

Nutrient Uptake and Utilization and Antioxidants of Fruits in Red Raspberry (*Rubus idaeus* L.) Cultivar ‘Autumn Bliss’ in response to Fertilization under Extended Photoperiod

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Abstract

Annual-fruited cultivars of red raspberry (*Rubus idaeus* L.) complete its entire cycle of vegetative growth, flowering, and fruiting in one growing season, which has not been well studied in response to treatments of photoperiod and fertilization. In this study, micro-propagated plantlets of ‘Autumn Bliss’ were planted in a greenhouse at Dalian, Northeast China. Some were treated with fertilizers at the rate of 12 g per plant (N-P₂O₅-K₂O, 14-14-14) under extended photoperiod of 17 h with PPFD of 240 μmol m⁻² s⁻¹ at dark-time (Pho.+Fert.), while others were treated with longer photoperiod (Pho.) or with the control. Compared to the control, both growth and biomass in shoot part of ‘Autumn Bliss’ were promoted by the Pho.+Fert. treatment, but foliar nutrients declined as the symptom of nutrient dilution. The Pho. treatment had no effect on either growth or nutrient uptake in leaves, resulting in the symptom of nutrient depletion compared to the Control. Soil P availability positively correlated with foliar P concentration but negatively correlated with root length. Only the Pho.+Fert. treatment resulted in the advance of fruiting by two months at July when fruits were measured to have acceptable fruit weight of about 3 g and contents of anthocyanin at ~26.4 mg cyaniding-3-glucoside equiv. 100 g⁻¹ Fw and total phenolic content at ~17.5 mg GAE 100 g⁻¹ Fw.

Keywords: autumn-bearing stock; caneberry; fruit phenology; prolonged illumination; vector analysis

Introduction

The red raspberry (*Rubus idaeus* L.) is a caneberry species with short-lived woody shoots borne on a long-lived perennial root system (Sønsteby and Heide, 2008, 2009). Red raspberry is an excellent source of natural antioxidants (Dragišić Maksimović *et al.*, 2013); hence it is widely grown across all temperate and cold regions of the world (Heide and Sønsteby, 2011). There have been more than 40 countries signed as a group member of red raspberry development with the overall area of about 2 × 10⁵ ha worldwide. Up to 2014, red raspberry cultivation has occupied ~6560 ha and ~5900 ha in the United States (Strik and Bryla, 2015; USDA, 2015) and China, respectively. Continuously increasing interest in the cultivation of red raspberry worldwide inspires better understanding of the environmental manipulation in annual-fruited cultivars.

Red raspberry has been commercially developed as two cultivars. Biennial-fruited cultivars have a 2-year life-cycle through vegetative growth, overwinter, flowering, and fruiting in summer. On the other hand, annual-fruited cultivars complete their whole life-cycle in a single growing season and fruit in autumn (Heide and Sønsteby, 2011). The environmental control of growth and development has been well understood in biennial-fruited red raspberry with manipulations on temperature and photoperiod (Heide and Sønsteby, 2011; Sønsteby *et al.*, 2013), but much less is known about annual-fruited cultivars. Literature reported that flowering and fruit maturation of annual-fruited cultivars were advanced at general temperatures of 15~25 °C (Carew *et al.*, 2003; Sønsteby and Heide, 2012) and sometimes their floral primordial can be initiated at temperature as high as 30 °C (Sønsteby and Heide, 2009). Photoperiod treatment from 8 h to 17 h seemed to have no consistent effect on their growth and flowering (Carew *et al.*, 2003; Sønsteby and Heide, 2009). For the annual-

cultivar 'Polka', the longer photoperiod tended to stimulate vegetative growth and advance flowering (Carew *et al.*, 2003; Sønsteby and Heide, 2009). Other manipulations have also been reported to affect annual-fruiting cultivars' growth and production, such as mycorrhiza inoculation (Jones and McGavin, 1998; Campos-Mota *et al.*, 2004), NaCl stress (Neocleous and Vasilakakis, 2007), chilling (Carew *et al.*, 2001; Neocleous *et al.*, 2005), and pruning (Oliveira *et al.*, 2004). However, most significance of these manipulations appeared to be lower than photoperiod and temperature on cane growth and flowering initiation of annual-fruiting raspberries.

Commercial caneberry growers are encouraged to develop fertilization programs based on initial nitrogen (N) recommendations with adjustments made to N and other nutrients as needed. Strik and Bryla (2015) summarized that N application may increase primocane number and advance growth and flowering, thus increasing yield of annual-fruiting raspberry cultivars, whose response of biomass, however, was not different to nitrate or ammonium forms of N. Other elements rather than N have also been added to red raspberries both as organic amendment and as mineral fertilizers (Horuz *et al.*, 2013). Boron (B) was found to be the main micronutrient that increased yield of raspberries, and tissue phosphorus (P) concentration in raspberry was found to significantly respond to N and B fertilizations (Kowalenko, 2006). In addition to B, P and potassium (K) were both found to tend to undergo deficiency risks in several raspberry cultivars (Horuz *et al.*, 2013). Dean *et al.* (2000) found that N fertilization lost most of its effect on growth and N uptake in raspberry unless soils were amended by manure. Compared to results about environmental control, our knowledge about fertilization of red raspberry is much less and most results in fertilizer experiments were obtained for biennial-fruiting cultivars with quite few studies supplying information about nutritional manipulation on red raspberry cultivars.

