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Approaches to investigate crop responses to ozone pollution: from O₃-FACE to satellite-enabled modeling

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SUMMARY

Ozone (O₃) is a damaging air pollutant to crops. As one of the most reactive oxidants known, O₃ rapidly forms other reactive oxygen species (ROS) once it enters leaves through stomata. Those ROS in turn can cause oxidative stress, reduce photosynthesis, accelerate senescence, and decrease crop yield. To improve and adapt our feed, fuel, and food supply to rising O₃ pollution, a number of Free Air Concentration Enrichment (O₃-FACE) facilities have been developed around the world and have studied key staple crops. In this review, we provide an overview of the FACE facilities and highlight some of the lessons learned from the last two decades of research. We discuss the differences between C₃ and C₄ crop responses to elevated O₃, the possible trade-off between productivity and protection, genetic variation in O₃ response within and across species, and how we might leverage this observed variation for crop improvement. We also highlight the need to improve understanding of the interaction between rising O₃ pollution and other aspects of climate change, notably drought. Finally, we propose the use of globally modeled O₃ data that are available at increasing spatial and temporal resolutions to expand upon the research conducted at the limited number of global O₃-FACE facilities.

Keywords: air pollution, free air concentration enrichment, oxidative stress response, genetic variation.

INTRODUCTION

Ozone (O₃) is a damaging air pollutant and potent greenhouse gas with both direct and indirect effects on vegetation and human health (Ainsworth et al., 2012; DeLang et al., 2021; Monks et al., 2015; Wedow et al., 2021a). As one of the most reactive oxidants known (Audran et al., 2018), O₃ forms in the lower atmosphere from chemical reactions of precursor gases including nitrogen oxides (NO_x), carbon monoxide, and volatile organic compounds. Ozone is lost by chemical destruction in the atmosphere and deposition to surfaces, including vegetation (Schultz et al., 2017). Local O₃ pollution levels and the atmospheric lifetime of O₃ vary with the relative concentrations of precursor molecules from local and long-range sources and meteorological conditions such as radiation, temperature, and atmospheric humidity (Goldberg et al., 2015; Lefohn et al., 2018; Young et al., 2013). The global average lifetime of O₃ is approximately 25 days in the troposphere (Young

et al., 2013) and decreases to under 5 days in the summertime at the surface boundary layer (Schultz et al., 2017). Maps of global O₃ pollution are based on surface observations (Cooper et al., 2014; Schultz et al., 2017), atmospheric chemistry models (e.g., Brauer et al., 2016), and most recently model-data fusion approaches (DeLang et al., 2021; Figure 1(a)). Current O₃ concentrations are highest in East Asia, South-Central Asia, central Africa, and western North America and lowest in Oceania (Figure 1(a)). In China and India, O₃ pollution has been exceptionally high in recent years. Summer monsoons influence the seasonality of O₃ pollution in Asia in addition to increasing anthropogenic, biogenic, and biomass burning emissions (Gao et al., 2020). While peak O₃ concentrations in Europe and North America have decreased since the 1980s in response to emission reduction legislation and strategies, O₃ exposure is increasing globally with significant impacts for human health (DeLang et al., 2021).
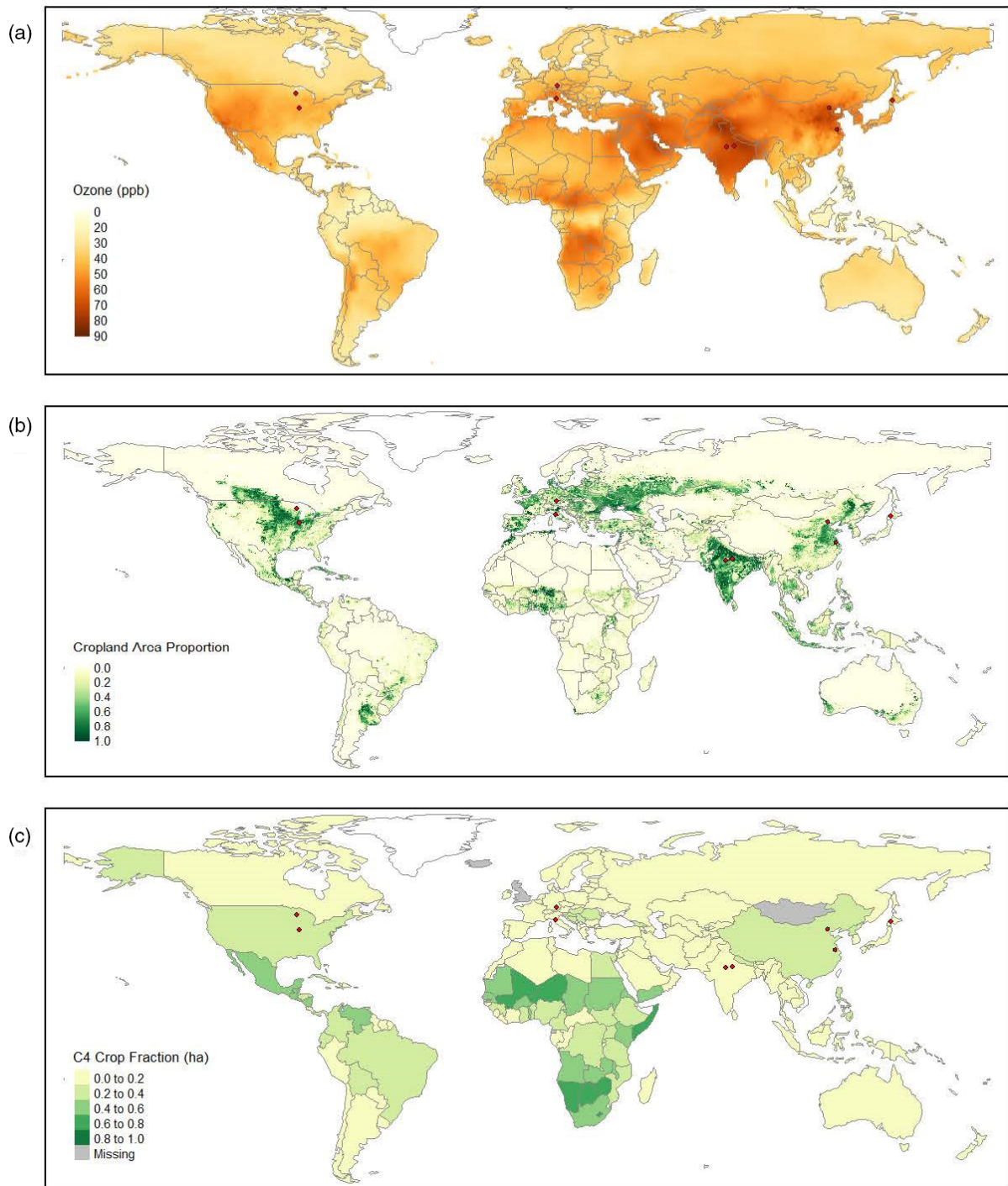
Figure 1. (a) 2017 yearly tropospheric O_3 distribution in parts per billion (ppb) at 0.1° resolution, calculated from combined surface O_3 observations and a composite of nine atmospheric chemistry models (DeLang *et al.*, 2021). (b) Global distribution of agricultural cropland in the year 2000 derived from remote land-cover and agricultural inventory data (Ramankutty *et al.* 2000). (c) The fraction of total harvested area in hectares (ha) comprising C_4 crop production in each country, obtained from 2010–2019 average global crop production data from the Food and Agriculture Organization of the United States (FAO). Red dots indicate the location of O_3-FACE experimental sites.

