being taken to the field, the 20 cm seedlings were selected and acclimatized according Pio (2018). Irrigation was performed daily, using drippers in pots for water supply.

The experimental design was randomized blocks with four replicates. The treatments consisted of six Si doses, applied to the soil, and one control treatment (without Si application). The Si doses were 0, 50, 100, 200, 400, 800, and 1,600 mg dm<sup>-3</sup> of soil. The source of Si was AgriSil (Agrobiológica: Soluções Naturais, Leme, SP, Brazil), at 98% SiO<sub>2</sub> concentration. The Si concentrations in the treatments were applied by surface incorporation to the soil 15 days after transplanting the seedlings.

Crop treatments normally required for the cultivation of raspberry were performed throughout the experiment, such as weed control, monitoring for possible attacks of pests and diseases, and topdressing fertilization (Moura et al., 2012; Curi et al., 2014). Three topdressing applications were performed at 30-day intervals, and 2 g of urea (45% N) and 2 g

of potassium chloride (58% K<sub>2</sub>O) were used for each application. All crop treatments, such as topdressing fertilizer application, were performed in all pots of the experiment.

At 90 days after transplanting the seedlings, which corresponded to the beginning of fruit production, the chlorophyll a and b concentrations were measured in the treatments on the 3rd and 4th newly developed leaves, in each pot. Readings were performed in the three plants in the plot, and their mean values were used. Levels of chlorophyll content indexes a and b were determined using a SPAD-502 portable chlorophyll meter (Minolta Camera Co., Osaka, Japan), which non-destructively and instantaneously measures the light transmittance through the leaf, in wavelengths with one peak at 650 nm, a region of high absorbance by chlorophyll molecules, and another peak at 940 nm, in which the absorbance by the leaf is low, serving as a correction factor for the water content or leaf thickness. Total chlorophyll was calculated by adding chlorophyll a and b.



**Figure 1.** Mean maximum and minimum temperatures and mean monthly cumulative rainfall between January 2020 and December 2020.

At 100 days after the seedling transplanting, leaf water potential was measured using a pressure chamber Scholander pump (PMS Instrument Company, Albany, OR, USA). Twelve leaves, immediately below the panicles, were collected for each treatment, and the mean of the readings was calculated. The measurements were performed when plants were already at the beginning of production. The measurements were performed in the morning, between 1:00 h and 6:00 h, when leaves would have their stomata closed, due to the low light intensity, which was considered the baseline potential.

Fruit were harvested three times a week throughout the experiment, time at which they were counted and weighed. At the end, the total production was calculated by adding all harvests performed throughout the production cycle.

Fruit were taken to the laboratory for color analysis, which was performed at three different points of the fruit using the CR-400 Minolta Colorimeter

(Minolta Camera Co., Osaka, Japan) in CIE mode, by determining the color ( $L^*$ ), chromaticity ( $C^*$ ), and hue angle (hue\*). The respiratory rate was analyzed using glass containers with one fruit of known mass, after the aliquots of the internal sample were removed with the aid of the PBI Dansensor gas analyzer (Ametek Mocon, MIN, USA). The results, which are expressed as percentage of  $CO_2$ , were converted to  $mL CO_2 kg^{-1} h^{-1}$ , considering the volume of the container, the mass and volume of fruit in each container, and the time that such containers remained closed. Firmness was measured by a manual digital fruit hardness tester (Instrutherm, PTR-300, São Paulo, Brazil), with a 3 mm diameter probe. The evaluations were performed at the center of the fruit surface, and the results were expressed in newtons (N). The content of total soluble solids (% °Brix) was determined in a digital refractometer according to the AOAC method (AOAC International, 2023). pH was determined with a Schott Handylab pH meter, according to the AOAC technique (Latimer Jr., 2023).

The results were subjected to the analysis of variance by the F-test, at 5% probability. When significant, a regression analysis was performed by the F-test, at 5% probability, using the statistical program R.

**Table 1.** Chemical and physical characteristics of the soil used in the experiment.

Characteristic	Unit	Result
pH in water - 1:2.5 ratio		4.4
Potassium (Mehlich-1)	mg dm <sup>-3</sup>	59.18
Phosphorus (Mehlich-1)	mg dm <sup>-3</sup>	0.61
Calcium (1 mol L <sup>-1</sup> KCl)	cmol <sub>c</sub> dm <sup>-3</sup>	1.08
Magnesium (1 mol L <sup>-1</sup> KCl)	cmol <sub>c</sub> dm <sup>-3</sup>	0.20
Aluminum	cmol <sub>c</sub> dm <sup>-3</sup>	0.53
Hydrogen + aluminum (extractor: SMP)	cmol <sub>c</sub> dm <sup>-3</sup>	6.52
BS = sum of exchangeable bases	cmol <sub>c</sub> dm <sup>-3</sup>	1.43
CEC (t) – effective cation exchange capacity	cmol <sub>c</sub> dm <sup>-3</sup>	1.96
CEC (T) – CEC at pH 7.0	cmol <sub>c</sub> dm <sup>-3</sup>	7.95
V – base saturation index	%	18.01
m – aluminum saturation index	%	27.04
Organic matter	g kg <sup>-1</sup>	2.95
Remaining phosphorus	mg L <sup>-1</sup>	10.0
Zinc (Mehlich-1)	mg dm <sup>-3</sup>	0.72
Iron (Mehlich-1)	mg dm <sup>-3</sup>	71.27
Manganese (Mehlich-1)	mg dm <sup>-3</sup>	43.75
Copper (Mehlich-1)	mg dm <sup>-3</sup>	3.05
Boron (hot water extractor)	mg dm <sup>-3</sup>	0.06
Sulfur (monocalcium phosphate in acetic acid)	mg dm <sup>-3</sup>	20.24
Si	mg kg <sup>-1</sup>	9.95
Clay	g kg <sup>-1</sup>	61
Silt	g kg <sup>-1</sup>	23
Sand	g kg <sup>-1</sup>	16
Texture	Soil type 3, clayey texture	

