C₄ bioenergy crops for cool climates, with special emphasis on perennial C₄ grasses

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Abstract

There is much interest in cultivating C₄ perennial plants in northern climates where there is an abundance of land and a potential large market for biofuels. C₄ feedstocks can exhibit superior yields to C₃ alternatives during the long warm days of summer at high latitude, but their summer success depends on an ability to tolerate deep winter cold, spring frosts, and early growth-season chill. Here, we review cold tolerance limits in C₄ perennial grasses. Dozens of C₄ species are known from high latitudes to 63 °N and elevations up to 5200 m, demonstrating that C₄ plants can adapt to cold climates. Of the three leading C₄ grasses being considered for bioenergy production in cold climates—Miscanthus spp., switchgrass (Panicum virgatum), and prairie cordgrass (Spartina pectinata)—all are tolerant of cool temperatures (10–15 °C), but only cordgrass tolerates hard spring frosts. All three species overwinter as dormant rhizomes. In the productive Miscanthus x giganteus hybrids, exposure to temperatures below –3 °C to –7 °C will kill overwintering rhizomes, while for upland switchgrass and cordgrass, rhizomes survive exposure to temperatures above –20 °C to –24 °C. Cordgrass emerges earlier than switchgrass and M. giganteus genotypes, but lacks the Miscanthus growth potential once warmer days of late spring arrive. To enable C₄-based bioenergy production in colder climates, breeding priorities should emphasize improved cold tolerance of M. ×giganteus, and enhanced productivity of switchgrass and cordgrass. This should be feasible in the near future, because wild populations of each species exhibit a diverse range of cold tolerance and growth capabilities.

Key words: Biofuels, chilling, cold tolerance, cordgrass, frost tolerance, Miscanthus, Panicum virgatum, switchgrass, Spartina pectinata.

Introduction

C₄ photosynthesis is the most productive photosynthetic pathway in warm to hot climates, due to its ability to concentrate CO₂ around Rubisco and suppress photorespiration. Because of this, the most productive crops on Earth are C₄ species, with peak biomass yields and radiation conversion efficiencies that are twice those of the most productive C₃ crops (Monteith, 1978; Snaydon, 1991; Long, 1999; Sheehy et al., 2007; Zhu et al., 2008). The superior productivity enabled by the C₄ pathway allows sugar cane, maize, and sorghum to dominate the list of existing, first-generation bioenergy crops, while perennial C₄ grass species such as those in the Miscanthus genus (M. sinensis, M. sacchariflorus, and their hybrid M. ×giganteus, or Mxg), switchgrass (Panicum virgatum), and Napier grass (Pennisetum purpureum) lead the list of second-generation bioenergy crops (Samson et al., 2005; Jones, 2011). In cool climates, C₄ photosynthesis is often thought to be maladapted, due to frequent chilling injury, low productivity, and inferior quantum yields compared with C₃ vegetation (Ehleringer, 1978; Berry and Björkman, 1980; Long, 1983). Consistently, there is a large drop in the contribution of C₄ photosynthesis to biomass production and regional floras as one goes from warmer (low latitude, low
elevation) to cooler climates (mid- to high latitude or high elevation; Teeri and Stowe, 1976; Sage et al., 1999). C₄ plants are uncommon in environments where the mean minimum temperature of the mid-growing season is <8–10 °C, which generally corresponds to latitudes above ~45 ° and USDA plant hardiness zones of ≤4 (Teeri and Stowe, 1976; Hattersley, 1983; Sage et al., 1999; Wan and Sage, 2001). This observation has been interpreted to indicate that C₄ plants cannot become major contributors to bioenergy feedstocks in northern climates, where much of the Earth’s landmass is present, and where the market for heat resources is greatest.

Like many paradigms, the idea that C₄ photosynthesis is maladapted for cold climates was generated by the initial wave of physiological and ecological studies that emphasized common patterns over exceptions that call the paradigm into question. With hindsight, the paradigm of C₄ cold sensitivity is overly simplistic. Most C₄ plants are indeed intolerant of cold, but not because they use C₄ photosynthesis; instead, they lack cold tolerance because they originated in warm climates and thus are generally maladapted to cold, as are C₃ species from warm climates of low latitude (Edwards and Still, 2008; Sage et al., 2012; Long and Spence, 2013). It is now recognized that C₄ photosynthesis per se does not exclude plants from cold climates, as there are hundreds of C₄ species that survive bitterly cold winters and episodic summer chill, and dozens have been identified growing in perennially cold climates such as foggy, cool temperate coastlines and alpine tundra (Long et al., 1975; Long, 1983, 1999; Schwarz and Redmann, 1988; Schwarz and Reaney, 1989; Sage et al., 1999, 2011; Pittermann and Sage, 2000, 2001; Sage and Sage, 2002; Long and Spence, 2013). At a leaf level, C₄ photosynthesis is generally inferior to C₃ photosynthesis at cooler temperatures (<15 °C; Sage et al., 2011). Despite this limitation, there are opportunities for superior C₄ performance, even in alpine ecosystems or at the relatively high, boreal latitudes of interior Canada and Eurasia (Pittermann and Sage, 2000, 2001; Sage and Sage, 2002; Kubien and Sage, 2003). For example, long, warm summer days of the boreal landscape can offset the short growing season and cooler temperatures at the beginning and end of a high-latitude growing season (Kubien and Sage, 2003, 2004a, b). While there are physiological mechanisms that predispose C₄ plants to have inferior performance relative to C₃ plants at cooler temperatures (Long, 1983; Sage, 2002), some C₄ species have evolved mechanisms that compensate for these liabilities, and thus can persist in cold environments (Schwarz and Redmann, 1988; Pittermann and Sage, 2000, 2001; Sage and Sage, 2002; Kubien and Sage, 2004a, b; Long and Spence, 2013). Humanity has also exploited C₄ plants to boost crop productivity in cool environments—the best examples are northern and high elevation races of maize and, more recently, Miscanthus, grown in Europe (Long, 1983; Goodman and Brown, 1988; Clifton-Brown et al., 2011). Miscanthus stands out because its chilling tolerance enables the superior C₄ photosynthetic potential to boost bioenergy production in cool temperate landscapes of Northern Europe, and potentially higher latitudes in Canada and Eurasia (Clifton-Brown et al., 2004; Long and Spence, 2013). Even in an unimproved state, Miscanthus routinely outperforms C₃ crops in temperate zone latitudes (Beale and Long, 1995; Heaton et al., 2010). While the cold tolerance and yield potential of Miscanthus photosynthesis are relatively well known (Long and Spence, 2013), the potential of other C₄ species to contribute genotypes, genes, or knowledge to the improvement of bioenergy production at high latitude is less clear.

If cold tolerance can be improved, exploitation of C₄ plants could provide new ways to enhance biomass production at latitudes of the cool temperate zone. Specifically, the regions that we envisage could be exploited by cold-tolerant C₄ perennial crops are the USDA hardiness zones 3–5 across North America, Europe, and Asia, which generally correspond to the sub-boreal to cool temperate regions (Fig. 1). USDA plant hardiness zones are based on winter minimum air temperatures, such that zones 3–5 would correspond to winter minimum temperatures of −23 °C to −40 °C (USDA, 2014). In addition, C₄ biofuel production should be feasible in cool climates of north-central and northwestern Europe that fall within plant hardiness zones 6–9 based on winter minimum temperatures (Fig. 1B; Clifton-Brown et al., 2008). Cold-tolerant C₄ photosynthesis could also contribute to biofuel production in the cold deserts of western North America and Central Asia, where high water use efficiency of the C₄ pathway could allow for commercial production of woody C₄ species in an agroforestry setting (see ovals in Fig. 1; Supplementary Fig. S1 available at JXB online).

Together, these landscapes account for a significant fraction of Earth’s landmass, and are often near population and industrial centres that could become significant markets for bioenergy. Moreover, there is much marginal land in these areas, and a considerable fraction has been abandoned (Field et al., 2008). A strong rationale therefore exists to develop bioenergy production at higher latitude, and cold-tolerant C₄ grasses such as Miscanthus may be advantageous because of their relatively high productive potential in cool temperate climates (Beale and Long, 1995). Winter survival, however, and tolerance of spring cold are critical if perennial C₄ crops are to become important feedstocks for bioenergy in plant hardiness zones 3–5 (Clifton-Brown and Lewandowski, 2000; Farrell et al., 2006).

In this review, we evaluate critical temperatures for winter survival and spring success of the leading C₄ perennial grasses now in development for bioenergy cultivation in the temperate zone. These are the Miscanthus genotypes (M. sacchariflorus, M. sinensis, and their Mxg hybrids, or collectively, Miscanthus), switchgrass (Panicum virgatum), and prairie cordgrass (Spartina pectinata). In our assessment, we assess the state of knowledge in light of numerous recent studies, which in combination with older work provide a better understanding of the particular challenges facing C₄-based bioenergy production in cold climates.

Patterns of cold exposure

To persist in a cold environment, perennial plants must tolerate the many ways that cold exposure inhibits physiological performance (Fig. 2). For one, they must survive winter
cold in the dormant state, which involves a number of aspects (Farrell et al., 2006; Ruelland et al., 2009; Bykova and Sage, 2012; Gusta and Wisniewski, 2013). The first is to survive sub-zero temperatures, through either deep supercooling or tissue freezing. The second is they must survive freeze–thaw events that could de-acclimate overwintering tissues, and, thirdly, below-ground tissues need to survive cold, near-freezing conditions in wet soil, where anoxia and root rot could harm the overwintering tissue. To complicate matters further, there is a strong time dependence of hardening and dehardening that can influence the critical threshold temperatures for survival, and age may be an important factor (Zub et al., 2012; Gusta and Wisniewski, 2013). The rate of temperature decline can influence the degree to which tissues acclimate to low temperature, and first-year plants are more vulnerable to winter cold than older, more established plants (Clifton-Brown and Lewandowski, 2002; Rosser, 2012; Yan et al., 2012; Gusta and Wisniewski, 2013; Peixoto, 2015). Moreover, an ability to cold-harden effectively in autumn is required if a perennial feedstock is to survive severe winters (see criterion #7 in Fig. 2; Ruelland et al., 2009; Bykova and Sage, 2012).

