

Targets for Crop Biotechnology in a Future High-CO₂ and High-O₃ World¹

Elizabeth A. Ainsworth^{2*}, Alistair Rogers², and Andrew D.B. Leakey²

Photosynthesis Research Unit, U.S. Department of Agriculture/Agricultural Research Service, Urbana, Illinois 61801 (E.A.A.); Environmental Sciences Department, Brookhaven National Laboratory, Upton, New York 11973 (A.R.); and Department of Plant Biology (E.A.A., A.D.B.L.), Department of Crop Sciences (E.A.A., A.R.), and Institute for Genomic Biology (E.A.A., A.D.B.L.), University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

Predictions of population growth outpacing agricultural production have been made for the past 200 years (Malthus, 1817; Ehrlich, 1968), and though world food supply has more than kept pace with demand, there are over 850 million malnourished people in the world, the vast majority in developing countries (FAO, 2006). In the next approximately 40 years, global demand for cereal production will increase by 60% (Rosegrant and Cline, 2003) as the global population rises from 6.6 billion today to 8.7 to 11.3 billion in 2050 (Bengtsson et al., 2006). Feeding the growing world population will be a significant challenge to agriculture. Simultaneously, global climate change will provide an additional challenge by significantly modifying the crop production environment. What are the additional challenges and opportunities that climate change will present for biotechnological improvement of crop yield and food supply?

Global climate change will alter many elements of the future crop production environment. Atmospheric carbon dioxide concentration ([CO₂]), average temperature, and tropospheric ozone concentration ([O₃]) will be higher, droughts will be more frequent and severe, more intense precipitation events will lead to increased flooding, some soils will degrade, and climatic extremes will be more likely to occur (IPCC, 2007). Drought, extreme temperatures, flooding, and soil quality have challenged agriculture since its inception. Therefore, there is a long history of investment in basic science and crop breeding to produce germplasm that sustains high yield under stressful conditions, and the opportunity for biotechnology to contribute to improved crop stress tolerance has been widely recognized. Advances in the understanding of crop-environment interactions at the molecular, biochemical, physiological, and agronomic scales, as well as their relevance to

biotechnological crop improvement, have been extensively reviewed. These include discussions of the response mechanisms and potential targets for improving crop response to drought (Wang et al., 2003; Chaves and Oliveira, 2004; Parry et al., 2005; Barnabás et al., 2008), flooding (Agarwal and Grover, 2006), low temperature (Wang et al., 2003; Nakashima and Yamaguchi-Shinozaki, 2006), high temperature (Iba, 2002; Wahid et al., 2007; Barnabás et al., 2008), and low nutrient availability (Hirel et al., 2007). It follows that a number of companies, including Monsanto, Syngenta, and Pioneer-DuPont, have drought-tolerant, heat-tolerant, cold-tolerant, or nitrogen (N)-use efficient germplasm in their research and development pipelines (<http://www.monsanto.com/pdf/investors/2008/01-08-08.pdf>; http://www.syngenta.com/en/about_syngenta/crop_protection_pipeline.aspx; http://www.pioneer.com/CMRoot/Pioneer/research/pipeline/DuPont_BG_Pipeline.pdf). However, two other major elements of global climate change, rising atmospheric [CO₂] and [O₃], have been widely recognized as important to crop production only in the last 30 to 40 years. Even with this recognition, there has so far been little effort to improve crop responses to these factors through breeding or biotechnology. Here we briefly outline the nature of global climate change and then discuss potential biotechnological targets for improving crop production in a future high-[CO₂] and high-[O₃] world. We conclude by reviewing some practical challenges to developing and testing biotechnology crops that are targeted to a changing production environment.

CLIMATE CHANGE: A 50-YEAR VIEW FROM THE PLANT PERSPECTIVE

In the last 250 years, atmospheric [CO₂] has risen from 280 μmol mol⁻¹ to 381 μmol mol⁻¹. This exceeds the [CO₂] at any time in the last 650,000 years and probably the last 23 million years (IPCC, 2007). Atmospheric [CO₂] is projected to continue rising to at least 550 μmol mol⁻¹ by 2050 (IPCC, 2007). Rising concentrations of CO₂ and other greenhouse gases have resulted in a 0.76°C increase in global surface temperature since the 1800s, and the mean global surface temperature is predicted to increase by an additional

¹ This work was supported by the U.S. Department of Energy Office of Science (contract no. DE-AC02-98CH10886 to Brookhaven National Laboratory; A.R.).

² These authors contributed equally to the article.

* Corresponding author; e-mail lisa.ainsworth@ars.usda.gov.

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiol.org) is: Elizabeth A. Ainsworth (lisa.ainsworth@ars.usda.gov).

www.plantphysiol.org/cgi/doi/10.1104/pp.108.117101

1.3°C to 1.8°C by 2050 (IPCC, 2007). Warming over land is expected to be greater than this average, and it is very likely that heat waves will be more intense, more frequent, and longer lasting. Daily minimum temperatures are predicted to rise more rapidly than daily maximum temperatures. The number of frost days will decrease, and in mid- to high latitudes, an extension of the growing season is likely (IPCC, 2007).

Warming will generally increase evaporation, total precipitation, and the spatial variability of precipitation, leading to less rainfall in the tropics and more rainfall at higher latitudes. However, the spatial and temporal boundaries between areas of projected increasing and decreasing precipitation are uncertain. Globally, the intensity of precipitation events is projected to increase, even in areas with a mean reduction in precipitation, and the time between precipitation events is also projected to increase, thereby increasing both the risk of flooding and drought (IPCC, 2007).