## Results and Discussion

The ratio of chlorophyll a and b was not affected by increasing Si doses applied to the soil. However, the Si doses significantly affected the leaf water potential (Table 2). Leaf water potential decreased with the increase in the first Si dose (Figure 2 A). The lowest leaf water potential (2.06 MPa) was obtained with at 800 mg dm<sup>-3</sup> Si dose.

The Si doses significantly influenced the harvest parameters, such as production, number of fruit, and mean fruit mass (Table 2). An important way by which Si could have contributed to the harvest parameters is related to the effect that this element has on other chemical elements that are key to plant nutrition and that are absorbed from the soil, such as phosphorus (Greger et al., 2018). The occurrence of this reaction throughout the crop cycle provides a significant amount of phosphorus to the plants, which leads to increased production. In a research carried out with physalis, the presence of silicon provided the improvement of leaf structures of seedlings of

physalis (*Physalis peruviana* L.), and improved also other phytotechnical characteristics (shoot length, root length, stem diameter, number of leaves, and number of shoots) (Lazzarini et al., 2020).

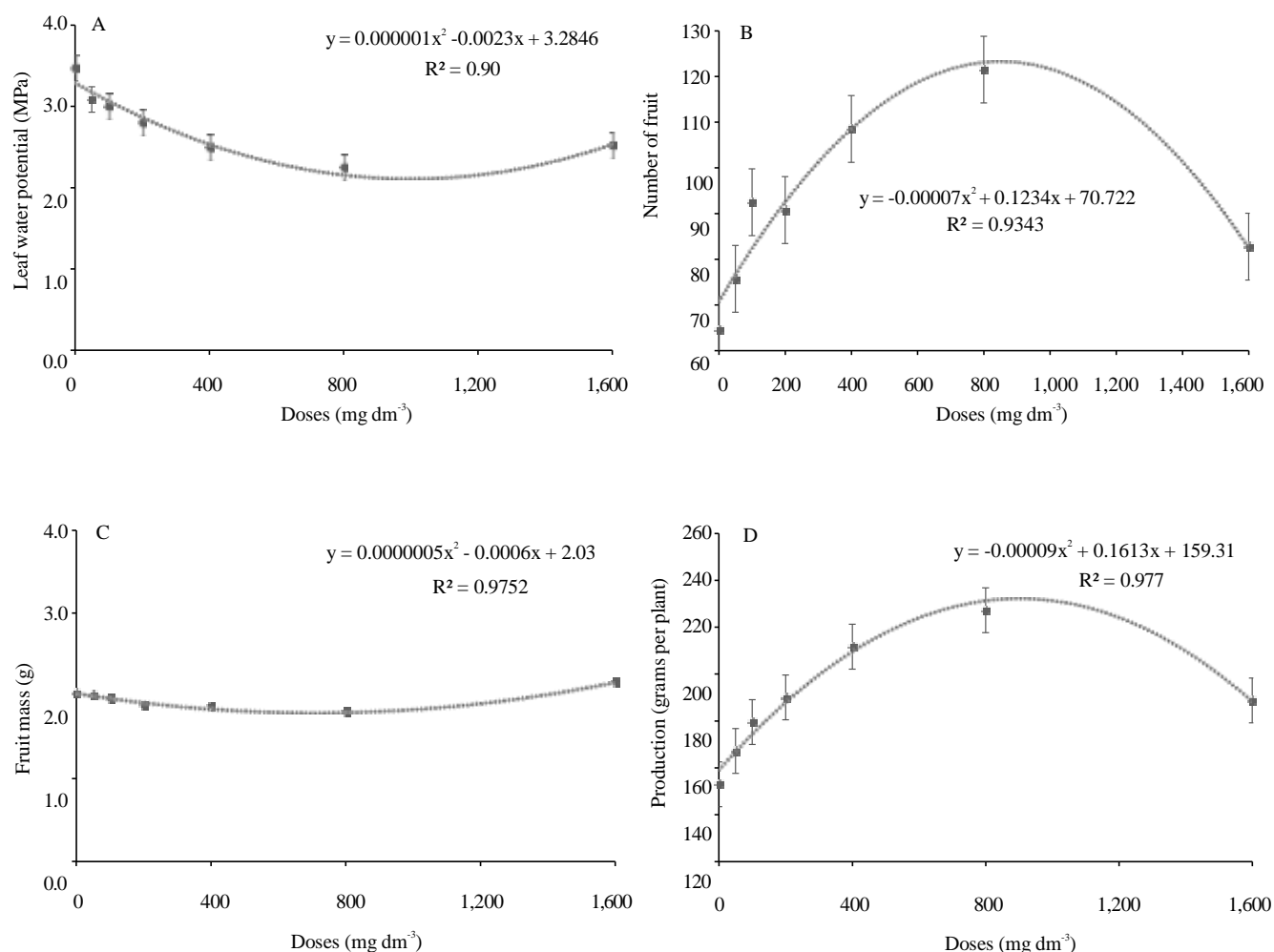
The leaf water potential reduction is another factor that may have contributed to the increased production

