# Influence of low temperatures on the growth and photosynthetic activity of industrial chicory, *Cichorium intybus* L. partim

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## Abstract

The cold stress effect on early vigour and photosynthesis efficiency was evaluated for five industrial chicory varieties with contrasting early vigour. The relationships between the growth and physiological parameters were assessed. The varieties were examined at three growth temperatures: 16 (reference), 8 (intermediate) and 4 °C (stress). The effect was measured using physiological processes (growth, photosynthesis, chlorophyll *a* fluorescence), and pigment content. The analysis of the measured growth parameters (dry leaf and root mass, and leaf area) indicated that temperature had a significant effect on the varieties, but the overall reaction of the varieties was similar with lowering temperatures. The photosynthesis and chlorophyll *a* fluorescence measurements revealed significant changes for the photosynthesis (maximum net photosynthesis, quantum efficiency, light compensation point and dark respiration) and chlorophyll *a* fluorescence parameters (photochemical and non-photochemical quenching) with lowering temperatures for Hera and Eva, two extremes in youth growth. No significant differences could be found between the extremes for the different temperatures. The pigment content analysis revealed significant differences at 4 °C in contrast to 16 and 8 °C, especially for the xanthophyll/carotenoid pool, suggesting a protective role. Subsequently, the relationship between the physiological processes was evaluated using principal component analysis. At 4 °C, 2 principal components were detected with high discriminating power for the varieties and similar classification of the varieties as determined in the growth analysis. This provides a preview on the possible relationships between photosynthesis and growth for industrial chicory at low temperatures.

*Additional key words*: chilling; chlorophyll *a* fluorescence; cold stress; early vigour; photoinhibition; photosynthesis.

## Introduction

Chicory roots (*Cichorium intybus* L. partim) are cultivated in Europe (mainly Belgium, the Netherlands, France) for the production of inulin, a linear fructose polymer with a terminal glucose molecule. Inulin is used as a probiotic soluble dietary fibre that stimulates the beneficial intestinal flora and calcium absorption. Inulin yield and quality (chain length) are therefore important parameters for the breeder and farmer (Baert 1997). High root yield, inulin content and longer inulin chains are the target. Although these factors are affected by sowing date, harvest date and genotype, in general, the quality of inulin is optimal in September while the highest root

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*Abbreviations*: AGR – average growth rate; $C_{ab}$ – total chlorophyll content; $C_a/C_b$ – chlorophyll a/b ratio; $C_{ab}/C_{x+c}$ – chlorophyll/carotenoid-xanthophyll pool ratio; Chl – chlorophyll; DM – dry mass; $DM_L$ – dry mass leaves; $DM_R$ – dry mass roots; DMF – N,N-dimethylformamid; $F'_m$ – maximum chlorophyll fluorescence yield in the light-adapted state; $F_s$ – steady-state chlorophyll fluorescence yield level; $F'_o$ – minimum chlorophyll fluorescence yield in the light-adapted state; $F_v$ – maximum variable chlorophyll fluorescence yield in the dark-adapted state; $F'_v$ – maximum variable chlorophyll fluorescence yield in the light-adapted state; $I_c$ – light compensation point; LA – leaf area; NPQ – non-photochemical quenching; PC – principal component; PCA – principal component analysis; $P_{max}$ – maximum net photosynthesis; $P_N$ – net photosynthesis; PSII – photosystem II; $q_N$ – non-photochemical quenching coefficient; $q_P$ – photochemical quenching; $R_D$ – dark respiration; RGR – relative growth rate; SD – standard deviation; SLA – specific leaf area; x+c – xanthophyll and carotenoid pool; $\alpha_c$ – quantum efficiency; $\Phi_{PSII}$ – quantum yield of photosystem II.
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biomass is reached a few weeks later. Sowing earlier would eventually result in a better synchronisation between these two parameters. Consequently, the chicory varieties used for early sowing should not only be resistant to bolting, but also be able to grow at limiting conditions, in this case low temperatures sometimes combined with high light intensities.

Low temperatures are expected to affect the growth rate of young plants (Wolfe 1991, Venema *et al*. 2000). This, in combination with high light intensities, can cause photoinhibition of photosystem II (PSII). Photoinhibition has been defined as (*1*) the inhibition of photosynthesis caused by excessive irradiance, damaging the photosynthetic apparatus irreversibly and as (*2*) a slow, reversible reduction of photosynthetic efficiency depending on irradiation and leading to partial loss of capacity to convert energy (Alves *et al.* 2002). Osmond (1994) defined the first process as "chronic photoinhibition" and the second process as "dynamic photoinhibition". This reduction in efficiency is due to damage and breakdown of the PSII reaction center protein D1 (Richter *et al.* 1990). Therefore, to select chicory genotypes able to withstand low temperatures at high light intensities, we can use parameters based on physiological processes such as photosynthesis and chlorophyll (Chl) *a* fluorescence (Fracheboud *et al*. 1999; Maxwell and Johnson 2000, Lootens *et al*. 2004). These physiological parameters, as described by Maxwell and Johnson (2000) and Roháček (2002), have previously been used to evaluate the effect of chilling on young tomato plants (Brüggeman *et al*. 1992) or to test cold-sensitive and -tolerant maize (Fracheboud *et al*. 1999).

