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Ecophysiological characteristics of two carrot (*Daucus carota* L.) cultivars in response to agroecological factors and nitrogen application

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Abstract

Plant density, planting time, harvest timing, and nitrogen influence on short-term gas-exchange properties of carrot cultivars, Topcut and Sugarsnax (*Daucus carota* L.) were investigated under field conditions. Net photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration rate (E) differed significantly with the cultivars studied. Both planting and harvest timing changed the midday P_N rates. P_N increased as harvest timing advanced regardless of planting time. Late planting combined with late harvesting registered the maximum P_N rates (4.5 μmol m^{-2} s^{-1}). The water-use efficiency (WUE) was altered by temperature at different harvest timings along with the choice of cultivar. Early harvested Sugarsnax had a higher WUE (2.29 mmol mol^{-1}) than TopCut (1.64 mmol mol^{-1}) as Sugarsnax exhibited more stomatal conductance than TopCut. These changes were principally governed by fluctuations observed with air temperature and photosynthetic photon flux density (PPFD) and altered by the sensitivity of the cultivars to ecological factors. Plant density did not affect the photosynthetic gas-exchange parameters. Our results suggest that carrots manage high population density solely through morphological adaptations with no photosynthetic adjustments. Carrot leaves responded to N application in a curvilinear fashion in both cultivars. N did not alter g_s, E, or WUE in carrots. N, applied at a rate of 150 kg N ha^{-1}, increased foliar N up to 2.98%. We conclude that 2.98% of foliar N is sufficient to achieve the maximum photosynthetic rates in processing carrots.

Additional key words: *Daucas carota;* foliar nitrogen; photosynthesis; processing carrots; water-use efficiency.

Introduction

Choice of population density, planting time and harvest timing impose diverse regimes of environmental and growth conditions, which can directly affect net photosynthesis. The two major environmental parameters that change with planting and harvesting time are ambient air temperature and photosynthetic photon flux density (PPFD). The relationship between PPFD (Kyei-Boahen *et al.* 2003), temperature (Thiagarajan *et al.* 2007) and P_N has been investigated in carrots (*Daucus carota* L.), nevertheless, under noncompetitive and/or controlled environments. High plant densities arising from high seeding rates under field conditions enforce resource limitations particularly, in terms of water, nutrients, and light (Rajasekaran *et al.* 2006). Currently, limited information is available on changes in photosynthetic parameters under competitive agroecological conditions for processing carrots. Interestingly, carrots sustain their productivity under competitive environments by altering their leaf orientation, root size and bulking (Benjamin and Sutherland 1992). While the morphological adaptations are well demonstrated, the underlying photosynthetic adjustments under various agroecological conditions are yet to be understood. Evidence from other crops such as American Pokeweed (*Phytolacca Americana*), suggest that high population densities cause a decline in g_s, E, and P_N even under elevated CO_2 levels (He and Bazzaz 2003).

Carbon-fixation ability of the leaves is linearly correlated to foliar N content in several C_3 species (Evans 1989). Nitrogen increases chlorophyll content, induces osmotic adjustments (Premachandra *et al.* 1990, Saneoka *et al.* 2004), and promotes CO_2 assimilation rates. Although increases in carrot foliar nitrogen concentrations to N application have been established, actual N

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Abbreviations: C_a – ambient CO_2 concentration; C_i – internal CO_2 concentration; E – leaf transpiration rate; g_s – stomatal conductance; P_N – net photosynthetic rate; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase; WUE – intrinsic water-use efficiency.

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requirements under field conditions still remains elusive or mixed (Westerveld *et al.* 2004, 2006, 2007). Carrots are well established to utilize nitrogen sources from deep root zone layers owing to their deep root system (Westerveld *et al.* 2004). This root system has been attributed to varying responses to nitrogen fertilizers. Alternatively, tissue N status can serve as a key indicator of optimal N requirement of carrots. Understanding the influence of N on gas-exchange parameters in carrots can help identify optimal N status for improved photosynthetic performance in carrots. Accordingly, this investigation addressed the following objectives: (*1*) to understand the photosynthetic response in carrots to changes in foliar N, (*2*) to examine the relationship between foliar N and P_N and (*3*) to identify the influence of plant density, abiotic conditions that prevailed during planting and harvesting times on the gas-exchange parameters in selected carrot cultivars.

Materials and methods

Two different experiments were conducted to achieve the objectives. The general description on the locations and field management practices are provided in the following section. The subsequent sections provide information on the specific treatment and design of the experiments.

Site locations and soil types: Research fields were established in Northville, King's County, NS, Canada (45º12'N, 64º57'E) and Lakeville, King's County, NS, Canada (45º10'N, 64º64'E) in two consecutive years. In 2007, the N trial was located in Lakeville while the planting- and harvest-timing trial was in Northville. In 2008, both trials were located in Northville. Soil types for both areas are characterized by 60–80 cm of well drained, loamy sand over loose, yellowish-brown sand. The slope ranges from 0.5%–2% in Lakeville and 2–5% in Northville.