At higher latitudes, a biofuel crop must be able to exploit the long photoperiods of late spring and early summer, which compensate for shorter growing seasons relative to
lower latitudes (Beale and Long, 1995; Farrell et al., 2006; Dohleman and Long, 2009; Dohleman et al., 2009; Głowiacka et al., 2014). Perennials are better able to exploit these long photoperiods than annuals because they can draw on carbohydrate and nutrient reserves stored in the rhizomes to support rapid canopy growth, whereas an annual crop must be planted and allowed to establish from seed (Samson et al., 2005; Dohleman and Long, 2009). To exploit the long photoperiods fully, rhizomes should break dormancy and begin new shoot growth by early to mid-spring (criterion #2 in Fig. 2). Early emergence, however, increases the risk of cold injury, and thus the newly emerged leaves must tolerate both frost and chilling exposure (criterion #3 in Fig. 2). Chilling conditions are defined here as low temperatures above the freezing point that cause injury, through, for example, photo-oxidation, membrane impairment, and dysfunction in gene expression (Buchanan et al., 2000; Naidu et al., 2003; Ruelland et al., 2009; Long and Spence, 2013). Throughout the growing season at higher latitudes, photosynthesis and growth can be slowed by below-par performance at ‘cool’ temperatures that are not low enough to cause chilling injury, but do reversibly slow photosynthetic capacity and growth (Sage et al., 2011). Thus, in addition to avoiding injury at low temperature, successful biofuel feedstocks need to maintain significant growth and photosynthesis rates at suboptimal temperatures in order to support rapid establishment of the leaf canopy (criterion #4 in Fig. 2), and to remain productive during episodic cold days of summer (criterion #5, Fig. 2). For C₄ plants in particular, the rapid, early-season growth is essential if they are to have a closed canopy in place to exploit fully the long, warm days of summer, when the C₄ advantage is greatest. Above 25 °C, C₄ species outperform potential C₃ alternatives in terms of maximum photosynthetic capacity, and the efficiency of light, water, and nitrogen use (Beale and Long, 1995; Ehleringer et al., 1997; Zhu et al., 2008; Soltani and Sinclair, 2012). As an example, an ability to close the canopy by late May is considered to be a key advantage of the productive Mxg in temperate zone landscapes (Dohleman and Long, 2009; Long and Spence, 2013). Maize, in contrast, has a greater photosynthetic capacity than Mxg in Illinois, but cannot close its canopy until later June and thus has lower yields (Dohleman and Long, 2009). C₃ crops can establish in early spring, but lack the productive potential of C₄ crops such as Mxg during the warmest part of the year, and thus tend to have lower yields in temperate climates with warm summers (Lewandowski et al., 2003; Samson et al., 2005; Byrt et al., 2011).

With the arrival of autumn chill, perennial grasses need to senesce their shoots properly and translocate the resident nutrients into below-ground rhizomes (criterion #6 on Fig. 2). This is particularly important in a bioenergy context because recycling of nutrients lowers fertilizer costs and allows the plants to remobilize the stored reserves to support rapid growth in the spring (Jørgensen, 1997; Heaton et al., 2010). Ideally, senescence would be timed to maximize the growing season length without exaggerating the probability that pre-senesced tissues will be killed by freezing temperatures. Effective senescence improves the combustion quality of biomass because it reduces ash content and nitrous oxide emissions (Jørgensen, 1997; Heaton et al., 2010; Kludze et al., 2013). Recycling of shoot nutrients also eliminates the need to recover nutrients from the soil, which is expensive for the plant, and reduces fertility decline should nutrients be leached from decomposing litter into groundwater and surrounding waterways. As an example of this issue, we observed mortality of green leaves from two putatively cold-tolerant lines of Miscanthus that failed to senesce before a hard frost killed the canopy (Fig. 3). Nitrogen concentrations in the green-killed leaves were >2%, some 4–5 times greater than in leaves from Miscanthus varieties that fully senesced the leaf canopy. Leaf nitrogen content in a summer-active stand of Mxg is 2–3% (Dohleman et al., 2009; Heaton et al., 2010), indicating that ≥ 60% of the leaf nitrogen remained in the green-killed leaves.

**Cold-climate C₄ plants**

To illustrate the taxonomic diversity of cold tolerance in C₄ plants, we list the major C₄ species being considered for bioenergy production in cold climates, along with a sampling of wild species from high altitudes or elevations (Table 1). Among these are the grass *Oryzopsis thyroldi*, the world’s highest-elevation C₄ species that has been collected in Tibet at 5200 m, and *Gomphrena meyeniana*, the world’s highest dicot that has been collected at 4600 m in the Andes (Wang et al., 2004; Sage et al., 2011). These and other ultrahigh-elevation C₄ plants are not fast-growing species, and often exhibit a tundra growth form with a low leaf canopy and large, belowground organs (Sage and Sage, 2002; Sage et al., 2011). In these high-elevation locations, night temperatures can dip >10 °C below freezing, while day temperatures can exceed 25 °C and thus favour C₄ photosynthesis (Sage and Sage, 2002). Although these species are not candidates for bioenergy production given their growth form, they could provide specific genes for improving cold tolerance in productive C₄ bioenergy crops. They also provide some understanding of how C₄ species perform in cold climates, as has been demonstrated by *Muhlenbergia* species from the mountains and boreal fens of western North America (Pittermann and Sage, 2001; Sage and Sage, 2002; Kubien and Sage, 2003, 2004a, b).

*Wild C₄ species: the Muhlenbergia case study and the value of Setaria viridis*

Above 3500 m, temperatures frequently drop below freezing, and snow and frost are common during the growing season (Körner, 2003). Despite these challenges, numerous species of the grass genus *Muhlenbergia* of the Chloridoideae subfamily occur above 4000 m, including *Mu. perwiana*, which is the highest C₄ grass in the Western Hemisphere, growing to at least 4800 m in the Andes, and *Mu. richardsonis*, which grows to near 4000 m in the White Mountains of California (Table 1; Sage et al., 2011). *Muhlenbergia* species are also noted for being among the most northern of C₄ species (*Mu. glomerata* and *Mu. richardsonis*), occurring up to 60 °N in Canada, and the earliest sprouting C₄ species (*Mu. sobolifera*) which begins growth in March in north temperate habitats.
(Schwarz and Redmann, 1988; Smith and Wu, 1994; Kubien and Sage, 2003). In these locations, the various Muhlenbergia species can be common rather than rare; for example, *Mu. richardsonis* can form extensive swards in the high Sierra Nevada and White Mountains of California (Sage and Sage, 2002; Sage et al., 2011). Muhlenbergia thus stands out as one of the most cold-tolerant genera of *C₄* photosynthesis.

In its high tundra environment, *Mu. richardsonis* forms mats growing low to the ground, leading to its common name ‘mat’ Muhly. This name has functional significance, because it reflects a key adaptation to survival in the tundra environment. During clear days, high solar insolation warms leaf canopies if they are near the ground and protected from the wind. The low canopy of *Mu. richardsonis* remains in the surface boundary layer of the ground, where solar heat is trapped and leaves can warm above air temperatures by 10–15 °C in direct sunshine (Sage and Sage, 2002). Since summer air temperatures in its high alpine habitat are generally <15 °C, the solar heating allows it frequently to experience leaf temperatures exceeding 25 °C that are considered favourable to *C₄* photosynthesis. At its thermal optimum of 30–35 °C, *Mu. richardsonis* has photosynthesis rates that are more than double the rates observed in its *C₃* competitors, such as the grass *Koeleria macrantha* (Sage et al., 2011). This can offset the time during the day when the *Mu. richardsonis* leaves operate below 10 °C, which is when the *C₃* grasses have twice the photosynthesis rate of *Mu. richardsonis*.

The microsite distribution of *Mu. richardsonis* in the alpine environment indicates that exposure to direct sunshine is critical to its ability to persist in the alpine tundra. In the tundra zone, it primarily occurs in microsites where solar heat can be trapped within the surface boundary layer and warm the plants well above air temperature (Sage and Sage, 2002). Such microsites are those with good solar exposure on south-facing slopes, and some protection from the wind. However, by residing in microsites where it can trap solar heat and warm above air temperatures during the day, *Mu. richardsonis* is prone to cooling below air temperature at night. This is because, at night, thermal emission of infrared radiation from the plants to the thin alpine atmosphere cools the leaves below air temperature by up to 10 °C, allowing for frost to form on the leaves (Sage and Sage, 2002). This ‘radiation-induced’ frost persists after sunrise, such that the leaves are exposed to a full solar load in the frosted condition. Frost does not harm the leaves, and the plants are tolerant of subzero temperatures down to approximately –14 °C (based on electrolyte leakage tests), which is equivalent to the cold tolerance limits observed in nearby *C₃* grass species (Sage and Sage, 2002; R.F. Sage, unpublished observations).

