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Evaluation of cold stress of young industrial chicory (*Cichorium intybus* L.) by chlorophyll *a* fluorescence imaging. II. Dark relaxation kinetics

P. LOOTENS[*,+], S. DEVACHT[*, **], J. BAERT[*], J. VAN WAES[*], E. VAN BOCKSTAELE[*,**], and I. ROLDÁN-RUIZ[*]

Institute for Agricultural and Fisheries Research (ILVO), Plant Sciences Unit, Caritasstraat 21, 9090 Melle, Belgium[*]
Ghent University, Faculty Bioscience Engineering, Department of Plant Production, Coupure Links 653, 9000 Gent, Belgium[**]

Abstract

Industrial chicory, *Cichorium intybus* L., has rather poor early vigour under the typical early spring morning conditions of low temperatures and high light intensity. Screening tools are being developed to assess the cold tolerance/sensitivity of young industrial chicory plants under these conditions. Refinement of such tools requires better understanding of the plants' physiological responses. In this paper we discuss the effects of growth temperature (GT), measurement temperature (MT), and measuring light intensity (ML) on the relaxation of the Kautsky curve. We chose the chicory variety 'Hera', as it is known to possess a good average early vigour. Young plants of the variety 'Hera' were grown at three temperatures (GT): 16°C (reference), 8°C (intermediate), and 4°C (cold stress). The dark relaxation kinetics were analyzed at different light intensities (ML) in combination with different measurement temperatures (MT). The three components of the nonphotochemical quenching process (NPQ_E, NPQ_T, and NPQ_I) were determined. NPQ_E was not affected by GT but was significantly affected by MT and ML. NPQ_T and NPQ_I were affected by all factors and their interactions. An acclimation effect for plants grown at low GT was detected. Acclimation resulted in lower NPQ_T and NPQ_I values. The halftime of the inhibition depending on NPQ (NPQ_I) was not affected by any of the factors investigated. Based on the data generated, we conclude that NPQ_I is a valuable parameter for screening the cold sensitivity of young industrial chicory plants.

Additional key words: chilling, energy-dependent quenching, nonphotochemical quenching, photoinhibition, state-transition-dependent quenching.

Introduction

Industrial chicory, *Cichorium intybus* L., is cultivated for the production of inulin, a linear fructose polymer with a terminal glucose molecule. Early sowing allows for a longer growth season and can contribute to improved yield (Baert 1997). However, the yield potential of the currently available varieties has not yet been completely exploited due to the slow youth growth that most chicory varieties display at low temperatures (Baert 1997).

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[+]Corresponding author; tel: +32 9 272 28 55, fax: +32 9 272 29 01, e-mail: peter.lootens@ilvo.vlaanderen.be
Abbreviations: ANOVA – analysis of variance; Ax – antheraxanthin; Chl – chlorophyll; EC – electrical conductivity; F_o – the minimum chlorophyll fluorescence in dark-adapted state; F_m – the maximum chlorophyll fluorescence in dark-adapted state; F_m' – maximum fluorescence after light induction; htE – halftime of the energy-dependent quenching; htI – halftime of the photoinhibition-dependent quenching; htT – halftime of the state-transition-dependent quenching; GT – growth temperature; k_P – rate constant for PSII photochemistry; LHC – light-harvesting complex; ML – measurement light intensity; MT – measurement temperature; NPQ – nonphotochemical quenching of the chlorophyll fluorescence signal; NPQ_E – energy-dependent quenching; NPQ_f – fast nonphotochemical quenching; NPQ_I – photoinhibition-dependent quenching; NPQ_T – state-transition-dependent quenching; PAM – pulse amplitude modulated; PAR – photosynthetically active radiation; PSI – photosystem I; PSII – photosystem II; q_E – energy-dependent quenching; q_I – photoinhibition-dependent quenching; q_N – nonphotochemical quenching coefficient of the Chl fluorescence signal; q_T – state-transition-dependent quenching; SE – standard error; Vx – violaxanthin; Zx – zeaxanthin.
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A more detailed description of the crop and its reaction to low temperatures can be found in Part I (Devacht *et al.* 2011). In Part I we described the response of young industrial chicory plants to cold stress at the level of the light induction curve. We concluded that parameters such as the PSII operating efficiency (including PSII maximum efficiency and PSII efficiency factor) and NPQ are important to evaluate the effect of stress in terms of severity, processes affected, and acclimation to lower GT. The results clearly demonstrated that young industrial chicory plants can adapt to lower GT. Although the results presented in part I provide information on the effect of the applied stress conditions on the PSII efficiency, a more detailed analysis of the dark relaxation parameters of the Chl *a* fluorescence signal is necessary to fully understand the plant response.

Relaxation of the Kautsky curve is dependent on three mechanisms: (*1*) pH- or energy-dependent quenching (q_E), (*2*) state-transition quenching (q_T), and (*3*) photoinhibition quenching (q_I) (Horton and Hague 1988, Walters and Horton 1991, Muller *et al.* 2001). These are correlated with the relaxation of F_m with approx. halftimes (ht) of 30–60 s, 5–10 min, and >30 min, respectively (Horton and Hague 1988, Hodges *et al.* 1989, Walters and Horton 1991, Roháček 2010). The ht at 20°C for the different components of q_N is nevertheless dependent on the actinic light level before the relaxation period (Walters and Horton 1991). q_E usually relaxes fully within about 10 min in darkness. q_I relaxes much slower (up to several hours) but is enhanced under low light (approx. 30 μmol m^{-2} s^{-1}) (Skogen *et al.* 1986, Leitsch *et al.* 1994).