Ozone has caused 0.4 W m^{-2} of radiative forcing since the industrial revolution (Myhre et al., 2013), thereby contributing to surface warming, which indirectly impacts rates of photosynthesis, transpiration, and respiration. Ozone directly damages plants by entering leaves through the stomata and reacting in the aqueous apoplast to

produce other reactive oxygen species (ROS) that subsequently accelerate senescence and at high enough concentrations lead to programmed cell death (Ainsworth, 2017; Hasan et al., 2021). Regions of high O_3 pollution (Figure 1(a)) correspond with global croplands (Figure 1(b)) resulting in significant negative effects on crop productivity (Fischer, 2019; Mills et al., 2018; Tai et al., 2021). Fischer (2019) estimated that O_3 pollution currently reduces wheat (*Triticum aestivum*) yields by more than 20% in India. Ozone pollution has been estimated to constrain global soybean (*Glycine max*) and maize (*Zea mays*) yields to a similar extent as nutrient stress, heat stress, or aridity stress (Mills et al., 2018). The estimated magnitude of global O_3 impacts on crop yields varies with assumptions about O_3 flux through stomata into leaves and the degree of detoxification within leaves. Still, conservative estimates suggest that current O_3 pollution reduces yield in major staple crops by 3.6% in maize, 2.6% in rice (*Oryza sativa*), 6.7% in soybean, and 7.2% in wheat (Tai et al., 2021).

To adapt agriculture to O_3 pollution, better understanding of the most vulnerable crops and growing regions along with mechanistic understanding of O_3 impacts on crops is needed. Manipulative experiments have tested the impacts of elevated O_3 concentrations ([O_3]) on crops, and for nearly 20 years, O_3 Free Air Concentration Enrichment (O_3-FACE) experiments have enabled testing of vegetation responses to elevated [O_3] under fully open, field conditions (Table 1). Many of these experiments focused on forest responses to elevated [O_3] with only facilities in the Midwest U.S., China, India and Italy having studied crop responses to elevated [O_3]. Both C_3 and C_4 crops including major grain and oilseed crops have been studied (Table 1). While manipulative O_3-FACE experiments have tested responses to increased [O_3], other approaches are used to estimate how current background O_3 impacts crop productivity. Cumulative metrics for O_3 exposure have been used along with assumptions about minimum thresholds for damage to estimate crop yield losses to O_3 (e.g., Avnery et al., 2011; Mauzerall and Wang, 2001; Mills et al., 2007; Van Dingenen et al., 2009). This approach is relatively simple and supported by improved global monitoring, remote sensing, and modeling of [O_3]. However, it is widely recognized that O_3 exposure is not always consistent with uptake into plants and the times and places of greatest O_3 exposure do not always coincide with greatest uptake of O_3 through stomata (Musselman et al., 2006). Therefore, O_3 flux-based models that consider environmental effects on stomatal conductance and subsequent O_3 flux into vegetation are used in combination with statistical relationships between O_3 uptake and crop yield loss to more accurately approximate the magnitude and cost of O_3 pollution to crop production (e.g., Emberson et al., 2000; Mills et al., 2018; Pleijel et al., 2007). The effects of O_3 on vegetation can also be incorporated into mechanistic crop and even earth-system models (Emberson et al., 2018; Lombardozzi et al., 2018). In this review, we describe the global O_3-FACE facilities that have been used to study crop responses to O_3 pollution and discuss some of the key lessons learned for adapting crops to O_3 pollution. We then discuss the potential for taking advantage of greater spatial resolution in both O_3 metrics and crop performance indicators to improve understanding of O_3 impacts on crops.

O_3-FACE EXPERIMENTS

FACE technology was originally developed to investigate plant responses to elevated [CO_2] and [O_3] in settings more natural than growth chambers or open top chambers (Lewin et al., 1994; Figure 2). FACE plots operate by releasing or blowing air enriched with O_3 into the wind which then increases [O_3] across an experimental plot (varying from approximately 2 m to 30 m in diameter). A sensor in the center of each O_3-FACE plot continuously monitors [O_3] in real-time and relays the information to a proportional-integral-derivative control system which automatically adjusts O_3 output to maintain a desired setpoint. While exposing plants to elevated [O_3] in growth chambers, greenhouses, or open-top chambers (OTCs) is simpler due to the ease of fumigation control in an enclosed system, plants grown in these controlled conditions often show different responses compared to plants grown in an open-field setting (De Graaf et al., 2006; Poorter et al., 2016). A meta-analysis of O_3-FACE and OTC experiments found that the relative yield loss of rice and wheat to increasing [O_3] was greater in FACE experiments than OTC experiments, while the opposite was true for soybean (Feng et al., 2018). Notable differences between enclosed systems and open-air growing environments are light attenuation, humidity or vapor pressure deficit, water availability, wind conditions, and constraints on root growth. These microenvironmental factors in the growing environment can lead to confounding experimental results, at times under- or overestimating the effect of the treatment (Ainsworth and Long, 2005; McLeod and Long, 1999).

O_3-FACE experiments mitigate many of these concerns by creating an environment which is as close to natural conditions as possible, only altering the concentration of the target gas and leaving the remainder of the environment unaltered. Despite these improvements, O_3-FACE technology still has several experimental limitations. The accuracy of fumigation control is dependent upon wind speed, so when wind speed is very low, control is poor. Also, because of the rapid reactions of O_3 on wet surfaces, most facilities do not fumigate plants when leaves are wet due to rain or dew. O_3 also varies diurnally in response to light levels, precursor pollutant concentrations, and other environmental conditions (Heath et al., 2009). Some O_3-FACE experiments track the diurnal variations in [O_3] and scale to a fold-change factor above the ambient [O_3], while

Table 1 Description of global ozone Free Air Concentration Enrichment (O₃-FACE) facilities. Superscripts identify reference manuscripts that tested different target [O₃] within a given O₃-FACE facility. Bold font indicates C₄ species tested in O₃-FACE facilities.