*Muhlenbergia richardsonis* thus demonstrates a number of important points for improving cold tolerance in *C₄* breeding stock destined for cold climates. First, access to daytime heat during the growing season is important if the superior benefits of the *C₄* photosynthetic pathway are to be realized. This may mean developing varieties with a leaf canopy that can effectively retain the heat from the sun, or by growing crops in locations where solar input is naturally enhanced, for example, on south-facing slopes. Secondly, the *C₄* photosynthetic pathway is not inherently incompatible with cold conditions during the growing season and *C₄* species should be able to exhibit cold tolerance as great as that of any *C₃* species. Thirdly, the very high photon flux densities that are common at high elevation and which illuminate the *Muhlenbergia* leaves early in the morning do not cause irreparable harm; instead, the *C₄* leaves appear to be well protected against these high radiation loads, probably through carotenoid-based photoprotection as has been described in cold-active *C₃* species (Kubien and Sage, 2004a, b; Farage et al., 2006; Demmig-Adams et al., 2012).

Exploiting the genes conferring cold tolerance in *Muhlenbergia* and other cold-adapted *C₄* species may thus be a useful route to increase cold tolerance rapidly in the more productive bioenergy feedstocks, unless there are unavoidable trade-offs between cold tolerance and growth potential. Dissecting the genetics of cold tolerance and yield should therefore be a priority for developing *C₄* crops with *Miscanthus*-like productivity and the cold tolerance of hardy *C₄* plants such as *Mu. richardsonis* (Jones et al., 2015). Along these lines, *Setaria viridis*, a weedy relative of the *C₄* crop *Setaria italica*, could be a valuable resource in which to study the genetic controls governing cold tolerance in *C₄* plants, as

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Leaf N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M161 (Illinois) (senesced)</td>
<td>0.33A</td>
</tr>
<tr>
<td>M116 (Nagara) (senesced)</td>
<td>0.53A</td>
</tr>
<tr>
<td>M116 (Nagara) (killed pre-senescence)</td>
<td>2.06E</td>
</tr>
<tr>
<td>M118 (killed pre-senescence)</td>
<td>2.14B</td>
</tr>
</tbody>
</table>

![Fig. 3. Autumn-killed pre-senesced leaves of Miscanthus×giganteus (Mxg) variety M118 (4n; top left) and properly senesced leaves of Mxg variety ‘Illinois’ (3n; bottom left) collected in November 2009 at the New Energy Farms plantation at Learnington, Ontario (www.newenergyfarms.com/). Leaf nitrogen data from the leaves are shown on the right. Data for leaf nitrogen from a nearby Mxg variety Nagara (3n) on senesced leaves and pre-senesced leaves are shown for a comparison.](Image 164x626 to 346x769)
Table 1. Cold- and/or chilling-tolerant C₄ plants of the world (Wang et al., 2004; Sage et al., 2011)

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proven bioenergy feedstock in cold climates</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miscanthus giganteus (and relatives)</td>
<td>Poaceae</td>
<td>Native to East Asian highlands</td>
</tr>
<tr>
<td><strong>High potential as a cold-climate feedstock</strong></td>
<td></td>
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<tr>
<td><strong>Perennials</strong></td>
<td></td>
<td></td>
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<tr>
<td>Andropogon gerardii (big bluestem)</td>
<td>Poaceae</td>
<td>North American prairie grass</td>
</tr>
<tr>
<td>Haloxylon ammodendron and H. aphyllum</td>
<td>Chenopodiaceae</td>
<td>Central Asian Chenopod shrubs</td>
</tr>
<tr>
<td>Miscane</td>
<td>Poaceae</td>
<td>Hybrid of Miscanthus and sugar cane, can tolerate snowfall</td>
</tr>
<tr>
<td>Panicum virgatum (switchgrass)</td>
<td>Poaceae</td>
<td>North American Prairie grass</td>
</tr>
<tr>
<td>Spartina pectinata (prairie cordgrass)</td>
<td>Poaceae</td>
<td>High-latitude C₄ grass</td>
</tr>
<tr>
<td><strong>Annuals</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zea mays (maize)</td>
<td>Poaceae</td>
<td>High latitude (Newfoundland), and Andean varieties to &gt;3600 m</td>
</tr>
<tr>
<td>Setaria italica (foxtail millet)</td>
<td>Poaceae</td>
<td>High-latitude cultivation</td>
</tr>
<tr>
<td>Salsola kali (tumbleweed)</td>
<td>Poaceae</td>
<td>High latitude to 63 °N, and high altitude</td>
</tr>
<tr>
<td><strong>Model species for feedstock improvement</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Setaria viridis (green foxtail)</td>
<td>Poaceae</td>
<td>Genetic model for C₄ grasses; high-latitude and early-season weed</td>
</tr>
<tr>
<td><strong>Wild, cold-tolerant C₄ species of note</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>High-latitude grasses and sedges</strong></td>
<td></td>
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<tr>
<td>Cyperus longus</td>
<td>Cyperaceae</td>
<td>High-latitude weedy sedge, Europe and N. America</td>
</tr>
<tr>
<td>Distichilis spicata (saltgrass)</td>
<td>Poaceae</td>
<td>High-latitude salt marshes and beaches, N. America</td>
</tr>
<tr>
<td>Muhlenbergia glomerata</td>
<td>Poaceae</td>
<td>Boreal zone grass of fens, N. America</td>
</tr>
<tr>
<td>Spartina anglica</td>
<td>Poaceae</td>
<td>High-latitude salt marsh grass, Europe</td>
</tr>
<tr>
<td>Spartina gracilis</td>
<td>Poaceae</td>
<td>High-latitude salt marsh grass, Europe</td>
</tr>
<tr>
<td><strong>High-latitude dicots</strong></td>
<td></td>
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<tr>
<td>Atriplex laciniata</td>
<td>Chenopodiaceae</td>
<td>Grows along the Norwegian coastline</td>
</tr>
<tr>
<td><strong>High-elevation grasses and sedges</strong></td>
<td></td>
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</tr>
<tr>
<td>Bulbostylis densa</td>
<td>Cyperaceae</td>
<td>Tibet, to 4300 m</td>
</tr>
<tr>
<td>Andropogon amethystinus</td>
<td>Poaceae</td>
<td>Mt Kenya to 4000 m</td>
</tr>
<tr>
<td>Aristida alpina</td>
<td>Poaceae</td>
<td>Tibet to 4500 m</td>
</tr>
<tr>
<td>Bouteloua gracilis</td>
<td>Poaceae</td>
<td>Rocky Mountains to 3200 m, N. America</td>
</tr>
<tr>
<td>Eragrostis soratensis</td>
<td>Poaceae</td>
<td>Andes to 4000 m</td>
</tr>
<tr>
<td>Miscanthus nudipes</td>
<td>Poaceae</td>
<td>Grows to 3600 m in the Himalayas</td>
</tr>
<tr>
<td>Muhlenbergia richardsonis</td>
<td>Poaceae</td>
<td>Grows to 4000 m in western North America</td>
</tr>
<tr>
<td>Muhlenbergia peruviana</td>
<td>Poaceae</td>
<td>Andes to 4800 m; highest C₄ plant in the Americas</td>
</tr>
<tr>
<td>Pennisetum clandestinum</td>
<td>Poaceae</td>
<td>Andes to 4000 m</td>
</tr>
<tr>
<td>Pennisetum centrasiacicum</td>
<td>Poaceae</td>
<td>Grows up to 4500 m on the Tibetan plateau</td>
</tr>
<tr>
<td>Pennisetum flaccidum</td>
<td>Poaceae</td>
<td>Western Asia to 5000 m</td>
</tr>
<tr>
<td>Paspalum bonplandianum</td>
<td>Poaceae</td>
<td>Andes to 4500 m</td>
</tr>
<tr>
<td>Ononis thoroldii</td>
<td>Poaceae</td>
<td>Tibet to 5200 m; world’s highest C₄ plant</td>
</tr>
<tr>
<td>Sporobolus indicus</td>
<td>Poaceae</td>
<td>Andes to 4000 m</td>
</tr>
<tr>
<td>Triopogon fiouae</td>
<td>Poaceae</td>
<td>Tibet, dry slopes to 4600 m</td>
</tr>
<tr>
<td><strong>High elevation dicots</strong></td>
<td></td>
<td></td>
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<tr>
<td>Amaranthus peruviana</td>
<td>Amaranthaceae</td>
<td>Andes to 4000 m</td>
</tr>
<tr>
<td>Atriplex pamirica</td>
<td>Chenopodiaceae</td>
<td>Pamir mountains (Asia) to 4500 m</td>
</tr>
<tr>
<td>Bassia dasyphylla</td>
<td>Chenopodiaceae</td>
<td>Tibet to 4350 m</td>
</tr>
<tr>
<td>Euphorbia serpens</td>
<td>Euphorbiaceae</td>
<td>Andes to 4500 m; latex-producing species</td>
</tr>
<tr>
<td>Climacoptera lanata</td>
<td>Chenopodiaceae</td>
<td>Pamir to 4125 m</td>
</tr>
<tr>
<td>Gomphrena meyeniana</td>
<td>Chenopodiaceae</td>
<td>Andes to 4600 m; classic alpine plant morphology</td>
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<tr>
<td>Halogeton glomeratus</td>
<td>Chenopodiaceae</td>
<td>Tibet to 4250 m</td>
</tr>
<tr>
<td>Portulaca fulgens</td>
<td>Portulacaceae</td>
<td>Andes to 4300 m</td>
</tr>
<tr>
<td>Salsola monoptera</td>
<td>Chenopodiaceae</td>
<td>Tibet to 4200 m</td>
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<tr>
<td>Early season active</td>
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<tr>
<td>Atriplex confertifolia</td>
<td>Chenopodiaceae</td>
<td>West N. American shrub, active from early spring</td>
</tr>
<tr>
<td>Haloxylon aphyllum and H. persicum</td>
<td>Chenopodiaceae</td>
<td>Central Asia, including saline and dry soils to northern Mongolia</td>
</tr>
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</table>

Arabidopsis thaliana has been for C₃ plants (Stitt and Hurry, 2002). Setaria viridis is currently being developed as a genetic model for C₄ photosynthesis given its rapid generation time, relatively small genome, and ease of growth (Li and Brutnell, 2011). Its widespread distribution across Eurasia and North America also indicates that it has high genetic diversity that can be exploited. Setaria viridis occurs from warm climates in the southern USA and China to cold climates of Asia and central Canada, to above 60°N (Malyschev and Peschkova, 2001; Rominger, 2003). In midwestern Canada, it is one of the more severe weeds of wheat and rapeseed, both of which are cool-climate crops (DeFelice, 2002).

