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## Influence of location and fertilization on antioxidant activity in highbush blueberries (*Vaccinium corymbosum* L.)

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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. Fruits were harvested at two picking dates and analyzed for their content of phenolic compounds and antioxidant activity. These data were compared with samples of two forest soil locations from the Brandenburg region (Beelitz and Klaitow).

The results showed significant differences between cultivars, both harvest times and different locations. The variations in fertilization and ground cover (with or without mulch) showed significant differences. Moreover, it is demonstrated that without ground cover and commercial fertilization higher contents of total phenolic compounds and an increase in antioxidant activity tendentiously occurred. This result paralleled the decline in vegetative growth and was associated with drought stress.

### Introduction

Secondary plant metabolites, especially phenolic compounds, which are responsible for flavour and colour, are being produced by all plant material for the protection of biotic and abiotic influences. Moreover it is known that phenolic compounds block free radicals, which are activated due to oxidative stress or environmental pollution (RICE-EVANS et al., 1995). Therefore, phenols have been suggested to reduce cellular damage and play a role in preventing harmful diseases such as cancer and coronary heart disease (KALT and DUFOUR, 1997).

Berry fruits are one of the richest sources of antioxidants in our diets (KÄHKÖNEN et al., 1999). Especially blueberries have received much attention due to high levels of plant phenolic compounds and thus high antioxidant activity (PRIOR et al., 1998).

Blueberry bushes originally grow on heathland or forest soil. However, in Germany there are attempts to cultivate blueberries on formerly used farmland. On such location soil condition are not favourable due to high soil pH, low organic matter content and low water storability. Moreover, there is a lack of symbiosis of mycorrhiza organism, which is essential for nutrition especially for nitrogen supply (YANG et al., 2002). Therefore, soil preparation (mulching) and fertilization are important features for cultivation on farmland soil. Furthermore, the foliar applications of boron support growing and fruiting of plants, as boron is known for enhancing fruit set and yield of many crops, e.g. for apple (WÓJCIK, 2006) and black currant (WÓJCIK, 2005) as well as fruit berry number of blueberries (BLEVINS et al., 1996). Boron also plays an important role as a drought stress inhibitor. WERMINGHAUSEN (1957) reported that plants with higher boron level showed a decreased transpiration rate under drought conditions.

Therefore, the aim of the present study was to investigate the effect of different nitrogen and boron supply as well as ground cover systems of formerly used farmland on changes in bioactive secondary plant metabolites of highbush blueberries.

### Materials and methods

#### Plant material

Plant material was obtained from the highbush blueberry cultivars 'Bluecrop' and 'Reka'. Bushes were planted in 1997 in peat filled holes on formerly used farmland in Berlin-Dahlem. In 2004 soil minerals data comprised of 31 mg P<sub>2</sub>O<sub>5</sub>, 62 mg K<sub>2</sub>O, 33 mg MgO per 100 g soil, soil acidity was 5.58 (CaCl<sub>2</sub>) with an organic matter content of 2.97%. The plants were cultivated in two ground cover (with and without pine bark mulch) and fertilization variants. Fertilization was conducted from May to mid July with a nutrient supply of 10 g N- 29 g P<sub>2</sub>O<sub>5</sub>- 83 g K<sub>2</sub>O- 13 g MgO- 66 mg B per plant in first fertigation variant (F1). Plants of second nutrient supply system (F2) received an increased nitrogen fertilization (14 g/ plant) and additionally boron foliar applications (400 mg/ plant).

Ripe berries were picked at two harvest dates providing 70-80% of total crop ('Reka': 19/07/04 and 29/07/04; 'Bluecrop': 28/07/04 and 04/08/04). Samples of each variant and cultivar were frozen immediately and stored at -20°C until further analysis. These samples were compared with blueberries of two forest soil locations from the Brandenburg region, Klaitow and Beelitz.