Northeast China (38~50°N) is one of the largest red raspberry plant area in China, where about 3000 ha of lands are used for red raspberry planting and total fruit production is estimated to over 2×10^4 ton. Annual-fruiting cultivar is the main red raspberry in Northeast China and accounts for about 70% of all planting reserve. Pruning technique is widely used as within the practical protocol of red raspberry culture to promote the fruit production in Northeast China. Generally, 10 to 15 canes per square meter are left by pruning primocanes emerged from crown buds (Oliveira *et al.*, 2004) so as to gain about 0.6 kg plant⁻¹ of yield from September to mid-October (Xu *et al.*, 2015). However, this region was found to suffer early autumn frost events in recent years (Yin *et al.*, 2016) which have caused significant impact on yield of red raspberry. Therefore, alternative harvest time is demanded to avoid the shock of chilling by some environmental controls.

According to the above summary, temperature and photoperiod appeared to be two major factors determining fruiting and production of annual-fruiting red raspberries. According to our former study (Wei *et al.*, 2013), however, manipulation of photoperiod may cause inherent nutrient dilution within a growing plant even at a proper

temperature. Over-decline of nutrient reserve could impair fruiting because both flowering and fruit production needed to utilize nutrients by translocation (Strik and Bryla, 2015). This contributes to the necessity to supply red raspberries with additional nutrients under the extended photoperiod to improve nutrient uptake and to warrant the potential of advanced fruiting.

Dalian City, Liaoning Province, Northeast China has an area of 330 ha for raspberry cultivation. Also, Dalian City has the crucial harbors for export red raspberry fruit productions to Korea, Japan, Russia, and the Americas. 'Autumn Bliss' (Great Britain, Plant Patent #6597) is early ripening red raspberry cultivar with large and highly flavored fruits. It ripens 10 to 14 days before heritage. Much of the crop is produced within the first two weeks of harvest, which is an advantage in Northern climates. It produces short canes with few spines. It is susceptible to raspberry bushy dwarf virus (Weber, 2012). Generally, several studies have been conducted on 'Autumn Bliss' (Carew *et al.*, 2003; Oliveira *et al.*, 2004; Neocleous *et al.*, 2005; Neocleous and Vasilakakis, 2007; Dragišić Maksimović *et al.*, 2013) and some have confirmed that the prolonged photoperiod stimulated shoot growth of 'Autumn Bliss', but the specific effects could not be conclusive from 8 h to 17 h (Carew *et al.*, 2003). In the present study, 'Autumn Bliss' was planted in a greenhouse in Dalian City with extended photoperiod and additional nutrient supply. It was hypothesized that: (i) fruiting of 'Autumn Bliss' cannot be advanced by long photoperiod unless additional nutrient supply was involved, and (ii) secondary metabolism in fruits would not change too much compared to in those under the ordinary condition.

Materials and Methods

Plant materials

The experiment was conducted in 2015 in a greenhouse at Pulandian Town of Dalian City (39°16' N, 121°49' E), Liaoning Province, Northeast China. 'Autumn Bliss' was supplied as plantlets from micro-propagation by Prof. Zhang from Chinese Academy of Sciences. Briefly, canes with buds from mature 'Autumn Bliss' plants were collected and divided into segments, sterilized, and cultured on the induction medium. The first generations were continuously cultured on the multiplication medium and induced for root egress. Rooted plantlets were moved to pots filled with sands and peat (Zhilunpudao® Agric. Tech. Ltd., Changchun, Jilin, China) in a greenhouse and cultured for 10 d. During this time, indoor temperature and relative humidity were controlled to be 13~23 °C and 50~70%, respectively. In April 2015, plantlet stocks were transplanted to planting beds of the greenhouse for this experiment for another shading-culture of 15 d until most plantlets grew to have new leaves.

Experiment conduction and design

The soils in the greenhouse corresponded to a clay-loamy texture with a maximum depth of 3 m. Soils at the top 30 cm were collected and analyzed to determine the pH of 5.2, organic matter content of 13%, bulk density of 1.1 Mg m⁻³, total N of 2.8 g kg⁻¹, total P of 0.5 g kg⁻¹, total K of

13 g kg⁻¹. Before transplant, soils of planting beds were amended by local poultry manure. Chemical analysis indicated contents as total N of 25.6 g kg⁻¹, organic matter of 499 g kg⁻¹, NH₄⁺-N of 4.6 mg kg⁻¹, organic carbon (C) of 184.3 g kg⁻¹, and C/N ratio of 7.67. Three planting beds were employed in this study and each had the length of 7 m and width of 0.5 m. Each planting bed received one photoperiod regime and two adjacent beds were separated by black-out curtains to eliminate lateral light impact.

