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Phenolic compounds, pectin and antioxidant activity in blueberries (*Vaccinium corymbosum* L.) influenced by boron and mulch cover

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Summary

Highbush blueberry cultivars 'Bluecrop' and 'Reka' were growing in two variants of mulching and fertilizing systems on formerly used farmland. Particular attention of this work was to study the effect of using pine bark as a mulch layer and foliar application with the plant stimulant Wuxal® Ascofol (3% Boron) on selected bioactive compounds (polyphenols and pectins) of blueberry fruits.

The results represented a stress-preventive effect of mulch application. Furthermore, these plants exhibited lower calcium content in fruits due to a reduced calcium uptake from the soil. With regard to the bioactive compounds, mulched plants showed a higher content of pectin which was in contrast to the phenolic compounds. They revealed reduced concentrations in fruits accompanied by a lower antioxidant activity. The foliar supply of boron was able to inactivate polyphenols presumably by complex formation and favoured the formation of pectins.

Introduction

Highbush blueberries (*Vaccinium corymbosum* L.) gain high popularity in Europe due to their proposed health-beneficial properties. Optimum locations for cultivation of blueberries are found on heathland, peat or forest soils. Nevertheless, such locations are not always available. However, it is tried to cultivate blueberries on formerly used farmland, which is primarily insufficient for blueberry cultivation. Unfavorable conditions are the high pH value; the low humus content and the reduced water holding capacity of the soil (Haynes, 1986). The improvement of blueberry production on formerly used farmland has been carried out in several investigations, all with the aim of increasing crop yield. Unfortunately, changes of the bioactive compound composition have not been investigated up to now. Blueberries offer a high content of pectin (Ternes et al., 2005) and phenolic compounds (Häkkinen, 2000). For both classes the health beneficial aspects are well known. Due to their antioxidant properties especially polyphenols are attributed with several positive physiological properties. They are able to quench free radicals, released by oxidative stress, and are implicated in prevention of cancer and cardiovascular diseases in the human organism (Bazzano et al., 2002).

In plants, secondary metabolites such as the phenolic compounds are accumulated as a protective defence mechanism against stress conditions (Dixon and Pavia, 1995). Also pectins, as compounds of the primary plant metabolism, are able to react on changed ecophysiological conditions (Lesniewska et al., 2004). Besides stress-induced effects on the primary and secondary plant metabolism, availability of nutrients can be an influencing factor for the biosynthesis of compound classes (Stewart et al. 2001). Thus, mineral availability may have an effect on both classes, phenolic compounds and pectins by e.g. nitrogen, phosphate (Steward et al., 2001; Juszczuk et al., 2004) or calcium availability (Naphun et al., 1997). As a result, there are different possibilities to influence the amount and composition of bioactive compounds by specific cultivation techniques. The aim of this study was to investigate whether mulch layer and foliar applications using pine bark and a plant stimulant based on 3% boron (Wuxal® Ascofol) might influence mineral availability of blueberries under open land conditions. Besides the two minerals boron and calcium, which were mostly affected due to treatments, phenolic compounds and pectins have been analyzed as they are closely related to mineral metabolism. Additionally, antioxidant activity was determined.

Materials and methods

Plant material

Plant material was obtained from the highbush blueberry cultivars 'Bluecrop' and 'Reka', which have been produced on open land conditions at the research site of the Humboldt-Universität zu Berlin. Bushes were planted in peat filled holes on formerly used farmland and cultivated in two ground cover (with [M] and without [oM] pine bark mulch) and fertilization variants. Fertilization was conducted from May to mid July with a nutrient supply of 10 g N - 15 g P_2O_5 - 24 g K_2O - 2 g MgO - 66 mg B per plant in first fertigation variant (F1). Plants of the second nutrient supply system (F2) received an increased nitrogen fertilization (14 g per plant) and additionally boron foliar applications (400 mg per plant). Boron foliar applications were given at the start of vegetation cycle (April), at the blossom (May) and at beginning of fruit development (June) in a concentration of 3 L ha^{-1} (= 340 mg boron per plant per year).

Samples of ripe berries of each variant and cultivar were frozen immediately after picking and stored at -20 °C. Approx. 100 g of fruits of each sample were lyophylized for 48 h (ALPHA 1-4, Fa. Christ, Osterode am Harz, Germany), ground and stored in a vaccuum desiccator for further analysis. The experiment was conducted with 6 replicates per variant.