#### Chemical analysis

Fifteen berries of each variant were carefully crushed and 1 ml of juice was filtered through filtrations tubes (20 µm pore size, Supelco, Deisenhofen, Berlin) and frozen immediately at -30°C until analysis. Antioxidant activity was determined by electron spin resonance (ESR) spectroscopy and trolox equivalent antioxidant capacity (TEAC) assay. ESR was performed as described by RÖSCH et al. (2003), using a table spectrometer Miniscope MS 100 (Magnetech, Berlin, Germany). The signal intensity of a stabilized synthetic radical (Potassium nitrosodisulfonate (Fremy's salt), Sigma- Aldrich, Steinheim, Germany) was obtained after 20 min, by which the time of reacting with blueberry antioxidants was completed and expressed as moles of Fremy's salt reduced by one mole antioxidant/l.

TEAC assay was carried out on a Specord 40 (Analytik Jena, Jena, Germany) spectrophotometer as described by RE et al. (1999) with ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid, Sigma-Aldrich, Steinheim, Germany) as free radical and TROLOX (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma-Aldrich, Steinheim, Germany) as a standard at a wavelength of 732 nm. Results were expressed as mmol TROLOX/100 ml.

Total soluble phenols were analyzed photometrically with Folin-Ciocalteu reagent (Sigma-Aldrich, Steinheim, Germany) by the method of Jennings (1981), using gallic acid (Serva, Heidelberg, Germany) as a standard at a wavelength of 765 nm. Results were expressed as mg gallic acid /100 ml.

#### Statistic calculations

The statistic calculations were performed with SPSS 11.0 by SPSS Inc., Chicago, USA (2001). Significances of differences were conducted with a Tukey test (P < 0.05).

## Results and discussion

### Influence of location on total phenol content and antioxidant activity

There were significant differences between the forest soil locations Beelitz (22.0 mmol Fremy's salt/l) and Klaistow (27.7 mmol Fremy's salt/l) when using ESR method (Fig. 1). TEAC assay determined differences between the locations Beelitz (14.4 mmol TROLOX/ 100 ml) and Klaistow (24.8 mmol TROLOX/ 100 ml), as well as Beelitz and Berlin-Dahlem (27.0 mmol TROLOX/ 100 ml). Significant differences in total phenol analysis were observed between the locations Beelitz (69.8 mg gallic acid/ 100 ml) and Berlin-Dahlem (125.9 mg gallic acid/ 100 ml). Plants of Berlin-Dahlem are more stressed by wind and unfavourable soil conditions. Furthermore open farmland berries were influenced by a high solar radiation in comparison to forest locations. Stress as well as increased light conditions increase phenolic metabolism (FEUCHT and TREUTTER, 1989; HÄKKINEN, 2000) and might explain the higher levels of bioactive compounds in Berlin-Dahlem. The forest soil location Beelitz showed the lowest content of total phenols as well as of antioxidants in all analysis, probably due to a higher berry weight and higher soil nitrogen content. High nitrogen levels decrease the content of phenolic compounds (FEUCHT and TREUTTER, 1989; HÄKKINEN, 2000). Berry weight again is negative correlated with the content of phenolic compounds (CONNOR et al., 2002b; MOYER et al., 2002).

However, variation between locations is depending on local micro climate (temperature, irradiation, moisture) and soil conditions in

blueberry cultivation (JONES, 1999 in HOWARD, 2003; HÄKKINEN, 2000; MOYER et al., 2002). In contrast, PRIOR et al. (1998) reported no differences between the blueberry growing locations Orgeon, New Jersey and Michigan.

### Influence of cultivation on total phenol content and antioxidant activity

ESR data ranged from 23.6 to 28.9 mmol Fremy's salt/l, for TEAC method 24.5 to 27.5 mmol TROLOX/ 100 ml and for total phenol content from 120.1 to 134.4 mg gallic acid/ 100 ml (Fig. 2). The present results demonstrate that blueberries without ground cover, lower nitrogen and boron fertilization (F1oM) had higher contents of total phenolic compounds and an increase in antioxidative activity. This result is assumed to be related to a stress mediated process, i.e. the non covered soil variant resulted in a low organic matter content, high soil ph and thus insufficient waterstorage. This might also explain the decline in vegetative growth. In contrast the F2oM variant revealed a lower content of bioactive compounds due to additional boron application. Boron could have restricted the influx of substrate into the pentose-phosphate pathway and the synthesis of phenols (LEE and ARONOFF, 1967 in MONDY and MUNSHI, 1993). MONDY and MUNSHI (1993) reported a decreased phenol concentration in potato tubers after boron treatment. Also AL-YOUSIF et al. (1994) determined a reduced phenol content in date palm and sorghum leaves due to an increased boron level.

