Variation in leaf-level photosynthesis among switchgrass genotypes exposed to low temperatures does not scale with final biomass yield

ANGEL CORDERO¹,² and BRUCE A. OSBORNE¹,²

¹UCD School of Biology and Environmental Science, University College Dublin, Belfield, Dublin, Ireland, ²UCD Earth Institute, University College Dublin, Belfield, Dublin, Ireland

Abstract

The effect of a 10-day low-temperature treatment and subsequent recovery of 13 genotypes of switchgrass, including upland and lowland types, resulted in significant variations in a number of leaf-level photosynthetic parameters. Whilst the most resistant genotype (Blackwell) is an upland type, there was no clear separation of upland and lowland genotypes in terms of the resistance of leaf photosynthesis ($A_{sat}$, $\theta_{CO2max}$, $V_p$ and $V_{pmax}$), respiration ($R_d$) or fluorescence (Fv/Fm) parameters to low temperatures, with lowland types also achieving higher biomass yields than upland types. Examination of the reason for differences in the impact of low temperatures on leaf photosynthesis indicated that this could be due to both PEP carboxylase activity and/or PEP regeneration, but with some support for PEP regeneration being the more important. However, variations in leaf photosynthesis were not related to the final biomass yield in either control or low-temperature-exposed plants, and the best correlation was found with leaf area. Low temperatures were found to have a post-treatment impact on leaf area development through a reduction in the formation of new leaves. Overall, these results suggest that more attention should be directed at variations in the effect of low temperature on leaf initiation and emergence to exploit the wider use of switchgrass as a biomass–bioenergy crop.

Keywords: abiotic stress, bioenergy crop, CO₂ assimilation, leaf initiation, leaf traits, Panicum virgatum

Received 31 October 2015; revised version received 4 February 2016 and accepted 8 February 2016

Introduction

In recent decades, there has been an increase in interest by governments and industry in the use of renewable energy sources. The decrease in fossil fuel availability and the increase in its costs have created an interest in crops as a potential source of bioenergy production (McKendry, 2002; Lemus & Lal, 2005). There are several candidate bioenergy crops, with the recent emphasis on second-generation biomass crops where all of the standing biomass is used for energy or fuel generation, as well as an ability to grow under marginal environmental conditions. Nonfood crops, such as perennial grasses, are considered to have a better potential production on marginal lands (Sims et al., 2010). Whilst there is no clear definition for marginal lands, these are often characterized on the basis that they are at the margin of economic viability (Strijker, 2005) due to significant environmental or policy-related constraints (Wiegmann et al., 2008), or are susceptible to degradation, and unsuitable for conventional agricultural production (Cai et al., 2010). One of the more significant environmental factors that constrains the geographical spread and/or productivity of bioenergy C4 crops is low temperature as many promising C4 species are relatively intolerant of low temperatures (Heaton et al., 2004). Even in areas considered more optimal for bioenergy production, increased tolerance to low temperatures could enhance productivity by increasing the potential growing season through earlier emergence and/or extended late season growth (Lewandowski et al., 2000).

Two of the main candidates for biomass-based bioenergy production are switchgrass (Panicum virgatum) and miscanthus (Miscanthus x giganteus), with switchgrass often described as more suitable for warmer climates, whilst miscanthus has proved to be particularly suitable for cool-temperate conditions (Dohleman & Long, 2009; Sage et al., 2015). However, switchgrass genotypes have been identified that have significant resistance to low temperatures (Hope & McElroy, 1990; Lemeziene et al., 2015). The switchgrass genus also has a wide adaptation range often resulting in a high productivity in different geographical regions (Lewandowski et al., 2003) although its photosynthetic capacity has been reported to be reduced under cold growing conditions (Mitchell et al., 2014). The existence of two major ecotypes of switchgrass, the so-called ‘upland’ and ‘lowland’ types...
(Stroup et al., 2003), with upland types reportedly showing greater tolerance to low temperatures (Ma et al., 2011; de Koff & Tyler, 2012), suggests that there is significant low-temperature-related variation that could be exploited, although this has not been fully explored. Further studies on low-temperature tolerance in switchgrass could not only increase the area where it can be grown but also contribute to increase yields in areas where it is currently grown through an extension in the length of the growing period.