Chemical analysis

Minerals

For the estimation of the calcium content freeze-dried fruit samples (0.5 g) were ashed 4 h at a temperature of 490 °C. Afterwards the ash was transferred in hydrochloric solution (5 mL of 10% HCl) and vaporized on a sand bath (type HC 42, GERHARDT, Bonn, Germany). The residue was heated up once more to 490 °C for 1 h. After addition of 5 mL of HCl (25%) the ash solution was filtered (Macherey-Nagel MN 615 ¼, Düren, Germany). Filtrate was filled up to a volume of 50 mL. The measurement of calcium was done using atomic absorption spectrometry (AAS, type 905 AA, GBC, Sydney, Australia). Results were expressed as mg Ca g^{-1} DW.

To analyze boron, freeze-dried fruit samples (1 g) were ashed for 6 h at a temperature of 550 °C. Afterwards, the ash was incubated with 0.36 N H_2SO_4 for 1 h. The solution was filtered (Macherey-Nagel MN 260, Düren, Germany) and the filtrate was filled up to a volume of 5 mL. The measurement of boron was conducted spectrophotometrically (SP8-300, Pye Unicam, OK, USA) at a wavelength of 420 nm according to the Azomethine-H method (Bingham, 1982). Hence, 1 mL of extracted sample was added to a

mixture of 1 mL buffer (pH 5.1) and 2 mL colour reagent (0.45 g of azomethine-H and 1 g of ascorbic acid in 100 mL of destilled water). Boric acid (Merck 10165) was used as a standard. Results were expressed as µg B g^{-1} DW.

Antioxidant Activity and Total Phenolic Content

For the determination of the antioxidant activity and total phenolic content 0.5 g freeze-dried fruit samples were extracted in 10 mL of the cooled solvent 0.1% HCl/methanol (v/v; 15/85) according to the method of CONNOR et al. (2002). The samples were diluted in 3 mL solvent, and centrifuged for 10 min at 3000 rpm (Heraeus Christian Labofuge GL, Hanau, Germany). Supernatants were decanted and filtered (Macherey-Nagel, MN 260, Düren, Germany). This process was repeated twice. Extracts were filled up to a 10 mL volume and stored at -20 °C until further analysis.

Antioxidant activity was estimated using electron spin resonance (ESR) spectroscopy and the trolox equivalent antioxidant capacity (TEAC) assay. ESR was performed as described by RÖSCH et al. (2003), using a Miniscope MS 100 spectrometer (Magnettech, Berlin, Germany). The signal intensity of a stable synthetic radical (Fremy's salt; Sigma- Aldrich, Steinheim, Germany) was obtained after 5 min. Results were expressed as mmol Fremy's salt g^{-1} DW.

TEAC assay was carried out spectrometrically as described by ROHN et al. (2004). Absorbance was measured on a Specord 40 spectrophotometer (Analytik Jena, Jena, Germany) at a wavelength of 732 nm. ABTS (2,2'-azinobis-3-ethylbenzothiazonline-6-sulfonic acid, Sigma-Aldrich, Steinheim, Germany) was used as a free radical and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma-Aldrich, Steinheim, Germany) as a standard. Results were expressed as mmol TROLOX g^{-1} DW.

Total phenolic content was analyzed spectrophotometrically (SP8-300, Pye Unicam, OK, USA) according to the Folin-Ciocalteu procedure. Absorbance was measured at a wavelength of 765 nm. Gallic acid (Serva, Heidelberg, Germany) served as a standard. Results were expressed as mg gallic acid (GAE) g^{-1} DW.

Pectic substances

For determination of pectic fractions (water-soluble pectin, alkali-EDTA soluble pectin, insoluble pectin) and the total pectin content, cell wall extraction (AIS, alcohol insoluble substance) was conducted according to the method by BLUMENKRANTZ and ASBOE-HANSEN (1973) und HUYSKENS (1991). An aliquot of 2 g of freeze-dried samples was diluted in 120 mL of an acetone/70% ethanol mixture (70/30) and boiled for 30 min. Thereafter, the cell wall material was washed under vacuum using glass frits (G3, Schott Duran, Mainz, Germany) with acetone and ethanol (70%). Glas frits were dried for 24 h at 70 °C (WTC Binder, Tuttlingen, Germany).