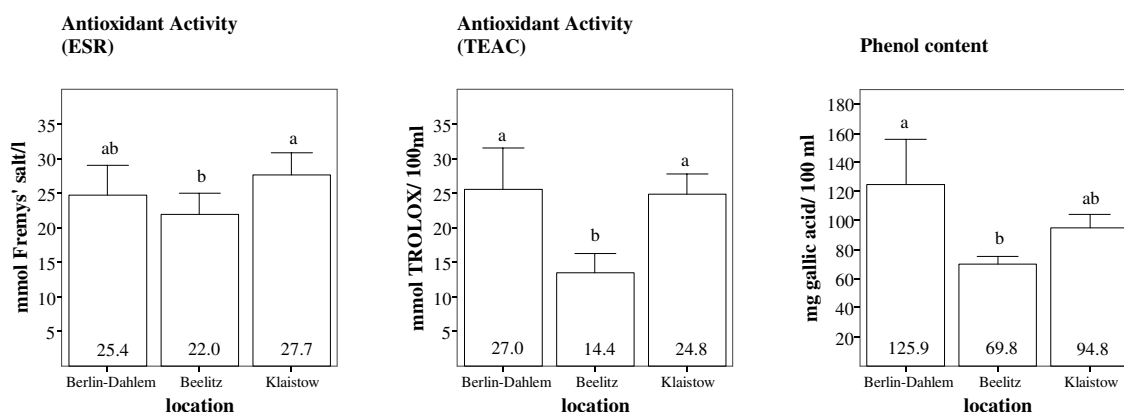


Fig. 1: Influence of location on total phenol content and antioxidant activity, Tukey Test ( $P < 0.05$ )

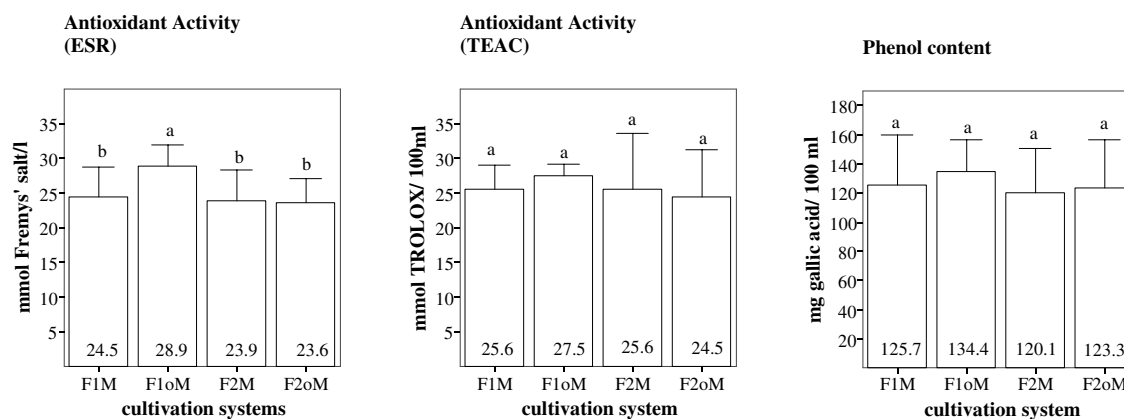


Fig. 2: Influence of cultivation on total phenol content and antioxidant activity; F1M= commercial fertilization with mulch, F1oM= commercial fertilization without mulch, F2M= increased nitrogen and boron supply with mulch; F2oM= increased nitrogen and boron supply without mulch; Tukey test ( $P < 0.05$ )