Facility name	Location	Operational	Ecosystem	Plant species	Target ozone concentration	References
China FACE	Jiangdu, Jiangsu, China	2007–2012	Crop	*Sativa oryza* *Triticum aestivum*	1.5× ambient [O₃]	Tang *et al.*, 2011
China O₃-FACE	Yanqing, Beijing, China	2018–present	Forest	*Populus deltoides* *Populus euramericana*	1.5× ambient [O₃]	Xu *et al.*, 2021
KROFEX	Freising, Germany	2000–2007	Forest	*Fagus sylvatica* *Picea abies*	2× ambient [O₃]	Werner and Fabian, 2002
FAOCE	New Delhi, India	2016–present	Crop	*Cicer arietinum* *Triticum aestivum* **Zea mays**	60–70 ppb	Yadav *et al.*, 2019
India O₃-FACE	Lucknow, Uttar Pradesh, India	2018–present	Forest	*Leucaena leucocephala*	+20 ppb above ambient [O₃]	Singh *et al.*, 2021
FO₃X	Florence, Italy	2015–present	Crop/forest	*Passiflora edulis* *Phaseolus vulgaris* *Phoenix dactylifera* *Populus* spp. *Punica granatum* *Quercus* spp. **Saccharum spp**.	1.2–1.4× ambient [O₃]	Paoletti *et al.* 2017
Sapporo Forest	Sapporo, Hokkaido, Japan	2011–present	Forest	*Betula platyphylla* *Fagus crenata* *Larix kaempferi* *Quercus mongolica* *Salix sachalinensis*	60–70 ppb[a] 2× ambient [O₃][b]	Watanabe *et al.*, 2013[a] Agathokleous *et al.*, 2017[b]
Tsukuba FACE	Tsukuba, Japan	2011–2019	Forest	*Betula platyphylla*	2× ambient [O₃]	Kitao *et al.*, 2021
Aspen FACE	Rhinelander, WI, USA	1998–2009	Forest	*Acer saccharum* *Betula papyrifera* *Populus tremuloides*	60–100 ppb	Dickson *et al.*, 2000
SoyFACE	Savoy, IL, USA	2002–present	Crop	*Glycine max* **Panicum virgatum** **Sorghum bicolor** **Zea mays**	1.2–2× ambient [O₃][a, b] 60-200 ppb[c]	Morgan *et al.* 2004[a] Gillespie *et al.*, 2012[b] Betzelberger *et al.*, 2012[c]

other experiments at O₃-FACE facilities raise the elevated treatment to a particular threshold and maintain the set concentration throughout the day (Table 1). The former treatment would more accurately simulate a natural environment by varying throughout the day (Heath et al., 2009), while the latter fixed treatment would ensure season-long concentrations above the thresholds for sensitivity (Fuhrer et al., 1997). Another limitation of O₃-FACE is the expense of setting up the infrastructure for fumigation.

To date, O₃-FACE experiments have investigated crop and forest ecosystems. Studies conducted at China FACE (Jiangdu, Jiangsu, China), FAOCE (New Delhi, India), and Soybean FACE (SoyFACE; Savoy, IL, USA) investigated annual cropping systems, focusing on locally relevant crops. Maize and soybean have been the major focus at SoyFACE, while rice and wheat have been investigated in China and India. The O₃-FACE (Beijing, China), KROFEX (Freising, Germany), Sapporo Forest FACE (Sapporo, Japan), and Aspen FACE (Rhinelander, WI, USA) facilities investigated forest responses to elevated [O₃] (Table 1).

Free air O₃ eXposure (FO₃X), an O₃-FACE site in Florence, Italy, investigates both crop and forest species. Historically, far fewer O₃-FACE facilities have been in operation at any point than CO₂ FACE facilities (for a comprehensive list of CO₂ FACE facilities see https://facedata.ornl.gov/global_face.html). Of the 10 O₃-FACE facilities established, six are currently in operation (Table 1). While CO₂ FACE facilities have been built in the Northern and Southern Hemispheres, all O₃-FACE facilities are in the Northern Hemisphere (Figure 1). For this review, we will focus on the O₃-FACE facilities largely investigating crop species, namely China FACE, SoyFACE, FO₃X, and FAOCE.

SoyFACE

The SoyFACE research facility (soyface.illinois.edu) is situated in one of the most productive maize and soybean regions in the United States and is the longest-running FACE facility investigating crop responses to elevated [CO₂] and [O₃] (Table 1). SoyFACE began in 2002 and has 24 FACE plots approximately 20 m in diameter, each capable of

Figure 2. Image of an elevated O_3 plot at the Soy-FACE facility. Soybean and C_4 grass species are grown at 100 ppb ozone. A retractable awning is located inside of the plot and is released to capture rainfall and study the interaction of elevated O_3 pollution and drought stress.

augmenting the local atmospheric concentrations of trace gases including O_3, CO_2, and the combination of both (Figure 2). Because of the prominence of maize and soybean as feed, fuel, and oil crops, these species have been the primary focus of research to date, but work at SoyFACE has also deepened our understanding of how other crops, like snap bean (*Phaseolus vulgaris*; Burkey et al., 2012), sorghum (*Sorghum bicolor* L.; Li et al., 2021), and switchgrass (*Panicum virgatum*; Li et al., 2019a), respond to elevated $[O_3]$.

The early SoyFACE O_3 experiments were conducted with limited numbers of soybean genotypes, and the elevated O_3 treatment was set at 1.2× ambient $[O_3]$. The primary findings from this early research were: (i) young leaves showed little measurable differences in gas exchange and other photosynthetic parameters under the elevated O_3 treatment (Morgan et al., 2004); (ii) significant differences in leaf photosynthetic traits appeared during reproductive development in older leaves (Morgan et al., 2004); (iii) total leaf area, plant height, stem diameter, and aboveground biomass were all significantly negatively affected by chronic O_3 exposure (Morgan et al., 2006); (iv) elevated $[O_3]$ increased the rate of senescence (Dermody et al., 2008); and (v) significant decreases in soybean yields were observed in elevated $[O_3]$ and reached as high as 25% (Morgan et al., 2006). Decreased yield was driven primarily by reduced seed size and number (Morgan et al., 2006). However, the extent of the O_3-induced yield loss varied substantially across growing seasons, with a 15% decrease in 2002 and a non-significant 4% decrease in 2004 (Christ et al., 2006).