Whilst much emphasis has often been placed on the impact of low temperatures on leaf-level photosynthesis of putative bioenergy species, such as miscanthus (Dohleman & Long, 2009; Glowacka et al., 2015a), there is less evidence on how this impacts on final biomass production, and whether this scales with a superior leaf-level photosynthetic response. Whilst the ultimate goal is to maximize plant productivity in response to low temperatures, the relationship between leaf-level photosynthesis and final yield or productivity remains controversial (Kruger & Volin, 2006; Long et al., 2006; Gu et al., 2014). For miscanthus, there is evidence, for instance, that biomass yields could be more affected by differences in leaf area duration and development, rather than variations in leaf-level photosynthetic properties (Dohleman & Long, 2009).

In order to examine variation in the performance of switchgrass in response to low temperatures, we assessed the effects on leaf-level photosynthesis (1) after exposure to a 10-day low-temperature screening protocol, using thirteen switchgrass cultivars, including upland and lowland ecotypes, (2) after transfer back to more optimal conditions to assess variability in recovery, and (3) assessed how exposure to low temperatures impacted on the final biomass yield, by subsequently growing plants to maturity. In turn, this allowed us to assess the relationship between the effects of low temperature on leaf photosynthetic responses and plant productivity.

Materials and methods

Plant material, treatment and harvest

Seeds of thirteen switchgrass genotypes were used for the experiments – Blackwell (B), Carthage (Ct), Cave-in-Rock (Cv), EG2101 (E2), Shawnee (Sh), Sunburst (Sb) and Trailblazer (T) are upland ecotypes; and Blackwell (B), Carthage (Ct), Cave-in-Rock (Cv), EG2101 (E2), Shawnee (Sh), Sunburst (Sb) and Trailblazer (T) are upland ecotypes; and Alamo (A), Cimarron (C), EG1101 (E11), EG1102 (E12) and Kanlow (K) are lowland ecotypes. Seeds were obtained from a variety of sources (C), EG1101 (E11), EG1102 (E12) and Kanlow (K) are lowland ecotypes (seeds were obtained from a variety of sources

Leaf gas exchange and chlorophyll fluorescence analysis

Leaf gas exchange measurements were performed at 25 °C (leaf temperature) on the last fully expanded leaf of three replicates per treatment per variety using a portable infrared gas analyser (LI-COR 6400, Lincoln, NE, USA). For each sampling date, 6 days were required to make the measurements. Plants were randomized with respect to time over the six measurement days. Light response (A/PPFD) curves and the response of assimilation to intercellular CO2 concentration (A/Ci curves) were conducted before exposing the plants to the low-temperature treatment (day 0), immediately after 10 days of exposure (day 10) and 5 days after the low-temperature treatment had finished (recovery) on both control and low-temperature-exposed plants. The average VPD used during the measurements varied from 1.37 to 1.55 kPa.

A/PPFD curves were measured starting at a PPFD of 2000 μmol photon m⁻² s⁻¹, and then, the values decreased in eleven steps (2000, 1500, 1000, 750, 500, 300, 150, 100, 50, 25, 0) to complete darkness following the protocol recommended by LI-COR. A nonrectangular hyperbola (Long & Hällgren, 1993) was fitted to the eleven data points for each leaf in order to calculate the following parameters: apparent quantum yield of photosynthesis determined from the initial slope of the light response curve (φCO2), light-saturated assimilation rate obtained from the asymptote of the upper part of the curve (Amax), and the dark respiration rate (Rd).

To measure the A/Ci curves, the PPFD was maintained at a constant level of 2000 μmol photon m⁻² s⁻¹ and an external CO2 concentration (Ca) set at 400 μmol CO2 m⁻² s⁻¹. After a stable reading of A was reached, Ca was decreased in six steps (400, 300, 200, 100, 50, 0) to 0 μmol CO2 m⁻² s⁻¹ and then returned to 400 μmol CO2 m⁻² s⁻¹ and increased stepwise (600, 800, 1000, 1200, 1600, 2000) to 2000 μmol CO2 m⁻² s⁻¹ for a total of 12 steps. Values of the in vivo capacity for phospho-
enolpyruvate (PEP) carboxylation ($V_{\text{pmax}}$) and the CO$_2$ saturated rate ($V_p$), determined by the capacity for PEP regeneration and leakage, were calculated from these curves using the method described by Bellasio et al. (2015). After 10 days of exposure to low temperatures, only values for $V_{\text{pmax}}$ for one genotype were calculated (Blackwell), as photosynthesis was reduced to such an extent for the remaining twelve that the model could not be reliably fitted to the data.