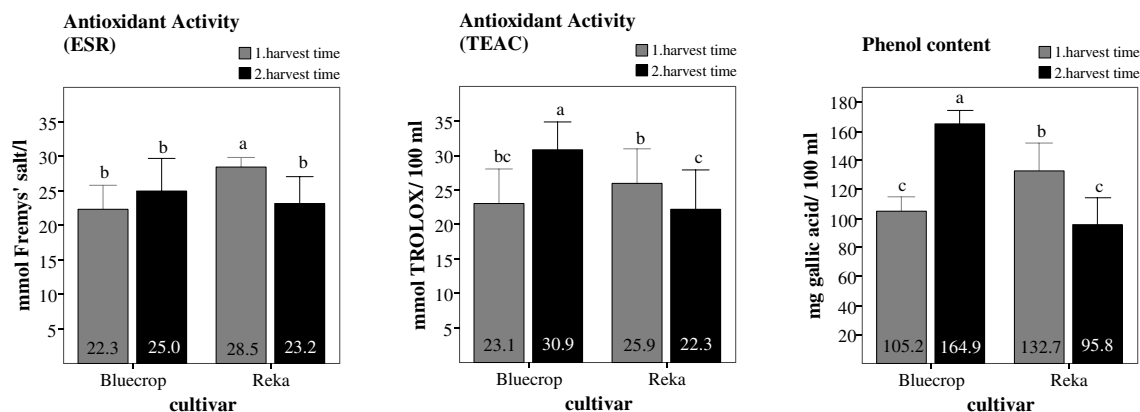


Fig. 3: Influence of cultivar and harvest time on total phenol content and antioxidant activity, Tukey ( $P < 0.05$ )

### Influence of cultivar and harvest time on total phenol content and antioxidant activity

Comparison of cultivars showed significant differences in antioxidant activity at the first harvest date between 'Bluecrop' (22.3 mmol Fremy's salt/l) and 'Reka' (28.5 mmol Fremy's salt/l) by ESR method (Fig. 3). In TEAC assay differences were found between cultivars of the second harvest date ('Bluecrop': 30.9 mmol TROLOX/ 100 ml; 'Reka': 22.3 mmol TROLOX/ 100 ml). In respect to total phenols significant differences were observed for the first harvest date between 'Bluecrop' (105.2 mg gallic acid/ 100ml) and 'Reka' (132.7 mg gallic acid/ 100ml) as well as for the second date ('Bluecrop': 164.9 mg gallic acid/ 100ml; 'Reka': 95.8 mg gallic acid/ 100ml).

Variation in bioactive compounds of different cultivars are affected genetically, also described by PRIOR et al. (1998), CONNOR et al. (2002a; 2002b), MOYER et al. (2002) and HOWARD et al. (2003).

Comparison of harvest times showed differences in the cultivar 'Reka' (first date: 28.5 mmol Fremy's salt/l; second date: 23.2 mmol Fremy's salt/l) using ESR method. Significant differences in TEAC assay of harvest times occurred at both harvest dates for 'Bluecrop' (first date: 23.1 mmol TROLOX/ 100ml; second date: 30.9 mmol TROLOX/ 100 ml) and 'Reka' (first date: 25.9 mmol TROLOX/ 100 ml; second date: 22.3 mmol TROLOX/ 100ml). Total phenol content differed significantly between harvest time and cultivar, i.e. 'Bluecrop' (first date: 105.2 mg gallic acid/ 100 ml; second date: 164.9 mg gallic acid/ 100 ml) as well as of 'Reka' (first date: 132.7 mg gallic acid/ 100 ml; second date: 95.8 mg gallic acid/ 100 ml).

However, antioxidant activity (TEAC) and total phenol analysis showed that bioactive components of the medium-ripened cultivar 'Bluecrop' were significantly higher in the second harvest in comparison to the first harvest, whereas in contrast the first harvest of the early-ripened cultivar 'Reka' had higher levels of total phenols and antioxidants. The results of the ESR method showed a similar trend.

Total phenolic content and antioxidant activity of blueberries decrease during fruit ripening (KALT, 2003). Although all berries were harvested at a fully ripe stage, fruits of 'Bluecrop' second crop showed a lower sugar/acid ratio, i.e. an earlier ripening stage (10.3) in comparison to the first harvest date (16.0). Moreover, in the cultivar 'Bluecrop' increased level of phenolic compounds and antioxidants of the second harvest date may also be caused by the decline in fruit weight from 1.9 g to 1.4 g, respectively. This is also consistent with findings by HOWARD et al. (2003), CONNOR et al. (2002b) and MOYER et al. (2002).

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