The primary steps in the light absorption process are performed by several pigments, such as chlorophylls, carotenoids and anthocyanins (Gitelson *et al*. 2001). Absorption of quanta is a critical step for the subsequent plant processes, where changes can be triggered by alterations in the pigment concentration, organisation and function. Evaluation of the pigment content can offer a glance in the plant reaction to changing environments. Chlorophylls are typical pigments of photosynthetic organisms. Carotenoids, often named accessory pigments, play an essential role in photoprotection. They are a sort of safety valve, venting excess energy before it can damage the organism (Niyogi *et al*. 1997a, b, Taiz and Zeiger 2002). Anthocyanins, red coloured pigments, accumulate in leaves and associate with abiotic and biotic factors. They are also presumed to play a photoprotective role (Holton and Cornish 1995, Close and Beadle 2003).

The objective of this study was to evaluate the response of five chicory varieties to cold stress conditions. We carried out a detailed analysis of the early vigour and photosynthesis efficiency of young chicory plants grown at different temperatures and assessed the relationship between early vigour and a number of physiological parameters.

## Materials and methods

**Plants**: In a preliminary experiment, we performed a growth analysis of 17 industrial chicory varieties and lines (8 plants per variety, five measuring points with an interval of 2 weeks) at 10 °C. Five chicory varieties 'synthetics' (Eva, Hera, Maurane, Melci and RegaloG) with contrasting growth rates at 10 °C were chosen for this study. Eva showed a slower early vigour than Hera, Maurane, Melci and RegaloG. The ranking order for early vigour (from good to moderate) was: Hera and RegaloG > Maurane and Melci > Eva (results not published). Seeds of these five varieties were sown in 5.5-cm pots containing universal soil and were placed in trays (51 cells). Fertilizer was added during daily irrigation (NPK + Mg (6/12/36 + 3), 0.5g/l). All plants were grown in growth chambers (Johnson Control, Brussels, Belgium) at 16 (reference), 8 (intermediate) or 4 °C (cold stress). The temperatures were selected from real conditions in the Typical Reference Year tables for Belgium (Dogniaux *et al.* 1978). The relative humidity was set at 60 % and the light intensity at 250 µmol (quanta) $m^{-2}$ $s^{-1}$ for 16 hours per day.

**Growth analysis**: For each variety, the dry mass (DM) of roots ($DM_R$) and leaves ($DM_L$) was estimated at five time points with an interval of 1, 2 and 4 weeks for 16, 8 and 4 °C, respectively, so that the plants were at the same growth stage (number of leaves formed) for each time point at the chosen growth temperature. Each estimation of DM was based on the individual measurements of ten plants per variety. The plant materials were dried during 24 hours at 75 °C (Binder 9010-0212, Binder, Tuttlingen, Germany). The leaf area (LA) was measured by image analysis (Devacht *et al*. 2007). Subsequently, the growth parameters were calculated using the equations of Hunt (1982).

**Photosynthesis and Chl *a* fluorescence**: Light response curves were measured at the respective growth temperature, constant $CO_2$ concentration [400 µmol($CO_2$) $mol^{-1}$] and 60 % relative humidity through an open gas exchange system (*LI-6400, LI-COR*, Lincoln, USA). The following irradiances were used: 800, 400, 200, 100, 50, 25 and 0 µmol(quanta) $m^{-2}$ $s^{-1}$. The irradiance response parameters - maximum net photosynthesis ($P_{max}$), quantum efficiency ($\alpha_c$), and light compensation point ($I_c$) - were calculated by fitting the curve $P_N = P_{max}$ [1–exp ($-\alpha_c(I-I_c)/P_{max}$)]. Dark respiration ($R_D$) was calculated by putting I = 0 (Lootens *et al*. 2004). Chl *a* fluorescence was measured simultaneously. We measured one leaf (third stage, after 3, 6 and 12 weeks of growth

at respectively 16, 8 and 4 °C) of eight plants per variety and growth temperature. The equations for the calculation of Chl *a* fluorescence parameters, photochemical ($q_P$) and non-photochemical ($q_N$) quenching, $q_P = (F_M'–F_S)/(F_M'–F_O')$, $q_N = (F_V–F_V')/F_V$, were as described by Roháček (2002). The quantum yield of PSII ($\Phi_{PSII}$) was calculated according to the formula $\Phi_{PSII} = 1–F_S/F_M'$ described by Roháček (2002).

**Pigment content**: The exact concentration of Chl *a* and Chl *b* and the total amount of carotenoid and xanthophyll (x+c) were measured spectrophotometrically (*Cary 50 conc UV-VIS, Varian*, Victoria, Australia) determined using the N,N-dimethylformamid (DMF) extraction method and the equations described by Wellburn (1994). The pigment measurements were performed on the same leaf used for the photosynthesis and Chl *a* fluorescence measurements.