Chlorophyll fluorescence measurements were made on the adaxial surface of leaves using a fluorometer (FMS 2, Hansatech, Norfolk, UK) and coincided with the gas exchange measurements. The maximum quantum efficiency of photosystem II (Fv/Fm) was calculated from fluorescence parameters after dark adaptation of the leaves for 20 min.

**Statistical analysis**

Linear regression analysis and one-way analysis of variance were performed in spss (IBM Corp, Armonk, NY, USA) to assess statistical differences for the studied parameters. Where a significant difference was found, the Levene’s test was used to check for homogeneity of variances and the Tukey-B post hoc test used to further study those differences.

**Results**

After 10 days of exposure to low temperature, there were significant genotype-related reductions in photosynthesis at saturating light levels ($A_{\text{sat}}$). Blackwell showed the smallest reduction (58%), whereas the rest of the genotypes showed much larger reductions of between 87 and 110%. Stomatal conductance (gs) also decreased drastically for all genotypes (Fig. 1). From the thirteen genotypes studied, only four (EG1101, Kanlow, Shawnee and Sunburst) showed a reduction in Fv/Fm. The ratio of intercellular to external CO$_2$ concentration (Ci/Ca) increased in all genotypes, but one (Blackwell) showed a strong negative correlation ($R^2 = 0.75$, $P < 0.001$) with the recorded Fv/Fm values. In contrast to the photosynthetic and fluorescence measurements, 10 days of low-temperature exposure had much smaller effects on the dark respiration rate ($R_d$), with only three genotypes (Cave-in-Rock, Shelter and Trailblazer) showing a significant reduction in $R_d$ (Table 1). Data from day 0 (not shown) showed no statistically significant differences with the control plants at day 10.

After allowing the plants to recover in the greenhouse for 5 days (day 15), all the measurements were repeated (Fig. 2). Recovery of $A_{\text{sat}}$ based on control plant values, after low-temperature exposure, occurred in the major-
Table 1  Apparent quantum yield ($\Phi_{\text{CO}_2\text{max}}$), dark respiration rate ($R_d$), in vivo capacity for phosphoenolpyruvate (PEP) carboxylation ($V_{\text{pmax}}$) and $\text{CO}_2$ saturated rate-PEP regeneration ($V_p$) for the control and low-temperature-exposed plants of the thirteen switchgrass genotypes at days 10 and 15 (recovery). Within each genotype, statistical differences are indicated by different letters ($P < 0.05$). Due to major reductions in photosynthesis, with negative values for some of the genotypes, some $V_{\text{pmax}}, \Phi_{\text{CO}_2\text{max}}$ and $V_p$ values could not be estimated for the low-temperature treatment at day 10.

