Effect of ontogeny, heterophylly and leaf position on the gas exchange of the hop plant

V. Hejnák, H. Hniličková, F. Hnilička

Department of Botany and Plant Physiology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic

ABSTRACT

This paper evaluates the influence of ontogeny and the position of bine and offshoot leaves on the rate of photosynthesis (P_n), transpiration (E) and stomatal conductance (g_s) in hop plants. In the ontogeny influencing P_n, E and g_s among hops. The highest P_n was measured in phase 81–89 BBCH and E and g_s in phase 61–69 BBCH. The P_n increased over the course of ontogeny from the 1st to 3rd level of the hop plants. From the 61 BBCH phase, the leaves of the first and second level achieved a photosynthetic maturity and P_n value no longer increased. The third-level leaves achieved photosynthetic maturity from the 81 BBCH phase. Higher E was measured in the upper parts of the hop plant. P_n and E were higher in the bine leaves in the third level of the hop plant over the entire course of the vegetation period. In the first and second level of the hop plant, higher values of P_n were measured in the offshoot leaves. Differences in g_s were not noteworthy among the leaf types. Results show that a significant factor affecting the differences in P_n and E in hops is the age of the leaves.

Keywords: *Humulus lupulus* L.; rate of photosynthesis; bine leaves; offshoot leaves; ontogenetic development

The ontogenetic development of the hop plant spreads over many years and includes repeated annual cycles. The annual cycle of hop plants consists of the period of cryptovegetation (dormancy) and period of vegetation. The period of vegetation starts in spring, with sprouting of the bines, and lasts until their total decay in the autumn. Among the main ontogenetic phases are: the period of linear growth of bines, the period of establishment, the period of shoots and shooting, period of butonization and inflorescence and period of cone formation (Rybáček 1991).

Gas exchange can also be influenced by many plant factors such as leaf position and age, sink effects, mutual shading, as well as environmental factors such as light, temperature, nutrition and water availability (Aighewi and Ekanayake 2004). Changes in the rate of photosynthesis are caused by the degree of development and the level of morphology and anatomy of the plant, particularly its development of foliage. Jiang et al. (2006) stated that the rate of photosynthesis gradually increases during development of the leaf and reaches its maximum in fully developed leaves. Greer (1999) demonstrated maximum rates of photosynthesis coincided with, or shortly followed, full leaf expansion, and photosynthetic development was dependent on the environmental history of the leaves. Young expanding leaves were characterized by low efficiency of photochemistry and photosynthesis, low capacity for both electron transport through photosystem II, low CO_2 fixation, high respiration, and a high capacity for non-radiative thermal dissipation (Greer and Halligan 2001).

Heterophylly, i.e. the existence of two or more types of leaves on the same plant, may be the result of a rigid development program (Kerstetter and Poeting 1998) or a plastic response of plants to different habitat conditions (Titus and Gary Sullivan 2001). Kubien et al. (2007) showed a higher rate of

photosynthesis in *Metrosideros excelsa* leaves in the juvenile phase, compared with mature leaves. Tanaka-Oda et al. (2010) reported differences in photosynthetic rates, light compensation point, respiration rate and water usage effectiveness in *Sabina vulgaris* with two types of leaves. The common hop is a perennial dioecious herbaceous plant with leaves of differing shapes. Hop leaves can be divided based on the place from which they grow to bine leaves and offshoot leaves. Young leaves are heart-shaped, while mature leaves have five to seven lobes. The offshoot leaves are often compact or with three to five lobes (Hejný and Slavík 1997).

The size of hop plants and the technical difficulties of measurement are apparently among the reasons behind the fact that detailed measurements of the gas exchange during the ontogenic development of individual leaf types in the entire vertical profile of the hop plants have so far been performed in minimal scope and almost unpublished. The main goal of our experiment was to monitor the rate of net photosynthesis (P_n), transpiration (E) and stomatal conductance (g_s) in the main ontogenic phases and evaluate the differences in bine and offshoot leaves from the perspective of their position on the hop plant during ontogeny. The goal of the study was to acquire new and missing information about the gas exchange of hop and thus contribute to other researchers and growers of hop plants.