Cold-tolerant C₄ perennial grasses (Miscanthus, switchgrass, Spartina, and Andropogon)

Miscanthus

The genus Miscanthus consists of 14–25 species, largely of eastern Asia and west into the high elevations of the Himalayan and Tibetan Plateaus (Shouliang and Renvoize, 2006; Clifton-Brown et al., 2011; Yan et al., 2012). Most species are of warm climates, although a number are noted to occur in cold climates, leading to the conclusion that the genus is subtropical in origin with secondary radiations into colder climates (Earnshaw et al., 1990; Sacks et al., 2013). Miscanthus sinensis is a widespread diploid species of East Asia that grows up to 2500 m and as far north as 48° in the Amur River Valley and the Kuril and Sakhalin Islands in the Russian Far East (Kharkevich et al., 2003; Sacks et al., 2013). Miscanthus sacchariflorus is diploid or tetraploid, and occurs in China, Japan, Korea, and the Russian Far East up to near 50°N, in the Amur River valley of Siberia (Kharkevich et al., 2003; Shouliang and Renvoize, 2006). Both M. sacchariflorus and M. sinensis extend into the equivalent of USDA plant hardiness zone 3 in eastern Siberia, although they predominantly occur in the equivalent of zones 4 and higher in eastern Asia (Fig. 1B; NAPPPAST, 2012; Sacks et al., 2013; Clark et al., 2014). Plant hardiness zone 3 is characterized by minimum winter air temperatures of −34°C to −40°C, while hardiness zone 4 corresponds to minimum winter temperatures of −29°C to −34°C (USDA, 2014). In addition to M. sinensis and M. sacchariflorus, three montane species of Miscanthus are of note: M. floridus grows to at least 3300 m in New Guinea where it has been shown to have good cold tolerance (Earnshaw et al., 1990); M. nepalensis occurs on mountain slopes up to 2800 m in the Himalayan and Tibetan plateaus; and M. nudipes occurs up to 3600 m in the same region (Shouliang and Renvoize, 2006; Clifton-Brown et al., 2011). The highest populations of these species probably have excellent cold tolerance, given the low night temperatures that can occur at these elevations; for example, it is probable that M. nudipes at 3600 m experiences subzero night temperatures during the growing season, as observed in Mt. richardsonis plants of similar elevations (Sage and Sage, 2002). Miscanthus sinensis and M. sacchariflorus naturally hybridize to produce triploid and tetraploid genotypes that are collectively termed M. ×giganteus (Clifton-Brown et al., 2008; Sacks et al., 2013).

Some of these hybrids were exported to Europe and America from Japan since the first half of the 20th century, where they have been grown as ornamentals. Since the 1970s, they have been studied as feedstock candidates for bioenergy production, initially in Europe (e.g. Clifton-Brown and Jones, 1997; Clifton-Brown et al., 2001, 2008, 2011; Jones, 2011), and in the USA and Canada over the past 15 years (Heaton et al., 2004, 2010; Deen et al., 2011).

Miscanthus giganteus lines stand out as having high yields in the cool temperate climates of western Europe and, more recently, the northern USA, with annual dry matter production routinely exceeding 20 t ha⁻¹ and peak yields exceeding 40 dry t ha⁻¹ (Lewandowski et al., 2003; Heaton et al., 2010; Vyn et al., 2012; Arundale et al., 2014). Physiologically, Mxg is noted to have good chilling tolerance and good photosynthetic capacity at suboptimal temperatures, which explains its high radiation conversion efficiency in northern climates (Beale and Long, 1995; Dohleman and Long, 2009; Long and Spence, 2013). It is not, however, regarded as being particularly hardy in cold winters, and thus is not considered a useful option in more northern locations such as Scandinavia, Eastern Europe, and Canada where mortality of young plants is high in the first winter after planting, and yields are generally <20 t ha⁻¹ (Clifton-Brown et al., 2001; Hastings et al., 2009a, b). Lee et al. (2014) suggest that the principle genotype of Mxg studied in North America, the ‘Illinois’ clone obtained from the Chicago Botanical Garden, would be suitable for USDA hardiness zones 5–8, or where minimum winter air temperatures do not fall below −29°C (USDA, 2014). This would preclude growth in the northeast and upper Midwest region of the USA, much of Canada, and Eastern Europe. To overcome this limitation and expand where Mxg can be grown, new genotypes are being bred for northern climates, such as the tetraploid Nagara lines and diploid Amuri lines developed by Tipplant Biotechnik in Germany (Sacks et al., 2013; Glowacka et al., 2015). These have been marketed in Canada by New Energy Farms (www.newenergyfarms.com) and are currently under trial at the University of Guelph, where second-year yields up to 20 dry t ha⁻¹ have been recorded (Deen et al., 2011).

Switchgrass (Panicum virgatum)

Switchgrass was an early favourite of grass-based bioenergy efforts in the USA due to its good productive potential, pre-existing use in a variety of applications, stress tolerance, and native habit (McLaughlin and Adams Kszos, 2005; Parrish and Fike, 2005). By the early 1990s, switchgrass was recognized as a model bioenergy feedstock by the US Department of Energy and was praised as a potentially important contributor to USA energy stocks by President George W. Bush in his 2006 State of the Union address (Parrish and Fike, 2005). Its stature has lagged behind Mxg in recent years due to inferior yields, but, with further breeding, its yield potential is increasing (Madakadze, 1977; Heaton et al., 2004; Parrish and Fike, 2005; Lee et al., 2014; Delaquis et al., 2015). The peak yield of switchgrass is reported to be 39 t ha⁻¹, although the large majority of reports indicate yields of 2–12 t ha⁻¹ for upland genotypes (mean of 8.7 t ha⁻¹) and 5–20 t ha⁻¹ (mean of 12.9 t ha⁻¹) for the more productive lowland genotypes.
In side by side trials in the central USA, switchgrass produced about one-third to a half the yield of the unimproved Mxg genotype ‘Illinois’ (Heaton et al., 2004, 2008; Dohlenman et al., 2009; Arundale et al., 2014). In northern sites, yields can exceed 9 t ha\(^{-1}\). At the University of Guelph farm in Elora, Ontario Canada, two leading switchgrass varieties bred for cooler climates (Cave-in-Rock and Shelter) produced just under 10 t ha\(^{-1}\) of dry biomass which was half of the yield exhibited by Nagara and Amuri lines of Mxg in second-year stands (Deen et al., 2011). Madakadze et al. (1998b) observed that productive switchgrass varieties such as Cave-in-Rock yielded up to 14 dry t ha\(^{-1}\) at a short-season location near Montreal, Canada, in USDA hardiness zone 4. Despite its yield deficit, switchgrass has a number of potential advantages over Miscanthus. It is native to the North America and is widespread, such that accessible genetic variation is high; it has good drought resistance, and it occurs up to 54 °N into central Manitoba and Alberta, indicating it may have excellent cold tolerance (Barkworth, 2003; Parrish and Fike, 2005). The northernmost switchgrass populations straddle the boundary between USDA hardiness zones 2 and 3, which correspond to minimum air temperatures of –40 °C. Thus, while Mxg may outperform switchgrass across the most productive regions of North America, switchgrass may be the superior species where drought and cold are major challenges. This has significance when considering the trade-off between biofuels and food production: the high yields of Mxg are best realized on good crop land, but, on dry or cold marginal lands, switchgrass, and other native prairie grasses such as Andropogon gerardii (big-bluestem) and prairie cordgrass (Spartina pectinata) may be preferred alternatives (Voigt et al., 2012).

Switchgrasses are segregated into two forms—upland and lowland (Parrish and Fike, 2005; Lee et al., 2014). The lowland varieties develop a bunchgrass growth habit that produces taller, more desirable stocks, while upland forms tend to be rhizomatous, and are less productive than lowland forms. Upland forms predominate in colder climates, extending into the upper US Midwest and central Canada (USDA growing zone 3), and are thus recognized as being more cold tolerant than the more southern, lowland forms that range from Mexico to Nebraska (Casler et al., 2004; Parrish and Fike, 2005; Lee et al., 2014).

Prairie cordgrass (Spartina pectinata)

Spartina species have long been recognized as cold-tolerant C\(_4\) grasses (Long et al., 1975; Long, 1983). In Britain, for example, the coastal saltmarsh species S. anglica grows to 58 °N in northwestern Scotland (Long, 1983). In eastern North America, the saltmarsh grasses S. alterniflora and S. patens can dominate saltmarshes along the northeast coast of North America up to 50 °N on the Island of Newfoundland (Barkworth, 2003). In August 2012, two of us (RFS and PF) noted that S. alterniflora is aggressively expanding its range in northern Newfoundland saltmarshes. In Canada, two species—S. gracilis and S. pectinata—have been collected as far north as great Slave Lake, above 60 ° latitude in USDA hardiness zone 2 or Canada hardiness zone 0–1, although the common distribution for each extends to 54 °N in central Canada in USDA hardiness zone 3 (Long, 1983; Barkworth, 2003; NRC, 2014; USDA, 2014).