| Genotype       | Parameter | Day | Treatment | Alamo | Cimarron | EG1101 | EG1102 | Kanlow | Blackwell | Carthage | Cave in Rock | EG2101 | Shawnee | Shelter | Sunburst | Trailblazer |
|----------------|-----------|-----|-----------|-------|----------|--------|--------|--------|-----------|----------|-------------|--------|---------|---------|----------|-------------|
| $\Phi_{\text{CO}_2\text{max}}$ | 10        | Control | 0.056 (±0.004) a | 0.058 (±0.010) a | 0.043 (±0.001) a | 0.062 ±0.006 a | 0.055 ±0.002 a | 0.046 (±0.001) a | 0.051 (±0.007) a | 0.052 (±0.001) a | 0.055 (±0.009) a | 0.045 (±0.001) a | 0.054 (±0.001) a | 0.046 (±0.005) a | 0.022 a |
| $R_d$ (µmol m$^{-2}$ s$^{-1}$) | 10        | Control | 1.24 ±0.131 a | 1.73 ±0.42 a | 2.54 ±0.28 a | 2.33 ±0.66 a | 2.00 ±0.27 a | 1.93 ±1.10 a | 2.72 ±0.93 b | 3.02 ±0.78 a | 3.01 ±2.78 a | 2.29 ±2.29 a | 2.58 ±2.03 a | 1.55 ±0.15 a | 1.46 ±0.31 a |
| $V_{\text{pmax}}$ (µmol m$^{-2}$ s$^{-1}$) | 10        | Control | 39 (±5.5) ab | 81.5 ±8.5 a | 34 (±5.8) a | 24 (±9.8) a | 95 ± (±4.0) a | 57 ± (±6.0) a | 94 ± (±16) a | 52 ± (±6.0) a | 23 ± (±6.3) a | 63.5 ±50.2 a | 92 ±48 a | 54 ±68.5 a |
| $\text{CO}_2$ saturated rate-PEP regeneration ($V_p$) (µmol m$^{-2}$ s$^{-1}$) | 10        | Control | 23.2 (±6.1) a | 26.2 (±6.1) a | 20.45 (±6.2) a | 23.9 (±6.2) a | 25.6 (±6.2) a | 26.8 ±3.4 a | 31.9 ±4.9 a | 15.9 (±6.0) a | 17.1 ±3.9 a | 21.7 ±3.9 a | 24.2 ±1.7 b | 19.35 ±27 b |
|                | 15        | Low temperature | 15.8 (±2.2) a | 24.7 (±3.1) a | 18.4 (±3.6) a | 22.8 (±5.7) a | 25.6 (±0.1) a | 27 ±3.3 a | 30.1 ±4.0 a | 21.9 ±3.5 a | 17.8 ±2.5 a | 19.2 ±2.5 a | 26.6 ±1.7 b | 16.9 ±28.9 b | 15.8 ±15.8 a |

© 2016 The Authors. Global Change Biology Bioenergy Published by John Wiley & Sons Ltd., 1.44-1.52
ity of varieties examined (Fig. 2a). Values of gs in low-temperature-exposed plants also increased during the recovery period, and only in two cases (Alamo and Blackwell) were statistically significant differences found, compared with the control plants (Fig. 2b). No significant differences were observed for Ci/Ca (except for EG2101) and Fv/Fm values (Fig. 2c,d).

In four of the varieties (Alamo, EG1101, Cave-in-Rock and Sunburst), φCO₂max values during recovery were lower for plants that were exposed to low temperatures (Table 1). Due to major reductions in photosynthesis, with negative values for some of the genotypes, twelve Vpmax values could not be estimated for the low-temperature treatment at day 10 (Table 1) and the model was only fitted when there were at least three reliable data points for calculating Vpmax and Vp. During recovery, seven genotypes showed a reduction in Vpmax values, whereas only three genotypes exhibited a decrease in Vp.

Significant effects of the low-temperature exposure on dry biomass yields were found in nine of the varieties (Fig. 3a), although Sunburst, EG2101, Shawnee and Kanlow produced the same amount of biomass in both control and low-temperature-treated plants. Overall, lowland ecotypes showed higher biomass production (control = 45.62 ± 2.2 g/plant, low temperature = 29.92 ± 4.7 g/plant) even after low-temperature exposure, compared with the upland ecotypes (control = 29.83 ± 3.6 g/plant, low temperature = 16.6 ± 3.1 g/plant), with a reduction in the yield of 34.4% for lowland ecotypes and 44.3% for upland ecotypes after exposing the plants to low temperatures. Total leaf area (Fig. 3b) was reduced in seven of the varieties after low-temperature exposure, with similar values for Alamo, Cimarron, EG1101, Kanlow, EG2101 and Sunburst. Significant reductions in leaf number were found (Fig. 3c) that paralleled the reductions in final biomass and leaf area for most of the varieties examined, although Alamo showed a reduction in yield that was not coupled with a reduction in leaf area.