Another set of O_3 experiments at SoyFACE aimed to quantify genetic variation in soybean sensitivity to elevated $[O_3]$ (2× ambient $[O_3]$) and to establish more nuanced exposure thresholds (ambient and eight set points ranging from 40 ppb to 200 ppb) (Betzelberger et al., 2010, 2012). In accordance with previous research, Betzelberger et al. (2010) reported significant differences under 2× ambient $[O_3]$ for leaf area index, photosynthesis, stomatal conductance, and chlorophyll content across 10 soybean varieties, with greater effects of elevated $[O_3]$ observed during reproductive development. Yield reductions varied from 8% to 37% across the different cultivars. While it had been previously known that U.S. and Canadian soybean germplasm displayed different sensitivities to O_3 pollution (Burkey and Carter, 2009), this was one of the first experiments to obtain field-grown estimates of yield sensitivity across multiple mid-maturity group soybeans under an elevated $[O_3]$ treatment. Betzelberger et al. (2012) later tested the dose response of seven soybean genotypes, and found linear reductions in harvest index, light interception efficiency, and photosynthetic capacity, all of which cumulatively influence seed yield. That study also discovered a linear reduction in seed yield of 37–39 kg ha^{-1} per ppb cumulative O_3 exposure over 40 ppb (Betzelberger et al., 2012).

More recent experiments at SoyFACE have tested the response of maize (Choquette et al., 2019, 2020; Yendrek et al., 2017a, 2017b) and other C_4 plants, specifically sorghum and switchgrass (Li et al., 2019a, 2021), to a step increase in elevated $[O_3]$ (100 ppb). Yendrek et al. (2017a,b) grew over 200 inbred and hybrid maize lines from 2013–2015 and directly measured and modeled multiple leaf traits including instantaneous gas exchange (A and g_s), the rate limiting steps of C_4 photosynthesis (V_{pmax} and V_{max}),

chlorophyll content, specific leaf area, and leaf nitrogen content. Similar to soybean, many of these traits in both inbred and hybrid lines showed significant O_3-induced decreases. Genotype by O_3 treatment interactions were also significant with large differences in the O_3 response across different maize lines. For example, photosynthesis in the leaf subtending the ear was reduced under elevated [O_3] by 0% to 59% during grain fill. Further study revealed that lower photosynthesis was associated with reduced Rubisco content and activity, but not activation state (Choquette et al., 2020).

Sorghum and switchgrass, two proposed bioenergy crops, showed greater tolerance to elevated [O_3] at Soy-FACE. Switchgrass, like maize, showed significant decreases in A, V_{max}, and PSII maximum efficiency, but maintained nutrient composition, leaf area, and total biomass in elevated [O_3] (Li et al., 2019a). Ten varieties of bioenergy sorghum also appeared to be robust against elevated [O_3] with significant decreases only found transiently for photosynthetic capacity, while total aboveground biomass remained largely unaffected (Li et al., 2021).

China FACE and FAOCE

Two O_3-FACE systems have been established in Asia to perform crop research, China FACE and FAOCE (Table 1). These O_3-FACE facilities are important because of increasing O_3 pollution in Asia in recent years (Kunchala et al., 2021; Li et al., 2019b). The China O_3-FACE facility was established in 2007 in Jiangsu, China, where rice and wheat cultivation has been in practice for over 1000 years (Shi et al., 2009). The O_3-FACE plots were 14-m diameter octagons and both rice and wheat were exposed to elevated [O_3] of approximately 25–50% above ambient (Tang et al., 2011). Five Chinese rice cultivars have been measured at China FACE and elevated [O_3] at 50% above ambient significantly reduced rice yields by 15–17.5% in two sensitive cultivars, but had no effect on two tolerant cultivars (Shi et al., 2009; Wang et al., 2012a). Further investigation of two contrasting cultivars attributed yield loss to a decrease in the number of spikelets per panicle in elevated [O_3] (Wang et al., 2012b). Another study tested the effects of plant density on the response of rice to elevated [O_3] and failed to detect any effect of plant density on O_3 response (Peng et al., 2018). The deleterious effects of elevated O_3 on rice grain quality were found in a sensitive rice hybrid Shanyou 63 (Wang et al., 2012a).

In wheat, a 25% increase in background O_3 concentration resulted in 10–35% decrease in yield in four Chinese cultivars (Zhu et al., 2011). Genetic variation in O_3 response in both rice and wheat (Shi et al., 2009; Zhu et al., 2011) were reported at the China FACE experiment, and further experiments suggested that antioxidative enzymes play a key role in determining sensitivity (Feng et al., 2011, 2016). In wheat, allocation to biomass belowground was reduced in elevated [O_3], and soil CO_2 and N_2O emissions increased in response to elevated [O_3] in a sensitive cultivar (Kou et al., 2018), but not in a resistant cultivar (Kou et al., 2015). Additionally, elevated [O_3] altered the soil microbial food web, with the soil biota changing more in response to elevated [O_3] in an O_3-tolerant wheat variety compared to a sensitive line (Li et al., 2012). Specifically, soil microbial communities in the rhizosphere of O_3-tolerant wheat shifted towards consumption of easily degradable carbon sources, while consumption of more complex carbon sources was associated with the microbial communities in the rhizosphere of O_3-sensitive wheat (Bao et al., 2015). Changes in the microbial community associated with specific wheat cultivars were suggested to alter their plant growth, nutrient uptake, and subsequent response to O_3 pollution.

The FAOCE was installed in 2016 in New Delhi, India, and analyzed the response of yield and grain quality to the combination of elevated [O_3] and [CO_2] in wheat, maize, and chickpea (*Cicer arietinum*) (Singh et al., 2021; Yadav et al., 2019, 2021). The FACE rings at FAOCE were 5 m in diameter and were divided into halves or quartiles to study different genotypes. Similar to results from China FACE, significant negative impacts of elevated O_3 (70 ppb) on photosynthesis and yield in two wheat cultivars were observed in a FAOCE study in India (Yadav et al., 2019). Chickpea was also sensitive to O_3 and growth at approximately 60 ppb decreased leaf area index, pod number per plant, pod and seed mass, and total yield (Singh et al., 2021).