The experiment was conducted as a random plot design with three treatments placed at three planting beds. In one bed, plantlets were cultured with additional fertilizers under extended photoperiod for 17 h by supplementing illumination from 18:00 to 1:00 the next day (labeled as Pho.+Fert.); in the second bed, plantlets were cultured with the same photoperiod regime without any additional fertilizer supply (labeled as Pho.); in the last bed, neither supplementary lighting nor additional fertilizer was applied (labeled as the control). Each planting bed was divided into seven plots with the area of 0.5 m × 1 m for each plot. Every two adjacent plots were separated by plastic cloth inserted into soils at the depth of 50 cm to eliminate lateral impact of fertilizers. Two plots at the two ends of the planting bed were left out of the experiment design as the buffer plot to eliminate possible edge effect by exogenous environment of the planting beds. The left five plots in each planting bed were involved as randomly placed replicates as treatment units.

Plantlet culture

Daytime light was only from the natural source unless the photosynthetic photon flux density (PPFD) declined to lower than 200 μmol m⁻² s⁻¹ in cloudy or rainy days when lamps were open. Supplementary light at dark-time was supplied at the PPFD intensity of 240 μmol m⁻² s⁻¹ at the apical floral bud level. This light intensity has been proven to promote flowering in ‘Autumn Bliss’ (Carew *et al.*, 2003). Artificial lighting was supplied by 200-W plant growth lamps (Zhilunpudao® Agric. Tech. Ltd., Changchun, Jilin, China). The light spectrum of these lamps has been tested to be effective for plant growth for several times (e.g. Wei *et al.*, 2013; Zhu *et al.*, 2016). One week after transplant, nutrients were delivered through Osmocote controlled release fertilizer (Scotts UK Ltd., Nottinghamshire, UK) at the rate of 12 g per plant (N-

P₂O₅-K₂O, 14-14-14, micronutrients plus). This fertilizer regime has also been employed for the culture of ‘Autumn Bliss’ by Carew *et al.* (2001) and Sønsteby and Heide (2009). Nutrients were broadcasted along two lateral furrows of each planting bed to the depth of 10 cm and then covered by soils.

Plantlets were transplanted to the density of 49 canes bed⁻¹ translating to 14 canes m⁻² and 7 canes m⁻¹ (with the width of 0.5 m). This density was practical for the culture of red raspberry locally in Dalian City. Also the density of 14 canes m⁻² was quite close to that generating maximum fruit yield following the treatment of pruning (Oliveira *et al.*, 2004). Canes were pruned for floral buds in crowns during vegetative growth period at mid-May, early June, and mid-June, 2015. About ten days after the final pruning flowers were initiated in the Pho.+Fert. treatment. Subsequently, at early July, 2015 fruits started to come out in this treatment until the late July when all fruits grew mature (Fig. 1). No plants in the Pho. treatment and the control had any fruits until late July, 2015; hence, the experiment was terminated at this time. Throughout the experiment, temperature for day/night was measured to be 25.8/17.1 °C, respectively. Highest/lowest relative humidity was 84.3/36 %, respectively. Weeds were controlled manually every week. Temperature inside the greenhouse was controlled with the ambient one by rolling the greenhouse-cover up and down at day and night, respectively.

Plantlets sampling and chemical analysis

Three plantlets were randomly sampled from each treatment plot and bulked as a replicate unit. Parameters were determined for each of these three samplings but their results were averaged as a mean for the replicate. When sampling, roots were carefully shovelled out to keep the root system out of harm. Sampled plantlets were firstly measured for morphological parameters of shoot (cane) length, diameter 3 cm above ground, root length at the steady status, and root volume by measuring the replaced water in a given volume. After morphological measurement, plantlets were divided into parts of leaves, stem, and root and oven-dried at 70 °C for a week. Dried samples were measured for biomass and determined for total concentrations of N, P, and K. Soils were also sampled at the top 30 cm from the plots where plantlets were shovelled. Sampled soils were smashed, air-dried for half a month, and prepared for



Fig. 1. Fruits of red raspberry (*Rubus idaeus* L.) ‘Autumn Bliss’ plantlets at early July, 2015 (A) and mature fruit at end of July, 2015 (B) after treated by additional fertilizer addition under extended photoperiod. (C) At harvest time, both cover over the greenhouse and black-out curtains between planting beds were removed to facilitate plantlet and fruit sampling

chemical analysis of available N, P, and K. Because fruits were only produced in the Pho.+Fert. treatment they did not take part in the comparison among treatments but harvested for three times at early, mid-, and end of July, 2015. Fruits were measured for length, diameter, weight, and number of setting fruits (fallen ones were not counted because they corrupted very soon and quite few have fallen). Harvested fruits were ice-stored until the laboratory and analyzed for contents of antioxidants therein, included anthocyanin, gallic acid, and total phenolics.

Total N, P, and K were determined according to the methodology described by Wei *et al.* (2013, 2014) and Wang *et al.* (2016). Soil available N, P, and K were determined by the method of Guo *et al.* (2016) and Zhu *et al.* (2016). Fruit antioxidants were analyzed according to the methods by Bobinaitė *et al.* (2012) and Dragišić Maksimović *et al.* (2013).