Unlike $[\text{CO}_2]$, tropospheric $[\text{O}_3]$ is spatially and temporally heterogeneous because O_3 is short lived and its synthesis is tied to the abundance of its pollutant precursors, and water vapor and sunlight. In industrialized countries of the northern hemisphere, daily 8-h tropospheric $[\text{O}_3]$ is estimated to have increased from approximately 10 nmol mol^{-1} prior to the industrial revolution to a current level of approximately 60 nmol mol^{-1} during summer months, and is predicted to increase 20% more by 2050 (IPCC, 2007). This is particularly relevant to agriculture because sensitive crops show a reduction in yield once the $[\text{O}_3]$ exceeds 40 nmol mol^{-1} for extended periods (Heagle, 1989).

The changes in temperature, precipitation, and tropospheric $[\text{O}_3]$ projected for 2050 are spatially and temporally variable, poorly constrained, and occurring in parallel. This moving and poorly defined target presents a significant challenge to a biotechnology industry hoping to provide cultivars tailored to regional production environments. In contrast, the increase in $[\text{CO}_2]$ is uniform, global, and unfortunately, committed. Even in the unlikely event that we stabilize CO_2 emissions at present-day levels, atmospheric $[\text{CO}_2]$ would still be $>500 \text{ } \mu\text{mol mol}^{-1}$ by 2050 (IPCC, 2007). Therefore, attempts to engineer crops to perform better under the conditions of increasing environmental stress associated with increased O_3 exposure, temperature, and changing precipitation patterns should be considered against the back drop of a guaranteed and ubiquitous increase in atmospheric $[\text{CO}_2]$.

ENGINEERING CROPS FOR A HIGH- CO_2 WORLD

Elevated $[\text{CO}_2]$ directly reduces stomatal conductance in C_3 and C_4 species, and also directly stimulates photosynthesis in C_3 species (Drake et al., 1997; Long et al., 2004; Ainsworth and Rogers, 2007). All other effects of elevated $[\text{CO}_2]$ on crops, including the stimulation in yield, are derived from these primary responses (Ainsworth and Rogers, 2007). How can we

engineer crops to maximize the advantages of rising $[\text{CO}_2]$? As Rubisco is the key carboxylating enzyme and frequently the rate-limiting factor for photosynthesis (Rogers and Humphries, 2000), it is worth asking: Can crop responsiveness to elevated $[\text{CO}_2]$ be improved by altering the properties of Rubisco, and are current efforts to improve Rubisco relevant to the production environment of 2050? In C_3 crops, increasing the affinity of Rubisco for CO_2 , and thereby avoiding photorespiratory carbon (C) losses, is a target for increasing yield potential (Reynolds and Borlaug, 2006; Ainsworth and Rogers, 2007), but the conserved inverse relationship between specificity and catalytic rate has hindered progress (Bainbridge et al., 1995). The kinetic properties of current C_3 Rubisco suggest that the affinity/specificity is optimal for a $[\text{CO}_2]$ of $200 \text{ } \mu\text{mol mol}^{-1}$, consistent with a Rubisco that evolved under such conditions. By 2050, the optimal Rubisco would actually need to have a lower, not higher affinity for CO_2 , and a higher catalytic rate (Zhu et al., 2004). Substituting current Rubisco for Rubisco from other species, particularly nongreen algae, which has a markedly lower specificity and higher catalytic rate, could dramatically increase C gain at current and elevated $[\text{CO}_2]$. Further gains could be maximized by engineering plants to express different types of Rubisco in sunlit and shade leaves (Zhu et al., 2004). This is not without precedent because *Hydrogenovibrio marinus*, an anaerobic microorganism, switches among its three forms of Rubisco depending on environmental cues (Yoshizawa et al., 2004). Thus, improving the catalytic properties of Rubisco will remain a viable target for the biotechnology industry. Although the focus of the research may need to shift away from improving specificity for CO_2 in crops grown in temperate environments, those grown in high-temperature conditions in tropical environments would still benefit from improved specificity because as temperature rises, the ratio of carboxylation to oxygenation is reduced (Long, 1991).

As $[\text{CO}_2]$ rises, photosynthesis in C_3 crops shifts from being limited by Rubisco to being limited by the capacity for regeneration of the CO_2 acceptor, ribulose-1,5-bisphosphate (RuBP; Ainsworth and Rogers, 2007). An alternative to reducing excess Rubisco content at elevated $[\text{CO}_2]$ is to increase the capacity for regeneration of RuBP and thereby match the increased carboxylation rates. Recent modeling analysis suggests that manipulating the partitioning of N resources among enzymes of C metabolism could greatly increase photosynthesis without any increase in the total N requirement (Zhu et al., 2007). Greater capacity for the regenerative phase of the Calvin cycle was identified as a key target to improve C acquisition. There is already some evidence that crops increase RuBP regeneration capacity at elevated $[\text{CO}_2]$. The level of Fru-1,6-bisphosphatase was increased in a forage crop (*Lolium perenne*) grown at elevated $[\text{CO}_2]$ with a high N supply (Rogers et al., 1998) and in tobacco (*Nicotiana tabacum*), Rubisco activity decreased and other Calvin cycle enzymes increased at elevated $[\text{CO}_2]$ (Geiger et al.,

1999). In addition, antisense technology coupled with flux analysis has shown that sedoheptulose-1,7-bisphosphatase exerts considerable control over photosynthesis, and plants overexpressing sedoheptulose-1,7-bisphosphatase had a marked increase in photosynthesis and growth (Raines, 2003; Lefebvre et al., 2005).

