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Impacts of abiotic stresses on the physiology and metabolism of cool-season grasses: A review

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Abstract

Grasslands cover more than 70% of the world's agricultural land playing a pivotal role in global food security, economy, and ecology due to their flexibility and functionality. Climate change, characterized by changes in temperature and precipitation patterns, and by increased levels of greenhouse gases in the atmosphere, is anticipated to increase both the frequency and severity of extreme weather events, such as drought, heat waves, and flooding. Potentially, climate change could severely compromise future forage crop production and should be considered a direct threat to food security. This review aimed to summarize our current understanding of the physiological and metabolic responses of temperate grasses to those abiotic stresses associated with climate change. Primarily, substantial decreases in photosynthetic rates of cool-season grasses occur as a result of high temperatures, water-deficit or water-excess, and elevated ozone, but not CO₂ concentrations. Those decreases are usually attributed to stomatal and non-stomatal limitations. Additionally, while membrane instability and reactive oxygen species production was a common feature of the abiotic stress response, total antioxidant capacity showed a stress-specific response. Furthermore, climate change-related stresses altered carbohydrate partitioning, with implications for biomass production. While water-deficit stress, increased CO₂, and ozone concentrations resulted in higher carbohydrate content, the opposite occurred under conditions of heat stress and flooding. The extent of damage is greatly dependent on location, as well as the type and intensity of stress. Fortunately, temperate forage grass species are highly heterogeneous. Consequently, through intra- and in particular inter-specific plant hybridization (e.g., *Festuca x Lolium* hybrids) new opportunities are available to harness, within single genotypes, gene combinations capable of combating climate change.

KEYWORDS

abiotic stresses, climate change, cool-season grasses, metabolism, physiology

1 | INTRODUCTION

The unprecedented increases in carbon dioxide and greenhouse gas levels due to the intensified use of fossil fuels, deforestation, and human activity are the driving force behind climate change. According to the Intergovernmental Panel on Climate Change (IPCC), current CO₂ levels of 403 μmol/L are projected to double by 2100, despite attempts at global policies intended to counter greenhouse gas emissions (Figure 1). As a result, significant direct and indirect changes in global temperature and hydrological cycle, accompanied by increases in ozone atmospheric levels, are anticipated by 2100. An average rise in global temperature of 6.4°C along with a 20% decrease in precipitation and soil moisture (Scheirmeier, 2008) is expected to intensify the frequency and severity of extreme climatic events (IPCC 2014).

Heat waves, droughts, and flooding can have a marked impact on global economy due to reductions in productivity and yield losses. The 188 recorded events of drought, flooding, and heavy storms in the United States since 1980 have resulted in economic losses of more than \$1 trillion (<https://www.ncdc.noaa.gov/cdo-web/>), while in the United Kingdom, the immediate impact of flooding in 2014 alone was estimated to be £40 million (ADAS 2014).

Such climatic events, depending on their duration and severity, can have instantaneous or long-lasting effects on plant physiology and productivity, since plant plasticity is increasingly challenged beyond the capacity to acclimate or recover (Jentsch, Kreyling, Boettcher-Treschkow, & Beierkuhnlein, 2009). Furthermore, climatic extremes are rarely observed in isolation, since under field conditions they occur typically in

combination with other climatic factors (Mittler, 2006). Such combinations of abiotic stresses are known to have an additive, negative effect and can generate more distinct and pronounced consequences on plant crop yields than when each stress is applied in isolation (Mittler, 2006; Rhizsky, Liang, & Mittler, 2002). Alternatively, exposure to a primary stress can confer some degree of tolerance to a subsequent stress (Ling et al., 2018; Ohama, Sato, Shinozaki, & Yamaguchi-Shinozaki, 2017; Tombesi, Frioni, Poni, & Palliotti, 2018). This is of particular relevance to cool-season grasses which are grown as a long-lived perennial crop. Consequently, investigations into the effects of climate extremes on plant physiology and metabolism are garnering considerable attention (Lobell & Gourdji, 2012; Prasad, Vu, Boote, & Allen, 2009).

Cool-season grasses, originating from and adapted to cool climatic zones, are economically and ecologically among the most valuable species due to their pivotal role in carbon fixation and as healthy fodder for livestock consumption (Falloon & Betts, 2010). Physiologically, cool-season grasses are categorized as C₃ species since they utilize the C₃ photosynthetic pathway (Beard, 1973) with optimum temperatures for shoot growth between 18 and 24°C while maximum root growth is attained between 10 and 18°C (Beard, 1973; Turgeon 2008). Cool-season grasses of major importance in agriculture include perennial ryegrass (*Lolium perenne*), Italian ryegrass (*Lolium multiflorum*), tall fescue (*Festuca arundinacea*), meadow fescue (*Festuca pratensis* Huds.), and their hybrids (*Festulolium* x), as well as Kentucky bluegrass (*Poa pratensis* L.) and creeping bentgrass (*Agrostis stolonifera* L.) (Beard, 1973, <https://npgsweb.ars-grin.gov/gringlobal/search.aspx>). The most cultivated species, deemed suitable for agriculture, is perennial ryegrass (Furet et al., 2012) as it combines high growth rates and provides nutritious forage. In contrast, despite their inclusion in cool-season grasses, fine fescues, as well as rough bluegrass (*Poa trivialis* L.) and supine bluegrass (*Poa supina*), are not so commonly cultivated (Beard, 1973).

Climate change is expected to have a significant impact on cool-season grass growth. The increases in CO₂ and the accompanying higher temperatures are projected to shift the adaptation regions for most species (Abberton, MacDuff, Marshall, & Humphreys, 2008) resulting in higher productivity (Lobell & Gourdji, 2012). However, the concomitant increases in the frequency of heat waves or warm periods during winter will have an adverse effect. Furthermore, the perturbation of the hydrological cycle, due to alterations of precipitation patterns, is projected to exacerbate the already existing problems of limited water supply. Additionally, the estimated increases in ozone escalation are anticipated to intensify plant injury and result in substantial decreases in plant productivity (IPCC 2014). This will include effects on cool-season turfgrass yields (Table 1). Previous reviews

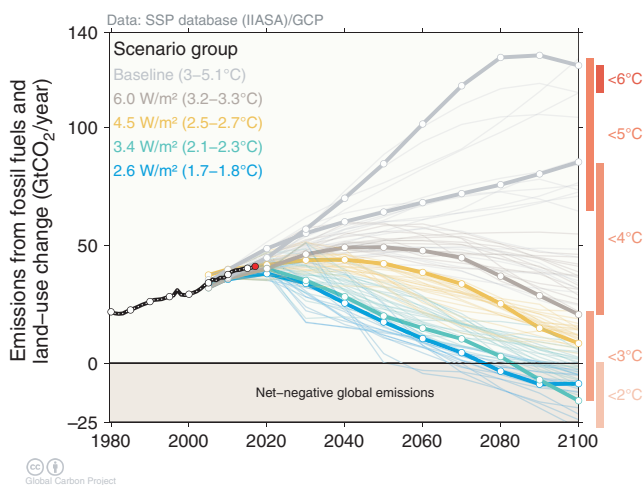


FIGURE 1 Predictions of emissions from fossil fuels and land-use change (GtCO₂/year) according to five shared socioeconomic pathways that have been developed to explore challenges to adaptation and mitigation and achieve target forcing levels (W/m²). Source: Global Carbon Budget 2017, (<http://www.globalcarbonproject.org/carbonbudget/index.htm>)

TABLE 1 Effects of increased CO₂ concentrations, drought, and flooding on various cool-season turfgrasses. Plus symbols (+) indicate yield increase, and minus symbols (–) indicate yield decrease

| Stress | Yield increase/decrease (%) | Species | Study |
|------------------------|-----------------------------|-----------------------|-------------------------------|
| Higher CO ₂ | +30 | <i>L. perenne</i> | Heibesen et al. (1997) |
| Drought | –20 | <i>L. perenne</i> | Fariaszewska et al. (2017) |
| | –15 | <i>L. multiflorum</i> | Fariaszewska et al. (2017) |
| | –11 | <i>F. pratensis</i> | Fariaszewska et al. (2017) |
| | –29 | <i>F. arundinacea</i> | Fariaszewska et al. (2017) |
| Flooding | –3 | <i>Festulolium</i> | Fariaszewska et al. (2017) |
| | –40 | <i>L. perenne</i> | Nie, Ward, and Michael (2001) |

have tended to focus on the impact of a relatively limited number of environmental factors, such as CO₂ and heat. However, it is clear that these are not the only potential effectors of plant productivity and the secondary stresses resulting from primary climate change also need consideration. This review was undertaken to address some of these gaps and in recognition of the need for increased knowledge of stress responses in the context of economically important plant species rather than by relying on extrapolation from models. Furthermore, by identifying commonalities and differences in physiological and metabolic stress responses it should be possible to identify which stress combination would have a more detrimental effect on plant physiology than single independent encounters would be. These findings will enable the development of crop plants better able to tolerate and thrive under future climatic conditions and so maintain production potential. The data used in this review have largely been collected from studies under controlled environments. Although field data could be considered to be more informative, reliably maintaining environmental conditions is difficult under field conditions and hence information is scarcer.

2 | ELEVATED CO₂ LEVELS

Levels of atmospheric CO₂ have increased by more than 100 ppm since the beginning of the industrialization era, and they are projected to reach 550 ppm by 2050 (IPCC 2014).

Previous research has indicated that C₃ photosynthesis is substrate-limited under the current CO₂ atmospheric levels. However, under optimal conditions, plant growth can be encouraged by elevated CO₂ levels due to the stimulation of photosynthesis, and concomitant reductions in

stomatal conductance, dark respiration, and photorespiration. Consequently, higher carbohydrate content and improved water-use efficiency can be attained (Ainsworth & Rogers, 2007; Prasad et al., 2009; Reddy, Rasimeni, & Raghavendra, 2010). Nevertheless, the magnitude of plant responses to changing CO₂ concentrations depends greatly on the availability of potentially limiting resources (Ziska & Bunce, 2007) as well as the type of soil (Nord, Jaramillo, & Lynch, 2015).

3 | EFFECTS OF ELEVATED CO₂ ON COOL-SEASON GRASS PHYSIOLOGY

For cool-season grasses, photosynthesis and stomatal conductance are the main plant physiological functions to be primarily affected by CO₂ levels (Long, Ainsworth, Rogers, & Ort, 2004). Significant decreases in stomatal conductance rates have been reported in a variety of grass species under conditions of increased CO₂ (Farfan-Vignolo & Asard, 2012; Burgess and Huang 2014, Song, Yu, & Huang, 2014). These decreases have been attributed either to alterations in stomatal aperture (Assmann, 1999) or in stomatal density (Lammertsma et al., 2011) (Figure 2). These alterations are usually accompanied by changes in evapotranspiration rates and water-use efficiency (He, Kirkham, Lawlor, & Kanemasu, 1992; Nijs, Ferris, Blum, Hendrey, & Impens, 1997; Nijs, Impens, & Behaeghe, 1989). An additional impact of exposing a currently CO₂-limited C₃ species to higher CO₂ levels is the inhibition of photorespiration, accompanied by increased carboxylation and electron-transfer rates (Leakey et al., 2006; Reddy et al., 2010). This is especially important for grasses since they have the largest potential for enhanced