**Discussion**

The main objective of the study was to assess variations in the impact of exposure to low temperatures on leaf photosynthesis in a range of switchgrass genotypes, as well as their ability to recover after the stress was removed and how this impacted on the final biomass yield. Whilst the approach used was primarily focused on screening for genetic variation in a large number of genotypes, a 10-day exposure period during early development and subsequent recovery may have some relevance to field situations as episodic low-temperature events are often a common occurrence during the early
establishment and growth of bioenergy crops in the spring (Friesen et al., 2015; Sage et al., 2015). Perennial species such as switchgrass can benefit from early emergence (Verdú & Traveset, 2005), but there is also evidence that the early establishment phase is a critical period in terms of achieving maximum biomass production (West & Kincer, 2011), partly due to the sensitivity of vegetative growth to temperature (Sanderson & Wolf, 1995). Whilst the majority of genotypes showed marked reductions in photosynthesis after exposure to low temperatures remarkably, Carthage and particularly Blackwell showed significant rates of photosynthesis after the treatment period. This indicates that an ability to maintain photosynthesis at low temperatures may not be a unique feature of the C4 bioenergy species miscanthus (Sage, 1999; Liu & Osborne, 2008; Glowacka et al., 2015b).

The elevated Ci/Ca values, combined with reductions in $V_p$ and $V_{pmax}$, suggest that the impact of low temperatures on photosynthesis is due to reductions in both PEP carboxylase activity and regeneration, although this may be confounded by the effects of CO$_2$ leakage from the bundle sheath cells (Głowacka et al., 2015b). The inverse correlation between Fv/Fm and Ci/Ca found in our study could point to PEP regeneration being the more important factor, given that this relies on photochemically generated ATP. Although there is a large body of evidence indicating a significant impact of low temperatures on a range of carbon assimilating enzymes, including PEP carboxylase (Kingston-Smith et al., 1997; Yamori et al., 2014), PEP regeneration may be the key limitation to photosynthesis in C4 plants under these conditions (Naidu & Long, 2004; Wang et al., 2008; Glowacka et al., 2015b).

There is some uncertainty, however, about the validity of Ci/Ca values $\gg$1 in the light given that it may be difficult to accurately determine Ci values when photosynthesis and stomatal conductance are significantly depressed. A positive relationship between $R_d$ and Ci/Ca ($R^2 = 0.27$, $P = 0.007$, data not shown) could indicate that this is due to higher respiration rates. This is also supported by the correlation between a higher $R_d$ (Table 1) and a significantly higher value for Ci/Ca (Fig. 2c) for the EG2101 genotype during the recovery period of 5 days after low-temperature exposure. For Blackwell, values for Ci/Ca were smaller in low-temperature-exposed plants at day 10 when compared to control plants. Photosynthesis and stomatal conductance for this variety were not as affected as for most of the other genotypes, and Blackwell was also one of the ecotypes that did not recover its assimilation rate fully after 5 days, possibly indicating a greater resistance to low temperatures but a slower ability to acclimate to the changed conditions after low-temperature exposure.
There is also a possibility that photoinhibition contributed to reductions in photosynthesis after plants previously exposed to low temperatures were transferred to ambient light conditions (Long, 1983), but the relatively small decrease in Fv/Fm may indicate that any impact was small. Full recovery of the assimilation rates and Fv/Fm in comparison with control plants after 5 days also indicated that there was no permanent impact of the low-temperature exposure.

Contrary to expectation, there was no clear difference overall in the temperature sensitivity of upland and lowland ecotypes, although the most resistant genotype (Blackwell) was an upland ecotype. Although upland genotypes have also been described to have inherently lower assimilation rates than lowland genotypes in the field (Wullschleger et al., 1996), there were no significant differences in A sat between upland (22.7 ± 1.08) and lowland (20.2 ± 1.46) ecotypes in this study for the control treatments and no evidence for any clear separation based on low temperature tolerance. It is therefore possible that the greater low temperature tolerance of upland ecotypes is related more to developmental differences than inherent differences in low temperature tolerance. This is supported by evidence that northern switchgrass populations grown at southern latitudes mature faster than those found in southern regions (Casler et al., 2007), although they may not be intrinsically more tolerant to low temperatures and further experiments under natural conditions would be required to verify this proposal.