MATERIAL AND METHODS

Experiment characteristics. The experiment was conducted in Stekník (Czech Republic) at the experimental hop gardens between 2003 and 2007. The characteristics of the experimental location are stated in Table 1. The experiments were focused on monitoring changes in the rate of net photosynthesis, transpiration and stomatal conductance during ontogeny and the influence of the position of leaves and the heterophylly of the Saaz hop (Os. No. 72). Saaz is semi-early red-bine hop. It is fine aroma-type cultivar, which originated in the Saaz (Žatec) and Auscha (Úštěk) hop growing areas as a landrace hop. Classic agro technology was used on the plants over the course of vegetation. The dates for measuring P_n were selected to reflect the main phases of growth and development of the hop plant: 21–29 BBCH forming bine sprouts, 31–39 BBCH offshoot extension, 61–69 BBCH blooming and 81–89 BBCH hop cone ripening.

Weather characteristics. The course of weather conditions in the experimental years 2003 through 2007 is shown in Table 2. The experimental years 2004 and 2005 were normal in the vegetative period (April through September) both in terms of temperature and precipitation. 2006 was warm and wet. Divergences from the long-term average were noted in 2003 and 2007, where 2003 was extraordinarily warm and normal in terms of precipitation and 2007 was very warm and very wet.

Measuring of gas exchange. The rate of net photosynthesis, transpiration and stomatal conductance were measured over the course of the vegetative period in four identical plants selected from a collection of fifty experimental plants. P_n, E and g_s were measured on three bine and three offshoot leaves in three levels of the hop plant. The gas exchange was identified in the intact leaves by means of a commercial portable gasometric infrared analyzer LCA-4 (ADC Bio Scientific Ltd., Hoddesdon, UK). The gas exchange was measured from 8:00 AM to 11:30 AM, Central European time. The irradiance was 650 µmol/m²/s PAR (photosynthetically active radiation), the temperature in the measurement chamber was 25°C, the CO_2 concentration was 420 ± 35 vpm (µmol/mol), the air flow rate was 205 ± 30 µmol/s and the duration of the measurement of each sample was 10 min after the establishment of steady-state conditions inside

Table 1. Experimental location characteristics

Location	Stekník – Hop-growing area of Žatec (Czech Republic)
Geographic position	50°19'30.633''N, 13°37'14.334''E
Altitude (m a.s.l.)	190
Mean temperature during vegetation (°C)	15.2
Mean precipitation during vegetation (mm)	237
Soil texture	Loamy to clay-loam soil
Soil type	Haplic Pheozem
P[1] (mg/kg)	225
K[1] (mg/kg)	385
Mg[1] (mg/kg)	354
Soil pH$_{KCl}$	6.2–7
Soil organic carbon (%)	2.5–3.5

[1]Melich III extractable solution; 1:10 w/v

Table 2. Average temperatures and amounts of precipitation between the months of April through September during the experimental years 2003–2007 (Stekník, Hop-growing area of Žatec, Czech Republic) and the intervals in the evaluation of air temperature and the intervals in the evaluation of precipitation

	Air temperature			Precipitation		
	(°C)	difference from long-term average[1]	intervals in the evaluation (Difference from long-term average) and evaluation	(mm)	% of long-term average[2]	intervals in the evaluation (% of long-term average) and evaluation
2003	17.5	+2.7	> 2.5 extraordinary warm	184.6	70	70–120 normal
2004	15.0	+0.2	−1.0–1.0 normal	269.2	102	70–120 normal
2005	15.4	+0.6	−1.0–1.0 normal	298.1	113	70–120 normal
2006	16.1	+1.3	1.1–1.5 warm	329.3	125	121–150 wet
2007	16.5	+1.7	1.6–2.5 very warm	438.5	167	151–180 very wet

[1]long-term average 14.8°C (for the period 1961 through 1990); [2]long-term average 263.0 mm (for the period from 1961 through 1990)

the measurement chamber. The stated values are indicated as an average over the experimental years in the individual levels of the plant and leaf types.

Statistical analysis. A statistical evaluation of the experiment was made using the analysis of variance (ANOVA) and the values obtained were compared in more detail, using the Tukey's test at the significance level $P < 0.05$. Statistical analyses were performed using Statistica 9.0 CZ (Tulsa, USA) for MS Windows software.

RESULTS

Photosynthesis. The highest measured average P_n in the 21–29 BBCH stage in the first level of the hop plant was 4.35 µmol $CO_2/m^2/s$. In the 31–39 BBCH phase, the highest achieved P_n was in the second level of the hop plant (5.25 µmol $CO_2/m^2/s$) and lowest was measured in the third level (4.05 µmol $CO_2/m^2/s$) (Figure 1a). The differences in P_n between the bine and offshoot leaves in the first and second level were not statistically significant. In the third level, the P_n was evidently higher in bine leaves (4.5 µmol $CO_2/m^2/s$) (Table 3).