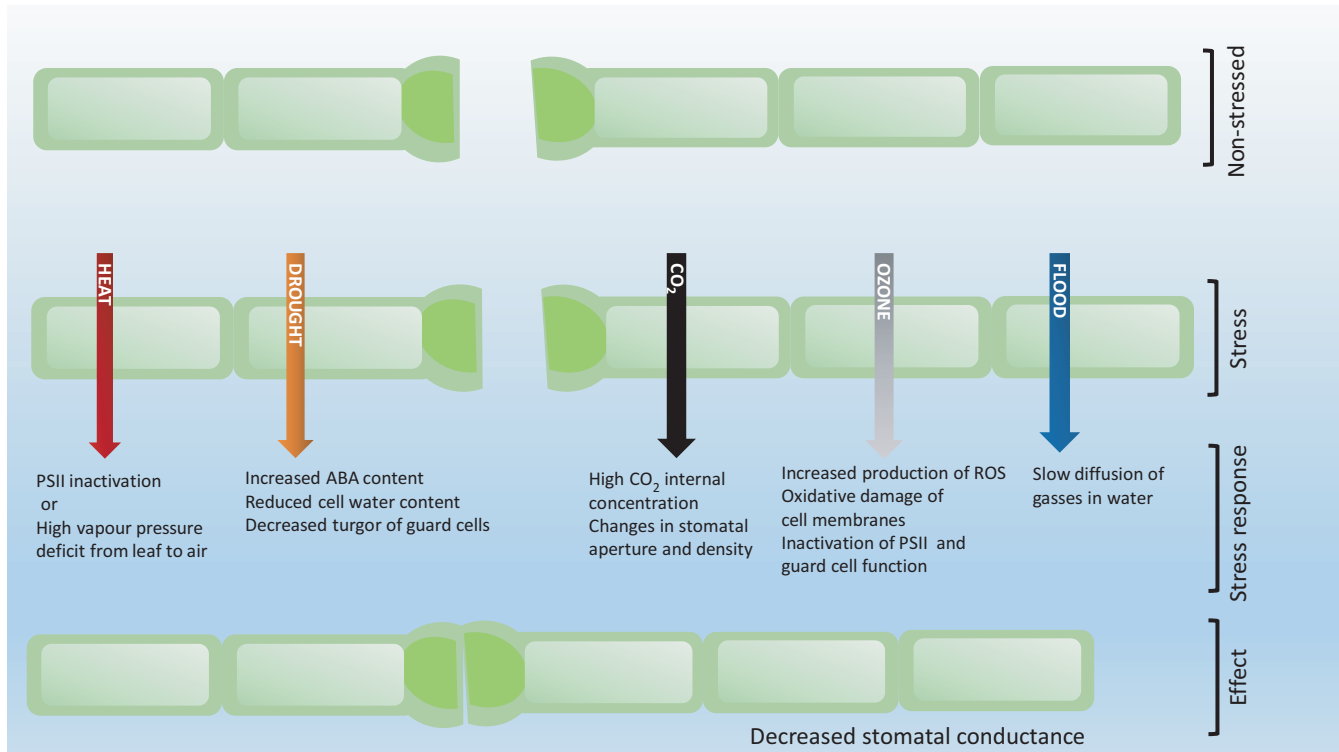


FIGURE 2 Effects of heat stress, drought stress, elevated CO_2 levels, increased ozone concentrations, and flooding on cool-season grasses stomatal conductance

photosynthesis (Ainsworth & Rogers, 2007). Substantial increases in leaf photosynthetic rates were reported in perennial ryegrass, tall fescue, and Kentucky bluegrass (Rogers et al., 1998; Song et al., 2014; Yu, Chen, Xu, & Huang, 2012) when CO_2 concentrations were doubled (800 ppm). Burgess and Huang (2014a,b) also noted significant increases in net photosynthetic rates of creeping bentgrass. These were attributed to either the higher availability of CO_2 or the improved activation state of Rubisco. Casella and Soussana (1997) observed a marked increase in leaf photosynthetic rates of perennial ryegrass after long-term exposure to elevated CO_2 levels, noticing, however, that the increases were dependent on nutrient availability and especially on nitrogen (N) input. Accordingly, a number of studies have indicated that initial positive effects of elevated CO_2 on photosynthesis are rather transient and N-limited, with photosynthetic acclimation observed in longer term experiments (Ainsworth & Rogers, 2007; Urban et al., 2012). Photosynthetic acclimation under elevated CO_2 conditions is associated with the inability of plants to increase their sink strength. Rather than being driven by acclimation of stomatal conductance (Nijs et al., 1997), this is mostly attributed to N-limitations (Rogers et al., 1998; Taub & Wang, 2008), with consequent reductions in Rubisco content in order to balance source activity with sink capacity (Aranjuelo, Cabrera-Bosquet, Araus, & Nogues, 2013). Furthermore, Ainsworth and Rogers (2007) reported that acclimation of photosynthesis in perennial ryegrass under

elevated CO_2 concentrations was due to the substantial limitations in the sink strength observed. Nevertheless, elevated CO_2 concentrations had no effect on light-saturated photosynthesis (A_{sat}) and photochemical efficiency of Photosystem II (PS II) in perennial ryegrass (Farfan-Vignolo & Asard, 2012) which is in contrast with Casella and Soussana (1997). We speculate that this discrepancy was due to the shorter duration to CO_2 exposure (weeks vs. years), since the CO_2 concentrations used in both studies were quite similar (620 ppm vs. 700 ppm).

While the effect of increased CO_2 on cool-season grasses is generally agreed to be beneficial to photosynthesis, the effect of elevated CO_2 concentrations on respiration rates has been more variable. Nijs et al. (1989) reported in perennial ryegrass that there were substantial increases in leaf dark respiration rates after exposure to doubled CO_2 concentrations ($\approx 650 \mu\text{l/L}$), while the opposite conclusion was reported by Bunce and Caulfield (1991) for whole-plant respiration rates. Casella and Soussana (1997) reported that increases in the dark respiration rates of perennial ryegrass were higher during summer than spring. This was attributed to the significant stimulation of the aboveground plant biomass. In contrast, Song et al. (2014) reported markedly lower leaf respiration rates were observed in Kentucky bluegrass when exposed to higher CO_2 than in the control due to suppression of respiratory activity. Root respiration has also been shown to respond to atmospheric CO_2 concentrations (Drake, Muehe,

Peresta, Gonzalez-Meler, & Matamala, 1997). However, the results from the few studies available are contradictory. Root respiration by perennial ryegrass was reported to increase after exposure to elevated CO₂ levels (Casella & Soussana, 1997), while the opposite was reported by Schapendonk and Goudriann (1995). Plant responses to abiotic stresses largely depend on species, the types of tissue investigated, and the growth stage at which the stress occurs (Couee, Sulmon, Gouesbet, & El Amrani, 2006; Gray and Brady 2016). Consequently, apart from the differences in the experimental design among the studies, the above factors should also be taken into consideration in the interpretation of conflicting results. Nevertheless, clearly, further investigation of shoot and root respiration responses to CO₂ concentrations are needed, in order to elucidate the exact effects on each species of cool-season grasses.

4 | EFFECTS OF ELEVATED CO₂ ON COOL-SEASON GRASS METABOLISM

Changes in ambient CO₂ levels are anticipated to result in significant alterations in plant metabolism since CO₂ constitutes the main substrate that drives plant photosynthesis and carbohydrate production.

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the chloroplast-based enzyme responsible for CO₂ fixation within the photosynthetic process, is affected greatly by atmospheric CO₂ levels (Prasad et al., 2009). General decreases in Rubisco content, activity, or activation state have been reported dependent on the extent of CO₂ increase (Galmes, Aranjuelo, Medrano, & Flexas, 2013) and N-availability. Additionally, the plant's capacity to adjust source-to-sink balance, with associated changes on leaf carbohydrate metabolism, also plays a significant role (Ainsworth, Rogers, Nelson, & Long, 2004; Aranjuelo et al., 2013). Further supporting these observations in perennial ryegrass, when grown under elevated CO₂ levels and low N-availability, Rogers et al. (1998) reported that significant decreases in Rubisco's large subunit and content were found. However, the decrease in Rubisco was not observed when perennial ryegrass was provided with high N, indicating a redistribution of resources away from Rubisco. Additionally, recent studies have remarked on the significant variation found in Rubisco quantity and catalytic turnover among and within cool-season grasses which should also be taken into consideration under conditions of elevated CO₂ concentrations (Ishikawa, Hatanaka, Misoo, & Fukayama, 2009; Khaembah et al., 2013).

Fischer et al. (1997) observed substantial increases in leaf carbohydrate content of perennial ryegrass grown under elevated CO₂ levels, irrespective of N-availability, and similar findings were reported by Rogers et al. (1998). In support of

these findings, Yu, Chen, et al. (2012), in experiments with tall fescue plants exposed to increased CO₂ levels, reported substantial increases in hexose and galactose. Accordingly, increases in insoluble (structural) carbohydrates, under similar conditions, were noted in perennial ryegrass (Farfan-Vignolo & Asard, 2012). Higher levels of total non-structural carbohydrates were also observed in perennial ryegrass under higher CO₂ levels by Isopp, Frehner, Long, and Nosberger (2000) with the authors indicating a more pronounced effect under low N-input. A partial explanation for the high levels of sucrose could be that the activity of sucrose phosphate synthase, the main enzyme involved in sucrose synthesis, increased under elevated CO₂ levels. However, the authors noted that sucrose phosphate synthase content was also influenced mainly by N-input, indicating the important role the source-to-sink ratio plays in the regulation of carbohydrate metabolism (Isopp et al., 2000). In accordance with the aforementioned positive results, the levels of non-structural carbohydrates of Kentucky bluegrass grown under optimal temperature and double CO₂ concentration were higher compared to the control (Song et al., 2014) and that was attributed to the positive carbon ratio.

Apart from carbon metabolism, changes in CO₂ atmospheric levels have been reported to have a positive impact on plant defense mechanisms against oxidative damage by increasing antioxidant capacity (Mishra & Agrawal, 2014; Zinta et al., 2014). Farfan-Vignolo and Asard (2012) reported in perennial ryegrass exposed to elevated CO₂ concentrations that both pronounced increases and decreases were observed in the components of the ascorbate/glutathione cycle. However, total antioxidant capacity along with tocopherol and polyphenol contents was unaffected and the authors suggested that this was due to acclimation of the system. Obviously, more studies on the effects of elevated CO₂ on the antioxidant production of cool-season grasses would be helpful to clarify the exact mechanism of the protective effect of CO₂ to oxidative damage.

In summary, increased CO₂ concentrations appear to enhance photosynthetic rates of cool-season turfgrasses despite the decreases observed in their stomatal conductance and Rubisco content and activity. Apart from photosynthesis, higher CO₂ levels also resulted in increases in respiration rates. However, this was not sufficient to reduce carbohydrate content which was higher for plants growing under elevated CO₂ (Figure 3).

5 | HEAT STRESS

Temperature, a key factor of plant growth and development, has been consistently rising annually. This is believed to be due to anthropomorphic effects including industrial emissions. The UK Met Office Hadley Centre reported record

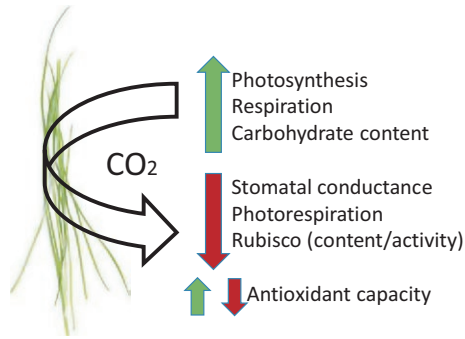


FIGURE 3 Effects of increased CO₂ concentrations on cool-season grasses

temperatures over the last 2 years (2015–2016) and that global temperatures had risen by 1.1°C compared to the 1850–1900 baseline (<http://www.metoffice.gov.uk/news/releases/2017/2016-record-breaking-year-for-global-temperature>). Observations have shown that average T_{\max} and T_{\min} have been increasing by approximately 0.3 and 0.2°C per decade, respectively (Sacks et al. 2010), while climatic models have predicted a further increase between 1.4°C and 5.8°C in global mean temperature by the end of the century (IPCC 2014).

Temperature plays a significant role in the growth, productivity, and distribution of cool-season grass species (Baron & Belangern, 2007). The optimum temperature range has been defined from 15 to 24°C for shoot growth and between 10 and 18°C for root growth (Paulsen, 1994). Increases beyond the optimum temperature threshold that result in damage to plant physiology, metabolism, and productivity are defined as heat stress (Porter 2005). The extent of heat stress damage is variable and dependent largely on the duration and intensity of exposure as well as the rate of increase in temperature. Heat stress effects also change depending on the plant growth stage, along with the place where it is manifested (air or soil) (Huang, Da Costa, & Jiang, 2014; Sung, Kaplan, Lee, & Guy, 2003). Considerable variability exists between and among species in terms of heat tolerance. Among the agricultural grasses, tall fescue is considered to have the greatest heat tolerance, followed closely by Kentucky bluegrass, while perennial ryegrass and creeping bentgrass are considered to be susceptible to heat stress (Bonos & Murphy, 1999; Fry & Huang, 2004). In amenity varieties of the latter two grass species, reductions in turf quality and production due to heat damage are often observed (Huang & Gao, 2000a). Among the ryegrass/fescue species' complex, the North African species Atlas fescue (*Festuca mairei*) has been considered the most heat tolerant and has been incorporated into ryegrass breeding programs in an attempt to introduce this trait to bolster survival and productivity (Humphreys, O'Donovan, Farrell, Gay, & Kingston-Smith, 2014).