in the treatments with Si doses (Table 2), as it reduced leaf transpiration. It is likely that this saving of untranspired water was used by plants in their various metabolic pathways, increasing their productive potential.

**Table 2.** Analysis of variance on chlorophyll a (Ca), chlorophyll b (Cb), total chlorophyll (CT), leaf water potential (WP), number of fruit (NF), mean fruit mass (FM) and production (P) of 'Batum' raspberry (*Rubus idaeus*) grown in a subtropical region as a function of Si dose.

Source of variation	DF	Mean square						
		Ca	Cb	CT	WP (MPa)	NF	FM (g)	Production (g)
Treatments	6	6.64 <sup>ns</sup>	3.36 <sup>ns</sup>	16.92 <sup>ns</sup>	0.69*	1,495.56*	0.59*	2,558.20*
Blocks	3	13.74	15.20	53.47	0.04	2.18	0.00	3.82
Residual	18	9.47	6.25	29.23	0.03	9.69	0.00	10.30
CV (%)	-	10.92	21.40	13.56	5.69	3.43	3.14	1.71
Mean	-	28.20	11.80	39.90	2.80	90.88	1.96	188.21

\*Significant at 5% probability. <sup>ns</sup>Nonsignificant by the F-test.



**Figure 2.** Characteristics of 'Batum' raspberry (*Rubus idaeus*) grown in a subtropical region, as a function of Si dose applications: A, leaf water potential; B, number of fruit per plant; C, mean fruit mass; and D, fruit production. Pesq. agropec. bras., Brasília, v.58, e03371, 2023 DOI: 10.1590/S1678-3921.pab2023.v58.03371

The maximum fruit production (230.39 g per plant) was obtained with Si supplied at the 793.13 mg dm<sup>-3</sup>, with about 28% increase over the production without Si application, which was only 180.07 g per plant (Figure 2 D). Curi et al. (2015) evaluated the productivity and quality of 'Batum' raspberry, in a subtropical region, and obtained 10.3 Mg ha<sup>-1</sup> production. The production of 13.2 Mg ha<sup>-1</sup> was estimated by using this Si dose. These results are similar to those obtained by Silva et al. (2013), who worked with strawberries, whose fertilizations with Si contributed to the increase of fruit production.

The number of fruit per plant followed the same trend as fruit production (Figure 2 B). The highest number of fruit (125) was obtained with 889.29 mg dm<sup>-3</sup> Si, which represented 78% increase of the number of fruit, in comparison with treatments without the addition of Si (70 fruit per plant). These results corroborate those found by Silva et al. (2013), who evaluated the effect of Si on the production and quality of strawberry fruit.

However, with the increase of production both in mass (total g per plant) and in number of fruit per plant, there was a reduction of the mean fruit mass (Figure 2 C). This reduction of mass per fruit unit can be explained by the leaf/fruit ratio (source/drainage); by increasing the number of fruit, considered a drainage, and maintaining the number of leaves the same, there was a distribution reduction of photoassimilates (Silva et al., 2013). As the number of fruit increased, the mass decreased as a function of the source/drainage ratio. A significant difference was observed in the respiratory rate of fruit (Table 3). As Si dose increased, the respiratory rate of fruit decreased. This reduction was 59.2% under the dose of 900 mg dm<sup>-3</sup> Si, in comparison with the control without Si application (Figure 3 A).

According to Galati et al. (2015), there was less CO<sub>2</sub> release from lettuce leaves in all treatments with Si than in the treatment without Si addition.

Fruit firmness was another very important characteristic analyzed in the postharvest period, especially for small fruit. Higher fruit firmness was obtained with increasing Si doses (Table 3, Figure 3 B). Since higher Si doses yielded firmer fruit, these fruit may be less sensitive to the attack by pests and diseases (Galati et al., 2015). The findings of the present work are similar to those reported for the increased pulp firmness in the post-harvest quality of 'Xavante' blackberry and 'Albion' strawberry fruit, which resulted from the positive effects of Si applications (Munaretto et al., 2018, 2020). This characteristic may be attributed to the fact that Si is part of the structure of the plant. In the plant cell walls, Si can increase the content of hemicellulose and lignin, thus increasing the rigidity of the cell walls (Munaretto et al., 2018). Silicon is also present in the lumen and intercellular spaces of plants as amorphous silica in the cellular epidermis, stomata, trichomes, and vessel elements, providing greater firmness to plant structures, including the fruit (Munaretto et al., 2018).