Accumulation of foliar carbohydrates is one of the most pronounced and universally observed responses of C_3 plants to elevated $[CO_2]$, even in field-grown plants where rooting volume is unrestricted (Long et al., 2004). Knowledge of the relationship between C status and growth is advancing (Raines and Paul, 2006; Smith and Stitt, 2007), but a clear picture of how carbohydrates are sensed by the plant and how they regulate the flux of C through different pathways, impacting growth and partitioning, remains a major challenge to developing crops that will respond maximally to rising $[CO_2]$. Large and sustained increases in Suc, starch, or fructan content that persist over a number of days are usually indicative of replete sinks (Rogers and Ainsworth, 2006). This has two important implications. First, crops could be manipulated to use the additionally available C to increase growth, seed yield, or stress tolerance (see below). Second, because foliar carbohydrates are known to have negative feedback on photosynthetic capacity (for review, see Long et al., 2004), ensuring adequate sink strength in crops will be an essential part of maximally exploiting rising $[CO_2]$. Evidence from a Free Air Concentration Enrichment (FACE) experiment supports this view. There was no reduction in maximum Rubisco activity at elevated $[CO_2]$ in an indeterminate soybean (*Glycine max*) cultivar, but in an otherwise identical isolate that had a single gene mutation resulting in a determinate growth habit and lower sink strength, carbohydrates accumulated and photosynthetic capacity was significantly reduced (Ainsworth et al., 2004). This illustrates that the capacity for utilization of photosynthate is critical to a crop's ability to sustain increased C acquisition at elevated $[CO_2]$. Further, a recent review concluded that at current $[CO_2]$, yields of maize (*Zea mays*), wheat (*Triticum aestivum*), and soybean were limited more by sink capacity than photosynthetic capacity (Borras et al., 2004). In contrast, results from elevated $[CO_2]$ research clearly show that increased photosynthesis does stimulate yield at elevated $[CO_2]$ (Kimball et al., 2002; Ainsworth and Long, 2005; Long et al., 2006), suggesting that increases in yield can also be source driven. However, it is likely that increases in photosynthesis will need to be balanced by commensurate increases in sink capacity if maximum yield potential is to be realized.

What are the components of yield that might be limiting the response of crops to elevated $[CO_2]$? Yields of soybean and rice (*Oryza sativa*) grown under elevated $[CO_2]$ in FACE experiments were 15% and 13% higher than yields of crops grown at ambient $[CO_2]$, despite significant declines in harvest index (HI; seed yield/total aboveground biological yield; Kim et al., 2003; Morgan et al., 2005). Genetic improve-

ments over the past century in grain yield in a number of crops, including soybean, wheat, and rice, have been closely associated with improvements in HI (Gifford et al., 1984); however, it has been argued that the scope for further increasing yield through increasing HI is limited (Austin, 1999). Still, at least maintaining current levels of HI at elevated $[CO_2]$ would improve productivity. Regardless of improvements to HI, a second way to increase yield is to increase total biomass at maturity. More aboveground biomass per area has been the major factor in yield improvements at elevated $[CO_2]$ in FACE experiments. In rice, elevated $[CO_2]$ decreased the productive tiller ratio (Kim et al., 2003) and reduced the percentage of fertile spikelets (Yang et al., 2006). However, because the tiller number (and panicle number) per area was significantly higher at elevated $[CO_2]$, yields were increased despite the negative effects on productive tiller ratio and spikelet fertility (Kim et al., 2003; Yang et al., 2006). Elevated $[CO_2]$ also has little effect on individual grain or seed mass (Morgan et al., 2005; Yang et al., 2006), which may not be surprising because individual grain weight has not substantially changed with genetic improvement in wheat, maize, or soybean over much of the last century (Morrison et al., 2000; Fischer, 2007). Therefore, efforts to target grain or seed mass may not be fruitful, but there appears to be the opportunity to improve biomass production, the number of reproductive sinks, and therefore yield.

In their research and development pipelines, the major biotechnology companies (Monsanto, Syngenta, and Pioneer-DuPont) all have investments aimed at improving N use efficiency. Although growth at elevated $[CO_2]$ improves N use efficiency (Drake et al., 1997), improved nutrition is essential to maximally exploit rising $[CO_2]$. The greater efficiency of carboxylation at elevated $[CO_2]$ makes it theoretically possible for C_3 crops in 2050 to reduce their Rubisco content by approximately 25% and maintain the same photosynthetic rate as they have today (Drake et al., 1997). Consistent with this theory, C_3 crops grown at 567 $\mu\text{mol mol}^{-1}$ $[CO_2]$ in FACE studies reduced their maximum Rubisco activity by approximately 20% (Ainsworth and Rogers, 2007). This reduction in Rubisco activity is most likely due to a reduction in Rubisco content (Long et al., 2004). Crops with the C_3 pathway invest up to 25% of their leaf N in Rubisco to compensate for its poor catalytic rate (Long et al., 2004). The large investment in Rubisco, coupled with a potential 25% reduction in Rubisco content, could release approximately 6% of leaf N at elevated $[CO_2]$. However, there is only benefit in reducing the amount of N in Rubisco when the resources can be usefully deployed elsewhere, either within the leaf, or to support sink development in the plant (Parry et al., 2003). When this 6% theoretical savings in N is compared to the predicted 10% to 40% increase in C acquisition (Long et al., 2004), the N acquisition gap at elevated $[CO_2]$ is readily apparent. Despite increased yields in