6 | EFFECTS OF HEAT STRESS ON COOL-SEASON GRASS PHYSIOLOGY

Photosynthesis is highly sensitive to temperature change and can be inhibited completely by high temperatures (Camejo et al., 2005). Substantial reductions have been observed in the photosynthetic rates of cool-season grasses when the air or soil temperatures have increased beyond the optimal thresholds (Cui, Li, Fan, Xu, & Zhang, 2006; Jiang & Huang, 2000, 2001a,b; Liu, Xie, Du, Sun, & Bai, 2008; Song et al., 2014; Xu & Huang, 2000; Zhao et al., 2008). Xu and Huang (2000) considered that high soil temperatures can be more detrimental to photosynthesis than exposures to high air temperatures. They reported that photosynthetic rates of creeping bentgrass exposed to high soil and cool air temperatures were increased when the conditions were reversed. Optimum temperature thresholds vary among grass species. Song et al. (2014) reported reductions in photosynthetic rates of Kentucky bluegrass at temperatures >25°C, while photosynthesis of creeping bentgrass was compromised by temperatures >23°C (Huang & Gao, 2000b). Reductions in photosynthetic rates under heat stress have also been reported in tall fescue, but in much higher temperatures (>30°C) compared to the rest of the cool-season grasses (Cui et al., 2006; Wang, Cui, Wang, & Li, 2009; Yu, Yang, Jespersen, & Huang, 2014; Zhao et al., 2008). In contrast, Yu, Chen, et al. (2012) observed no decline in photosynthetic rates of tall fescue when grown under 30/25°C (day/night) temperatures. The authors speculated that the applied heat treatment was not enough to result in decreases in photosynthetic rates.

Photosynthetic reductions under heat stress have been attributed to stomatal and non-stomatal limitations with differential responses observed among and within cool-season grass species. For example, stomatal conductance of tall fescue has been reported to decrease under conditions of heat stress (Cui et al., 2006; Wang et al., 2009; Yu et al., 2014; Zhao et al., 2008) with concomitant decreases in leaf net photosynthetic rates. In contrast, the opposite effect was observed by Yu, Chen, et al. (2012) with leaf photosynthetic rates remaining unaffected. Increases in stomatal conductance rates following heat stress were also reported for perennial ryegrass (Farfan-Vignolo & Asard, 2012) accompanied by decreases in leaf photosynthetic rates. Wang et al. (2009) demonstrated that during early stages of heat stress reductions in photosynthesis were due to decreases in stomatal conductance (Figure 2), which in later stages of heat stress was replaced by other non-stomatal limitations. These can be associated with structural changes in the chloroplasts that lead to alterations in energy distribution and carboxylation efficiency of the photosynthetic apparatus (Camejo et al., 2005). Additionally, PS II has been reported

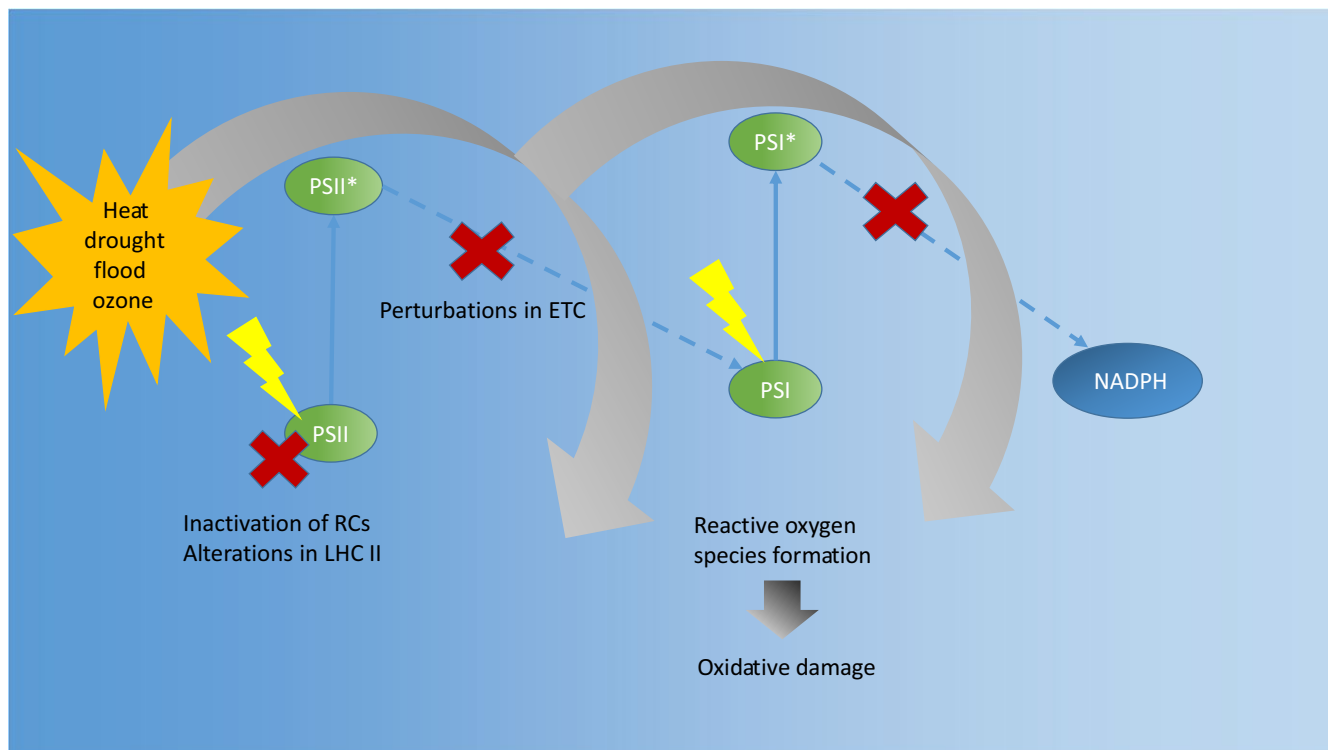


FIGURE 4 Effects of abiotic stresses on PS II and PSI of cool-season grasses. (RC: reaction center, LHC II: light harvest complex II, ETC.: electron-transport chain)

to be the main target of oxidative damage under heat stress (Cui et al., 2006). Specifically, its inhibition has been attributed to alterations in its light-harvesting complex (LHC II), inactivation of its reaction center, and perturbations to the electron-transport chain (Figure 4). As a consequence, reductions in PS II maximum photochemical efficiency and quantum yield under conditions of heat stress have been widely reported in cool-season grasses (Cui et al., 2006; Du, Wang, Yu, Liu, & Huang, 2011; Jespersen, Zhang, & Huang, 2016; Jiang & Huang, 2001a,b; Song et al., 2014; Xu & Huang, 2000). Nevertheless, Yu, Chen, et al. (2012) observed that heat stress had no effect on the maximum photochemical efficiency of PS II of tall fescue and similar results were also reported in perennial ryegrass (Farfan-Vignolo & Asard, 2012). We speculate that this lack of effect was due to the temperatures applied as heat stress ($\leq 30^{\circ}\text{C}$) in those two studies being lower than those applied to the rest of the heat stress studies. It should be noted, however, that significant variability has been reported in the extent of the impacts found among and within the cool-season grass species (Jiang & Huang, 2001a,b; Zhao et al., 2008). In another interesting note, Chen et al. (2014) indicated that lower photochemical efficiency of PS II was positively associated with thermotolerance in tall fescue since oxidative damage was avoided through the limited energy, but further research was needed in order to elucidate the exact mechanism of defense.

In addition to their effect on photosynthesis, higher than optimum temperatures significantly alter plant respiration (Bunce, 2007; Salvucci & Crafts-Brandner, 2004). Increased respiration rates under elevated air temperatures have been reported in Kentucky bluegrass (Song et al., 2014), tall fescue (Yu, Chen, et al., 2012; Zhao et al., 2008), perennial ryegrass (Wehner & Watschke, 1981), and creeping bentgrass (Xu & Huang, 2000). Furthermore, it has been suggested that increased heat tolerance is associated closely with the maintenance of lower respiration rates under raised temperatures (Deal, Raulston, & Hinesley, 1990). Similarly to photosynthesis, high soil temperatures have greater effects than air temperature on plant respiration rates. When compared with high air temperatures, a high soil temperature results in larger increases of both whole-plant and root respiration rates, indicating the root's greater sensitivity to temperature changes. Rachmilevitch, Lambers, and Huang (2006) in studies of rough bentgrass (*Agrostis scabra*), which grows on soil temperatures of 20–50°C with air temperatures ranging between 15 and 27°C, reported that thermotolerance was achieved through downregulation of root respiration which in turn enabled cell membrane thermostability.

A decrease in membrane thermostability is a common observation under heat stress conditions. Inhibition of photosynthesis is known to lead to perturbations in the electron-transfer chain and in overproduction of reactive oxygen species. As a result, peroxidation of membrane lipids is

enhanced and increases in electrolyte leakage occur (Wahid, Gelani, Ashraf, & Foolad, 2007). Chen et al. (2014) reported significant increases in both hydrogen peroxide and the superoxide radical in tall fescue plants exposed to heat stress. Furthermore, Cui et al. (2006) reported that high-temperature stress increased malondialdehyde content, an indication of lipid peroxidation, and electrolyte leakage in both tall fescue cultivars studied in their experiment. Comparable increases in lipid peroxidation and electrolyte leakage, with significant variations among and within species, were also observed in perennial ryegrass (Jiang & Huang, 2001a; Soliman, Fujimori, Tase, & Sugiyama, 2011; Xu, Li, Zhang, Wei, & Cui, 2006), Kentucky bluegrass (Du et al., 2011), and creeping bentgrass (Jespersen et al., 2016) under conditions of heat stress. A linear relationship between hydrogen peroxide content and physiological damage was demonstrated in perennial ryegrass (Soliman et al., 2011). The authors suggested that heat tolerance was associated with critical concentrations of hydrogen peroxide rather than general tolerance to hydrogen peroxide. In support, Farfan-Vignolo and Asard (2012) reported that the onset of a short-term mild heat stress (3°C increase) had no effect on lipid or protein oxidation of perennial ryegrass, indicating the significant role that duration and extent of heat stress play on the manifestation of negative effects on plant physiology.

7 | EFFECTS OF HEAT STRESS ON COOL-SEASON GRASS METABOLISM

Temperature plays a crucial role in all aspects of biological activity since it affects enzyme activity and activation state. For example, Rubisco specificity for CO₂/O₂ is influenced by temperature (Drake et al., 1997) with higher than optimum temperatures leading to increases in photorespiration (Salvucci & Crafts-Brandner, 2004). Accordingly, Wang et al. (2009) reported significant increases in photorespiration by tall fescue when under conditions of heat stress. In addition, substantial decreases in Rubisco activity and Rubisco activase, the enzyme responsible for the catalytic activation of Rubisco (Spreitzer & Salvucci, 2002), were found in creeping bentgrass when exposed to heat stress with concomitant reductions in carboxylation efficiency (Xu and Huang 2001; Liu et al., 2008). Reduced carboxylation efficiency due to lower Rubisco activity was also reported for tall fescue (Cui et al., 2006; Yu et al., 2014) and Kentucky bluegrass (Song et al., 2014). Liu et al. (2008) indicated in creeping bentgrass plants that deactivation of Rubisco is the main reason for the reductions in photosynthesis under heat stress conditions. This was justified by observation that pre-stress heat acclimation resulted in substantially smaller decreases in Rubisco activity and activation state as well as photosynthesis

compared to non-acclimated plants. Regeneration of ribulose 1,5-bisphosphate (RuBP) has also been suggested to be especially sensitive to heat stress (Salvucci & Crafts-Brandner, 2004). In support of this suggestion, Yu et al. (2014) using tall fescue under conditions of heat stress reported significant decreases in the maximum electron-transport rate (J_{max}) which controls the regeneration of RuBP.