For the extraction of water-soluble pectins (WSP) 100 mg of AIS were mixed with 20 mL of distilled water and dissolved by stirring for 1 h. Thereafter, samples were adjusted to a pH value of 4.5 (Microprocessor pH meters 526, Fa. WTW, Weilheim, Germany) and 0.1 mL pectinase (= 20 µg) (Pectinex ultra SP L, Novo Nordisk Ferment, Switzerland) was added. Subsequently, samples were stirred for 1 h prior to filtration (Miracloth filter, Calbiochem, Frankfurt, Germany) and centrifugation for 10 min at 4°C and 11000 rpm (Biofuge 22R, Heraeus Sepatech, Osterode am Harz, Germany). The remaining pellets were frozen at -20 °C for extracting further pectin fractions. The filtrates were filled up with 0.5% EDTA buffer (pH 4.5) to a 50 mL volume and stored until the spectrophotometric measurement. The extraction of the alkali-soluble pectins (ESP) was conducted by adding 20 mL of 0.5% EDTA solution (pH 6.0) to the pellets of WSP fraction, the insoluble pectin fraction (ISP) by adding 20 mL of 0.5% EDTA solution (pH 11.5) to the pellets of ESP fraction similary to the extraction of the water-soluble pectins.

The measurement of each pectin fraction was performed spectrophotometrically at a wavelength of 520 nm using MHDP (m-hydroxydiphenyl) as a color reagent (MCCOMP and MCCREADY (1952). As a standard, D-galacturonic acid (5 to 80 µg mL^{-1}, Sigma D 4288) was used. Results were expressed as mg galacturonic acid (Gal) g^{-1} DW.

Statistics

The statistic evaluation was performed using SPSS 13.0 (SPSS Inc., Chicago, USA 2001). Significance of differences was conducted with a Tukey-B test ($p < 0.05$).

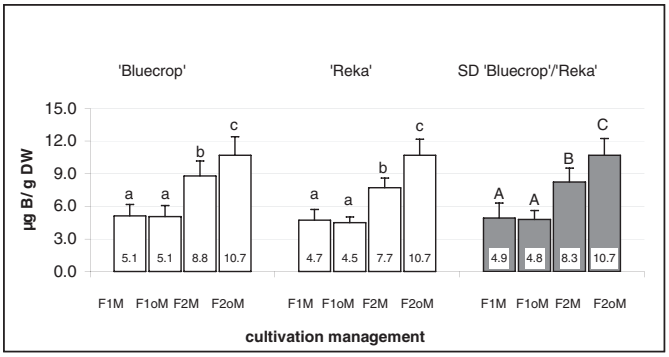
Results and discussion

Mulch covering (with pine bark) in blueberry cultivation influences soil physical properties primarily, especially soil temperature and evaporation (KREWER et al., 1997). Furthermore, it enriches the soil organic matter, and therefore, helps conserving soil moisture (PLIZKA et al., 1997). On the other hand, soil water availability affects the mineral nutrition of plants. All these factors might have an effect on the biosynthesis of bioactive compounds in fruits. Moreover, as far as minerals concerned mulch covering revealed the greatest impact on calcium which will be discussed in this work in detail.

Boron and calcium content of blueberry fruits as influenced by different cultivation practices

In blueberry fruits boron content ranged from 4.5 - 10.7 µg g^{-1} DW. Calcium contents varied between 0.85 - 1.43 mg g^{-1} DW (Fig. 1-2). Boron application and mulch cover had a significant influence on the boron content of blueberry fruits (Fig. 1). Boron treated plants revealed clearly higher boron concentrations than plants without additional boron supply. Furthermore, differences could be observed between the mulched and the unmulched boron variant, i.e. the unmulched boron variant showed the highest boron content in fruits.

The calcium content of the fruits was predominantely influenced by the mulch treatment (Fig. 2). Results revealed significantly higher calcium content in fruits of the unmulched variants than in fruits of the mulched variants. However, boron application did not affect the calcium content of blueberry fruits.

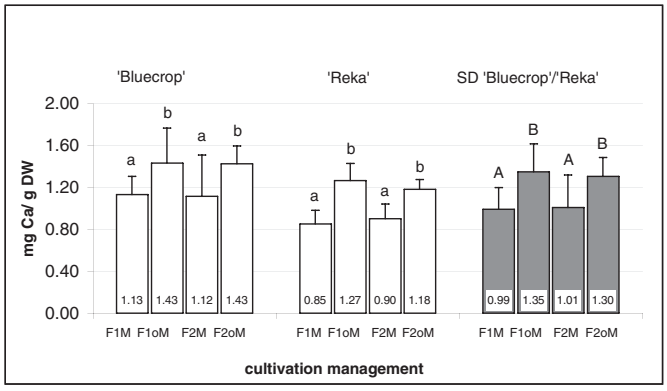


SD 'Bluecrop'/'Reka'= standard devision of both cultivars

Fig. 1: Boron content of fruits of different blueberry cultivars [µg B/g DW], (n ± SD, Tukey-B Test, $p < 0.05$); F1M-mulched without B; F1oM-unmulched without B, F2M-mulched with B, F2oM-unmulched with B.