C₃ crops at elevated [CO₂], a recent meta-analysis of the protein concentration in food crops shows that the N acquisition gap at elevated [CO₂] manifests itself as a 10% to 15% reduction in grain protein content, even in field-grown crops (Taub et al., 2008). This is notable because the major biotechnology companies are attempting to increase protein content in grain crops and will have to work against the effects of rising [CO₂] to achieve this goal (Stafford, 2007). Legumes might be expected to avoid the N acquisition gap at elevated [CO₂] because they can trade their excess C for N with their bacterial symbionts. Evidence from soybean supports this hypothesis because grain protein content showed only a 1.4% decrease at elevated [CO₂] (Taub et al., 2008) and soybean grown at elevated [CO₂] using FACE technology avoided N limitation (Rogers et al., 2006). Introducing the capacity for N fixation into nonleguminous plants to improve nutrition and reduce demand for fertilizer has always been desirable, but closing the N acquisition gap at elevated [CO₂] and maximizing increases in yield will make this goal even more attractive in 2050.

These targets for biotechnological improvement of crop responses to elevated [CO₂] should be selected with consideration for the projected increase in temperature, drought stress, and [O₃] that will accompany rising [CO₂]. Because C supply is increased at elevated [CO₂], it may be possible to partition a greater fraction of photosynthate into C-rich metabolites associated with stress resistance. For instance, pinitol, mannitol, and raffinose are examples of osmolytes that support maintenance of turgor under drought and high temperatures, whereas antioxidants such as ascorbate have been implicated in tolerance of crops to drought, heat, salt, and oxidative stresses (Streeter et al., 2001; Bartels and Sunkar, 2005; Busch et al., 2005). Larger pool sizes of these metabolites could extend the benefits of growth at elevated [CO₂] in germplasm marketable to farmers in poor growing areas, not just prime agricultural zones.

Greater productivity of C₄ crops at elevated [CO₂] has been reported from some chamber studies (for review, see Ghannoum et al., 2000). However, evidence from FACE studies suggests that stimulated photosynthesis at elevated [CO₂] is an indirect effect of reduced stomatal conductance and water use, leading to amelioration of drought stress (Ottman et al., 2001; Wall et al., 2001; Leakey et al., 2004, 2006). There have not been FACE studies on C₄ crops in nutrient poor soils, but it appears that the benefits of elevated [CO₂] to crops such as maize, millet (*Panicum miliaceum*), and sorghum (*Sorghum bicolor*) will not be universally observed, but instead limited to times and places of drought. In addition, because elevated [CO₂] is only ameliorating stress in C₄ species and not directly stimulating productivity, there is no gain in yield potential that can be realized by pursuing the targets listed above for C₃ plants. Thus, climate change presents a challenge to C₄ crop improvement, rather than an opportunity.

ENGINEERING CROPS FOR A HIGH-O₃ WORLD

The variability of [O₃] over time and space, as well as the dose-specific nature of plant responses to [O₃] have made it difficult to estimate the average agronomic impacts of elevated [O₃] or to develop a cohesive synthesis of the mechanisms of response. Nevertheless a number of studies estimate present-day crop losses to O₃ damage in the range of \$1 to \$3 billion in the U.S. (Murphy et al., 1999) and \$2 billion in China (Mauzerall and Wang, 2001). There is wide qualitative agreement that elevated [O₃] in the future will further reduce crop yields as a result of oxidative stress in foliar and reproductive tissues (Black et al., 2000; Fiscus et al., 2005).

Recent reviews of the effects of elevated [O₃] on plants have identified distinct mechanisms of response to chronic and acute O₃ exposure (Long and Naidu, 2002; Fiscus et al., 2005; Kangasjärvi et al., 2005). Exposures to daily peak [O₃] of <120 nmol mol⁻¹ for days, weeks, or months are typically considered to be chronic, whereas exposures to daily peak [O₃] of >120 to 150 nmol mol⁻¹ for as little as a few hours are considered acute (Long and Naidu, 2002; Fiscus et al., 2005). Distinguishing between chronic and acute responses has helped fair comparison of experiments imposing shock treatments versus those simulating current or future field conditions. However, the threshold concentrations characterizing acute versus chronic experiments are arbitrary and the distinction between plant responses is subjective. With monitoring stations in locations such as China reporting hourly maximum [O₃] >200 nmol mol⁻¹ and average annual concentrations of 74 nmol mol⁻¹ (Wang et al., 2007), plants in the field will likely experience both acute and chronic O₃ conditions simultaneously. This situation is more likely to occur in the future and determining the threshold [O₃] required for induction of various molecular, biochemical, and physiological responses will be increasingly important.