**Statistical analysis**: The different process parameters were analyzed using *STATISTICA* (*Statsoft*, USA). The following statistical analyses were performed: basic statistics, *Levene* test, *Duncan Post-Hoc* test, One-way *ANOVA*, factorial *ANOVA* and Principal component analysis (PCA), which combines the calculated and correlated variables from the photosynthesis and chlorophyll fluorescence measurements in unrelated variables or principal components to obtain a smaller amount of variables.

## Results

**Growth analysis**: The results of growth after 28 days (a common point in the growth curve to explain the data) are summarized in Table 1. A factorial *ANOVA* indicated that temperature had a significant effect on the growth parameters and that the different varieties reacted similarly to a decrease in temperature. Average decreases by 93.6, 90.3, 92.2 and 15.3 % were found for $DM_L$, $DM_R$, LA and specific leaf area (SLA), respectively, when the growth temperature was lowered from 16 to 4 °C. In general, Hera, Maurane, Melci and RegaloG showed a better early vigour than Eva for all temperatures tested. At 16 °C, no significant differences were found between the different varieties for any of the parameters studied. However, with decreasing temperatures we could detect significant differences among varieties. This was especially the case at 4 °C. If we take the contrasting pair Eva/Hera as an example, at 16 °C differences for $DM_L$ and $DM_R$ were respectively 14.0 and 27.0 % lower and, LA and SLA respectively 1.0 and 11.4 % higher for Eva than for Hera. At 4 °C, the difference became even clearer as $DM_L$, $DM_R$, LA and SLA were respectively –41.0, –50.0, –35.0 and 21.5 % lower for Eva than for Hera.

**Photosynthesis and Chl *a* fluorescence**: The results of the light response curves are presented in Fig. 1 and Table 2. In Fig. 1, only the light response curves of the contrasting pair Hera and Eva are presented. The conductance for $H_2O$ during the photosynthesis measurements was on average 0.146 mol $H_2O$ $m^{-2}$ $s^{-1}$ with an SD of 0.025. The differential response of the varieties to decreasing temperatures became especially apparent at high light level. A decrease in $P_{max}$ was detected as temperature decreased (Table 2). At 16 °C, Hera and Eva responded similarly. At 8 and 4 °C, we can see a decrease in $P_{max}$ in comparison to 16 °C. This decrease is larger for

Table 1. The results for dry leaf mass ($DM_L$), dry root mass ($DM_R$), leaf area (LA) and the specific leaf area (SLA) are shown for the cultivars Eva, Hera, Maurane, Melci and RegaloG after 28 days at different growth temperatures (T) ($n$=10, mean ± SD). [a, b]: indicate significant difference at the 0.05 level per growth temperature (*Duncan Post-Hoc* test).

| T [°C] | Cultivar | $DM_L$ [g] | $DM_R$ [g] | LA [$cm^2$] | SLA [$m^2$ $g^{-1}$] |
|---|---|---|---|---|---|
| 16 | Eva | 0.37±0.13[a] | 0.27±0.13[a] | 120±36[a] | 0.033±0.005[b] |
| | Hera | 0.44±0.14[a] | 0.37±0.16[a] | 119±32[a] | 0.029±0.009[ab] |
| | Maurane | 0.44±0.10[a] | 0.33±0.11[a] | 123±40[a] | 0.028±0.005[ab] |
| | Melci | 0.47±0.11[a] | 0.35±0.12[a] | 145±30[a] | 0.031±0.002[ab] |
| | RegaloG | 0.48±0.11[a] | 0.40±0.16[a] | 128±37[a] | 0.027±0.006[a] |
| 8 | Eva | 0.16±0.05[a] | 0.11±0.03[a] | 34±9 | 0.021±0.002[a] |
| | Hera | 0.19±0.07[a] | 0.14±0.06[ab] | 37±9[ab] | 0.020±0.003[a] |
| | Maurane | 0.20±0.07[a] | 0.20±0.08[b] | 44±11[ab] | 0.024±0.004[b] |
| | Melci | 0.20±0.04[a] | 0.18±0.05[b] | 58±7[b] | 0.024±0.003[b] |
| | RegaloG | 0.19±0.10[a] | 0.15±0.10[ab] | 38±19[ab] | 0.020±0.002[a] |
| 4 | Eva | 0.03±0.01[a] | 0.02±0.01[a] | 7±3[a] | 0.028±0.003[a] |
| | Hera | 0.05±0.03[b] | 0.03±0.02[ab] | 11±5[ab] | 0.022±0.003[b] |
| | Maurane | 0.04±0.02[ab] | 0.02±0.01[ab] | 9±4[ab] | 0.026±0.004[a] |
| | Melci | 0.04±0.02[ab] | 0.02±0.01[ab] | 10±4[ab] | 0.025±0.003[ab] |
| | RegaloG | 0.06±0.04[b] | 0.03±0.02[b] | 12±6[b] | 0.025±0.005[ab] |