The increased boron content of boron treated fruits demonstrated the uptake of foliar-applied boron by leaves (data not shown) followed

by its translocation to the fruit which was also reported by HAHNFELD et al. (2004). Moreover, bushes of the unmulched boron variant showed a stronger accumulation of boron in the fruit tissue in comparison to plants of the mulched boron variant. It is assumed that these bushes could have undergone drought stress due to the unprotected ground. HETHERINGTON and WOODWARD (2003) reported that during persistent drought conditions leaves developed a higher number of small stomata on the leaf lower surface in order to regulate the gas exchange. An increased number of small stomata could explain the increased boron uptake of the leaves (data not shown), and therefore, the higher content of boron in fruits of the unmulched variant.



SD 'Bluecrop'/'Reka'= standard devision of both cultivars

Fig. 2: Calcium content of fruits of different blueberry cultivars [mg Ca/g DW], (n ± SD, Tukey-B Test, $p < 0.05$); F1M-mulched without B; F1oM-unmulched without B, F2M-mulched with B, F2oM-unmulched with B.

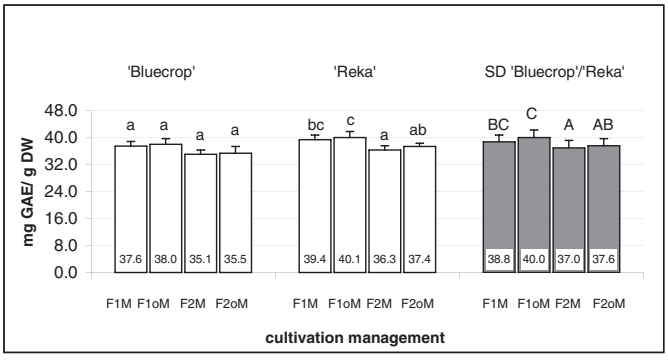
Fruit calcium contents of the unmulched variants were significantly higher than in mulched variants. However, LI et al. (2006) observed a higher calcium content in leaves after ground covering with organic matter due to a higher calcium content being released during the rotting process. In the present work the soil (below the ground cover) exhibited increased calcium contents in the mulched variants (data not shown). However, calcium was transported to the leaves (data not shown) as well as to the fruits of the mulched variants to a much lower extent. This might be explained by the fact that calcium was fixed in the rhizosphere, and thus, uptake of calcium was inhibited. Hence, the plants of the mulched variants rooted predominantly in the ground cover (own observations) where they probably absorbed nutrients preferably from this layer. On the basis of a few random samples (roots with adherent mulch and/or soil particles) a clear pH decline was found in the mulched variants ([oM]: pH = 5.6; [M]: pH = 4.5). This acidification could have occurred by exudation of organic acids in the rhizosphere resulting from a symbiosis with mycorrhizal fungi or a direct root secretion. Though, a mycorrhizal infection could not be found in this work (based on few random samples), it can not be excluded completely.

Despite high calcium availability, the plants of the mulched variants were able to inhibit an excessive calcium uptake. In contrast, the plants of the unmulched variants completely rooted in the peat bed because mineral soil was generally avoided for rooting (own observation). Due to the fact that peat was almost decomposed, these conditions apparently did not inactivate calcium uptake in plants.

Influence of boron and calcium on total phenolic compounds and antioxidant activity (TEAC, ESR) of blueberry fruits