The process of conventional breeding, integrating the results of thousands of plots across years and environments, would be expected to enable exploitation of some of the potential benefits of elevated [CO₂], be it with a significant time lag. In contrast, the variability in [O₃] will not lead to the consistent selection pressure that is needed to make breeding advances. To date, discussions of improving tolerance to elevated [O₃] have focused on three general strategies: (1) controlling O₃ entry into the leaf, (2) improving detoxification within cells, and (3) altering the signal transduction pathway. Stomata regulate the flux of O₃ into leaves, and the protective value of stomatal closure in response to O₃ has long been recognized (Hill and Littlefield, 1969). Therefore, reducing stomatal conductance is an obvious biotechnological strategy to increase O₃ tolerance (Lin et al., 2001). However, the fundamental trade-off of lower stomatal conductance leading to lower photosynthesis must be recognized, and the balance between reducing stress versus reducing productivity evaluated. Elevated [CO₂] will reduce stomatal con-

ductance and O₃ uptake in the future, while increasing rather than decreasing photosynthesis. This will reduce the negative impacts of elevated [O₃] (Booker and Fiscus, 2005). In many growing regions experiencing moderate increases in [O₃], the gain from targeting reduced stomatal conductance to ameliorate stress would likely be small, as the decrease in productivity would outweigh any reduction of O₃-induced stress. The wheat genotypes released in China between 1945 and today provide an example of net gain in yield, despite increased sensitivity to elevated [O₃]. Breeding for higher C uptake, shorter stalks, and improved HI has improved the yield potential from approximately 1,000 kg ha⁻¹ to approximately 8,000 kg ha⁻¹, despite greater O₃ uptake from greater stomatal conductance (Biswas et al., 2008). Although there is not always a simple relationship between stomatal conductance and O₃ tolerance, targeting stomatal conductance as a means to improve crop production under elevated [O₃] seems unlikely to be an effective strategy for maintaining productivity, other than in regions with the highest [O₃]. Nonetheless, it should be noted that the relative concentrations of CO₂ necessary to protect crops from O₃ damage are very poorly defined, and variable for species and genotypes. Furthermore, any direct effect of elevated [O₃] on reproductive structures will probably not be mitigated by elevated [CO₂], and loss of reproductive sinks may constrain the response to elevated [CO₂] (McKee et al., 1997; Ashmore, 2005). In addition, elevated [CO₂] did not prevent acceleration of senescence that is characteristic of O₃ fumigation at ambient [CO₂] (Fiscus et al., 2005). Without further experimentation, it is not possible to precisely predict where and when the stress of elevated [O₃] will be matched or exceeded by the benefits of elevated [CO₂].

A second strategy for improving crop tolerance to O₃ has focused on improving detoxification of O₃-induced reactive oxygen species (ROS; Fiscus et al., 2005). Ozone passes through the stomata, and is rapidly degraded in the apoplast forming various ROS. The ROS burst following O₃ exposure is similar to the oxidative burst associated with the hypersensitive response, which leads to programmed cell death (Overmyer et al., 2003). Ascorbate is one of the major antioxidants thought to govern O₃ tolerance in plants, and total ascorbate content has been correlated with O₃ tolerance in a wide range of species (for review, see Conklin and Barth, 2004). Localization of ascorbate or other antioxidants to the apoplastic space, where interception and detoxification occurs, appears to be important in protection from O₃ damage, as is recycling of ascorbate back to the reduced "anti-oxidant" state (Burkey et al., 2003; Conklin and Barth, 2004). However, not all studies have demonstrated that O₃ tolerance correlates with ascorbate content (e.g. D'Haese et al., 2005), and apoplastic ascorbate concentrations in some plants may be too low to play a significant role in detoxification. Still, increasing the apoplastic antioxidant capacity of crops may be a fruitful target for limiting O₃-induced ROS damage.

Components of the O₃ sensing and signaling pathways seem to be good potential targets for biotechnological manipulation to improve crop productivity. For instance, ethylene-insensitive mutants of *Arabidopsis* (*Arabidopsis thaliana*) and birch (*Betula* spp.) are more tolerant to O₃ than wild-type control genotypes (Vahala et al., 2003; Kangasjärvi et al., 2005). However, it is important to note that the signaling pathway associated with acute O₃ damage has considerable overlap with the signaling pathway that limits pathogen spread (Overmyer et al., 2003). In addition, interactions among ROS, jasmonic acid, salicylic acid, and ethylene are involved in the cell death cycle (Kangasjärvi et al., 2005), as well as regulation of normal leaf senescence (Lim et al., 2007). Therefore, successful improvement of crop tolerance to O₃ by altering sensing, signaling, or regulatory pathways will depend on identifying targets for modification that do not disrupt other vital processes (Conklin and Barth, 2004).

CHALLENGES TO IMPROVING PRODUCTION UNDER GLOBAL CLIMATE CHANGE

Although some aspects of global change like rising [CO₂] will be uniform around the globe, others such as rising tropospheric [O₃] and altered precipitation will vary regionally. To meet the demands of a growing population, the next era of biotechnology and future crop breeding strategies will be challenged with identifying genotypes that can maximally exploit rising [CO₂] for yield enhancement and have improved stress tolerance traits (Reynolds and Borlaug, 2006). Global climate change will add at least three new dimensions to this challenge: (1) the production environment will be more variable and more stressful, (2) climatic variation will be greater between years and locations of field trials, and (3) the environment for which crops are being designed will be a rapidly moving target. Successfully understanding the genotype x environment interactions and closing the phenotype gap, i.e. understanding the function of crop genes in their production environment (Mifflin, 2000), will be a major hurdle in the race to release lines capable of improved yield in the rapidly changing climate. This emphasizes the necessity of field screening for CO₂-responsive and O₃-tolerant germplasm in the production environment, and recognizing that crops entering research and development pipelines today will emerge in 10 to 20 years into a different climate. Over the last decade, transgenic crop technology has been adopted faster than any other technology in the history of agriculture (Chassy, 2007). The approximately 250 million acres of biotechnology-engineered maize (*Zea mays*), canola (*Brassica napus*), cotton (*Gossypium hirsutum*), soybeans, papaya (*Carica papaya*), sugar beets (*Beta vulgaris*), sweet corn (*Zea mays* var. *rugosa*), and squash (*Cucurbita pepo*) have increased global farmer profits by approximately \$27 billion, reduced pesticide application by approximately 224 million kg, reduced the environmental impact of pesticide