FO₃X

FO₃X, located near Florence, Italy, is the only O_3-FACE facility in a Mediterranean climate and has been used to study a wide variety of plant species (Paoletti et al., 2017; Table 1). The construction of the FO₃X experimental plots differs from SoyFACE and China FACE in that O_3 is delivered in a three-dimensional structure with piping above and on all four sides of a 5 × 5 × 2 m box (Paoletti et al., 2017). Plants are grown in pots, which allows for a mixture of different species to be investigated within the FACE rings with a range of other treatments, but has the drawback of restriction of root growth and access to the natural rhizosphere. Both woody crops (pomegranate [*Punica granatum*], date palm [*Phoenix dactylifera*], passion fruit [*Passiflora edulis*]) and herbaceous crops (snap bean, sugarcane [*Saccharum* spp.]) have been studied at the FO₃X facility (Cotrozzi et al., 2020; Pellegrini et al., 2021). The foliar application of agrochemicals as a protective measure against O_3 stress was studied in snap bean. Ethylenediurea and cytokinin applications minimized many of the negative effects of elevated [O_3] on foliar injury, photosynthesis, and stomatal conductance (Zhang et al., 2018). Crosstalk between salinity and O_3 stress was measured in pomegranate, which was sensitive to O_3. Studies focusing on sugarcane varieties found that exposure to elevated O_3 reduced

aboveground biomass and photosynthetic capacity relative to ambient [O_3] (Moura et al., 2018). Susceptibility to O_3 varied between sugarcane varieties with varieties showing early reductions in stomatal conductance having less long-term O_3-induced decreases in biomass. There are trade-offs in all FACE experiments, especially when studying perennial crops for a single season, and long-term experiments are needed to fully understand how these crops respond to elevated [O_3].

LESSONS LEARNED FROM FACE EXPERIMENTS

C_3 versus C_4

Previous studies mostly focused on how elevated [O_3] affected plant growth, development, and productivity in C_3 species because of a lower ratio of stomatal conductance to photosynthesis in C_4 species hypothesized to limit O_3 flux into the leaf (Treshow and Anderson, 1989). Early studies in open-top chambers also suggested C_4 species were less sensitive (Heagle, 1989). The global importance of C_4 plants, which contribute approximately 25% of global terrestrial primary production (Sage et al., 1999; Still et al., 2003), has led to a re-evaluation of their sensitivity to elevated [O_3]. Most C_4 plants are grasses, including important crops such as maize and sorghum, and are widely distributed from the tropics to the temperate regions (Still et al., 2003). Maize is one of the most important food sources in the world, providing more than 30% of the food calories to over 4.5 billion people in 94 developing countries (Palacios-Rojas et al., 2020; Shiferaw et al., 2011). Other C_4 crops, including switchgrass and miscanthus are considered as major sources of bioenergy and ethanol production in North America (Heaton et al., 2008; Schmer et al., 2008), while sugarcane is a primary energy crop in South America (Manochio et al., 2017). C_4 crops are most abundant in North and South America, East Asia, and Africa (Figure 1(c); Still et al., 2003), where the yearly peak O_3 concentrations can exceed 60 ppb or more (Figure 1 (a)). It is estimated that background O_3 concentrations reduced maize yields by 10% in the United States between approximately 1980 and 2010 (McGrath et al., 2015) and by 6.1% worldwide (Mills et al., 2018). However, more recent empirical estimates suggest that other pollutants, namely particulate matter and nitrogen dioxide, are more damaging to maize than O_3 (Lobell and Burney, 2021). Clearly, a better understanding of O_3 effects on plant physiology and production in C_4 plants is essential to predict how O_3 may threaten global food and energy security and provide a new perspective on vegetation responses to O_3.

So far, only four C_4 crops have been studied for O_3 response using O_3-FACE facilities (Table 1). Research from SoyFACE showed elevated O_3 concentrations (approximately 100 ppb) significantly reduced leaf photosynthetic capacity in maize (Choquette et al., 2019, 2020; Sorgini et al., 2019; Wedow et al., 2021b; Yendrek et al., 2017a,b), but had little or no effect on photosynthesis and biomass yield in switchgrass (Li et al., 2019a) and sorghum (Li et al., 2021). These studies also reported considerable genotypic variation in O_3 sensitivity among maize and sorghum lines (Choquette et al., 2019; Li et al., 2021; Yendrek et al., 2017a,b). In a study at the FO$_3$X facility, Moura et al. (2018) exposed two potted sugarcane genotypes to three levels of O_3 concentrations and found a significant reduction in plant biomass at increased [O_3]. Overall, the existing FACE studies have shown interspecific and genotypic variation in O_3 response among C_4 crops like maize and sugarcane, but there is also evidence that many genotypes of C_4 species are more tolerant to relatively high concentrations of chronic O_3 (Li et al., 2021).