Field management practices: Field preparation consists of one deep chisel plough (30 cm) during the fall followed by spring chisel plough, s-tine (twice) and bed-forming. Pre-plant basal fertilizer custom mix was applied at the rate of 407 kg ha^{-1} which consisted of N:P:K:Mg: Zn:B nutrients in the ratio of 0:0:4:3:0.2:0.3 (*Cavendish AgriServices Canada,* Nova Scotia). A top dress of N:P:K (ratio of 34:0:0) fertilizer was applied at a rate of 224 kg ha^{-1} based on soil tests (*Cavendish AgriServices Canada,* Nova Scotia). Carrots were seeded on beds that were 176 cm wide. Each bed was comprised of 3 rows spaced 54 cm on center. The spacing between outside rows on adjacent beds was 68 cm to allow for wheel spacing of the bed-former. Pest control was based on weekly integrated pest management reports and varied between years with applications of *Bravo* (*Syngenta Crop Protection Canada, Inc*., Ontario, Canada) 2.5 L ha^{-1} and *Sevin XLR* (*Bayer CropScience*, Canada) *Plus* 2.5 L ha^{-1} for insect and disease control.

Planting and harvest timing (Exp. 1): The treatments consisted of two cultivars, three seeding rates, three planting time and three harvesting times. Each treatment unit was replicated three times and hence total of 54 plots were used in each year of investigation.

Sugarsnax and TopCut (*Nunhems seeds*, The Netherlands) are two of the principal cut and peel varieties grown in Atlantic Canada. They are both imperative type carrots preferring cool climate and typically require 95 to 115 days to attain maturity. The early and late harvest timings were determined based on this average maturity period. These two cultivars were sown at 40, 50 and 60 seeds 30 cm^{-1} using a 9-row *Stanhay-Webb Stars* seeder (*Stanhay Webb Ltd*., Grantham, UK) on 3 different dates to attain the different population densities. During 2007, plots were sown on 8 May (early), 15 May (mid) and 23 May (late). In 2008, seeding occurred on 13 May (early), 5 June (mid) and 19 June (late). Each plot was 9 rows (5.4 m) wide by approximately 30-m length. There were 3 replications per treatment and gas-exchange measurements were taken randomly throughout the plot. Watermark soil moisture probes (*Spectrum Technologies,* IL, USA) were installed in each experimental plot to measure the soil moisture potential (10 cm deep) prior to gas exchange measurements. Multiple harvests were made after 60 days of planting (DAP) which marks the initiation of active bulking stage. The growth stages of the experimental units during harvest were 98, 111, and 128 DAP for early-planted; 91, 104, and 121 DAP for mid-planted and 83, 96, and 113 DAP for late-planted fields in 2007. In 2008, the growth stages were 99, 115, and 127 DAP for early, 76, 92, and 104 DAP for mid- and 62, 78, and 90 DAP for late-planted fields. The delay in the planting date during 2008 was inevitable due to continuous rainfall events.

Foliar nitrogen and photosynthesis (Exp. 2): Sugarsnax and Top Cut, two cut and peel varieties were sown at 50 seeds 30 cm^{-1} using a 9-row *Stanhay-Webb Stars* seeder (*Stanhay Webb Ltd*., Grantham, UK) on 15 May, 2007 and 13 May, 2008. A total of 40 plots (2 varieties, 7 fertilizer treatments, 3 replicates) were used for this study. Each plot was 1.8 m wide and 10 m long and flanked by 3 guard rows on each side. Gas-exchange measurements were performed between 11:00 and 13:00 h each time at all treatments. Each treatment was replicated 3 times. In 2007, plots were harvested on 4 September and in 2008, harvest occurred on 9 September. From the harvested plants, 3 to 4 root portions were randomly selected, cut, washed and freeze-dried until analysis. The foliage from each replication was dried in an oven at 60°C prior to analysis. Foliar and root nitrogen contents were analyzed employing an N gas analyzer utilizing induction furnace and thermal conductivity (*LECO FP-528*) by AOAC method 990.03 (1997).

Fertility treatments: Initial soil N levels on the plots were 0.098% in 2007 and 0.255% in 2008. Plots received N (34:0:0) fertilizer *viz*., 0, 50, 100, 150, 200, 300, and 400 kg of N ha^{-1} in a 60% (pre-emergence), 40% (8 weeks after emergence) split application. The ammonium nitrate fertilizer (*Cavendish AgriServices* Canada, Nova Scotia) was broadcast carefully and uniformly over the entire plot area.

Gas-exchange measurements were made on the newest fully expanded leaf using a portable open-flow gas analyzer connected to a portable leaf chamber unit (*LCA-4, Analytical Development Company*, Hoddesdon, UK). Observations were made between 11:00 and 13:00 h on each harvest date. An interval of 1 min was allowed before measurements were taken from each leaf to enable gas exchange to reach steady state conditions. Leaf area was calculated using image analysis software (*CIAS 2.0, Jandel Scientific*, CA, USA). Gas-exchange measurements were calculated based on their respective leaf areas. The WUE was calculated as the ratio between P_N and E. The carboxylation efficiency was calculated as the ratio between the ambient CO_2 (C_a) and the internal CO_2 (C_i), expressed as C_a/C_i.