In phase 61–69 BBCH, a notable P_n took place in all levels of the hop plant. The highest average values of P_n were measured in the second level of the hop plant (7.42 µmol $CO_2/m^2/s$) and lowest in the first level (6.48 µmol $CO_2/m^2/s$) (Figure 1a). There were evident differences in P_n in each level between both types of leaves. The highest values were measured in offshoot leaves of the second

level (7.84 µmol $CO_2/m^2/s$) and bine leaves of the third level of the hop plant (7.43 µmol $CO_2/m^2/s$), while the lowest in bine leaves of the first level (6.13 µmol $CO_2/m^2/s$) (Table 3).

In phase 81–89 BBCH, the highest average values of P_n were measured in the third level of the hop plant (7.73 µmol $CO_2/m^2/s$) and the lowest in the first level (6.48 µmol $CO_2/m^2/s$) (Figure 1a). The highest P_n values were measured in bine leaves of the third level (7.95 µmol $CO_2/m^2/s$). The P_n differences between both types of leaves in the first level were statistically insignificant. In the second level, the P_n value was evidently higher in offshoot leaves (Table 3).

Transpiration. In the 21–29 BBCH phase, an evident difference in E was measured between the second and third level (1.02 mmol $H_2O/m^2/s$) of the hop plant. In phase 31–39 BBCH, the highest average E was achieved in the third level of the hop plant (1.68 mmol $H_2O/m^2/s$) while the lowest E rate was measured in the first level (0.90 mmol $H_2O/m^2/s$) (Figure 1b). The differences in E between bine and offshoot leaves in the individual levels are statistically significant in the first and third level (Table 3).

In the 61–69 BBCH phase, the highest average E values were measured in the second level of the hop plant (3.26 mmol $H_2O/m^2/s$) while the lowest were in the first level (2.51 mmol $H_2O/m^2/s$) (Figure 1b). The highest E values were measured in the 61–69 BBCH phase. The differences between E in the bine and offshoot leaves in the individual levels were statistically significant (Table 3).

In the 81–89 BBCH phase, the highest average values of E were measured in the third level of the
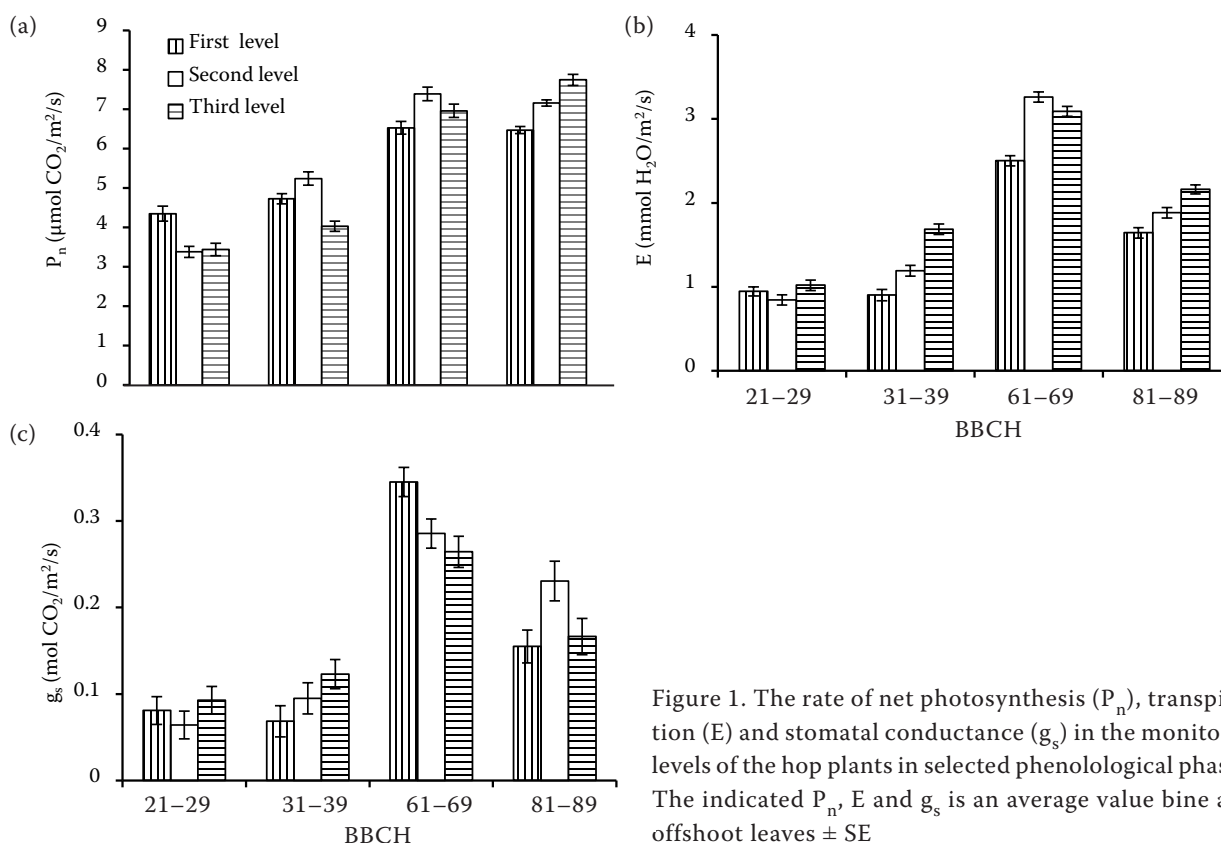
Figure 1. The rate of net photosynthesis (P_n), transpiration (E) and stomatal conductance (g_s) in the monitored levels of the hop plants in selected phenolological phases. The indicated P_n, E and g_s is an average value bine and offshoot leaves ± SE