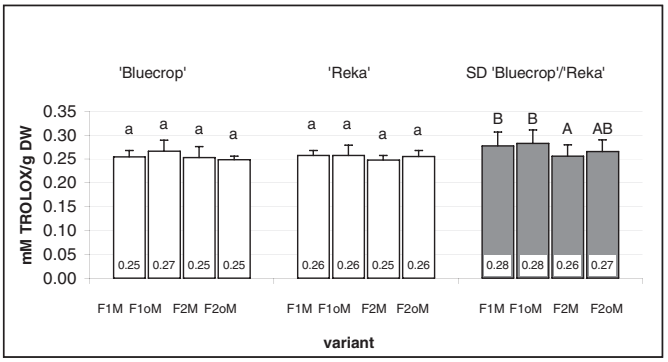
Higher values of the total phenolic content were determined in the present study (35.1 - 40.1 mg of GAE g^{-1} DW or 702 - 802 mg GAE 100 g^{-1} FM), exceeding the contents reported by MOYER et al. (2002) with 444 mg GAE 100 g^{-1} FW (Fig. 3). This might be due to different local growing conditions. An own comparative study on fruits cultivated on forest soils confirmed this assumption (EICHHOLZ et al., 2007). In particular, the suboptimal growing conditions on farmerly used farmland presumably might have caused stress mediated responses in plants with a subsequently increase of the phenol synthesis and therefore higher values of antioxidant activity. Similary, for the TEAC assay higher values were determined (0.25-0.27 mmol TROLOX g^{-1} DW) in comparison to the literature where an average value of 0.19 mmol TROLOX g^{-1} DW was reported by GARCIA-ALONSO et al. (2004) (Fig. 4).

The antioxidant activity of the fruits, being measured by ESR-analysis, ranged between 0.38 - 0.51 mmol Fremy's salt g^{-1} DW (Fig. 5). In the literature, no data are available on the ESR analysis in order to compare the results of the present study. However, the high correlation of this analysis to TEAC assay ($r = 0.76$; $p < 0.01$) suggested that the ESR method is suitable for the determination of the antioxidant activity in blueberries. The high amount of anthocyanins in blueberries often leads to difficulties in spectrophotometrical tests. Thus, the ESR method indicated to be a good alternative for samples with a high coloration.



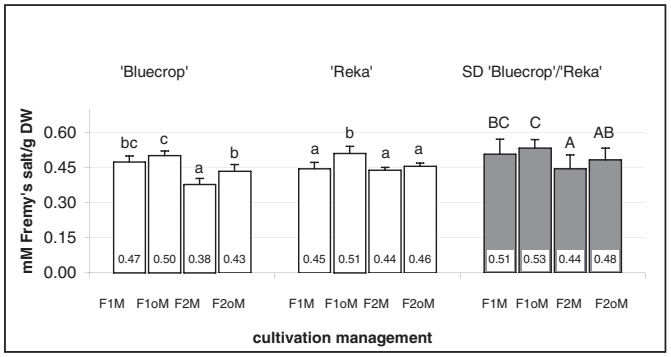
SD 'Bluecrop'/'Reka'= standard devision of both cultivars

Fig. 3: Total Phenolic Content of different blueberry cultivars as measured by the Folin-Ciocalteu method [mg GAE/g DM], (n ± SD, Tukey-B Test, $p < 0.05$); F1M-mulched without B; F1oM-unmulched without B, F2M-mulched with B, F2oM-unmulched with B.



SD 'Bluecrop'/'Reka'= standard devision of both cultivars

Fig. 4: Antioxidant Activity of different blueberry cultivars as measured by TEAC assay [mM TROLOX/g DM], (n ± SD, Tukey-B Test, $p < 0.05$); F1M-mulched without B; F1oM-unmulched without B, F2M-mulched with B, F2oM-unmulched with B.



SD 'Bluecrop'/'Reka'= standard devision of both cultivars

Fig. 5: Antioxidant Activity of different blueberry cultivars as measured by ESR method [mM Fremy's salt/g DM], (n ± SD, Tukey-B Test, $p < 0.05$); F1M-mulched without B; F1oM-unmulched without B, F2M-mulched with B, F2oM-unmulched with B

Statistical evaluation revealed an influence of boron application on total phenol content as well as on antioxidant activity (ESR; TEAC) of fruits (Fig. 3-5). In the boron treated variants lower values were observed in comparision to the untreated plants. Furthermore, ground covering tended to a decline in total phenolic content and in the antioxidant activity. Although single statistical calculation of each cultivar did not always exhibit any differences (no significances in TEAC assay for both cultivars and in total phenol assay for 'Bluecrop'), results showed a similarity in all analysis due to the treatments (Boron, Mulching).