Given that leaf anatomical features differ between C_4 and C_3 species, a key question is, do C_4 and C_3 species have different mechanisms of response to O_3? Ozone is thought to instantly react with molecules within the intercellular air space after entry through stomata to produce a variety of ROS (Figure 3). In C_3 leaves, ROS can directly damage mesophyll cells and chloroplasts where photosynthesis occurs (Figure 3(a)) (Ainsworth, 2017). In the O_3-FACE experiments, many C_3 plants show decreased Rubisco activity and content in elevated [O_3], often associated with accelerated senescence (Betzelberger et al., 2010; Feng et al., 2011; Morgan et al., 2004). However, in C_4 leaves, there is a physical separation between the initial assimilation of atmospheric CO_2 in mesophyll cells via phosphoenolpyruvate (PEP) carboxylase and the photosynthetic carbon reduction cycle. After initial assimilation in mesophyll cells, the fixed carbon is then decarboxylated and refixed in the bundle sheath cells where the photosynthetic carbon reduction cycle occurs (Hatch, 1987; Sage, 2004). By surrounding bundle sheath cells, mesophyll cells may protect bundle sheath cells from oxidative damage caused by ROS and prolong the ROS diffusion pathway from the intercellular air space to the bundle sheath cells (Figure 3(b)). A greenhouse study of four sugarcane hybrids suggested that PEP carboxylase was more sensitive than Rubisco to O_3 (Grantz et al., 2012), again providing some support for the hypothesis that C_4 species may respond differently to elevated O_3. However, in the O_3-FACE experiments to date, there is inconclusive evidence for greater sensitivity of PEP carboxylase to O_3. Gas exchange studies of maize varieties reported that both maximum PEP carboxylase activity and Rubisco activity were significantly reduced by elevated [O_3] (Choquette et al., 2020), and to a greater degree as leaves aged. Similarly, carboxylation efficiency was reduced by elevated [O_3] in sugarcane after 75–85 days of exposure (Moura et al., 2018). In switchgrass and sorghum, the effects of O_3 on photosynthesis were less consistent, and neither species showed reduced biomass at elevated [O_3] (Li et al., 2019a,
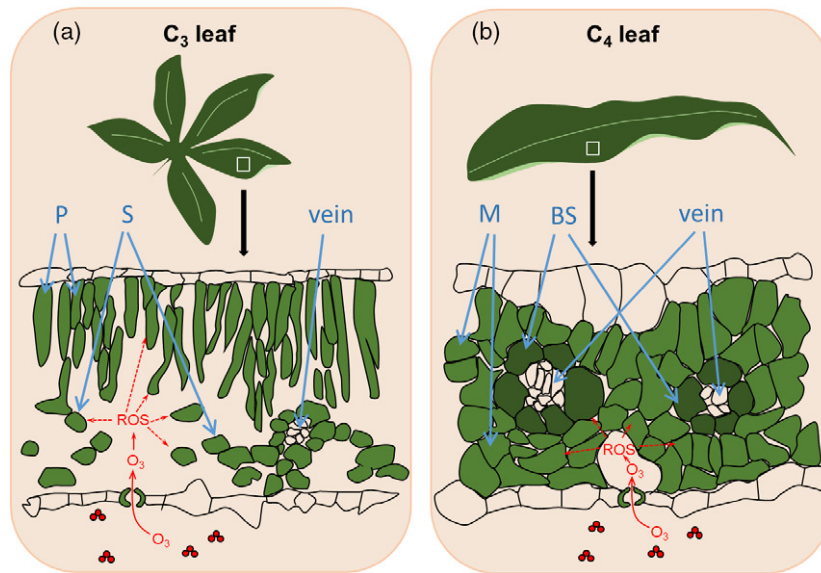
Figure 3. Representative cross-sections of a typical C_3 (a) and C_4 (b) leaf. (a) C_3 leaves generally have two types of mesophyll cells: palisade and spongy. Palisade mesophyll cells, located below the adaxial surface, are elongated cells containing many chloroplasts, which absorb a major portion of the light energy used for photosynthesis. Spongy mesophyll cells are close to the abaxial surface and composed of rounded cells with few chloroplasts. (b) C_4 leaves typically exhibit Kranz anatomy, in which mesophyll cells surround bundle sheath cells and bundle sheath cells further surround vascular bundles. Phosphoenolpyruvate (PEP) carboxylase is localized in the mesophyll cells, but Rubisco and many other Calvin cycle enzymes are found in the bundle sheath cells. In both C_3 and C_4 leaves, O_3 diffuses through stomata into the leaf intercellular airspaces and instantly forms a variety of reactive oxygen species (ROS), including hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2), superoxide radicals ($O_2^{\bullet-}$), singlet oxygen ($^1O_2$), and nitric oxide (NO). In the C_3 leaf (a), ROS can directly damage both palisade and spongy mesophyll cells, leading to reductions in photosynthesis. In the C_4 leaf (b), the surrounding mesophyll cells may protect bundle sheath cells from direct ROS damage and maintain photosynthetic capacity under O_3 stress. P, palisade mesophyll cell; S, spongy mesophyll cell; M, mesophyll cell; BS, bundle sheath cell.

2021). While some C_4 species appear to be more tolerant to elevated $[O_3]$ than many C_3 species, others appear to be similarly affected with reduced photosynthetic capacity, especially in aging leaves. O_3-FACE experiments have only scratched the surface of investigating C_4 species and future studies are needed to quantitatively characterize how C_4 and C_3 species respond to elevated $[O_3]$ and to determine the anatomical, physiological, and genetic bases for variation in O_3 tolerance.

Protection versus productivity during O_3 stress

FACE studies have revealed several hallmarks of growth under elevated $[O_3]$, mainly decreased photosynthetic carbon assimilation and accelerated senescence that ultimately leads to reduced biomass production and yield (Bernacchi et al., 2006; Burkey et al., 2012; Feng et al., 2011; Morgan et al., 2006; Singh et al., 2021). Plants chronically exposed to elevated $[O_3]$ face the challenge of maintaining development and productivity while protecting against O_3-induced oxidative damage. The phytotoxicity of O_3 is generally thought to be initiated by oxidative stress caused by the formation of ROS upon O_3 entry into the intercellular space (Grimes et al., 1983). Decreasing stomatal conductance to limit O_3 entry into the leaf and increasing antioxidant capacity to alleviate O_3-induced oxidative stress are mechanisms for protecting against O_3 damage,

but both have potential costs on plant productivity (Bechtold et al., 2018). Multiple O_3-FACE studies have indicated trade-offs between biochemical protection and crop productivity under elevated $[O_3]$. Studies conducted at Soy-FACE have investigated this trade-off in soybean at metabolomic, transcriptional, and proteomic levels.

Betzelberger et al. (2010) found a negative correlation between productivity (photosynthesis or seed yield) and total antioxidant capacity in soybean exposed to twice the ambient $[O_3]$, suggesting a trade-off between carbon gain and antioxidant metabolism under O_3 stress. A later study by Betzelberger et al. (2012) investigated soybean growth under a range of elevated $[O_3]$ and again found a linear decrease in photosynthetic carbon assimilation, biomass, and yield under elevated $[O_3]$, while a linear increase in total antioxidant capacity was observed. Further, during reproductive growth a negative linear response to increasing $[O_3]$ was found in the levels of glucose, fructose, sucrose, total protein, and total starch in soybean leaves. At the metabolite level, this demonstrates a trade-off between protection and productivity whereby increasing antioxidant capacity and metabolism may divert resources away from primary metabolism. Gillespie et al. (2012) correlated O_3-induced reductions in photosynthesis and increases in antioxidant capacity with transcriptional reprogramming of antioxidant, chlorophyll, and respiratory metabolism in

soybean. Most notable among the transcriptional responses to elevated [O_3] was increased abundance of transcripts associated with these three metabolic processes. Increasing respiratory capacity could help meet the energy and carbon demand for increases in antioxidant and chlorophyll metabolism to protect against O_3-induced oxidative stress. Consistent with this shift in energy demand under O_3 stress, Galant et al. (2012) found notable changes in the O_3 proteome associated with reductions in photosynthetic carbon assimilation. Changes in the abundance and oxidation of proteins involved in photosynthesis, antioxidant pathways, and carbon metabolism were found, including an increase in abundance and oxidation of proteins involved in sugar mobilization and conversion of starch to sugar.

Though not studied as robustly as in soybean, interesting relationships between protection and productivity during season-long O_3 exposure have been found in other species as well. For instance, changes in activity of enzymes involved in ROS metabolism and detoxification (namely catalase [CAT], superoxide dismutase [SOD], and ascorbate peroxidase [APX]) have been found under chronic O_3 exposure in wheat (Feng et al., 2016) and sugarcane (Moura et al., 2018), and these changes were linked to differential photosynthetic and yield sensitivities to O_3 among genotypes. Wedow et al. (2021b) investigated differential sensitivity between two inbred maize lines (B73 and Mo17) and the hybrid cross (B73 x Mo17) in relation to the accumulation of defense metabolites in leaf tissue. Senescence was accelerated in the hybrid compared to the inbred lines under elevated [O_3] and O_3-induced reductions in photosynthetic carbon assimilation during grain filling were greater in the hybrid line, leading to significantly greater yield loss. The greater negative response to O_3 in the hybrid line was exacerbated as leaves aged and associated with an increase in the accumulation of phytosterols and α-tocopherol, metabolites that serve protective roles against oxidative stress, again demonstrating a negative correlation between productivity and protection under O_3 stress. Together this research has provided noteworthy insight into the complex interactions that coordinate the O_3 stress response in multiple crop species and the mechanisms involved in the physiological and biochemical balance between defense, growth, and productivity of crops during O_3 stress.