q_E, the fastest of the above components, is a build-up of thylakoid ΔpH generated by photosynthetic electron transport. The decrease in pH within the thylakoid lumen is an immediate signal of excessive light that triggers the feedback regulation of light harvesting by q_E (Müller *et al.* 2001). It involves the light-induced formation of the carotenoid zeaxanthin (1990, Demmig-Adams and Adams 1992). At lumen pH values below 6 the violaxanthin de-epoxidase that transforms violaxanthin (Vx) to antheraxanthin (Ax) and zeaxanthin (Zx) is activated (Eskling *et al.* 1997). Discussion continues about the exact mechanism by which energy is dissipated and Chl fluorescence is quenched (Krause and Jahns 2004).

q_T is due to the phenomenon of state-transition, the uncoupling of light-harvesting complexes (LHCs) from PSII (Walters and Horton 1991). This involves the reversible phosphorylation of light-harvesting proteins and detachment of peripheral light-harvesting complexes of PSII (LHCIIb). It is thought to be important in balancing the distribution of light energy between PSI and PSII at low light intensities (Demmig and Winter 1988, Horton and Hague 1988, Somersalo and Krause 1990, Lunde *et al.* 2000, Maxwell and Johson 2000). This component is rather negligible in most plants during exposure to excess light (Müller *et al.* 2010). Under such conditions, phosphorylation of a mobile LHCIIb appears to be inhibited by the high trans-thylakoid ΔpH resulting in dephosphorylation (Walters and Horton 1991, 1993).

For most applications, q_E and q_T are regarded as photoprotective processes and are not quantified separately for the following reasons: (*1*) they are difficult to distinguish based on their relaxation kinetics, (*2*) q_T is only important at low light intensities, and (*3*) they both relax over a time-scale of a few minutes following the cessation of illumination (Demmig and Winter 1988, Somersalo and Krause 1990, Maxwell and Johson 2000). However, Krause and Jahns (2004) disagree that q_T can be regarded as a photoprotective mechanism as it is suppressed under excessive illumination.

q_I, the photoinhibition quenching, generally refers to both protective processes as well as damage to the reaction centres of PSII (Osmond 1994). Photoprotective processes are related with zeaxanthin and are thought to occur in the light-harvesting antenna of PSII. Damage to PSII reaction centres results in quenching occurring within the PSII reaction center. This distinction between antenna and reaction center is important as the former causes changes in the F_0 level of quenching and the latter does not. The two processes can thus be distinguished under strict conditions (Demmig and Winter 1988, Somersalo and Krause 1990, Maxwell and Johnson 2000). With the partial inactivation of PSII, a decline is caused in the optimal quantum yield of photosynthetic CO_2 assimilation and O_2 evolution (Krause and Jahns 2004). Under prolonged exposure to high-light stress in combination with conditions that restrict energy use by the dark reactions, q_I becomes a large component of q_N (Krause 1988). As an alternative for q_I, the potential quantum efficiency of PSII can be measured after 10 min of darkness, when q_E has relaxed. The parameter $(1/F_o) - (1/F_m)$ has also been used as an indicator of photoinactivation of PSII.

The different components of q_N can be calculated by the repeated extrapolation of distinct phases of recovery back to the point of the *y* intercept from the remaining points (Horton and Hague 1988, Walters and Horton 1991, Johnson *et al.* 1993, Scholes *et al.* 1997). The following formula is used here: $(1 - q_N) = (1 - q_E)(1 - q_T)(1 - q_I)$ (Krause and Jahns 2004).

Adams and Demmig-Adams (2004) and Oxborough (2004) point out the following problem with the determination of q_N (and consequently q_E, q_T, and q_I): given that they are calculated as the normalized variable fluorescence (F_v) at the point of measurement, any change in the value of the rate constant for PSII photochemistry (k_P) will cause a decrease in the value of the supposedly nonphotochemical quenching coefficient being calculated. This may occur when a healthy plant is subjected to photoinhibitory conditions. Under these conditions, the rate at which PSII centres are inactivated exceeds the rate at which they are replaced, with

a consequent decrease in the effective rate constant for PSII photochemistry (Baker and Oxborough 2004). Recently, Roháček (2010) also described a method for the quantification of the components of the nonphotochemical quenching based on q_N.

NPQ, in contrast, as based on the Stern-Volmer quenching calculated as $(F_m/F_m') - 1$ (Bilger and Björkman 1990), is unaffected by changes in k_P. This makes it a better parameter for quantifying nonphotochemical quenching processes. Furthermore, this parameter has the advantage of being directly proportional to the level of energy dissipation activity, and is thus linearly related to thermal dissipation in a leaf (Maxwell and Johnson 2000). In this case, the Stern-Volmer quenching formula becomes $NPQ = NPQ_E + NPQ_T + NPQ_I$ (Krause and Jahns 2004). NPQ is widely used to represent nonphotochemical quenching (Baker and Oxborough 2004). Lambrev *et al*. (2007) also calculated NPQ to evaluate the nonphotochemical quenching at low temperature and high light levels. We also chose this method to evaluate the variation in cold sensitivity of young plants of the chicory variety 'Hera'. We have examined the influence of temperature and light stress, either combined or not, on the relaxation kinetics parameters described above.

Materials and methods

Plant materials and growth conditions: All the experiments were carried out using plants of the industrial chicory variety 'Hera'. This variety is known to possess a good early vigour (Devacht *et al.* 2007) and displays a high level of genetic variability. Seeds were sown in perforated multi-well plates (4 × 6) filled with a mixture of peat and perlite (EC = 250 µS cm^{-1}), and placed in trays with an irrigated underlay. These trays were first kept for 6 days at 16°C to allow germination. They were then transferred to the corresponding growth temperature (GT). For the reference situation, the seedlings were further grown during 4 days at 16°C. For the stress situations the seedlings were kept either at 8 or 4°C during 10 days. It was necessary to keep the plants for a longer period at 8 and 4°C before screening to allow them to reach the same developmental stage (cotyledons) as was reached after 4 days of growth at 16°C. These growth temperatures were chosen based on the average temperatures during early spring listed in the typical reference year tables for Belgium (Dogniaux *et al.* 1978). In all cases, the air relative humidity was set at 60% (the vapour pressure deficit was not controlled) and the light intensity at 220 µmol m^{-2} s^{-1} for 16 h per day (*TL-D 58W/840, Philips*, the Netherlands). All plants were grown in growth chambers (*1600US, Weiss*, Reiskirchen, Germany).