Statistical analyses: Gas-exchange data from both trials were analyzed using *SAS* software (*SAS* 1999, NC, USA). The "proc Mixed" statement in *SAS* was used to test the treatment effects. For data from Exp. 1, population density, planting time, harvest timing and cultivar were considered as main effects and year was included as a block factor. For Exp. 2, nitrogen application rate was included as main factor along with cultivar. Since the data did not show constant variance, transformations were carried out on gas-exchange parameters to meet normality assumptions. However, only nontransformed values are reported in this paper to ensure clarity. When differences were significant, *Tukey*'s LSD was used to separate the means by using the macro developed by Saxton (1998).

Results and discussion

Gas-exchange responses to abiotic conditions at planting and harvest timing: Abiotic conditions prevailing at planting time along with harvest timing greatly influenced P_N. The PPFD, temperature and rainfall changed significantly with planting and harvesting times (Fig. 1). In general, maximum chamber temperature and PPFD were observed at mid planting time throughout the study. The cumulative rainfall amounts decreased as the planting day was delayed. P_N generally increased from early to late harvest (Fig. 1). The trends observed in this

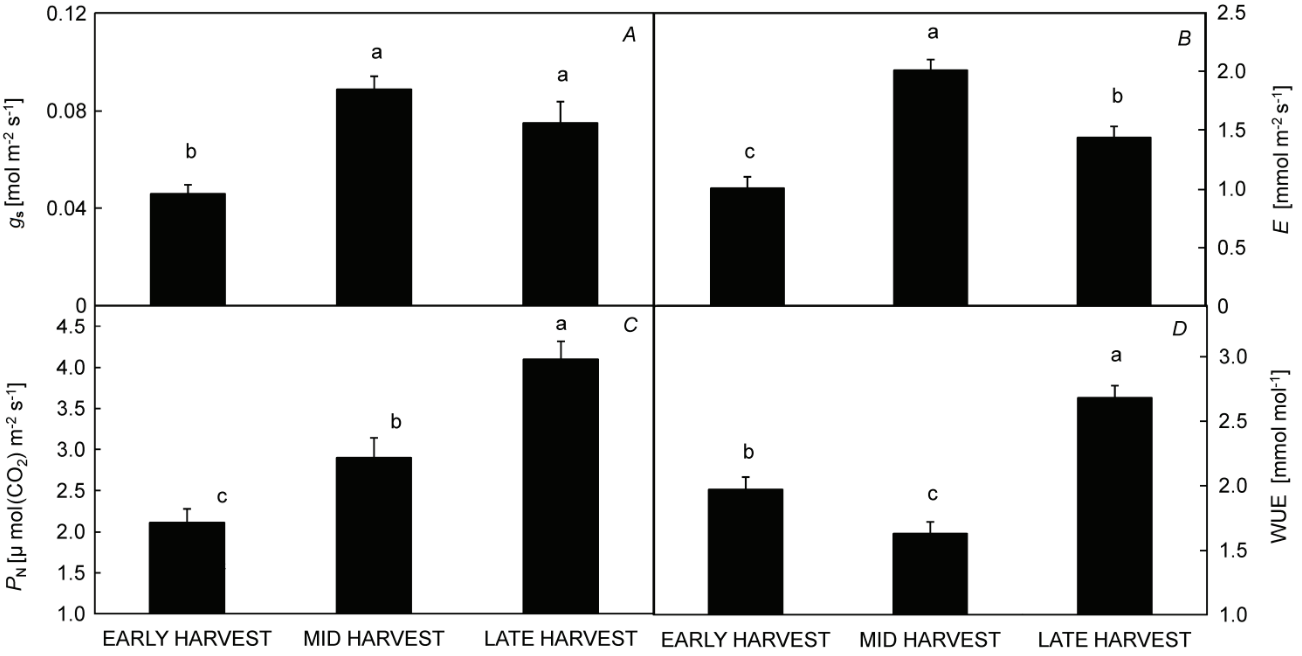

Fig. 1. Mean values of stomatal conductance (g_s) (*A*), transpiration (*E*) (*B*), net photosynthetic rate (P_N) (*C*), and intrinsic water-use efficiency (WUE) (*D*) of carrot (*Daucus carota* L.) cultivars influenced by harvest timing (early, mid, and late). LSD means ± SE followed by *the same letters* are not significantly different. $n = 18$, $p<0.05$.