Utilizing genetic variation

The O_3-FACE experiments discussed in this review have allowed researchers to observe different sensitivities to elevated [O_3] within and across the four major staple crop species – maize (Yendrek et al., 2017b), rice (Shi et al., 2009), wheat (Feng et al., 2018), and soybean (Betzelberger et al., 2010). This same variation in O_3 response may potentially help adapt our major crops to be less susceptible to current and future O_3 concentrations because genetic variation is the foundation of evolutionary adaptation as well as plant breeding. With O_3-induced reductions in yield across all four staple crops, a concerted effort should be made to breed and select crops for O_3 tolerance (Mills et al., 2018). This is especially true because it is believed that indirect selection of O_3-tolerant varieties by plant breeders has not occurred due to the substantial temporal and regional variation in ground-level [O_3] (Ainsworth et al., 2008; Biswas et al., 2009). Some of the relevant literature suggests that modern varieties of these staple crops appear more sensitive (Pleijel et al., 2006; Osborne et al., 2016) and current weather patterns leave crops more prone to O_3-induced yield losses (Mills et al., 2018; Ronan et al., 2020); however, this hypothesis has not been substantiated universally (Pleijel et al., 2018).

To date, efforts have been made to leverage variation in O_3 sensitivity to better understand the mechanisms involved and map genomic regions associated with O_3 tolerance (Frei, 2015). The logistical hurdles of screening large mapping populations under elevated [O_3] are a major challenge of this research. Ideally, selection for plant improvement is performed where the crops are grown (Poorter et al., 2016; Rapacz et al., 2015; Rouphael et al., 2018), and FACE technology has allowed for more realistic simulations of future atmospheric environments. However, the size of mapping populations with meaningful statistical power from either traditional linkage mapping or genomewide association studies usually exceed the space available in O_3-FACE plots. Yet, genetic studies have successfully mapped leaf damage in elevated [O_3] in maize to chromosome 2 (161 Mb) using chromosome segment substitution line (CSSL) populations (Sorgini et al., 2019). Choquette et al. (2019) also estimated heritability and combining abilities for leaf traits associated with O_3 damage using a half-diallel design with 45 F_1 hybrid crosses from a diverse set of 10 maize inbred accessions. These two experiments show that meaningful genetic mapping studies can identify mechanisms of the O_3 response within O_3-FACE systems.

Additionally, creative techniques have been developed to rapidly survey large populations or selectively chosen representative germplasm to target O_3 response traits in younger plants in growth chambers or glasshouses with increased O_3 concentrations (Burkey and Carter, 2009; Manigbas et al. 2010; Ueda et al., 2015; Burton et al., 2016; Begum et al., 2020). Many of these glasshouse studies are short-term experiments measuring and genetically mapping O_3-induced leaf damage. While unable to measure several cumulative traits negatively impacted by O_3, including early senescence and yield, these studies provide an important framework to further our understanding of the genetic controls of O_3 susceptibility (Mashaheet et al., 2020; Waldeck et al., 2017; Whaley et al., 2015).

Performing experiments at O_3-FACE facilities and in controlled environments has resulted in exciting advances in mapping O_3 tolerance traits in rice (Frei, 2015). Frei et al. (2008) mapped significant quantitative trait loci (QTLs) for leaf bronzing in a traditional linkage mapping population using a susceptible and a tolerant variety of rice for population development. Upon confirming the location of the QTL in a CSSL population from the same parental varieties, breeding lines were developed by introgressing two O_3-tolerant QTLs from the tolerant parent into the sensitive parent (Wang et al., 2014). The breeding lines outperformed the sensitive parent during a season-long O_3 treatment, demonstrating less severe leaf bronzing and decreased O_3-induced reductions in total biomass and yield while also maintaining higher photosynthetic rates and chlorophyll content. Ideally, the results from these controlled glasshouse and chamber studies would be validated in the field using FACE technology. Additionally, greater understanding of genetic mechanisms of plant O_3 response will inform transgenic approaches for improving O_3 tolerance.

Interactions with other climate change factors

Rising tropospheric $[O_3]$ is coincident with greater drought and temperature stress as well as rising atmospheric $[CO_2]$. The interaction between drought stress and O_3 stress is not well understood, and potential overlap exists between the signaling pathways involved in O_3 and drought stress responses (Wilkinson and Davies, 2010). Experiments imposing both drought and O_3 stress on tree species have shown mixed results (Cotrozzi, 2021). In some experiments severe drought events increased plant sensitivity to O_3 (Pollastrini et al., 2014), while in others drought ameliorated adverse effects of O_3 (Cotrozzi, 2021; Li et al., 2015). O_3-FACE studies are well situated to create experimental conditions with interactive stressors in a realistic setting. At the FO_3X facility, where plants are grown in pots, irrigation is used to vary the levels of drought stress (Hoshika et al., ; Pellegrini et al., 2019). Ongoing research at SoyFACE is conducted to investigate the interactive effects of drought and O_3 stress in soybean (Figure 2). Drought experiments are being conducted using large awnings to exclude rainfall and reduce water availability in sections of the FACE ring (Gray et al., 2016) (Figure 2). The interactive drought and O_3 experiments at FO_3X and Soy-FACE are currently the only research investigating the ways drought and O_3 stresses interact in field-grown crops. In rainfed regions, large-scale drought experiments could be conducted using rain-out shelters designed to have retractable roofs and walls to precisely control rainfall amounts on plants (Kant et al., 2016). It is feasible to envision a system whereby O_3 is pumped into these buildings while all walls and roofs are open, allowing for mixing in a relatively open-air system similar to FACE studies. This would allow for larger-scale studies to occur in rainfed

environments, where a major constraint on current drought studies is the restricted size of the awnings.