Chl *a* fluorescence was measured using a Chl fluorescence imager (*CFImager, Technologica*, UK). This was done at different measurement light intensities (ML) (50, 100, 200, 400, 800 and 1,200 µmol m^{-2} s^{-1}, *i.e*., the actinic light levels used in the Chl fluorescence measurements) in combination with various measurement temperatures (MT) (2–16°C, at intervals of 2°C). The measurement procedure took 2.5 h, as illustrated in Fig. 1*A*. The first step was to adapt the plants to the dark during 30 min. F_0 was measured using a measurement light level of 0.52–0.85 µmol m^{-2} s^{-1}. F_m was measured with a saturation pulse of 4,947 µmol m^{-2} s^{-1} for 800 ms. After 20 s, the plants were exposed to actinic light, which corresponds to the ML for 1 h. At the end of the actinic light period, a saturation pulse was given for the quenching analysis. Then the actinic light was switched off. During this dark period a saturation pulse was given twice after 2.5 min and every five minutes thereafter during 1 h to determine the different components of the relaxation process (NPQ_E, NPQ_T, and NPQ_I). The first two pulses after 2.5 min are important to determine NPQ_E, the "fast" component of the relaxation process (Horton and Hague 1988, Müller *et al.* 2001, Bruce *et al.* 2004). Subsequent pulses were applied with longer intervals, *i.e*., every 5 min, because the procedure can have an influence on the relaxation of NPQ (Walters and Horton 1991). The application of a saturation pulse every 5 min allowed us to maintain the influence of the pulses on the relaxation kinetics as low as possible, without any loss of information about the dark recovery.

Within each plate 14 plants were selected at random. This corresponds to the maximum number of traces that the used Chl fluorescence imaging system can record simultaneously. Chl fluorescence measurements were done on the adaxial side of the matures cotyledons. The Chl signals for each of the 14 plants result from the average values of all the pixels of that individual plant.

For the calculation of the relaxation parameters, a regression analysis similar to that described by Horton and Hague (1988) was used. However, we based the calculation of the parameters on F_m' (maximum Chl fluorescence in light-adapted state) instead of q_N (nonphotochemical quenching coefficient of the Chl fluorescence signal), the basis of the method of Horton and Hague (1988). This provides the possibility to work directly with the fluorescence signal measured by the Chl fluorescence imaging device. But it has also the advantage of using NPQ instead of q_N. For each component of the relaxation kinetics separately, a regression of F_m' was performed from the end of each phase of the dark relaxation curve back to the start of the dark period. Based on those F_m' values at the start of the dark relaxation period, the NPQ components: NPQ_E (energy-dependent quenching), NPQ_T (state-transition-dependent quenching), and NPQ_I (photoinhibition-dependent quenching) were calculated.

Besides the relaxation parameters, the halftime of the photoinhibition-dependent quenching (htI, [min]) was determined. This was calculated based on the time needed to reach a F_m' value half-way between F_m' related to the photoinhibition dependent quenching (calculated based on the regression) at the start of the dark relaxation and the maximum F_m' during the dark relaxation.

Statistical analyses: The effects of GT, MT and ML on the different Chl *a* fluorescence parameters were analyzed by one-way and factorial analysis of variance (*ANOVA*; significance level $p \leq 0.05$). To determine whether the conditions applied (GT, MT, and ML) had a significant effect on the Chl *a* fluorescence parameters a *Duncan Post-Hoc* test was performed. Multiple linear regression analysis was used to compute a relationship and to determine which condition or combination of conditions (GT, MT, and ML) had the highest influence on the parameters considered. For the regression analyses all factors were included as well as their second degree interactions. All statistical calculations were conducted in *STATISTICA v9* (*Statsoft*, USA).

Results

The relaxation of F_m' differs greatly in function of MT and ML (Fig. 1). Comparing Fig. 1*A* and *C,* the effect of an increased ML becomes clear at a MT = 16°C. Lowering the MT to 2°C results in a reduction of the relaxation (Fig. 1*B,D*). In the most severe of the situations presented (Fig. 1*D,* MT = 2°C and ML = 800 µmol m^{-2} s^{-1}), F_m' hardly relaxes and photoinhibition appears.