use by approximately 14%, and reduced greenhouse gas emissions by approximately 960 million kg of CO₂ (Brookes and Barfoot, 2006). These improvements have come from engineering single genes into crops; however, crop responses to elevated [CO₂] and [O₃] are complex traits and will be much more difficult to control and engineer. Nonetheless, germplasm with greater abiotic stress resistance is making progress through research and development pipelines. Knowledge of crop responses to elevated [CO₂] and elevated [O₃] could match that of drought, flooding, temperature, and nutrient stress with greater investment in research, and here we have identified a number of potential targets for biotechnological improvement in a high-[CO₂] and high-[O₃] world.

ACKNOWLEDGMENT

We thank Damian Allen for useful discussions and helpful comments on a draft of this manuscript.

Received January 30, 2008; accepted February 28, 2008; published May 7, 2008.

LITERATURE CITED

- Agarwal S, Grover A (2006) Molecular biology, biotechnology and genomics of flooding-associated low O₂ stress response in plants. *Crit Rev Plant Sci* **25**: 1–21
- Ainsworth EA, Long SP (2005) What have we learned from 15 years of free air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO₂. *New Phytol* **165**: 351–372
- Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising [CO₂]: mechanisms and environmental interactions. *Plant Cell Environ* **30**: 258–270
- Ainsworth EA, Rogers A, Nelson R, Long SP (2004) Testing the ‘source-sink’ hypothesis of down-regulation of photosynthesis in elevated [CO₂] in the field with single gene substitutions in Glycine max. *Agric For Meteorol* **122**: 85–94
- Ashmore MR (2005) Assessing the future global impacts of ozone on vegetation. *Plant Cell Environ* **28**: 949–964
- Austin RB (1999) Yield of wheat in the United Kingdom: recent advances and prospects. *Crop Sci* **39**: 1604–1610
- Bainbridge G, Madgwick P, Parmar S, Mitchell R, Paul M, Pitts J, Keys AJ, Parry MAJ (1995) Engineering Rubisco to change its catalytic properties. *J Exp Bot* **46**: 1269–1276
- Barnabás B, Jäger K, Fehér A (2008) The effect of drought and heat stress on reproductive processes in cereals. *Plant Cell Environ* **31**: 11–38
- Bartels D, Sunkar R (2005) Drought and salt tolerance in plants. *Crit Rev Plant Sci* **24**: 23–58
- Bengtsson M, Shen Y, Oki T (2006) A SRES-based gridded global population dataset for 1990–2100. *Popul Environ* **28**: 113–131
- Biswas DK, Xu H, Li YG, Sun JZ, Wang XZ, Han XG, Jiang GM (2008) Genotypic differences in leaf biochemical, physiological and growth responses to ozone in 20 winter wheat cultivars released over the past 60 years. *Glob Change Biol* **14**: 46–59
- Black VJ, Black CR, Roberts JA, Stewart CA (2000) Impact of ozone on the reproductive development of plants. *New Phytol* **147**: 421–447
- Booker FL, Fiscus EL (2005) The role of ozone flux and antioxidants in the suppression of ozone injury by elevated CO₂ in soybean. *J Exp Bot* **56**: 2139–2151
- Borras L, Slafer GA, Otegui ME (2004) Seed dry weight response to source-sink manipulations in wheat, maize and soybean: a quantitative reappraisal. *Field Crops Res* **86**: 131–146
- Brookes G, Barfoot P (2006) Global impact of biotech crops: socio-economic and environmental effects in the first ten years of commercial use. *AgBioForum* **9**: 139–151
- Burkey KO, Eason G, Fiscus EL (2003) Factors that affect leaf extracellular ascorbic acid content and redox status. *Physiol Plant* **117**: 51–57
- Busch W, Wunderlich M, Schoff F (2005) Identification of novel heat shock factor-dependent genes and biochemical pathways in *Arabidopsis thaliana*. *Plant J* **41**: 1–14
- Chassy BM (2007) The history and future of GMOs in food and agriculture. *Cereal Foods World* **52**: 169–172