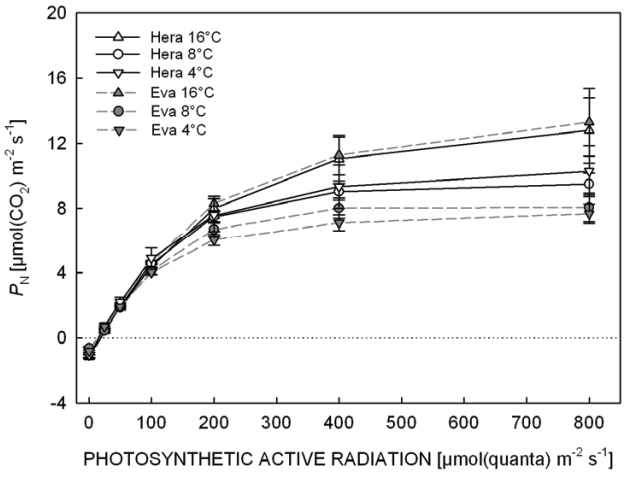


Fig. 1. Light response curves of photosynthesis ($P_N$) for Hera and Eva at 16 °C, 8 °C and 4 °C ($n$=8, mean ± SE).

Eva than for Hera. However, no differences were found between 8 and 4 °C for the light response curves of both varieties. The quantum efficiency ($\alpha_c$), which is highly correlated with the light reactions, was significantly lower for Eva, 5.8 and 9.9 % at 8 and 4 °C, respectively, when compared to the 16 °C situation. For Hera no significant response was found for this parameter. $R_D$ decreased by 22.0 % for Eva when temperature was lowered from 16 to 4 °C, and 4.0 % for Hera. The behaviour of these two parameters indicates that Hera keeps a higher photosynthetic efficiency and more efficient dark reactions at lower temperatures than Eva. The light compensation point ($I_c$) showed a decrease in both varieties as the temperature was lowered from 16 to 4 °C. Both varieties responded in a similar way, as the decrease was 14.4 % for Eva and 11.4 % for Hera.

In Table 3, the results are presented for $q_P$ and $q_N$, at 200 and 400 µmol(quanta) m$^{-2}$ s$^{-1}$ (respectively the normal growth and stress light intensities according to the Typical Reference Year tables) at the different growth temperatures. No significant differences for $q_P$ and $q_N$ were found among varieties at the different growth temperatures for none of the light intensities. At 200 µmol(quanta) m$^{-2}$ s$^{-1}$, $q_P$ decreased by 11.1 % and $q_N$ increased by 25.9 %, as average across the varieties, when the temperature was lowered from 16 to 4 °C. At 400 µmol(quanta) m$^{-2}$ s$^{-1}$, the decrease in $q_P$ was even larger (24.4 %) but the increase for $q_N$ was less pronounced (10.3 % as average). In general, $q_P$ decreases and $q_N$ increases for the different chicory varieties at lower temperatures, except for $q_N$ at 400 µmol(quanta) m$^{-2}$ s$^{-1}$, which showed no significant difference between 16 and 4 °C. The results indicate that the energy conversion processes become less efficient as the temperature decreases. At higher light intensities the process becomes even clearer, but the increase in $q_N$ was not as high as expected, indicating that the excess energy could cause damage to PSII.

Table 2. The parameters related to the light response curve of photosynthesis, the maximum net photosynthesis ($P_{max}$), the quantum efficiency ($\alpha_c$), the light compensation point ($I_c$) and the dark respiration ($R_D$), are shown for the cultivars Eva, Hera, Maurane, Melci and RegaloG at different growth temperatures (T) ($n$=8, mean ± SD). [a, b]: indicate significant difference at the 0.05 level per growth temperature (*Duncan Post-Hoc* test).