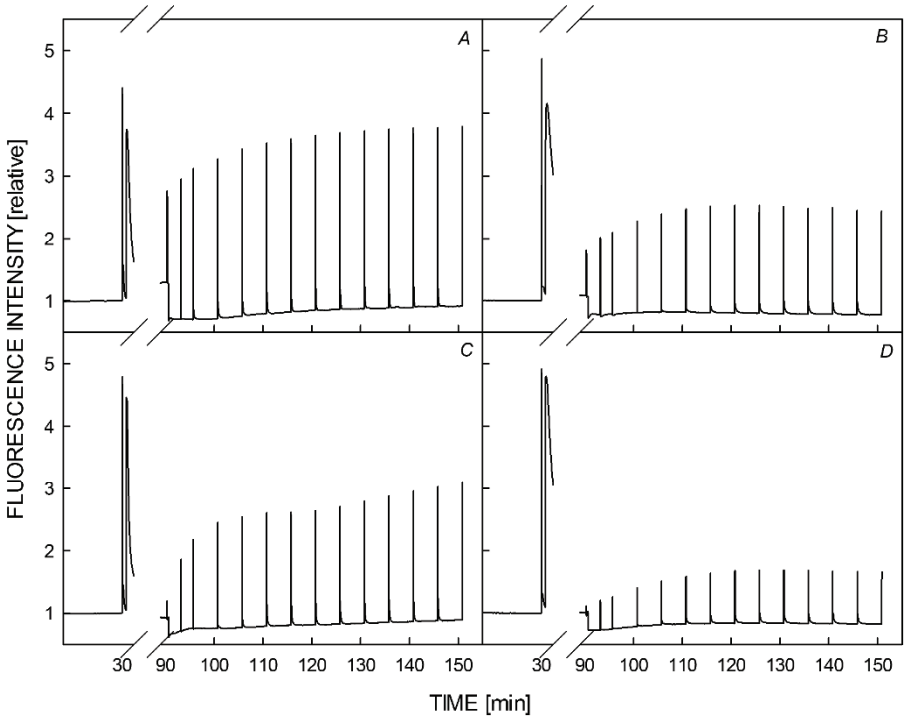

Fig. 1. Chl fluorescence signal registered with a Chl fluorescence imaging system for plants (n = 14) grown at 16°C (reference situation) and measured under different conditions: (*A*) 16°C and 100 µmol m^{-2} s^{-1}, (*B*) 2°C and 100 µmol m^{-2} s^{-1}, (*C*) 16°C and 800 µmol m^{-2} s^{-1}, and (*D*) 2°C and 800 µmol m^{-2} s^{-1}. The fluorescence has been normalized to the initial fluorescence intensity (F_o).

Based on the standardized regression coefficients of the multiple linear regression analysis for NPQ and its components (Table 1), where GT, MT, and ML were included as well as the second degree interactions, we found that all factors and interactions were highly significant except for NPQ_E. ML is the most important factor contributing to all the models. GT plays an important role for NPQ and NPQ_I. The interaction GT × ML is important for NPQ and NPQ_T while the interaction MT × ML is important for NPQ_E and NPQ_I. NPQ_E was not affected by either GT or MT, even though an interaction between ML and MT was found. Given these results, further statistical analysis was carried out per GT, except for NPQ_E. Multiple linear regressions per GT based on MT, ML, and their interaction showed a significant effect for all factors and interactions for NPQ, NPQ_T, and NPQ_I. This was not the case for NPQ_T at GT = 8°C (results not shown).

Table 1. Standardized regression coefficients of the multiple linear regressions of the nonphotochemical quenching (NPQ) and its components [energy-dependent quenching (NPQ_E), state-transition-dependent quenching (NPQ_T) and the photoinhibition-dependent quenching (NPQ_I)] for plants of the industrial chicory variety 'Hera' grown at 16°C, 8°C, and 4°C (growth temperature, GT [°C]) for different measurement temperatures (MT [°C]) and measuring light intensities (ML [μmol m^{-2} s^{-1}]). Interactions up to the second degree were included in the model. *Underlined coefficients* contribute the most to the model. ** – significant at $p<0.01$.

Factor	NPQ	NPQ_E	NPQ_T	NPQ_I
GT	0.2934**	–0.0371	0.2528**	0.3960**
MT	–0.0743**	–0.0008	–0.0889**	–0.0733**
ML	0.5310**	–0.1664**	0.5016**	0.7486**
GT × MT	–0.2363**	0.0102	–0.2619**	–0.2583**
GT × ML	0.3122**	0.0028	0.3656**	0.3146**
MT × ML	0.1086**	1.0028**	–0.1259**	–0.3692**

The most important change for NPQ was caused by changes in ML (Fig. 2, Table 1). As ML increases, more energy needs to be dissipated almost regardless of MT. At high ML, NPQ clearly drops as GT becomes lower (Fig. 2*A* and *B* compared to Fig. 2*C*). This indicates a positive acclimation effect for plants grown at low GT.

NPQ_E was not significantly influenced by GT (Table 1). A summary graph is shown for all GT together (Fig. 3). NPQ_E was activated at high MT in combination with high ML, reaching average levels of 1.6 at MT = 16°C and ML = 1,200 μmol m^{-2} s^{-1}. At high ML in combination with low MT and at low ML in combination with all MT, NPQ_E levels were low (on average 0.1–0.2). NPQ_T was the highest at high ML in combination with MT = 6–8°C (depending on GT) (Fig. 4). Finally, NPQ_I (Fig. 5) was more activated at low MT and high ML.

The acclimation effect of lowering the GT from 16 to 8 and 4 is illustrated in Fig. 6. NPQ and its components