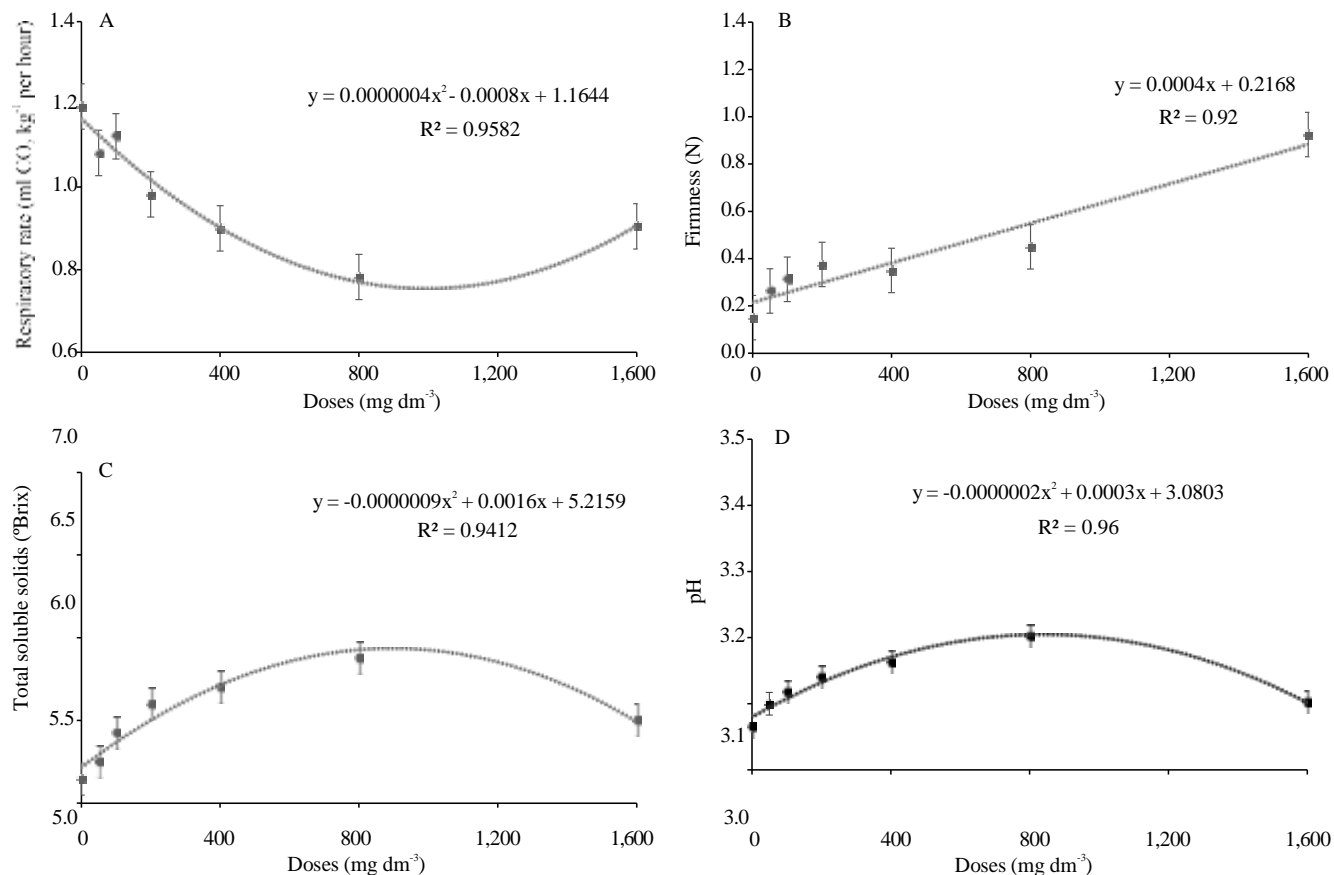
Another point to be considered is that firmer fruit tend to promote greater durability postharvest. This is an important factor in the cultivation of raspberry trees in the subtropical region, given that high temperatures at the time of harvest correlate with lower fruit durability. It is assumed that firming up the fruit is a strategy to increase the durability of fruit and the time they last in the postharvest period.

The contents of soluble solids (°Brix) changed significantly because of the Si applications (Table 3), however this reduction was small (Figure 3 C). This

**Table 3.** Analysis of variance for color (L\*), chroma (C\*), hue angle (Hue\*), fruit respiration rate (FR), firmness (N), soluble solids (SS), and pH of 'Batum' raspberry (*Rubus idaeus*) grown in a subtropical region as a function of Si dose.

Source of variation	DF	Mean square							
		L*	C*	Hue*	FR (mL CO <sub>2</sub> kg <sup>-1</sup> per hour)	Firmness (N)	SS (°Brix)	pH	
Treatment	6	0.79 <sup>ns</sup>	5.08 <sup>ns</sup>	24.34 <sup>ns</sup>	0.08*	0.36*	0.26*	0.08*	
Block	3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Residual	18	6.64	40.86	159.88	0.00	0.01	0.01	0.00	
CV (%)	-	11.66	23.17	45.77	2.68	18.77	1.35	0.49	
Mean	-	22.10	27.59	27.63	1.00	0.42	5.49	3.13	

\*Significant at 5% probability. <sup>ns</sup>Nonsignificant by the F-test.