| T [°C] | Cultivar | $P_{max}$ [µmol(CO$_2$) m$^{-2}$ s$^{-1}$] | $\alpha_c$ µmol(CO$_2$) µmol$^{-1}$ (quanta) | $I_c$ [µmol quanta m$^{-2}$ s$^{-1}$] | $R_D$ [µmol(CO$_2$) m$^{-2}$ s$^{-1}$] |
|---|---|---|---|---|---|
| 16 | Eva | 13.3±5.1$^a$ | 0.071±0.002$^b$ | 15.1±3.4$^a$ | –1.1±0.3$^a$ |
| | Hera | 12.8±4.9$^a$ | 0.069±0.006$^{ab}$ | 16.0±3.9$^a$ | –1.2±0.3$^a$ |
| | Maurane | 9.4±2.8$^a$ | 0.066±0.008$^{ab}$ | 14.6±3.0$^a$ | –1.0±0.2$^a$ |
| | Melci | 13.2±5.3$^a$ | 0.074±0.005$^b$ | 12.9±3.8$^a$ | –1.0±0.3$^a$ |
| | RegaloG | 9.3±4.4$^a$ | 0.056±0.021$^a$ | 17.8±10.1$^a$ | –0.9±0.2$^a$ |
| 8 | Eva | 8.4±2.3$^a$ | 0.067±0.007$^a$ | 14.0±5.7$^a$ | –1.0±0.3$^a$ |
| | Hera | 9.7±1.9$^a$ | 0.072±0.003$^a$ | 13.1±2.5$^a$ | –1.0±0.2$^a$ |
| | Maurane | 8.3±2.9$^a$ | 0.073±0.003$^a$ | 12.1±4.2$^a$ | –0.9±0.3$^a$ |
| | Melci | 7.0±5.6$^a$ | 0.071±0.007$^a$ | 12.0±5.9$^a$ | –1.0±0.6$^a$ |
| | RegaloG | 9.4±5.0$^a$ | 0.069±0.007$^a$ | 12.3±3.0$^a$ | –0.9±0.2$^a$ |
| 4 | Eva | 7.6±1.7$^a$ | 0.064±0.005$^a$ | 12.9±2.4$^a$ | –0.9±0.2$^a$ |
| | Hera | 10.2±4.4$^a$ | 0.076±0.024$^a$ | 14.2±4.5$^a$ | –1.1±0.4$^a$ |
| | Maurane | 8.6±2.7$^a$ | 0.066±0.005$^a$ | 11.8±2.7$^a$ | –0.8±0.2$^a$ |
| | Melci | 8.0±1.7$^a$ | 0.062±0.005$^a$ | 14.0±2.4$^a$ | –0.9±0.2$^a$ |
| | RegaloG | 9.4±4.4$^a$ | 0.072±0.032$^a$ | 13.4±3.6$^a$ | –1.0±0.3$^a$ |

**Pigment content**: In Table 4, the contents of Chl *a* ($C_a$), Chl *b* ($C_b$), xanthophyll-carotenoid pool ($C_{x+c}$), total chlorophyll content ($C_{ab}$), Chl *a*/*b* ratio ($C_a/C_b$) and Chl/carotenoid-xanthophyll pool ratio ($C_{ab}/C_{x+c}$) are presented. At 16 and 8 °C, no significant differences were found among varieties for the different pigment

Table 3. The chlorophyll fluorescence parameters, the photochemical quenching ($q_P$) at 200 µmol quanta $m^{-2}$ $s^{-1}$ ($q_{P200}$), the photochemical quenching at 400 µmol quanta $m^{-2}$ $s^{-1}$ ($q_{P400}$), the non-photochemical quenching at 200 µmol quanta $m^{-2}$ $s^{-1}$ ($q_{N200}$), the non-photochemical quenching at 400 µmol quanta $m^{-2}$ $s^{-1}$ ($q_{N400}$), are shown for the cultivars Eva, Hera, Maurane, Melci and RegaloG at different growth temperatures (T) ($n$=8, mean ± SD). No significant difference was found when the data were analysed per growth temperature. However, significant differences were found between the growth temperatures for the various industrial chicory varieties.

| T [°C] | Cultivar | $q_{P200}$ | $q_{N200}$ | $q_{P400}$ | $q_{N400}$ |
|---|---|---|---|---|---|
| 16 | Eva | 0.87±0.03[a] | 0.52±0.08[a] | 0.72±0.05[a] | 0.71±0.10[a] |
| | Hera | 0.89±0.03[a] | 0.52±0.13[a] | 0.74±0.05[a] | 0.72±0.12[a] |
| | Maurane | 0.86±0.02[a] | 0.55±0.10[a] | 0.72±0.06[a] | 0.75±0.06[a] |
| | Melci | 0.87±0.04[a] | 0.53±0.12[a] | 0.70±0.07[a] | 0.72±0.10[a] |
| | RegaloG | 0.84±0.09[a] | 0.57±0.14[a] | 0.67±0.11[a] | 0.76±0.07[a] |
| 8 | Eva | 0.83±0.03[a] | 0.72±0.04[a] | 0.46±0.10[a] | 0.83±0.02[a] |
| | Hera | 0.84±0.02[a] | 0.64±0.06[a] | 0.49±0.10[a] | 0.79±0.04[a] |
| | Maurane | 0.81±0.06[a] | 0.69±0.10[a] | 0.45±0.07[a] | 0.82±0.04[a] |
| | Melci | 0.77±0.11[a] | 0.69±0.15[a] | 0.44±0.11[a] | 0.80±0.08[a] |
| | RegaloG | 0.82±0.06[a] | 0.65±0.11[a] | 0.41±0.09[a] | 0.77±0.05[a] |
| 4 | Eva | 0.76±0.06[a] | 0.75±0.06[a] | 0.52±0.10[a] | 0.73±0.02[a] |
| | Hera | 0.80±0.05[a] | 0.70±0.06[a] | 0.58±0.09[a] | 0.67±0.03[a] |
| | Maurane | 0.76±0.12[a] | 0.71±0.07[a] | 0.54±0.14[a] | 0.69±0.03[a] |
| | Melci | 0.77±0.07[a] | 0.75±0.03[a] | 0.53±0.08[a] | 0.72±0.02[a] |
| | RegaloG | 0.75±0.07[a] | 0.71±0.06[a] | 0.52±0.09[a] | 0.69±0.03[a] |