Whilst many studies have focussed on leaf-level photosynthetic responses as an indicator of the final biomass yield after exposure to low temperatures, or other environmental constraints, the link between these has always been a matter of debate (Sanderson et al., 1996; Harrington et al., 2004; Kruger & Volin, 2006; Long et al., 2006). Ultimately, however, it is the impact of low temperatures on the final biomass yield that is of most interest in terms of the identification of suitable genotypes or species as biomass/bioenergy crops. In the current study, differences in leaf-level photosynthesis did not explain variation in final biomass production in either control or low-temperature-exposed plants. Kanlow, which showed marked reductions in all leaf-level photosynthetic parameters, had a final biomass yield that was unaffected by low-temperature exposure. Sunburst, which had one of the lowest final biomass values, had photosynthetic rates and Fv/Fm values comparable to the higher yielding genotypes such as Alamo and EGI101. Even reductions in $\Phi_{\text{CO}_2}$ max which are often indicative of an intolerance to low temperatures (Long & Spence, 2013) and are related to damage to PSII or to mechanisms associated with damage avoidance (Głowacka et al., 2015b), did not scale with final yield. The long-term impact of the low-temperature treatment on the final yield varied between −1.4% (Kanlow) and 70.4% (Blackwell). Calculation of the impact of a 10-day period, where there was little or no carbon gain, indicated that this cannot account for the 60–70% reduction in the final biomass as observed for Blackwell and Cave-in-Rock. What appears to be more important is the long-term impact on leaf area development, which was correlated with the final biomass yield both in control plants and in plants subjected to low temperatures (Fig. 4). A strong relationship between canopy development and final yields has been previously reported for switchgrass (Redfearn et al., 1997). Factoring leaf photosynthesis into this correlation through the product of photosynthesis times leaf area did not improve the relationship indicating that higher rates of leaf photosynthesis are offset by reductions in leaf area per plant. However, other factors in addition to leaf area per se could contribute to final biomass production, including leaf architecture/leaf display and canopy duration, as well as source–sink balance.

Examination of the underlying reasons for differences in leaf area indicated that this was due to variations in leaf number and not leaf size. The generally lower final biomass after low-temperature exposure was therefore due to lower leaf numbers (Fig. 3c), and any variations were due to differences in the effects of low temperature on the initiation of new leaves. The low-temperature sensitivity of leaf initiation and leaf emergence has previously been reported in a number of maize varieties (Padilla & Otegui, 2005) and grass species (Peacock, 1976; Duru & Ducrocq, 2000; Berone et al., 2007). These
results suggest that low-temperature-related limitations on meristematic activity associated with leaf initiation and emergence (Berone et al., 2007) could significantly constrain the yield of biomass crops. Although there was some evidence of increases in leaf area in Kanlow (Fig. 3b), these values were not significantly different from control plants. However, the evidence that in some genotypes leaf initiation may be relatively more resistant to low temperatures (Alamo, EG1101, Kanlow, EG2101 and Sunburst) suggests that this could be a target for plant breeding. An ability to maintain leaf area/leaf number could compensate for the period where there was little or no carbon gain.

In conclusion, this work indicates significant variation in the leaf-level photosynthetic responses of switchgrass genotypes to low-temperature exposure. Whilst the observed variation in photosynthesis was significant and might have some potential for enhancing the performance of switchgrass, the absence of a direct link with the final biomass yield may not result in the expected gains in plant productivity. Based on our results, more attention should perhaps be directed at leaf initiation and canopy development as a more direct way of increasing the productivity of switchgrass for its wider exploitation as a bioenergy crop. The fact that canopy characteristics, particularly leaf area duration, rather than leaf-level photosynthetic traits better explained the superior performance of Miscanthus over maize (Dohleman & Long, 2009) suggests that such an approach may be applicable to other putative bioenergy crops.

Acknowledgements

This work was funded by the FP7 OPTIMA project ‘Optimisation of Perennial Grasses for Biomass production (Grant Agreement 289642)’.

References

Bellasio C, Boerling Df, Griffiths H (2015) Deriving C4 photosynthetic parameters from combined gas exchange and chlorophyll fluorescence using an Excel tool: theory and practice. Plant, Cell & Environment. doi: 10.1111/pce.12626.

Berone GD, Lattanzi FA, Colabelli MR, Agnusdei MG (2007) A comparative analysis of the temperature response of leaf elongation in Bromus stamineus and Lolium perenne plants in the field: Intrinsic and size-mediated effects. Annals of Botany, 100, 813–820.

Cai X, Zhang X, Wang D (2010) Land availability for biofuel production. Environmental Science & Technology, 45, 334–339.