If plants undergo stress like drought, high light intensity or nutrient deficiency, they are affected by oxidative damage resulting from reactive oxygen species (ROS). ROS are formed in plants primarily during the photosynthetic electron transport and the activation of membrane-engaged NADPH-oxidases (MØLLER, 2001). To protect themselves from a cellular damage, plants mobilise compounds which inactive ROS – the so-called antioxidant compounds. In particular in blueberries, phenolic compounds are responsible for their high antioxidant properties (HÄKKINEN, 2000). This was also confirmed by the high correlations between the total phenolic content and both analytic assays used for the antioxidant activity (ESR: $r = 0.86$: TEAC: $r = 0.82$; $p < 0.01$) in the present study. Hence, due to these correlations the following conclusions, which were stated for the total phenolic content, can also be considered for TEAC-and ESR analysis.

The statistical calculation represented a significant decrease of total phenolic content in the boron treated variants. A close relationship between the boron content and the polyphenol metabolism in plants is already known. The accumulation of polyphenols is a typical symptom of boron deficient plants. Boron deficiency influences the polyphenol metabolism which is proved by its impact of the enzymes phenylalanine-ammonialyase (PAL) and the polyphenoloxidase (PPO) (CAMACHO-CRISTÓBAL et al., 2002). In addition, due to the high complex binding properties boron easily binds polyphenols (BROWN et al. 2002). During boron deficiency the content of free boron molecules in plant tissue is low. Hence, a complexation and an inactivation of the polyphenols do not occur. Furthermore, an increase in phenolic compounds could be associated with the accumulation of carbohydrates induced by boron deficiency. Here, the polyphenol synthesis might be stimulated by a reinforced supply via pentose phosphate cycle (PPP). According to GOMEZ-RODRIGUEZ et al. (1987) boron deficiency resulted in an activation of the glucose-6-phosphates dehydrogenase. This enzyme is known as the starting point of the PPP and oxidizes glucose-6-phosphate to 6-phosphogluconolacton. In addition, it was also reported that boron deficiency increases activities of the enzyme 6-phosphogluconat-dehydrogenase which is responsible for the 2nd reaction step of the PPP (DUGGER, 1983).

With an adequate boron supply a high portion of free boron ions would be available, also being able to bind polyphenols. This would lead to a depression of PAL and also to a degraded phenol oxidation by PPO (BROWN et al., 2002), possibly explaining the decline of total phenol content of the boron variants in the present study.

Numerous investigations have been carried out to provoke an activation of the polyphenol metabolism due to moderate stress induction by abiotic and biotic orgins (SCHREINER and HUYSKENS-KEIL, 2006). In the present work stress mediated responses of plants might have been caused, primarily, by the lack of ground cover resulting in a low ground water capacity, a low portion of organic matter and an increased calcium uptake of the roots. Thus, a trend to higher contents of antioxidant activity and of total phenolic content became evident in the unmulched variants.

Concerning the minerals a pronounced influence should be expected from calcium as a stressor, mainly because blueberries are calcifuge plants. However, apart from the effect of the ground cover and boron availability, several other factors might have had an impact on the calcium uptake. From the present results it is concluded that the stimulation of the polyphenol synthesis might have been a result of stress caused by a high content of calcium in blueberries as it was measured in the unmulched variants. An excess of calcium is known to cause phosphate deficiency as well as decreased iron availability and uptake in fruit tissue. This could have been stimulated polyphenol synthesis, as it was found in beans (*Phaseolus vulgaris* L.) (JUSZCZUK et al., 2004) and grapes (*Vitis vinifera* L.) (PIAGNANI et al., 2003) revealing phosphate and iron deficiency, respectively.

Influence of boron and calcium on pectic substances of blueberry fruits

Contents of total pectin of berries were found to range between 29.4-39.1 µg Gal g^{-1} DW. The single pectin fractions exhibited average contents of 20.1-30.3 µg Gal g^{-1} DW for water-soluble pectins (WSP), 5.5-7.0 µg Gal g^{-1} DW for alkali-soluble pectins (ESP) and 1.7-3.6 µg Gal g^{-1} DW for insoluble pectins (ISP) (Tab. 1).

No significant differences of WSP were determined in ground cover and boron application treatments, although both cultivars showed a significant increase of WSP in the boron treated variants compared to the untreated variants. Furthermore, variants without boron application (F1) of 'Reka' revealed significantly higher contents of WSP in the mulched variants in contrast to the unmulched bushes. In the ESP fraction was a trend to higher contents in the boron treated variants, especially, in the mulched variant compared to the unmulched variant were established. Moreover, for the ISP fraction only the cultivar 'Bluecrop' resulted in significant differences in the mulched boron variant when compared to the mulched untreated variant. Thus, the statistical calculation in respect to total pectin content showed clear differences in the variants treated with boron, primarily to unmulched untreated variants.