Table 4. An overview of the pigment parameters, the chlorophyll (Chl) *a* concentration ($C_a$, µg $cm^{-2}$), the Chl *b* concentration ($C_b$, µg $cm^{-2}$), the carotenoid/xanthophyll pool concentration ($C_{x+c}$ µg $cm^{-2}$) and the Chl *a*/*b* ratio concentration ($C_a/C_b$) are shown for the cultivars Eva, Hera, Maurane, Melci and RegaloG at the different growth temperatures (T, °C) ($n$=8, mean ± SD).
[a, b]: indicate significant difference at the 0.05 level per growth temperature (*Duncan Post-Hoc* test).

| T [°C] | Cultivar | $C_a$ | $C_b$ | $C_{x+c}$ | $C_a/C_b$ |
|---|---|---|---|---|---|
| 16 | Eva | 30±7[a] | 7.6±2.1[a] | 6.7±1.2[a] | 4.0±0.3[a] |
| | Hera | 30±6[a] | 7.4±1.7[a] | 6.7±1.3[a] | 4.0±0.1[a] |
| | Maurane | 31±2[a] | 7.7±0.9[a] | 6.8±0.4[a] | 4.0±0.2[a] |
| | Melci | 27±3[a] | 6.6±0.9[a] | 6.0±0.7[a] | 4.2±0.3[a] |
| | RegaloG | 27±4[a] | 6.8±1.0[a] | 6.1±0.8[a] | 4.0±0.1[a] |
| 8 | Eva | 19±7[a] | 4.1±1.5[a] | 5.2±1.6[a] | 4.8±0.3[a] |
| | Hera | 25±4[a] | 5.2±1.0[a] | 6.4±1.0[a] | 4.9±0.5[a] |
| | Maurane | 23±9[a] | 5.1±2.1[a] | 5.8±1.9[a] | 4.6±0.4[a] |
| | Melci | 21±5[a] | 4.6±1.3[a] | 5.3±1.2[a] | 4.6±0.4[a] |
| | RegaloG | 22±4[a] | 4.9±1.1[a] | 5.7±0.9[a] | 4.7±0.5[a] |
| 4 | Eva | 27±4[a] | 5.3±1.0[a] | 7.4±1.1[a] | 5.2±0.2[a] |
| | Hera | 35±5[b] | 7.3±1.4[b] | 9.5±1.5[b] | 4.9±0.4[a] |
| | Maurane | 32±8[ab] | 6.5±1.7[ab] | 8.6±1.9[ab] | 5.0±0.3[a] |
| | Melci | 30±6[ab] | 5.8±1.4[ab] | 8.0±1.6[ab] | 5.2±0.4[a] |
| | RegaloG | 33±6[ab] | 6.6±1.5[ab] | 8.6±1.6[ab] | 5.1±0.4[a] |

parameters. At 4 °C, we detected significant between-variety differences for $C_a$, $C_b$, $C_{x+c}$ and $C_{ab}$. The level of $C_{x+c}$ increased less in Eva than in Hera, Maurane, Melci or RegaloG when the temperature was lowered from 16 to 4 °C. An average increase of 9.6 % was measured for Eva and an average increase of 26.3 % was detected for the other cultivars. This could be related to the protective role of the xanthophyll cycle, as described by Haldimann (1999). However, when considering the SLA, which is inversely proportional to the thickness of the leaf, we need to make a carefully balanced appraisal. Hera shows a higher xanthophyll-carotenoid pool and has a thicker leaf than Eva at lower temperatures. Gonzalez *et al.* (2002) previously described that pigments such as carotenoids could be interacting with leaf morphology to protect the photosynthetic machinery in an environment with high solar radiation, and that increase in leaf thickness is one of the most common responses of higher plants to help prevent damage to the photosynthetic apparatus.