Of these Spartina species, S. pectinata has a number of characteristics that encourage development into a biofuel feedstock. It produces culms to 2.5 m in the wild (>3 m in cultivation), whereas S. gracilis culms extend to 1 m (Barkworth, 2003; Boe et al., 2013). It is not strictly a marsh grass as many of the coastal Spartina species are, as it can occur in both wet and dry prairies (Voigt et al., 2012). It is salinity and flooding tolerant, and has a wide distribution across North America, indicating excellent diversity in the species gene pool (Barkworth, 2003; Lee et al., 2014). In yield trials in Illinois, wild accessions from South Dakota and Illinois produced an estimated 15–25 t ha\(^{-1}\), while in the UK, unimproved lines of S. pectinata exhibited yields up to 18 t ha\(^{-1}\) (Potter et al., 1995; Boe et al., 2013). A northern population of S. pectinata from North Dakota exhibited yields equivalent to 9 t ha\(^{-1}\) at the Illinois site, while in South Dakota, S. pectinata outproduced switchgrass with yields up to 9.7 t ha\(^{-1}\) (Boe and Lee, 2007; Boe et al., 2013). These results demonstrate a high potential for substantial yields in northern locations, particularly with genetic improvement.

Andropogon gerardii (big-bluestem)

Andropogon gerardii is a native grass of the North American Tall and Mixed Grass Prairies, with a natural range extending from Texas to 54 °N in south-Central Manitoba (Campbell, 2003). It is noted for producing dominant swards that are 2–3 m high, but is largely unimproved for bioenergy production. It has superior drought tolerance relative to switchgrass, and occurs where minimum winter air temperatures are as low as –50 °C (Boe et al., 2013). Yields can be respectable, approaching 10 t ha\(^{-1}\) in northern locations such as North Dakota, Minnesota, and Montreal, Quebec, but are generally below those of switchgrass when grown at the same sites (Madakadze, 1977; Deen et al., 2011; Boe et al., 2013). Big-bluestem exhibits many traits suited for bioenergy production, including tall culms and stress tolerance, but is not currently a leading candidate given the higher yields noted for Mxg, switchgrass, and prairie cordgrass.

Winter cold tolerance in three leading candidate bioenergy crops: Miscanthus, switchgrass, and Spartina pectinata

With the emphasis on Miscanthus development in Europe and, more recently, in Illinois, a good database is present for Miscanthus cold tolerance, such that critical temperatures are known for many genotypes and forms of cold exposure. In switchgrass and Spartina, the physiological cold tolerance database is incomplete, although a number of recent studies have contributed to our understanding of winter and spring survival in S. pectinata. Switchgrass, despite the high emphasis placed upon it, lacks a well-developed physiological database of cold tolerance, and its tolerance thresholds of winter and spring cold have to be inferred from a few physiological studies and a much richer agronomy database, as will be discussed below.
Miscanthus cold tolerance has largely been examined in polyploid Mxg hybrids and their parent species, the diploid M. sinensis and M. sacchariflorus. Once established, these species overwinter as a dense, underground mass of rhizomes, with new springtime growth arising from the rhizomes (Fig. 4A, B; Anderson et al., 2011). Most rhizomes occur along the rhizome clump at a depth of 0–12 cm. Switchgrass exhibits a rhizome mass similar in morphology to Mxg, but rhizomes tend to be above 10 cm depth (Fig. 4C, D). In contrast, S. pectinata forms dormant shoots that overwinter at the soil surface, in addition to underground rhizomes that can be 0–12 cm deep (Fig. 4E, F).

In Miscanthus, genotypes vary in the morphology of the rhizome mass, with some exhibiting tighter, more compact rhizome masses while others have less compact morphologies in which individual rhizomes extend further into the soil from the central mass (Withers, 2014). This may have significance for cold tolerance by affecting the insulation properties of the soil/plant mass above the rhizomes. Stem biomass appears to be a good insulator compared with soil, such that rhizomes within a tightly packed rhizome mass may be better protected than rhizomes in loosely packed rhizome masses. This hypothesis, however, remains to be tested, but, if true, it could be one mechanism explaining genotype variation in cold tolerance. In addition, rhizomes in first-year plantations may be more vulnerable to cold injury than rhizomes from older stands because the first-year plants may lack the added insulation provided by larger stem masses of older plants.

Due to soil and snow insulation, air temperature is rarely a good indication of lethal exposure, and it is necessary to know rhizome temperature during cold events in order to explain causes of winter mortality. Soil type, density, water content, organic matter content, plant residue cover, and wind all affect the probability that lethal cold will penetrate to the depth of the rhizomes (Sturm et al., 2005; Henry, 2007). Of the potential factors affecting the probability that lethal cold will penetrate into the soil, snow cover is probably the most important, because it acts as an effective insulator (Henry, 2007). At our field sites at Elora, Ontario and Leamington, Ontario, soil temperature at 2–5 cm depth were near 0 °C when covered by snow, despite air temperatures that varied between 0 °C and –30 °C (Friesen et al., 2015; Peixoto et al., 2015). In contrast, in the absence of snow, soil temperatures at rhizome depth declined to –4 °C to –6 °C on cold winter days, particularly when a strong wind was present (Friesen et al., 2015). This observation is significant because models predicting Miscanthus survival and productivity across Europe use a Fourier heat conduction formula to predict cold penetration into the soil, assuming that a lengthy period (30 d) of air temperature below –6 °C will cause rhizome mortality (Hastings et al., 2009a, b). Snow cover will affect such predictions by slowing heat flux, and thus should be accounted for when modelling where bioenergy crops might be cultivated.

Studies that use a constant rate of cooling the rhizomes to treatment temperatures consistently show that Miscanthus genotypes are killed when rhizome temperatures fall below –7 °C. Mean temperatures causing 50% mortality (LT50) of rhizomes harvested from Miscanthus genotypes in late autumn or winter range between –3.4 °C and –6.7 °C, based on regrowth trials and electrolyte leakage assays (Clifton-Brown and Lewandowski, 2000; Heaton et al., 2010; Friesen

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**Fig. 4.** The rhizome mass from (A, B) Miscanthus x giganteus (Mxg) variety Illinois, (C, D) switchgrass (Panicum virgatum variety Carthage), and (E, F) prairie cordgrass (Spartina pectinata variety Red River). Plants were harvested and photographed by Professor Bill Deen at the University of Guelph experimental farm in Elora, Ontario on 27 November 2014. Mxg and switchgrass are 4-year-old plants, while S. pectinata is 2 years old. Arrows indicate above-ground dormant stems in S. pectinata. The horizontal bars indicate 2 cm in panels A, C and E and 1 cm in panels B, D and F.
Rhizomes from triploid and tetraploid hybrids show a 2–3 °C warmer LT$_{50}$ when harvested in summer, indicating that there is modest cold acclimation during the autumn hardening period. Diploid ‘Amuri’ hybrids of *M. sinensis* and *M. sacchariflorus* show no shift in LT$_{50}$ between summer and winter (Peixoto et al., 2015). *Miscanthus* genotypes exhibiting the lowest LT$_{50}$ values (between –6 °C and –7 °C) were one diploid *M. sinensis* variety and two diploid Amuri lines (Clifton-Brown and Lewandowski, 2000; Peixoto et al., 2015). All Mxg lines examined showed LT$_{50}$ values of –3 °C to –6 °C when harvested in late autumn to winter, including the well-studied Illinois triploid line (LT$_{50}$ = –4 °C to –5 °C) and a triploid Nagara genotype (LT$_{50}$ = –4.6 °C) (Clifton-Brown and Lewandowski, 2000; Friesen et al., 2015; Peixoto et al., 2015). Clifton-Brown and Lewandowski (2000) observed 1–3 °C higher LT$_{50}$ values than Friesen et al. (2015) and Peixoto et al. (2015), which could reflect differences in cooling rate, genotype, or exposure temperatures in the field before harvest. The LT$_{50}$ values observed in rhizomes harvested in Ontario, Canada by Friesen et al. (2015) and Peixoto et al. (2015) were colder than in Germany (Clifton-Brown and Lewandowski, 2000). Heaton et al. (2010) also noted that Mxg survives winter soil temperatures to –6 °C. Though subtle, these LT$_{50}$ differences are notable because the mean critical temperature of –3.4 °C determined by Clifton-Brown and Lewandowski (2000) is used to predict Mxg rhizome survival in potential growing regions of Europe using the MISCANFOR model (Hastings et al., 2009a, b). Modelled winter survival based on a –3.4 °C threshold may be too conservative if the North American values are accurate, particularly since minimum soil temperatures recorded in Canadian *Miscanthus* plantations are below the LT$_{50}$ values determined for Mxg genotypes (Clifton-Brown and Lewandowski, 2000; Clifton-Brown et al., 2001; Friesen et al., 2015; Peixoto et al., 2015).