hop plant (2.16 mmol $H_2O/m^2/s$) (Figure 1b). The differences between the bine and offshoot leaves in the individual levels were statistically significant in the first and second level, where the highest E values were measured in offshoot leaves (Table 3).

Stomatal conductance. In the 21–29 BBCH phase, the differences in g_s values measured in between bine and offshoot leaves were statistically insignificant (Table 3). In the 31–39 BBCH phase, the highest average g_s was in the third level of the hop plant

(0.12 mol $CO_2/m^2/s$). The differences between g_s values of bine and offshoot leaves in terms of levels were statistically insignificant (Table 3).

In the 61–69 BBCH phase, the highest average g_s was reached in the first level of the hop plant (0.35 mol $CO_2/m^2/s$) (Figure 1c). The differences of g_s values between bine and offshoot leaves in the individual levels were statistically significant in the third level. The highest g_s values were measured in this developmental stage (Table 3).

Table 3. The rate of net photosynthesis (P_n), transpiration (E) and stomatal conductance (g_s) in the individual levels in bine (BL) and offshoot leaves (OL) of the hop plant in selected ontogenic phases in Saaz hop (Os. No. 72)

| Level | Leaves | BBCH | | | | | | | | | | | |
| | | 21–29 | | | 31–39 | | | 61–69 | | | 81–89 | | |
		P_n	E	g_s	P_n	E	g_s	P_n	E	g_s	P_n	E	g_s
First	BL	4.35[a]	0.95[ab]	0.08[a]	4.54[cb]	1.06[c]	0.09[ab]	6.13[d]	2.71[c]	0.36[a]	6.39[d]	1.36[cd]	0.16[a]
	OL				4.93[b]	0.74[d]	0.04[b]	6.83[c]	2.30[d]	0.33[a]	6.56[d]	1.93[b]	0.15[a]
Second	BL	3.38[b]	0.84[b]	0.07[a]	5.08[ab]	1.16[c]	0.09[ab]	6.99[c]	3.08[bc]	0.25[ab]	6.91[c]	1.69[c]	0.24[a]
	OL				5.42[a]	1.22[c]	0.10[ab]	7.84[a]	3.44[a]	0.32[a]	7.40[b]	2.07[ab]	0.22[a]
Third	BL	3.44[b]	1.02[a]	0.10[a]	4.51[c]	1.81[a]	0.14[a]	7.43[b]	3.29[a]	0.19[b]	7.95[a]	2.17[a]	0.18[a]
	OL				3.59[d]	1.55[b]	0.10[ab]	6.53[c]	2.89[c]	0.34[a]	7.50[b]	2.15[a]	0.15[a]

Values within a column marked with the same letter are not significantly different ($P \le 0.05$)

In the 81–89 BBCH phase, the highest average g_s was in the second level of the hop plant (0.23 mol $CO_2/m^2/s$) (Figure 1c). The differences in the g_s values between the bine and offshoot leaves in terms of the levels were statistically insignificant (Table 3).