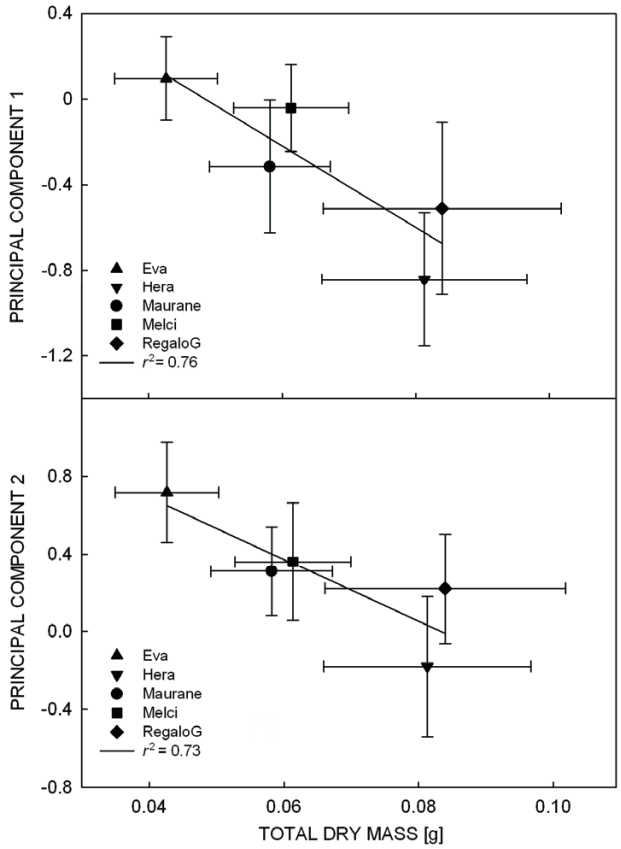


Fig. 2. The regression plot of the relation between the total dry mass and principal components 1 and 2 after the principal component analysis of the photosynthesis, chlorophyll *a* fluorescence, pigment content and growth parameters for the five cultivars at 4 °C.

**PCA**: A relationship between growth (early vigour) and individual photosynthesis parameters was not found, probably due to the high within-variety variability, as a chicory variety is a collection of different genotypes. PCA was used to transform all the data of the photosynthetic response. The resulting principal components (PCs) were analysed for their relation with early vigour. The PCs of the data measured at 16 and 8 °C showed no clear relationship between growth and photosynthetic response. At 4 °C, however, the first and the second principal component (PC) showed a significant difference between varieties, corresponding to their difference in early vigour (Fig. 2). The first PC is negatively correlated with $P_{max}$, $\alpha_c$ and $q_P$ at 200 µmol(quanta) $m^{-2}$ $s^{-1}$, and is positively correlated with the non-photochemical quenching parameters, NPQ and $q_N$ at 200 µmol(quanta) $m^{-2}$ $s^{-1}$. The second PC is positively correlated with the Chl *a*/*b* ratio. Eva, the variety with the lowest early vigour, has a higher value for the first and second PC. This corresponds with a lower $P_{max}$, more energy input in the non-photochemical processes and a higher Chl *a*/*b* ratio. Hera, on the other hand, a variety with a better early vigour, has a lower value for the first and second PC, corresponding with a higher $P_{max}$, more energy input in the photochemical processes and a lower Chl *a*/*b* ratio.

## Discussion

**The basic processes**: The growth analysis demonstrated that the five cultivars react similarly to a lowering of the growth temperature. A decrease in $DM_L$, $DM_R$, LA, RGR and AGR was observed for each of the varieties. A similar reduction of the RGR parameter was described previously for *Phaseolus vulgaris* L., *Zea mays* L., *Pisum sativum* L., *Spinacia oleracea* L. and *Lycopersicon esculentum* L. grown under cooler conditions in comparison to controls (Wolfe 1991, Venema *et al*. 2000). These results demonstrate clearly the influence on growth. At high light intensities, $P_{max}$ decreased as the temperature did. This was expected as this phase of the light response curve depends on temperature-based processes (dark reactions) in comparison to the light controlled part of the curve (light reactions), which is situated at low light levels. The large standard error is due to the fact that the cultivars we used were synthetics, which are a collection of different genotypes. Similar results were obtained by Fracheboud *et al.* (1999) and Lootens *et al*. (2004) for maize hybrids and inbred lines. The efficiency of the photosynthesis process can be evaluated through the Chl *a* fluorescence. Janda (1998) wrote that $q_P$ could be considered as a suitable indicator for the ability of the photosynthetic apparatus to tolerate suboptimum temperatures. The values indicate that at higher light intensities more energy is dissipated through heat and radiation (higher values of $q_N$) than at the lower ones. This means that the lower the photosynthesis efficiency, the higher $q_N$ and the lower $q_P$ will be. This effect became even clearer as the growth temperature was lowered from 16 to 4 °C. Brüggeman *et al.* (1992), studying the effect of chilling on young tomato plants, discovered that after chilling $q_N$ increased and $q_P$ decreased for both stress conditions (10 and 6 °C). After recovery none of the parameters returned to the control-values, measured before the stress was applied. Similar results were described by Fracheboud *et al.* (1999) in their study of cold-tolerant and cold-sensitive maize lines. Fracheboud and Leipner (2003) detected that the decreases on $q_P$ were even more pronounced at lower temperatures combined with high light intensities. An increase in the xanthophyll-carotenoid pool was detected at 4 °C, compared to the control (16 °C). The xanthophyll cycle is known for its possible function in the photoprotection of the photosynthetic apparatus (Koroleva *et al.* 1994). Similar results were described by Haldimann

(1997, 1998, 1999) for *Zea mays*. In cold-tolerant compared to cold-sensitive maize higher total carotenoid content was observed as the temperature decreased. Fracheboud *et al.* (2000) proved that maize grown at 14 °C had a higher xanthophyll-carotenoid pool than maize grown at 25 °C, and that the zeaxanthin pigment specifically increased in this pool. Lidon *et al.* (2001) described the same phenomenon for chilling-stressed wheat and maize. However, the growth analysis revealed that Hera had a thicker leaf than Eva. Gonzalez *et al*. (2002) declared that one of the most common responses of higher plants is to increase their leaf thickness to help prevent damage to the photosynthetic apparatus. Further specific xanthophyll pigment analyses should provide more insight.