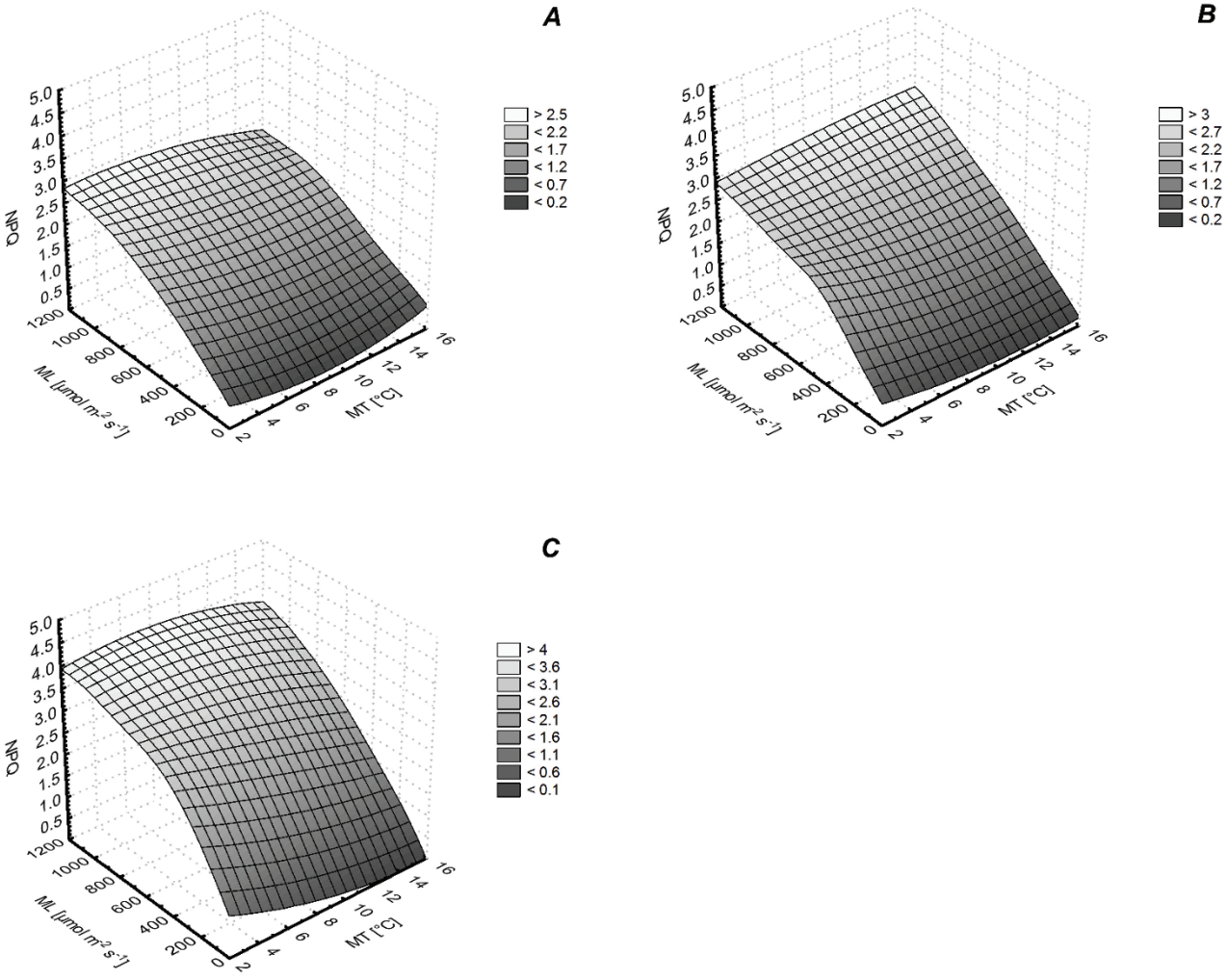

Fig. 2. 3D spline plot of the average value of the nonphotochemical quenching (NPQ) measured at different measuring light intensities (photosynthetic active radiation, ML) and measurement temperatures (MT) for plants of the industrial chicory variety 'Hera' grown at GT of 4°C (*A*), 8°C (*B*), or 16°C (*C*).

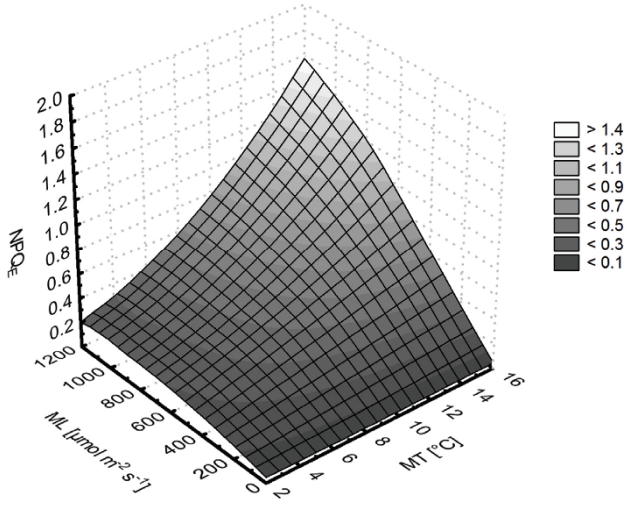

Fig. 3. 3D spline plot of the average value of the energy-state-dependent quenching (NPQ_E) at different measuring light intensities (photosynthetic active radiation, ML [µmol m^{-2} s^{-1}]) and measurement temperatures (MT [°C]) for plants of the industrial chicory variety 'Hera' grown at temperatures (GT = 4, 8, and 16°C). Values obtained at different GT have been averaged.

are represented in relation to ML as this is the most influential factor (Table 1). Above 200 µmol m^{-2} s^{-1}, NPQ decreased with 32–40% when plants were grown at 4°C compared to plants grown at 16°C (Fig. 6*A,E)*. For 8°C this relative change was between 22–42%. For the NPQ components different reactions upon a GT decrease were observed. NPQ_E displayed no significant differences between the different GT (Fig. 6*B,F*). For NPQ_T the decrease was significant but similar responses were detected for both 4°C and 8°C in relation to 16°C (Fig. 6*C,G*). A decrease of between 29–40% was found for light levels above 200 µmol m^{-2} s^{-1}. Finally for NPQ_I, a decrease in relation to a GT of 16°C was found but was more pronounced for 4°C (42–57%) than for 8°C (28–57%) at ML > 200 µmol m^{-2} s^{-1} (Fig. 6*D,H*).

NPQ consists of three processes: (*1*) energy-dependent quenching, (*2*) state-transition quenching and (*3*) photoinhibition quenching. These three processes all have different rate constants and consequently different halftimes (ht). At start of the relaxation, F_m' was only measured at 2.5, 5, and 10 min after the initiation of a dark period to reduce the influence of the measuring procedure on the kinetics of the relaxation. As a consequence, the resolution was insufficient for a correct determination of the half times of NPQ_E (htE) and NPQ_T (htT). Nevertheless, we were able to calculate the halftime of NPQ_I (htI). From the regression analysis with all factors and their interactions we concluded that for htI none of the factors had a significant effect. For all measurements an average htI of 29.1 ± 0.1 minutes (mean ± SE, n = 1,799) was calculated.