Casler MD, Vogel KP, Taliaferro CM et al. (2007) Lattitudinal and longitudinal adaptation of switchgrass populations. Crop Science, 47, 2249–2260.

Dohleman FG, Long SP (2009) More productive than maize in the midwest: how does Miscanthus do? Plant Physiology, 150, 2104–2115.

Duru M, Ducrocq H (2000) Growth and senescence of the successive grass leaves on a tiller. Ostoegonic development and effect of temperature. Annals of Botany, 85, 635–643.

Friesen PC, Peixoto MM, Lee DK, Sage RF (2015) Sub-zero cold tolerance of Spartina pectinata (prairie cordgrass) and Miscanthus × giganteus: candidate bioenergy crops for cool temperate climates. Journal of Experimental Botany, 66, 4403–4413.

Glowa ska K, Ahmed A, Sharma S, Abbott T, Comstock JC, Long SP, Sacks EJ (2015a) Can chilling tolerance of C4 photosynthesis in Miscanthus be transferred to sugarcane? GCB Bioenergy, 8, 407–418.

Glowa ska K, Jørgensen U, Kjeldsen JB, Kerpuk K, Spitz I, Sacks EJ, Long SP (2015b) Can the exceptional chilling tolerance of C4 photosynthesis found in Miscanthus × giganteus be exceeded? Screening of a novel Miscanthus Japanese germplasm collection. Annals of Botany, 115, 981–990.

Gu J, Yin X, Stomph T-J, Struik PC (2014) Can exploiting natural genetic variation in leaf photosynthesis contribute to increasing rice productivity? A simulation analysis. Plant, Cell & Environment, 37, 22–34.

Harrington RA, Fownes JH, Cassidy TM (2004) Japanese barberry (Berberis thunbergii) in forest understory: leaf and whole plant responses to nitrogen availability. The American Midland Naturalist, 151, 206–216.

Heaton E, Voigt T, Long SP (2004) A quantitative review comparing the yields of two candidate C4 perennial biomass crops in relation to nitrogen, temperature and water. Biomass and Bioenergy, 27, 21–30.

Hope HJ, McElroy A (1990) Low-temperature tolerance of switchgrass (Panicum virgatum L.). Canadian Journal of Plant Science, 70, 1091–1096.

Kingston-Smith AH, Harbison J, Williams J, Foyer CH (1997) Effect of chilling on carbon assimilation, enzyme activation, and photosynthetic electron transport in the absence of photo-inhibition in maize leaves. Plant Physiology, 114, 1039–1046.

de Koff JP, Tyler DD (2012) Improving switchgrass yields for bioenergy production. Cooperative Extension Faculty Research, 40, 2–8.

Kruger EL, Volin JC (2006) Reexamining the empirical relation between plant growth and leaf photosynthesis. Functional Plant Biology, 33, 421–429.

Lemeziene N, Nokrekienevi E, Liutakas Z, Dabkeviciene G, Cecceviene J, Butkute B (2015) Switchgrass from North Dakota – an adaptable and promising energy crop for northern regions of Europe. Acta Agriculturae Scandinavica, Section B - Soil & Plant Science, 65, 118–124.

Lemus R, Lal R (2005) Bioenergy crops and carbon sequestration. Critical Reviews in Plant Sciences, 24, 1–21.

Lewandowski I, Clifton-Brown JC, Srclocickimo, Huisman W (2000) Miscanthus: European experience with a novel energy crop. Biomass and Bioenergy, 19, 209–227.

Lewandowski I, Srclocickimo, Lindvall E, Christou M (2003) The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. Biomass and Bioenergy, 25, 335–361.

Liu M-Z, Osborne CP (2008) Leaf cold acclimation and freezing injury in C3 and C4 grasses of the Mongolian Plateau. Journal of Experimental Botany, 59, 4161–4170.

Long SP (1983) C4 photosynthesis at low temperatures. Plant, Cell & Environment, 6, 345–363.

Long SP, Hallgren JE (1993) Measurement of CO2 assimilation by plants in the field and the laboratory. In: Photosynthesis and Production in a Changing Environment: A field and laboratory manual (eds Hall DO, Srlocickimo, Bolhar-Nordenkampf HR, Leegood RC, Long SP), pp. 129–179. Chapman and Hall, London.