**Figure 3.** Parameters of fruit of 'Batum' raspberry (*Rubus idaeus*) grown in a subtropical region, as a function of Si doses: A, respiratory rate; B, firmness; C, total soluble solids; and D, pH.

result can be explained by the increase of the number of fruit with the increase in Si, which made for a lower amount of photoassimilates per fruit.

Fruit pH increased as Si increased (Table 3). There was a maximum pH of 3.15 at the 800 mg dm<sup>-3</sup> Si dose. In general, the pH tends to increase with the increase of soluble solids and the advancement of the maturation stage of fruit.

### Conclusions

1. The doses of silicon influence the productive and physicochemical characteristics of 'Batum' raspberry (*Rubus idaeus*) fruit grown in subtropical regions.

2. The dose of 800 mg dm<sup>-3</sup> Si favors the reduction of leaf water potential, increasing the number of fruit and production of 'Batum' raspberry; in return, there is a reduction of the fruit fresh mass.

3. The dose of 800 mg dm<sup>-3</sup> Si decreases the respiratory rate of fruit and the total soluble solids content and increases the pH.

4. The gradual increase in silicon doses promotes a greater fruit firmness.

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**ARTICLE 2 – CALCIUM ACCUMULATION IN DEVELOPING FRUITS OF  
RASPBERRY AND BLACKBERRY**

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# Calcium Accumulation in Developing Fruits of Raspberry and Blackberry

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## Abstract

Calcium (Ca) is an essential macronutrient associated with fruit quality across many horticultural crops. Growers frequently apply Ca to promote fruit quality, but there are no studies reporting the best stages for Ca fertilizer application in caneberry. The objective of this study was to determine the timing and peak uptake periods of Ca in the fruits of raspberry (*Rubus idaeus*) and blackberry (*Rubus* sp.) to inform fertilizer application strategies. Fruits were collected at seven stages of development from three floricane-fruiting raspberry ('Meeker', 'WakeField', and 'WakeHaven') and three trailing blackberry ('Marion', 'Black Diamond', and 'Columbia Star') cultivars in 2022. Fruit samples were analyzed for Ca using an inductively coupled plasma optical emission spectrometer. Scanning electron microscopy (SEM) was also performed on a subset of 'Meeker' raspberries to explore micro-surface morphology that could influence Ca uptake dynamics. The peak period of Ca uptake was from stage S4 (half developed/"immature green") to S6 (immature fruit/"white fruit") across the raspberry cultivars and from stage S4 to S5 (mature green) in 'Black Diamond' and S4 to S6 in 'Columbia Star' and 'Marion' trailing blackberry. Little to no uptake of Ca was observed in the fruit or receptacle tissues before these stages. Fruit Ca uptake appeared greatest in 'Meeker' through S6, but 'WakeHaven' retained more Ca than 'Meeker' or 'WakeField' at S7 ("ripe fruit"). Unlike raspberries, blackberries continued to accumulate Ca between stages S6 and S7, which was attributed to retention of the receptacle in ripe fruit. SEM micrographs revealed a greater density of filamentous, epidermal hairs at S5 when compared to S6 and S7, which may impact Ca uptake via foliar fertilizers. Overall, findings from this study suggest that Ca fertilizer applications should be targeted between stages S4 to S6 to coincide with peak periods of plant uptake.

**Keywords:** *Rubus*, plant nutrition, tissue analysis, foliar feeding, accumulation of nutrients, march of nutrient absorption, dry matter accumulation

## INTRODUCTION

Raspberries and blackberries are soft fruits susceptible to softening and crumbling in the fresh and processed individually quick frozen (IQF) markets, where firm and intact fruits command price premiums. To increase fruit firmness and overall quality, raspberry and blackberry growers routinely apply calcium (Ca) fertilizers. Calcium is an essential macronutrient needed for plant growth and development under both non-stressed and stressed conditions through its structural, signaling, and enzymatic roles. One important structural function of Ca is cell wall strengthening and promotion of tissue and organ integrity by serving as a key constituent of the middle lamella between cells through pectin binding (Demarty et al., 1984; Zamil and Geitmann, 2017). The structural

function of Ca is frequently associated with fruit firmness and overall quality of multiple horticultural crops, as insufficient Ca levels can cause declines in fruit quality (de Freitas and Mitcham, 2012). Multiple studies have documented the consequences of insufficient Ca on fruit quality, such as bitter pit in apple (*Malus domestica*), blossom end rot in tomato (*Solanum lycopersicum*), internal flesh breakdown in mango (*Mangifera indica*), and internal tissue browning and premature fruit drop in ‘Draper’ northern highbush blueberry (*Vaccinium corymbosum*) (Ferguson and Watkins, 1989; Ho and White, 2005; Gerbrandt et al., 2019; Ma et al., 2023).