Discussion

Chl *a* fluorescence has been used extensively for years as a noninvasive method to evaluate the photosynthetic efficiency of plants. In particular, the use of Chl *a* fluorescence imaging to generate objective data on the influence of diverse environmental stresses such as cold stress has been examined (Daley *et al.* 1989, Sayed 2003, Baker and Rosenqvist 2004). This technology has proven useful to detect differences in cold sensitivity in *Solanum lycopersicum* L. among others (Brüggemann *et al.* 1992), *Quercus* (Cavender-Bares *et al.* 1999, Gimeno *et al.* 2009), wheat (Rapacz and Wozniczka 2009) and *Zea mays* L. (Earl and Tollenaar 1999, Fracheboud *et al.* 1999, Lootens *et al.* 2004).

To evaluate the response of plants to chilling and light stress conditions, often F_v/F_m or F'_v/F'_m is measured after the stress period (Groom *et al.* 1992, Andrews *et al.* 1995, Fracheboud *et al.* 1999). Although these parameters can be measured very quickly and are particularly useful for high throughput screening purposes, they do not allow us to quantify how protection mechanisms such as energy-dependent quenching or state-transition work, nor how they change in response to specific growth conditions or treatments.

Our results clearly show that both MT and ML have a large impact on the Kautsky curve itself (Devacht *et al*. 2011; part I) and the relaxation kinetics (this paper). This should be kept in mind when defining a procedure to estimate plant responses derived from Chl fluorescence measurements, but even more when comparing Chl fluorescence data obtained in growth chambers and those obtained under field conditions, where the light environment and temperature change continuously and at different temporal scales.

The dark relaxation process consists of three parts: a "fast", "middle", and "slow" part (Quick and Stitt 1989, Lambrev *et al.* 2007). These are the energy-dependent quenching, the state-transition-dependent quenching and the photoinhibition-dependent quenching stages, respectively. Maxwell and Johnson (2000) proposed to combine the first two parts into one parameter, NPQ_f. However, to evaluate the effect of the abiotic stress applied, the different components of the relaxation curve need to be taken into consideration, as each of these parts provides complementary information on the way in which biological systems respond to stress conditions. The relaxation parameters NPQ, NPQ_T, and NPQ_I were significantly affected by the GT, ML, and MT applied. For NPQ_E only ML and MT played a significant role. The high NPQ_E values found at high ML and high MT and the low NPQ_E obtained at high ML and low MT correspond with Lambrev *et al.* (2007).

Quick and Stitt (1989) described that the "fast"

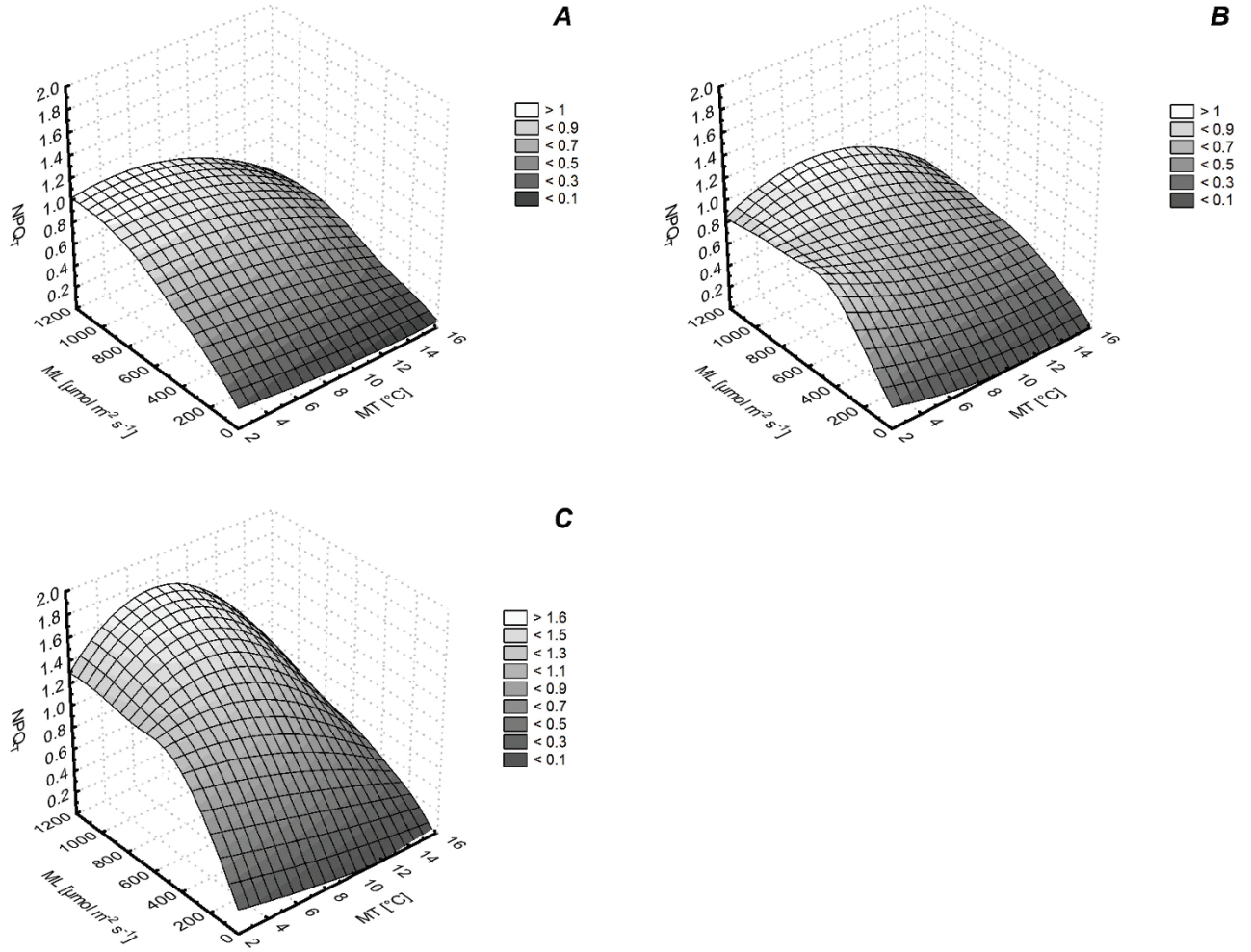

Fig. 4. 3D spline plot of the average value of the-state-transition-dependent quenching (NPQ_T) at different measuring light intensities (photosynthetic active radiation, ML [µmol m^{-2} s^{-1}]) and measurement temperatures (MT [°C]) for plants of the industrial chicory variety 'Hera' grown at different temperatures: GT of 4°C (*A*), 8°C (*B*), and 16°C (*C*).