Statistical analysis

Nutrient utilization index (*NUI*) was involved to estimate nutrient utilized by red raspberry which was calculated as (Hawkins, 2007):

$$NUI = \text{Mass}_{\text{leaf}} / N_{\text{leaf}}$$

where, $\text{Mass}_{\text{leaf}}$ and N_{leaf} were the biomass (g) and nutrient (N, P, and K) concentration (mg g^{-1}), respectively. Vector analysis was also employed to evaluate the status of nutrient content and concentration according to the method described by Wei *et al.* (2013) and Wang *et al.* (2016). Regression analysis was performed between soil nutrients and plantlet parameters about root growth and foliar nutrient concentration. ANOVA analysis was conducted on all parameters when significant effect was indicated results were compared and arranged according to LSD test at the 0.05 level. All data were analyzed by SAS 9.2 (SAS Institute, Cary, NC, USA). Temperature and humidity data in this study were all obtained by the web of Weather Underground (<https://www.wunderground.com/>).

Results

Plantlet growth

The Pho.+Fert. treatment resulted in greater shoot length, shoot diameter, and root length than the Pho. treatment and the control (Table 1). Compared to the control, shoot height, shoot diameter, and root length increased by 40%, 45%, and 79%, respectively in the Pho.+Fert. treatment. While compared to the Pho. treatment, these parameters increased by 34%, 31%, and 76%, respectively. The Pho.+Fert. treatment also increased root volume by 98% relative to the control, but results between Pho.+Fert. treatment and Pho. treatment was not

statistically different (Table 1).

Plantlet biomass

The Pho.+Fert. treatment resulted in the increase of foliar biomass by 112% and 85% relative to the Pho. treatment and the control, respectively ($P=0.0056$) (Fig. 2). However, the Pho.+Fert. treatment did not increase stem biomass relative to the Pho. treatment although it increase stem biomass relative to the control by 94% ($P=0.0265$). Root biomass did not respond to any treatment ($P=0.8426$). Root to shoot biomass ratio did not respond to any treatments ($P=0.2750$) which ranged from 0.5 to 0.8.

Plantlet nutrient concentration and utilization

Both treatments of Pho.+Fert. and Pho. resulted in the decline of foliar nutrients for N, P, and K (all three, $P<0.0001$) (Fig. 3). Relative to the control, average declines for N, P, and K in treated plantlets ranged in 17~22 %, 20~28%, and 30~36%, respectively. Stem N and K concentrations were higher in the Pho.+Fert. treatment by 36% and 40%, respectively compared to those in the control, while stem P concentration in the Pho.+Fert. treatment decreased by 19%. In contrast to results about foliar N concentration, root N concentration was increased by 45% in the Pho.+Fert. treatment relative to the control.

Nutrient utilization index (*NUI*) was higher in the Pho.+Fert. treatment than the Pho. treatment and the control for N ($P=0.0058$), P ($P=0.0179$), and K

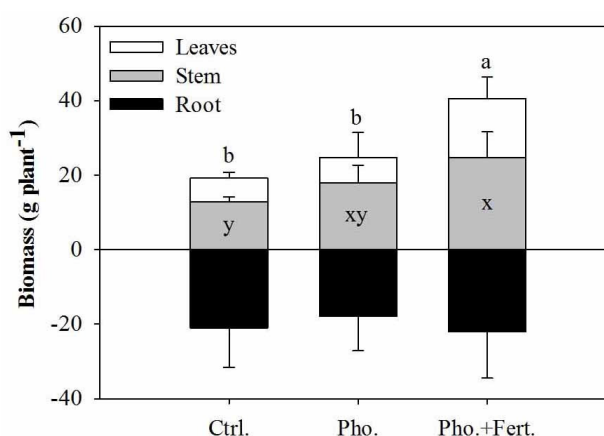


Fig. 2. Biomass in leaves, stem, and root of red raspberry (*Rubus idaeus* L.) 'Autumn Bliss' plantlets in response to the control, the Extended photoperiod (Pho.) treatment, and the additional fertilization plus extended photoperiod (Pho.+Fert.) treatment. Different letters indicate significant difference among treatments at the 0.05 level. For biomass results a and b indicate for leaves; x and y indicate for stem biomass

Table 1. Morphological parameters of raspberry plantlets in response to the control (Ctrl.), and treatments of supplementary photoperiod (Pho.) and Pho. plus fertilizer (Pho.+Fert.)

Treatments	Shoot length (cm)	Shoot diameter (mm)	Root length (cm)	Root volume (cm ³)
Ctrl.	107.56±12.76b	9.75±1.65b	18.80±2.20b	34.20±5.36b
Pho.	112.58±18.81b	10.84±0.78b	19.20±6.63b	53.60±23.66ab
Pho.+Fert.	150.64±27.43a	14.17±2.08a	33.70±10.19a	67.80±18.34a

Note: different letters in a column indicate significant difference at 0.05 level.