In the literature a total pectin content of 0.51 g Gal 100 g^{-1} FM in blueberries was reported by SILVA et al. (2005) which underlines the data upraised in the present work (29.4-39.1 µg Gal g^{-1} DW = 0.58-0.78 g Gal 100 g^{-1} FW). Furthermore, WSP fraction exhibited the highest portion of pectic substances, followed by ESP and ISP fraction.

The majority of cellular boron is associated with pectin in the cell wall (BROWN et al., 2002), where the cell wall bound boron forms a tetravalent borate-diol-ester. Here, two molecules rhamnogalacturonan-II (RG II) are cross-linked with each other to form a stable dimer in the primary cell wall (ISHII and MATSUNAGA, 1996). MATSUNAGA and ISHII (2006) reported during boron deficiency

cell wall thickening of leaves is closely related with the formation of a dimeric RG-II-B complex. Adding boric acid to boron deficient leaves of pumpkin (*Cucurbita maxima* Duch.) led to the formation of dimeric RG-II-B from monomeric RG-II as well as to the formation of a thinner cell wall. In contrast, boron deficient plants form a huge, thick cell wall.

Boron applications also influenced the content of pectins in blueberry fruits of the present study. Nevertheless, the influence of boron due to additionally formed RG-II-B complexes seems to be of minor importance, because according to BROWN et al. (2002) such complexes stabilize primarily the protopectin. Hence, it is assumed that the presence of boron allowed an improved transport of carbohydrates, the source of pectin synthesis, and/or exerted a direct influence on the carbohydrate synthesis. The synthesis of pectin "backbones", which are mainly galacturonic acid molecules, depends on the availability of carbohydrates (MOHNEN, 1999). However, an improved transport of sugars by boron is reported to be controversial. The chemical structure of the respective sugar influences the stability and the transport ability of those complexes. Carbohydrates which could easily react with boric acid are riboses, apiose, sorbitol and other polyols (BROWN et al., 2002). However, in most plants sucrose is the main transport sugar (WILLIAMS et al., 2000) and thus, the chemical structure does not allow a stable complex because sucrose shows a trans-diol position of the hydroxyl group (BROWN et al., 2002).

Furthermore, HAN et al. (2008) determined a lower content of sucrose in boron deficient leaves of citrus seedlings (*Citrus sinensis* L.), while the content of glucose and fructose strongly increased. These authors associated a direct impact of boron on the sucrose synthesis. Nevertheless, currently it is unclear whether boron is involved in the synthesis processes of this sugar or if it reduces its degradation. DUGGER and HUMPHREY (1960) had already suggested a support of sucrose synthesis from glucose-1-phosphate and fructose in the presence of boron. They proved a support of the UDPG-pyrophosphorylase with simultaneous inhibition of the UDPG-transglycosylase in leaves of peas and sugar cane seedlings (*Pisum sativum* L.; *Saccharum officinarium* L.). Also TEARE (1974) reported an accumulation of UTP- and a decrease of UDP-glucose in bean roots (*Phaseolus vulgaris* L.) during boron deficiency. However, HAN et al. (2008) referred to reinforced sucrose degradation by an increased invertase activity in the absence of boron.

The higher content of boron in berries studied here might have led to a reinforced construction of pectins, based on a presumably higher availability of transport sugars. In the presence of boron this would become predominantly for the construction of pectins as cell wall material where boron plays a primary role in the synthesis (BROWN et al., 2002). With an increase in protopectin the single pectin fractions also increased in the boron treated variants. As a result of ongoing fruit development and maturation the content of protopectin was decomposed increasingly to soluble pectin fractions, as it was also reported by VICENTE et al. (2007).

Moreover, an increase of pectins by enhanced calcium content was also proved by other research studies, for example, in strawberry fruits (*Fragaria x ananassa* Dutch.) (NAPHUN et al., 1997). In general, the element calcium is able to link galacturonic acid molecules in the HG complex with each other. A decline of calcium results in a dissolution of the gel, leading to a break-down of calcium bridges and to a loss of the cell wall integrity (WILLATS et al., 2001).