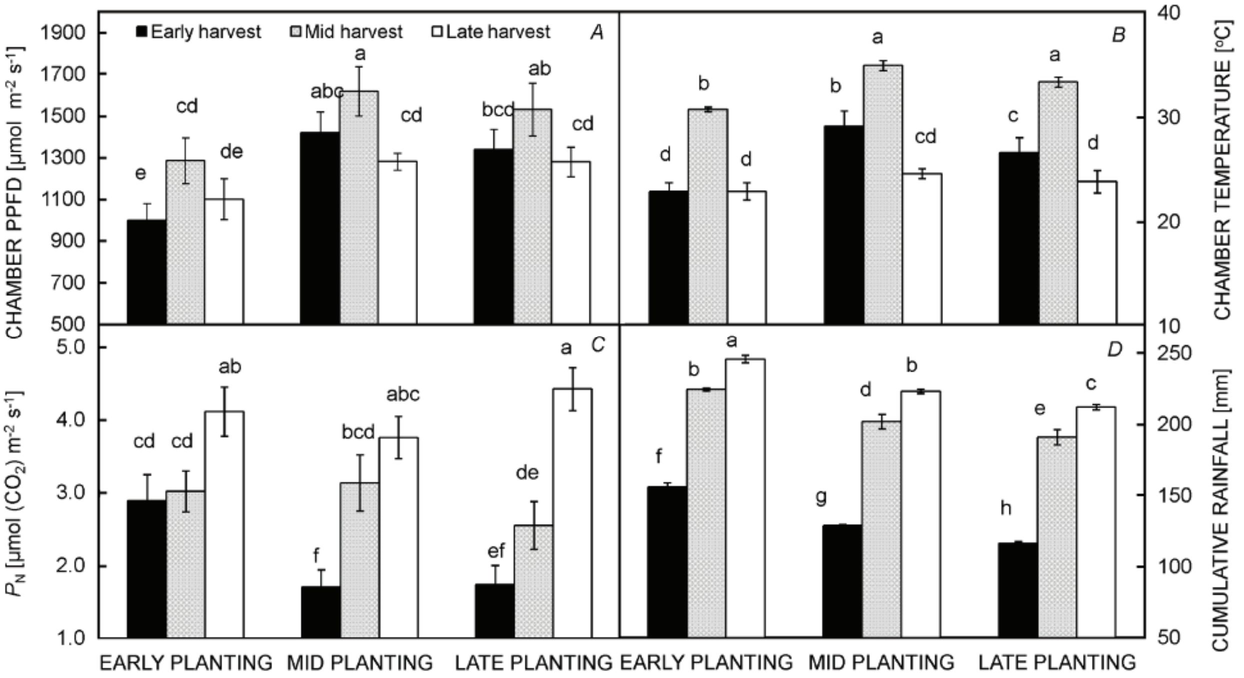

Fig. 2. Average photosynthetic photon flux density (PPFD) (*A*), chamber temperature (*B*), net photosynthetic rate (P_N) (*C*), and cumulative rainfall (*D*) of carrot (*Daucus carota* L.) cultivars influenced by planting time (early, mid and late) and harvesting times (early, mid and late). LSD means ± SE followed by *the same letters* are not significantly different. $n = 6$, $p<0.05$.

study were comparable to those observed during our previous studies (Thiagarajan *et al.* 2007).

As the soil moisture potential never reached below 25 kPa, effects of rainfall on gas-exchange parameters were considered negligible (Thiagarajan *et al.* 2007). When seeded early or late, P_N was not affected by fluctuations in PPFD or temperature. However, when mid-seeded, a two fold increase in P_N in response to the rise in temperature and PPFD from early to mid harvesting (Fig. 2) was observed. With regard to cultivar influence, our results corroborate with previous findings (Kyei-Boahen *et al.* 2003) that two Chantenay-type cultivars, Cascade and Red Core Chantenay differ in their photosynthetic characteristics.

With regard to harvest times, the early harvest registered the lowest P_N (2.09 µmol m^{-2} s^{-1}) (Fig. 2). There was a 38% increase in P_N at mid harvest and a 95% increase at late harvest over the P_N for the early harvest. Mild temperatures (21–23°C) at late-harvest stages combined with the matured physiological stage (>100 DAP) likely contributed to high P_N. Relatively warmer temperatures (24–30°C) during the mid-harvest stages may have led to reduction in P_N. Carrots typically reach their P_{Nmax} around 1,100 µmol m^{-2} s^{-1} (Kyei-Boahen *et al.* 2003). High temperatures (>25°C) are well known to induce heat stress and reduce Rubisco activity (Jensen 2000) and restrict electron transport capacity in the PSII system. Despite higher PPFD at the mid harvest, the occurrence of lower P_N may imply the following: (*1*) an optimal temperature around 20°C leads to a maximum P_N (Hussain *et al.* 2008) and (*2*) higher temperature (>25°C) negates the beneficial influence of PPFD on P_N (Thiagarajan *et al.* 2007).

Cultivars significantly differed in their g_s and *E* values (Fig. 3). Sugarsnax had 25% higher g_s and 10% higher *E* than TopCut (0.063 mol m^{-2} s^{-1}, 1.41 mmol m^{-2} s^{-1}) (Fig. 3). Differences in stomatal conductance between cultivars are reported in several C_3 species including carrots (Kyei-Boahen *et al.* 2003, Tekalign and Hammes 2005). The g_s was lowest at early-harvest conditions (0.04 mol m^{-2} s^{-1}) and highest at mid-harvest conditions (0.09 mol m^{-2} s^{-1}) along with the temperature (Fig. 1). At a fixed ambient CO_2 concentration (C_a), increases in P_N are often accompanied by an increase in g_s as the stomata maintain a constant supply of CO_2 across the epidermis (Jarvis and Davies 1998, Kyei-Boahen *et al.* 2003). Our results exhibited this typical response. *E* closely followed g_s and no statistically significant differences were observed between mid and late harvest. *E* is principally regulated by the fluctuation in vapor pressure deficit. The high temperature that prevailed during mid and late harvest likely increased the vapor pressure deficit and consequently *E*.