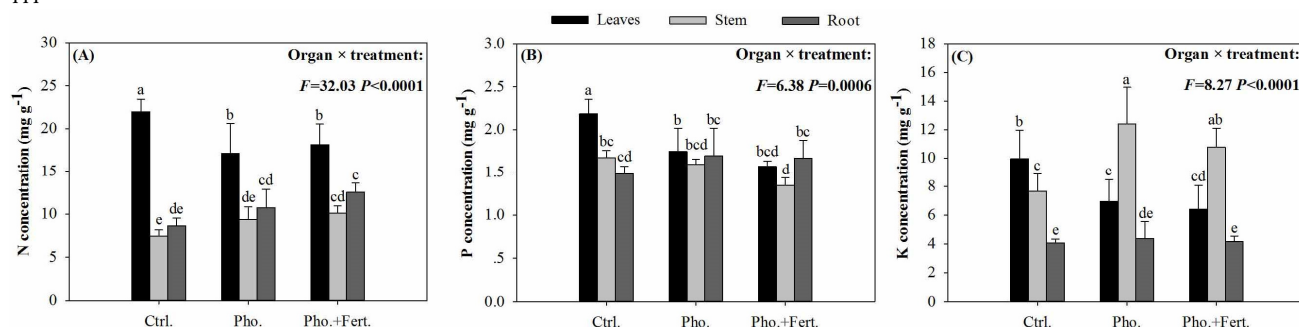


Fig. 3. Nutrient concentrations of N (A), P (B), and K (C) in leaves, stem, and root of red raspberry (*Rubus idaeus* L.) ‘Autumn Bliss’ plantlets treated with the control, the extended photoperiod (Pho.) treatment, and the additional fertilization plus extended photoperiod (Pho.+Fert.) treatment. Different letters indicate significant difference among treatments at the 0.05 level

Table 2. Nutrient utilization indices for N, P, and K in raspberry plantlets in response to the control (Ctrl.), and treatments of supplementary photoperiod (Pho.) and Pho. plus fertilizer (Pho.+Fert.)

Treatments	N	P	K
Ctrl.	0.35±0.07b	3.62±1.06b	0.83±0.31b
Pho.	0.40±0.14b	3.83±1.19b	0.97±0.26b
Pho.+Fert.	0.85±0.48a	9.26±4.50a	2.45±1.94a

Note: different letters in a column indicate significant difference at 0.05 level.

($P=0.0076$) (Table 2). Relative to the control, the Pho.+Fert. and the Pho. treatments increased *NUI* for N, P, and K by 110~141%, 142~156%, and 153~194%, respectively.

Soil nutrient availability

Neither soil available N nor soil available K responded to any treatment ($P=0.9837$ and $P=0.3051$, respectively), which ranged around 0.31 mg kg^{-1} and 0.14 mg kg^{-1} , respectively. However, soil available P concentration responded to treatments ($P=0.0026$). Relative to the control, soil available P concentration declined by 31% and 19% in the Pho.+Fert. and the Pho. treatments, respectively (Fig. 4).

Pearson correlation indicated no relationship between soil and foliar nutrient concentrations except for P element ($R=0.7159$, $P=0.0027$). The relationship between soil available P concentration and foliar P concentration can also be described by a spearman correlation ($R=0.7250$, $P=0.0022$). Likewise, spearman correlation was also retained in the relationship between soil P concentration

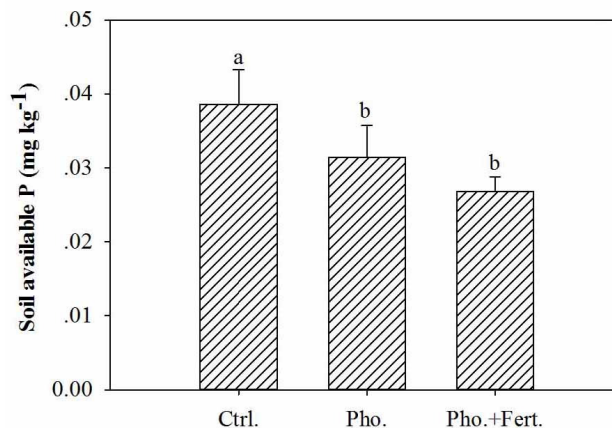


Fig. 4. Soil P content for the culture of red raspberry (*Rubus idaeus* L.) ‘Autumn Bliss’ plantlets in response to the control, the Extended photoperiod (Pho.) treatment, and the additional fertilization plus extended photoperiod (Pho.+Fert.) treatment. Different letters indicate significant difference among treatments at the 0.05 level