*Miscanthus* rhizomes typically will not see continuous cooling as used in artificial cooling trials, but would see a gradual cooling with periods of stasis as autumn progresses into winter. To evaluate whether a slow, more natural cooling rate could promote greater cold hardening in the field, Peixoto et al. (2015; see also Peixoto, 2015) treated five *Miscanthus* varieties harvested from the field during winter with a slow cooling rate of 1 °C h$^{-1}$ with 24 h holds at a sequential series of subzero temperatures (–5, –7, –10, –12, –14, –18, and –22 °C). Following warming and a regrowth period, LT$_{50}$ values were observed to be near –6.5 °C in triploid and tetraploid Mxg lines, but had declined to –12.6 °C and –14.4 °C in two diploid Amuri lines (Peixoto et al., 2015). The Mxg Illinois genotype exhibited an LT$_{50}$ of –6.3 °C when cooled in stages (Peixoto, 2015). These results demonstrate that diploid Amuri lines have greater acclimation potential than the triploid and tetraploid *Miscanthus* hybrids when experiencing a gradual, staged cooling that allows enough time for acclimation: a reduction in LT$_{50}$ of 1–2 °C is apparent in the Mxg genotypes, but can be 6–8 °C in the Amuri diploids. It can be concluded from this study that even with a lengthy acclimation period, the more productive polyploid *M. ×giganteus* hybrids lack the ability for significant low temperature hardening, and would be vulnerable to winter kill in more extreme locations. Of note, rhizomes from Mxg genotypes bred for cold climates, such as the Nagara lines, are not significantly more cold tolerant than others such as the Illinois clone. This supports the general conclusion of Clifton-Brown and Lewandowski (2000) that Mxg is unsuited for more northern locations, although the critical temperature is probably 2–3 °C below the –3.4 °C value they propose. Instead, diploids such as the Amuri lines, or *M. sinensis* as suggested by Clifton-Brown and Lewandowski (2000), may be more suited for northern sites such as Scandinavia and plant hardiness zones 3–4 in Canada.

It has been observed that young, first-year *Miscanthus* plantations can suffer significant winter mortality in cold locations (Heaton et al., 2008, 2010). Reported losses exceed 80% of first-year plants at sites in Ontario, Canada (Deen et al., 2011; Rosser, 2012) and Scandinavia (Clifton-Brown and Lewandowski, 2000; Clifton-Brown et al., 2001). The cause of this mortality is uncertain, although in Scandinavia, minimum soil temperatures at 5 cm depth were below the LT$_{50}$ of Mxg rhizomes, but not *M. sinensis* rhizomes which suffered less mortality (Clifton-Brown and Lewandowski, 2000; Clifton-Brown et al., 2001). It is possible that first-year rhizomes were more exposed to cold than older rhizomes because they were not as deep as or insulated by the rhizome mass (Withers, 2014). Consistently, we observed 20% rhizome mortality in first-year rhizomes of the Mxg Illinois variety after the winter of 2013–2014, when soil temperatures at 2 cm depth were recorded to have fallen to –3 °C on average, close to the –4 °C LT$_{50}$ recorded for this genotype; most of the dead rhizomes were in the top of the rhizome mass (Friesen et al., 2015). Physiological improvements in cold tolerance of older versus young rhizomes do not appear to explain the first-year mortality, because critical temperatures do not vary between rhizomes collected from first- and third-year plantations (Friesen et al., 2015; Peixoto et al., 2015).

Winter kill could also result from dehardening due to freeze–thaw cycles, precocious sprouting during winter to early spring warm events, and rhizome rot in cold, saturated soils (Clifton-Brown and Lewandowski, 2000; Christian and Haase, 2007; Deen et al., 2011). First-year plantings would be more susceptible to such stress given that there are fewer rhizomes and carbohydrate reserves in the smaller plants (Clifton-Brown and Lewandowski, 2002; Withers, 2014). To clarify mortality patterns and their cause, it will be necessary to monitor rhizome temperatures, health, and LT$_{50}$ values carefully during the various types of cold exposure that can occur before emergence in the spring.

Canada, like Eurasia, has an abundance of marginal land at high latitude that could be suitable for *Miscanthus* production if cold tolerance is sufficient (Field et al., 2008). Given the knowledge of the threshold temperatures of –4 °C to –6 °C for polyploid hybrids, and down to –15 °C for diploids, Peixoto et al. (2015) examined the soil temperature database compiled by Environment Canada between 1984 and 2006 to determine where soil temperatures in winter would pose a risk for *Miscanthus* production in Canada. Soil temperatures were recorded at 73 sites across Canada at 5 cm depth.
(Environment Canada, 2014). In southwest Ontario, between Lake Erie and Lake Huron in USDA plant hardiness zone 5, the lowest soil temperatures recorded were above the −5 °C to −7 °C lethal threshold for rhizomes (Peixoto et al., 2015). Further north, at sites across Canada from the Rockies to the Maritime Provinces, many stations in hardiness zones 4 and less recorded soil temperatures that would be cold enough to kill overwintering Mxg rhizomes. Field observations support these possibilities; in Kemptville, Ontario, at the boundary of USDA hardiness zones 4 and 5, winter mortality of first-year Mxg plants was near 100% in all lines studied except one, while at more southern locations within zone 5, the first-year winter mortality of the same lines was largely between 0% and 50% (Rosser, 2012). Most of Canada lies within USDA hardiness zone 4 or lower (Fig. 1), indicating that the country is largely unsuited for Miscanthus cultivation. Using the MISCANFOR model and regional climate data, Hastings et al. (2009a, b) similarly conclude that low soil temperatures would harm Mxg plantations in all of Scandinavia, Poland, and most of European Russia. These areas fall into USDA hardiness zones 4 and 5, which could reflect their use of the warmer, −3.4 °C LT50 for Mxg mortality.

It is possible that breeding with a wider variety of accessions may significantly reduce the LT50 and enable cultivation of Miscanthus in more northern locations; however, as a lineage of tropical origin, the Miscanthus gene pool may lack the genes required to confer extreme cold tolerance. Because the genetic diversity in the current Mxg breeding pool is considered to be relatively narrow (Sacks et al., 2013; Glowacka et al., 2015), it is possible that greater cold tolerance is present in the highest and most northerly populations of the Miscanthus gene pool (Yan et al., 2012). If this is the case, then enhanced cold hardiness could be obtained when high-elevation or northern genomes are incorporated into new Mxg lines. If not, then alternative C4 species may be needed to exploit more northerly locations, such as S. pectinata and switchgrass.

Spartina pectinata and switchgrass

Spartina species have been noted since the 1970s to have good cold tolerance, leading to the suggestion that they are a ‘New World parallel to Miscanthus’ (Long and Spence, 2013). Spartina gracilis rhizomes can withstand winter cold to −27 °C (Schwarz and Reaney, 1989), indicating that its more productive sister, S. pectinata, also can tolerate extreme winter cold. This was confirmed by Friesen et al. (2015), in side by side trials examining cold tolerance of three diverse S. pectinata accessions and the Illinois genotype of Mxg. In all three S. pectinata accessions, rhizomes harvested from the field in late autumn and winter exhibited LT50 values near −24 °C, which is sufficient to ensure tolerance of mid-winter soil temperatures across most of Canada. In late April, when plants were beginning to sprout and presumably were no longer in the cold-hardened state, the LT50 of S. pectinata rhizomes had risen to −10 °C, while in Mxg, the LT50 remained at −4 °C, the same value as observed in mid-winter (Friesen et al., 2015). Seasonal changes in cold tolerance are common in plants adapted to cold climates, but weakly developed in species with limited cold tolerance (Huner and Williams, 1988).

In switchgrass, strong cold tolerance is expected, given that the species naturally grows into central Canada (Freckmann and Lelong, 2003); however, cold physiology has not been extensively examined as it has for Miscanthus. Two studies demonstrate that winter cold hardiness of switchgrass approaches that of S. pectinata. In field-grown plants near Ottawa, Ontario, the LT50 of rhizome masses declined from −4 °C in summer to −19 °C to −23 °C by early December (Hope and McElroy, 1990). The genotypes examined were chosen from seed of six Pathfinder cultivars that had been subject to selection for tolerance of extreme winter cold. These results mirror those reported from China, where switchgrass survived winter cold to near −20 °C (Ichizen et al., 2002; Parrish and Fike, 2005).

Emergence date and frost tolerance

In addition to winter cold, a major criterion for the success of C4 bioenergy crops in cold climates is an ability to begin leaf growth early enough in the spring so the canopy can close before the long photoperiods of late spring commence (Beale and Long, 1995; Dohleman and Long, 2009). This requires an ability to emerge early and to tolerate spring frosts, which can be common well into May in southern Canada and subboreal Europe. For example, Farrell et al. (2006) modelled that a 1 °C reduction in LT50 for spring leaves and a 2.5 °C reduction in the minimum temperature for leaf emergence in Mxg could increase the growing season by a month in Germany, thereby improving yield by up to 25% in the absence of frost.

Emergence dates depend on soil temperature, and will thus vary with season, location, slope aspect, and surface cover by crop residues or mulch. In northern locations, surface cover could be particularly important and require more attention by growers. For example, insulation provided by mulch could protect rhizomes from winter cold, but then slow soil warming and delay emergence in the spring. In a controlled exposure study, Farrell et al. (2006) determined that the minimum emergence temperature is ~6 °C for M. sinensis line H9, and near 9 °C for Mxg. Consistently, in 2014, we observed Mxg emergence at the University of Guelph Research Station near Elora, Ontario to occur first on about 9 May when 2 cm deep soil temperatures warmed above a daily minimum of 6 °C, and the daily maximum air temperature exceeded 10 °C (Friesen et al., 2015). These values are similar to the 10–12 °C daily air temperatures used by productivity models to predict growth initiation of Mxg (Hastings et al., 2009a, b; De Laporte et al., 2014). At the Elora site, the spring of 2014 was cool compared with previous years, when emergence and active growth of Miscanthus varieties occurred by late April (in 2009–2010; Deen et al., 2011). At the University of Illinois Energy Farm, emergence and rapid growth of Mxg were underway by the first week of May in 2003, 2007, and 2008, which allowed for high canopy light interception efficiency (ei >0.8) by the end of May (Heaton et al., 2004; Dohleman and Long, 2009).
Late April emergence of *Mxg* has been documented in Nebraska and New Jersey in 2009, but shifted at these sites to the second week of April in the warmer spring of 2010, as it did in Illinois (Maughan et al., 2012). In Europe, *Mxg* emergence varies from late April in the mild climate of southern England and southern Germany, to early May in northern Germany (Clifton-Brown and Jones, 1997; Clifton-Brown and Lewandowski, 2002; Farrell et al., 2006).