- Chaves MM, Oliveira MM (2004) Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. *J Exp Bot* **55**: 2365–2384
- Conklin PL, Barth C (2004) Ascorbic acid, a familiar small molecule intertwined in the response of plants to ozone, pathogens, and the onset of senescence. *Plant Cell Environ* **27**: 959–970
- D’Haese D, Vandermeiren K, Asard H, Horemans N (2005) Other factors than apoplastic ascorbate contribute to the differential ozone tolerance of two clones of *Trifolium repens* L. *Plant Cell Environ* **28**: 623–632
- Drake BG, Gonzalez-Meler MA, Long SP (1997) More efficient plants: a consequence of rising atmospheric CO₂? *Annu Rev Plant Physiol Plant Mol Biol* **48**: 609–639
- Ehrlich PR (1968) *The Population Bomb*. Ballantine Books, New York
- FAO (2006) *The State of Food Insecurity in the World*. Food and Agriculture Organization of the United Nations, Rome
- Fischer RA (2007) Understanding the physiological basis of yield potential in wheat. *J Agric Sci* **145**: 99–113
- Fiscus EL, Booker FL, Burkey KO (2005) Crop responses to ozone: uptake, modes of action, carbon assimilation and partitioning. *Plant Cell Environ* **28**: 997–1011
- Geiger M, Haake V, Ludewig F, Sonnewald U, Stitt M (1999) The nitrate and ammonium nitrate supply have a major influence on the response of photosynthesis, carbon metabolism, nitrogen metabolism and growth to elevated carbon dioxide in tobacco. *Plant Cell Environ* **22**: 1177–1199
- Ghannoum O, von Caemmerer S, Ziska LH, Conroy JP (2000) The growth response of C₄ plants to rising atmospheric CO₂ partial pressure: a reassessment. *Plant Cell Environ* **23**: 931–942
- Gifford RM, Thorne JH, Hitz WD, Giaquinta RT (1984) Crop productivity and photoassimilate partitioning. *Science* **225**: 801–808
- Heagle AS (1989) Ozone and crop yield. *Annu Rev Phytopathol* **27**: 397–423
- Hill AC, Littlefield N (1969) Ozone. Effect on apparent photosynthesis, rate of transpiration and stomatal closure in plants. *Environ Sci Technol* **3**: 52–56
- Hirel B, Le Gouis J, Ney B, Gallais A (2007) The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *J Exp Bot* **58**: 2369–2387
- Iba K (2002) Acclimative response to temperature stress in higher plants: approaches of gene engineering for temperature tolerance. *Annu Rev Plant Biol* **53**: 225–45
- IPCC (2007) *Climate change 2007: the physical science basis*. In S Solomon, D Qin, M Manning, Z Chen, M Marquis, KB Averyt, M Tignor, HL Miller, eds, *Contribution of Working Group I to the Fourth Annual Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK, pp 996
- Kangasjärvi J, Jaspers P, Kollist H (2005) Signalling and cell death in ozone-exposed plants. *Plant Cell Environ* **28**: 1021–1036
- Kim HY, Lieffering M, Kobayashi K, Okada M, Mitchell MW, Gumpertz M (2003) Effects of free-air CO₂ enrichment and nitrogen supply on the yield of temperate paddy rice crops. *Field Crops Res* **83**: 261–270
- Kimball BA, Kobayashi K, Bindi M (2002) Responses of agricultural crops to free-air CO₂ enrichment. *Adv Agron* **77**: 293–368
- Leakey ADB, Bernacchi CJ, Dohleman FG, Ort DR, Long SP (2004) Will photosynthesis of maize (*Zea mays*) in the US corn belt increase in future [CO₂] rich atmospheres? An analysis of diurnal courses of CO₂ uptake under free-air concentration enrichment (FACE). *Glob Change Biol* **10**: 951–962
- Leakey ADB, Uribealarea M, Ainsworth EA, Naidu SL, Ort DR, Long SP (2006) Photosynthesis, productivity, and yield of maize are not affected by open-air elevation of CO₂ concentration in the absence of drought. *Plant Physiol* **140**: 779–790
- Lefebvre S, Lawson T, Zakhleniuk OV, Lloyd JC, Raines CA (2005) Increased sedoheptulose-1,7-bisphosphatase activity in transgenic tobacco plants stimulates photosynthesis and growth from an early stage in development. *Plant Physiol* **138**: 451–460