Heat stress has been reported to negatively affect chlorophyll and carotenoid content through either suppression of synthesis or acceleration of degradation. Jespersen et al. (2016) indicated that decreases observed in the chlorophyll content, found in hybrids of common bentgrass (*Agrostis capillaris*) x creeping bentgrass (*Agrostis stolonifera*), were mainly due to increases in chlorophyll degradation rather than decreases in chlorophyll synthesis. Substantial decreases in chlorophyll content under increased temperatures have been reported in tall fescue (Cui et al., 2006; Wang et al., 2009; Yu et al., 2014) as well as in creeping bentgrass, Kentucky bluegrass, and perennial ryegrass (Huang, Liu, & Fry, 1998). Nevertheless, Liu et al. (2008) observed that despite the significant decreases in chlorophyll and carotenoid contents of creeping bentgrass under heat stress, the reductions were smaller for heat-acclimated plants. Xu and Huang (2000) also reported that heat stress in the roots had a detrimental effect on total chlorophyll content, even when the leaves were exposed to optimum temperatures. Differential effects of heat stress on chlorophyll a (Chl a) and chlorophyll b (Chl b) content have also been reported with Cui et al. (2006) noticing that heat stress increased Chl a/b ratio in tall fescue but that was due to a larger effect on Chl b content than on Chl a. Increases in Chl a/b ratio have been associated with alterations in the light-harvesting complex system of PS II in order to reduce photooxidative damage (Spundova et al., 2003). However, the opposite effect was observed in a thermotolerant cultivar of Kentucky bluegrass where Chl a remained unaffected while Chl b was significantly increased under heat stress when compared with the control (He & Huang, 2010). The differential responses observed could be attributed not only to the variable temperature thresholds among grass species but also to the varying degrees of thermotolerance within grass species. Together, these results indicate that further investigation of the pathways of chlorophyll synthesis and degradation would be helpful in elucidating the exact mechanism of heat stress response.

Decreases in photosynthesis, with concomitant increases in respiration under conditions of heat stress, have been reported in grasses to result in disturbances in carbohydrate accumulation and a negative correlation between temperature and total non-structural carbohydrate content (Youngner & Nudge, 1976). Previous research in creeping bentgrass (Huang & Gao, 1999; Huang et al., 1998; Liu & Huang, 2000), Kentucky bluegrass (Song et al., 2014; Watschke, Schmidt, & Blaser, 1970), perennial ryegrass (Watschke

et al., 1970), and tall fescue (Zhao et al., 2008) has indicated that higher than optimal growth temperatures have led to significant decreases in total non-structural carbohydrates and fructan contents. In contrast, a different response was recorded for leaf carbohydrates of Kentucky bluegrass under conditions of heat stress (Du et al., 2011). In this work, glucose, fructose, sucrose, galactose, xylose, and mannose increased significantly at the initial stages of stress. However, by the end of the stress period, all the monitored carbohydrates assessed, with the exception of maltose and xylose, had significantly decreased compared to the control (Du et al., 2011). Additionally, significant variations have been found within plant in carbohydrate content when exposed to heat stress conditions. In creeping bentgrass, a reduction in total non-structural carbohydrates and fructans was found to be greater in the roots than in the shoots, while the reverse effect was observed in starch content (Liu & Huang, 2000).

Xu and Huang (2000) reported that in creeping bentgrass an increased soil temperature appears more detrimental for carbon metabolism than are increased air temperatures since reducing soil temperature enhances carbohydrate accumulation under higher than optimum air temperatures. Furthermore, assimilate translocation was suppressed to a greater extent under high soil temperatures than under high air temperatures, indicating root sensitivity to increased temperatures in cool-season grasses (Xu & Huang, 2000). Carbohydrates function as energy reserves either for catabolic or anabolic reactions as well as osmoprotectants under stress conditions (Guy, Kaplan, Kopka, Selbig, & Hincha, 2008). A correlation between heat tolerance and carbohydrate content has been suggested and investigated by Liu and Huang (2000) in creeping bentgrass cultivars differing in thermotolerance. They found higher accumulations of total non-structural carbohydrates and fructans in a heat-tolerant cultivar than in a heat-sensitive cultivar. Further supporting that indication, higher accumulations of carbohydrates in thermotolerant cultivars were also observed in Kentucky bluegrass (Song et al., 2014) and in tall fescue (Sun, Hu, Xie, & Fu, 2014). The observed upregulation of sucrose synthase in the thermotolerant rough bentgrass (*Agrostis scabra*) was also associated with increased root thermotolerance (Xu & Huang, 2008). The authors suggested that the upregulation of sucrose synthase assisted in the maintenance of a positive carbon balance under conditions of heat stress. Together, this indicates that enzymes participating in primary and secondary carbohydrate metabolism are significantly involved in grasses' response to heat stress; however, their precise roles are not yet fully described.

Under conditions of heat stress, the antioxidant mechanisms of the plants are activated and several studies have investigated the effect of heat stress on antioxidant enzyme activities. Variable responses, however, have been reported depending on specific enzymes, extent and duration of stress

as well as the rate of the increase in temperature and the plant species. Liu and Huang (2000) observed that long-term heat stress substantially depressed leaf superoxide dismutase and catalase activities and increased peroxidase activity in creeping bentgrass. Comparable results were also obtained when the heat stress was applied in the root zone (Wang, Pote, & Huang, 2003). Furthermore, Xu et al. (2006) indicated that ascorbate and glutathione concentrations were significantly decreased in both tall fescue and perennial ryegrass under heat stress conditions. Transient increases in catalase, ascorbate peroxidase, glutathione reductase, and superoxide dismutase at the beginning of the heat stress regime were reported in Kentucky bluegrass and tall fescue followed, nevertheless, by significant decreases once the stress had become overwhelming (Jiang & Huang, 2001b). Farfan-Vignolo and Asard (2012) reported that a mild increase in temperature had no effect on total antioxidant capacity of perennial ryegrass; however, significant increases were observed in total polyphenol concentrations as well as peroxidase activities while concentrations of glutathione reductase significantly decreased. Since all of the above studies were conducted under controlled conditions with potted plants, we speculate that the varying responses observed were due to differences among grass species as well as the extent and duration of heat stress.

Maintenance of higher antioxidant enzyme activities under conditions of higher than optimum temperatures has been suggested to be correlated positively with heat tolerance. In support of that observation, Xu et al. (2006) reported that a moderately heat-tolerant tall fescue cultivar had higher concentrations of ascorbate and glutathione under heat stress conditions compared with the concentrations found in the less heat-tolerant perennial ryegrass. Apart from among species, significant variation in thermotolerance is also observed within species. Accordingly, He and Huang (2010) reported in Kentucky bluegrass that higher activities of superoxide dismutase and ascorbate peroxidase, for singlet oxygen and hydrogen peroxide scavenging, respectively, were associated with increased heat tolerance. Similarly, Li, Zhan, Xu, and Zhang (2014) and Du, Zhou, and Huang (2013) observed that the thermotolerant cultivar of Kentucky bluegrass in their studies had higher activities of superoxide dismutase, ascorbate peroxidase, peroxidase, and catalase compared with that found in the heat-sensitive cultivar for the duration of the stress. Higher activities of superoxide dismutase and catalase were also recorded in a heat-tolerant creeping bentgrass cultivar compared to one that was heat-sensitive (Huang, Liu, & Xu, 2001; Liu & Huang, 2000; Wang et al., 2003).

Summarizing, heat stress is detrimental for cool-season grasses physiology and metabolism since it results in substantial decreases in photosynthesis due to stomatal and non-stomatal limitations (Figure 5). Concomitant increases in ROS production result in significant oxidative stress and

cell membrane damage; however, there is consistent evidence that high antioxidant concentrations are positively associated with thermotolerance. Similarly, accumulation and maintenance of high levels of carbohydrates are linked to thermotolerance, probably due to their additional roles as ROS scavengers and osmoprotectants (Couee et al., 2006; Keunen, Peshev, Vangronsveld, van den Ende, & Cuypers, 2013). Nevertheless, even though the majority of studies reported higher rates of respiration and photorespiration coupled with lower photosynthetic rates, results regarding carbohydrate content of cool-season grasses under heat stress are inconclusive. Aside from differences in experimental design, including variable duration and extent of stress, the inherent variation that exists among and within grass species could be another reason for those discrepancies. Furthermore, the type of tissue or organ that is investigated as well as the plant's growth stage at which the stress is applied appear to play a pivotal role to the plant's response to heat stress.

8 | DROUGHT STRESS

The stress from water-deficit is a major abiotic factor restricting plant growth and crop productivity around the world (Kramer, 1983). Despite water covering about three quarters of the planet, fresh water accounts for only 2.5%, and of this, only 0.6% is available and suitable for use by living organisms (Staniak & Kocon, 2015). According to recent statistics, one-third of cultivated areas suffer from inadequate supplies of water, either due to low precipitation or from insufficient irrigation. Furthermore, water-deficit stress is expected to be intensified not only due to the projected climate trends, but also due to the population increases and water requirements for increased agricultural production (IPCC 2014).

In physiological terms, plant water stress is defined as the state where the plant's water potential and turgor is reduced to a sufficient extent to inhibit normal plant function (Hsiao, 1973). This is determined by both the supply of water to the soil, which is influenced by soil type, texture and hydraulic conductivity, and the evaporative demand of the atmosphere.

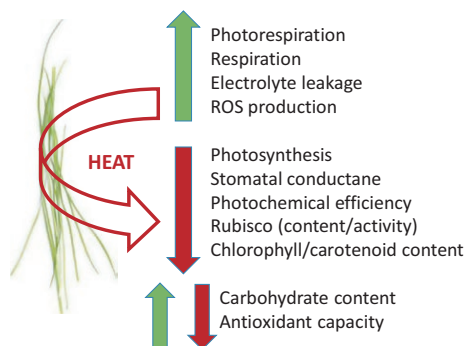


FIGURE 5 Effects of heat stress on cool-season grasses

Additionally, the ability of plants to extract water effectively from soils, which is largely dependent on the extent and depth of their root systems, has a significant effect. Since water is the primary component of plants, ranging from 70 to 90% of plant fresh biomass (Gardner & Gardner, 1983), water availability, and quality play fundamental roles in plant morphology, physiology, and metabolism (Hsiao, 1973). However, the extent of water-deficit effects depends on the severity and duration of stress, the developmental stage at which stress is imposed as well as the genotype of the plant (Kramer, 1983).

Drought escape, avoidance, and tolerance are the main strategies plants have developed in order to continue growth or ensure survival under conditions of limited water availability (Levitt, 1980). Drought escape is associated with plants that accelerate their growth rates when water supply is adequate and enter dormancy when water becomes scarce (Kramer 1980). Examples include grasses such as the Mediterranean fescue, (*Festuca arundinacea* var *glaucescens*) (Humphreys, Harper, Armstead, & Humphreys, 2005), which moderates its growth according to the available water (Assuero et al., 2000). Kentucky bluegrass, which can sustain growth through periods of limited water supply due to its rhizomes, is also included in this category (Fry & Huang, 2004).

Drought avoidance is employed by plants that maintain high tissue water potential under conditions of limited water supply by reducing leaf water loss while maintaining a high water uptake rate through the development of a larger root system (Levitt, 1980). Drought-tolerant plants, on the other hand, sustain low tissue water potential and maintain turgor under conditions of water-deficit. This is accomplished by accumulating variable compatible solutes, such as carbohydrates, amino acids, and mineral ions and consequently decreasing the plants' cell osmotic potential (Levitt, 1980). Drought avoidance plants, such as tall fescue, are characterized by various morphological alterations, including decreased number of stomata and leaves, increased leaf pubescence, increased root plasticity, and extension (Qian, Fry, & Upham, 1997). On the other hand, drought-tolerant plants, such as Kentucky bluegrass and creeping bentgrass, maintain growth through alterations in physiology and metabolism (Nilsen & Orcutt, 1996). For agricultural and livestock production, in the absence of extreme soil water-deficit, some continued growth when under suboptimal water supplies is necessary.

9 | EFFECTS OF DROUGHT STRESS ON COOL-SEASON GRASS PHYSIOLOGY

Water-deficit stress affects several plant physiological processes in complex and interrelated ways. Cellular water content plays a pivotal role in plants' responses to limited

water supply since it largely controls stomatal aperture and consequently CO₂ diffusion and photosynthetic carbon fixation.

Cellular water content, monitored either as relative water content or leaf water potential, has been reported to substantially decrease in cool-season grasses including tall fescue, Kentucky bluegrass, perennial ryegrass, and creeping bentgrass (Bian & Jiang, 2009; Jiang & Huang, 2000; Kosmala, Perlikowski, Pawlowicz, & Rapacz, 2012; Thomas, 1987, 1991; Xu, Yu, Han, & Huang, 2013; Zwicke, Picon-Cochard, Morvan-Bertrand, Prud'homme, & Volaire, 2015). Nevertheless, differential responses in relative water content within species have been observed. For example, in potted experiments with Kentucky bluegrass cultivars differing in drought tolerance, Abraham, Huang, Bonos, and Meyer (2004) reported that the more tolerant cultivars suffered less severe decreases in relative water content compared to the drought-sensitive ones. Thomas (1991), additionally, reported on differences in osmotic potential between bases and laminae in perennial ryegrass under conditions of water-deficit. Furthermore, Huang and Fu (2000), in potted experiments under controlled conditions, observed that tall fescue and Kentucky bluegrass plants were able to maintain leaf water potentials similar to an unstressed control when drought was applied only to the upper (20 cm) soil profile. However, that was reversed under conditions of full drought (40 cm), indicating not only the important role that the spatial distribution of roots plays in drought tolerance but also the significance of the way the stress is imposed.