The responses in g_s to planting time varied with cultivars (Fig. 4). Sugarsnax exhibited 38% higher g_s than that registered by TopCut (0.06 mol m^{-2} s^{-1}) at mid planting with no significant differences in other planting times (Fig. 4). The high g_s recorded by Sugarsnax can be attributed to its increased sensitivity to temperature and/or potentially high stomatal density on its leaf surface.

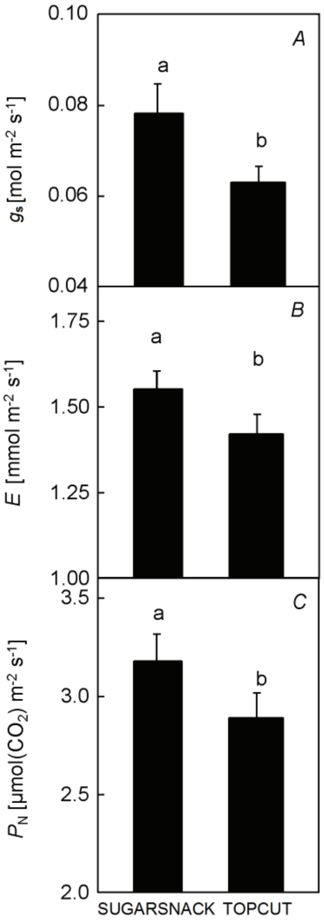

Fig. 3. Mean values of stomatal conductance, (g_s) (*A*) and transpiration, (*E*) (*B*) and net photosynthetic rate, (P_N) (*C*) of carrots (*Daucus carota* L.) influenced by individual cultivars. LSD means ± SE followed by *the same letters* are not significantly different. $n = 27$, $p<0.05$.

Evidence exists for stomatal density differences across genotypes in several crops such as faba beans (*Vicia faba* L.) (Darwish and Fahmy 1997) and carrots (Gonzalez *et al.* 2009). The increase in P_N observed with TopCut also supports this assumption. Nevertheless, these differences were masked by temperature fluctuations observed at planting time with respect to *E* losses. The highest *E* was registered at mid planting (2.0 mmol m^{-2} s^{-1}) followed by early (1.44 mmol m^{-2} s^{-1}) and late planting (1.02 mmol m^{-2} s^{-1}). These values corresponded to the increase in the temperature. Based on our results, g_s responses were likely manifestations of both transpiration demands and morphology governed by the vapor pressure deficit imposed by the ambient temperature.

The WUE was also directly influenced by harvest timing and changed with the choice of cultivar (Fig. 4). WUE was highest at late harvest (2.77 mmol mol^{-1} in TopCut and 2.59 mmol mol^{-1} in Sugarsnax) while the lowest was observed at the mid harvest, (1.49 in TopCut and 1.76 mmol mol^{-1} in Sugarsnax) irrespective of cultivars. At early harvest, WUE of Sugarsnax was 1.3 × higher than Topcut. Lack of difference in *E* between mid and late harvests suggests that the increase in WUE in plants at late harvest was caused by an increase in P_N. An optimum combination of temperature and developmental stage of the plants influenced P_N at this time. The same trend was shown in both cultivars however, WUE between cultivars was significantly different when harvested early. Sugarsnax registered higher WUE than TopCut but both cultivars did not differ in WUE at mid- and late harvest. The WUE of Topcut did not change at the first two harvests and similarly the WUE of Sugarsnax at the early and late harvest were not different. Such cultivar differences in WUE have been documented before (Klus *et al.* 2001). This phenomenon demonstrates that TopCut is more efficient than Sugarsnax for early harvesting practices owing to its' sensitivity to temperature fluctuations.

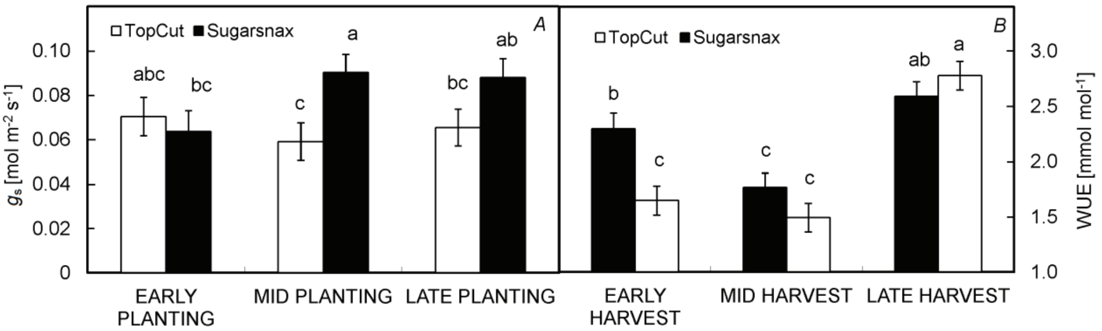

Fig. 4. Interactive effect of cultivar and planting timing on stomatal conductance (g_s) (*A*) and interactive effects of cultivar and harvest timing on the intrinsic water-use efficiency (WUE) (*B*) of carrots (*Daucus carota* L.) (B). LSD means ± SE followed by *the same letters* are not significantly different. $n = 9$, $p<0.05$.