Long SP, Spence AK (2013) Toward cool C4 crops. Annual Review of Plant Biology, 64, 701–722.

Long SP, Zhu XG, Naidu SL, Ort DR (2006) Can improvement in photosynthesis increase crop yields? Plant, Cell & Environment, 29, 315–330.

Ma Y, An Y, Shui J, Sun Z (2011) Adaptability evaluation of switchgrass (Panicum virgatum L.) cultivars on the Loess Plateau of China. Journal of Plant Sciences, 181, 638–643.

McKendry P (2002) Energy production from biomass (part 1): overview of biomass. Bioresource Technology, 83, 37–46.

Mitchell LB, Halter M, Stewart CN Jr, Nilsen ET (2015) Switchgrass from North Dakota: an adaptable and promising energy crop for northern regions of Europe. Acta Agriculturae Scandinavica, Section B - Soil & Plant Science, 65, 118–124.

Naidu S, Long S (2004) Potential mechanisms of low-temperature tolerance of C4 photosynthesis in Miscanthus × giganteus: an in vivo analysis. Planta, 220, 145–155.

Padilla JM, Ortegui ME (2005) Co-ordination between leaf initiation and leaf appearance in field-grown maize (Zea mays): genotypic differences in response of rates to temperature. Annals of Botany, 96, 997–1007.

Peacock JM (1976) Temperature and leaf growth in four grass species. Journal of Experimental Botany, 27, 225–232.

Redfearn DD, Moore KJ, Vogel KP, Waller SS, Mitchell RB (1997) Canopy architecture and morphology of switchgrass populations differing in forage yield. Agronomy Journal, 89, 262–269.

Sage RF (1999) Why C4 photosynthesis? In: Annual Review of Plant Biology (eds Sage RF, Monson RK), pp. 3–16. Academic Press, San Diego, CA, USA.

Sage RF, De Melo Peixoto M, Friesen P, Deen B (2015) C4 bioenergy crops for cool climates, with special emphasis on perennial C4 grasses. Journal of Experimental Botany, 66, 4195–4212.
Sanderson MA, Wolf DD (1995) Morphological development of Switchgrass in diverse environments. Agronomy Journal, 87, 908-915.

Sanderson MA, Reed RL, McLaughlin SB et al. (1996) Switchgrass as a sustainable bioenergy crop. Bioresource Technology, 56, 83-93.

Sims REH, Mabee W, Saddler JN, Taylor M (2010) An overview of second generation biofuel technologies. Bioresource Technology, 101, 1570-1580.

Strijker D (2005) Marginal lands in Europe—causes of decline. Basic and Applied Ecology, 6, 99-106.

Stroup JA, Sanderson MA, Muir JP, McFarland MJ, Reed RL (2003) Comparison of growth and performance in upland and lowland switchgrass types to water and nitrogen stress. Bioresource Technology, 86, 65-72.

Verdú M, Traveset A (2005) Early emergence enhances plant fitness: a phylogenetically controlled meta-analysis. Ecology, 86, 1385-1394.

Wang D, Portis AR, Moose SP, Long SP (2008) Cool C4 photosynthesis: pyruvate Pi dikinase expression and activity corresponds to the exceptional cold tolerance of carbon assimilation in Miscanthus x giganteus. Plant Physiology, 148, 557-567.

West DR, Kincer DR (2011) Yield of switchgrass as affected by seeding rates and dates. Biomass and Bioenergy, 35, 4057-4059.

Wiegmann K, Hennenberg KJ, Fritsche UR (2008) Degraded Land and Sustainable Bioenergy Feedstock Production. Joint International Workshop on High Nature Value Criteria and Potential for Sustainable Use of Degraded Lands, Paris.

Wullschleger SD, Sanderson MA, McLaughlin SB, Biradar DP, Rayburn AL (1996) Photosynthetic rates and ploidy levels among populations of switchgrass. Crop Science, 36, 306-312.

Yamori W, Hikosaka K, Way DA (2014) Temperature response of photosynthesis in C3, C4, and CAM plants: temperature acclimation and temperature adaptation. Photosynthesis Research, 119, 101-117.