Stomatal aperture controls water loss from the plant, and a positive correlation has been suggested between leaf water potential and stomatal conductance (Socias, Correia, Chaves, & Medrano, 1997) (Figure 2). Supporting this view in the grasses, Kentucky bluegrass (Xu et al., 2013), perennial ryegrass (Abdelgawad, Farfan-Vignolo, de Vos, & Asard, 2015; Farfan-Vignolo & Asard, 2012), creeping bentgrass (Huang et al., 2014), and tall fescue (Yu, Chen, et al., 2012), suboptimal water supplies have generated marked decreases in stomatal conductance and consequently in transpiration. Nevertheless, considerable variation in the extent of the physiological responses to water-deficit exists both among and within cool-season grass species. Specifically, drought-tolerant cultivars and species demonstrate substantially smaller reductions in their cellular water content, stomatal conductance, and transpiration rates (Abraham et al., 2004; Hu, Wang, & Huang, 2010; Kosmala et al., 2012; Yu, Chen, et al., 2012) when compared directly to their drought-sensitive counterparts. Further research, however, is needed in order to elucidate the exact pathways that plants use to maintain such high relative water content and achieve higher drought tolerance.

Relative water content and stomatal behavior have been reported to play a major role in photosynthetic responses of plants under water-deficit stress (Chaves, 1991; Cornic, 1994; Lawlor & Corninc, 2002). Even though photosynthesis is not as sensitive to drought stress as it is to heat stress, marked reductions in photosynthetic rates have been reported in tall fescue (Huang & Fu, 2000; Kosmala et al., 2012; Yu, Chen, et al., 2012), Kentucky bluegrass (Bian & Jiang, 2009; Huang & Fu, 2000; Xu et al., 2013), perennial ryegrass (Abdelgawad, Farfan-Vignolo, et al., 2015; Farfan-Vignolo & Asard, 2012), and creeping bentgrass (Fu & Dernoeden, 2008). These reductions were attributed to both stomatal and non-stomatal limitations. Non-stomatal limitations, including reductions in mesophyll conductance and photochemical efficiency, have been widely reported under conditions of water-deficit stress (Farfan-Vignolo & Asard, 2012; Hu et al., 2010; Jiang & Huang, 2000; Yu, Chen, et al., 2012) (Figure 4). Significant decreases in electron-transport rate, photochemical efficiency, and yield of PS II were also recorded in certain *Festulolium*, a ryegrass/fescue species' hybrid, with concomitant increases in non-photochemical quenching (Koscielniak, Filek, & Biesaga, 2005). Stress level and duration appear to exert significant control on the outcomes following limited water supply on photochemical efficiency of PS II resulting in variable responses. Bian and Jiang (2009), using Kentucky bluegrass, reported that a moderate water-deficit stress had little effect on photochemical efficiency. Yu, Chen, et al. (2012) observed that photochemical efficiency of tall fescue was greatly compromised following a prolonged period of water stress (28 days), but not following a short-term stress (7 days), probably due to the higher drought tolerance of tall fescue. In contrast, Kosmala et al. (2012) observed a significant compromise to the photochemical efficiency of PS II in a drought-sensitive tall fescue cultivar following a short (6 days) mild drought treatment, indicating the significant variability that exists within grass species.

Respiration is another physiological function regulated by plant relative water content and consequently by water supply. Past research indicated that respiration has a biphasic response to drought stress with decreases observed at the initial phases of stress, followed by substantial increases once the stress becomes more severe (Flexas, Bota, Galmes, Medrano, & Ribas-Carbo, 2006). Regarding cool-season grasses, Yu, Chen, et al. (2012) observed significant increases in respiration rates of tall fescue after prolonged water-deficit stress and similar results were obtained in Kentucky bluegrass (Hu et al., 2010). In contrast, Huang and Fu (2000) reported substantial decreases in canopy and root respiration rates of tall fescue and Kentucky bluegrass plants exposed to drought at different layers of the soil profile. They observed that dry conditions at the upper soil layers (20 cm) were not as detrimental as fully dried conditions leading us to conclude that

this was due to the active living roots of the grasses present at depth in the soil profiles. Fu and Dernoeden (2008) found no significant effect of mild water stress on whole-plant respiration rates of tall fescue plants. However, in a later study, Burgess and Huang (2014a,b) reported that metabolites, involved in the tricarboxylic acid cycle of dark respiration, were downregulated in roots of drought-stressed creeping bentgrass plants, indicating a decrease in root dark respiration rates under limited water supply conditions.

Increases in membrane damage are often observed in drought-stressed plants, and significant increases in electrolyte leakage have been reported in cool-season grasses, including creeping bentgrass (Burgess & Huang, 2014a,b; Merewitz, Gianfagna, & Huang, 2011; Xu, Burgess, Zhang, & Huang, 2016), tall fescue (Huang & Gao, 1999; Yu, Chen, et al., 2012), perennial ryegrass (AbdElgawad, Farfan-Vignolo, et al., 2015; Farfan-Vignolo & Asard, 2012), and Kentucky bluegrass (Abraham, Meyer, Bonos, & Huang, 2008; Jiang & Huang, 2001b; Yang, Xu, Yu, DaCosta, & Huang, 2013). However, Yu, Chen, et al. (2012) emphasized the importance of stress duration to the extent of cell membrane damage caused by drought stress. They indicated that a short-term stress had no effect on electrolyte leakage of tall fescue in contrast to a prolonged stress which resulted in substantial increases. Abraham et al. (2008) pointed out that drought-tolerant cultivars of Kentucky bluegrass had better membrane stability under conditions of drought stress compared to those that are drought sensitive, and similar observations were also made in tall fescue (Sarmast, Salehi, & Niazi, 2015).

10 | EFFECTS OF DROUGHT ON METABOLISM OF COOL-SEASON GRASSES

Plant responses to drought stress include changes in various metabolic processes such as photosynthesis and carbohydrate accumulation (Nilsen & Orcutt, 1996). Decreases in photosynthesis due to non-stomatal limitations that become more prominent under conditions of severe drought stress are associated mainly with metabolic impairments of Rubisco activity (Feller, 2016; Galmes et al., 2013) and decreases in chlorophyll content. Significant decreases in Rubisco activity and activation state have been reported in cool-season grasses under conditions of limited water supply, including Kentucky bluegrass (Hu et al., 2010; Xu et al., 2013), tall fescue (Yu, Chen, et al., 2012) and creeping bentgrass (Burgess & Huang, 2016). Xu et al. (2013) reported that drought-tolerant cultivars of Kentucky bluegrass suffered much lower reductions in Rubisco activity and activation state compared to those that were drought-susceptible, indicating the important relationship between Rubisco metabolism and drought

tolerance. Hu et al. (2010) observed that Rubisco activity of drought-tolerant Kentucky bluegrass plants after re-watering returned to similar levels to that found in their irrigated controls. On the other hand, in drought-sensitive plants, Rubisco activity never fully recovered, and as a consequence, their photosynthetic rates remained at a consistently lower rate than their controls.

Decreases in chlorophyll and carotenoid content have been widely reported to occur under conditions of limited water supply, ultimately leading to significant inhibitions in leaf photosynthetic rates (Farooq, Wahid, Kobayashi, Fujita, & Basra, 2009). However, differential responses have been observed in the chlorophyll content of cool-season grasses under conditions of limited water supply. Fu and Huang (2001) observed that surface soil drying had no significant effects on chlorophyll content of tall fescue and Kentucky bluegrass. However, chlorophyll content was significantly decreased under conditions of full drought. Additionally, Jiang and Huang (2001b) reported transient increases in chlorophyll content of both tall fescue and Kentucky bluegrass early into drought stress treatments. This was followed by substantial reductions in chlorophyll contents once the stress had become more severe. Similar results with significant increases in chlorophyll content under mild water-deficit were also reported in tall fescue by Ebrahimiyan, Majidi, Mirlohi, and Noroozi (2013). Nevertheless, under conditions of severe drought stress, chlorophyll concentrations were markedly decreased, and the total carotenoid content and Chl a:Chl b ratio were substantially increased. In contrast, AbdElgawad, Farfan-Vignolo, et al., 2015 observed a consistent and significant reduction in chlorophyll a content in perennial ryegrass exposed to limited water supply. Furthermore, Sarmast et al. (2015), in potted experiments with eleven commercial fescue cultivars, found there to be a gradual but consistently decreasing chlorophyll and carotenoid content in drought-sensitive genotypes of fescue when exposed to water stress. In contrast, in drought-tolerant genotypes, the changes in chlorophyll and carotenoid content were minimal.

Carbon metabolism is affected strongly by water-deficit, and several studies have demonstrated a positive correlation between drought tolerance and carbohydrate concentrations (Livingston, Hinch, & Heyer, 2009; Voltaire et al., 1998). Water-soluble carbohydrates, hexose, and sucrose have been shown to enhance osmotic adjustment and cell membrane stabilization, while storage carbohydrates, fructans, and starch act as energy reserves (Kaur, Gupta, & Kaur, 2007; Livingston et al., 2009). Significant increases in total non-structural carbohydrates under conditions of limited water supply have been reported in several cool-season grass species (Busso et al. 1990; Huang & Fu, 2000; Huang et al., 2001; Huang & Gao, 2000a; Da Costa & Huang, 2006; Fariaszewska et al., 2017). Thomas (1991) in studies with perennial ryegrass under drought stress conditions, remarked

on the differential pattern observed between leaves and bases, with the latter containing substantially more carbohydrates. In addition, Fu, Huang, and Fry (2010) indicated that water-deficit had a significant impact on leaf carbon metabolism in tall fescue by markedly increasing sucrose phosphate synthase and sucrose synthase activities. Concomitantly, acid invertase activity was suppressed, which ultimately resulted in a marked increase in leaf sucrose content. Analogous results were observed in a drought-tolerant Kentucky bluegrass cultivar under conditions of drought stress, while at the same time, total non-structural carbohydrate concentrations and activities of sucrose-metabolizing enzymes, remained unaffected in a drought-susceptible cultivar (Yang et al., 2013). The authors concluded that there was a positive correlation between sucrose concentration and drought resistance. They also indicated that fructan availability had a vital role in plant regrowth during recovery (Yang et al., 2013). Furthermore, Zwicke et al. (2015) found that under mild drought stress, sucrose concentrations increased in leaf meristems of tall fescue and Kentucky bluegrass, indicating the importance of sucrose in osmotic adjustment and dehydration avoidance. However, when these grasses were exposed to severe drought conditions, the leaf sucrose and fructan contents remained unaffected and root sucrose content significantly decreased. The better survival rates of Kentucky bluegrass compared to tall fescue under conditions of severe drought stress were attributed to the higher concentrations of high DP (degree of polymerization >5) fructans in their storage organs (Zwicke et al., 2015).

Oxidative damage, due to overproduction of reactive oxygen species such as superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) and manifested through lipid peroxidation, protein degradation, and carbohydrate consumption, has been closely associated with drought stress (Halliwell, 2006; Smirnov, 1993). Increased lipid and protein peroxidation under conditions of drought stress have been recorded in several grass species (Da Costa & Huang, 2007; Farfan-Vignolo & Asard, 2012; Fu & Huang, 2001; Jiang & Huang, 2001a,b; Merewitz et al., 2011; Xu et al., 2016). Nevertheless, the extent of damage is strongly dependent on plant species, duration, and timing of stress, as well as the type of tissue (Jiang & Huang, 2001a,b; Xu et al., 2016), with substantial variation existing also within species (Kosmala et al., 2012).