- Lim PO, Kim HJ, Nam HG** (2007) Leaf senescence. *Annu Rev Plant Biol* **58**: 115–136
- Lin DJ, Lur HS, Chu C** (2001) Effects of abscisic acid on ozone tolerance of rice (*Oryza sativa* L.) seedlings. *Plant Growth Regul* **35**: 295–300
- Long SP** (1991) Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO₂ concentrations: Has its importance been underestimated? *Plant Cell Environ* **14**: 729–739
- Long SP, Ainsworth EA, Leakey ADB, Nösberger J, Ort DR** (2006) Food for thought: lower than expected crop yield stimulation with rising CO₂ concentrations. *Science* **312**: 1918–1921
- Long SP, Ainsworth EA, Rogers A, Ort DR** (2004) Rising atmospheric carbon dioxide: Plants FACE the future. *Annu Rev Plant Biol* **55**: 591–628
- Long SP, Naidu SL** (2002) Effects of oxidants at the biochemical, cell and physiological levels, with particular reference to ozone. In JNB Bell, M Treshow, eds, *Air Pollution and Plant Life*. John Wiley & Sons, West Sussex, UK, pp 69–88
- Malthus TR** (1817) *An Essay on the Principle of Population*. Murray, London
- Mauzerall DL, Wang XP** (2001) Protecting agricultural crops from the effects of tropospheric ozone exposure: reconciling science and standard setting in the United States, Europe, and Asia. *Annu Rev Energy Environ* **26**: 237–268
- McKee IF, Bullimore JF, Long SP** (1997) Will elevated CO₂ concentrations protect the yield of wheat from O₃ damage? *Plant Cell Environ* **20**: 77–84
- Mifflin B** (2000) Crop improvement in the 21st century. *J Exp Bot* **51**: 1–8
- Morgan PB, Bollero GA, Nelson RL, Dohleman FG, Long SP** (2005) Smaller than predicted increase in aboveground net primary production and yield of field-grown soybean under fully open-air [CO₂] elevation. *Glob Change Biol* **11**: 1–10
- Morrison MJ, Voldeng HG, Cober ER** (2000) Agronomic changes from 58 years of genetic improvement of short-season soybean cultivars in Canada. *Agron J* **92**: 780–784
- Murphy JJ, Delucchi MA, McCubbin DR, Kim HJ** (1999) The cost of crop damage caused by ozone air pollution from motor vehicles. *J Environ Manage* **55**: 273–289
- Nakashima K, Yamaguchi-Shinozaki K** (2006) Regulons involved in osmotic stress-responsive and cold stress-responsive gene expression in plants. *Physiol Plant* **126**: 62–71
- Ottman MJ, Kimball BA, Pinter PJ, Wall GW, Vanderlip RL, Leavitt SW, LaMorte RL, Matthias AD, Brooks TJ** (2001) Elevated CO₂ increases sorghum biomass under drought conditions. *New Phytol* **150**: 261–273
- Overmyer K, Broshe M, Kangasjärvi J** (2003) Reactive oxygen species and hormonal control of cell death. *Trends Plant Sci* **8**: 335–342
- Parry MAJ, Andralojc PJ, Mitchell RAC, Madgwick PJ, Keys AJ** (2003) Manipulation of Rubisco: the amount, activity, function and regulation. *J Exp Bot* **54**: 1321–1333
- Parry MAJ, Flexas J, Medrano H** (2005) Prospects for crop production under drought: research priorities and future directions. *Ann Appl Biol* **147**: 211–226
- Raines CA** (2003) The Calvin cycle revisited. *Photosynth Res* **75**: 1–10
- Raines CA, Paul MJ** (2006) Products of leaf primary carbon metabolism modulate the developmental programme determining plant morphology. *J Exp Bot* **57**: 1857–1862
- Reynolds MP, Borlaug NE** (2006) Applying innovations and new technologies for international collaborative wheat improvement. *J Agric Sci* **144**: 95–110
- Rogers A, Ainsworth EA** (2006) The response of foliar carbohydrates to elevated [CO₂]. In J Nösberger, SP Long, RJ Norby, M Stitt, GR Hendrey, H Blum, eds, *Managed Ecosystems and CO₂. Case Studies, Processes and Perspectives*. Springer-Verlag, Berlin, pp 293–310
- Rogers A, Fischer BU, Bryant J, Frehner M, Blum H, Raines CA, Long SP** (1998) Acclimation of photosynthesis to elevated CO₂ under low N nutrition is effected by the capacity for assimilated utilization. Perennial ryegrass under Free-Air CO₂ Enrichment. *Plant Physiol* **118**: 683–692
- Rogers A, Gibon Y, Stitt M, Morgan PB, Bernacchi CJ, Ort DR, Long SP** (2006) Increased carbon availability at elevated carbon dioxide concentration improves N assimilation in a legume. *Plant Cell Environ* **29**: 1651–1658
- Rogers A, Humphries SW** (2000) A mechanistic evaluation of photosynthetic acclimation at elevated CO₂. *Glob Change Biol* **6**: 1005–1011
- Rosegrant MW, Cline SA** (2003) Global food security: challenges and policies. *Science* **302**: 1917–1918
- Smith AM, Stitt M** (2007) Coordination of carbon supply and plant growth. *Plant Cell Environ* **30**: 1126–1149
- Stafford N** (2007) Future crops—the other greenhouse effect. *Nature* **448**: 526–528
- Streeter JG, Lohnes DG, Fioritto RJ** (2001) Patterns of pinitol accumulation in soybean plants and relationships to drought tolerance. *Plant Cell Environ* **24**: 429–438
- Taub DR, Miller B, Allen H** (2008) Effects of elevated CO₂ on the protein concentration of food crops: a meta-analysis. *Glob Change Biol* **14**: 565–575
- Vahala J, Ruonala R, Keinänen M, Tuominen H, Kangasjärvi J** (2003) Ethylene insensitivity modulates ozone-induced cell death in birch (*Betula pendula*). *Plant Physiol* **132**: 185–195
- Wahid A, Gelani S, Ashraf M, Foolad MR** (2007) Heat tolerance in plants: an overview. *Environ Exp Bot* **61**: 199–223
- Wall GW, Brooks TJ, Adam R, Cousins AB, Kimball BA, Pinter PJ, LaMorte RL, Triggs L, Ottman MJ, Leavitt SW, et al.** (2001) Elevated atmospheric CO₂ improved sorghum plant water status by ameliorating the adverse effects of drought. *New Phytol* **152**: 231–248
- Wang X, Manning W, Feng Z, Zhu Y** (2007) Ground-level ozone in China: distribution and effects on crop yields. *Environ Pollut* **147**: 394–400
- Wang W, Vinocur B, Altman A** (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta* **218**: 1–14
- Yang L, Huang J, Yang H, Zhu J, Liu H, Dong G, Liu G, Han Y, Wang Y** (2006) The impact of free-air CO₂ enrichment (FACE) and N supply on yield formation of rice crops with large panicle. *Field Crops Res* **98**: 141–150
- Yoshizawa Y, Toyoda K, Arai H, Ishii M, Igarashi Y** (2004) CO₂-responsive expression and gene organization of three ribulose-1,5-bisphosphate carboxylase/oxygenase enzymes and carboxysomes in *Hydrogenovibrio marinus* strain MH-110. *J Bacteriol* **186**: 5685–5691
- Zhu XG, de Sturler E, Long SP** (2007) Optimizing the distribution of resources between enzymes of carbon metabolism can dramatically increase photosynthetic rate: a numerical simulation using an evolutionary algorithm. *Plant Physiol* **145**: 513–526
- Zhu XG, Portis AR, Long SP** (2004) Would transformation of C₃ crop plants with foreign Rubisco increase productivity? A computational analysis extrapolating from kinetic properties to canopy photosynthesis. *Plant Cell Environ* **27**: 155–165