Calcium may be deficient for multiple reasons, including an overall lack of Ca in the soil solution or imbalances with other nutrients (K, Mg, etc.) in the rhizosphere. The nutrient is relatively mobile in the soil and accumulation of Ca in plant tissues is driven by transpiration and the concentration of Ca in the xylem sap. Accumulation and distribution of Ca in fruits is therefore also highly dependent on water availability, cell wall interactions in the apoplast, and transpiration, which in turn influences the concentration of Ca in the xylem (Hocking et al., 2016; Yang et al., 2020). However, xylem vessels have been shown to constrict during later stages of fruit development and impede the flow of Ca dissolved in xylem sap to developing fruits (Ho and White, 2005). Prior research with other crops, including highbush blueberry, grape (*Vitis vinifera*), kiwifruit (*Actinidia deliciosa*), tomato, and apple, support this notion and show key periods of Ca accumulation in fruits occurs early in their development (Cline et al., 1991; Ho and White, 2005; Rogiers et al., 2006; Montanaro et al., 2015; Gerbrandt et al., 2019). Accumulation of epicuticular wax can further reduce transpiration and overall xylem flow to developing fruits (Rogiers et al., 2004).

To mitigate deficiencies and imbalances, growers often apply Ca fertilizers to soil and/or plant canopies (i.e., “foliar feeding”). Yet, the overall efficacy of these applications is mixed (Brown et al., 1996; Arrington and DeVetter, 2017; Vance et al., 2017; Davarpanah et al., 2018; Morton et al., 2018; Gerbrandt et al., 2019). Inconsistent results in Ca fertility studies may be attributed to timing of fertilizer application, fertilizer formulation and rate, and application method. Regarding foliar applied fertilizers, these nutrients must pass through the cuticle and/or stomata of leaves and fruits before entering plant tissues. Cuticle thickness increases as fruit ripen and increasing layers of epicuticular wax may also cover stomata, limiting both transpiration and movement of foliar applied nutrients into target tissues (Yang et al., 2019 and 2020). Therefore, fruits have a limited period of effective nutrient uptake, making it important to align Ca fertilizer application during key periods of uptake. Key periods of Ca accumulation in raspberry and blackberry fruits are presently unknown and was the knowledge gap addressed in this study. Specifically, the objective of this study was to determine the timing and peak uptake periods of Ca in the fruits of raspberry and blackberry to inform fertilizer application strategies. In addition, raspberry fruit micro-surface characteristics were evaluated to assess their role in affecting Ca uptake.

## **MATERIAL AND METHODS**

### **Sampling location**

Fruit and leaf samples were collected from three cultivars of floricane-fruiting red raspberry, [‘Meeker’, ‘WakeField’ (marketed as ‘Wake™Field’), and ‘WakeHaven’ (marketed as ‘Wake™Haven’)] located in a single commercial field site in Lynden, Washington, USA (lat. 48°59’ N, long. 122°20’ W). The field was managed conventionally and established in 2011 for ‘WakeField’, 2020 for ‘WakeHaven’, and 2021 for ‘Meeker’. Soil type was a Kickerville silt loam. Sampling occurred on both sides of three, permanent transects per cultivar with transects beginning and ending 15 m from the field edge. Fruits were also sampled from three cultivars of trailing blackberry (‘Black Diamond’, ‘Columbia Star’, and ‘Marion’) located in an experimental planting at the Oregon State University North Willamette Research and Extension Center in Aurora, Oregon, USA (lat. 45°16’ N, long. 122°45’ W). The blackberry plants were likewise managed conventionally and established in 2018 on Willamette silt loam soil. Again, sampling occurred on both sides of the plants from three replicated

plots of three plants each. Fertilizer rates were the same across cultivars in the Washington and Oregon sites, although rates differed between the two states. No foliar fertilizers were applied during the study period.

### Sample collection

Seven different stages of reproductive development were sampled every 2 weeks following a scale developed by Kozhar and Peever (2018) (Table 1). All samples were collected between May to August and each sampling event included all predominant stages of development. The total number of fruits per replicate varied by sampling date in order to attain a minimum of 1 g of dried, ground tissue per replicate due to changes in mass during development.

**Table 1.** Developmental stages of red raspberry and blackberry sampled and number of fruits per replicate for each stage. Stages were identified in accordance with Kozhar and Peever (2018).

Stage ID	Stage of development	Number of samples per replicate (No.)
S1	Closed green buds	80
S2	Half open flowers	80
S3	Open flowers	80
S4	Immature green fruit	50
S5	Mature green fruit	30
S6	White fruit	30
S7	Mature, red or black fruit <sup>a</sup>	30

<sup>a</sup>Blackberry retained the receptacle at S7, whereas raspberry did not.

### Sample processing and analysis

Within 24 h of sample collection, remaining pedicles were removed, and wet and dry weights of the samples were determined. Samples were then milled and analyzed at the USDA-ARS Horticultural Crops Research Laboratory in Corvallis, OR, USA. Samples were digested using 70% (v/v) nitric acid and analyzed for Ca using an ICP-OES (inductively coupled plasma optical emission spectrometer) (Optima 3000DV; Perkin Elmer, Wellesley, MA, USA). Calcium content and dry weight of each stage and cultivar were calculated and accumulation patterns by stage of development determined.