Plant density and net photosynthesis: Plant density had no influence on any of the gas-exchange parameters (Table 1). Based on our results, we suggest that the competition for water and nutrients imposed by high densities may have solely been compensated through morphological adjustments. Earlier studies with different seed spacing and densities found that under limiting conditions, shoot to root ratio is decreased, plants grew smaller and roots became thinner and longer than those grown under optimal conditions (Lazcano *et al.*1998, Rajasekaran *et al.* 2006). Physiologically, plant densities do not appear to alter P_N in carrots.

Table 1. Probability values for the main and interactive effects of cultivar, planting time and harvest timing on net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration (E), and intrinsic water-use efficiency (WUE). * – significant difference at P=0.05.

Source of variation	P_N [μmol m^{-2} s^{-1}]	g_s [mol m^{-2} s^{-1}]	E [mmol m^{-2} s^{-1}]	WUE [mmol mol^{-1}]	C_i/C_a
Year	<0.716	<0.0001*	0.811	0.480	<0.0001*
Cultivar	0.008*	0.005*	0.022*	0.131	0.569
Planting time	0.854	0.812	0.015*	0.049	0.523
Harvest timing	<0.0001*	<0.0001*	<0.0001*	0.007*	0.059
Plant density	0.179	0.285	0.127	0.721	0.442
Cultivar × seed timing	0.600	0.036	0.126	0.321	0.616
Cultivar × harvest timing	0.120	0.539	0.879	0.035*	0.292
Planting time × harvest timing	0.010*	0.095	0.119	0.285	0.064
Cultivar × planting time × harvest timing	0.574	0.787	0.411	0.373	0.313

Table 2. Probability values for the main and interactive effects of cultivar and nitrogen on net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration (E), intrinsic water-use efficiency (WUE), C_i/C_a ratio, soil N, and root N of carrot (*Daucus carota* L.) cultivars. * – significant difference at P=0.05.

Source of variation	P_N [μmol m^{-2} s^{-1}]	g_s [mol m^{-2} s^{-1}]	E [mmol m^{-2} s^{-1}]	WUE [mmol mol^{-1}]	C_i/C_a	Soil N [%]	Root N [%]
Year	0.040*	0.637	0.0013*	<0.0001*	0.002*	<0.0001*	0.039*
Cultivar	0.594	0.914	0.040*	0.123	0.262	0.0723	0.076
Nitrogen [kg ha^{-1}]	0.049*	0.517	0.083	0.0008*	0.033*	<0.001*	<0.0001*
Cultivar × nitrogen [kg ha^{-1}]	0.141	0.649	0.112	0.950	0.778	0.951	0.182

Nitrogen and gas exchange: Application of N did not appear to affect g_s or E (Table 2). Based on previous reports, responses of g_s to N are mixed. For instance, N application increased g_s in *Picea glauca* (Livingston *et al.* 1999), decreased g_s in *Phaseolus vulgaris* (Lima *et al.* 1999) and caused no response in grasses (Chen *et al.* 2005). From this study, the lack of influence on g_s confirms that N supply increased P_N principally through nonstomatal factors such as, increased allocation of N towards chlorophyll content and/or PSI in carrots increasing the carboxylation efficiency. C_i/C_a, an indirect measure of carboxylation efficiency, was strongly influenced by the N application. The lowest C_i/C_a (0.56) values were observed at the highest N rate [400 kg(N) ha^{-1}] (Fig. 5), which was on par with those observed at rates beyond 50 kg(N) ha^{-1}. The control plot, 0 kg(N) ha^{-1}, registered the significantly high C_i/C_a (0.67) than any other N application rates. The high values may indicate that either the Rubisco enzyme complex is over-saturated or replenishment rates are too low to compensate for the influx of CO_2. In our study, since the g_s was nonresponsive to N and remained at near constant levels, high inflow of CO_2 did not occur. Hence, high C_i/C_a ratio observed at 0 kg(N) ha^{-1} was most likely a consequence of low content and activity of Rubisco which is linearly related to foliar N. Conversely, high P_N and low C_i/C_a observed above 50 kg(N) ha^{-1} suggest that N is invested in Rubisco protein complexes which would have enhanced P_N. Previous studies also provide support to this assertion. Beyond threshold levels, the increasing response of P_N was limited by other CO_2 fixation factors, such as supply of inorganic phosphate (P_i) and certain feedback inhibition mechanisms (Kyei-Boahen *et al.* 2003). Our data suggest that N supply of 100 kg(N) ha^{-1} is essential to attain the maximum carboxylation efficiency in carrots.