Despite higher calcium contents in leaves and fruits the plants of the unmulched variants were not able to link calcium in the pectin fractions of the fruits in absence of boron (Tab. 1). The reason might be associated with the characteristics of the plant as a calcifuge species. Calcium preferentially bounds other elements like P, Fe or Mn and causes a deficiency of these elements in the tissue (ZOHLEN and TYLER, 2004). In the presence of boron, calcium would be also required for the linkage of pectin, in particular in the low esterified fraction. Calcium is also required for stabilizing RG-II-B-complexes.

Tab. 1: Pectin content (WSP, ESP, ISP, Total pectin) of different blueberry cultivars [µg Gal/ g DW], (n ± SD, Tukey-B Test, $p < 0.05$); F1M-mulched without B; F1oM-unmulched without B, F2M-mulched with B, F2oM-unmulched with B.

	'Bluecrop'			'Reka'			SD 'Bluecrop'/'Reka'		
WSP	F1M	29.21	± 0.73 ab	21.88	± 1.05 b		25.54	± 3.93	A
	F1oM	28.27	± 0.29 a	20.09	± 1.57 a		24.18	± 4.59	A
	F2M	30.10	± 1.16 b	23.92	± 0.58 c		27.01	± 3.34	A
	F2oM	30.30	± 0.82 b	24.83	± 0.97 c		27.57	± 2.98	A
ESP	F1M	6.44	± 0.57 ab	5.55	± 0.78 a		6.00	± 0.80	A
	F1oM	5.55	± 0.31 a	6.10	± 0.48 ab		5.83	± 0.47	A
	F2M	6.96	± 0.57 c	6.87	± 0.43 b		6.91	± 0.48	B
	F2oM	6.66	± 0.66 c	6.01	± 0.78 ab		6.36	± 0.76	AB
ISP	F1M	1.48	± 0.20 a	2.84	± 0.21 a		2.10	± 0.74	A
	F1oM	1.71	± 0.07 ab	3.19	± 0.49 a		2.45	± 0.86	A
	F2M	2.05	± 0.29 b	3.63	± 0.46 a		2.84	± 0.90	A
	F2oM	1.81	± 0.17 ab	3.29	± 0.66 a		2.55	± 0.90	A
Total pectin	F1M	37.13	± 1.26 ab	29.96	± 1.42 a		33.55	± 3.96	A
	F1oM	35.53	± 0.47 a	29.38	± 0.64 a		32.46	± 3.40	A
	F2M	39.10	± 1.41 b	34.58	± 0.63 b		36.84	± 2.58	B
	F2oM	38.78	± 1.06 b	33.13	± 2.87 b		35.95	± 3.60	B

SD 'Bluecrop'/'Reka'= standard devision of both cultivars

BROWN et al. (2002) reported that the RG-II-B complex consists of two calcium molecules, apart from two boron molecules and two monomere RG-II chains. However, the pectin content increased in the mulched variants where calcium content of fruits was lower than in unmulched variants. This leads to the conclusion that there are other influencing factors. For example, higher potassium contents were found in leaves and fruits of the mulched variants (data not shown). Potassium is closely integrated into the regulation of stomata, and therefore enhances its turgor pressure, causing an opening of stomata (HUMBLE and RASCHKE, 1971). However, this regulatory mechanism assumes optimum leaf water potential. In the present study, a higher soil water capacity and accordingly higher leaf water potential is suggested in the mulched variants in contrast to the unmulched variants. The shortage of stomata opening under drought stress could decrease the uptake of CO_2, and could led to a limited photosynthesis activity and a lower production of ribulose biphosphat as reported from FLEXAS and MEDRANO (2002). Ribulose biphosphat is responsible for the rebuilding of CO_2 to glucose in the Calvin cycle.

In conclusion, it could be shown that plants absorbed foliar applied boron, leading to higher boron contents of fruits. Application of boron induced an increase in the pectin content of all fractions (water-soluble, alkali-soluble, and insoluble). However, the content of phenolic compounds decreased and respectively the antioxidant acticvity. In contrast, the increased calcium content in the unmulched variants did not influence the biosynthesis of pectin substances, probably as blueberries avoid high calcium content. Therefore, they cannot benefit high calcium content for further pectin synthesis. Plants preferred to root in mulch layer benefiting from a lower pH and reduced calcium uptake in the rhizosphere. Mulching appeared to improve growth conditions due to the high amount of organic matter and higher soil water availability. This circumstance tended to higher contents of pectin. However, polyphenol accumulation in fruits was inhibited by mulching. Finally, this work indicates that moderate stress conditions during cultivation might improve fruit quality in terms of bioactive compounds, however only to a limited extent.

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