Scanning electron microscopy (SEM) was also performed on a subset of ‘Meeker’ raspberry at S5 to S7 to explore micro-surface characteristics. Samples were prepared using the methods outlined by Bray et al. (1993) and El Kayal et al. (2017) and sputter coated with gold/palladium (Polaron SC7640 sputter coater, Quorum Technologies, East Sussex, UK). Images were observed using a Tescan Vega 3 thermionic scanning electron microscope (Brno-Kohoutovice, Brno, Czech Republic) at an accelerating voltage of 10 kV.

## RESULTS AND DISCUSSION

Patterns of Ca uptake and accumulation varied by species and cultivar, which could have corresponding effects on optimal fertilizer timing and fruit quality. All raspberry cultivars were observed to have peak periods of uptake between stages S4 (half developed/“immature green”) and S6 (immature fruit/“white fruit”) (Figure 1). Raspberry cultivar differences in Ca uptake and retention were observed with ‘Meeker’ having greater uptake through S6. By S7 (“ripe fruit”), however, ‘WakeHaven’ retained greater Ca compared to ‘Meeker’ and ‘WakeField’. Periods of Ca uptake in ‘Columbia Star’ and ‘Marion’ blackberry followed a similar trend with peak periods of uptake between S4 through S6 (Figure 2). Interestingly, the peak period of uptake in ‘Black Diamond’ was constrained between S4 to S5 (“mature green”). Blackberry fruit continued to accumulate Ca between stages S6 and S7, which was likely due to retention of the receptacle. Uptake of Ca was negligible before S4 across all species and cultivars. Despite species and cultivar differences, our

results suggest that Ca fertilizer applications should be targeted between stages S4 to S6 to coincide with peak periods of Ca uptake.

Cultivar differences in fruit Ca concentrations may be due to a variety of interacting factors, including xylem conductivity, nutrient allocation patterns, canopy size, and root growth. Fertilizer availability was not considered to be an influencing factor in our study, as fertilizer rates were consistent within our sampling sites. While xylem conductivity and flow of Ca dissolved in xylem sap has been shown to decline during later stages of fruit development due to restriction of xylem vessels, blackberry may maintain conductivity during later stages of fruit development as evidenced by increases in fruit Ca content through S6 in all cultivars and S7 in 'Black Diamond' and 'Columbia Star' (Figure 2). The same trend was not observed in raspberry, but this may be due to exclusion of the receptacle in our raspberry samples. Cultivars may also differ in fruit Ca concentrations due to uptake and allocation differences between growing leaves and fruits, which has been observed in northern highbush blueberry (Strik and Vance, 2015).

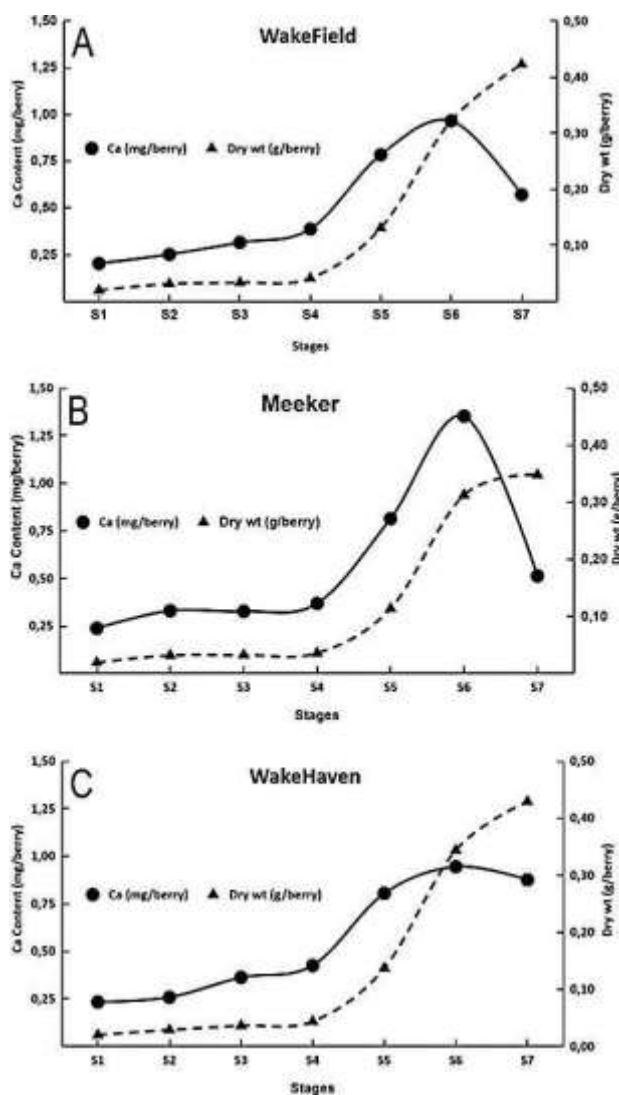


Figure 1. Calcium content (mg/berry; left y-axis) and dry weight (g/berry; right y-axis) in 'Meeker' (A), 'WakeField' (B), and 'WakeHaven' (C) floricane raspberry fruits.