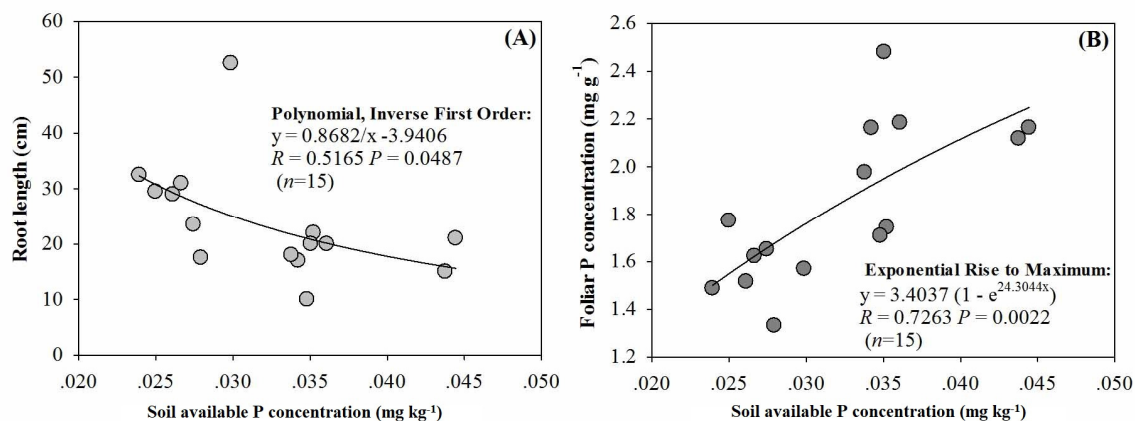


Fig. 5. Non-linear regression analysis of soil available P concentration on root length (A) and foliar P concentration (B) red raspberry (*Rubus idaeus* L.) ‘Autumn Bliss’ plantlets

and root length ($R=-0.6041$, $P=0.0171$). Regression analysis indicated a non-linear negative relationship of polynomial between soil P concentration and root length (Fig. 5a) and a non-linear positive relationship of exponential-rise between soil P concentration and foliar P concentration (Fig. 5b).

Vector analysis

When compositing nutrient content, nutrient concentration and biomass in leaves for vector analysis, ‘Autumn Bliss’ plantlets in the Pho.+Fert. treatment showed apparent symptom of nutrient dilution for all three elements relative to the control due to stimulated biomass with the decline of nutrient concentration (Fig. 6). Otherwise, the Pho. treatment induced the foliar symptom of nutrient depletion for all three elements due to unchanged biomass but decreased nutrient concentration.

Fruit traits

Fruit length and diameter increased since the developed fruiting stage but difference was not significant between the developed and end fruiting stages (length, $P=0.0018$;

diameter, $P=0.0001$) (Table 3). The ratio of fruit length to diameter ratio did not change with time which ranged between 1.1 ± 0.03 and 1.14 ± 0.05 . In contrast, setting fruit number declined with time ($P=0.0003$). Single fruit weight increased from the initial fruiting stage to the developed one by 19% then declined by 11% ($P<0.0001$). Similar trends emerged also for all parameters of antioxidants. Apparently, gallic acid content ($P=0.0003$) was higher than anthocyanin ($P<0.0001$) and total phenolic content ($P<0.0001$).

Discussion

Generally in Northeast China, annual-fruiting cultivars, such as ‘Autumn Bliss’, initiate flowering in July and start to fruit since September to October following the common treatment of floral bud pruning. However, ‘Autumn Bliss’ plantlets in our study advanced their flowering and fruiting phenology by about two months in the combined treatments of extended photoperiod and additional fertilizer application. Therefore, it is reasonable to accept our first hypothesis. In Dalian City, the maximum and

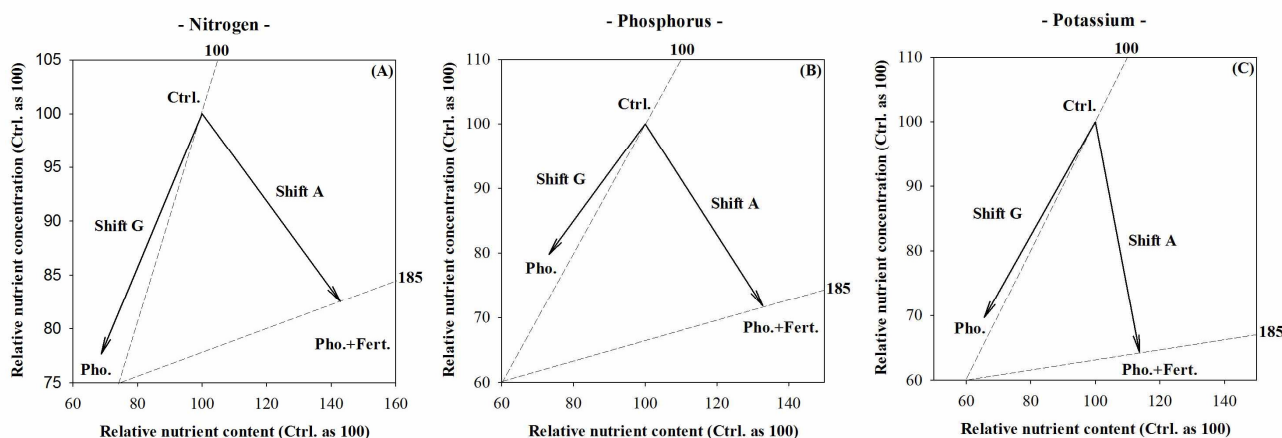


Fig. 6. Vector nomogram of relative change in nutrient content, nutrient concentration and biomass in leaves of red raspberry (*Rubus idaeus* L.) ‘Autumn Bliss’ plantlets in response to the control, the extended photoperiod (Pho.) treatment, and the additional fertilization plus extended photoperiod (Pho.+Fert.) treatment. The type of nutritional response induced by enrichment is characterized by shift (or vector) direction and magnitude. Shift A indicates the symptom of nutrient dilution possibly caused by growth stimulation without sufficient nutrient supply; shift G indicates the symptom of nutrient decline possibly caused by nutrient depletion