The highest WUE (2.69 mmol mol^{-1}) was recorded at 100 kg(N) ha^{-1} which did not significantly differ from any N rates other than the lowest value found at 0 kg(N) ha^{-1} (1.81 mmol mol^{-1}). As N had no influence on g_s or E the WUE is principally a manifestation of N supply augmenting the P_N. Foliar and root N tissue percentage both revealed a typical curvilinear response to N application (Fig. 6). Foliar N increased linearly up to 2.98% until 250 kg(N) ha^{-1} and did not improve with additional N application. Foliar N content in conventionally grown carrots was found to be between 3.0 to 3.3% (Warman and Havard 1997). Even following an application of 200 kg(N) ha^{-1}, foliar N was still only approximately 2.95% in carrots (Pettipas *et al.* 2008). Many plants are known to adjust tissue N influx as a part of nutrient re-allocation strategies (Glass *et al.* 2002) which, likely occurs in carrots. This coincided with accumulation of N in roots. Root N stabilized at 150 kg(N) ha^{-1} rate indicating that the re-allocation of N to foliar growth occurs at this point. Results from Exp. 2 revealed a significant influence of N application on P_N at the cultivar level. P_N increased with foliar N only until 150 kg(N) ha^{-1} (foliar N of 2.65%) and further increases in foliar N had no significant effect on P_N. More than 75% of the foliar N is allocated towards Calvin cycle enzymes, pigment-protein

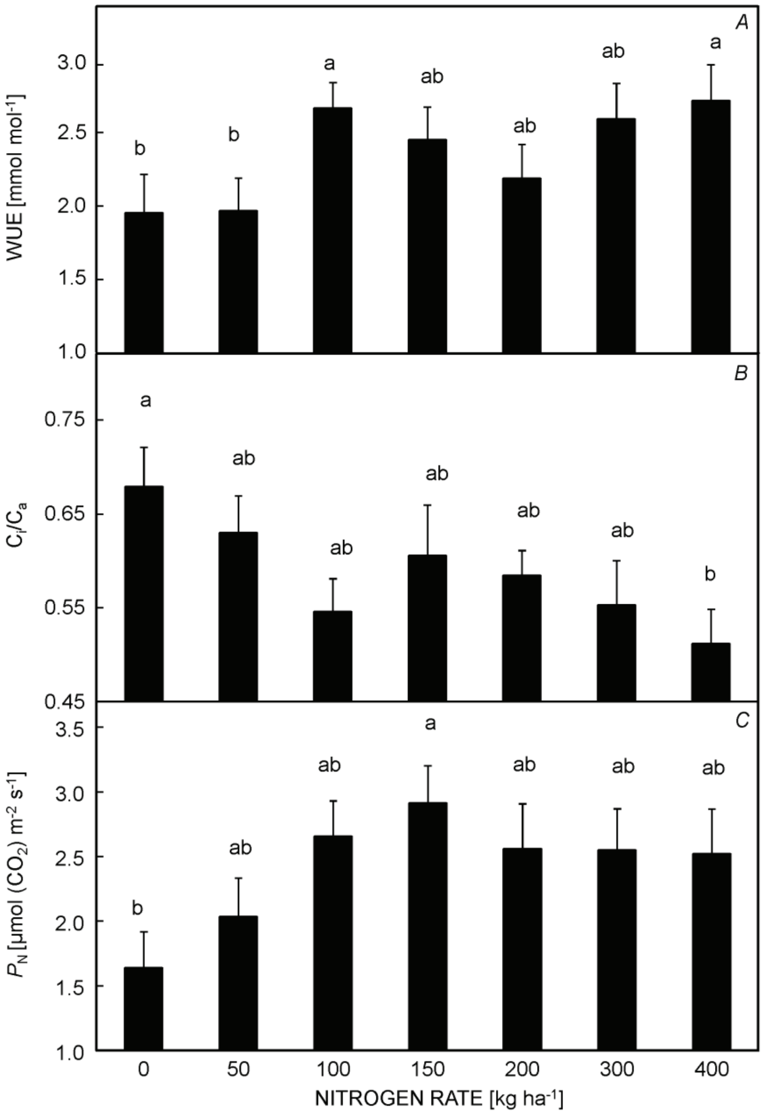

Fig. 5. Intrinsic water-use efficiency (WUE) (*A*), C_i/C_a ratio (*B*), and net photosynthetic rate (P_N) (*C*) response of *Daucus carota* L. as influenced by nitrogen rate. LSD means ± SE followed by *the same letters* are not significantly different. $n = 12$, $p<0.05$.