Previous studies have indicated that higher activity levels of antioxidant enzymes may contribute to providing for a better drought tolerance by increasing a plant's protection capacity against oxidative damage (Larkindale & Huang, 2004). Cool-season grass species vary in response by their antioxidative machinery, dependent on their genotype and the intensity of the stress. Farfan-Vignolo and Asard (2012) observed significant increases in total antioxidant capacity and content of tocopherols in drought-stressed perennial ryegrass. In contrast, AbdElgawad, Farfan-Vignolo et al. (2015)

reported that drought stress had no significant effect on either total antioxidant capacity or content of tocopherols in perennial ryegrass. However, the stress imposed in their study was far more gradual and less severe compared to Farfan-Vignolo and Asard (2012). Nevertheless, significant increases in antioxidant capacity were observed in the more drought-tolerant species of Kentucky bluegrass AbdElgawad, Farfan-Vignolo, et al., 2015;. Fu and Huang (2001) reported that in Kentucky bluegrass and tall fescue superoxide dismutase activity was increased in leaves of water-stressed plants, while catalase and peroxidase activity remained similar to that observed in the controls under conditions of mild drought. However, superoxide dismutase, catalase, and peroxidase activity decreased once the stress had become more severe. Significant increases in superoxide oxidase and ascorbate peroxidase content were also noted in different genotypes of fescue under water-deficit; again, the extent of the increase was dependent on the extent of their drought resistance (Sarmast et al., 2015). Kentucky bluegrass, in contrast, showed no significant effect of drought stress on leaf superoxide dismutase activity (Zhang & Schmidt, 1999). Da Costa and Huang (2007) reported a consistent decrease in both superoxide dismutase and catalase activities in the leaves of creeping bentgrass when exposed to prolonged water-deficit, and similar decreases in superoxide dismutase expression were reported in the roots of drought-stressed creeping bentgrass (Xu et al., 2016). Root catalase activity was substantially higher and peroxidase activity was decreased, while glutathione and total ascorbate content remained unaffected (Xu et al., 2016). Apart from the variable antioxidant responses between different tissue types, Xu et al. (2015) also reported the contrasting antioxidant activities they found between the root elongation and maturation zones in tall fescue plants under conditions of drought stress.

Aside from their inherent antioxidant capacity, infection of cool-season grasses with mutualistic fungal endophyte strains of *Neotyphodium* species, such as *Epichloe cenophiala* and *Epichloe festucae*, has been reported to increase their protection against oxidative damage under water-deficit stress conditions through increases in their antioxidant capacity (Malinowski, Alloush, & Belesky, 1998; Malinowski, Kligel, & Pinchack, 2009; Zhou, Gao, & Ma, 2003). In studies with tall fescue, Fike et al. (2001) reported that endophyte-infected plants had higher activity of superoxide dismutase. Comparable results were also noted by Briggs, Crush, Ouyang, and Sposen (2013) in endophyte-infected perennial ryegrass plants subjected to drought stress, while Leuchtman, Bacon, Schardle, White, and Tadych (2014) observed higher ascorbate peroxidase activity in perennial ryegrass plants infected with *E. festucae* var. *lolii*.

Apart from changes in antioxidant concentrations, drought stress is also associated with the production of compatible solutes such as proline, in order to sustain turgor and

maintenance of cell membrane stability. Fariaszewska et al. (2017) observed substantial increases in proline content of meadow fescue under conditions of mild drought stress, whereas in tall fescue, leaf proline content was increased under both mild and severe water-deficit stress (Ebrahimiyan et al., 2013). Sarmast et al. (2015) also using tall fescue reported significant increases in leaf proline content; however, the increases were observed only in drought-susceptible cultivars, and in contrast, the changes were only minimal in the drought-tolerant cultivars tested. Substantial increases in leaf proline content were also reported in perennial ryegrass, with leaf bases, in particular, accumulating significantly higher quantities of proline when under water-deficit stress conditions. In contradiction to this finding, AbdElgawad et al. (2015b) observed proline content of perennial ryegrass when exposed to drought stress to remain largely unaffected. However, they found that proline did increase significantly in Kentucky bluegrass.

Summarizing, drought stress adversely affects photosynthesis, due to stomatal or non-stomatal constraints, depending on the drought's severity and extent of duration. Furthermore, under limited water availability, production of reactive oxygen species is intensified, leading ultimately to substantial oxidative damage to the plants, despite the significant increases in carbohydrate levels that double in their role as ROS scavengers (Figure 6). The variable responses observed to the total antioxidant capacity of plants under water-deficit stress, comparable to those under heat stress and elevated CO₂ levels. Since all experiments were conducted under controlled conditions with potted plants, we speculate that differing outcomes were due to differences in the experimental procedures including different ambient temperatures, growth mediums, duration, and severity of stress. Additionally, considerable variation, in terms of drought tolerance, exists among and within grass species. This variation, in conjunction with the differential responses observed among tissues (e.g., roots vs. leaves, leaves vs. bases) and within tissues (e.g., root elongation zone vs. root maturation zone), makes the comparison and interpretation of results quite difficult.

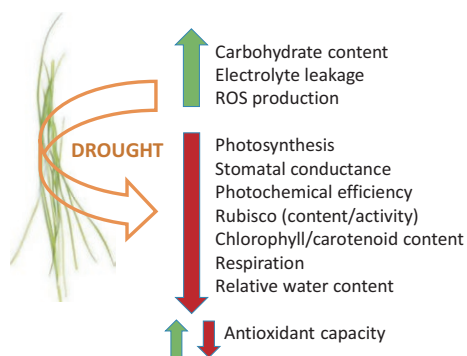


FIGURE 6 Effects of drought stress on cool-season grasses

11 | FLOODING

Flooding affects almost 10% of the global land area (Setter & Waters, 2003), and future climate projections are anticipating increases in the frequency and intensity of extreme precipitation events (IPCC 2014). Depending on the extent of water involved, flooding includes both waterlogging and submergence. In the case of waterlogging, only the roots are exposed to wet conditions, whereas in the case of submergence, partial or whole immersion of the shoots is observed. In both cases, over-saturation of the soil with water has a negative impact on the whole ecosystem since all available oxygen in the soil is rapidly consumed by soil microbial organisms and plant root respiration (Vashisht et al., 2011). Since gas diffusion into the soil is severely inhibited by the presence of water, anoxia sets in and soil redox potential significantly decreases. As a result, increases in denitrification, manganese, and iron reduction accompanied by the elimination of carbon reserves and increases in sulfide content are observed (Adams & Akhtar, 1994; Sasidharan & Voesenek, 2015). Waterlogged plants can still supply oxygen to the roots through shoot photosynthesis; however, the supply rate is largely dependent on sink strength, tissue porosity, and root respiratory demand (Vashisht et al., 2011). Nevertheless, more often than not, the anoxic soil conditions force roots into anaerobic mode in order to maintain energy production and plant function. Eventually, root growth and function compromises are observed, along with inhibition of water and nutrient uptake and translocation. As a result, leaf senescence and, ultimately, plant death occur (Bailey-Serres, Lee, & Brinton, 2012; Sasidharan & Voesenek, 2015).

In the event of submergence, where photosynthesis and, consequently, oxygen supply to the roots, are inhibited (Voesenek & Bailey-Serres, 2015), plants unavoidably enter into either escape or quiescence mode (Bailey-Serres et al., 2012), depending on the extent of their immersion. The escape mode is usually activated under partial immersion. It includes rapid shoot growth to ensure maintenance of photosynthesis, and production of aerenchyma in order to enable the successful transfer of oxygen to the roots (Colmer, 2003; Pierik, Djakovic-Petrovic, Keuskamp, de Wit, & Voesenek, 2009; Sasidharan & Voesenek, 2015). When totally immersed, plants enter into a quiescence mode by restricting cellular metabolism and growth (Bailey-Serres et al., 2012).

Plant growth reductions under conditions of excess water, due to physiological and metabolic limitations, have been reported to vary between 15 and 80% of optimum yield. The extent of the damage incurred is dependent on several factors, including the soil type, duration and depth of the flood, and temperature, as well as plant species and growth stage (Bailey-Serres et al., 2012; Setter et al., 2009; Striker & Colmer, 2016). Waterlogging tolerance is dependent on the

extent of oxygen limitation as well as morphological, physiological, and metabolic alterations (Setter & Waters, 2003). Considerable variability in terms of flooding tolerance exists among and within cool-season grasses (Jiang & Wang, 2006; Wang & Jiang, 2007; Yu, Nuo, et al., 2012). Therefore, there is considerable potential for increased yield loss due to climate change-related flooding, but there is also potential for mitigation through identification and exploitation of adaptive germplasm. Considering that grasslands often frequent areas of high rainfall and are prone to flooding, the impacts of climate change are likely to make flooding incidents more severe. Thus, further investigation into the physiology, metabolism, and morphology, necessary for flood tolerance in cool-season grass cultivars, is a priority and is urgently needed for future plant breeding.

12 | EFFECTS OF FLOODING ON COOL-SEASON GRASS PHYSIOLOGY

Plant physiological functions are affected greatly by flooding with the extent of the damage highly dependent on the sensitivity of plants to prolonged exposures to excess water.

Waterlogging has been shown to affect plant nutrient availability (Ashraf & Harris, 2013) due to alterations in element solubility in the soil when under anoxic conditions. Additionally, changes in the ion selectivity by root cells, under waterlogged conditions, can lead to reductions in nutrient uptake, translocation, and distribution (Bailey-Serres et al., 2012; Setter et al., 2009). Accordingly, Adams and Akhtar (1994) observed significantly higher levels of manganese and iron in the leaves of waterlogged perennial ryegrass when compared to the control, while nitrogen uptake was markedly decreased due to increased denitrification in the soil.

The significant reductions observed in photosynthetic rates of waterlogged plants have been partly attributed to nutrient deficiencies (Smethurst, Garnett, & Shabala, 2005). However, the principal explanation likely relates to the fact that gas diffusion through water is 10^{-3} times slower than through air. The outcome is that plant gas exchange functions are severely impaired (Bailey-Serres et al., 2012; Sibbernsen & Mott, 2010).

A rapid decline in stomatal conductance under conditions of excess water has been reported in several plant species (Folzer, Dat, Capelli, Rieffel, & Badot, 2006; Kozłowski & Pallardy, 1984) leading to significant reductions in leaf photosynthetic rates. McFarlane, Civarella, and Smith (2003) observed marked reductions in stomatal conductance rates in perennial ryegrass when under waterlogged conditions, followed by concomitant decreases in leaf photosynthetic rates (Figure 2). Comparable results

were also reported in creeping bentgrass (Huang et al., 1998). Reductions in photosynthetic rates of perennial ryegrass under conditions of submergence were also reported by Yu, Nuo, et al., (2012), but in this instance, these were attributed to compromises in the photochemical efficiency of PS II (Figure 3). Contrastingly, Ploschuk, Grimoldi, Ploschuk, and Striker (2017), in experiments with tall fescue and *Phalaris aquatica* plants, reported that 15 days of waterlogging had no effect on stomatal conductance and net photosynthesis of either species. Furthermore, photosynthetic rates of submerged *Phalaris arundinacea* plants remained unaffected after 30 days of submergence (Vervuren, Beurskens, & Blom, 1999); however, stomatal conductance rates were not estimated. Clearly, further investigation and more detailed measurements are needed in order to elucidate the effect of water-excess on the photosynthetic mechanism of the plants.

Serious compromises to cell membrane properties and stability have been reported under conditions of suboptimal oxygen supply (Shabala, 2011). Wang and Jiang (2007) in Kentucky bluegrass under waterlog conditions noted substantial increases in root electrolyte leakage. However, maintenance of root cell membrane stability is positively associated with tolerance to flooding since electrolyte leakage of flood-tolerant cultivars were less affected compared to flood-sensitive cultivars.

Under waterlogging conditions, oxygen supply to the roots is severely inhibited leading to significant reductions in root respiration. Decreases in root aerobic respiration rates is one of the earliest responses of plants when under flooded conditions (Colmer, 2003; Liao & Lin, 2001) since mitochondrial function is highly dependent on oxygen availability (Vartapetain, Andreeva, & Nuritdinov, 1978). Substantial mitochondrial swelling was observed in the root cells of waterlogged Kentucky bluegrass (Jiang & Wang, 2006); however, root respiration rates were not determined. Leaf respiration responses to waterlogging are also under-reported, but Huang et al. (1998) did measure significant increases in leaf respiration rates in creeping bentgrass when grown under low aeration conditions.

13 | EFFECTS OF FLOODING ON COOL-SEASON GRASS METABOLISM

Rubisco content and activity have been reported to be adversely affected by excess water, leading to significant reductions in photosynthesis in various plant species (Ahsan et al., 2007; Lin, Lin, Chen, Shen, & Lo, 2015). Substantial decreases in soluble protein content were observed in Kentucky bluegrass and creeping bentgrass under flood conditions (Jiang & Wang, 2006; Wang & Jiang, 2007; Wang et al.,

2009); however, more research needs to be dedicated to understanding responses of Rubisco under flooding conditions in cool-season grasses.