Table 3. Fruit parameters of raspberry plantlets treated with supplementary photoperiod plus fertilizer application at three fruiting stages

Fruit parameters	Fruiting stage		
	Initial	Developed	End
Growing trait			
Length (cm)	1.71±0.05b	1.81±0.03a	1.85±0.05a
Diameter (cm)	1.50±0.05b	1.62±0.04a	1.67±0.03a
Setting fruit number	24±1.77a	20±1.46b	18±1.46b
Fruit weight (g)	2.77±0.08c	3.30±0.07a	2.92±0.12b
Antioxidants			
Anthocyanin (mg cyaniding-3-glucoside equiv. 100 g ⁻¹ Fw)	25.40±1.44b	38.66±5.88a	15.12±1.01c
Ellagic acid (mg 100 g ⁻¹ Fw)	80.42±7.62b	97.60±10.04a	60.68±9.64c
Total phenolic content (mg GAE 100 g ⁻¹ Fw)	18.18±1.59b	22.53±2.60a	11.90±0.90c

Different letters in a horizon row indicate significant difference at 0.05 level.

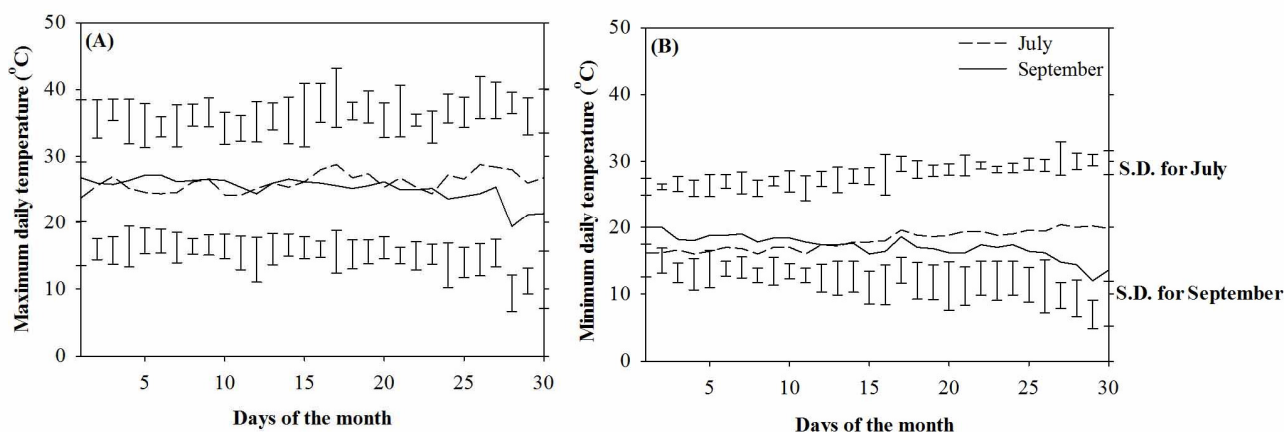


Fig. 7. Dynamics for maximum (A) and minimum temperatures (B) in July and September at Dalian City with data from the most recent five years (2012~2016)

minimum temperatures in July were 26.13 ± 2.27 °C and 18.4 ± 1.47 °C, respectively, and they were 25.22 ± 2.13 °C and 17.13 ± 2.17 °C, respectively in September (Fig. 7). Hence both maximum and minimum temperature in July was higher than those in September (Max, $P=0.0058$; Min, $P=0.0011$). However, the increment of temperature between the two months was only ± 1 °C. The general temperature in both months fell in the most proper temperature range for the culture of annual-fruited red raspberry (Carew *et al.*, 2003; Sønsteby and Heide, 2012). Temperature during fruiting the greenhouse of our study was higher than ambient one by about 5~6 °C, which touched the highest temperature of floral primordial initiation of 30 °C (Sønsteby and Heide, 2009). Therefore, summer temperature in Dalian City is suitable for the culture of annual-fruited red raspberry, which does not need further examination.

Our results showed that the single effect of extended photoperiod was unable to change any parameters of plantlet growth and biomass unless combined with additional fertilization to 'Autumn Bliss'. These results disagreed with findings by Sønsteby and Heide (2009; 2012) where the main effect of photoperiod was quite significant on shoot elongation of annual-fruited cultivars. This might be related to the interaction with temperature, which was much lower in Norway (~60 °N) and varied in the experiments therein. When temperature was not limited, main effect of photoperiod on red raspberry growth may be impaired as a response. However, when additional fertilization was involved growth was promoted. This suggested that fertilizer addition had significant effect on growth of red raspberry under a given photoperiod. Similar results were also obtained by Lockshin and Elfving (1981) and DeGomez *et al.* (1986).

Although root biomass in our study did not respond to treatments, root length showed significantly decline trend with the increase of soil P availability while foliar P concentration increased accordingly. These results together suggested that 'Autumn Bliss' is sensitive to soil P availability by the response of root proliferation and P uptake to leaves. Actually, different plant species showed divergent morphological response to P availability and some crops allocated more sources to support root elongation

hence enhance P uptake and some others showed reverse response (Wang *et al.*, 2015). According to our study, 'Autumn Bliss' chose the strategy to facilitate shoot P concentration at the cost of root morphology when P availability was enhanced by fertilization.