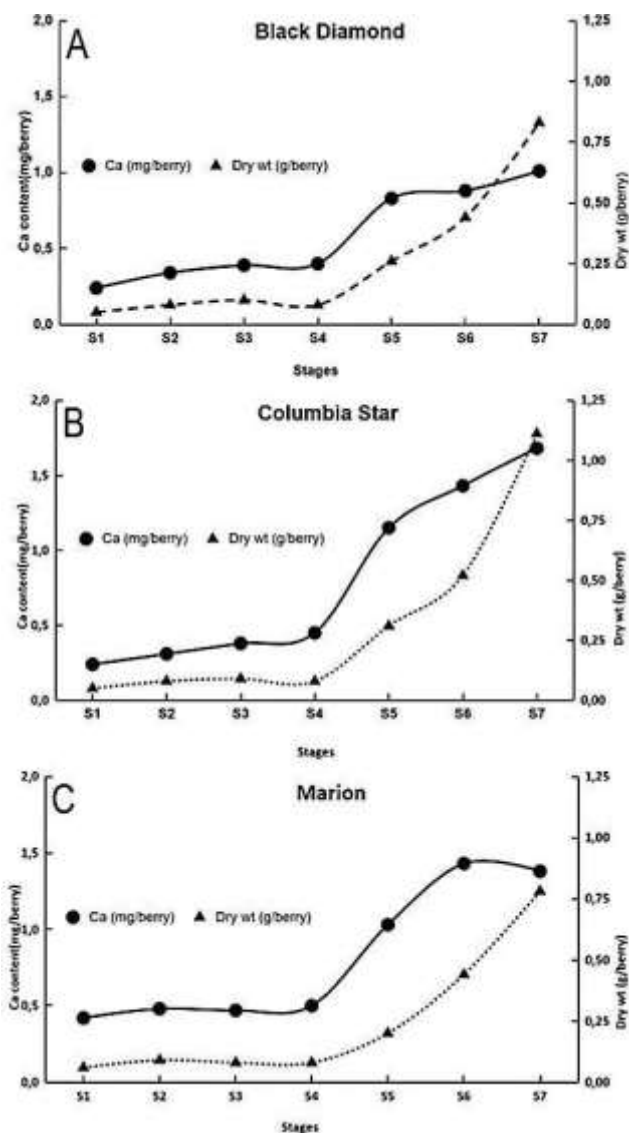


Figure 2. Calcium content (mg/berry; left y-axis) and dry weight (g/berry; right y-axis) in 'Black Diamond' (A), 'Columbia Star' (B), and 'Marion' (C) trailing blackberry fruits.

Micro-surface characteristics differed by stage of development with a high density of filamentous, epidermal hairs observed at S5 (Figure 3). The density and rigidity of these epidermal hairs appeared to decrease as the fruit expanded to S6 and S7. These epidermal hairs may increase the functional surface area of fruit and enhance the retention time of foliar-applied nutrients, particularly when combined with surfactants. Furthermore, epidermal hairs may impact fruit transpiration. In kiwi, Xiloyannis et al. (2006) reported that as fruit mature and expand, epidermal hair viability declines and thereby increases the boundary layer around fruit, which effectively reduces transpiration. This in turn could reduce flow of mineral nutrients in the xylem sap. However, few stomata were observed on the surface of the raspberries, and further characterization of stomata density and conductance should be explored in both raspberry and blackberry. Further examinations of fruit cuticle and epicuticular wax formation are also recommended, in addition to characterizing the relationship between fruit Ca concentrations and structural integrity that may impact quality.

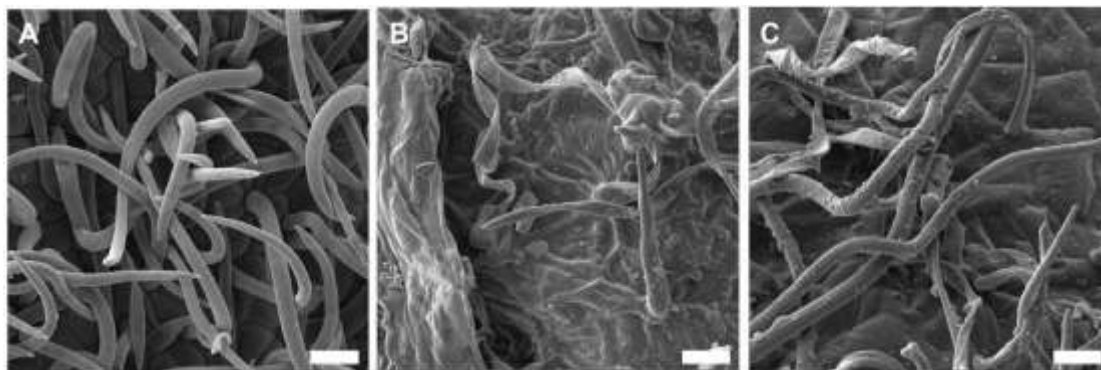


Figure 3. Scanning electron micrographs depicting surface characteristics of ‘Meeker’ raspberries at S5 (“mature green”; A), S6 (“white fruit”; B), and S7 (red fruit; C) stages of development. White scale bar denotes 20  $\mu\text{m}$ .

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