component, NPQ_E, appears after illumination with high actinic light and makes a very variable, and often small, contribution to the nonphotochemical quenching in barley leaves. Our results demonstrate a more complicated behaviour, as the level of NPQ_E depended on ML, MT, and their interaction. Furthermore, Maxwell and Johnson (2000) stated that the state-transition-dependent quenching makes only a small contribution to overall quenching and is only relevant when low light intensities are applied. This contrasts with our findings, as NPQ_T was on average responsible for about 30% of NPQ for all situations tested, and was significantly influenced by all factors considered.

Finally, NPQ_I is expected to increase as the stress becomes more severe. Indeed, as ML increased and MT decreased, higher values of NPQ_I were reached in our experiments. From this, it is clear that NPQ_I is probably the most interesting parameter to evaluate the severity of (photoinhibition) stress conditions. For htI, no significant effects of GT, MT and ML were found and the average value 29.1 ± 0.1 minutes corresponded well with values found in literature (30–40 minutes) (Horton and Hague 1988, Hodges *et al.* 1989, Walters and Horton 1991, Roháček 2010). For screening purposes, a procedure similar to the one described here could be used, but instead of measuring the entire relaxation curve, F_v/F_m could be measured in plants after a period of 30 min or 1 h of dark adaptation. This would have some important advantages: (*1*) the throughput of the measurements can be much higher and (*2*) as the recovery of F_v/F_m caused by temperature and light treatment is stopped in darkness, the potential impact of the saturating pulses on the relaxation is eliminated.

Overall, ML at the range tested was the most important parameter influencing NPQ and its components in the experiments discussed here. Young chicory plants were clearly able to acclimate when grown at low temperatures. Plants grown at lower GT displayed lower values of NPQ, particularly in NPQ_T and NPQ_I. NPQ_E was not significantly influenced by GT. These low NPQ

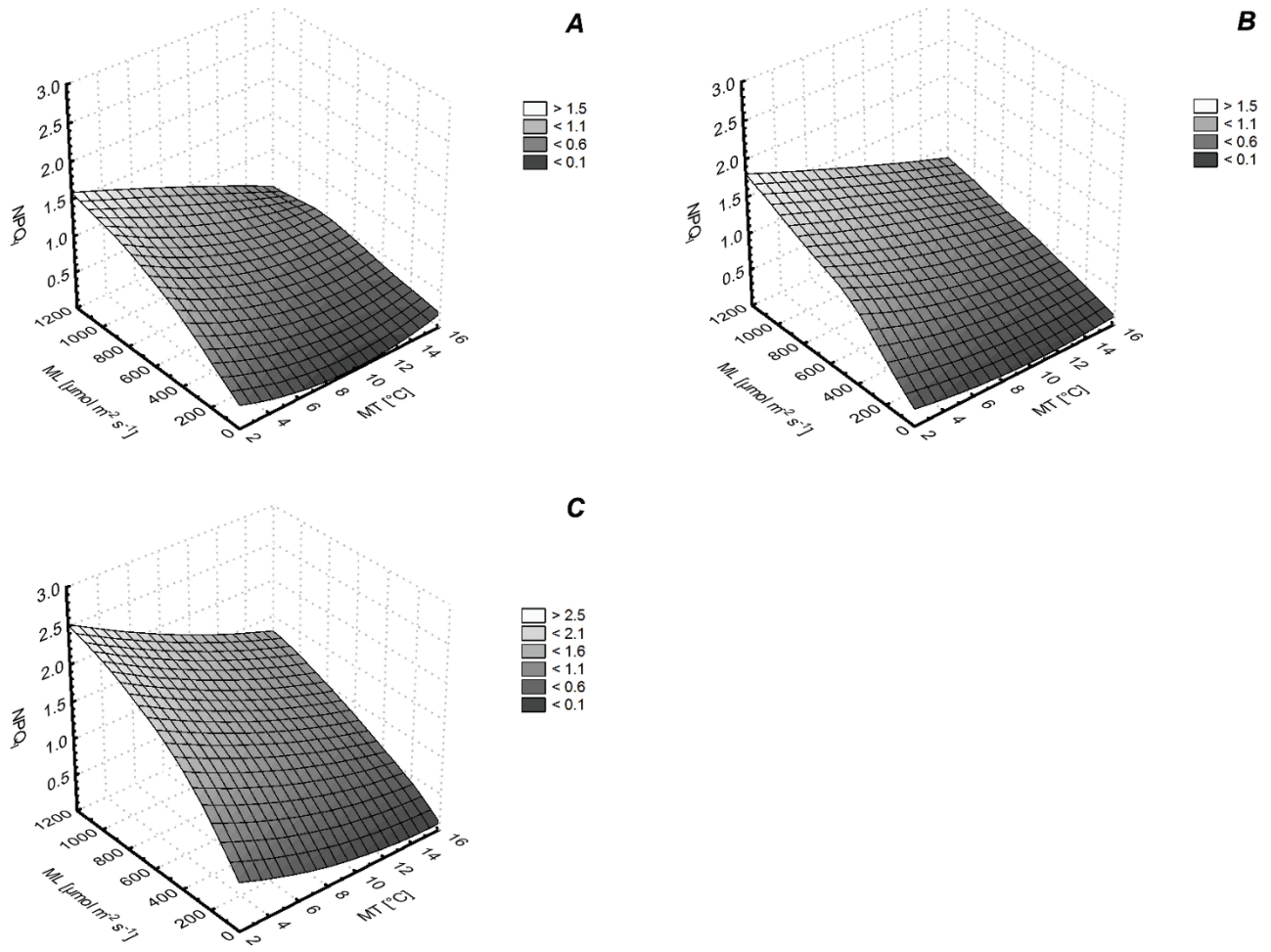

Fig. 5. 3D spline plot of the average value of the inhibition dependent quenching (NPQ_I) at different measuring light intensities (photosynthetic active radiation, ML [µmol m^{-2} s^{-1}]) and measurement temperatures (MT [°C]) for plants of the industrial chicory variety 'Hera' grown at different temperatures: GT of 4°C (*A*), 8°C (*B*), and 16°C (*C*).