Another major stressor to crop systems is increasing temperatures, both through season-long warming and high temperature stress events, both of which are expected to occur at frequencies in the future (Dai, 2011; Fuhrer, 2009). As with drought stress, increasing temperatures are expected to coincide with higher tropospheric $[O_3]$, posing the potential for these stresses to interact and alter the plant's response to the cumulative stress (Suzuki et al., 2014). To date, interactive studies of season-long warming, heat waves, and altered $[CO_2]$ have been conducted at CO_2 FACE sites (Köhler et al., 2017, 2018; Thomey et al., 2019), but none have been conducted with crops investigating the interaction between heat and O_3 stress. Lee et al. (2020) investigated interactive effects in *Brassica juncea* using growth chambers, finding that the O_3-induced decrease in biomass is greater under high temperature stress. In another interactive chamber experiment conducted by Hansen et al. (2019), three varieties of spring wheat were grown and exposed to varying levels of O_3, CO_2, and temperature. They found that O_3 stress reduced yield more noticeably at lower temperature, and that at higher temperatures the yield reduction was largely attributable to the effects of heat stress. Given that results from growth chamber experiments are often different from those of FACE studies (McLeod and Long, 1999), finding a way to integrate both O_3 fumigation and heating arrays could provide greater insight into how these two stressors may interact under future climate conditions.

Unlike temperature and drought stress, elevated $[CO_2]$ has the potential to mitigate the negative impacts of O_3 exposure (Ainsworth and Long, 2021; Suzuki et al., 2014). Bernacchi et al. (2006) and Dermody et al. (2008) included a combination treatment of elevated $[CO_2]$ and $[O_3]$ and showed that elevated $[CO_2]$ ameliorated many of the negative effects of chronic O_3 stress in part because stomatal conductance is significantly lower in elevated $[CO_2]$, and therefore O_3 flux is reduced. At FAOCE, researchers found that the interactive effect of elevated $[O_3]$ and $[CO_2]$ compensated for O_3 damage in wheat and chickpea by maintaining yields similar to those in ambient conditions (Singh et al., 2021; Yadav et al., 2019). Work in maize at the same research facility found that elevated $[CO_2]$ helped to maintain yield when the plant was exposed to elevated O_3 (Yadav et al., 2021). The FACE experiments overall provide evidence that elevated $[CO_2]$ partially mitigates the negative effects of O_3 pollution.

PRIORITIZING CROPS FOR ADAPTION TO OZONE POLLUTION

The O_3-FACE experiments provided key insights into mechanisms of crop responses to O_3 pollution and helped define thresholds and dose response functions. However,

O_3-FACE experiments cover a very limited geographical range and number of crop species studied (Figure 1; Table 1). Other approaches for prioritizing which crops and regions most urgently require O_3 adaptation are needed. One alternative to experimental studies is to use statistical models of historical data using long-term records of yield and air pollution (Burney and Ramanathan, 2014; Fishman et al., 2010; Hong et al., 2020; Lobell and Burney, 2021; McGrath et al., 2015). This is similar to the approach used to estimate the global burden of human disease from air pollution (Cohen et al., 2017). By combining estimates of O_3 exposure from satellite data, chemical transport models and surface measurements with epidemiological studies to specify theoretical minimum risk exposure levels and relative risks to populations, Cohen et al. (2017) estimated that O_3 pollution caused 254 000 deaths and 4.1 million disability-adjusted life years in 2015.

A similar statistical approach to link O_3 exposure to crop yield loss has been used by Burney and Ramanathan (2014) to test air pollution impacts on wheat and rice yields in India. They examined the relationship between O_3 precursor emissions (NO_x, VOCs), rather than O_3 itself, and estimated that wheat yields were reduced by 33% by air pollution and rice yields by 23%. Data from the U.S. Environmental Protection Agency's Air Quality System were used to test O_3 impacts on U.S. maize and soybean yields (McGrath et al., 2015) and perennial crop yields in California (Hong et al., 2020). In these studies, the effects of temperature and precipitation were modeled along with O_3 effects, and it was estimated that from 1980 to present, O_3 has reduced rainfed U.S. maize and soybean yields by 10% and 5% (McGrath et al., 2015) and cost roughly \$1 billion per year to Californian perennial agriculture (Hong et al., 2020). Another recent analysis suggested that improvements to air quality in the U.S. since 1999 have contributed to roughly 20% of yield gains over the past two decades and currently particulate matter pollution and nitrogen dioxide are more damaging to crop yields than O_3 pollution (Lobell and Burney, 2021). Such statistical models require a long-term record of O_3 data and make assumptions about how O_3 concentrations vary spatially between sensors. The lack of O_3 monitoring and long-term yield records in many parts of the world have prohibited this approach from identifying key regions for adaptation to O_3 pollution. However, advances in modeling, machine learning, and data fusion now produce high-resolution surface O_3 datasets that could be used for statistical modeling of crop responses to O_3 (DeLang et al., 2021). Additionally, high-resolution remotely sensed estimates of crop photosynthesis (Bodesheim et al., 2018) could be used in combination with surface O_3 datasets to test O_3 impacts during a growing season, and not just on end-of-season crop yield.

The statistical approach described above assumes that yield loss is associated with cumulative exposure to O_3 above a certain threshold and does not mechanistically account for environmental variation in stomatal conductance or genetic variation in O_3 detoxification that would alter the effective O_3 flux (Clifton et al., 2020). The notion of a phytotoxic O_3 dose above a threshold 'y' (POD_y) and its incorporation into models accounts for both environmental variation in stomatal conductance and detoxification capacity of leaves (Musselman et al., 2006; Mills et al., 2011). POD_y values have been estimated for major 2011crop species and their use in global scale crop damage assessments suggests significant effects of O_3 on yields (Emberson, 2020). Wheat has been identified as particularly vulnerable to O_3, with yields in China 6–15% lower because of O_3 pollution, and Indian wheat yields 8–22% lower (Tang et al., 2013). Yet, even POD_y approaches to quantify O_3 impacts fail to account for seasonal variation in detoxification capacity, and there is likely significant variation even within species for the threshold value (e.g., Feng et al., 2011). Therefore, greater mechanistic understanding of O_3 stress and its interaction with other environmental variables is critical to incorporate into process-based models (Emberson, 2020).

CONCLUSION

Ozone remains a damaging air pollutant in many crop growing regions and new approaches are needed to understand and adapt agriculture to O_3 stress. Combining the power of field experiments with high-spatial resolution atmospheric and yield datasets has the potential to improve our understanding of the mechanisms of the O_3 response and to enable prioritization of the crops and regions of greatest need. Future O_3-FACE experiments can combine O_3 fumigation with other abiotic stresses, including drought, high temperature, nutrient deficiency, and elevated [CO_2]. These interactions are currently poorly understood at all scales, from molecular mechanisms to crop productivity. Satellites provide high-temporal and spatial resolution crop yield datasets that can be coupled with improved models of air quality to test how current pollution levels and mixtures of pollutants are impacting crop yields around the globe. Both of these approaches are useful for guiding future crop adaptation strategies.

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