Emergence dates for switchgrass generally reflect those for *Miscanthus* in that emergence and growth commence in mid-April to early May across northern study sites (Heat on et al., 2004, 2008; Dohleman et al., 2009, 2012). At the Elora, Ontario site, switchgrass was observed to emerge several days after *Mxg* (Deen et al., 2011). *Spartina pectinata*, in contrast, emerged and commenced growth ~2 weeks earlier than *Mxg* at the Elora site in 2014, such that by 17 May, *S. pectinata* ‘Red River’ shoots were double the height of shoots from *Mxg* ‘Illinois’ (Friesen et al., 2015). Of note, the *Mxg* shoots caught up with the *S. pectinata* shoots by late June, highlighting the superior growth potential of *Mxg* in warmer conditions. In a comparison of *Mxg* and *Spartina cynosuroides* in southern England, Beale and Long (1997) also observed earlier emergence of the *Spartina* genotype, by ~10 d in April.

In a comparison of one *S. pectinata* genotype (CWNC) with 12 switchgrass lines, two big-bluestem lines, and five lines of the C₄ grass prairie sandreed (*Calamovilfa longifolia*) at a site near Ottawa, Ontario, the *S. pectinata* line showed the earliest emergence, on 5 April in 1995, and on 30 April in the cooler spring of 1994 (Madakadze, 1977; Madakadze et al., 1998a). These dates were at least 5 d earlier than most of the other lines in 1995, and 1–2 weeks earlier than most lines in 1994.

Once emerged, frost tolerance becomes critical, because it can cause a loss of the developing canopy, and can be lethal for young plants emerging from their first winter (Farrell et al., 2006). Loss of the early canopy due to frost injury restarts the development timetable, thereby offsetting the advantages of early season emergence and elongation rate (Farrell et al., 2006). The published data on frost tolerance in *Miscanthus* are inconsistent, however. At Elora, newly emerged leaves of nine *Miscanthus* hybrids were killed by a hard frost on 9–10 May 2010, including the Illinois *Mxg*, numerous Amuri diploid lines, and the Nagara line; some *M. sinensis* leaves survived this frost (Friesen et al., 2015; Peixoto et al., 2015). Controlled freezing studies of *Mxg* ‘Illinois’ leaves harvested in late May and June of 2014 showed the critical temperature for lethal electrolyte leakage to be near −5 °C, which is consistent with the probable leaf temperatures occurring during the frost event of May 2010 (Friesen et al., 2015). In contrast, Farrell et al. (2006) observed the LT₅₀ for leaves of *Mxg* to be near −8 °C, while *M. sinensis* and *M. sacchariflorus* leaves had LT₅₀ values between −6 °C and −9.3 °C. We have no explanation for the differences except to note that protocols, leaf age, genotypes, and the presence of ice nucleating agents could account for variation in critical temperatures (Zub et al., 2012; Gusta and Wisniewski, 2013; Peixoto, 2015). Old *Miscanthus* leaves, for example, are reported to be more frost tolerant than young leaves (Zub et al., 2012). Jørgensen and Muhs (2001) reported low critical leaf temperatures of −5 °C to −9 °C in one trial of five genotypes, and −11 °C to −14.7 °C in a second using seven outdoor-grown genotypes. These values are unreliable because the rapid cooling rate (30–60 °C h⁻¹) is too fast to allow tissue adjustments, and the method used to score leaves (the appearance of a freezing exotherm) will not reflect injury prior to tissue freezing, nor will it reflect critical temperatures if leaves can freeze without injury, as can many cold climate species (Peixoto, 2015). Given the reported variation in frost tolerance, we recommend further field studies to identify exact points of frost injury, and thus substantiate the general impression that *Miscanthus* species are frost intolerant (Christian and Haase, 2007).

Switchgrass is also frost intolerant (Gu et al., 2008), although a lack of experimental studies prevent us from evaluating the LT₅₀ of early spring leaves. In *S. pectinata*, substantial cold tolerance of winter-dormant shoots is apparent (to −24 °C), allowing them to overwinter just above the soil surface (Friesen et al., 2015). Once they break dormancy and begin growth, they de-harden, and exhibit an LT₅₀ of −9 °C to −10 °C in April and June, indicating that they can tolerate severe springtime frost (Friesen et al., 2015).

### Early leaf expansion

Minimal basal temperatures for leaf expansion are reported to range between 5 °C and 9 °C for a number of *Mxg* varieties, and slightly below this for *M. sinensis* lines (Clifton-Brown and Jones, 1997; Farrell et al., 2006). Switchgrass seedlings are reported to initiate growth above ~2.8–7.3 °C, with the lower basal temperatures for growth being observed in the more northern accessions (Madakadze, 1977; Parrish and Fike, 2005). The base temperature for *S. pectinata* growth is unknown, but is probably similar to northern switchgrass lines and other north temperate prairie grasses such as big-bluestem and prairie sandreed (*Calamovilfa longifolia*), namely between 2.6 °C and 5 °C (Madakadze, 1977). Basal temperatures are important for growth models as they are used to predict when growth initiates (Parrish and Fike, 2005; Hastings et al., 2009a, b); however, they can be misleading because the temperature response of leaf growth varies substantially between genotypes, and there can be a trade-off between a good elongation rate at low temperature and rapid growth at warm temperatures (Madakadze, 1977; Farrell et al., 2006; Glowacka et al., 2014). For example, a cold-hardy *M. sinensis* line (H9) that exhibits a superior leaf extension rate at 9 °C relative to Gig-2 *Mxg* lags behind Gig-2 above 20 °C (Farrell et al., 2006). In *Mxg* hybrids, leaf extension rates increase exponentially from 7 °C to 15 °C, such that leaf elongation rates of *Mxg* at 25 °C are >9-fold greater than at 12 °C/5 °C (Farrell et al., 2006; Dohleman and Long, 2009; Long and Spence, 2013; Jones et al., 2015). In switchgrass, northern genotypes whose relative growth rates increase linearly above 5 °C have slower growth at 25 °C than southern genotypes whose growth rates have a weak thermal response below 10 °C but a strong thermal response above 15 °C (Madakadze, 1977). In side by side trials of the Illinois variety of *Mxg* and the Cave-in-Rock variety of switchgrass, both exhibited similar emergence times, both had slow rates of leaf
extension early in the growing season, which was followed by rapid canopy expansion when the warm days of May arrived; however, Mxg showed leaf expansion rates at warm temperatures superior to those of switchgrass, which explained the greater Mxg yield (Heaton et al., 2008; Dohleman and Long, 2009; Dohleman et al., 2012). These observations highlight the need to improve the leaf expansion rate in warmer conditions if cold-tolerant switchgrass is to match the yields of productive Mxg lines.

The leaf expansion rate in chilly conditions is highly variable in the Miscanthus gene pool. In a detailed comparison of the leaf extension rate for 51 Miscanthus accessions grown in growth chambers at 10 °C/5 °C or 25 °C/25 °C day/night temperatures, Głowacka et al. (2014) observed that mean leaf growth rates in the chilling treatment ranged from 16% to <1% of the rates observed at 25 °C. Miscanthus×giganteus ‘Illinois’ was among the best performing Mxg lines in the chilling treatment, with an extension rate at 10 °C/5 °C that was 11% of the rate at 25 °C/25 °C. One diploid M. sinensis line and one tetraploid M. sacchariflorus line performed poorly, with leaf extension rates that were near 15% of values in the warm treatment. Most of the M. sinensis lines and a handful of the Mxg hybrids performed poorly, with leaf extension rates in chilling conditions that were similar to those of chilling-intolerant sugar cane and maize (Głowacka et al., 2014). Most of the chilled M. sacchariflorus lines exhibited leaf extension rates that were near 10% of rates at 25 °C, similar to Mxg Illinois, leading Głowacka et al. (2014) to hypothesize that the M. sacchariflorus parent contributed the superior low temperature performance to the Mxg ‘Illinois’ germline. Purdy et al. (2013), in a European study, also noted that an Mxg line (311) showed the best leaf expansion rate after a 4 d chilling shock at 12 °C, relative to two M. sinensis and one M. sacchariflorus line. These studies indicate that M. sacchariflorus is the more important contributor to low temperature performance of Mxg, which is consistent with its more northern occurrence in the Amur River valley of the Russian Far East. Głowacka et al. (2014) note that the northernmost populations of M. sinensis and M. sacchariflorus have not been examined, and many of the highest elevation resources within the Miscanthus genus also have not been exploited, such that there is probably superior, unexploited cold performance with the Miscanthus gene pool.

**Photosynthesis**

To support early-season growth, photosynthesis rates also need to be substantial at suboptimal temperatures. This would generally mean light-saturated rates >10 μmol m⁻² s⁻¹ at temperatures >10 °C, which is what productive cool-season C₄ crops exhibit (Fig. 5; Labate and Leegood, 1988; Ferrar et al., 1989; Yamasaki et al., 2002; Naidu et al., 2003; Yamori et al., 2005; Sage et al., 2011). For C₄ plants, this can be problematic, because they are prone to severe photoinhibition, and may be limited by enzymatic bottlenecks unique to their physiology (Long, 1983; Sage, 2002; Sage and Kubien, 2007). Miscanthus is often noted as having excellent chilling tolerance, which is attributed to its ability at cool temperatures (10–15°C) to maintain protein synthesis, produce a well-developed photoprotection capacity using zeaxanthin and other carotenoids, and repair its photosystems under high light conditions (Naidu et al., 2003; Naidu and Long, 2004; Farage et al., 2006; Wang et al., 2008; Spence, 2012; Long and Spence, 2013; Jones et al., 2015). In contrast, Zea mays and other warm-season grasses such as sugar cane cannot maintain photosynthetic protein and suffer severe photoinhibition below ~15 °C, and as a result exhibit a steady degradation of photosynthetic capacity during chilling exposure (Du et al., 1999; Naidu et al., 2003; Naidu and Long, 2004; Głowacka et al., 2014). Similar patterns are also noted in warm-season C₃ plants such as tomato and bean, and probably reflect a general maladaptation of warm-climate species for chilling conditions (Coolbear et al., 1984; Sassenrath et al., 1990; Brüggemann et al., 1992; Yoshida, 1994).