Oxygen depletion due to submergence has a major impact on root metabolism, since plants are forced to switch from aerobic to anaerobic respiration in order to maintain energy production and growth. Depending on the severity and the extent of flooding stress, plant anaerobic catabolism may enter one of two pathways, namely alcoholic fermentation or lactic acid fermentation. Alcoholic fermentation is controlled by alcohol dehydrogenase and pyruvate decarboxylase, while lactic fermentation is controlled by lactate dehydrogenase (Drew, 1997). Wang et al. (2009) indicated that short-term waterlogging stress mainly activated the alcoholic fermentation pathway in the roots of Kentucky bluegrass. Root oxidase activity, an indicator of oxygen diffusion and oxidation capacity in the root, was initially increased under waterlogging conditions in Kentucky bluegrass, but was decreased when under prolonged stress conditions (Wang & Jiang, 2007). This was associated with the root carbohydrate content which increased initially but later decreased after prolonged stress.

Severe decreases in chlorophyll and carotenoid content have also been reported to occur under conditions of excess water (Bailey-Serres et al., 2012). With the exception of *P. arundinacea*, which was reported to maintain its chlorophyll content even after 30 days of submergence (Vervuren et al., 1999), a decreasing pattern in chlorophyll and carotenoid content appears in temperate grasses. For example in both creeping bentgrass and Kentucky bluegrass, significant decreases in chlorophyll content have been noted at various levels of waterlogging stress (Huang et al., 1998; Jiang & Wang, 2006; Puyang, An, L., Han, & Zhang, 2015; Wang & Jiang, 2007). The authors indicated that water depth, as well as stress duration, played an important role in chlorophyll concentration responses to excess water. Liu and Jiang (2015) observed that both waterlogging and submergence had a detrimental effect on the chlorophyll content of perennial ryegrass, with the waterlogging effects being less severe compared to those of total submergence. Contrastingly, the carotenoid content remained unaffected by waterlogging but was decreased significantly under submergence conditions, and the authors indicated that further research is needed in order to elucidate the mechanism responsible for those differential responses. Interestingly, chlorophyll content of *Phalaris arundinacea* remained constant after 30 days of full submergence (Vervuren et al., 1999).

Anaerobic metabolism demands higher consumption of substrate compared to aerobic metabolism, and as a result, carbohydrate content and metabolism are affected greatly by water-excess (Bewley & Black, 1982). Past research has demonstrated that pre-stress stored carbohydrate content is

positively correlated with improved survival under flooding conditions. That has been attributed not only to their positive effects on maintenance of anaerobic respiration (Albrecht, Biemelt, & Baumgarten, 1997; Vriezen, Zhou, & van der Straeten, 2003) under flooding conditions but also due to their ability to recover after the end of stress (He, Patick Bentley, & Scott Holaday, 2011). For example, the high flood tolerance of *Phalaris arundinacea*, a known invasive grass species, has been ascribed to its high content of fructose polymers in its rhizomes (Tamura & Moriyama, 2001) as well as its more efficient use of carbohydrates under flooding conditions (Qin, Li, Chen, & Xie, 2013).

Yu, Nuo, et al. (2012) reported that flood-tolerant genotypes of perennial ryegrass, as part of their flood escape mode, had relatively higher water-soluble carbohydrate and fructan concentrations when compared to more flood-sensitive genotypes. Nevertheless, in all cases, submergence conditions substantially reduced water-soluble carbohydrates and fructan levels (Yu, Nuo, et al., 2012; Liu & Jiang, 2015). In contrast, Liu and Jiang (2015) observed that waterlogging had no significant effect on shoot water-soluble carbohydrate (hexose, sucrose, fructans) content, suggesting that maintenance of carbohydrate content was positively associated with waterlogging tolerance. Jiang and Wang (2006) reported increases in carbohydrate concentrations in the shoots of waterlogged creeping bentgrass plants, while the opposite outcome was observed in the roots, leading to their suggestion that waterlogging impeded carbohydrate translocation to the roots. In waterlogged Kentucky bluegrass plants, a two-phased response was observed with shoot carbohydrate concentrations decreasing early in the onsets of stress (10 days), but later increasing as the stress was allowed to persist (30 days). Wang et al. (2009) observed no significant effects of short-term waterlogging stress on root water-soluble carbohydrates in Kentucky bluegrass but conceded that the lack of response they found was probably due to the short duration of the stress.

Production of reactive oxygen species (ROS) is enhanced under conditions of excess water (Mittler, 2006). Consequently, substantial increases in membrane lipid peroxidation, as indicated by malondialdehyde and hydrogen peroxide levels, have been recorded in roots of perennial ryegrass (Liu & Jiang, 2015) and in shoots of Kentucky bluegrass (Puyang et al., 2015). In contrast, Wang and Jiang (2007b) reported that various levels of waterlogging stress for 28 days in roots of creeping bentgrass had no effect on the malondialdehyde or hydrogen peroxide concentrations. Even though the stresses, described in the last two studies, had the same stress duration, we speculate that the differential responses were due to the different grass species.

The balance between ROS production and scavenging efficiency is critical for plant survival under adverse

environmental conditions, and plants' antioxidant mechanisms are activated readily in order to mitigate the oxidative stress resulting from the anaerobic soil conditions (Kato, Collard, Septiningsih, & Ismail, 2014). Nevertheless, variable responses have been reported among and within cool-season grass species, depending on stress duration and severity as well as the tissue type. For example, Liu and Jiang (2015) reported in perennial ryegrass that submergence had a more severe effect on shoot catalase and ascorbate peroxidase activities than waterlogging, while root antioxidant response was considerably different compared to that of the shoots either under waterlogging or submergence conditions. In waterlogged Kentucky bluegrass plants, Puyang et al. (2015) reported significant decreases in shoot superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase. The decreases in these were significantly smaller in flood-tolerant compared to flood-susceptible cultivars, making recovery possible in flood-tolerant genotypes following the end of exposure to the stress. Wang and Jiang (2007) reported that in the roots of waterlogged creeping bentgrass plants, glutathione reductase, and peroxidase activity remained unaffected, while superoxide dismutase and ascorbate peroxidase activities increased and decreased, respectively. Evidently, more research needs to be dedicated toward the antioxidant mechanisms of cool-season grass species under flooded conditions, while the establishment of a standard experimental protocol would facilitate the interpretation of results.

Water-excess, either as partial submergence or full submergence, results in substantial injuries on cool-season turfgrasses. Gas exchange functions of the plants are significantly inhibited, leading to marked decreases in photosynthesis and nutrient uptake. The activation of anaerobic metabolism, which requires much larger consumption of carbohydrates, in combination with the lowered photosynthetic rates, leads in marked reductions of plant carbohydrate levels. Production of ROS is enhanced under conditions of water-excess, with substantial damages in cell membrane while the antioxidant response, was again variable and dependent on the length and severity of stress (Figure 7).

14 | ELEVATED OZONE CONCENTRATIONS

Ozone (O_3), a ground-level (tropospheric) pollutant, is produced through sunlight's reaction with air containing pollutants formed through industrial activity (Fowler et al., 1999). Current ozone levels are reported to be around 35 ppb, compared to 10 ppb during the pre-industrial era, and are expected to reach 70 ppb by 2050, while regional spikes as high as

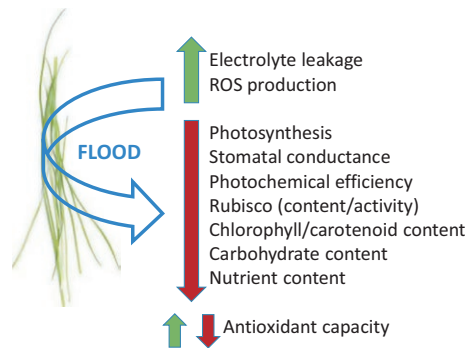


FIGURE 7 Effects of flooding on cool-season grasses

200 ppb have become rather frequent (Ainsworth, Yendrek, Sitch, Collins, & Emberson, 2012).

Due to its highly unstable and reactive nature, ozone is considered as the most toxic air pollutant and can have grave consequences on plant physiology, metabolism, and morphology (Fiscus, Booker, & Burkey, 2005). From an agricultural perspective, experiments with grass monocultures and species' mixtures (the most widely used farming practice) have indicated that ozone can have significant effects on plant species distribution and balance within an ecosystem (Fuhrer and Booker 2003; Hayes, Mills, & Ashmore, 2009). The extent of the damage depends on several factors, including ozone concentrations, rate of entry into the plant, canopy architecture, environmental conditions, and growth stage of the plants, making the assessment of exact agronomic impact extremely difficult (Fuhrer, 2009). However, research has shown that exposure of plants to increased ozone concentrations results in increased leaf senescence, reductions in photosynthesis, and assimilates availability as well as increased oxidative damage. As a consequence, substantial compromises in yield output (Fiscus et al., 2005; Fuhrer, 2009) are observed.

15 | EFFECTS OF OZONE ON COOL-SEASON GRASS PHYSIOLOGY

Reductions in photosynthesis are commonly observed in several plant species after exposure to high ozone concentrations (Booker et al., 2009; Hayes et al., 2009; Plazek, Hura, & Rapacz, 2001). Such reductions have been attributed to both stomatal and non-stomatal limitations (Ainsworth et al., 2012). Stomatal limitations have been associated with alterations in guard cell ion channel function, calcium homeostasis, and hormone regulation (Mills, Hayes, Wilkinson, & Davies, 2009; Torsethaugen, Pell, & Assmann, 1999) as well as decreases in internal CO_2 concentration (Fiscus et al., 2005) (Figure 2). Non-stomatal

limitations, on the other hand, are mostly linked to compromises in photochemical and carboxylation efficiency of PS II (Catalayud & Barreno, 2001; Meyer, Kollner, Willenbrink, & Krause, 1997). Hayes et al. (2009) reported that exposure of perennial ryegrass to ozone (30 ppb + peaks) had no effect on the photosynthetic capacity or carboxylation efficiency early into the exposure period. Nevertheless, substantial decreases in both photosynthetic capacity and carboxylation efficiency were later observed in older leaves after 10 weeks of exposure, suggesting the negative cumulative effects of ozone exposure on perennial ryegrass (Figure 4). Significant reductions in net photosynthetic rates were also reported by Plazek et al. (2001) in meadow fescue (*Festuca pratensis*), but they were attributed to stomatal closure, since photochemical efficiency remained unaffected.

Ozone is a problem because after entering the apoplast through the stomata, it is quickly degraded to form reactive oxygen species such as hydrogen peroxide, superoxide anion, and singlet oxygen, that in turn react with cell wall components and compromise membrane stability (Pell, Schlagnhauser, & Arteca, 1997). Decreased membrane stability, as indicated by increases in electrolyte leakage and lipid peroxidation, has been recorded consistently after exposure to high ozone levels (Catalayud & Barreno, 2001; Iglesias, Catalayud, Barreno, Primo-Millo, & Talon, 2006; Plazek et al., 2001) with further consequences on plasmodesmata connections and assimilate translocation (Grantz & Farrar, 2000; Landolt et al., 1997). However, Plazek et al. (2001) reported that exposure of meadow fescue plants to high ozone concentrations did not alter membrane permeability. In contrast, Pasqualetti et al. (2015) using Italian ryegrass (*Lolium multiflorum*) reported a marked increase in hydrogen peroxide levels after exposure to increased ozone concentrations.

16 | EFFECTS OF OZONE ON COOL-SEASON GRASS METABOLISM

The responses of Rubisco content and activity to high ozone concentrations have been variable depending on the species, the growth stage of the leaf and the plant, as well as the duration of the exposure (Pell et al., 1997). Nevertheless, the majority of studies have shown a decreasing trend in Rubisco content and activity after ozone exposure (Galmes et al., 2013; Pell et al., 1997). These decreases have been attributed to either increased protein degradation or decreased protein synthesis (Andersen, 2003; Pell et al., 1997). However, in cool-season grasses, Rubisco content and activity after exposure to ozone have received little attention.

Decreases in chlorophyll and carotenoid content have also been reported under conditions of elevated ozone

concentrations (Catalayud & Barreno, 2001; Endress, Endress, & Iverson, 1999; Iglesias et al., 2006). Those decreases were, again, attributed to the oxidative nature of ozone that results in increased protein degradation and metabolic disruptions. Meyer et al. (1997) using wheat plants (*Triticum aestivum*) indicated that the plant growth stage at which ozone exposure occurs plays a pivotal role in the extent of damage. Chlorophyll content of wheat plants remained unaffected when the plants were exposed to higher ozone levels during their tillering phase, but was decreased significantly when exposure occurred during anthesis. Similar impacts may occur in cool-season forage and turf grasses, but this has yet to be investigated.