DISCUSSION

The position, age and heterophylly of the leaves influenced P_n, transpiration and stomatal conductance (Greer and Weedon 2012). All monitored physiological parameters showed a gradual increase of measured values over the course of the ontogeny. The lowest P_n was measured in the 21–29 BBCH phase, while maximum P_n was measured in the 81–89 BBCH phase. Pokorný et al. (2011) stated that the Premiant, Saaz hop (Os. No. 72) and selected new cultivars show statistically evident increase of P_n of the hop plant during the phase 61–69 BBCH. Lieth and Pasian (1990) also found that young, expanding leaves had low P_n. Young expanding leaves were characterized by low efficiency of photochemistry and photosynthesis, low capacity for both electron transport through photosystem II, low CO_2 fixation, high respiration, and a high capacity for non-radiative thermal dissipation (Greer and Halligan 2001). Kenny (2005) stated that the older leaves reach lower values of photosynthesis. On the contrary, Skládal and Kafka (1975) state that old, semi-yellow hop leaves show considerably higher photosynthesis. Maximum values of E and g_s conductance were measured already in the 61–69 BBCH phase and subsequently begun decreasing. Salmon et al. (2011) stated that g_s changes together with the development of the leaf, initially increasing but generally decreasing with the age of the leaf.

The influence of the position of the leaves on the monitored physiological parameters in the vertical profile of the hop plant is apparent from the obtained results. With the increasing ontogenic development of the hop plant, the highest values of P_n are measured in the leaves of the upper parts of the hop plant. In the 21–29 BBCH phase, P_n values were lower in leaves positioned higher, meaning also in younger leaves and leaves less mature in terms of photosynthesis. In this stage of development, the bine leaves contribute to the overall photosynthetic assimilation because the offshoot leaves have not yet created. From 61 BBCH phase, the leaves of the first and second level reached photosynthetic maturity and their P_n no longer

increased. The leaves of the third level reached photosynthetic maturity from 81 BBCH phase. Greer and Weedon (2012) stated that earliest emerging leaves had the highest P_n and E, and these were achieved early in the growing season. As the season progressed, P_n declined in the basal leaves and increased in leaves towards the apical meristem. Skládal and Kafka (1975) stated that, in the first part of the vegetation, the higher P_n values were measured in the bottom third to bottom half of the hop plant and, in the second half of the vegetation, higher P_n values were measured in the upper half to upper third of the hop plant.

Higher E was measured in the upper parts of the hop plant. E increases with decreasing temperature and higher air movement, which are characteristics describing the upper parts of the hop plant. These physical conditions apply in case the number of stomata does not change (Larcher 2003).

According to Leigh et al. (2011), the heterophyllous nature of the leaves could lead to significant environmental and developmental heterogeneity, which shows in hops in our measurements as differences in P_n and E in bine and offshoot leaves in the vertical profile of the hop plans over the course of ontogeny. Higher P_n and E were measured in the upper bine leaves throughout the entire vegetation period. In the first and second level of the hop plant, higher P_n values were measured in the offshoot leaves. Nielsen and Sand-Jensen (1993) stated that the differences identified in photosynthetic assimilation are caused by morphological and anatomic differences. Despite Larcher (2003) stating that there are differences in the stomata behavior (opening and closing) between the individual leaves of the plants and their position on the shoot, the differences in g_s were not noteworthy.

The results show that a significant factor influencing the differences in P_n and E in hops is the age of the leaves. The hop plant grows up to 8 m in height, resulting in the presence of leaves of different age on the plant. The leaves in the lower parts achieved photosynthetic maturity earlier than leaves in the upper parts of the hop plants, reaching senescence earlier, which results in a lower rate of gas exchange (Ananieva et al. 2008). In the lower parts, the offshoot leaves were formed at a time when the hop plant had not yet reached its final height and the bine leaves were still unformed. It is assumed that differences of P_n and E between bine and offshoot leaves are influenced by the age of the leaves rather than their heterophyllic nature.

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Corresponding author:

Ing. Helena Hniličková, PhD., Česká zemědělská univerzita v Praze, Fakulta agrobiologie, potravinových a přírodních zdrojů, Katedra botaniky a fyziologie rostlin, Kamýcká 129, 165 21 Praha-Suchdol, Česká republika
e-mail: hnilickova@af.czu.cz