The common chilling treatments used in Mxg research range between 10 °C and 14 °C daytime temperatures. These growth conditions do not produce prolonged declines in carbon assimilation in Mxg Illinois, as observed in maize and sugar cane (Naidu et al., 2003; Naidu and Long, 2004; Spence, 2012; Purdy et al., 2013; Friesen et al., 2014; Głowacka et al., 2014). Mxg Illinois plants grown at 14 °C/10 °C day/night temperatures exhibit identical responses of net CO₂ assimilation rate (A) to measurement temperatures <20°C as plants grown near 25 °C; these measured values of A are similar to...
or slightly less than values observed in productive C₃ plants from cool-season habitats (Fig. 5; Naidu et al., 2003; Friesen, 2015). Notably, while the photosynthetic capacity of Mxg is not superior to that observed in cool-season C₃ crops below 15 °C, it is greater than C₃ photosynthesis rates above 25 °C (Fig. 5). This leads us to conclude that the special advantage of Mxg as a bioenergy crop is not superior low temperature performance relative to the C₃ alternative, but the ability to establish in cool conditions such that when the warm days of late spring arrive, Mxg plants have a well-developed canopy that can fully exploit the superior productive potential of C₄ photosynthesis in warm environments. If C₄ plants are denied access to this warmth, or the warmth corresponds to summer drought, then they typically do not perform as well as the C₃ competition (Sage et al., 1999; Sage and Sage, 2002; Kubien and Sage, 2003, 2004a, b; Lewondowski et al. 2003). This explains, for example, the superior yields observed in the C₃ Arundo donax relative to Mxg in Italy, which has a Mediterranean climate characterized by cool, moist winters and dry summers that do not allow for high summer productivity (Angelini et al., 2009).

While 10–14 °C is a good approximation of chilling conditions during spring in Western Europe, where much of the Miscanthus research has occurred, this treatment can be too mild to represent springtime chill in Canada and more continental areas of Eurasia, where temperatures routinely fall below 10 °C in April and May. Under these more severe conditions, significant signs of stress are apparent in many Miscanthus genotypes, including Mxg Illinois, as indicated by sustained decline of carbon assimilation rate in the days after chilling exposure, and incomplete recovery of photosynthesis when plants are returned to warmer conditions. To evaluate tolerance of more severe chilling conditions, Friesen et al. (2014) measured A in plants of five Miscanthus and two Saccharum genotypes that were transferred from 25 °C/20 °C day/night growth conditions to 12 °C/5 °C day/night conditions for 6 d, and then returned to the warm growth regime. The most tolerant line was the Illinois genotype of Mxg, which had the least reduction in net CO₂ assimilation rate (A) after 6 d of chilling, showed no decline in A between day 1 and day 6 of chilling, and, when returned to warm growth conditions, showed only a 17% reduction in A when compared with values recorded in pre-chilled plants at a common measurement temperature of 27 °C. In a diploid Amuri line and a tetraploid hybrid of M. sinensis and M. sacchariflorus, A was inhibited close to 80% by the chilling treatment, with the degree of inhibition increasing between the first and sixth day. Upon return to warm conditions, A at 27 °C was almost 40% lower than observed in pre-chilled plants; these patterns are similar to those observed in chilling-sensitive sugar cane (Friesen et al., 2014; Friesen, 2015). This was somewhat surprising because the Amuri hybrid (M115) had a lower LT₃₀ of winter-dormant rhizomes than the Mxg lines (Peixoto et al., 2015), indicating that winter freezing and spring chilling tolerance are not coupled traits. In a similar study examining the response of 13 Miscanthus genotypes following transfer to chilling conditions (10 °C/5 °C day/night), Glowacka et al. (2014) observed a marked reduction of A in the first 1–5 d of chilling in 12 of the 13 genotypes; Mxg Illinois was one of the best performers of the 13, with a slight (10%) reduction in A in the days following the chilling. All M. sinensis and half of the M. sacchariflorus lines showed >40% inhibition of A in the week following the transfer to 10 °C/5 °C, as did two new bred Mxg triploid lines. Based on their results, Glowacka et al. (2014) concluded that available M. sacchariflorus lines have greater cold tolerance than M. sinensis lines, and recommend that new, more cold-tolerant accessions of M. sinensis are needed to improve low temperature A in new Mxg hybrids.

European Spartina species (S. anglica, S. townsendii, and S. cynosuroides) exhibit lower photosynthetic rates at cool temperatures relative to Mxg (Long and Woolhouse, 1978; Long, 1983; Beale et al., 1996; Long and Spence, 2013). Spartina pectinata, in contrast, exhibits photosynthetic capacities that are close to those of Mxg at 14 °C (Fig. 5; Spence, 2012). Photosynthesis of S. pectinata, however, appears to be more tolerant of chilling conditions than that of Mxg, particularly severe chilling (<5 °C). In a recent study, plants of Mxg Illinois and the Red River variety of S. pectinata were transferred from 25 °C/20 °C to 14 °C/10 °C (day/night) and the response of A was measured over 10 d (Spence, 2012). After 3 d of chilling, A had declined by 40% in S. pectinata and by >50% in Mxg Illinois, relative to the pre-chilled values. Friesen et al. (2015) noticed clear differences in photosynthetic frost tolerances between field-grown Mxg Illinois and S. pectinata. On the day after a mild frost event on 17 May 2014, which exposed leaves to temperatures near 0 °C, three S. pectinata genotypes, including Red River, exhibited mean light-saturated A values >15 μmol m⁻² s⁻¹ at 11 °C, some three times the mean light-saturated A of Mxg Illinois at the same measurement temperature. These results indicate that while Mxg can be considered tolerant of moderate chill, it is not tolerant of severe chill as often occurs during spring in USDA hardiness zones 3–5. Spartina pectinata, in contrast, is little affected by severe chilling.

Summary and conclusion: optimizing bioenergy feedstocks for cold climates

In summary, Miscanthus, switchgrass, and prairie cordgrass show promise for cold climate production, but each has limitations that will need to be addressed before widespread cultivation can be expected in northern landscapes, specifically those corresponding to USDA hardiness zones 3–4. Of the three, Miscanthus has received the most physiological scrutiny, which provides insight into mechanisms of chilling tolerance and shows clear directions for future improvement. Switchgrass has received relatively little physiological scrutiny, while recent work with S. pectinata indicates that it is the hardiest of the three in terms of winter cold tolerance and an ability to withstand frost and severe spring chill. All Miscanthus varieties examined to date have relatively modest levels of cold tolerance. LT₃₀ values for the productive hybrids are between –3 °C and –7 °C, which is close to expected soil temperatures in hardness zone 5, and
above expected soil temperatures in hardiness zones 3 and 4. Diploid hybrids, and perhaps *M. sinensis* lines, can survive below –10 °C with a prolonged subzero acclimation period, but this does not ensure survival in hardiness zones 3 and 4. While there is some uncertainty regarding frost tolerance, no *Miscanthus* line maintained living leaves following a typical hard frost that can occur in May in hardiness zones 5 or less. Even the most chilling-resistant variety of *Miscanthus*, the Illinois genotype of *Mxg*, showed severe photosynthetic inhibition when exposed to a mild frost. We therefore conclude that *Miscanthus* is currently a risky proposition in hardiness zones 3 and 4, which encompasses most of Canada, and vast stretches of Eurasia (Fig. 1). *Spartina pectinata* and upland switchgrass have the pre-requisite cold tolerance, as shown by –20 °C to –25 °C values of LT50 for dormant rhizomes, and, in the case of *S. pectinata*, good frost hardness of leaves and photosynthesis; however, they lack the rapid growth that the *Mxg* hybrids exhibit once conditions warm in later spring, and thus do not exhibit the yield potential the *Miscanthus* lines.

Ideally, it would be possible to merge the superior cold tolerance of *S. pectinata* and switchgrass with the greater growth potential of *Miscanthus*. There are many options to meet this objective. One is to improve cold tolerance in *Mxg*, which may happen soon if the northernmost, or highest, *M. sinensis* and *M. sacchariflorus* populations have superior cold tolerance and are available for breeding. In the case of *S. pectinata* and switchgrass, the necessary cold tolerance is evident; however, growth capacity will need to improve without compromising cold tolerance. This will probably require accelerating the leaf expansion rate at the warmer temperatures, which is where the yield advantages of the C4 crops such as *Mxg* arise. Trade-offs between growth and cold tolerance may be a barrier, but, if not, then faster growing southern genotypes could be bred with cold-tolerant northern varieties to achieve optimal results. Identifying specific traits governing cold tolerance and high growth rate, and employing effective screening methods for each, should accelerate the breeding process and bring the day closer when C4 perennials can enhance bioenergy heat production in colder regions where it may be needed the most.

**Supplementary data**

Supplementary data are available at *JXB* online.

Figure S1. *Haloxylon aphyllum* plants growing in a plantation in Kazakhstan and an experimental plot in Reno, Nevada.

**References**


grass (Phalaris arundinacea), maize (Zea mays) and perennial ryegrass (Lolium perenne). Global Change Biology Bioenergy 3, 375–386.


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