values probably reflect the photosynthetic systems' optimisation to work at lower temperatures or the activation of other energy dissipative systems. As all the plants were grown under the same light conditions, it was impossible for us to estimate whether young chicory plants are able to adapt their photosynthetic apparatus under different light regimes. Future research could test the response of plants grown under different light levels, as plants should ideally be able to acclimate to the continuous changes in temperature and light conditions typically present in the field.

Conclusion: In the first part of this study (Devacht *et al.* 2011), where the light-adapted state of the Kautsky curve was evaluated, we found that parameters such as the PSII operating efficiency (including PSII maximum efficiency and PSII efficiency factor) and NPQ are important to evaluate the effect of stress in terms of severity, processes that are affected, and acclimation to low GT. Analysis of the light-induction curves clearly demonstrated that young industrial chicory plants can adapt to low GT. The plants seem to react by protecting their primary processes, which subsequently guarantees growth. This was confirmed by the analysis of the dark relaxation kinetics presented here, where we found significant effects of GT, MT, and ML. The photoinhibition-dependent quenching parameter, NPQ_I, seems to be a particularly interesting parameter to examine.

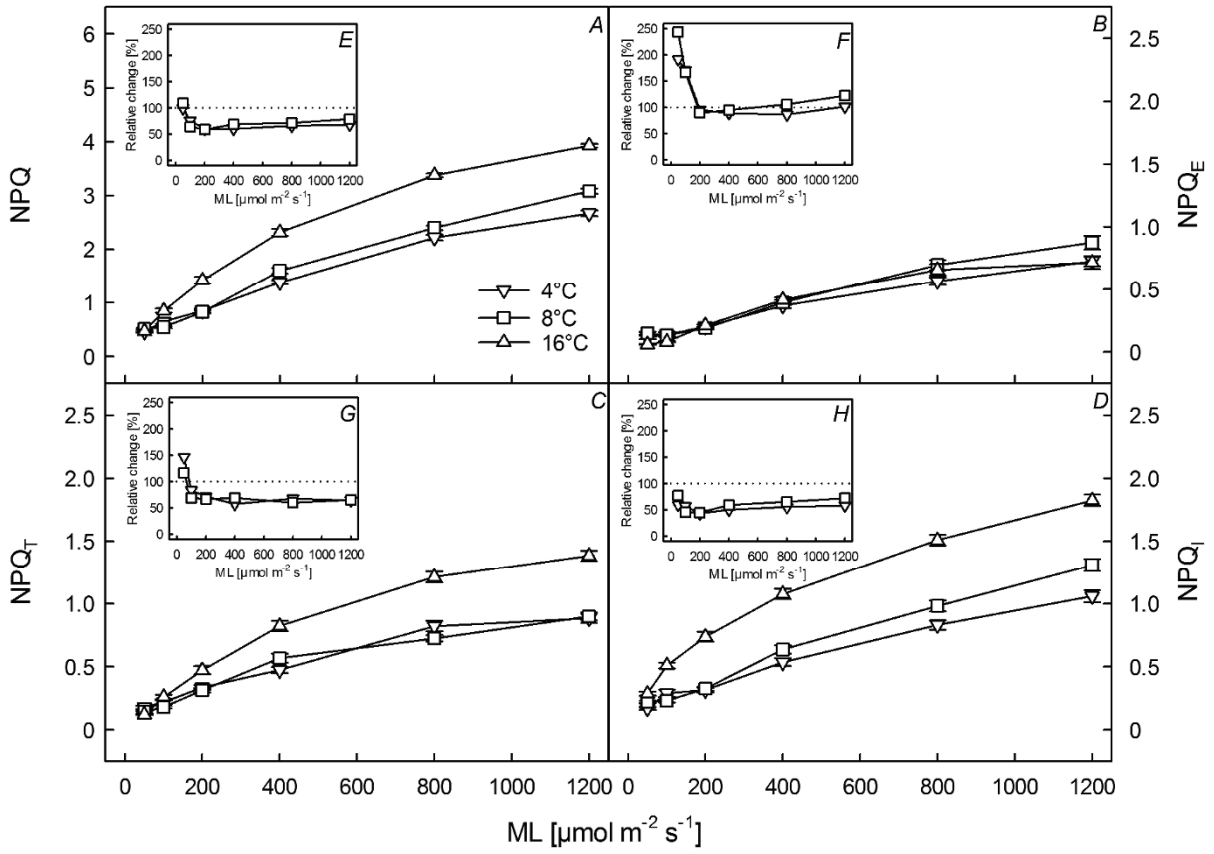

Fig. 6. Nonphotochemical quenching (NPQ; *A*) and its components [NPQ_E (*B*), NPQ_T (*C*), NPQ_I (*D*)] averaged by measurement temperature (MT) for plants of the industrial chicory variety 'Hera' (mean ± SE, $n = 100$) grown at different temperatures (GT = 4, 8, and 16°C) and measuring light intensities (ML, [µmol m^{-2} s^{-1}]). The corresponding relative change for the growth temperatures 4 and 8°C related to 16°C for the respective NPQ components are shown in *E*, *F*, *G*, *H*.

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