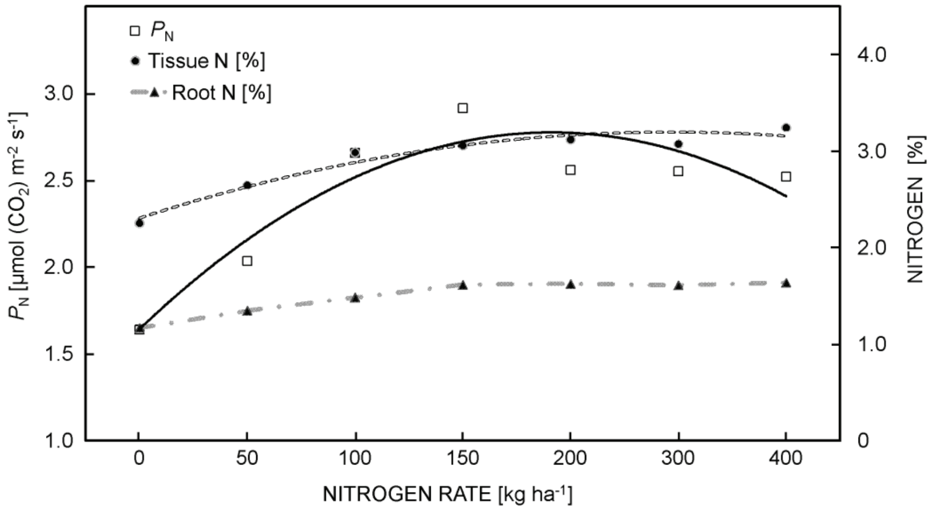

Fig. 6. Average foliar nitrogen, corresponding net photosynthetic rates (P_N), and root nitrogen of carrot (*Daucus carota* L.) cultivars influenced by nitrogen rate.

complexes and electron transport components (Evans 1989). As expected, N application increased P_N activity. Carrots N tissue levels that are required to induce maximal P_N rates were achieved at 100–150 kg(N) ha^{-1} which coincides with the foliar N saturation levels. Beyond 150 kg(N) ha^{-1}, a lack of significant improvement in P_N showed that carrots may avoid overinvestment in the Rubisco enzyme complexes and invest perhaps in promoting source activity through foliar growth. A lack of increase in N % in carrot roots at N rates above the threshold (Fig. 6) also supports this hypothesis. This study suggests that foliar N up to 2.98% was necessary to ensure maximum P_N. Therefore, application of N up to 150 kg(N) ha^{-1} can be recommended to optimize CO_2 assimilation.

To summarize, carrot cultivars TopCut and Sugarsnax differed in their stomatal conductance and thus, exhibited differential P_N and WUE responses. P_N unequivocally increased with physiological maturity of the crop regardless of planting time. Changes in temperature and PPFD during planting and harvesting times interactively influenced P_N. Physiological age of the plants as reflected by planting time altered the ability of the plant to cope with changes in PPFD and temperatures. Suboptimal and supraoptimal temperatures superseded the positive influence of PPFD levels. Plant density did not alter gas-exchange characteristics in both cultivars and therefore, compensatory strategies in carrots do not occur at gas-exchange level. Both cultivars responded similar to N application. Neither of the cultivars showed any response to N in terms of g_s or E. Foliar N and P_N in carrots responded to N application in a curvilinear fashion up to 150 kg(N) ha^{-1}. This N level indicates a maximum threshold in carboxylation efficiency.

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The book is a second edition of a successful and frequently used first edition. The new version is supplemented with some new parts. This book is presented in the series Methods in Molecular Biology (684 – Springer Protocols). The book belongs to very precious books because it contains detailed description of laboratory procedures. Most of scientific books concentrate only on the results and their discussion, this book is a methodological one.

The methods described are mostly of biochemical and molecular biology character, supplemented with biophysical and spectroscopic methods (for instance infrared and fluorescence spectroscopy, EPR spectroscopy or mass spectrometry, CD spectroscopy, chlorophyll fluorescence, *etc*.). The book contains 30 chapters written by specialists in specific fields, altogether 72 authors from 13 countries of the whole world. Each chapter is composed of standard parts: Abstract, Introduction, Materials, Methods, Notes, and References. The Introduction usually presents condensed, up-to date information about the studied subject (*e.g.* PSI complex). This may serve as very valuable information on the state-of-the-art of the studied object.

The described methods are presented in such a detail as to provide research protocols for concrete laboratory work. Usually no previous knowledge of the method is necessary. So the book is intended for the people starting their work in a photosynthesis laboratory or for those starting a new direction in their photosynthesis research.

The most frequently used model plants are *Arabidopsis thaliana* and it´s numerous mutants and spinach. Among lower plants the cyanobacterium *Synechocystis* sp. or the green alga *Chlamydomonas reinhardtii* should be named.

Some of the frequently used themes: expression of genes, gene deletion, genetic engineering.

A reader may find information about isolation of PSII- or PSI-enriched membranes, PSII reaction center complexes, cytochrome b_6f complexes, chloroplast lipids, CP43 and CP47 antenna complexes, LHCII complex, and PHCII-aggregates, *etc*.

Other interesting topics are *e.g.* transfer of proteins across the chloroplast membrane, proteomics of thylakoid membrane, thylakoid phosphoproteins, PSII reconstitution, and immobilization methods of photosynthetic materials. Besides purification, also crystallization of some complexes is described.

The work with most important photosynthetic enzymes comprises rapid isolation of intact chloroplasts from spinach, bundle sheath cells from C_4 plants, isolation of Rubisco and quantification of its amount and activity, purification and activity assays of Rubisco activase.

Some chapters may be of special interest for plant ecophysiologists. For instance a chapter devoted to direct detection of free radicals and reactive oxygen species in thylakoids using the spin-trapping method, or the assay of photoinhibition and heat inhibition of PSII in higher plants.

This book should be in the library of each laboratory dealing with molecular biology, biochemistry, and biophysics of photosynthetic systems.

J. NAUŠ (*Olomouc*)