**Early vigour and photosynthesis**: Photosynthesis is a highly regulated and integrated process. It aims to maximize the use of the light, optimize the use of carbon resources and minimize the damaging effects of excess energy. Consequently, the photosynthetic process is highly sensitive to any change in the environment and low temperatures could cause an imbalance between the source of energy and the metabolic sink (Ensminger *et al*. 2006). Disturbances of the photosynthetic process could therefore influence the secondary metabolic processes such as growth. The experiments described here did not allow us to define a straightforward relationship between individual parameters, probably because of the genetic variability within the varieties. Through PCA, however, we were able to assess the relationship between the early vigour and the basic physiological process parameters. At 4 °C, PCA revealed two PCs with a high discriminating power and a similar classification of varieties as determined for the early vigour. The growth analysis and PCA both showed a classification in three groups. Hera displayed the highest $P_{max}$, less energy input in the non-photochemical processes and a lower Chl *a*/*b* ratio. Growth and photosynthesis are linked to each other *via* mechanisms that are still not fully understood (Schurr *et al.* 2006 and Long *et al*. 2006). To connect the two processes we should look at the links between the carbohydrate metabolism, the transportable energy currency produced in photosynthesis and growth dynamics of leaves and other tissues. Oliveira and Penuelas (2004), studying the effect of temperature on growth and photosynthesis for *Cistus albidus* L. and *Quercus ilex* L., described a reduction in photosynthetic efficiency and in growth rate during winter conditions (lower temperatures) and a reduction of the ratio starch:soluble sugars, suggesting insufficient photo-assimilation to support processes such as growth and maintenance. Similarly, Verheul *et al.* (1996) reported that in maize a more efficient shoot dry matter accumulation and leaf area expansion were related to a greater photosynthetic capacity per unit leaf, under chilling conditions.

**Photosynthesis and Chl *a* fluorescence**: Chl *a* fluorescence is a useful technique to monitor non-invasively the photosynthetic performance of plants and can be used for screening (Baker and Rosenqvist 2004). For Hera and

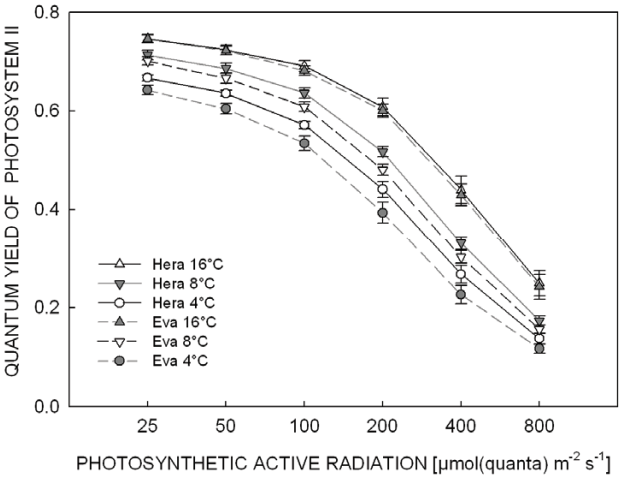


Fig. 3. The light response curve of the quantum yield of photosystem II ($\Phi_{PSII}$) for Hera and Eva at the different growth temperatures – 16 °C (reference), 8 °C (intermediate) and 4 °C (cold stress). $n$ = 8, mean ± SE.

Eva (two representatives of varieties with respectively faster and slower early vigour) a decline in $\Phi_{PSII}$ was detected under moderate and high light intensities. This decrease is visually more pronounced for Eva than for Hera (Fig. 3). This is in agreement with the results obtained by Janda (1998). Fracheboud *et al.* (1999) evaluated the use of Chl fluorescence as a selection tool for cold tolerance of photosynthesis in maize. They discovered that cold-tolerant maize genotypes maintained higher electron transport rates than cold-sensitive maize using $\Phi_{PSII}$ as a monitoring tool. They suggested that this was probably due to the ability of the tolerant plants to keep higher efficiency of excitation energy capture by open PSII reaction centers. The decline in $P_{max}$ at lower growth temperature and the lower maximum quantum yield of photosynthesis found in industrial chicory probably suggests that the cold stress situation affects the activity of the enzymes involved in $CO_2$ fixation or the availability of $CO_2$ for photosynthesis, as described by Fracheboud and Leipner (2003).

The objective of this study was to evaluate the effect of cold stress on the early vigour and the photosynthesis efficiency of industrial chicory plants, and subsequently to assess the possible relationship(s) between the early vigour and the physiological parameters. The results allowed us to relate the primary process, photosynthesis, to the secondary process, youth growth, under the influence of low growth temperatures. The analysis of the basic physiological processes revealed that all parameters are influenced by the growth temperature. However, some varieties are more sensitive to the lower growth

temperature than others. We were able to classify the varieties into different classes according to the growth rate and the photosynthetic and Chl *a* fluorescence parameters.

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