Carbon metabolism has been reported to be affected significantly by increased ozone concentrations due to reductions in photosynthetic rates and changes in carbon partitioning observed under conditions of increased ozone concentrations (Andersen, 2003; Meyer et al., 1997). The effect of ozone exposure on leaf carbohydrate content varies considerably, depending on the plant species and growth stage, as well as duration and extent of exposure (Blum et al. 1982; Fuhrer, Shariatmadari, Perler, Tschannen, & Grub, 1994; Meyer et al., 1997; Iglesias et al., 2006; Ainsworth et al., 2012). Nevertheless, increases in leaf hexose and sucrose concentrations are the most commonly reported observations (Grantz & Farrar, 1999; Landolt et al., 1997). As reported throughout this review, carbohydrate accumulation is consistently implicated in increased tolerance to abiotic stresses (Rosa et al., 2009). A similar effect following ozone exposure might explain how in Italian ryegrass higher ozone concentrations substantially increased leaf fructose content and altered fructan polymerization (Moretto, Sandrin, Itaya, Domingos, & Figueiredo-Ribeiro, 2009; Pasqualetti et al. 2015). Indeed, substantial increases in leaf sucrose and starch content were reported in wheat (Meyer et al., 1997) following exposure to high ozone levels. In this case, the authors suggested an impact of ozone that led to impediments to phloem loading. Schoene, Franz, and Masuch (2004) using perennial ryegrass reported that double the ambient ozone concentration repressed starch accumulation in the pollen grains; however, starch metabolism was not further explored.

Exposures to increased concentrations of ozone enhance production of reactive oxygen species (Rao & Davis, 2001), and a positive association has been suggested between plant's antioxidant capacity and tolerance to ozone (Booker et al., 2009). Ascorbic acid has, especially, been acknowledged to play a pivotal role in plant defense against ozone-induced oxidative damage, due to its presence in the apoplast, the point of entrance for ozone (Conklin & Barth, 2004). Levels of total ascorbic acid have been correlated positively with ozone resistance among several plant species (Conklin & Barth, 2004) especially early into the exposure period. However, in cases of prolonged exposure to ozone, such correlations fail to exist

TABLE 2 Summarized effects of elevated CO₂ levels, heat stress, drought stress, flooding stress, and O₃ levels on the physiology and metabolism of cool-season grasses. Plus symbols (+) indicate increase, and minus symbols (–) indicate decrease

| | CO ₂ | Heat | Drought | Flood | O ₃ |
|------------------------------------|-----------------|------|---------|-----------|----------------|
| Photosynthesis | + | – | – | – | – |
| Stomatal conductance | – | – | – | – | – |
| Photorespiration | – | + | | | |
| Respiration | + | + | – | Anaerobic | |
| Electrolyte leakage | | + | + | + | + |
| Photochemical efficiency | | – | – | – | |
| Rubisco (content/activity) | – | – | – | | – |
| Chlorophyll/carotenoid content | | – | – | – | – |
| Total soluble carbohydrate content | + | | + | – | + |
| Total antioxidant capacity | | | | | |
| Reactive oxygen species | | + | + | + | + |
| Relative water content | | | – | | |
| Nutrient content | | | | – | |

(Burkey and Eason 2002; Foyer & Noctor, 2005). In addition to ascorbic acid, glutathione, glutathione reductase, superoxide dismutase, and ascorbate peroxidase also participate in the defense mechanisms against oxidation from exposure to high ozone levels (Bassin, Volk, & Fuhrer, 2007; Sharma & Davis, 1997). Pasqualetti et al. (2015) reported that in Italian ryegrass ascorbate peroxidase activity was significantly decreased after exposure to ozone stress, indicating that the antioxidative mechanism of the plants was overwhelmed.

In summary, photosynthetic rates of cool-season turfgrasses were decreased under conditions of elevated ozone concentrations. Stomatal and metabolic constraints as well as increased oxidative damage due to ozone's highly reactive nature were associated with those reductions. Nevertheless, carbohydrate content was reported to increase, while variable responses were observed for the total antioxidant capacity of grasses under increased ozone levels. Evidently, more research is required on the carbohydrate and antioxidant metabolism of cool-season turfgrasses in order to clarify the mechanisms that are affected under conditions of higher ozone concentrations (Figure 8).

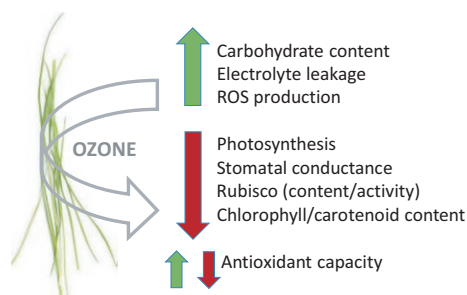


FIGURE 8 Effects of elevated ozone levels on cool-season grasses

17 | COMMON EFFECTS OF ABIOTIC STRESS ON THE PHYSIOLOGY AND METABOLISM OF COOL-SEASON GRASSES

Reported herein and elsewhere are the impacts of individual abiotic stresses on the plant physiology and metabolism of cool-season grasses. By comparing the extensive literature on the subject, it is possible to see that similar physiological responses can be invoked by different, non-related stresses (Table 2). The general response of temperate grasses to stress involves decreases in the abundance of photosynthetic pigments together with a tendency for defense via the accumulation of carbohydrate. ROS concentrations are consistently increased under conditions of all abiotic stresses with concomitant increases in electrolyte leakage, an indication of membrane damage. Nevertheless, the responses of grass species in terms of total antioxidant capacity and specific enzyme activation are variable. Activation and efficiency of specific enzymes are mostly dependent on the type and the intensity of the stress imposed, as well as the type of tissue investigated, the growth stage at which the stress occurs, and the grass species. Additionally, total antioxidant capacity appears to have a biphasial behavior during the stress period, by increasing at the onset of the stress and decreasing once the stress becomes more severe. Furthermore, reductions in stomatal conductance and Rubisco content or activity appear to be the standard responses under all stress conditions. In contrast, other responses appear to be more specific to particular stresses; photosynthesis is upregulated by CO₂ and downregulated by heat, whereas the opposite is true for photorespiration (Table 2). Similarly, even though total carbohydrate content generally increases under stress, probably due to

carbohydrates functioning as ROS scavengers, under flooding conditions total carbohydrate concentrations decrease.

18 | CONCLUSIONS AND FUTURE RESEARCH

Increased CO₂ concentrations, as well as heat and drought stress, are clearly the stresses most extensively studied in cool-season grasses. Nevertheless despite extensive work by many groups, it is clear that the data set is incomplete and further investigations are still needed to understand the causes of differences and complementarities in responses. Photorespiration, for example, has not been studied under conditions of water-deficit stress and information on photochemical efficiency and chlorophyll content under conditions of high CO₂ levels are missing (Table 2). Additionally, more emphasis should be given to carbohydrate and fructan metabolism of cool-season grasses. The majority of studies have focused on the effects of the key stresses on leaf carbohydrate and fructan levels, relatively few of them have concentrated on their effects on the primary and secondary carbohydrate metabolism. Information on responses of enzymes, involved in carbohydrate metabolism, would aid the interpretation of the observed results. In addition, most of the studies have focused on leaf carbohydrate levels, and as a consequence completely ignoring root responses. Root physiology and metabolism, under conditions of abiotic stresses, are largely under-investigated, mainly due to practical difficulties. However, technologies, such as phenomics, can provide significant help in elucidating the extent of roots' involvement in plant response to abiotic stresses. Furthermore, hormonal metabolism of cool-season grasses has not received great attention even though hormones play a pivotal role in regulating plant responses to abiotic stresses. The number of studies on hormonal metabolism of grasses under stress conditions is extremely limited and addresses mainly heat and drought stress (Alam et al., 2018; Krishnan & Merewitz, 2015; Li et al., 2014; Ma, Xu, Meyer, & Huang, 2016; Man, Bao, & Han, 2011). The small number of studies in this subject is understandable due to the highly complex nature of hormonal crosstalk in plants. However, new technologies, such as metabolomics, proteomics, and transcriptomics, provide great opportunities to expand our understanding of on the role of hormones in plants' abiotic stress responses.

Lastly, in terms of abiotic stresses, flooding in particular and increased ozone concentrations have received considerably less attention to date than other stress conditions. Even though general responses to those stresses have been recorded (Table 2), the extent of literature dedicated to them is significantly smaller compared to heat and drought stress. A similar amount of effort should be dedicated to the physiological and metabolic responses of flooding and high ozone

concentrations since they are both likely to be implicated in future forage production and threaten food security given climate change.

In order, however, to gain a full perspective of the impact of abiotic stresses future research should not consider them in isolation since in practice these stresses may occur concurrently or sequentially throughout the plant's life cycle. For example, heat and drought stresses are usually observed simultaneously under field conditions. The need to conduct research designed accordingly is increasingly being recognized. While this subject would form the basis of a separate review, there are some general points. Research on two-way stress combinations such as heat and drought stress or increased CO₂ levels and temperatures (Jiang & Huang, 2001a,b; Song et al., 2014) and three-way stress combinations, such as high CO₂ levels, temperature, and drought stress (Farfan-Vignolo & Asard, 2012; AbdElgawad et al. 2014; AbdElgawad, de Vos et al., 2015a,b; AbdElgawad, Farfan-Vignolo, et al., 2015), has already been conducted on some grass species. Interestingly, the results from these studies have indicated that combinational stress effects are not always additive, as is generally predicted, and in some cases can even be ameliorative. Furthermore, increased consideration must be given to implications of exposure to sequential stress. Sequential stresses occur more often than not under field conditions. For instance, a grassland field may encounter a flood during the spring followed by a drought during the summer. As long-lived perennials, growth conditions will vary between years. Hence, chronic and acute exposure to varying stresses are particularly relevant for grasses and are likely to result in modulation of a particular stress response by epigenetic as well as metabolic factors (Ohama et al., 2017). Hence, while understanding the effects of individual stresses remains the baseline for interpretation of the effects of stress combinations nevertheless, these stress conditions are rather under-explored and there is a risk from extrapolating from the individual stress effects which might not always be applicable. In particular, realistic simulations of such scenarios need to be constructed and the "tipping points" for crop failure, be determined.

It is clear from the above that one of the challenges facing attempts to improve grassland resilience under stress is that experimental conditions vary greatly. In order to attain realistic simulations and pinpoint thresholds for yield decreases one solution would be to establish a platform based on standard experimental protocols. While this would increase comparability and ensure the accuracy of the collected data, there will still be experimental error derived from use of local genotypes; significant differences are already observed among and within grass species, due to their obligate outbreeding nature and their genetic diversity. Ideally, in order to eliminate discrepancies due to experimental methods, the standardized procedures should be closely aligned to real-field conditions.

Furthermore, plant responses to abiotic stresses depend greatly on the plant's growth stage at which the stress is imposed (Bailey-Serres et al., 2012; Setter et al., 2009; Striker & Colmer, 2016). As a result, differential results are reported under similar experimental conditions, due to the differential developmental stages of the plants. Moreover, differential responses to abiotic stresses are observed among and within types of tissue which also plays a significant role in the interpretation of results. For instance, leaf respiration rates of perennial ryegrass were found to increase under conditions of high CO₂ levels, while whole-plant respiration decreased (Nijs et al., 1997; Bunce 2007). Consequently, experimental design should take into consideration those factors not only in order to attain meaningful results but also to further expand our knowledge on grass physiology and metabolism.

Alternatively, there is an opportunity to use data mining to greater effect to extract the full potential in the data already in existence. As we saw from the studies presented here, experimental procedures undertaken by researchers vary greatly in their design which results in large variability in and between trial outcomes and hinders considerably data interpretation. Better characterization of meta-data and the establishment of a suitable repository for such information would be extremely useful in identifying where the future focus of stress research should be. Cool-season grass species harbor vast genetic variation with ecotypes found growing under consistently climatically stressed conditions having evolved many adaptations of potential use for future incorporation in variety development. However, since these non-domesticated ecotypes have not been studied in as much detail as other crop or model species further research is required on their physiology and metabolism of non-domesticated ecotypes, in order to elucidate the exact mechanisms used to confer increased tolerance to abiotic stresses. Furthermore, application of -omics technologies (metabolomics, proteomics, transcriptomics, etc.) alongside physiological assessment could provide valuable insight to crop adaptation to stress and routes to sustain/enhance production in a climate-changed world.

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