It is interesting that under the extended photoperiod foliar biomass was stimulated but nutrients declined which was evaluated to be the symptom of nutrient dilution. Similar results have also been mentioned and reported on tree seedlings (Wei *et al.*, 2013). Foliar dilution of nutrients in our study was unlikely caused by nutrient leaching impact because according to Zhu *et al.* (2016) where photoperiod appeared to have very weak influence on leachate events. Unlike tree crops whose termination was ended with vegetative growth, red raspberries' termination is with fruiting. Hence, foliar nutrient dilution in our study should be partly caused by the high utilization of nutrient for biomass accumulation and more tendentially used for fruiting. This was reasonable because fruiting was advanced by the extended photoperiod and additional fertilization after all. On the other hand, in the Pho. treatment where no additional fertilizer was applied foliar biomass did not change from that in the control while foliar nutrients declined (Figs. 2 and 5). These results together suggested that photoperiod stimulated biomass consumption without any contribution to growth or biomass accumulation under the condition without sufficient nutrient supply in 'Autumn Bliss'.

Fruits were only produced in the Pho.+Fert. treatment, hence they were compared with those from other studies. Although fruit weight in our study was comparable to that in Dai and Liao (2011) (3g plant^{-1}), the total yield per plantlet for 'Autumn Bliss' in our study ($\sim 55\text{g plant}^{-1}$) was only one tenth of that in the cultivar 'Heritage' in Beijing ($\sim 550\text{g plant}^{-1}$) (Xu *et al.*, 2015). Recent studies revealed that fruit yield in annual-fruited cultivars in Norway was even much greater at the level of 1~3 kg per plant (Sønsteby *et al.*, 2013). Therefore, although fruiting of 'Autumn Bliss' was successfully advanced by combined photoperiod and fertilization treatments, the fruiting yield was still limited by number of setting fruits.

Phenolics are important bioactive compounds and antioxidant, which can be divided into simple phenols,

phenolic acids, phenylpropanoids and flavonoids (Takahama and Oniki, 2000). The anthocyanins are natural pigments belonging to the flavonoid family. In berries, a multiple of phenolic compounds have been detected, including flavanols and phenolic acids (Pantelidis *et al.*, 2007). Ellagic acid released after hydrolysis was the main phenolic compound in the berries belonging to the genus *Rubus*, constituting 77-88% of the total phenolic acids (Häkkinen *et al.*, 1999). In our study, anthocyanin content in fruit extracts during developed fruiting stage (Mid-July) of 'Autumn Bliss' in our study (Table 3) concurs with that in the same cultivar of Pantelidis *et al.* (2007), and our anthocyanin results at the end of fruiting stage (late July) was comparable with those in Dragišić Maksimović *et al.* (2013). Ellagic acid content in our study was generally about two thirds of that in Bobinaitė *et al.* (2012). In addition, our total phenolic content was comparable with that in fruit of 'Autumn Bliss' in Pantelidis *et al.* (2007) (~10-20 mg GAE 100 g⁻¹ Fw) but much lower than that in Bobinaitė *et al.* (2012) and Dragišić Maksimović *et al.* (2013). Hence, it seemed that combined treatments of extended photoperiod and additional fertilization in our study increased flavonoids components by the promotion of anthocyanin contents but showed little effect on phenolic acids according to ellagic acid content. Unlike the traditional vitamins, anthocyanins are not essential for short-term well-being but can modulate human metabolism in a manner of long-term manner of intakes to prevent the health risk. These together suggested that total phenolic contents in fruits of 'Autumn Bliss' of our study can be acceptable for the usage of antioxidation due to the fair content of anthocyanin.

Conclusions

Our results showed that fruiting of red raspberry cultivar 'Autumn Bliss' can be advanced from September to July in temperate region at the latitude of about 40°, such as Dalian City, Liaoning Province, Northeast China. Temperature throughout the experiment was measured to range about 15-26 °C, which has been determined to be the proper for growth and reproduction of red raspberry by former studies. Manipulations contributing to advanced fruiting mainly included the additional fertilization at the rate of 12 g per plant (N-P₂O₅-K₂O, 14-14-14, micronutrients plus) under extended photoperiod of 17 h with PPFD of 240 μmol m⁻² s⁻¹ at dark-time. Both growth and biomass in shoot part of 'Autumn Bliss' were promoted by these treatments, but foliar nutrients declined as the symptom of nutrient dilution. Single treatment of extended photoperiod without additional fertilization affected neither growth nor nutrient uptake in leaves, resulting in the symptom of nutrient depletion. Both weight and anthocyanin content for fruit can be acceptable but fruit number per plant was much fewer, resulting yield to be quite lower compared to that from ordinary culture. In conclusion, combined treatments of extended photoperiod and additional fertilization can advance fruiting time of 'Autumn Bliss' and anthocyanin acted as the main antioxidant therein, but yield was limited by fruit number which needs more studies to improve in future.

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